The effect of crude glycerin in broiler rations on performance, excreta moisture, footpad dermatitis, blood parameters, ileum histopathology and intestinal microbiology

Yahya Işık[1*] and Ergün Demir[2]

[1]Kepsut Vocational School, Balıkesir University, Balıkesir, Turkey
[2]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Balıkesir University, Altıeylül, Balıkesir, Turkey

---

IŞIK, Y., E. DEMİR: The effect of crude glycerin in broiler rations on performance, excreta moisture, footpad dermatitis, blood parameters, ileum histopathology and intestinal microbiology. Vet. arhiv 96, 195-211, 2026.

ABSTRACT

Alternatives to corn, the primary energy source in poultry farming, are being sought. The aim of this study was to determine the effects in broiler diets of crude glycerin, considered an alternative energy source, on performance, footpad dermatitis, blood parameters, intestinal histopathology and microbiology, and to evaluate its use as a substitute for corn. The study included seven groups: one control group and six treatment groups. Different amounts of crude glycerin were added to the diets of these groups for different durations. The rations were isonitrogenic and isocaloric. Feed intakes were increased over 11-21 and 5-21 days with 10% crude glycerin ($P<0.05$). In all periods, 5% crude glycerin consumption resulted in higher body weight gain ($P<0.05$) compared to 0% crude glycerin consumption. Feed conversion ratios were better with 5%, and 10% crude glycerin for 5-10 and 5-21 days ($P<0.05$). 10% crude glycerin increased excreta moisture, and 5% crude glycerin caused less moist excreta ($P<0.05$) than in the control group. As consumption increased, serum triglyceride decreased, the footpad dermatitis degree-score-incidence increased ($P<0.05$). There was an increase in coliform count with an increase in consumption ($P<0.05$). With increasing consumption, crypt width, villus height, villus surface area, and villus height/crypt depth increased, and crypt depth and villus width decreased ($P<0.05$). These results show that if the ration is prepared in a balanced way, crude glycerin can be used in broilers diets up to 10% without impairing health and performance.

Key words: biodiesel; broiler; crude glycerin; excreta moisture; intestinal microbiology; intestinal histopathology

---

Introduction

The depletion of fossil fuels that currently meet energy needs, the harmful effects of greenhouse gas emissions, unstable oil prices, and increasing energy demand are encouraging governments to find alternative, more economical, environmentally friendly, renewable, and sustainable energy sources. This is because the rising energy demand due to the growing global population could pose a significant global issue (ŞAHİN and SURAL, 2020; BÖLÜKBAŞ and KAYA, 2022). The interest in biodiesel as an alternative energy source is gradually increasing. Biodiesel is obtained by catalyzed transesterification. Crude glycerin (CG) is obtained

* Corresponding author:
Yahya Işık, Kepsut Vocational School, Balıkesir University, Balıkesir, Turkey, phone: +905071374595, e-mail: yahya.isik@balikesir.edu.tr

ISSN 0372-5480
Printed in Croatia

as a by-product, as approximately 10% of the final product (BÖLÜKBAŞ and KAYA, 2022). Corn is used as the main energy source in poultry nutrition and can comprise up to 65% of diets (TAVERNARI et al., 2022). The apparent metabolizable energy (AMEn) of CG has been reported to be 3434 kcal/kg for broilers, which is approximately 92-95% of its gross energy (DOZIER et al., 2008). In general, purified glycerin has over 1500 applications across various industries, including personal care, cosmetics, pharmaceuticals, chemicals, food, and weapons (THOMPSON and HE, 2006). However, production is increasingly exceeding the demand in these sectors. Additionally, these industries predominantly require high-purity raw materials (99% and above). Achieving this level of purity for crude glycerin incurs additional costs. This situation necessitates finding continuous and viable applications for the surplus crude glycerin, without further processing (PAPADOMICHELAKIS et al., 2015; BÖLÜKBAŞ and KAYA, 2022; IŞIK and DEMİR, 2023). Therefore, it may be possible to reduce traditional energy sources and use CG in diets (DOZIER et al., 2011; DE SOUZA et al., 2020). CG may contain varying amounts of glycerol and impurities. Therefore, it is important to be aware of the chemical composition and nutritional properties of CG before use (BÖLÜKBAŞ and KAYA, 2022; ŞAHİN and SURAL, 2022). Previous studies have investigated the effects of dietary supplementation of CG on performance (CERRATE et al., 2006), excreta moisture (PAPADOMICHELAKIS et al., 2015), footpad lesions (SANTOS et al., 2019), blood parameters (DE SOUZA et al., 2020), and intestinal histopathology and microbiology (OZDOGAN et al., 2014). The aim of our study was to determine the effects of CG added to broiler diets at different rates and for different periods of time on feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), excreta moisture (EM), footpad dermatitis (FPD), blood parameters, and intestinal histopathology and microbiology.

Materials and methods

The study was carried out on a total of 350, mixed gender Cobb 500 broiler chicks at the Broiler Research Unit of Balıkesir University Livestock Research and Application Center. For the first 4 days, all the chicks were fed with a broiler pre-starter feed based on corn-soybean meal. On the 5th day of the study, the broilers were randomly distributed into seven experimental groups of 10 chicks each, with five replications according to a completely randomized design (the ratio of males to females was equal in each replication) (Table 1). All the chicks had access to water and formulated feed ad libitum throughout the trial. This research was approved by Balıkesir University Animal Experiments Local Ethics Committee (HADYEK) (Decision no: 2023/1-3).

Table 1. Experimental groups and treatments

Group I (Control)	Day 1 to 42 Based on corn-soybean meal
Group II	Day 5 to 21 5% CG + d 22 to 42 0% CG
Group III	Day 5 to 21 0% CG +d 22 to 42 5% CG
Group IV	Day 5 to 42 5% CG
Group V	Day 5 to 21 10% CG + d 22 to 42 0% CG
Group VI	Day 5 to 21 0% CG + d 22 to 42 10% CG
Group VII	Day 5 to 42 10% CG

The content of CG used was determined according to formula of the Association of Official Analytical Chemists AOAC (1997) as: 100 = %glycerol + %moisture + %ash + %matter organic non-glycerol (MONG). The content of the CG used was: 87.48% glycerol, 6.82% moisture, 4% ash, and 1.7% MONG. The CG used in the study was obtained from sunflower and canola. The methanol percentage was found to be 0.3% by the method specified in DOZIER et al. (2011). The gross energy of the CG was determined as 15.83 MJ/kg (3784.4 Kcal/kg) by an adiabatic bomb calorimeter (IKA, C6000 global standard, Germany). The AMEn value of CG has been accepted as 95% of its gross energy (3595 Kcal/kg) (DOZIER et al., 2008). The ether extract (EE) and crude protein (CP) percentages of the CG used were determined as 0.1% and 0.07%, respectively (AOAC, 1997). The %Na+ and %Cl- ratios in CG are 1.29% and 1.99%, respectively (AOAC, 1997). The pH of the CG used was measured as 4.35 with a pH meter (Orion Star™ A111, Thermo Scientific, USA). The diets were prepared as isonitrogenous and isocaloric according to NRC (1994) for use on days 1-4, 5-10, 11-21, and 22-42. Dry matter (DM), crude ash, EE, CP and crude fiber (CF) analyses of the diets were carried out according to AOAC (1997) and VAN SOEST et al. (1991). The proportions of the components in the diets (mash form) and their nutrient contents are given in Table 2. To calculate daily BWG and FCR, body weight (BW) and FI were recorded on days 5, 11, 22 and 42. To determine excreta moisture (EM), excreta samples were collected into sterile, leak-proof plastic excreta containers at regular intervals in the morning, at noon and in the evening on the 10th, 15th, 20th, 25th, 30th, 35th and 40th days for each replicate in study. The excreta samples taken were pooled. Samples taken on the 10th day were expressed as the 5th-10th day value, samples taken on the 15th and 20th days were expressed as the 11th-21st day value, samples taken on the 25th, 30th, 35th, 40th days were expressed as 22nd-42nd day value. Samples were kept at -20°C until analysis. Excreta samples were analyzed for %DM according to PAPADOMICHELAKIS et al. (2015). To determine the FPD

Table 2. Ingredients and nutrient composition of prestarter (d 1 to 4), starter (d 5 to 10), grower (d 11 to 21) and finisher (d 22 to 42) diets (as-fed basis)

