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Fatty Acid Profile, Performance and Quality of Eggs from Laying Hens Fed with Crude Vegetable Glycerine

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Abstract: The goal of this experiment was to evaluate the performance, quality and fatty acid profile of eggs from laying hens fed with diets containing Crude Vegetable Glycerine (CVG) originating from biodiesel production. Two hundred and forty 35-week-old Hy-Line W36 laying hens were used. The experiment lasted 112 days, split into 4 28-day cycles. The birds were distributed in a completely randomized experimental design to six treatments with CVG inclusion of 0, 1.5, 3.0, 4.5, 6.0 and 7.5%, with 5 replicates and 8 birds per experimental unit. With increased CVG inclusion, the egg-laying percentage linearly increased ($P<0.05$). The percentage of linoleic and behenic acid, Polyunsaturated Fatty Acids (PUFAs) and omega-6 fatty acids in eggs from laying hens fed with CVG increased as the levels of inclusion ($P<0.05$). According to the results of the present study, CVG can be included in the diet of laying hens up to the level of 7.5% of inclusion, considering the improvement in performance and lipid profile of the eggs.

Key words: Egg quality, fatty acids, glycerine, glycerol, lipid profile

INTRODUCTION

There is a constant search for feed that can replace soy beans and maize in animal feed to reduce production costs and contribute to a decrease in the environmental impacts of production. This has raised the interest of the scientific community in studying the use of agroindustrial by-products.

The production of biodiesel is currently being discussed as a method of reducing the environmental impact of fossil-fuels. For this reason, the worldwide production of biodiesel has experienced an accelerated growth over recent years, with an estimated production of approximately 28.8 million litres in 2013 (OECD-FAO, 2013). Biodiesel is produced from plant oils and animal fat, either through basic catalysis using sodium hydroxide or potassium hydroxide as catalysts, or through esterification in the presence of acid catalysts, during which triglycerides react to form diglycerides, monoglycerides and crude glycerine (the glycerol contents of these glycerines vary between 80 and 95%) as a by-product.

Although the glycerine resulting from biodiesel production can be used in the cosmetic, pharmaceutical and food industries (Thompson and He, 2006), there is a great concern regarding its final destination. One possible use for this glycerine which is recognized by the Food and Drug Administration of the U.S.A. (FDA, 2006, 21 CRF 582.1320), is as a component of animal feed. Glycerine can be used as an energy source because glycerol can be converted into a glycolytic substrate, via gluconeogenesis, or oxidized for energy production through glycolysis and the citric acid cycle (Robergs and Griffin, 1998; Dozier *et al*., 2011).

Glycerol can be used in the feed of laying hens due to its high energy content, with 4,305 kcal of crude energy per kg of pure glycerol and 3,805 kcal/kg of nitrogen-corrected apparent metabolizable energy (AMEn) (Lammers *et al*., 2008). Dozier *et al*. (2011) determined the AMEn for broiler chickens for 10 glycerine samples, originating from biodiesel industries that used different lipid sources and found AMEn values varying between 3,254 and 4,134 kcal/kg. According to the authors, this variation depends on fatty acid, methanol and water content of the glycerine.

Furthermore, considering the high level of fatty acids remaining in the glycerine and the high absorption coefficient of Polyunsaturated Fatty Acids (PUFAs) in birds, the use of glycerine in the feed of laying hens may enrich the eggs in PUFAs. This enrichment is of great interest because PUFAs can promote human health through a possible decrease in the occurrence of illnesses, such as cancer and diabetes, among others (Woods and Fearon, 2009).

The consumption of eggs from hens fed with high amounts of PUFAs could therefore provide considerable amounts of DHA to humans which is notable because

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the conversion of ALA into DHA is close to insignificant in humans. The alpha-linolenic Acid (ALA) ingested by the hens is readily allocated to the egg yolk and a small part is converted to long chain PUFAs, such as docosahexaenoic acid (DHA), in the liver (Beynen, 2004). The goal of the present study was to evaluate the effects of utilizing Crude Vegetable Glycerine (CVG) in the feed of commercial laying hens on the performance, quality and fatty acid profile of eggs and on the excreta moisture.

MATERIALS AND METHODS

Animals and handling: Two hundred and forty 35-week-old Hy-Line W36 laying hens were used and were randomly housed in galvanized wire cages (25 x 40 x 45 cm) divided into four sections with two birds each, totalizing eight birds per cage. The birds were distributed in a completely randomized experimental design to six treatments with 6 levels of CVG dietary inclusion: 0, 1.5, 3.0, 4.5, 6.0 and 7.5%, with 5 replicates each and 8 birds per experimental unit. The experiment lasted 112 days, split into 4 28-day cycles.

