Livestock Science 129 (2010) 129–134

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Effects of glycerol on performance, egg traits, some blood parameters and antibody production to SRBC of laying hens

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ARTICLE INFO

Article history:
Received 11 June 2009
Received in revised form 19 October 2009
Accepted 21 January 2010

Keywords:
Glycerol
Laying hen
Performance
Egg traits
Blood parameters

ABSTRACT

This study was designed to investigate the effects of the usage of glycerol from biodiesel production from soybean oil in laying hen diets on laying performance, egg traits, heterophils to lymphocytes ratio (H/L), some blood parameters and antibody production to SRBC. A total of 180 Lohmann Brown laying hens 39 weeks of age were allocated to four dietary treatments with one control group and three treatment groups and fed for 16 weeks. Each group was divided into five replicates as subgroups, comprising of 9 hens each. Glycerol was used at the level of 2.5, 5 and 7.5% in the diets of the first, second and third treatment groups, respectively. The diets were formulated to be isocaloric and isonitrogenous. Dietary treatments did not significantly affect body weight, egg production, egg weight, feed efficiency, mortality, egg albumen index, egg yolk index and egg Haugh unit, yolk weight percentage, exterior egg quality characteristics, excreta moisture, H/L ratio, blood parameters and antibody production to SRBC. Hens fed diets with 7.5% glycerol consumed significantly less feed than those of the other groups. Egg yolk cholesterol concentration was significantly higher for hens fed diets with 5 and 7.5% glycerol as compared to those of the other groups ($P<0.01$). The ratio of monounsaturated fatty acids (MUFA) to saturated fatty acids (SFA) in eggs was decreased ($P<0.01$) with dietary glycerol supplementation. The inclusion of glycerol had no significant effects on blood parameters, H/L ratio, antibody titers to SRBC and excreta moisture. It is concluded that glycerol can be used at 2.5% in the diets of laying hens without adverse effects on the measured parameters. Dietary glycerol at the levels of 5 and 7.5% increased egg yolk cholesterol and decreased the ratio of MUFA/SFA without affecting performance, other egg traits, immune response, H/L ratio, blood parameters and excreta moisture.

1. Introduction

Biodiesel, a mixture of methyl esters of fatty acids, is an alternative diesel fuel that is produced by chemically reacting a vegetable oil or animal fat with an alcohol, usually methanol. As a by-product 1 mol of glycerol is produced for every 3 mol of methyl esters, which is equivalent to approximately 10% of the total product (Karinen and Krause, 2006). Chemically pure glycerol is a valuable industrial compound for use in many applications including food and consumer products such as cosmetics and pharmaceuticals. Recently crude glycerol may become available for use as livestock feed. Some studies on glycerol for broilers (Simon et al., 1996; Cerrate et al., 2006), turkey hens (Rosebrough et al., 1980) and pigs (Kijora et al., 1995) have shown that glycerol from biodiesel production can

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doi:10.1016/j.livsci.2010.01.014

be used as a source of energy. Cerrate et al. (2006) reported that glycerol can be used in broiler diets up to 5.0%.

Some researchers (Mourot et al., 1994; Kijora et al., 1997; Lammers et al., 2008b) investigated the effects of dietary glycerol on cholesterol and fatty acid profile of pork lipid and meat. However, no studies will be seen to determine the effects of dietary glycerol on lipid composition and profile of egg yolk in laying hens. Therefore, the present study was aimed to examine the effects of different levels of glycerol on laying performance, egg external and internal quality characteristics, cholesterol concentration and fatty acid profile of egg yolk, excreta moisture, H–L ratio, antibody production to SRBC and some blood parameters in laying hens.

2. Materials and methods

2.1. Animals and diets

A total of 180 Lohmann Brown laying hens 39 weeks of age were allocated to four dietary treatments with one control group and three treatment groups. Each group was divided into five replicates as subgroups, comprising 9 hens each. They were housed in 60 laying cages (30 cm × 44 cm × 44 cm) with 3 hens per cage in a windowed poultry house at a light regimen of 17 h light. Feed in mash form and water were provided ad libitum during the entire 16 week experimental period. All animal-use protocols were in accordance with the regulations outlined by the Ankara University Committee on Laboratory Animals.