	Prestarter (d 1 to 4).	Starter (d 5 to 10)			Grower (d 11 to 21)			Finisher (d 22 to 42)		
Ingredients %										
Corn grain	55.53	58.91	53.00	47.00	59.00	52.49	46.00	61.40	56.99	48.73
Soybean meal, 46% CP	36.60	33.54	34.65	35.73	31.15	32.17	33.19	28.90	30.185	30.20
Sunflower Seed	-	-	-	-	-	-	-	-	-	2.87
Corn oil	2.74	2.24	2.14	2.06	3.35	3.37	3.38	4.00	3.62	3.00
Razmol	1.45	1.41	1.50	1.68	3.00	3.66	4.30	2.62	1.31	2.50
Crude glycerin	-	-	5.00	10.00	-	5.00	10.00	-	5.00	10.00
Dicalcium phosphate	1.63	1.68	1.69	1.69	1.505	1.50	1.49	1.31	1.34	1.31
Limestone	1.06	1.05	1.05	1.04	1.02	1.02	1.02	0.94	0.92	0.92
Sodium chloride	0.38	0.38	0.22	0.06	0.38	0.22	0.06	0.39	0.23	0.05
DL-Methionine	0.23	0.27	0.27	0.27	0.21	0.21	0.21	0.16	0.16	0.16
L-Lysine HCL	0.11	0.21	0.19	0.17	0.12	0.10	0.08	0.02	-	-
L-Threonine	0.01	0.06	0.06	0.05	0.02	0.01	0.01	-	-	-
Vit.-min.premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated chemical composition										
Dry matter (DM)	90.02	89.96	90.15	90.34	89.99	90.18	90.38	89.99	90.17	90.29
ME (kcal/kg)	3035	3035	3035	3035	3108	3108	3108	3180	3180	3180
CP (%)	22.02	21.04	21.04	21.04	20.04	20.04	20.04	19.02	19.02	19.02
EE (%)	4.51	4.06	3.84	3.63	5.19	5.08	4.96	5.85	5.34	5.70
Ash (%)	6.31	6.19	6.22	6.25	5.89	5.93	5.97	5.50	5.51	5.55
Methionine+Cystine (%)	0.98	0.98	0.98	0.98	0.89	0.89	0.89	0.82	0.82	0.82
Lysine (%)	1.32	1.32	1.32	1.32	1.19	1.19	1.19	1.05	1.05	1.05
Calcium (%)	0.90	0.90	0.90	0.90	0.84	0.84	0.84	0.76	0.76	0.76
Available phosphorus (%)	0.45	0.45	0.45	0.45	0.42	0.42	0.42	0.38	0.38	0.38
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Chlorine (%)	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27

[1] Contained per kilogram; vitamin A:9.000 000 IU, vitamin D_3: 5.000 000 IU, vitamin E: 70. 000 IU, vitamin K_3: 3.000 mg, vitamin B_1: 3.000 mg; vitamin B_2: 6.000 mg; vitamin B_3: 45.000 mg; B_6: 4.000 Mn: 100 g, Fe 60 g: Zn 60 g, Cu: 2 g, Co: 2 g, I: 2 g, Se: 0.35 g

score, both feet of four randomly selected chickens from each replicate were examined for lesions by two experts on the 41st day of the study, and the lesions were scored according to EKSTRAND et al. (1998). The FPD score (Score 0-200), which can take values between 0-200, and the FPD frequency were determined according to SORIN et al. (2013). On the 41st day of the study, for blood analysis, approximately 5 ml of blood samples were collected from one chicken from each replicate from the brachial wing vein into a serum biochemistry tube, following the usual blood collection procedures. The samples were quickly transported to the laboratory and all blood samples were centrifuged at 1008 RCF (3000 RPM) for 5 minutes, and the separated serum was transferred to plastic Eppendorf tubes. The samples were stored at -20°C until biochemical analysis. Serum ALT, AST, triglyceride (TG), glucose, creatinine and cholesterol were measured using a biochemical autoanalyzer (Olympus AU400 device (Beckman Coulter, USA)) and its accompanying kits. For intestinal analyses, the intestines of two broilers from each replicate were ligated from both ends (duodenum and cloaca). For microbiological analysis (coliform bacteria counts in the intestine), the intestinal contents of chickens were collected in sterile containers and stored at -20°C until analysis was performed. For histopathological analysis, the intestines of five broilers were randomly selected from each group and analyses were performed according to OZDOGAN et al. (2014). Villus height (VH), villus width (VW), crypt depth (CD), crypt width (CW) and epithelial cell thickness (ECT) measurements were performed. Bacterial and histopathological analyses were performed according to OZDOGAN et al. (2014) (Fig. 1.). Villus surface area (VSA) was calculated using the formula: 2π x (mean villus width/2) x villus height. In addition, the ratio of villus height to crypt depth (VH/CD) was also calculated.

Statistical analysis. In the study, seven treatment groups, each consisting of five replicates, were distributed according to the completely randomized design. The mathematical model of the experiment was $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$. Here Yijk represents: the observation value of the j-th treatment in the i-th block, the observation value of the k-th repetition, μ = General population mean, αi = i-th block effect, βj = j-th treatment effect, (αβ)ij = the interaction effect of j-th treatment

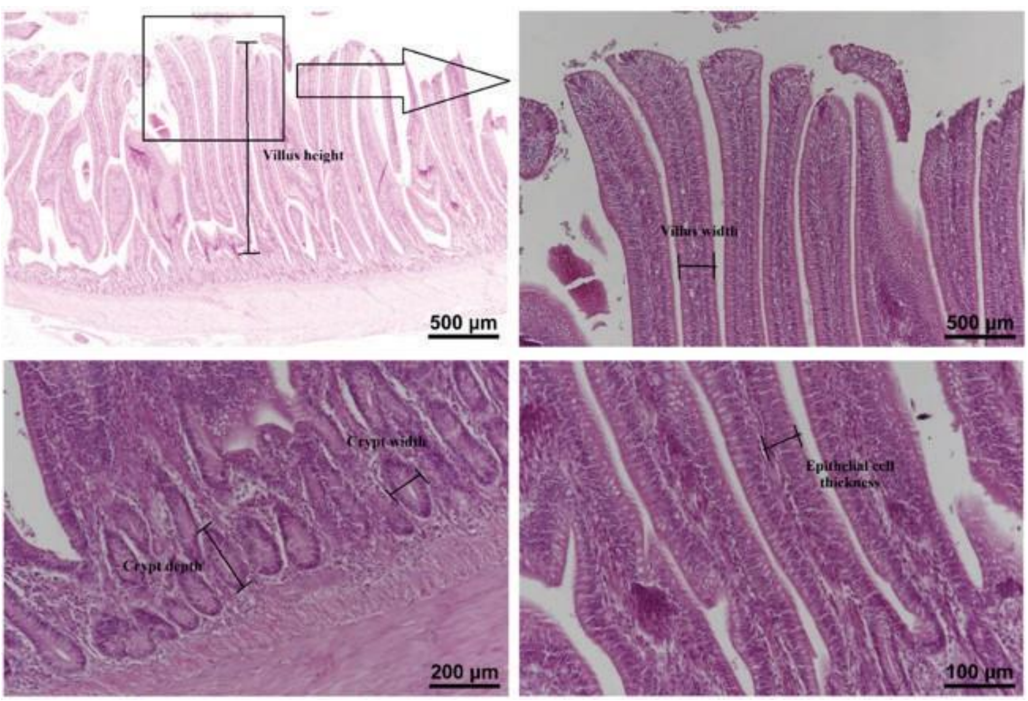

Fig. 1. a) villus height; b) villus width; c) crypt depth, crypt width; d) epithelial cell thickness

in i-th block, eijk = random error. Statistical analysis of the data was performed by IBM SPSS version 25.0. Normality and homogeneity analyses were conducted using the Kolmogorov-Smirnov and Levene's tests. Comparisons between groups with normal and homogeneous data distributions were performed using parametric one-way ANOVA and the post-hoc Tukey method. Comparisons between groups with non-normal and non-homogeneous data distributions were conducted using one-way ANOVA and the post-hoc Dunnett T3 method. Variance analysis for non-parametric values was carried out using the Kruskal-Wallis test. The data on the interaction between time and ration in repeated measures were analyzed using the general linear model (GLM). Relationships between the parameters were identified using the parametric Pearson or Spearman correlation and linear regression analyses. The significance levels of the analysis results are stated according to a 95% confidence interval (P<0.05).