The protocol for this experiment was approved and birds were cared according to the guidelines of the Universidade Estadual de Maringá, Maringá (UEM), Maringá, Paraná, Brazil.

Diets: The diets were formulated according to the recommendations of the production manual for laying hens in the production stage (Hy-Line, 2009). These diets were based on soy bran and maize and were isonutritious and isoenergetic (Table 1). The glycerine used in this study originated from biodiesel produced from soy oil and were supplied by the Biopar company (Rolândia, PR, Brazil). The fatty acid profiles of the glycerine and control diet, determined at the department of Chemistry and Physics of the UEM are shown in Table 2. The metabolizable energy of the glycerine used in the formulation of the feeds was 3, 751 kcal/kg, according to previous digestibility assay (data not published).

Productive performance and egg quality: During the experiment, egg production was registered daily by cage of 8 hens and the feed intake was recorded every four weeks. Feed conversion ratio was calculated using the egg production and egg mass (feed intake/egg production and feed intake/mass production).

The total eggs from each cage were collected over 3 d at the end of each cycle. Egg density was determined by weighing eggs in air and then sequentially placed in 5 saline solutions of 1.070 to 1.086 g/cm^3 (with 0.004 g/cm^3 increments between solutions), according to Hamilton (1982). Haugh unit and shell weight and

Table 1: Ingredients and nutritional composition of the experimental diets (as fedbasis)

		Crude Vegetable Glycerine levels (%)				
Ingredient kg	Control	1.5	3.0	4.5	6.0	7.5
Corn	65.32	63.5	61.69	59.98	58.42	56.6
Soybean meal 45%	20.86	21.22	21.57	21.83	21.97	22.33
Soybean oil	1.42	1.39	1.35	1.3	1.22	1.18
Dicalcium phosphate	2.1	2.1	2.11	2.11	2.12	2.12
Limestone	9.36	9.35	9.35	9.35	9.34	9.34
NaCl	0.3	0.3	0.3	0.3	0.3	0.3
Supplement min. and vitam[1]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Met 98%	0.19	0.19	0.19	0.19	0.2	0.2
L-Lys HCL 78.5%	0.18	0.17	0.17	0.16	0.16	0.16
L-Thr	0.03	0.03	0.03	0.03	0.03	0.03
L-Trp	0.01	0	0	0	0	0
Glycerine	0	1.5	3	4.5	6	7.5
Calculated composition						
ME (kcal/kg)	2.850	2.850	2.850	2.85	2.850	2.850
CP (%)	15.4	15.4	15.4	15.37	15.3	15.3
Calcium (%)	4.2	4.2	4.2	4.2	4.2	4.2
Chloride (%)	0.23	0.26	0.29	0.32	0.35	0.38
Potassium (%)	0.58	0.57	0.58	0.58	0.58	0.58
Sodium (%)	0.14	0.16	0.18	0.2	0.22	0.24
Available phosphorus (%)	0.48	0.48	0.48	0.48	0.48	0.48
Digestible Lys (%)	0.8	0.8	0.8	0.8	0.8	0.8
Digestible Met+Cys (%)	0.63	0.63	0.63	0.63	0.63	0.63
Digestible Thr (%)	0.53	0.53	0.53	0.53	0.53	0.53
Digestible Trp (%)	0.16	0.159	0.159	0.16	0.16	0.161

[1]The vitamin and mineral supplement contained per kg of premix: vitamin A: 8,000,000 IU; vitamin D_3: 2,200,000 IU; vitamin E: 6,200 mg; vitamin K_3: 2,000 mg; vitamin B_1: 2,000 mg; vitamin B_2: 3,000 mg; vitamin B_6: 6,000 mg; vitamin B_{12}: 10,000 µg; Calcium pantothenate: 6,000 mg; Niacin: 25,000 mg; folic acid: 400 mg; Se: 100 mg; Mn: 65,000 mg; Fe: 40,000 mg; Cu: 10,000 mg; Zn: 50,000 mg; I: 1,000 mg.