The ingredients and chemical composition of the diets are presented in Table 1. The diets were formulated to be isocaloric and isonitrogenous. The glycerol from biodiesel production (Biopet Company, Ankara-TURKEY) from soybean oil was used at the level of 2.5, 5 and 7.5% in the diets of the first, second and third treatment groups, respectively. As shown in Table 1, the amount of corn decreased slightly and the amounts of soybean meal and fullfat soya increased slightly as the level of glycerol in the diet increased. The metabolizable energy value of 3350 kcal/kg, which is the same energy level as corn, was used for glycerol to formulate the diets. Characterization of glycerol used is given in Table 2.

Table 1
Ingredients and chemical composition of the diets.

	Glycerol supplementation (%)			
	0	2.5	5	7.5
Ingredients (%)				
Corn	59.0	55.9	52.7	49.5
Soyabean meal	19.9	20.2	20.4	20.6
Full fat soyabean	7.0	7.3	7.8	8.3
Glycerol	0.0	2.5	5.0	7.5
Meat and bone meal	4.0	4.0	4.0	4.0
Limestone	8.4	8.4	8.4	8.4
Dicalcium phosphate	1.0	1.0	1.0	1.0
Salt	0.25	0.25	0.25	0.25
Vitamin–mineral premix [a]	0.25	0.25	0.25	0.25
DL-methionine	0.15	0.15	0.16	0.16
Lysine	0.05	0.05	0.04	0.04
Chemical composition (analyzed)				
Metabolizable energy [b], kcal	2790	2788	2780	2794
Crude protein, %	17.90	17.94	17.92	17.94
Calcium, %	4.05	4.07	4.00	4.02
Phosphorus, %	0.77	0.77	0.76	0.76

[a] Composition per 2.5 kg: 12 000 000 IU vitamin A, 2 400 000 IU vitamin D_3, 30 g vitamin E, 2.5 g vitamin K_3, 2.5 g vitamin B_1, 6 g vitamin B_2, 4 g vitamin B_6, 20 mg vitamin B_{12}, 25 g niacin, 8 g calcium-D-panthotenate, 1 g folic acid, 50 g vitamin C, 50 mg D-biotin, 150 g choline chloride, 1.5 g canthaxanthin, 0.5 g apo carotenoic acid esther, 80 g Mn, 60 g Zn, 60 g Fe, 5 g Cu, 1 g I, 0.5 g Co, and 0.15 g Se.

[b] Metabolizable energy content of diets was estimated according to the equation of Carpenter and Clegg (Leeson and Summers, 2001).

2.2. Traits measured

Nutrient composition of diets was determined according to the AOAC (2000). The sample of diets was ashed in a muffle furnace prior to the analysis of Ca and P (Farese et al., 1967; ADAS, 1981). Metabolizable energy content of the diets was estimated using the equation of Carpenter and Clegg (Leeson and Summers, 2001):ME, kcal/kg = 53 + 38 [(crude protein,%) + (2.25 × ether extract, %) + (1.1 × starch, %) + (sugar, %)].

Hens were weighed individually at the beginning and at the end of the experiment. Mortality was recorded as it occurred. Eggs were collected daily and egg production was expressed on a hen-day basis. All the eggs laid during the last two consecutive days of every week were collected and weighed individually to determine the egg weight. Feed intake was biweekly recorded and calculated as g per hen per day. Feed efficiency was calculated as kg feed per kg egg and kg feed per one dozen egg.

To determine the egg internal and shell quality characteristics 15 eggs were collected randomly from each group (3 eggs from each replicate) on the first day of the 4th, 8th, 12th and 16th weeks of the experiment (as a total 60 eggs per group during the experiment). Each egg was weighed and their shape index was measured with a special instrument (B.V. Apparatenfabreik Van Doorn, No: 75 135/2, De Bilt, Holland). Egg shell breaking strength was measured by using an egg breaking tester (static compression device, Dr -Ing. Georg Wazau Mess-+Pruftechnick, Berlin, Germany). The egg content was broken onto a glass-topped table. Egg shell thickness was measured in three different parts (upper and lower ends and middle) using a micrometer (Mitutoya, No. 1044N, 0.01–5 mm; Kawasaki, Japan). Then the height of the albumen and the yolk was measured with a tripod micrometer (Mitutoya, No. 2050-08, 0.01–20 mm; Kawasaki, Japan). The length and width of the albumen and the diameter of the yolk were measured using a digital caliper. By using these values yolk index, albumen index and Haugh units were calculated (Card and Nesheim, 1972). Egg internal quality and shell quality analyses were completed within 24 h of the eggs being collected. At the end of the

Table 2
Characteristics of glycerol fed to laying hens (as is basis).