Results

The feed intake, BWG and FCR of the groups are presented in Table 3. The feed intake, BWG and FCR of the groups according to their CG consumption rates are presented in Table 4. When these tables were examined; throughout the study period (days 5-10, days 11-21, days 22-42, days 5-21, and days 5-42), the effects of the dietary treatments on the broiler chickens' feed intake did not result in statistically significant differences among the groups (P>0.05). However, during the 11-21 day and 5-21 day periods of the experiment, when the average feed intake was evaluated on the basis of the CG intake of the groups, the average feed intake of the groups fed with diets containing 10% CG was significantly higher (P<0.05) than that of the groups fed with diets containing 0% CG. During the 5-10 day period of the study, the body weight gains of group VII and group II were statistically significantly higher (P<0.05) than those of group I, group III, and group VI. During the 5-10 day period of the experiment, when evaluating body weight gains on the basis of the CG ratios given to the groups, the average body weight gains of the animals in the groups fed with diets containing 5% and 10% CG were statistically significantly higher (P<0.05) than those of the groups fed with diets containing 0% CG. During the 5-10 day period of the experiment, the feed conversion ratios of group I, group III, and group VI were statistically significantly higher (P<0.05) than those of group II, group IV, group V, and group VII, indicating poorer feed utilization in these groups. Additionally, when evaluated on the basis of the CG ratios added to the mixed feed, the average feed conversion ratio of the groups fed with diets containing 0% CG during the 5-10 day period was statistically significantly higher (P<0.05) than that of the groups fed with diets containing 5% and 10% CG. During the 5-21 day period of the study, the feed conversion ratio of the group fed with diets containing 5% CG was statistically significantly lower (P<0.05) than the average value of the groups fed with diets containing 0% CG. There were statistically significant differences (P<0.05) in feed conversion ratios among the groups during the 5-21 day period. The feed conversion ratio of group I was statistically significantly higher (P<0.05) than that of group II, group IV, group V, and group VII. Additionally, the feed conversion ratios of group III and group VI were significantly higher (P<0.05) than that of group II. When considering the average feed conversion ratios of the groups fed with diets containing 0% CG, 5% CG, and 10% CG during the 5-21 day period, a statistically significant difference (P<0.05) was observed. No relationship was found between CG consumption of broilers and FI and FCR (P>0.05). A positive correlation of 34.9% (P<0.05) and a regression of 12.9% (P<0.05) were found between CG consumption and BWG in the groups.

The effect of the amount of CG consumed in different periods of the study on EM are presented in Table 5. A 48.8% correlation and 23.8% regression were determined between CG consumed and EM content (P<0.05). In the study, 42.8% correlation and 17.9% regression were found between CG consumption and FPD (footpad dermatitis) degree; 66.4% correlation and 40.9% regression were found for the FPD score (0-200); and 66% correlation and 40.8% regression were found for FPD incidence (P<0.05). In addition, the FPD scores (0-200) of the groups were found to be 2.5, 0, 0, 2.5, 5, 15, and

Table 3. Feed intake of groups, g/day/animal, body weight gain, g/day/animal and feed conversion ratios, g feed: g body weight gain

Group	d 5-10	d 11-21	d 22-42	d 5 21	d 5-42
	FI±SEM	FI±SEM	FI±SEM	FI±SEM	FI±SEM
I	26.31±0.39	67.89±1.15	125.05±1.23	53.21±0.63	92.91±0.83
II	27.00±0.58	68.73±1.53	126.22±1.74	54.00±1.09	93.91±1.29
III	26.52±0.22	67.91±0.77	128.29±1.72	53.30±0.44	94.74±0.81
IV	26.31±0.39	69.40±1.62	129.26±3.21	54.19±1.11	95.67±2.21
V	26.25±0.61	71.35±1.52	126.49±2.47	55.43±1.10	94.70±1.55
VI	25.62±0.30	66.23±2.87	129.15±2.22	51.89±1.94	94.59±1.91
VII	26.75±0.44	71.35±1.05	128.82±0.84	55.61±0.79	96.07±0.66
P value	>0.05	>0.05	>0.05	>0.05	>0.05
	BWG±SEM	BWG±SEM	BWG±SEM	BWG±SEM	BWG±SEM
I	17.81±0.62bc	43.35±0.52	84.14±0.72	34.33±0.25	61.85±0.48
II	20.33±0.59a	45.38±0.70	84.63±0.94	36.54±0.57	63.11±0.62
III	17.67±0.40bc	44.34±0.79	88.33±0.82	34.93±0.64	64.44±0.57
IV	19.29±0.45ab	46.06±0.99	86.11±2.23	36.61±0.76	63.97±1.45
V	19.19±0.53ab	45.01±1.33	83.70±2.40	35.90±0.96	62.33±1.39
VI	17.12±0.92c	43.36±1.43	87.56±0.81	34.10±1.23	63.64±0.71
VII	20.15±0.37a	45.81±0.96	86.84±1.23	36.75±0.65	64.43±0.70
P value	<0.05	>0.05	>0.05	>0.05	>0.05
	FCR±SEM	FCR±SEM	FCR±SEM	FCR±SEM	FCR±SEM
I	1.48±0.03a	1.57±0.02	1.49±0.03	1.54±0.02a	1.50±0.02
II	1.33±a0.02b	1.52±0.04	1.49±0.02	1.45±0.02c	1.49±0.02
III	1.50±0.03a	1.53±0.04	1.45±0.02	1.52±0.03ab	1.47±0.01
IV	1.37±0.02b	1.51±0.03	1.50±0.05	1.46±0.03bc	1.50±0.04
V	1.37±0.01b	1.59±0.02	1.51±0.03	1.51±0.02bc	1.52±0.02
VI	1.51±0.08a	1.53±0.03	1.48±0.02	1.52±0.02ab	1.49±0.02
VII	1.33±0.01ab	1.56±0.02	1.48±0.02	1.48±0.01bc	1.49±0.02
P value	<0.05	>0.05	>0.05	<0.05	>0.05

FI±SEM: feed intake ± standard error of mean, g/day/animal; BWG±SEM: body weight gain ± standard error of mean, g/day/animal; FCR±SEM: feed conversion ratio ± standard error of mean, g feed: g body weight gain

a, b, c: differences between data shown with different letters in the same column are significant (P<0.05)

Table 4. Feed intake (g CG/day/bird), body weights (g CG/bird), body weight gains (g CG/day/bird) and feed conversion ratio (g feed: g body weight gain) according to their crude glycerin consumption

Group	d 5-10	d 11-21	d 22-42	d 5 21
	FI±SEM	FI±SEM	FI±SEM	FI±SEM
0% CG	26.15±0.20	67.34±1.00a	125.92±1.02	52.80±0.67a
5% CG	26.66±0.35	69.06±1.06ab	128.77±1.72	54.10±0.73ab
10% CG	26.50±0.37	71.35±0.87b	128.98±1.12	55.52±0.64b
P value	>0.05	<0.05	>0.05	<0.05
	BWG±SEM	BWG±SEM	BWG±SEM	BWG±SEM
0% CG	17.53±0.37b	43.68±0.54b	84.15±0.83b	34.45±0.44b
5% CG	19.81±0.39a	45.72±0.58a	87.22±1.18a	36.57±0.45a
10% CG	19.67±0.35a	45.41±0.78ab	87.20±0.71a	36.33±0.57a
P value	<0.05	<0.05	<0.05	<0.05
	FCR±SEM	FCR±SEM	FCR±SEM	FCR±SEM
0% CG	1.50±0.03a	1.54±0.02	1.50±0.01	1.53±0.01a
5% CG	1.35±0.01b	1.51±0.03	1.48±0.03	1.45±0.02b
10% CG	1.35±0.01b	1.57±0.01	1.48±0.01	1.49±0.01ab
P value	<0.05	>0.05	>0.05	<0.05

FI±SEM: feed intake ± standard error of mean, g/day/animal, BWG±SEM: body weight gain ± standard error of mean; FCR±SEM: feed conversion ratio ± standard error of mean, g feed: g body weight gain

a, b, c: differences between data shown with different letters in the same column are significant (P<0.05)

47.5, respectively, and FPD incidences were found to be 5%-0%-0%-5%-10%-40%-50%, respectively. In the experiment, it was seen that there was a negative and very highly (90.9%) significant correlation between CG consumption and blood triglyceride level. Similarly, a high level (82.5%) regression were emerged between CG consumption and blood triglyceride level (P<0.05). This shows that increasing CG consumption causes a decrease in blood triglyceride levels. However, no statistically significant correlation-regression was detected between CG consumption and the levels of other blood serum parameters. A 72.4% correlation and 50.2% regression were found between CG consumption and the coliform bacteria count of the groups (P<0.05).

