Effect of dietary glycerin supplementation in the starter diet on broiler performance

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ABSTRACT: This study was conducted to evaluate the performance, carcass composition, and litter moisture of broiler chicks fed crude glycerin for 1–21 days. The study used 1056 male birds distributed in a completely randomized design, with six treatments (0, 3, 6, 9, 12, and 15% crude glycerin from 1 to 10 days), with eight replicates. After the 10th day, each treatment group was divided into two groups out of which one continued to receive the same glycerin level and the second group started to receive a glycerin-free diet. The parameters weight gain, feed intake, feed : gain, and livability during the first 10 days exhibited a quadratic response (P < 0.05), which predicted higher values at crude glycerin levels of 9.01, 9.02, 9.03, and 6.43%, respectively. From day 1 to day 21, the group receiving crude glycerin throughout the experiment showed a quadratic effect (P < 0.05) for weight gain, feed intake, feed : gain, and livability, with higher values at crude glycerin levels of 6.06, 7.97, 13.11, and 7.69%. As glycerin levels increased, the litter moisture increased linearly (P < 0.05) for both periods. The protein and fat deposition rates and dry matter of the carcasses were not affected (P > 0.05). Considering the period from day 1 to day 21, inclusion of up to 6.06% crude glycerin in the diet provided the best weight gain without affecting the birds' performance, the rate of protein and fat deposition on the carcass, or litter moisture compared with birds fed a glycerin-free diet.

Keywords: biodiesel; byproducts; carcass composition; glycerol

INTRODUCTION

The use of renewable fuels is notably expanding. Reports indicate that approximately 10% of the total weight of sources (plant or animal) used as raw material for biodiesel production becomes crude glycerin, a byproduct of this process (Dasari et al. 2005).

This byproduct has a low market value due to impurities, therefore filtration and refining processes are necessary for its further exploitability, especially in pharmaceutical, cosmetics, and food industries. However, these additional procedures increase the cost (Min et al. 2010). Thus, several studies on the inclusion of glycerin in its raw form in animal diets have been carried out to reduce the cost of production without affecting the performance (Jung and Batal 2011; Retore et al. 2012). Additional advantages deriving from the use of crude glycerin in animal diets have also been reported, such as the improvement of growth, economic performance, and feed conversion ratio (Sehu et al. 2013).

The energy content and absorption rate of glycerol, a major component of glycerin, are considered high, thus facilitating the production of energy by animals and serving as a potential alternative source of energy in animal diets (Swiatkiewicz and Koreleski 2009). In broilers, Dozier et al. (2008) found that the efficiency of energy use was approximately 95% of gross energy, depending on methanol, fatty acid, and glycerol contents of the sample (Dozier et al. 2011), and the efficiency might vary according to the manufacturing process adopted (Retore et al. 2012).

Although several authors have demonstrated beneficial effects of glycerol from the viewpoint of weight gain, feed intake, and feed conversion ratio (Simon et al. 1996; Suchy et al. 2011), there are still few studies correlating these effects with changes in body composition of these animals, as well as the existence of possible interference of age and levels of inclusion on the nutritional value of glycerin. There is evidence that the efficiency of utilization of glycerin is directly related to the species and age of the animal and inclusion levels of glycerin (Cerrate et al. 2006; Hanczakowska et al. 2010).

Thus, the aim of this study was to evaluate the performance, carcass composition, and litter moisture of broiler chicks fed different levels of crude glycerin, during a 21-day period.

MATERIAL AND METHODS

The study was performed in Marechal Cândido Rondon, Brazil, at 24°31'53''S latitude and 54°01'23''W longitude, at 377 m a.s.l., and lasted for 21 days. In all, 1056 1-day-old male COBB 500®

