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Metabolizable energy value of crude glycerin and effects on broiler performance and carcass yield

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HIGHLIGHTS

- The AMEN of crude glycerin is 2651 kcal/kg and 3013 kcal/kg for the initial and growth phases, respectively.
- Pelleted diets with crude glycerin improved broiler performance and weight gain in the pre-initial phase.
- Up to 46.30 g/kg of crude glycerin improves carcass yield and up to 120 g/kg reduces fat deposition.

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ABSTRACT

Two experiments were carried out to determine the metabolizable energy of crude glycerin (CG) and to evaluate the effect of adding different levels of CG on broiler performance and yield of carcass and cuts. The first consisted of a metabolism assay using total excreta sampling method aiming to determine the apparent metabolizable energy corrected for nitrogen balance (AMEN). This assay was performed in two phases: 10–18 days (initial phase) and 25–33 days of age (growth phase). The broilers were alloted in a randomized design with two treatments (reference diet and reference diet + 80 g/kg of CG inclusion) and nine replications, with ten birds per cage. The AMEN was 2651 kcal/kg and 3013 kcal/kg, for the initial and growth phases, respectively. In the second experiment, 1600 Cobb 500 male broilers from 1 to 42 days were alloted in a randomized block experimental design, with 8 treatments and 8 replications of 25 birds per experimental unit, according to factorial arrangement (2 feed forms × 4 levels of CG: 0, 40, 80 and 120 g/kg). At 43 days of age, three birds from each cage were slaughtered to evaluate yield of carcass and cuts in each treatment. There was a quadratic effect ($P < 0.01$) on feed intake and weight gain of glycerin fed birds, but no difference in feed conversion ($P>0.05$). Carcass and cut weights presented quadratic behavior ($P < 0.01$), but regarding yield, only quadratic effect ($P < 0.01$) was observed for carcass, linear increase ($P < 0.01$) for wings and linear reduction ($P < 0.01$) for fat. Using CG improved weight gain. Up to 46.30 g/kg CG improved carcass yield and up to 120 g/kg reduced fat deposition.

1. Introduction

Crude glycerin (CG) comes from the production of biodiesel (formed by glycerol, water, and methanol) and can be considered an energy by-product. For poultry diets CG can be used to minimize dependence on conventional ingredients in poultry diets, and reduce the environmental impact of agro-industrial waste. Arruda et al. (2007) reported that glycerin is regularly sold as a wetting additive, but many studies demonstrate the possibility of its use as an energy source in animal diets (Cerrate et al., 2006; Henz et al., 2014; Mandalawi et al., 2014; Papadomichelakis et al., 2015).

The potential use of CG as an energy ingredient to formulate diets destined to poultry is important as the metabolizable energy value of CG is proportional to its glycerol content. Guerra et al. (2011) found a linear

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decreasing effect on body fat as the inclusion of CG increased, while Mandalawi et al. (2014) observed that feed conversion ratio improved linearly as the CG content of the diet increased (2.5, 5.0, 7.5, and 10%) when using CG with up to 87% glycerol. However, increasing dietary glycerin above 10% has been shown to adversely affect growth performance and meat yield of broiler chickens (Simon et al., 1996; Cerrate et al., 2006). Dozier et al. (2011) when evaluating the effect of 10 different sources of glycerin, verified that glycerin is a good source of energy for broilers (ranging from 3254 to 4134 kcal/kg), and the AMEN of glycerin is dependent on glycerol, fatty acid, methanol, and water contents. Carcass yield has also influenced meat quality, for instance, Boonwong et al. (2018) evaluated up to 10.0% of CG from palm oil and verified that breast yield decreased from 25.8% to 23.2% comparing the control diet with the one added by 10% CG, while bone yield behaved differently by decreasing linearly from 27.8 to 31% with increasing CG levels. However De Souza et al. (2020) did not observe variation in carcass yield and cuts, regardless of CG levels added (0, 1, 3, 5, 7, and 9%).

By encouraging the increase of biofuel production in Brazil, and considering the fluctuating costs of commodities such as corn used in broiler diets, research about CG use is needed in order to reduce the dependence on corn use. Corn accounts for 65% of the diet, therefore by partially replacing corn by glycerin could reduce costs.

Although pelleting and the use of alternative sources, such as CG, may be a common practice adopted by the feed industries, requirement and feed evaluation research are mainly focused on mash diets, not corresponding to the reality in the field. As glycerin has the ability to improve the pellet, the aim of this study was to evaluate the performance and carcass yield of broilers fed pelleted or mash diets with the inclusion of increasing levels of CG.