The coliform bacteria counts in the intestine are presented in Table 6. When these tables were examined, it was found that, when examining the entire intestinal content from the gizzard exit to the cloaca for the coliform bacteria count, group VI had the statistically highest (P<0.05) coliform bacteria count compared to the other groups. Group V had the lowest coliform bacteria count, and there was no statistically significant difference (P>0.05) between group V and group I. The coliform bacteria counts of group VI and group VII were statistically higher (P<0.05) than those of the other groups.

The relationship between the study groups' VH, VW, CD, CW, and ECT and CG consumption are presented in Table 7. When the groups were evaluated in terms of ileum VH, it was determined that group VII had statistically significantly higher VH compared to the other groups (P<0.05). The VH of group VI was statistically higher than that of group I and group III (P<0.05). Similarly, the VH of group V was statistically higher than that of group I; the VH of group IV was statistically higher than that of group I, group II, and group III; and the VH of group III was statistically higher than that of group I (P<0.05). When the groups were evaluated in terms of ileum VW, it was found that group I had a numerically higher VW compared to the other groups, although this difference was not statistically significant. Additionally, comparisons of group I with group II, group VI, and group VII revealed that group I had a statistically significantly higher VW. When considering ileum CD, it was observed that the CD of group I was statistically significantly higher (P<0.05) than that of the other groups, while the differences between the other experimental groups were not statistically significant (P>0.05). The ileum CW in group VII was statistically significantly higher (P<0.05) than in group I. Similarly, the crypt widths of group V and group VI were higher (P<0.05) than that of group I. The ileum crypt widths of the other experimental groups were

Table 5. Effect of the amount of crude glycerin consumed in different periods of the study on excreta moisture, (%)

Group	d 5-10	d 11-21	d 22-42
	EM±SEM	DN±SEM	DN±SEM
0% CG	82.51±0.14b	82.67±0.10b	83.78±0.52b
5% CG	82.55±0.05b	81.84±0.38c	82.31±0.20c
10% CG	85.86±0.16a	87.03±0.10a	86.45±1.29a
P value	<0.05	<0.05	<0.05

EM±SEM: excreta moisture ± standard error of mean
a, b, c: differences between data shown with different letters in the same column are significant (P<0.05)

Table 6. Effects of CG intake on coliform bacteria count in the intestine (cfu/g)

$x10^7$	G I	G II	G III	G IV	G V	G VI	G VII	P value
Coliform± SEM	2.2±0.09d	5.6±0.15bc	5±0.09c	5.6±0.18bc	1.5±0.09d	9.6±0.2a	6.5±0.04ab	<0.05

G: group, SEM: standard error of mean
a, b, c, d, e, f: differences between data shown with different letters on the same line are significant (P<0.05)

found to be similar (P>0.05). It was determined that the differences in ileum ECT between the experimental groups were insignificant (P>0.05). Between CG consumption and ileum VH, there was 82.2% correlation, and 67.6% regression; with VW, 38.9% correlation, 15.1% regression, and with CW, 66% correlation, 43.6% regression were detected (P<0.05). The comparison of VSA and VH/CD between the groups is presented in Table 8. When the groups were evaluated the ileum villus absorptive surface area in group I was found to be statistically significantly lower (P<0.05) than in group IV and group VII. The differences between the other groups were found to be similar (P>0.05). In terms of the villus height to crypt depth ratio, group I had a statistically significantly lower value (P<0.05) compared to all the other groups. Additionally, the villus height to crypt depth ratio of group III was statistically significantly lower (P<0.05) than that of group VII (Table 8). The differences observed between the other groups were not statistically significant (P>0.05). A 49% correlation and 24% regression were found between CG consumed and VSA, and a 63.7% correlation and 46.2% regression were found between consumed CG and VH/CD (P<0.05).

Discussion

Our results show no difference in FI between the groups at any period of the study (P>0.05), which is consistent with the findings of SEHU et al. (2013) who found no differences at any point in their study. On the corresponding days examined in the relevant studies, no significant differences were

Table 7. Effects of using crude glycerin in the diet on ileum histopathology, (µm)

Group	Villus height ±SEM	Villus width ±SEM	Crypt depth ±SEM	Crypt width ±SEM	Epithelial cell thickness ±SEM
I	530.32±57.19e	167.07±44.64a	389.70±97.64a	49.32±5.65c	43.42±6.07
II	1205.36±144.31cd	101.37±18.25b	237.31±31.03b	56.20±10.18bc	49.62±10.43
III	1081.80±161.61d	153.35±35.34ab	269.29±28.22b	66.22±8.73abc	57.78±9.31
IV	1507.638±72.53ab	115.52±20.58ab	272.60±50.99b	67.95±8.00abc	61.33±12.07
V	1158.40±51.39cd	143.65±34.72ab	247.73±45.72b	76.47±12.56ab	58.67±7.83
VI	1394.38±150.13bc	107.38±23.43b	255.92±65.82b	75.26±11.79ab	46.79±8.50
VII	1636.93±138.20a	107.60±16.83b	250.92±35.75b	85.15±14.70a	53.57±7.15
P value	<0.05	<0.05	<0.05	<0.05	>0.05

SEM: standard error of mean
a, b, c, d: differences between data shown with different letters in the same column are significant (P<0.05)

Table 8. Effects of CG intake on villus surface area (mm^2) and villus height/crypt depth ratio

Group	Villus surface area (mm^2) ±SEM	Villus height/Crypt depth±SEM
I	0.28±0.03a	1,41±0.15a
II	0.39±0.03ab	5,20±0.55bcd
III	0.53±0.09ab	4,07±0.37b
IV	0.55±0.05b	5,69±0.50bcde
V	0.52±0.06ab	4,82±0.42bc
VI	0.48±0.07ab	5,80±0.81bcdef
VII	0.55±0.05b	6,62±0.43cdef
P value	<0.05	<0.05

SEM: standard error of mean
a, b, c, d, e, f: differences between data shown with different letters in the same column are significant (P<0.05)

observed, similar to our findings. Therefore, the study we present is consistent with the existing literature of MANDALAWI et al. (2014) on days 1-7 and 7-14, DE SOUZA et al. (2020) in the first 21 days, and SILVA et al. (2012) on days 1-42. On days 11-21 and 5-21 of our study, the FI of those consuming 10% CG was higher than those consuming 0% CG (P<0.05). Since CG was not included in the diets for the first 4 days before the trial, the positive or negative effects of glycerin on the gastro intestinal (GI) system may have been prevented during this period. After the 5th day of the study, CG may have prevented dust formation in the feed, resulting in improved palatability and increased FI. After the 21st day of the study, no difference in FI may have occurred due to the balanced diets containing similar nutrients in the GI system that completed its development. In all periods of our study, the mean BWG of those consuming 5% CG was higher than those consuming 0% CG (P<0.05). In the present study, BWG of those consuming 10% CG was higher than those consuming 0% CG on days 5-10, 22-42, and 5-21 (P<0.05). No difference was detected in BWG between those consuming 5% and 10% CG at any time during the study (P>0.05). On days 5-10, 5-21 and 22-42 of our study, the BWG of those consuming 5% and 10% CG was higher than those consuming 0% CG (P<0.05). On the 11th-21st days of the study, the BWG of those consuming 5% CG was higher than those consuming 0% CG (P<0.05). This result is parallel to the results of SILVA et al. (2012) and TAVERNARI et al. (2022) regarding the improvement in BWG due to an increase in CG consumption. MOUSA et al. (2018) reported that BWG on days 0-14 was higher in the group consuming 5% (P<0.05), and the optimum CG level for broiler feeds was 5%. However, if the ration is designed in a balanced manner, up to 10%. can be added. It has also been reported that young chickens have a better capacity to utilize the energy content in CG than older ones (LIMA et al., 2013). The results regarding BWG obtained on days 5-10 of our study may be related to the physical benefits caused by the addition of CG to the diet despite the diets being isoenergetic, because CG can improve the texture of powder feed in particular. In addition, it can prevent dustiness of feed by reducing the finely ground particles in feed. Although there was no statistical difference in this period, the BWG of the group consuming 5% CG was numerically higher. This is consistent with the study by ROSTAGNO et al. (2011) who reported that broilers should be given diets containing no more than 5% CG during the starter period and no more than 8% CG during the grower period, and HENZ et al. (2014) who found that the optimum level was 6.06%. It is also stated that in the early stages of life, the GI organs secrete insufficient amounts of digestive enzymes, the villi are not developed enough to function regularly, and the GK enzyme activity is not yet functional (MIN et al., 2010). Exceeding the capacity of the glycerin kinase (GK) enzyme may impair physiological and biochemical functions, such as limiting the absorption of glycerol and promoting a higher transit rate (FERREIRA et al., 2015). This information can explain why the BWG of those consuming 5% CG in present study was always numerically higher than that of those consuming 10% CG. Although there was no difference in FI on days 5-10 of the study (P>0.05), the BWG of those consuming 5% and 10% was higher than those consuming 0% CG (P<0.05), which improved their FCR. It has been reported that supplementation of up to 5% CG during the diet starter period (MOUSA et al., 2018) and 9.6% CG at 1-7 days of age resulted in the best FCR (TAVERNARI et al., 2022). SILVA et al. (2019) reported that there was a linear improvement in FRC with increasing amounts of CG. On the 5th-10th days of our study, those consuming 5% and 10% CG had better FCR than those consuming 0% CG (P<0.05), which is consistent with these studies. In the present study, this situation may have been caused by the fact that no chickens consumed CG for the first 4 days and the GI system organs were allowed to develop normally. Our results show that the FI of those consuming 10% was higher than the FI of those consuming 0% CG on the 11th-21st days (P<0.05). During this period, the BWG of those consuming 5% was higher than the BWG of those consuming 0% CG (P<0.05). However, there was no difference between those consuming 5% and 10% CG in terms of these parameters (P>0.05). This may have caused the lack of difference between the groups in