Characteristics	
Moisture (%)	3.4
Glycerol (%)	90.2
Methanol (%)	Not detected
Crude protein (%)	0.15
Crude fat (%)	0.23
Ash (%)	6.5
NaCl (%)	5
Arsenic (mg/kg)	0.03
Iron (mg/kg)	0.003
Specific gravity (at 15.6 °C)	1.26

experiment 20 eggs per group (4 eggs from each replicate) were randomly chosen to determine yolk cholesterol and fatty acid composition. Eggs were boiled for 5 min. They were allowed to cool and then broken and their constituent parts were separated and weighed. The shells were weighed after being air-dried for 24 h. The percentage values of shell weight, yolk weight and albumen weight were calculated. Egg yolk was blended with isopropyl alcohol with a volume of 10 ml per g of yolk (Waldroup et al., 1986). Cholesterol content of this extract was determined according to the enzymatic method of TECO (TECO, 2001). Yolk cholesterol was calculated and expressed as mg per g yolk and mg per yolk.

Yolk lipids were extracted by using the method of Folch et al. (1957). After alkaline hydrolysis, fatty acids were methylated with BF_3 (AOCS, 1997). The obtained fatty acid methyl esters (FAME) were analyzed by gas chromatography (HP 6890, Agilent, New Jersey, USA) using an HP-88 column for FAME (100 m × 250 μm × 0.25 μm) (Agilent, USA). The gas chromatography conditions were as follows: injector temperature, 250 °C; detector temperature, 280 °C, carrier gas, H_2; split ratio, 1/50 to 1/25; temperature program, 120 °C for 1 min, followed by an increase of 10 °C/min to 175 °C, 175 °C for 10 min., followed by an increase of 5 °C/min to 210 °C, 210 °C for 15 min., then the temperature raised at a rate of 5 °C/min to 230 °C, 230 °C for 5 min. Peaks were identified by comparison of retention times with those of the corresponding standards of FAMEmix-37 (Supelco, Bellefonte, PA, USA) and FAMEmix-C8-C24 (Supelco, Bellefonte, PA, USA). Identification of the peaks included fatty acids between 4:0 and 24:0. Amounts of fatty acids were expressed as a weight percentage of total methyl esters of fatty acids. Fatty acids of diets as a weight percentage of total methyl esters of fatty acids were also determined as in the methods in yolk.

The fresh excreta samples from each replicate in each group were collected using a plastic tray at the first and second day of the 16th week of the experiment. Care was taken to collect fresh excreta which had no contact with drinking water. All samples were dried in an air-forced oven at 60 °C until reaching constant weight then moisture of samples were determined according to the AOAC (2000).

At the 15th week of the experiment, 15 hens were randomly selected from each group (3 from each replicate) and injected with 0.1 ml of 0.25% suspension of sheep erythrocytes (SRBC) in phosphate buffer saline. Circulating anti-SRBC antibody titers were determined by the microhemagglutination technique from samples taken at 5 days after the immunization. All titers were expressed as the $\log_2$ of the reciprocal of the serum dilution (Onbaşılar and Aksoy, 2005).

At the end of the experiment 15 hens from each group (3 from each replicate) were randomly selected and bled from the brachial vein. Blood samples were taken in two tubes, one contained EDTA for estimating the H–L ratio and the other had no anticoagulant for estimating some blood parameters. The bleeding procedure was limited to 1 min or less to minimize the influence of handling stress. Samples from blood containing EDTA were smeared on to a glass slide for the determination of the H–L ratio. After drying, the smears were stained with May–Grünwald–Giemsa stain (Gross and Siegel, 1983). One hundred leucocytes were counted on each slide, using a light microscope at ×1000 magnification. The H–L ratios were determined by dividing the number of heterophils by the number of lymphocytes. Tubes containing blood without anticoagulant were centrifuged. Serum was collected and stored at −20 °C for determination of total protein, uric acid, triglyceride, cholesterol and levels of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) by Vitros 350 autoanalyser (New York, USA; Product code 680-2153) using their accompanying commercial kits (Vitros Chemistry Products, Ortho-Clinical Diagnostics, Johnson-Johnson Company, New York, USA).