2. Material and methods

All procedures involving the use of animals were submitted to the Ethics Committee on the Use of Animals (CEUA) of *Embrapa Suínos e Aves*, located at the National Center of Research for Swine and Poultry, CNPSA, in Concordia, SC, Brazil, and approved under N. 009/2011.

2.1. Experiment I – Metabolism

The first experiment was a metabolism assay for the determination of the apparent metabolizable energy corrected for nitrogen balance (AMEN) of the crude glycerin. The crude glycerin used was from soybean oil origin and its composition was 800 g/kg glycerol, 870 g/kg dry matter, 40 g/kg Cl (chlorine), 24 g/kg Na (sodium) and 3228 kcal/kg of gross energy. The experiment was carried out with male broiler chicks of the Cobb-500 strain at Embrapa Suínos e Aves, within the metabolism laboratory, in two phases: 10–18 days old (initial phase) and 25–33 days old (growth phase).

The birds were distributed in a completely randomized design, with two treatments, reference diet (RD; 0 inclusion of CG on Table 1 for each phase) and RD + 80 g/kg of CG inclusion in the mash diets and nine replicates, with ten birds per cage. In each step, the first four days were destined bird's adaptation to the experimental diets and then for five days, the total excreta took place. The excreta were stored in plastic bags, properly identified, weighed and stored in a freezer. At the end of the collection period, the samples were homogenized, and aliquots were withdrawn and placed in a forced circulation oven at 55°C for predrying. Subsequently, analyzes of excreta and animal diet were performed according to techniques described by Silva and Queiroz (2002).

At the end of the assay, the amount of diet consumed per experimental unit during the five days of collection was determined. Once the results of diet composition were obtained, the values of AMEN were calculated using equations proposed by Matterson et al. (1965).

The data were evaluated using descriptive analysis to verify the presence of outliers. Data analysis was carried out using SAS statistical software v. 9.4 (SAS Institute, Cary, NC, USA).

2.2. Experiment II – Performance and carcass quality

In this experiment, the effects of CG inclusion on the performance and carcass characteristics of broilers were evaluated. For that, 1600

Table 1
Experimental diets for the initial and growth phases.

Ingredients, g/kg	Crude glycerin levels, g/kg							
	Initial phase				Growth phase			
	0	40	80	120	0	40	80	120
Corn	573.3	529.3	485.4	436.6	620.1	576.1	531.6	482.7
Soybean meal	362.9	370.2	377.6	385.8	312.4	319.8	327.2	335.4
Glycerin	0.0	40.0	80.0	120.0	0.0	40.0	80.0	120.0
Soybean oil	25.4	24.5	23.6	24.3	34.3	33.3	32.6	33.4
Calcitic limestone	9.2	9.2	9.1	9.1	8.3	8.2	8.2	8.1
Dicalcium phosphate	15.0	15.0	15.1	15.1	11.8	11.8	11.9	12.0
Salt	4.8	2.4	0.0	0.0	4.5	2.1	0.0	0.0
L-Lysine HCl	1.7	1.6	1.4	1.3	1.7	1.5	1.4	1.3
DL-Methionine	2.7	2.8	2.8	2.9	2.4	2.4	2.5	2.5
L-Threonine	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3
Mycotoxin adsorber[a]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral Premix[b]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin Premix [c]	1.0	1.0	1.0	1.0	0.8	0.8	0.8	0.8
Choline chloride 60%	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
BHT [d]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calculated composition								
AMEN, kcal/kg	3,000	3,000	3,000	3,000	3,120	3,120	3,120	3,120
CP, g/kg	212.6	212.5	212.4	212.2	193.2	193.0	192.9	192.7
Digestible lysine, g/kg	11.7	11.7	11.7	11.7	10.5	10.5	10.5	10.5
Crude Fiber, g/kg	29.1	28.8	28.4	28.0	27.3	26.9	26.5	26.1
Na, g/kg	2.1	2.1	2.1	3.0	2.0	2.0	2.1	3.0

[a] Mycosorb®
[b] Mineral Premix (kg): Fe, 100 g; Cu, 20 g; Co, 2 g; Mn, 160 g; Zn, 100 g; I, 2 g.
[c] Vitamin Premix (kg): vit. A, 9000000 U.I.; vit. D_3, 2500000 U.I.; vit. E, 20000 U.I.; vit B_1, 1.5 g; vit B_2, 6.0 g; vit B_6, 3.0 g; vit B_{12}, 0.012 g; Pantothenic acid, 12.0 g; Biotin, 0.06 g; vit K_3, 2.5 g; Nicotinic acid, 25.0 g; Se, 250.0 mg.
[d] Butylhydroxytoluene (antioxidant).

male broiler chicks (approximately 44.5 g of initial body weight) of the Cobb-500 strain were used. The experiment started with a 1-day-old chick and lasted up until broilers achieved 42 days of age. For the treatments, 4 experimental diets were formulated for each phase (Table 1) to meet the nutritional requirements recommended by Rostagno et al. (2011). The diets were based on corn and soybean meal, with increasing levels of CG (0, 40, 80 and 120 g/kg) added in the diet. The values of AMEN from CG considered here were those calculated in Experiment I - Metabolism.