Table 1. Ingredients and nutrient composition of the experimental diets

	1–10 days						11–21 days					
	0%	3%	6%	9%	12%	15%	0%	3%	6%	9%	12%	15%
Ingredients (%)												
Corn	54.10	51.06	47.89	44.58	40.85	37.23	53.89	50.90	47.80	44.27	40.60	36.99
Soybean meal (45%)	39.14	39.70	40.27	40.85	41.52	42.18	38.60	39.14	39.69	40.32	40.98	41.63
Crude glycerin	0.00	3.00	6.00	9.00	12.00	15.00	0.00	3.00	6.00	9.00	12.00	15.00
Soybean oil	2.36	2.05	1.85	1.70	1.77	1.74	3.80	3.45	3.20	3.20	3.23	3.20
Limestone	0.91	0.91	0.90	0.90	0.90	0.89	0.96	0.95	0.95	0.94	0.94	0.93
Dicalcium phosphate	1.87	1.88	1.88	1.89	1.89	1.90	1.48	1.50	1.50	1.50	1.51	1.51
NaCl	0.51	0.31	0.12	0.00	0.00	0.00	0.48	0.29	0.10	0.00	0.00	0.00
DL-Methionine (98%)	0.361	0.363	0.366	0.370	0.373	0.376	0.289	0.292	0.295	0.298	0.301	0.304
L-Lysine HCl (78.5%)	0.276	0.265	0.254	0.243	0.230	0.217	0.157	0.147	0.136	0.124	0.111	0.099
L-Threonine (98%)	0.102	0.102	0.101	0.101	0.101	0.100	0.040	0.040	0.040	0.039	0.039	0.038
L-Valine	0.071	0.070	0.069	0.069	0.068	0.067	–	–	–	–	–	–
Supplementary minerals and vitamins[1]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride (60%)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Anticoccidial[2]	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Growth promoter[3]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Antioxidant[4]	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Calculated composition												
Metabolizable energy (kcal/kg)	2.960	2.960	2.960	2.960	2.960	2.960	3.050	3.050	3.050	3.050	3.050	3.050
Crude protein (g/kg)	225.50	225.50	225.50	225.50	225.50	225.50	220.40	220.40	220.40	220.40	220.40	220.40
Ca (g/kg)	9.20	9.20	9.20	9.20	9.20	9.20	8.41	8.41	8.41	8.41	8.40	8.41
Avaiable P (g/kg)	4.70	4.70	4.70	4.70	4.70	4.70	4.01	4.01	4.01	4.01	4.01	4.01
Na (g/kg)	2.20	2.20	2.20	2.48	3.25	4.01	2.10	2.10	2.10	2.48	3.25	4.01
Digestible lysine (g/kg)	13.24	13.24	13.24	13.24	13.24	13.24	12.17	12.17	12.17	12.17	12.17	12.17
Digestible methionine + cysteine (g/kg)	9.53	9.53	9.53	9.53	9.53	9.53	8.76	8.76	8.76	8.76	8.76	8.76
Digestible threonine (g/kg)	8.61	8.61	8.61	8.61	8.61	8.61	7.91	7.91	7.91	7.91	7.91	7.91
Digestible valine (g/kg)	10.20	10.20	10.20	10.20	10.20	10.20	9.38	9.40	9.40	9.40	9.41	9.42
Dietary electrolyte balance	21.88	22.13	22.33	22.55	22.86	23.16	21.61	21.84	22.03	22.27	22.83	22.88

[1]content per kg of premix: Mg 16.0 g, Fe 100.0 g, Zn 100.0 g, Cu 2.0 g, Co 2.0 g, I 2.0 g, Se 0.25 g, vit. A 10 000 000 IU, vit. D_3 2 000 000 IU, vit. E 30 000 IU, vit. B_1 2.0 g, vit. B_6 4.0 g, pantothenic acid 12.0 g, biotin 0.10 g, vit. K_3 3.0 g, folic acid 1.0 g, nicotinic acid 50.0 g, vit. B_{12} 15 000 µg

[2]Salinocycin 12%, [3]Avilamycin 10%, [4]hydroxy butyl toluene

chicks with average initial body weight of 46.03 ± 0.07 g were used in this study. The birds were cared according to the Ethical Principles for Animal Experimentation established by the Brazilian Society of Laboratory Animal Science (SBCAL) and National Council for the Control of Animal Experimentation (CONCEA).