FCR during this period (P>0.05). This is similar to the study conducted by TAVERNARI et al. (2022) who reported no difference in FCR between days 8 and 21. In the present study, the performance that showed improvement with CG supplementation on days 5-10, started to deteriorate after day 11, which may be due to the fact that GK activity was still insufficient and this situation disrupted the biochemical and physiological functions related to the relatively high amount of glycerin consumed. MOUSA et al. (2018) reported that there was no difference between the groups in terms of FCR on days 15-28 (P>0.05), and those consuming 0% and 5% CG had the best FCR. Although there was no difference in the present study (P>0.05), the best FCR was in chickens consuming 5%, 0% and 10% CG, respectively. The reasons for this may be due to the high FI in chickens consuming CG, the pleasant taste of the feed, and the improvement in absorption and utilization in the small intestine with GK activity. Although there was no difference in FI between the groups on days 22-42 of our study (P>0.05), the BWG of those consuming 0% CG was lower than that of the other groups (P<0.05). However, there was no difference in FCR (P>0.05). This may be explained by the changing CG levels in the groups after day 22. It was reported that there was no difference in FCR between the 22nd-42nd days in the study conducted by TAVERNARI et al. (2022) and between the 29th-42nd days in the study by MOUSA et al. (2018). Although the FI of those consuming 10% CG was higher than the FI of those consuming 0% on days 5-21 of our study (P<0.05), the BWG of those consuming both 5% and 10% CG was higher than that of those consuming 0%. This may have resulted in the FCR of those consuming only 5% being better than that of those consuming 0% CG (P<0.05). It was reported that FCR was not affected in the first 21 days, but numerically the best FCR was in the group consuming 5% CG (PAPADOMICHELAKIS et al., 2015). Our results show that the best FCR on days 5-21 was in those consuming 5% (P<0.05). This may be due to the higher FCR on days 5-10. This is similar to the study by HENZ et al., 2014, in which it was explained that the FCR of broilers consuming CG for only the first 10 days was better compared to those consuming CG for 21 days, and therefore feeding CG for only the first 10 days of life had a positive effect on FCR. SEHU et al. (2013) reported that there was no difference in FCR between the 1st and 42nd days. There was no difference in FCR between the groups on the 5th-42nd days of our study (P>0.05). This may be attributed to the effect of the changing CG levels in the groups after the 22nd day. The lack of difference in FCR between the 22nd-42nd days of our study (P>0.05) may be due to the effect of the changing CG levels.