The experimental design applied was in blocks (based on the initial weight) at random with 8 treatments and 8 repetitions of 25 birds, according to a 2 × 4 factorial arrangement (2 feed forms × 4 CG inclusion levels: 0, 40, 80 and 120 g/kg). Animal handling was conducted according to the specific Cobb strain manual, and the mortality record was used to correct the data. For the pelleted treatment, the chicks received a crumbled diet until 10 days old and the pellet size after that time was approximately 1.0 cm. The birds and diets were weighed at 7, 21 and 42 days to evaluate feed intake, weight gain, coefficient of variation (CV) of the final weight of the birds and feed conversion. To evaluate carcass yield (in relation to live weight) and cuts (in relation to carcass weight) in each treatment either when broilers were fed mash or pelleted diets at increasing CG levels, at 43 days of age, three birds from each cage were slaughtered. Then, each cut (breast, fillet, thigh, drumstick, wings, back, fat, liver, gizzard, and heart) was weighed and divided by the whole carcass weight. Data analysis was carried out using PROC GLM SAS (SAS, 2012), and means were compared by SNK's test at the 5% probability level. When significant, data referring to the different inclusion levels were evaluated by polynomial regression.

3. Results

3.1. Experiment I – Metabolism

In the first experiment, the glycerin AMEN was 2651 kcal/kg in the initial phase and 3013 kcal/kg in the growing phase (Table 2).

3.2. Experiment II – Performance and carcass quality

There was interaction ($P < 0.05$) between the feed form and CG inclusion levels in the diet for feed conversion (FC) in the first week; feed intake (FI) at the 2nd and 3rd weeks and at FI and weight gain (WG) considering the whole experimental period (1–42 days), showing different responses with glycerin inclusion according to the processing type (Table 3).

Although the interaction was significant, the FI in the total period (1–42 days) was better adjusted by quadratic equations with maximum point for both mash diets ($Y = 4776.88621 + 36.86502X - 4.4977X^2$; $R^2 = 0.9999$) and pelleted diets ($Y = 5127.13858 + 16.38161X - 4.463945X^2$; $R^2 = 0.9973$). The derivation of the equations allowed to estimate the maximum FI with the levels of 41 and 18 g/kg of glycerin inclusion for mash and pelleted diets, respectively. Although the interaction was significant, the WG was better adjusted by quadratic equations with a maximum point for both mash diets ($Y = 2989.78437 + 47.7364X - 4.54727X^2$; $R^2 = 0.9933$) and pelleted diets ($3231.79747 + 25.46384X - 4.20839X^2$; $R^2 = 0.9961$). The derivation of the equations allowed to estimate the maximum WG with CG inclusion levels of 53 and 30 g/kg for mash and pelleted diets, respectively. The FC showed adjusted significant interaction by quadratic equations with optimal value ($Y = 1.59274 - 0.01024X + 0.00077199X^2$; $R^2 = 0.8643$). The derivation of the equation allowed to estimate the best gains in FC with the level of 66 g/kg of CG inclusion for both diet types.

There was a quadratic effect ($P < 0.01$) with a minimum point for CV of the bird's final weight ($Y = 7.72636 - 0.36211X + 0.04381X^2$; $R^2 = 0.8796$), the lowest CV was estimated with the level of 41 g/kg of CG inclusion.

3.4. Pre-initial phase

In the pre-initial phase (1 to 7 days old), the pelleted diets promoted greater WG and better FC ($P < 0.05$) (Table 4). Glycerin levels promoted linear increase ($Y = 134.16196 + 0.48648X$; $R^2=0.7904$) on WG for both treatments. However, the interaction effect of the glycerin levels on FC was not observed in the pelleted diets, while for mash diets this interaction was better adjusted by quadratic equations with optimum point ($Y = 1.16758 - 0.01563X + 0.00081831X^2$; $R^2=0.9025$). The derivation of the equation allowed estimating the best gain in FC with a level of 96 g/kg of CG inclusion.