2.3. Statistical analyses

Statistical analyses were done using SPSS programme (SPSS Inc., Chicago, IL, USA). The normality of data distribution was checked using the Kolmogorov–Smirnov test. One-way ANOVA was performed to examine differences among groups. The significance of mean differences between groups was tested by Duncan. Values were given as mean ± standard error. Only egg internal and external quality characteristics were compared with ANCOVA (difference and repeated contrast method) and values were given as estimated marginal means ± standard error of mean with adjusting egg weight (Dawson and Trap, 2001). Level of significance was taken as $P<0.05$.

3. Results and discussion

Glycerol used in the present study had high content of glycerol (Table 2). Methanol was removed from glycerol completely during processing. Fatty acid profile of diets as % of total methyl esters of fatty acids was given in Table 3. Diets are rich in linoleic and oleic acids. The ratios of monounsaturated fatty acids to saturated fatty acids and polyunsaturated fatty acids to saturated fatty acids were 1.30–1.39 and 2.82–2.91, respectively.

During the experimental period one hen died in each of the groups fed with diets containing glycerol at the levels of 0,

Table 3
Fatty acid profile of the diets (% of total methyl esters of fatty acids).

	Glycerol supplementation (%)			
	0	2.5	5	7.5
Myristic acid (C14:0)	0.29	0.30	0.29	0.29
Palmitic acid (C16:0)	12.78	12.86	13.02	13.04
Palmitoleic acid (C16:1)	0.42	0.44	0.35	0.39
Heptadecanoic acid (C17:0)	0.23	0.24	0.23	0.22
Heptadesenoic acid (C17:1)	0.11	0.12	0.10	0.12
Stearic acid (C18:0)	5.20	5.28	5.45	5.59
Oleic acid (C18:1)	25.49	25.16	24.88	24.69
Linoleic acid (C18:2)	49.46	49.22	49.19	48.73
Linolenic acid (C18:3)	5.50	5.72	5.87	6.37
Eikosenoic acid (C20:1)	0.16	0.20	0.19	0.15
Behenic acid (C22:0)	0.26	0.32	0.31	0.30
Lignoseric acid (C24:0)	0.10	0.14	0.12	0.11
Saturated fatty acids (SFA)	18.86	19.14	19.42	19.55
Unsaturated fatty acids (USFA)	81.14	80.86	80.58	80.45
Mono unsaturated fatty acids (MUFA)	26.18	25.92	25.52	25.35
Poly unsaturated fatty acids (PUFA)	54.96	54.94	55.06	55.10
MUFA/SFA	1.39	1.35	1.31	1.30
PUFA/SFA	2.91	2.87	2.84	2.82

The symbols used – SFA, USFA, MUFA and PUFA – refer to saturated, unsaturated, monounsaturated and polyunsaturated fatty acids, respectively.

5 and 7.5% and mortality was not treatment related. These data are consistent with the study of Cerrate et al. (2006).

Dietary treatments did not significantly affect body weight, egg production, egg weight and feed efficiency of hens (Table 4). These data are consistent with the findings of researches involving laying hens (Coşkun et al., 2007; Lammers et al., 2008a; Swiatkiewicz and Koreleski, 2009) and turkey hens (Rosebrough et al., 1980). In the present study hens fed diets with 7.5% glycerol consumed significantly less feed than those of other groups. The inclusion of 2.5 and 5% glycerol in diets had no significant effects on feed intake during the 16 week experimental period. These data are consistent with the study of Swiatkiewicz and Koreleski (2009), who, in hens found that feed intake was not affected when 2, 4 or 6% crude glycerol was incorporated into the diet. However, Lammers et al. (2008a) reported that feed consumption was not affected by the diets with 5, 10 and 15% crude glycerol in laying hens aged 40 week old. Coşkun et al. (2007) observed that feed intake in laying hens was significantly increased by the usage of 5% pure glycerol, but the usage of 5% and 10% crude glycerol had no significant effect on feed intake of laying hens.

The inclusion of glycerol in the diet of laying hens had no significant effect on the mean values of egg breaking strength, egg shape index, egg shell thickness, shell weight percentage, albumen height, albumen index, yolk index, yolk weight percentage and Haugh unit (Tables 5 and 6). Similar results were reported by Swiatkiewicz and Koreleski (2009), who observed no effect of the inclusion of 2, 4 and 6% crude glycerol in the diets on egg quality parameters (albumen height, Haugh unit, egg shell thickness, and egg shell breaking strength). In the present study the percentage weight of albumen was significantly higher ($P<0.05$) in the groups fed diets with 5.0 and 7.5% glycerol than that of the control group (Table 6). In the study of Coşkun et al. (2007) the values of yolk index, albumen index and Haugh unit of eggs were not affected by the inclusion of glycerol at the levels of 5 and 10% in the diets but the shell thickness of egg was decreased with the usage of 10% glycerol.