Table 2: Fatty acid profile (%) of control diet and crude vegetable glycerine (CVG)

	Control diet	CVG
Myristic acid	0.07	0.30
Palmitic acid	12.88	11.52
Palmitoleic acid	0.10	<0.1
Stearic acid	2.88	4.25
Oleic acid	27.22	24.01
Vaccenic acid	1.03	1.47
Linoleic acid	51.61	52.67
Alpha-Linolenic acid	0.19	5.95
Saturated fatty acids (SFA)	16.41	16.07
Unsaturated fatty acids (USFA)	83.57	84.10
Monounsaturated fatty acids (MUFA)	28.35	25.48
Polyunsaturated fatty acids (PUFA)	55.22	58.62
MUFA/SFA ratio	1.73	1.58
PUFA/SFA ratio	3.37	3.65

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thickness were measured in three eggs from replicate. The albumen height was measured using a digital calliper and the Haugh units were calculated as indicated by Brant and Shrader (1958). Three measures of shell thickness was recorded using a micrometer (Mitutoyo, Tokyo, Japan) with a precision of 0.01 mm and the mean of three measurements was calculated.

Egg yolk and control feed lipid profiles: On the last cycle, six eggs were collected per replicate and the respective yolks were stored in two pools. One of the pools contained yolks from two sets of replicates and the other contained the yolks from the three remaining sets of replicates. The yolk pools were subsequently frozen for the determination of the lipid profile through gas chromatography. Total lipid was extracted using methanol:chloroform:water according to Bligh and Dyer (1959).

For the transesterification of the triacylglycerides, to obtain the fatty acid methyl esters, the triacylglycerides were transmethylated, according to the ISO 5509 method (ISO, 1978). The fatty acid methyl esters were separated with a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific Inc., Suwanee, GA, USA), equipped with a fused-silica capillary column (100 m long, 0.25 mm internal diameter and 0.20 μm CP-Sil88 (ChromPack, Middelburg, The Netherlands) and a flame ionization detector. The column temperature was programmed to be kept at 140°C for 10 minutes and then increased to 240°C at a rate of 5°C min^{-1}. The temperatures of the injector and detector were maintained at 220 and 240°C, respectively. The rates of gas flow were: 1.4 mL min^{-1} for the carrier gas, H_2; 30 mL min^{-1} for the auxiliary gas. N_2; 30 mL min^{-1} for the combustion gas, H_2 and 300 mL min^{-1} for the synthetic air. The sample split-ratio was 1:100. The peak areas were determined according to the normalisation method, with the use of a CG-300 Integrator-Processor (CGS Instrumentação Analítica, São Paulo, SP, Brazil). The identification of the peaks was performed by comparison with the retention times for fatty acid methyl esters standards (Sigma Aldrich, St. Louis, MO, USA). The lipid profile of the control feed was determined using the chromatograph from the laboratory of the Chemistry Department of UEM (Table 2).

Excreta moisture: The moisture percentage of the excreta of the each replicate with 8 hens was obtained at the end of the fourth cycle. Plastic sheets were placed under the cages for 8 hours to collect the excreta. Following the collection period, the samples were weighed and dried in a forced air oven at 55°C for 72 hours and weighed again for the determination of the moisture percentage, according to Ribeiro *et al.* (2005).

Statistical analysis: The data were examined in relation to the CVG levels using analysis of variance followed by Dunnett's test and regression analysis. SAEG (System for Statistical and Genetic Analysis, Universidade Federal de Viçosa, 1997) software was utilized for the analyses and a probability $P<0.05$ was considered significant.

RESULTS

Productive performance and egg quality: The birds that were fed with CVG showed a linear increase in the egg-laying percentage with increasing levels of glycerine inclusion ($P<0.05$, Table 3). Feed intake and feed conversion were not affected by the CVG inclusion ($P>0.05$).

The egg weight exhibited a quadratic response ($P<0.05$, Table 4) which predicted a lower weight at 4.9 % of CVG inclusion. The egg weight were lower in eggs from laying hens fed with 3 and 4.5 % of inclusion than eggs from control animals (Dunnett's test, $P<0.05$). The remaining parameters were not affected by the inclusion of CVG at any of the inclusion levels ($P>0.05$).

Fatty acid profile: The percentages of linoleic and behenic acids, PUFAs and omega-6 fatty acids in eggs from CVG-fed laying hens increased linearly with increasing glycerine inclusion levels ($P<0.05$, Table 5). The percentages of oleic acid, unsaturated (USFA) and monounsaturated (MUFA) fatty acids and the MUFA/SFA ratio exhibited quadratic responses to the level of glycerine inclusion ($P<0.05$), with highest values observed at 3.61, 5.12, 3.61 and 3.82% of CVG inclusion, respectively. The remaining fatty acids studied did not vary according to the level of CVG inclusion ($P>0.05$).