3.5. Initial phase

In the initial phase, 8 to 21 days of age, there was no effect ($P > 0.05$) of the treatments for FC, while for FI and WG were higher ($P < 0.05$) for pelleted diets (Table 5). The increase in CG inclusion in the diets promoted a quadratic response, with a maximum point for FI in the mash diet ($Y = 1079.91999 + 12.05608X - 0.96825X^2$; $R^2 = 0.9943$) and pelleted diet ($Y = 1204.53041 + 7.48487 - 1.25086X^2$; $R^2 = 0.9329$). The derivation of the equations made it possible to estimate the maximum FI points at 62 and 30 g/kg of CG for mash and pelleted diets, respectively. Like the FI, the WG in the phase was better adjusted with the quadratic model with maximum point ($Y = 820.66846 + 13.18302X - 1.22827X^2$; $R^2 = 0.9683$). The derivation of the equation allowed estimating the maximum WG, with the inclusion of 54 g/kg CG in both diet types.

3.6. Growth phase

In the growth phase, 22 to 42 days of age, the responses obtained showed similar behavior to the previous phases with positive effects of the pelleted diet for FI and WG ($P < 0.05$) (Table 6). In that phase, the use of CG in the diets promoted a quadratic effect with a maximum point for FI ($Y = 3658.08277 + 17.38116X - 3.50881X^2$; $R^2 = 0.9999$) and WG ($Y = 2156.18942 + 22.75892X - 3.13525X^2$; $R^2 = 0.9790$). The maximum FI and WG points were estimated with the inclusion of 25 e 36 g/kg of CG in the diets.

3.7. Carcass characteristics

Carcass yield showed significant interaction (Table 7) adjusted with quadratic equations with an optimal point ($Y = 75.84521 + 0.16325X - 0.02156X^2$; $R^2 = 0.9930$). The derivation of the equation allowed to estimate the best gains in carcass yield with the inclusion level of 38 g/kg CG in the diets. Glycerin levels promoted linear increase ($Y = 9.75457 + 0.02604X$; $R^2 = 0.8588$) in wing yield, and linear reduction ($Y = 2.18009 - 0.04889X$; $R^2 = 0.9350$) in body fat.

Similar to carcass yield, gizzard yield showed significant interaction adjusted by quadratic equations with an optimal point ($Y = 1.42728 - 0.02644X + 0.00262X^2$; $R^2 = 0.9923$). The derivation of the equation allowed estimating the maximum gizzard yield, with the inclusion of 51 g/kg CG in both diet types. The interaction of CG on heart yield was better adjusted by quadratic equations with an optimal point ($Y = 0.65223 - 0.00969X + 0.00145X^2$; $R^2 = 0.8481$). The derivation of the

Table 2
Apparent metabolizable energy corrected for nitrogen balance (AMEN) of reference diet and crude glycerin (on as-fed basis).

	Initial Phase	Growth phase
	AMEN, kcal/kg	
Reference Diet	2992[a] ± 19	3116[b] ± 10
Crude Glycerin	2651 ± 20	3013 ± 13

[a] Diet formulated with 3,000 kcal/kg
[b] Diet formulated with 3,120 kcal/kg

Table 3
Performance of broilers with increasing levels of crude glycerin (CG) (g/kg) and different feed forms (FF) from 1 to 42 days old.

	0	40	80	120	Mean	Effects		
	Feed intake (g)					FF	CG	FF × CG
Mash	4777 ± 30	4852 ± 37	4784 ± 52	4572 ± 19	4746 ± 26			
Pelleted	5123 ± 24	5132 ± 22	4948 ± 54	4660 ± 53	4966 ± 39			
Mean	4950 ± 48	4992 ± 42	4866 ± 42	4616 ± 29		<0.001	<0.001	0.005 QMP
	Weight Gain (g)							
Mash	2993 ± 31	3099 ± 12	3089 ± 21	2905 ± 21	3022 ± 18			
Pelleted	3235 ± 22	3255 ± 20	3177 ± 32	2928 ± 37	3149 ± 27			
Mean	3114 ± 36	3177 ± 23	3133 ± 22	2916 ± 21		<0.001	<0.001	0.001 QMP
	Feed conversion (g/g)							
Mash	1.60 ± 0.02	1.57 ± 0.01	1.55 ± 0.02	1.57 ± 0.01	1.57 ± 0.01			
Pelleted	1.58 ± 0.01	1.58 ± 0.01	1.56 ± 0.01	1.59 ± 0.02	1.59 ± 0.01			
Mean	1.59 ± 0.01	1.57 ± 0.01	1.55 ± 0.01	1.58 ± 0.01		0.501	0.034 Q	0.646
	Weight variation coefficient (%)							
Mash	7.27 ± 0.70	7.55 ± 0.70	7.25 ± 0.50	9.36 ± 0.80	7.86 ± 0.40			
Pelleted	7.85 ± 0.50	7.41 ± 0.50	7.01 ± 0.50	10.36 ± 1.00	8.16 ± 0.40			
Mean	7.56 ± 0.40	7.48 ± 0.40	7.13 ± 0.40	9.86 ± 0.60		0.515	0.001 Q	0.739

[Q] Quadratic
[QMP,] Quadratic Mash and Pelleted.