Sodium chloride content greater than 3% in their diet may cause ionic imbalance in birds and can ultimately lead to an increase in water consumption, feces production and litter wetness. It has been reported that increased water consumption negatively affects litter quality by increasing EM (CERRATE et al., 2006; MIN et al., 2010). Some studies (CERRATE et al., 2006; PAPADOMICHELAKIS et al., 2015) have linked this situation to the amount of sodium in CG, and to exceeding the recommended upper limit of dietary sodium. It has also been reported that up to 10% CG can be added to diets of monogastric animals without compromising health, as long as the recommended levels of sodium are not exceeded (GHAYAS et al., 2023). However, in a study where pure glycerin was used, GIANFELICI (2009) reported that litter wetness increased with increasing glycerin amount, independent of sodium, and that this was due to GK saturation and the excess was excreted through the kidneys. In a study conducted by DOZIER et al. (2011), the EM of those consuming 0, 7, 14 and 21% CG on days 1-14 was examined and it was reported that the EM increased with increasing CG consumption (P<0.05). In another study conducted by CERRATE et al. (2006), it was reported that the litter of broiler chickens consuming 10% glycerol was wetter than that of those consuming 0% and 5% glycerol. It has been reported that litter moisture increases linearly with increasing CG levels at 10 days of age (P<0.05) (HENZ et al., 2014) and that the incidence of diarrhea increases in chickens fed with glycerol (GHAYAS et al., 2023). According to this study, the reason for the increase in the amount of water in the feces is not due to retention of water in the intestine, but excessive urine production. The ability to metabolize glycerol in diets is limited due to its dependence on GK enzyme activity. Upon reaching the threshold value, an increase in serum glycerol levels occurs, necessitating its excretion in the urine (GIANFELICI, 2009). Since the sodium level did not exceed the normal limit in any repetition in the present study, it can be said that there was an increase in EM due to GK saturation in those consuming 10% CG. The finding that the EM of those consuming 10% CG on days 5-10 was higher than those consuming 0% and 5% CG ($P<0.05$) is consistent with the study by PAPADOMICHELAKIS et al. (2015), who reported that there was no difference between the EM of those consuming 0% and 5% CG ($P>0.05$). On the 11th-21st days of our study, the EM of those consuming 10% CG was higher than the other groups ($P<0.05$). During this period, the EM levels of those consuming 5% CG was lower than of those consuming 0% CG ($P<0.05$), indicating the positive effect of rations containing 5% CG on the 5th-21st days of the study. The decrease in EM percentage on the 11th-21st days in those consuming 5% CG compared to the 5th-10th days of the study supports this finding. This is consistent with the studies by PAPADOMICHELAKIS et al. (2015), who reported that the EM was lower ($P<0.05$) in those consuming 7% CG on the 15th-28th days, and SILVA et al. (2012) who reported that litter moisture increased in broilers consuming 10% glycerin after the 3rd week of their study. In this study, the CG consumption rates changed in some groups on days 22-42. During this period of the study, EM levels remained lower in those consuming 5% CG ($P<0.05$). The results are consistent with the study by PAPADOMICHELAKIS et al. (2015) who used feeds containing 0%, 7% and 14% CG. In addition, it has been reported that the litter quality of broiler chickens consuming 10% CG was worse than that of broiler chickens consuming 0% and 5% (DE SOUZA et al., 2020). Considering that this result is related to EM, the increase in EM on day 42 in those consuming 10% CG ($P<0.05$) is consistent with these results. The increase in EM as CG consumption increased in present study supports these studies. It has been reported that the incidence of lesions was higher in broilers consuming 7% glycerol feeds compared to those consuming glycerol-free feeds on days 1-42 of the study (SANTOS et al., 2019). Similarly, it has been reported that chickens exhibited severe lesions as glycerin levels increased (MOUSA et al., 2018). In the present study, a correlation of 42.8% and 66.4% and a cause-and-effect relationship of 17.9% and 40.9% were found between CG consumption and FPD degree and FPD scores, respectively, which is consistent with the literature. In addition, the threshold value for FPD score was reported as “50” (EU, 2005). The highest value in present study was 47.5. In this respect, footpad health can be considered good for all the experimental groups. In fact, this situation strengthens the hypothesis that the wet litter image seen in those consuming CG in the present study may be due to excess glycerin excreted in the urine due to GK saturation. Glycerin in the litter material may have served as a cushion/support material. This situation may explain the absence of severe footpad lesions. This hypothesis is supported by the fact that FPD scores did not exceed the threshold value of fifty. USTUNDAG et al. (2013a) reported a statistically significant ($P<0.05$) decrease in triglyceride levels in female quails. Similarly EROL et al. (2009) and MOUSA et al. (2018) observed comparable reductions in broilers when compared to the control group. In the present study, in parallel with these findings, a statistically significant decrease ($P<0.05$) in triglyceride levels occurred as CG consumption increased. This may be due to increased tissue storage of triglycerides or lipid utilization. USTUNDAG et al. (2013a) reported that blood glucose levels were not affected by glycerin levels in the ration at 21 days of age in Japanese quail, YALÇIN et al. (2010) in laying hens, and MOUSA et al. (2018) in broilers ($P>0.05$). The findings in our study are consistent with the literature. Our study is similar to the studies of YALÇIN et al. (2010) in laying hens, USTUNDAG et al. (2013a) in Japanese quails, and ROMANO et al. (2014) in broiler chickens, which reported that blood cholesterol levels in broiler chickens were not affected by glycerin consumption. In the present study, the fact that ALT, AST, and creatinine levels were not affected by CG consumption indicates that 5% and 10% CG levels do not cause liver or kidney damage in broiler chickens. These findings are similar to the finding of MOUSA et al. (2018) and YALÇIN et al. (2010) that glycerin consumption had no statistically significant effect (P>0.05). Diet composition and the interaction between diet and microflora may influence the mucus composition of the GI tract, mucosal structure, and intestinal development (OZDOGAN et al., 2014). In the present study, it was observed that the number of coliform bacteria increased linearly with the amount of CG consumed at the rate of 72.4% (P<0.05), and there was a moderate relationship (50.2%) between CG consumption and the number of coliform bacteria. The highest number of coliform bacteria was found in those who consumed 10% CG on the 22nd-42nd days of our study, indicating that consuming 10% CG only between the 22nd-42nd days may lead to an increase in the number of coliform bacteria compared to consuming 10% CG throughout the entire study. It has been reported that microorganisms use glycerol as an energy source and convert it into fumaric acid, succinic acid, and propionic acid. It has been suggested that beneficial microorganisms in the digestive organs (e.g. *Bifidobacterium* spp. and *Lactobacillus* spp.) convert glycerol into useful metabolites, and put pressure on some microorganisms in the intestine (DA SILVA et al., 2009). This may support our finding that there was no death or illness despite the increased coliform bacteria count due to increased CG consumption. USTUNDAG et al. (2013b) reported that there was no difference in the number of coliform bacteria between the groups on the 21st day (P>0.05) in male quails, while in female quails, group II (that consumed 5% CG on the 1st-35th days) had the highest number of coliform bacteria (P<0.05), and group IV (that consumed 5% CG on the 21st-35th days) had the lowest number of coliform bacteria (P<0.05). In the present study, group II had a numerically higher coliform bacteria count than group I, which is similar to this study. In addition, in the same study (USTUNDAG et al., 2013b), it was reported that group IV (5% CG consumption on days 21-35) had the lowest (P<0.05) coliform bacteria count on the 35th day in male quails, while groups II and III had the highest counts (P<0.05). In the present study, group IV and group II had a numerically higher coliform bacteria count than group III. In a study conducted by OZDOGAN et al. (2014), it was reported that the group consuming 4% CG had a lower (P<0.05) duodenum coliform bacteria count than the other groups. The reason for the difference between this study and our study may be factors such as breed, the glycerin composition, the rate and duration of use, gender, the age at which CG was started, and the small intestine section. In the present study, it was concluded that coliform bacteria increased in broiler chickens with a diet containing 10% CG until the 42nd day of feeding. However, there are not enough studies showing the relationship between different amounts of CG and the number of intestinal microorganisms in poultry at different ages. Therefore, more studies are needed to determine the effects on the intestine of the amounts of CG used in poultry diets and the duration of use. Histological changes in the intestine have been reported to be related to intestinal functions and are stimulated by diet (OZDOGAN et al., 2014). The microscopic structure of the small intestines is considered a main index of intestinal development, functionality and health (MARCHEWKA et al., 2021). The ileum is where mineral and water absorption occurs, while starch, fat and protein absorption occurs primarily in the jejunum (SVIHUS et al., 2002). There is evidence that the ileum plays a role in starch and fat digestion, especially in fast-growing broiler chickens (RICHARDS-RIOS et al., 2020). In general, an increase in VH is associated with increased nutrient absorption, while deep crypts are associated with higher tissue turnover rates (DE VERDAL et al., 2011). It has been stated that VH and VW may change with feeding, and that long villi increase the absorption of most nutrients in the small intestine (OZDOGAN et al., 2014). However, a study conducted by MAGAYA et al. (2023) showed that although substituting sorghum for corn increased VH, diets containing whole sorghum had negative effects on body weight and carcass weight. A similar situation occurred in the present study with CG. In the present study, ileum VH increased due to increased CG levels (P<0.05), but when the 42nd day BWs were examined, no difference was observed between the groups (P>0.05). In the present study, the ileum VHs of those consuming 5% and 10% CG were higher than those consuming 0% CG (P<0.05) and the ileum VHs increased with increasing CG levels (P<0.05), which is similar to the findings of OZDOGAN et al. (2014). This may be due to the fact that microorganisms that use glycerol as a food source produce short-chain fatty acid metabolites, and these products affect their VHs. In our current study, unlike OZDOGAN et al. (2014), a tendency for the VW to decrease with increasing amounts of CG was observed (P<0.05). In our study, the highest VW was in group I (P<0.05). This difference may be due to the composition of the CG used, race, and using CG on different days. Intestinal crypts are the areas between the villi where epithelial cells proliferate and secrete mucus (KARAMIK, 2019). In the present study, the ileum CD in group I was higher than in the other groups (P<0.05). This finding is different from the study of OZDOGAN et al. (2014) in which it was reported that there was no difference between the groups in ileum CD, while duodenum CD was greater in the groups receiving CG (P<0.05). This difference may be due to factors such as the composition of the glycerin used in the study, the rate and duration of use, and the age at which CG was started. Crypt width is a measure of the depth of the intestinal glands that play a role in the renewal and differentiation of intestinal cells. In the present study, the CW of group VII was higher than in group I (P<0.05). In addition, the increase in CW with the increase in CG rate is consistent with the findings of OZDOGAN et al. (2014). In the present study, it was observed that the use of CG had no effect on ileum ECT (P>0.05), which is consistent with the study by OZDOGAN et al. (2014). However, the relationship between CG and ECT needs to be investigated in further studies. An decrease in the thickness of the jejunum (P<0.001) and duodenum (P<0.05) were also reported (OZDOGAN et al., 2014). The surface area of the small intestine is affected by intestinal VH and VW. Digestion and absorption of nutrients can be affected by the surface area of the intestines. Therefore, the increase in poultry productivity can be associated with the increase in the absorption activity in the intestines. In the present study, the ileum VSA was higher in those consuming 5% and 10% CG than those consuming 0% CG throughout the study period. Although there was no difference in FCRs on day 42 in the present study (P>0.05), CG consumption led to numerical improvement in the group consuming 10% CG. Since our study is the first to evaluate the effect of CG on VSA, more studies are needed. Intestinal mucosal cell renewal can be measured by the ratio between intestinal VH and CD. This ratio indicates the life span of intestinal mucosal cells. The higher the ratio between intestinal VH and CD, the longer the life span of intestinal mucosal cells (KARAMIK, 2019). In a study conducted on chickens (MARCHEWKA et al., 2021), it was reported that the epithelial cell renewal and nutrient absorption were associated with the VH/CD ratio. Intestinal VH and CD may vary depending on various factors such as nutrition, age, stress, infection, inflammation, hormones and genetic factors (KARAMIK, 2019). In the present study, the VH/CD ratio increased parallel to CG consumption (P<0.05). This may be the reason for the numerical increase in FI, BW and BWG, and the numerical improvement in the FCR of broiler chickens on days 5-42 of the study. The present findings indicate that VH/CD increased with the increase in CG consumption, but these results did not cause any difference in final BWG, FI or FCR (P>0.05). Since this is the first study to evaluate the effects of CG on VH/CD ratio, more studies are needed.