The chicks were distributed in a completely randomized design, with six treatments with different inclusion levels of crude glycerin (0, 3, 6, 9, 12, and 15%) in the diet at pre-starter stage (1–10 days old) and eight replicates of 22 birds per experimental unit. After 10 days, each treatment group in the pre-starter stage was divided randomly into two groups – one continued to receive the same level of crude glycerin as in the previous phase and the other started to receive the control diet (without inclusion of crude glycerin), making up 11 treatments with four replicates of 22 birds per experimental unit. The experimental diets (Table 1) were isonitrogenous and isoenergetic and were formulated to meet the nutritional requirements proposed by Rostagno et al. (2011).

The broilers and feed were weighed at 1, 10, and 21 days of age for performance evaluation, which was measured as the feed intake, weight gain, feed : gain, and livability. Broiler mortality was recorded daily to correct the feed conversion per pen.

The average temperature and relative air humidity monitored daily inside the shed were 26.5°C and 56%, and 24.7°C and 61% for the periods from 1 to 10 and 11 to 21 days, respectively.

At the end of the experimental period (21 days), two birds per experimental unit with representative weight (mean ± 10%) were slaughtered by cervical dislocation and plucked for protein and fat deposition calculations according to methodology adapted from Fraga et al. (2008). Carcasses were ground in an industrial meat grinder, and samples were taken to perform pre-drying, pre-degreasing, ball mill grinding, and subsequent analysis of dry matter, crude protein, and ether extract according to the methodology described by Silva and Queiroz (2002). The protein and fat deposition rates were measured by comparing the birds slaughtered at the end of the experimental period with an additional group of 10 broilers slaughtered at 1 day of age.

At 10 and 21 days of age, litter was sampled from five different points in each experimental unit for dry matter analysis according to the methodology described by Silva and Queiroz (2002).

Data were examined in relation to the glycerin levels using the analysis of variance followed by Dunnett's test and regression analysis by polynomial decomposition of the degrees of freedom. Differences between the average results of the two groups (the diet with inclusion of crude glycerin and the diet without crude glycerin) at 21 days were tested by *F*-test. SAEG (System for Statistical and Genetic Analysis) software (Version 9.1, 2007) was used for the analyses, and a probability of $P < 0.05$ was considered significant.

RESULTS

The weight gain, feed intake, feed : gain, and livability varied quadratically ($P < 0.05$) with the inclusion of crude glycerin at the pre-starter stage (1–10 days), and the quadratic equation predicted higher values for animals fed at a crude glycerin level of 9.01, 9.02, 9.03, and 6.43%, respectively. At 10 days of age, the litter moisture increased linearly ($P < 0.05$) with increasing crude glycerin levels (Table 2).

Dunnett's test showed a higher feed intake ($P < 0.05$) in the groups fed 6, 9, and 12% levels of crude glycerin compared with the control group. The control group showed a better ($P < 0.05$) feed : gain than the groups receiving 3, 6, 9, and 12% crude glycerin in the diet. For livability, however, a difference ($P < 0.05$) compared with the control group was only found in birds fed the highest level of crude glycerin. In addition, the birds consuming 12 and 15% crude glycerin showed increased ($P < 0.05$) litter moisture compared with the control group. For the weight gain parameter, differences were not detected ($P > 0.05$) between the groups receiving crude glycerin and the control group (Table 2).

Considering the phase of 1–21 days of age, it was observed in the groups receiving crude glycerin throughout the experimental period that crude glycerin inclusion had a quadratic effect ($P < 0.05$) on weight gain, feed : gain, feed conversion, and livability, with higher values observed with inclusion levels of 6.06, 7.97, 13.11, and 7.69%, while the litter moisture showed a linear increase ($P < 0.05$) (Table 3). Dunnett's test showed that groups supplemented with 12 and 15% crude glycerin throughout the experimental period had a lower ($P < 0.05$) weight gain, impaired ($P < 0.05$) feed : gain, and higher ($P < 0.05$) litter moisture compared with the control group. For feed intake and

Table 2. Weight gain, feed intake, feed : gain, livability, and litter moisture ± standard error of broiler chicks fed diets containing different levels of crude glycerin (period 1–10 days of age)