Table 4
Performance of broilers with increasing levels of crude glycerin (CG) (g/kg) and different feed forms (FF) from 1 to 7 days old.

	0	40	80	120	Mean	Effects		
	Feed intake (g)					FF	CG	FF × CG
Mash	149 ± 2	148 ± 2	148 ± 1	150 ± 1	148.7 ± 1			
Pelleted	154 ± 3	155 ± 2	152 ± 3	156 ± 3	154 ± 1			
Mean	152 ± 2	151 ± 2	150 ± 2	153 ± 2		0.001	0.648	0.851
	Weight Gain (g)							
Mash	127 ± 2	134 ± 2	134 ± 1	137 ± 1	133 ± 1[b]			
Pelleted	140 ± 2	142 ± 2	140 ± 2	144 ± 2	141 ± 1[a]			
Mean	133 ± 2	138 ± 2	137 ± 1	140 ± 2		<0.001	0.012 L	0.467
	Feed conversion (g/g)							
Mash	1.17 ± 0.01	1.11 ± 0.01	1.11 ± 0.01	1.09 ± 0.01	1.12 ± 0.01[b]			
Pelleted	1.10 ± 0.01	1.09 ± 0.01	1.09 ± 0.01	1.09 ± 0.01	1.09 ± 0.01[a]			
Mean	1.14 ± 0.01	1.10 ± 0.01	1.10 ± 0.01	1.09 ± 0.01		<0.001	<0.001	0.009 QM

[a,b] Means followed by different lowercase letters, in the same column, differ from each other by the SNK test (P<0.05).
[QM,] Quadratic Mash.

Table 5
Performance of broilers with increasing levels of crude glycerin (CG) (g/kg) and different feed forms (FF) from 8 to 21 days old.

	0	40	80	120	Mean	Effects		
	Feed intake (g)					FF	CG	FF × CG
Mash	1079 ± 8.2	1114 ± 5.0	1113 ± 3.8	1086 ± 10.7	1098 ± 4.5[b]			
Pelleted	1200 ± 13.3	1229 ± 6.9	1170 ± 13.0	1119 ± 8.8	1179 ± 8.9[a]			
Mean	1140 ± 17.3	1171 ± 15.3	1142 ± 9.9	1102 ± 8.0		<0.001	<0.001	<0.001 QMP
	Weight Gain (g)							
Mash	776 ± 24	817 ± 19	823 ± 16	794 ± 17	802 ± 10[b]			
Pelleted	862 ± 16	901 ± 15	863 ± 14	814 ± 16	860 ± 9[a]			
Mean	819 ± 18	859 ± 16	843 ± 12	804 ± 11		<0.001	0.007 Q	0.113
	Feed conversion (g/g)							
Mash	1.40 ± 0.05	1.37 ± 0.03	1.37 ± 0.02	1.37 ± 0.04	1.38 ± 0.02			
Pelleted	1.40 ± 0.03	1.37 ± 0.03	1.36 ± 0.03	1.30 ± 0.04	1.38 ± 0.02			
Mean	1.40 ± 0.03	1.37 ± 0.02	1.36 ± 0.02	1.38 ± 0.03		0.98	0.627	0.996

[a,b] Means followed by different lowercase letters, in the same column, differ from each other by the SNK test (P<0.05).
[Q] Quadratic.

equation allowed estimating the best heart yield with an inclusion level of 33 g/kg of CG.

4. Discussion

4.1. Glycerin composition

On the basis of the considerable variation in nutritional composition between the crude glycerin (CG) products, it is critical that confirmatory nutritional analyses be conducted on every glycerin sample before it is given in animal diet (Jung and Batal, 2011). Our glycerin samples showed 800 g/kg glycerol. However, many studies evaluated different sources of CG such as palm oil (Boonwong et al., 2018), soybean oil (Cufadar et al., 2016; Dozier et al., 2011; Mandalawi et al., 2014), tallow, yellow grease or poultry fat (Dozier et al., 2011) or even mixed CG 80:20 vegetable fat:animal fat (Duarte et al., 2014). Dozier studied six sources of CG from soybean oil and found small variation in glycerin content (83.9% on average), however animal-origin samples from

Table 6
Performance of broilers with increasing levels of crude glycerin (CG) (g/kg) and different feed forms (FF) from 22 to 42 days old.