Feeding diets with 5 and 7.5% glycerol resulted in a significant increase in egg yolk cholesterol concentration as mg per g yolk and mg per yolk ($P<0.01$) compared to the diet of control group. However, there was no significant increase in yolk cholesterol concentration when laying hens were fed the diet with 2.5% glycerol. In the study of Mourot et al. (1994), cholesterol levels of liver and *Semimembranosus* muscle of pigs were not affected by the 5% dietary glycerol.

Table 5
The effects of dietary supplementation of glycerol on some egg traits of hens (mean ± standard error).

	Glycerol supplementation (%)			
	0	2.5	5	7.5
Egg breaking strength	2.61 ± 0.07	2.48 ± 0.06	2.43 ± 0.05	2.49 ± 0.04
Egg shape index	77.4 ± 0.3	76.8 ± 0.3	77.2 ± 0.4	77.1 ± 0.4
Egg shell thickness, μm	374 ± 4	374 ± 3	367 ± 3	366 ± 3
Egg albumen height, mm	6.18 ± 0.08	6.16 ± 0.08	6.03 ± 0.07	5.98 ± 0.08
Egg albumen index	7.86 ± 0.15	7.81 ± 0.14	7.59 ± 0.14	7.53 ± 0.15
Egg yolk index	42.1 ± 0.3	42.3 ± 0.3	42.4 ± 0.4	42.0 ± 0.3
Egg Haugh unit	77.4 ± 0.6	76.9 ± 0.6	76.1 ± 0.5	76.1 ± 0.6

$n = 60$ per group.
No significant differences ($P>0.05$) among groups.

The effects of dietary supplementation of glycerol on yolk methylated fatty acids are shown in Table 7. Total monounsaturated fatty acid content of egg yolk fat was lower in the group fed diet containing 7.5% of glycerol than that of the control group ($P<0.05$). Inclusion of 5 and 7.5% of glycerol increased the percentage of myristic acid ($P<0.01$), palmitic acid ($P<0.05$), palmitoleic acid ($P<0.01$) and linolenic acid ($P<0.01$) and reduced the percentage of oleic acid ($P<0.01$) when compared with eggs from hens fed control diet. However glycerol supplementation had no significant effect on total saturated, total unsaturated fatty acids and the ratio of polyunsaturated fatty acids to saturated fatty acids. The ratio of monounsaturated fatty acids to saturated fatty acids was decreased significantly ($P<0.01$) with dietary glycerol supplementation. As seen in Table 3, the ratio of MUFA to SFA in the diets was also decreased with dietary glycerol supplementation. In the study with growing pigs of Lammers et al. (2008b), fatty acid profile of the loin muscle was slightly changed by diet with the loin muscle from pigs fed 10% crude glycerol having less linoleic acid ($P<0.01$) and more eicosapentaenoic acid ($P=0.02$) than pigs fed the 0 and 5% crude glycerol diets. Some researchers (Mourot et al., 1994; Kijora et al., 1997) reported that crude glycerol supplementation had been shown to slightly increase oleic acid at the

Table 4
The effects of dietary supplementation of glycerol on laying performance (mean ± standard error).

	Glycerol supplementation (%)				*P*
	0	2.5	5	7.5	
Initial body weight, g	1997 ± 19	1967 ± 20	1979 ± 10	1973 ± 17	NS
Final body weight, g	2090 ± 13	2090 ± 21	2096 ± 27	2082 ± 15	NS
Feed intake, g/hen-day	116.5 ± 0.8a	116.3 ± 0.6a	116.1 ± 0.5a	113.3 ± 0.9b	<0.05
Hen-day egg production, %	89.9 ± 1.4	91.9 ± 1.2	91.9 ± 1.4	90.6 ± 1.4	NS
Egg weight, g	62.4 ± 0.2	62.8 ± 0.3	62.6 ± 0.1	62.7 ± 0.4	NS
Feed efficiency, kg feed/kg egg	2.08 ± 0.02	2.02 ± 0.03	2.02 ± 0.04	2.00 ± 0.03	NS
Feed efficiency (kg feed/dozen egg)	1.56 ± 0.02	1.52 ± 0.03	1.52 ± 0.03	1.50 ± 0.02	NS

$n = 5$ per group.
NS: no significant differences.
a,b: means within a row followed by the same superscript are not significantly different ($P>0.05$).