Inclusion	Weight gain (g)	Feed intake (g)	Feed : gain	Livability (%)	Litter moisture (%)
Control	207.8 ± 3.38	226.9 ± 1.75	1.092 ± 0.017	99.4 ± 1.14	37.0 ± 2.22
3%	202.6 ± 4,47	227.9 ± 1.54	1.125 ± 0.024*	100 ± 0.00	37.8 ± 4.64
6%	210.2 ± 4.98	242.1 ± 2.82*	1.152 ± 0.018*	100 ± 0.00	37.8 ± 4.43
9%	213.8 ± 2.68	245.1 ± 3.96*	1.146 ± 0.006*	100 ± 0.00	39.9 ± 3.80
12%	212.3 ± 3.84	243.7 ± 3.24*	1.148 ± 0.015*	98.9 ± 0.74	41.9 ± 3.10*
15%	205.8 ± 2.61	228.7 ± 0.99	1.111 ± 0.013	94.4 ± 1.87*	42.3 ± 2.27*
Dunnett's *P*-values	0.3000	0.0280	0.0400	0.0006	0.0350
CV (%)	5.93	6.10	3.30	2.61	17.67
Regression	quadratic[1]	quadratic[2]	quadratic[3]	quadratic[4]	linear[5]
P-values	0.0015	0.0141	0.0099	0.0010	0.0021

*significant difference between control group and experimental group (Dunnett's test, $P < 0.05$)

[1] $Y = 187.1741 + 5.96642x - 0.3311x^2$ ($R^2 = 0.96$); max. point: 9.01%

[2] $Y = 205.04406 + 9.010279x - 0.49943x^2$ ($R^2 = 0.97$); max. point: 9.02%

[3] $Y = 1.06934 + 0.01728x - 0.000956358x^2$ ($R^2 = 0.87$); max. point: 9.03%

[4] $Y = 97.31725 + 1.02811x - 0.07999x^2$ ($R^2 = 0.37$); max. point: 6.43%

[5] $Y = 36.08577 + 0.42928x$ ($R^2 = 0.90$)

livability, only the highest level of crude glycerin inclusion (15%) showed effects ($P < 0.05$) compared with control group (Table 3).

Regarding the performance of birds fed a glycerin-free diet during the phase of 11–21 days of age, only the livability variable showed the quadratic response ($P < 0.05$), with the highest livability predicted to occur at a level of 6.39% of crude glycerin (Table 3).

At 21 days of age, comparing the average results obtained in the two groups independent of crude glycerin levels, it was observed that birds fed a diet free of crude glycerin had higher ($P < 0.05$) performance after initial 10 days than birds that continued to receive the same level of crude glycerin as in the previous phase (Table 3).

The rate of protein and fat deposition as well as of dry matter on the carcasses did not differ ($P > 0.05$) among different crude glycerin levels (Table 4).

DISCUSSION

The crude glycerin supplementation increased feed intake of broiler chicks and consequently affected feed : gain during the pre-starter stage of development (1–10 days). According to Min et al. (2010), the increase in feed intake may be partly associated with the sweet taste of glycerin. On the other hand, considering that glycerol, a major component of glycerin, can interfere with the rate of digesta passage, reducing nutrient utilization (Barteczko and Kaminski 1999), energy uptake in the animals may be impaired, resulting in increased feed intake as a compensatory response.

The reduced performance in the early stages of chicks was not observed if considering the whole experimental period (days 1–21), making it clear that crude glycerin supplementation had an effect on performance only in the first 10 days of life; the performance parameters subsequently recovered, with a compensatory gain. It can be assumed that birds in the early stages have an immature digestive-absorptive apparatus, which may reduce crude glycerin utilization, considering the fact that glycerin absorption is dependent on glycerol kinase enzyme activity (Mourot et al. 1994). Moreover, this enzyme has probably limited activity at higher levels of glycerin supplementation (Min et al. 2010).

Considering the results obtained in the phase of 1–21 days, supplementation with 6.06% crude glycerin showed a maximum weight gain that did not differ from that for control group. This indicates that this percentage can be used without compromising productive parameters, allowing reduction in production costs, because the ingredient in question is a byproduct of biodiesel production.