	0	40	80	120	Mean	Effects		
	Feed intake (g)					FF	CG	FF × CG
Mash	3548 ± 36	3590 ± 37	3523 ± 52	3336 ± 16	3499 ± 25[b]			
Pelleted	3769 ± 20	3749 ± 18	3626 ± 47	3385 ± 49	3632 ± 33[a]			
Mean	3,659 ± 35	3,670 ± 29	3,574 ± 36	3,361 ± 25		<0.001	<0.001 Q	0.1137
	Weight gain (g)							
Mash	2,090 ± 32	2,149 ± 28	2,133 ± 34	1,974 ± 32	2,086 ± 19[b]			
Pelleted	2,234 ± 25	2,212 ± 22	2,175 ± 37	1,970 ± 43	2,148 ± 24[a]			
Mean	2,162 ± 27	2,181 ± 19	2,154 ± 25	1,972 ± 26		0.007	<0.001 Q	0.130
	Feed conversion (g/g)							
Mash	1.70 ± 0.02	1.67 ± 0.02	1.65 ± 0.04	1.63 ± 0.03	1.68 ± 0.01			
Pelleted	1.69 ± 0.02	1.70 ± 0.02	1.67 ± 0.02	1.72 ± 0.03	1.69 ± 0.01			
Mean	1.69 ± 0.01	1.68 ± 0.01	1.66 ± 0.02	1.71 ± 0.02		0.406	0.270	0.834

[a,b] Means followed by different lowercase letters, in the same column, differ from each other by the SNK test (P<0.05).
[Q] Quadratic.

Table 7
Yield (%) of cuts of broilers fed with increasing levels of crude glycerin (CG) (g/kg) and different feed forms (FF)

		Crude Glycerin levels, g/kg					Effects			
		0	40	80	120	Mean	FF	CG	FF × CG	CV (%)
Carcass	Mash	76.19 ± 0.46	75.97 ± 0.42	75.64 ± 0.20	73.98 ± 0.28	75.44 ± 0.23				
	Pelleted	75.54 ± 0.48	76.22 ± 0.29	76.02 ± 0.24	75.37 ± 0.34	75.79 ± 0.18				
	Mean	75.87 ± 0.33	76.09 ± 0.25	75.83 ± 0.16	74.68 ± 0.28		0.168	0.001 Q	0.046	1.53
Breast	Mash	40.33 ± 0.34	40.12 ± 0.43	40.48 ± 0.43	40.34 ± 0.55	40.32 ± 0.21				
	Pelleted	39.81 ± 0.29	40.76 ± 0.75	41.04 ± 0.35	40.69 ± 0.47	40.57 ± 0.25				
	Mean	40.07 ± 0.23	40.44 ± 0.43	40.76 ± 0.28	40.51 ± 0.35		0.441	0.518	0.578	3.23
Fillet	Mash	31.25 ± 0.32	31.09 ± 0.38	31.15 ± 0.48	30.76 ± 0.65	31.06 ± 0.23				
	Pelleted	30.55 ± 0.33	31.93 ± 0.80	31.91 ± 0.41	31.72 ± 0.46	31.53 ± 0.27				
	Mean	30.90 ± 0.24	31.51 ± 0.44	31.53 ± 0.32	31.24 ± 0.41		0.199	0.564	0.323	4.57
Thigh	Mash	12.74 ± 0.17	12.95 ± 0.25	12.80 ± 0.11	13.11 ± 0.26	12.90 ± 0.10				
	Pelleted	12.99 ± 0.19	12.68 ± 0.19	12.63 ± 0.21	13.10 ± 0.26	12.85 ± 0.11				
	Mean	12.87 ± 0.13	12.81 ± 0.16	12.72 ± 0.12	13.10 ± 0.18		0.736	0.298	0.615	4.58
Drumstick	Mash	18.43 ± 0.20	18.66 ± 0.22	18.35 ± 0.26	18.54 ± 0.17	18.49 ± 0.10				
	Pelleted	18.84 ± 0.16	18.64 ± 0.28	18.60 ± 0.14	18.43 ± 0.28	18.63 ± 0.11				
	Mean	18.63 ± 0.13	18.65 ± 0.17	18.47 ± 0.15	18.48 ± 0.16		0.349	0.710	0.533	3.26
Wing	Mash	9.77 ± 0.10	9.94 ± 0.11	10.08 ± 0.10	10.30 ± 0.12	10.02 ± 0.06[a]				
	Pelleted	9.83 ± 0.13	9.67 ± 0.13	9.76 ± 0.09	9.93 ± 0.13	9.80 ± 0.06[b]				
	Mean	9.80 ± 0.08	9.81 ± 0.12	9.92 ± 0.05	10.11 ± 0.20		0.009	0.037 L	0.257	3.60
Back	Mash	16.14 ± 0.15	16.22 ± 0.24	16.36 ± 0.23	16.13 ± 0.15	16.21 ± 0.09				
	Pelleted	16.03 ± 0.13	15.95 ± 0.25	15.94 ± 0.14	16.08 ± 0.27	16.00 ± 0.10				
	Mean	16.09 ± 0.10	16.09 ± 0.17	16.15 ± 0.14	16.11 ± 0.15		0.132	0.988	0.781	3.43
Fat	Mash	2.09 ± 0.16	1.98 ± 0.16	1.92 ± 0.14	1.43 ± 0.19	1.85 ± 0.09				
	Pelleted	2.33 ± 0.12	1.83 ± 0.11	1.81 ± 0.21	1.71 ± 0.10	1.92 ± 0.08				
	Mean	2.21 ± 0.10	1.91 ± 0.10	1.87 ± 0.12	1.57 ± 0.11		0.562	0.003 L	0.393	25.53
Liver	Mash	2.38 ± 0.04	2.28 ± 0.06	2.41 ± 0.06	2.49 ± 0.09	2.39 ± 0.04				
	Pelleted	2.39 ± 0.08	2.40 ± 0.06	2.31 ± 0.08	2.41 ± 0.07	2.38 ± 0.04				
	Mean	2.38 ± 0.04	2.34 ± 0.05	2.36 ± 0.05	2.45 ± 0.06		0.814	0.440	0.431	8.26
Gizzard	Mash	1.49 ± 0.05	1.42 ± 0.03	1.47 ± 0.06	1.65 ± 0.06	1.51 ± 0.03[a]				
	Pelleted	1.36 ± 0.04	1.32 ± 0.04	1.29 ± 0.04	1.33 ± 0.05	1.33 ± 0.02[b]				
	Mean	1.43 ± 0.03	1.37 ± 0.033	1.38 ± 0.04	1.49 ± 0.06		<0.001	0.037 Q	0.074	11.82
Heart	Mash	0.65 ± 0.03	0.64 ± 0.02	0.67 ± 0.04	0.77 ± 0.05	0.68 ± 0.02				
	Pelleted	0.64 ± 0.03	0.68 ± 0.04	0.62 ± 0.03	0.73 ± 0.02	0.67 ± 0.02				
	Mean	0.64 ± 0.02	0.66 ± 0.02	0.64 ± 0.02	0.75 ± 0.03		0.464	0.001 Q	0.288	14.34