Table 6
The effects of dietary supplementation of glycerol on weight percentages of egg parts and egg yolk cholesterol (mean ± standard error).

	Glycerol supplementation (%)				*P*
	0	2.5	5	7.5	
Shell weight percentage, %	13.0 ± 0.2	12.6 ± 0.3	12.5 ± 0.2	12.6 ± 0.2	NS
Albumen weight percentage, %	59.8 ± 0.4b	60.6 ± 0.3ab	61.2 ± 0.3a	61.4 ± 0.6a	<0.05
Yolk weight percentage, %	27.2 ± 0.4	26.8 ± 0.4	26.3 ± 0.3	26.0 ± 0.5	NS
Yolk cholesterol, mg/g yolk	13.1 ± 0.5b	14.4 ± 0.8b	17.5 ± 0.8a	17.7 ± 0.8a	<0.01
Total yolk cholesterol, mg/yolk	226.5 ± 10.9b	247.2 ± 12.9b	288.2 ± 14.9a	295.2 ± 15.9a	<0.01

$n = 20$ per group.
NS: no significant differences.
a,b: means within a row followed by the same superscript are not significantly different ($P > 0.05$).

expense of linoleic and linolenic acids. Mourot et al. (1994) reported also declines in myristic acid in backfat ($P < 0.01$) and linolenic acid in backfat ($P < 0.001$) and *Semimembranosus* muscle ($P < 0.001$) when pigs were fed 5% glycerol. However Kijora et al. (1997) did not observe these changes in backfat from pigs fed diets having 10% glycerol. The differences in fatty acid profile among literatures may be due to the differences in the amount and profile of fatty acids remaining in crude glycerol or the reduction in corn due to the addition of glycerol (Lammers et al., 2008b).

Mean values of excreta moisture of groups were 81.09, 80.40, 80.35 and 80.53%, respectively (data were not shown). Excreta moisture was not significantly affected by the inclusion of dietary glycerol. Lammers et al. (2008a) reported that the excreta from hens fed the 15% crude glycerol diet was considerably wetter than those from hens fed diets with 0, 5 and 10% glycerol because of the higher content of sodium in the diets with 15% glycerol. Cerrate et al. (2006) observed that litter in pens where birds were fed diets with 10% glycerol was much wetter than that of litter in pens where birds were fed the control diet or diet with 5% glycerol. Cerrate et al. (2006) also reported that diets with 10% glycerol contained approximately 0.15% more potassium than did the control diets because of higher content of potassium in glycerol.

It was observed in the present study that there were no significant differences among groups in H–L ratio, serum levels of total protein, uric acid, triglyceride, cholesterol, ALT, AST and ALP (Table 8). In the study of Mourot et al. (1994) a significant increase in plasma cholesterol level was observed in pigs fed diets containing 5% glycerol but triglyceride level was unaffected by glycerol treatment. Thacker et al. (1994) also reported that glycerol supplementation did not affect serum cholesterol level of broilers. Antibody titers (as $\log_2$) against SRBC of groups were 4.93, 4.93, 5.00 and 5.33, respectively (data were not shown). Antibody titers against SRBC were not affected by feeding diets with 2.5, 5 and 7.5% glycerol.

Different responses to supplementary glycerol among the literatures might be due to the species, composition of glycerol used, composition of the diet, levels of glycerol in the diet and the duration of supplementation.

Table 7
The effects of dietary supplementation of glycerol on yolk fatty acids (g/100 g total methyl esters of fatty acids) of laying hens (mean ± standard error).