The effects of dietary supplementation of crude glycerin in broiler chickens are still uncertain. However, several authors have indicated the po-

Table 3. Weight gain, feed intake, feed : gain, livability, and litter moisture ± standard error of broiler chicks at 21 days of age fed diets containing different levels of crude glycerin (periods 1–10 and 1–21 days of age)

Inclusion	Weight gain (g)		Feed intake (g)		Feed : gain		Livability (%)		Litter moisture (%)	
	days 1–10	days 1–21	days 1–10	days 1–21	days 1–10	days 1–21	days 1–10	days 1–21	days 1–10	days 1–21
Control	818.9 ± 15.1		1181.6 ± 17.79		1.444 ± 0.010		97.10 ± 1.20		39.55 ± 2.24	
3%	853.3 ± 15.7	809.2 ± 13.5	1227.8 ± 11.2	1176.9 ± 21.6	1.440 ± 0.016	1.455 ± 0.027	96.59 ± 1.1	89.77 ± 3.4	39.60 ± 3.4	40.80 ± 3.2
6%	831.2 ± 11.5	797.6 ± 11.1	1198.1 ± 13.4	1174.5 ± 19.2	1.441 ± 0.007	1.472 ± 0.002	93.18 ± 2.9	94.32 ± 2.2	39.70 ± 3.2	40.90 ± 2.6
9%	840.7 ± 16.7	826.1 ± 11.9	1224.0 ± 18.1	1237.7 ± 15.4	1.457 ± 0.014	1.498 ± 0.004	97.73 ± 2.3	95.45 ± 2.6	40.40 ± 4.0	43.55 ± 4.5
12%	843.1 ± 5.1	761.4 ± 12.1*	1233.6 ± 15.5	1197.6 ± 9.4	1,463 ± 0.013	1.573 ± 0.014*	92.05 ± 3.4	94.32 ± 2.9	41.80 ± 3.4	47.10 ± 3.4*
15%	817.4 ± 15.1	718.9 ± 5.4*	1162.9 ± 24.1	1086.8 ± 11.9*	1.423 ± 0.020	1.512 ± 0.009*	79.55 ± 2.3*	72.73 ± 1.9	42.50 ± 3.8	48.00 ± 2.1*
Average	837.2 ± 6.0[a]	782.6 ± 9.8[b]	1209.3 ± 9.1[a]	1174.7 ± 13.0[b]	1.445 ± 0.007[a]	1.502 ± 0.011[b]	91.82 ± 1.8[b]	89.32 ± 2.2[a]	40.80 ± 2.1[b]	44.07 ± 3.6[a]
Dunnett's *P*-values	0.4150	0.00001	0.0550	0.0002	0.3740	0.0004	0.0010	0.0001	0.3210	0.0010
CV (%)	3.10		3.59		2.00		5.45		17.37	
Regression	ns	quadratic[1]	ns	quadratic[2]	ns	quadratic[3]	quadratic[4]	quadratic[5]	ns	linear[6]
P-values	0.4050	0.0001	0.5230	0.0004	0.3860	0.0043	0.0001	0.0001	0.3480	0.0010

ns = non-significant, CV = coefficient of variation

*significant difference between control group and experimental group (Dunnett's test, $P < 0.05$)

[a,b]significant difference by F test ($P < 0.05$)

[1]$Y = 770.11605 + 14.92113x - 1.23009x^2$ ($R^2 = 0.66$); max. point: 6.06%

[2]$Y = 1061.8863 + 40.46326x - 2.53888x^2$ ($R^2 = 0.56$); max. point: 7.97%

[3]$Y = 1.38365 + 0.02269x - 0.00086508x^2$ ($R^2 = 0.41$); max. point: 13.11%

[4]$Y = 88.3305 + 2.83003x - 0.22155x^2$ ($R^2 = 0.52$); max. point: 6.39%

[5]$Y = 72.27305 + 6.6558x - 0.4329x^2$ ($R^2 = 0.67$); max. point: 7.69%

[6]$Y = 38.44 + 0.68643x$ ($R^2 = 0.84$)