CVG inclusion levels of 1.5 % or higher increased the percentages of oleic and linoleic acids, the percentages of USFA, MUFA and PUFA, the MUFA/SFA and

Table 3: Performance of laying hens fed with different levels of crude vegetable glycerine (CVG)

Level of CVG (%)	Egg production (%)	Feed intake (g/bird/day)	Feed conversion (kg/kg)	Feed conversion (kg/dz)
Control	83.76±0.10	103.31±0.55	2.010±0.02	1.481±0.01
1.5	80.62±0.72*	102.62±1.12	2.046±0.02	1.525±0.02
3.0	84.08±0.61	100.80±2.57	1.962±0.02	1.433±0.03
4.5	82.68±0.41	100.15±1.25	1.993±0.02	1.469±0.02
6.0	83.50±0.71	102.03±1.39	2.003±0.02	1.497±0.01
7.5	85.04±0.47	101.14±1.07	1.978±0.03	1.453±0.02
Regression	L^1	ns	ns	ns
CV (%)	1.51	3.21	2.39	3.17

n = 5 replicates with 8 birds each. *indicates significant difference from the control group within the same column (Dunnett´s test, P<0.05); ns = not significant (P>0.05). L^1 = 0.58099x+80.9453, R^2 = 0.87

Table 4: Egg quality of eggs from laying hens fed with different levels of crude vegetable glycerine (CVG)

Level of CVG (%)	Egg weight	Specific gravity	Haugh unit	Shell (%)	Shell thickness (mm)
Control	62.29±0.31	1.082±0.00	95.17±0.40	8.99±0.08	0.433±0.00
1.5	62.76±0.47	1.083±0.00	95.07±0.43	8.97±0.07	0.427±0.00
3.0	60.60±0.50*	1.083±0.00	96.26±0.15	8.90±0.07	0.425±0.00
4.5	60.88±0.07*	1.083±0.00	95.10±0.26	9.01±0.05	0.426±0.00
6.0	61.43±0.42	1.082±0.00	95.38±0.65	8.96±0.10	0.423±0.00
7.5	61.45±0.28	1.082±0.00	96.32±0.64	8.86±0.06	0.423±0.00
Regression	Q^1	ns	ns	ns	ns
CV (%)	1.34	0.06	1.07	1.84	1.78

n = 5 replicates with 8 birds each. *indicates significant difference from the control group within the same column (Dunnett´s test, P<0.05); ns = not significant (P>0.05). Q^1 = 64.2670-1.4382x+0.1467x2, R^2 = 0.67, (Min. point: 4.90)

Table 5: Fatty acids composition (%) of yolk egg from laying hens fed with different levels of crude vegetable glycerine (CVG)

	Level of CVG (%)								
Fatty acids	Control	1.5	3.0	4.5	6.0	7.5	Mean	Reg.	CV (%)
Myristic	0.330	0.323	0.333	0.336	0.328	0.322	0.328	ns	3.85
Palmitic	29.61	29.02	28.12	29.28	29.94	28.44	28.97	ns	8.96
Heptadecanoic	0.572	0.565	0.571	0.568	0.581	0.565	0.57	ns	4.47
Stearic	10.32	10.65	10.15	10.12	10.02	10.48	10.28	ns	3.57
Oleic	36.79	39.08*	38.85*	39.81*	38.51*	38.40*	38.91	Q^1	1.09
Linoleic	14.04	15.71*	16.08*	16.19*	16.26*	17.20*	16.24	L^2	4.54
Linolenic	2.37	2.24	2.48	2.48	2.42	2.11	2.35	ns	11.16
Eicosanoic	1.56	1.59	1.59	1.57	1.59	1.57	1.58	ns	4.54
Behenic	0.517	0.511	0.526	0.623*	0.624*	0.692*	0.588	L^3	3.36
Arachidonic	0.199	0.185*	0.199	0.195	0.206	0.208	0.198	ns	3.95
Lignoceric	1.18	1.85	1.63	1.45	1.52	1.54	1.61	ns	16.40
DHA (cervonic)	0.121	0.128	0.126	0.118	0.120	0.125	0.123	ns	7.61
SFA	44.08	44.52	42.93	43.96	44.60	43.60	43.50	ns	3.10
USFA	53.52	57.35*	57.73*	58.79*	57.52*	58.05*	57.77	Q^4	1.04
MUFA	36.79	39.08*	38.85*	39.81*	38.51*	38.40*	38.91	Q^5	1.09
PUFA	16.73	18.27*	18.88*	18.98*	19.01*	19.65*	18.86	L^6	3.79
MUFA/SFA	0.834	0.878*	0.905*	0.906*	0.871*	0.881*	0.887	Q^7	0.30
PUFA/SFA	0.379	0.410*	0.440*	0.435*	0.427*	0.451*	0.431	ns	3.68
Omega-3	2.49	2.37	2.60	2.60	2.54	2.24	2.47	ns	10.70
Omega-6	14.24	15.90*	16.28*	16.38*	16.49*	17.41*	16.44	L^8	4.50
Omega-6/Omega-3 ratio	5.71	6.72	6.28	6.52	6.54	7.77	6.72	ns	11.18