Conclusions

Alternative energy sources to corn are being sought in poultry production. Due to its increasing supply and decreasing price, CG is being considered as an alternative energy source. In this study, we investigated whether it is possible to use CG at different rates in different growing periods while reducing corn in broiler diets, and how this will affect performance, EM, FPD, intestinal microbiology and ileum histopathology. As a result, it was understood that up to 10% CG can replace corn in well-balanced broiler diets. However, it is thought that using 5% CG will have many advantages as less watery excreta will be formed due to the decrease in EM, and therefore less wet litter and foot-pad problems will be seen. Despite the increase in the number of coliform bacteria in the small intestines due to the increase in the amount of CG consumed, the absence of death or illness in the study supports the hypothesis that glycerol is converted into useful metabolites by beneficial microorganisms in the digestive organs, thereby suppressing some pathogenic bacteria. This may also lead to improvement in intestinal villi and crypts, and improved performance as a result of increased villus surface area. Crude glycerin prices may decrease due to the increase in the amount of CG produced. In addition, performance may even be improved by producing the feed in pelleted form. It is thought that the findings obtained and the ideas suggested will shed light on subsequent studies by other researchers.

Ethics approval

This research was approved by Balıkesir University Animal Experiments Local Ethics Committee (HADYEK) (Decision no: 2023/1-3).

Financial support statement

This research received no external funding.

Authorship contribution statement

This article was extracted from the 1st author's doctorate dissertation entitled: "The Effects of Crude Glycerin Used in Broiler Mixed Feeds on Performance, Small Intestine Histopathology and Footpad Lesions".

Declaration of competing interest

No potential conflict of interest was reported by the authors.

Authors ORCID iD

Y. IŞIK: https://orcid.org/0000-0001-7654-1565

E. DEMİR: https://orcid.org/0000-0001-8350-3479

References

AOAC (1997): Official Methods of Analysis of AOAC international. 16th Edition, Gaithersburg, Maryland, USA.

BÖLÜKBAŞ, B., İ. KAYA (2022): Utilization of crude glycerin in ruminant diets. Erciyes Üniv. Vet. Fak. Derg. 19, 74-82.
https://doi.org/10.32707/ercivet.1084972

CERRATE, S., F. YAN, Z. WANG, C. COTO, P. SACAKLI, P. W. WALDROUP (2006): Evaluation of glycerine from biodiesel production as a feed ingredient for broilers. Int. J. Poult. Sci. 5, 1001-1007.
https://doi.org/10.3923/ijps.2006.1001.1007

DA SILVA, G. P., M. MACK, J. CONTIERO (2009): Glycerol: a promising and abundant carbon source for industrial microbiology. Biotechnol. Adv. 27, 30-39.
https://doi.org/10.1016/j.biotechadv.2008.07.006

DE SOUZA, C., J. BROCH, C. EYNG, C. DE SOUZA, J. H. OXFORD, R. V. NUNES (2020): Influence of the gross dietary level of glycerin on performance, blood parameters, carcass yield, and quality of meat of broilers. J. Appl. Poult. Res. 29, 204-219.
https://doi.org/10.1016/j.japr.2019.10.007

DE VERDAL, H., A. NANCY, D. BASTIANELLI., H. CHAPUIS, N. MÊME, S. URVOİX., E. LE BIHAN-DUVAL, S. MIGNON-GRASTEAU (2011): Improving the efficiency of feed utilization in poultry by selection. Genetic parameters of anatomy of the gastro-intestinal tract and digestive efficiency. BMC Genetics 12, 59.
https://doi.org/10.1186/1471-2156-12-59

DOZIER, W. A., B. J. KERR, S. L. BRANTON (2011): Apparent metabolizable energy of crude glycerin originating from different sources in broiler chickens. Poult. Sci. 90, 2528-2534.
https://doi.org/10.3382/ps.2011-01510

DOZIER, W. A., B. J. KERR, A. CORZO, M. T. KIDD, T. E. WEBER, K. BREGENDAHL (2008): Apparent metabolizable energy of glycerin for broiler chickens. Poult. Sci. 87, 317-322.
https://doi.org/10.3382/ps.2007-00309

EKSTRAND, C., T. E. CARPENTER, I. ANDERSSON, B. ALGERS (1998): Prevalence and control of foot-pad dermatitis in broilers in Sweden. Br. Poult. Sci. 39, 318-324.
https://doi.org/10.1080/00071669888845

EROL, H., S. YALÇIN, M. MİDİLLİ (2009): The effects of dietary glycerol on growth and laying performance, egg traits and some blood biochemical parameters in quails. Rev. Med. Vet. 160, 469-476.

EU (2005): European Union, Commission of the European Communities. Proposal 221 final a Council Directive/ Laying down minimum rules for the protection of chickens kept for meat production, 2005. Brussels, Belgium.

FERREIRA, G. J. B. DE C., D. C. SOUSA DE, N. L. A. OLIVEIRA DE, L. R. B. DOURADO, (2015): Digestive system poultry and glycerol in the diet of broiler chickens - Review. PubVet 9, 369-380. (in Portuguese)

https://doi.org/10.22256/pubvet.v9n8.369-380

GHAYAS, A., J. HUSSAIN, S. AHMAD, H. M. ISHAQ, Z. KAMRAN, M. YOUSAF, S. AHMAD, W. ISHAQ, S. MUNIR, I. AHMED, H. I. AHMAD (2023): Glycerine: an alternative energy source for poultry. World's Poult. Sci. J. 79, 467-484.

https://doi.org/10.1080/00439339.2023.2225796

GIANFELICI, M. F. (2009): Use of glycerın as energy source for broıler. Masters diss., Federal University of Rio Grande Do Sul Faculty Of Agronomy Postgraduate Program in Animal Scıence, Porto Alegre, Brasil. (in Portuguese)

HENZ, J. R., R. V. NUNES, C. EYNG, P. C. POZZA, R. FRANK, R. A. SCHONE, T. M. M. OLIVEIRA (2014): Effect of dietary glycerin supplementation in the starter diet on broiler performance. Czech J. Anim. Sci. 59, 557-563.

IŞIK, Y., E. DEMİR (2023): Use of crude glycerin in broiler mixed feeds. F. Ü. Sağ. Bil. Vet. Derg. 37, 269-274. (in Turkish)

KARAMIK, Ş. (2019): Effects of in ovo or early post-hatch feeding of l-glutamine on digestive system, muscle development, meat quality and performance in broiler chicks. Masters diss., Eskişehir Osmangazi University, Türkiye. (in Turkish)

LIMA, E. M. C., P. B. RODRIGUES, R. R. ALVARENGA, V. M. P. BERNARDIO, L. MAKIYAMA, R. R. LIMA, V. S. CANTARELLI, M. G. ZANGERONİMO (2013): The energy value of biodiesel glycerine products fed to broilers at different ages. J. Anim. Physiol. Anim. Nutr. (Berl) 97, 896-903.

https://doi.org/10.1111/j.1439-0396.2012.01335.x

MAGAYA, R. P., T. MUTIBVU, S. NCUBE, E. NYAHANGARE, P. MAPFUMO, F. MTAMBANENGWE, E. NYAKUDYA, A. NHAMO (2023): Ileal villi morphological characteristics of cobb 500 broilers fed phytase and tannase treated sorghum based diets. J. Poult. Res. 20, 32-41.

https://doi.org/10.34233/jpr.1317771

MANDALAWI, H. A., M. V. KIMIAEITALAB, V. OBREGON, D. MENOYO, G. G. MATEOS (2014): Influence of source and level of glycerin in the diet on growth performance, liver characteristics, and nutrient digestibility in broilers from hatching to 21 days of age. Poult. Sci. 93, 2855-2863.

https://doi.org/10.3382/ps.2014-04156

MARCHEWKA, J., P. SZTANDARSKI, Ż. ZDANOWSKA-SĄSIADEK, D. ADAMEK-URBAŃSKA, K. DAMAZIAK, F. WOJCIECHOWSKI, A. B. RIBER, S. GUNNARSSON (2021): Gastrointestinal tract morphometrics and content of commercial and indigenous chicken breeds with differing ranging profiles. Animals 11, 1881.

https://doi.org/10.3390/ani11071881

MIN, Y. N., F. YAN, F. Z. LIU, C. COTO, P. W. WALDROUP (2010): Glycerin-a new energy source in poultry. Int. J. Poult. Sci. 9, 1-4.

https://doi.org/10.3923/ijps.2010.1.4

MOUSA, B. H., H. H. NAFA´A, Y. T. AL-RAWI, R. K. AL-DULAIMY (2018): Effect of partial substitution of crude glycerol as an alternative energy source to diets in productive performance and some blood parameters of broiler. J. Pharm. Sci. Res. 10, 2907-2911.

NRC (1994): National Research Council. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.