	Glycerol supplementation (%)				*P*
	0	2.5	5	7.5	
14:0	0.337 ± 0.008b	0.379 ± 0.011a	0.411 ± 0.010a	0.412 ± 0.016a	<0.01
16:0	24.47 ± 0.19b	25.09 ± 0.23ab	25.44 ± 0.26a	25.61 ± 0.35a	<0.05
16:1	2.957 ± 0.113c	3.149 ± 0.118bc	3.588 ± 0.177ab	3.659 ± 0.202a	<0.01
17:0	0.193 ± 0.008	0.175 ± 0.011	0.181 ± 0.006	0.191 ± 0.008	NS
17:1	0.170 ± 0.008	0.164 ± 0.008	0.179 ± 0.006	0.181 ± 0.008	NS
18:0	7.809 ± 0.116	7.871 ± 0.090	7.601 ± 0.123	7.539 ± 0.204	NS
18:1	44.20 ± 0.52a	42.84 ± 0.44ab	42.36 ± 0.38bc	41.27 ± 0.65c	<0.01
18:2	16.72 ± 0.51	17.21 ± 0.49	16.97 ± 0.36	17.807 ± 0.57	NS
18:3	0.548 ± 0.030c	0.636 ± 0.026bc	0.671 ± 0.020ab	0.760 ± 0.050a	<0.01
20:0	0.137 ± 0.006	0.120 ± 0.004	0.135 ± 0.006	0.131 ± 0.006	NS
20:1	0.132 ± 0.004	0.137 ± 0.009	0.130 ± 0.005	0.147 ± 0.013	NS
22:0	1.637 ± 0.078	1.536 ± 0.071	1.656 ± 0.039	1.606 ± 0.043	NS
24:0	0.011 ± 0.001	0.012 ± 0.001	0.012 ± 0.001	0.011 ± 0.001	NS
22:6	0.683 ± 0.061	0.690 ± 0.035	0.667 ± 0.077	0.683 ± 0.062	NS
SFA	34.60 ± 0.22	35.18 ± 0.22	35.44 ± 0.29	35.50 ± 0.43	NS
MUFA	47.46 ± 0.50a	46.29 ± 0.44ab	46.25 ± 0.36ab	45.25 ± 0.57b	<0.05
PUFA	17.95 ± 0.56	18.53 ± 0.51	18.31 ± 0.37	19.25 ± 0.62	NS
USFA	65.40 ± 0.22	64.82 ± 0.22	64.56 ± 0.29	64.50 ± 0.43	NS
MUFA/SFA	1.37 ± 0.02a	1.32 ± 0.01b	1.31 ± 0.02b	1.28 ± 0.03b	<0.01
PUFA/SFA	0.52 ± 0.02	0.53 ± 0.02	0.52 ± 0.01	0.54 ± 0.02	NS

$n = 15$ per group.
The symbols used – SFA, USFA, MUFA and PUFA – refer to saturated, unsaturated, monounsaturated and polyunsaturated fatty acids, respectively.
NS: no significant differences.
a,b, c: means within a row followed by the same superscript are not significantly different ($P > 0.05$).

Table 8
The effects of dietary supplementation of glycerol on blood serum parameters and H–L ratio of laying hen (mean ± standard error).

	Glycerol supplementation (%)			
	0	2.5	5	7.5
Total protein, g/L	54.9 ± 1.2	54.1 ± 1.7	51.6 ± 1.9	51.4 ± 2.0
Uric acid, mg/L	49.9 ± 1.8	51.6 ± 2.1	48.2 ± 2.2	48.4 ± 2.1
Triglyceride, g/L	14.9 ± 0.5	15.4 ± 0.5	15.4 ± 0.6	14.7 ± 0.5
Cholesterol, g/L	1.13 ± 0.05	1.10 ± 0.04	1.12 ± 0.05	1.12 ± 0.06
ALT, U/L	6.13 ± 0.52	6.67 ± 0.47	6.73 ± 0.66	6.80 ± 0.50
AST, U/L	178 ± 6	176 ± 6	165 ± 5	160 ± 8
ALP, U/L	408 ± 35	363 ± 30	409 ± 44	324 ± 18
H–L ratio	0.94 ± 0.08	0.98 ± 0.12	1.11 ± 0.10	1.23 ± 0.07

$n = 15$ per group.
ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, H–L ratio: heterophils to lymphocytes ratio.
No significant differences among groups.

4. Conclusion

As a result, the inclusion of glycerol obtained from biodiesel production from soybean at the level of 5 and 7.5% in diets had increased egg yolk cholesterol content. The ratio of MUFA to SFA in eggs was significantly reduced with glycerol supplementation. No adverse effects were seen in laying performance, egg quality characteristics, total saturated and total unsaturated fatty acids in eggs, blood serum parameters, H/L ratio, excreta moisture, and immune response with dietary glycerol supplementation.

Acknowledgement

The authors express their appreciation to Biopet Company (Ankara-Turkey) for providing the glycerol.

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