*indicates significant difference from the control group within the same column (Dunnett´s test, P<0.05); ns = not significant (P>0.05).
Q^1 Y = 38.433+0.4626x-0.064x^2 (R^2 = 0.46; Max. point: 3.61).
L^2 Y = 15.343+0.2103x (R^2 = 0.82).
L^3 Y = 0.4572+0.0307x (R^2 = 0.93).
Q^4 Y = 56.52+0.658x-0.0643x^2 (R^2 = 0.34; Max. point: 5.12).
Q^5 Y = 38.433+0.4626x-0.064x^2 (R^2 = 0.46; Max point: 3.61).
L^6 Y = 18.091+0.1926Xx (R\ = 0.87).
Q^7 Y = 0.8664+0.0153x-0.002x^2 (R^2 = 0.27; Max. point: 3.82).
L^8 Y = 15.5621+0.203072x (R^2 = 0.83).

PUFA/SFA ratios and the omega-6 percentage (P<0.05), when compared to the control.

Excreta moisture: This parameter was not affected by the inclusion of glycerine in the diet (P<0.05).

DISCUSSION

The results of performance and egg quality obtained in the present study indicate that the CVG can be used up to 7.5%. Although several authors have shown that the inclusion of glycerine in the diet does not influence the egg-laying percentage (Rosebrough *et al*., 1980; Lammers *et al*., 2008; Swiatkiewicz and Koreleski, 2009), in the present study, this parameter increased linearly in CVG-fed birds with increasing levels of CVG inclusion. The inclusion of 1.5% was the only level that was lower than control group.

Although some authors have shown that glycerol presents in the glycerine has a sweet taste which could increase the feed intake (Min *et al*., 2010; Piesker and Dersjant-Li, 2006), feed intake was not affected by the CVG inclusion in the diet. Moreover, feed intake could have been reduced by methanol that remains in glycerine after biodiesel production because it can cause adverse effects such as gastrointestinal disorders (Dozier *et al*., 2011); therefore, it can be considered a problem for glycerine utilization (Skrzydlewska, 2003). However, in this study the presence of methanol (10.96%) in the CVG did not compromise the feed intake and productive performance, thus, can not be considered toxic to laying hens.

There were no differences in productive performance between the control and any of the glycerine treatments, with the exception of the egg production from hens fed 1.5% of CVG, indicating that the levels used were suitable. In fact, the results support the inclusion of up to 10% glycerol in bird diets (Min *et al*., 2010). The absence of any effect on productive performance when glycerine is included in the diet is likely because glycerine constitutes an energy source similar to carbohydrates (Francois, 1994) and can be absorbed either actively or passively in the intestine (Ohta *et al*., 2006).

Regarding the egg quality, although no differences in Haugh units were found among treatments, Erol *et al*. (2009) reported a decrease in egg albumen height and Haugh units in Japanese quail eggs from quails that were fed with 10% glycerol. In other studies, however, no effects of the inclusion of glycerine were observed on this parameter (Swiatkiewicz and Koreleski, 2009; Yalçin *et al*., 2010). The results reported so far are still controversial, due to the differences in the chemical compositions of the glycerines used which originate from several different plant sources (Min *et al*., 2010).

The differences in the fatty acid profile, namely the higher percentages of linoleic and behenic acids, PUFAs and omega-6 found in eggs from CVG-fed hens according to the increase of inclusion levels were expected because the amounts of linoleic acid and PUFAs present in the composition of CVG (Table 2). The fatty acid profile of eggs is highly dependent on the diet of the laying hen (Yannakopoulos *et al*., 2005; Sosin *et al*., 2006). In fact, according to Poureslami *et al*. (2012), the percentages of linoleic and linolenic acids in eggs originate from the diet of the chickens.