OZDOGAN, M., E. TOPAL, E. P. PAKSUZ, S. KIRKAN (2014): Effect of different levels of crude glycerol on the morphology and some pathogenic bacteria of the small intestine in male broilers. Animal 8, 36-42.

https://doi.org/10.1017/S1751731113001833

PAPADOMICHELAKIS, G., A. C. PAPPAS, E. ZOIDIS, K. C. MOUNTZOURIS, K. FEGEROS (2015): Impact of feeding increasing crude glycerine levels on growth performance, glycerine kinase gene expression, nutrient digestibility and litter quality in broiler chickens. Livest. Sci. 181, 89-95.

https://doi.org/10.1016/j.livsci.2015.10.005

RICHARDS-RIOS, P., J. FOTHERGILL, M. BERNARDEAU, P. WIGLEY (2020): Development of the ileal microbiota in three broiler breeds. Front. Vet. Sci. 7, 17.

https://doi.org/10.3389/fvets.2020.00017

ROMANO, G. G., J. F. M. MENTEN, L. W. FREITAS, M. B. LIMA, R. PEREIRA, K. C. ZAVARIZE, C. T. S. DIAS (2014): Effects of glycerol on the metabolism of broilers FED increasing glycerine levels. Braz. J. Poul. Sci. 16, 97-106.

https://doi.org/10.1590/S1516-635X2014000100014

ROSTAGNO, H. S., L. F. T. ALBINO, J. L. DONZELE, P. C. GOMES, R. F. D. OLIVEIRA, D. C. LOPES, A. S. FERREIRA, S. L. D. T. BARRETO, R. F. EUCLIDES (2011): Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements. 3rd ed., Universidade Federal de Viçosa-Departamento de Zootecnia.

SANTOS, E. T. D, L. R. B. DOURADO, F. F. D. SILVA, R. F. D. SOUSA, S. P. B. D. LIMA, J. S. D. SIQUEIRA (2019): Feeding plans with the use of glycerin for broilers. Comun. Sci. 10, 254-261.

https://doi.org/10.14295/cs.v10i2.2254

SEHU, A., S. KUCUKERSAN, B. COSKUN, B. H. KOKSAL (2013): Effects of graded levels of crude glycerine addition to diets on growth performance, carcass traits and economic efficiency in broiler chickens. Kafkas Üniv. Vet. Fak. Derg. 19, 569-574.

https://vetdergikafkas.org/abstract.php?id=1365

SILVA, M. C. D., R. G. M. V. VAZ, K. F. RODRIGUES, J. H. STRINGHINI, L. F. SOUSA, F. L. R. FONSECA, W. F. AUGUSTO, L. D. S. BEZERRA (2019): Purified glycerin in balanced diets of broiler chickens treated from 1 to 42 days of age. Rev. Bras. Zootec. 48.

https://doi.org/10.1590/rbz4820180205

SILVA, C. L. S., J. F. M. MENTEN, A. B. TRALDI, R. PEREIRA, K. C. ZAVARIZE, J. SANTAROSA (2012): Glycerine derived from biodiesel production as a feedstuff for broiler diets. Braz. J. Poult. Sci. 14, 159-232.

https://doi.org/10.1590/S1516-635X2012000300006

SORIN, M. C., V. ILIE, C. GEORGETA, G. ANCA, M. TEODOR (2013): Influence of the dietary protein level on the incidence of footpad dermatitis in broiler chickens. Indian J. Anim. Sci. 83, 1084-1089.

SVIHUS, B., H. HETLAND, M. CHOCT, F. SUNDBY (2002): Passage rate through the anterior digestive tract of broiler chickens fed on diets with ground and whole wheat. Br. Poult. Sci. 43, 662-668.

https://doi.org/10.1080/0007166021000025037

ŞAHİN, T., T. SURAL (2020): Use of biodiesel byproducts in animal nutrition. J. Nat. Appl. Sci. 3, 199-206. (in Turkish)

https://doi.org/10.47495/okufbed.767249

TAVERNARI, F. DE C., A. R. S. V. DE SOUZA, V. FEDDERN, L. D. S. LOPES, C. J. D. S. TEIXEIRA, J. A. MULLER, D. SUREK, D. PAIANO, T. G. PETROLLI, M. M. BOIAGO (2022): Metabolizable energy value of crude glycerin and effects on broiler performance and carcass yield. Livest. Sci. 263, 105017.

https://doi.org/10.1016/j.livsci.2022.105017

THOMPSON, J. C., B. B. HE (2006): Characterization of crude glycerol from biodiesel production from multiple feedstocks. Appl. Eng. Agric. 22, 261-265.

https://doi.org/10.13031/2013.20272

USTUNDAG, A. O., A. E. TUZUN, M. OZDOGAN (2013a): Effect of glycerol supplemented diet fed different ages on growth performance and some blood parameters in japanese quails. J. Int. Sc. Publ. 1, 4-10.

USTUNDAG, A. O., A. E. TUZUN, M. OZDOGAN, G. B. OZDEMİR (2013b): Effect of glycerol supplemented diet at different ages on japanese quails on cecal microflora. Maced. J. Anim. Sci. 3, 81-85.

https://doi.org/10.54865/mjas1331081u

VAN SOEST, P. J., J. B. ROBERTSON, B. A. LEWIS (1991): Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

https://doi.org/10.3168/jds.S0022-0302(91)78551-2

YALÇIN, S., H. EROL, B. ÖZSOY, I ONBAŞILAR, S. YALÇIN, A. ÜNER (2010): Effects of glycerol on performance, egg traits, some blood parameters and antibody production to srbc of laying hens. Livest. Sci. 129, 129-134.

https://doi.org/10.1016/j.livsci.2010.01.014

Received: 14 November 2024
Accepted: 14 May 2025
Online publication: 10 October 2025

YAHYA IŞIK, Y., E. DEMİR: Utjecaj primjene sirovog glicerina u hranidbi brojlera na proizvodne pokazatelje, vlažnost fecesa, kontaktni dermatitis nogu te na pokazatelje u krvi, histopatološki nalaz ileuma i mikrobiološki nalaz crijeva. Vet. arhiv 96, 195-211, 2026.

SAŽETAK

U peradarskoj proizvodnji traže se alternativni izvori energije koji bi mogli poslužiti kao zamjena za kukuruz. Cilj istraživanja bio je utvrditi učinke sirovog glicerina, kao alternativnog izvora energije u obrocima za brojlere, na proizvodne parametre, vlažnost fecesa, kontaktni dermatitis nogu te na pokazatelje u krvi, histopatološki nalaz ileuma i mikrobiloški nalaz u crijevima. Temeljem navedenog, procijenila se mogućnost uporabe sirovog glicerina kao zamjene za kukuruz. Istraživanje je uključilo sedam skupina: jednu kontrolnu skupinu i šest pokusnih skupina. U prehranu navedenih skupina uvrštene su različite količine s različitim trajanjem prehrane sirovim glicerinom. Obroci su bili izonitrogeni i izokalorični. S dodatkom 10% sirovog glicerina povećan ($P<0,05$) je unos hrane u razdobljima 11.-21. dana i 5.-21. dana. Tijekom svih razdoblja konzumacije obroka s 5% sirovog glicerina došlo je do porasta ($P<0,05$) tjelesne mase u usporedbi s skupinom u kojoj sirovi glicerin nije konzumiran. Omjer konverzije hrane bio je bolji za 5% u skupini koja je uzimala sirovi glicerin 5.-10. dan i 10 % u skupini 5.-21. dan ($P<0,05$). 10% sirovoga glicerina povećalo je vlažnost izlučevina, dok je 5% sirovoga glicerina uzrokovalo manje vlažne izlučevine ($P<0,05$) od kontrolne skupine. Kako se potrošnja povećavala, trigliceridi u serumu su se smanjivali, a kontaktni dermatitis nogu se povećavao ($P<0,05$). Zabilježeno je povećanje broja koliformnih bakterija s povećanjem konzumacije sirovog glicerina ($P<0,05$). S povećanjem konzumacije sirovog glicerina visina resica, širina kripte, površina resica i visina/dubina resica u ileumu su se povećale, dok su se širina resica i dubina kripte smanjile ($P<0,05$). Ovi rezultati pokazuju da ako je obrok pripremljen na uravnotežen način, sirovi se glicerin može upotrijebiti u obrocima brojlera do količine od 10% bez narušavanja proizvodnosti i zdravlja.

Ključne riječi: biodizel; brojleri; sirovi glicerin; mikrobiologija crijeva; histopatologija crijeva