[a,b] Means followed by different lowercase letters, in the same column, differ from each other by the SNK test (P <0.05).
[L] Linear.
[Q] Quadratic.

tallow, yellow grease, and poultry fat showed high variation in glycerin content (51.5–93.9%). Also, different glycerin composition is found in the literature, with varying concentrations of glycerol, water, and fatty acid profile. For instance, no effect on performance was observed with the addition to the diet of 10% CG that contained 3.10% methanol (Jung and Batal, 2011) whereas the addition of 10% of a different CG product with a much lower methanol content (< 0.01% methanol) significantly depressed BW gain but did not affect feed intake or G:F. The levels of CG inclusion also matter and cause implications in different variables such as increased excreta moisture with increasing levels of CG inclusion (Duarte et al., 2014); decreased feed intake and BW gain in certain periods of birds live (0–15 days; 35–42 days; 0–42 days) by including up to 7.5% CG while also increasing BW (Jung and Batal, 2011); decreased effect on body fat as the inclusion of CG increased (Guerra et al., 2011); feed conversion ratio improvement with crescent CG inclusion containing up to 87% glycerol (Mandalawi et al., 2014).

4.2. Apparent metabolizable energy values

The value of the AMEN determined for the initial phase (2651 kcal/kg) and growth phase (3013 kcal/kg) corresponded to 0.82 and 0.94 of the CG gross energy (3228 kcal/kg), which confirms the potential of this ingredient as an energy source in broiler diets. The AMEN for CG are close to the AMEN values reported for corn (3381 kcal/kg) (Rostagno et al., 2011), which is the main energy component of broiler diets. The AMEN value determined for the initial phase was lower than the one mentioned by Henz et al. (2014), who evaluated different levels of crude glycerin inclusion in the initial phase (11–20 days) and found an average value of 3262 kcal/kg. As found in the initial phase, the AMEN value determined for the growth phase was lower than that mentioned by Henz et al. (2014), that found an average value of 3713 kcal/kg, when evaluating different levels of CG inclusion in the growth phase (21–30 days). The lower metabolization of CG in the initial and growth phases is due to broiler immaturity, provoking renal reabsorption reduction, once it is not reabsorbed, it is excreted together in the urine (Henz et al., 2014). However, the variation of raw materials used in biodiesel production and the lack of standardization in this process influence the chemical composition of crude glycerin (Zavarize et al., 2014), which hinders the establishment of expected standards for this ingredient.