The alpha-linolenic and linoleic acids are precursors of the omega-3 and omega-6 PUFAs, respectively (Simopoulos, 2000). Omega-3 and omega-6 are essential fatty acids because mammals and birds are incapable of synthesizing them and, therefore, have to acquire them through their diet. Following ingestion, linoleic acid is converted to arachidonic acid and ALA is converted to eicosapentaenoic acid (EPA) and DHA. These are important for normal growth and development, as they provide benefits for human health in general. However, the conversion of ALA into EPA and DHA in humans is almost non-existent, whereas hens readily allocate a part of the ingested ALA to eggs and convert the other part to DHA, prior to allocating this DHA to the eggs, although this conversion is limited in the cases of linseed and glycerine (Goldberg *et al*., 2012). For this reason, many studies have been conducted with the aim of increasing the PUFA content of eggs through the nutritional manipulation of the diet of laying hens, with the use of marine oils and products obtained from oilseeds (Carrillo-Domínguez *et al*., 2005; Calchadora *et al*., 2006; Nain *et al*., 2012). In the present study, only linoleic acid percentage increased in the eggs from CVG-fed laying hens, when compared to the control group, with consequent increase in the omega-6 content in these eggs (Table 5).

Although, omega-6 content in the eggs from laying hens fed with CVG had increased, the omega-6/omega-3 ratio was not affected by the levels of inclusion. The ratios found in the eggs of all groups are above 5 but similar to the control group. According to Simopoulos (2002), human nutrition with a low omega-6/omega-3 ratio (near 1) is recommended for the reduction of many chronic diseases. The enrichment of the diet of laying hens with fish and linseed oils promotes a reduction in the omega-6/omega-3 ratio of eggs to 6.6 and 1.6, respectively, when compared to non-enriched eggs (Simopoulos, 2002). Although the percentage of saturated fatty acids (SFA) in CVG is high, the values obtained for the eggs were not affected by the inclusion of glycerine in the diets of the laying hens. In fact, some authors have shown that dietary changes have almost no effect on SFAs, such as an enrichment in n-3 PUFAs or PUFAs (Naber, 1979; Milinsk *et al*., 2003). This is a positive result because SFAs are associated with an increase in cardiovascular diseases.

Considering the findings of performance, egg quality and fatty acid profile, up to 7.5% of CVG can therefore be added to the diets of laying hens. However, a major problem that can be associated to the use of glycerine in bird feeds is an increase in excreta moisture. Lammers *et al*. (2008) reported that laying hens had a higher level of excreta moisture when fed 15% glycerol compared with those fed 5 or 10% glycerol. However, in the present

Table 6: Excreta moisture from laying hens fed with different levels of crude vegetable glycerine (CVG)

Level of CVG (%)	Excreta moisture (%)
Control	74.72±0.76
1.5	73.38±1.19
3.0	74.39±0.45
4.5	73.42±1.51
6.0	74.67±1.07
7.5	73.93±0.84
Regression	ns
CV (%)	2.90

ns = not significant (P>0.05)

study, the inclusion of CVG at the tested levels did not affect excreta moisture levels (Table 6). Considering the amount of sodium chloride (3.52%) and potassium (0.174%) in the composition of CVG, higher excreta moisture levels in hens was expected. Furthermore, the higher inclusion levels could also result in higher excreta moisture levels due to a higher salt intake, such as sodium and potassium which would increase the water intake and excretion (Hooge *et al*., 1999). Similar results to the findings obtained in the present study were reported by Yalçin *et al*. (2010), who included 2.5 to 7.5% of glycerol in the diets of laying hens, without an increase in excreta moisture levels.

Conclusion: The results of this study indicate that crude vegetable glycerine can be effectively used in the diets of laying hens up to a 7.5% level of inclusion considering that the CVG inclusion improves the egg production and fatty acid profile of eggs.

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ABBREVIATIONS

ALA : Alpha-linolenic acid
AMEn : Nitrogen-corrected apparent metabolizable energy
CVG : Crude vegetable glycerine
DHA : Docosahexaenoic acid
EPA : Eicosapentaenoic acid
MUFA : Monounsaturated fatty acid
PUFA : Polyunsaturated fatty acid
SFA : Saturated fatty acid;
USFA : Unsaturated fatty acid

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