Table 4. Protein deposition rate (PDR), fat deposition rate (FDR), and dry matter (DM) ± standard error of broiler chicken carcasses at 21 days of age; chick diets contained different levels of crude glycerin (periods 1–10 and 1–21 days of age)

Inclusion	PDR (g/day)		FDR (g/day)		DM (%)	
	days 1–10	days 1–21	days 1–10	days 1–21	days 1–10	days 1–21
Control	4.16 ± 0.13		2.52 ± 0.17		27.17 ± 0.43	
3%	4.60 ± 0.14	3.94 ± 0.17	2.44 ± 0.16	2.24 ± 0.14	27.01 ± 0.53	27.02 ± 0.35
6%	4.00 ± 0.16	4.03 ± 0.18	2.28 ± 0.24	2.35 ± 0.14	26.54 ± 0.65	26.85 ± 0.34
9%	4.02 ± 0.19	4.08 ± 0.12	2.54 ± 0.19	2.41 ± 0.19	26.12 ± 0.35	26.08 ± 0.73
12%	4.24 ± 0.21	4.10 ± 0.17	2.49 ± 0.22	2.13 ± 0.16	26.67 ± 0.44	27.54 ± 0.46
15%	4.32 ± 0.08	3.71 ± 0.15	2.22 ± 0.20	1.98 ± 0.18	26.12 ± 0.58	26.71 ± 0.46
Average	4.24 ± 0.11	3.97 ± 0.10	2.39 ± 0.12	2.22 ± 0.10	26.49 ± 0.32	26.84 ± 0.31
Dunnett's *P*-values	0.3260	0.6078	0.0700	0.3029	0.1818	0.2444
CV (%)	11.83		23.15		5.45	
Regression	ns	ns	ns	ns	ns	ns
P-values	0.6100	0.5900	0.3000	0.2300	0.2400	0.1000

ns = non-significant, CV = coefficient of variation

*not significant differences between control group and experimental group were detected (Dunnett's test, $P < 0.05$)

tential of this ingredient for animal feed as a useful energy source with apparent metabolizable energy corrected by nitrogen balance ranged from 3254 to 4134 kcal/kg with no adverse effects on body weight, feed intake, or feed conversion (Cerrate et al. 2006; Min et al. 2008; Dozier et al. 2011).

One of the points to be considered by the crude glycerin inclusion in animal diets is the increase in litter moisture, which was confirmed by the results of this study, in agreement with studies reported in the literature (Cerrate et al. 2006; Silva et al. 2012). Gianfelici et al. (2011) observed diarrhea cases in broilers fed glycerol. According to these authors, the increase of water in the excreta was due to excessive urine production, not due to water retention by the intestine. As stated previously, glycerin utilization by the animal is dependent on enzyme activity, with a limited metabolism. When this limit is reached, there is an increase in serum glycerol, requiring its excretion via urine. Excretion of water-soluble glycerol automatically increases the excretion of water.

Besides this metabolic factor, the high sodium content of crude glycerin was responsible for the high concentration of this mineral in the experimental diets, which exceeded the dietary recommendations for broilers proposed by Rostagno et al. (2011). This could be the cause for increased water consumption, with a consequent increase in urine output. The high levels of sodium present in crude glycerin are due to sodium hydroxide utilization as a catalyst in biodiesel production. The inefficient recovery of this catalyst leads to the presence of impurities (Lammers et al. 2008). In fact, Simon et al. (1996) reported that when glycerol is included in animal diets, higher water requirements of the animals should be considered.

With respect to the rate of deposition of protein and fat, Cerrate et al. (2006) suggested that glycerol may increase protein deposition by decreasing gluconeogenesis from amino acids by inhibiting the activity of some enzymes such as phosphoenolpyruvate carboxylase. However, as observed in this study, the protein and fat deposition rates were not affected by inclusion of crude glycerin in the diet. Likewise, Lessard et al. (1993) reported that 5% glycerol supplementation did not affect the protein and fat contents of carcasses.

CONCLUSION

The inclusion of up to 6.06% of crude glycerin in the diet of broilers during the period of 1 to 21 days of age had no adverse effect on animals' performance during this stage.

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