Zavarize et al. (2014) carried out a metabolism test to determine the AMEN of four CG samples from different raw materials, in broilers from 21 to 29 days of age, due to the need to know the nutritional composition of this alternative ingredient. The authors noted that AMEN was 3,145, 5,026, 2,828 e 2,892 kcal/kg, being this variation attributed to the composition of glycerol and fatty acids present in each sample. Henz et al. (2014) still points out that the sodium content and the glycerol amount may favor the increase of the excreta produced, which can lead to a decrease in the values of AMEN.

4.3. Performance

The linear increase in WG in the first week is similar to the results reported by Silva et al. (2012), that included up to 100 g/kg of CG in the broiler diets and observed linear increase in FI and WG at the pre-initial phase (1–7 days), corroborating with the findings of the present study. The results obtained in the pre-initial phase may be associated with the physical benefits caused by CG inclusion once the diets were calculated to be isoenergetic. The WG response can be attributed to glycerol concentration in the diet, because it improves texture and reduces ground thin particles in diets (Silva et al., 2012).

The use of CG in pelleted diets up to 4 g/kg did not alter the other performance variables of the birds. Similar results were found by Silva et al. (2019) when evaluating increasing levels of CG inclusion (0, 20, 40 and 60 g/kg) in broiler diets. These authors did not verify effects on performance variables. According to Silva et al. (2012), 50 g/kg glycerin inclusion in the diet did not affect broiler performance during the breeding period (1–42 days). Papadomichelakis et al. (2015) revealed that CG addition to broiler diets affected growth performance in a quadratic manner when they were given CG at 70 g/kg in the diet showing the greatest final body weight, 15% greater than those fed the control diet and 33% greater than broiler fed 210 g/kg of CG in the diet. Attention should be given to the inclusion of glycerin in broiler diets because the maximum recommended levels are 50 and 80 g/kg for the initial and growth phases, respectively (Rostagno et al. 2011). Elevated levels of glycerin in the diet may affect triglyceride metabolism, impair biochemical and physiological functions, such as surpass the capacity of glycerol kinase enzyme, limiting its absorption and promoting the highest rate of digestion passage (Sousa et al., 2015). This characteristic can explain the quadratic behaviors with maximum points obtained for the variables from the 2nd week onwards, which showed excellent points followed by worsening. However, other studies should be conducted to evaluate the rate of diet passage with increasing glycerol concentration to confirm the hypothesis presented herein.

4.4. Carcass characteristics

From all the evaluated cuts, the significant variables that were affected by the CG inclusion in a linear way were fat and wings. Also, the pelletization process reduced the yield of wings (from 10 to 9.8%) and gizzard (from 1.5 to 1.3%). As CG was increased from 0 to 120 g/kg, the fat yield decreased linearly from 2.2 to 1.6% while wing yield increased from 9.8 to 10.1%. This same effect of CG on fat was also observed by other authors. Guerra et al. (2011) found a linear decreasing effect on body fat as the inclusion of crude glycerin increased, the authors attributed this factor to an underestimation of glycerin energy value. Another issue to be considered is the amount and type of residual glycerin fat (according to the source or production process), which can result in different results for the fat content in broiler carcasses. Legawa et al. (2018) in a study with the inclusion of crude glycerin from palm oil, which has higher amounts of palmitic (C16:0), stearic (C18:0) and oleic (C18:1) acids, when compared to soybean oil (Machado et al., 2008), identified that the body fat of broilers increased due to the inclusion of palm oil glycerin in the diet. Boonwong et al. (2018) evaluated 0, 2.5%, 5.0%, 7.5% and 10.0% of CG from palm oil and evaluated yields of some broiler cuts, noticing that breast yield decreased from 25.8% to 23.2% only when 10% CG was given in the diet, although between 0 and 7.5% CG no significant difference was observed. Bone yield behaved differently by decreasing linearly from 27.8 to 31% with increasing CG levels.

5. Conclusion

The AMEN of crude glycerin for broilers at 10–18 days of age is 2651 kcal/kg and for 25–33 days of age is 3013 kcal/kg. Pelleted diets with crude glycerin improved broiler performance in the pre-initial phase, improved weight gain and decreased fat deposition. Therefore, crude glycerin can be recommended to compose up to 46.30 g/kg of the mash or pelleted diets, without affecting broiler performance.

Author statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in Livestock Science.

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