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Effects of Dietary Supplementation of Glycerol on Performance, Egg Quality and Egg Yolk Fatty Acid Composition in Laying Hens

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Abstract

This study evaluated the performance and the quality and fatty acid profile of eggs from laying hens, fed with diets, containing different levels of glycerol replaced by acidulated sunflower soap stock. A total of 60 44-week-old Hy-Line W36 laying hens were distributed according to a completely randomized experimental design into four treatments, consisting of glycerol substituted of acidulated sunflower soap stock dietary inclusion levels (0, 25, 50, and 75 %), with five replicates of three birds each. Dietary treatments had no significant effect on egg production, feed intake, feed conversion ratio, egg weight and egg mass of laying hens. The inclusion of glycerol in the diet of laying hens had no significant effect on egg specific gravity, egg shell breaking strength, egg shell weight and egg shell thickness, egg shape index, albumen index, haugh unit, albumen pH, yolk pH and egg yolk color values L and b but to add glycerol in diets decreased a values in egg yolk . The inclusion of glycerol in the diet of laying hens had no significant effect on stearic, oleic, linoleic and linolenic acid contents of egg yolk. Palmitic acid content of egg yolk was significantly decreased and palmitoleic acid content of egg yolk significantly increased with the higher levels of dietary glycerol supplementation.

Keywords: Glycerol; Performance; Egg Quality; Laying Hens; Fatty Acid

INTRODUCTION

Lipids are necessary for normal growth and reproduction. The composition of lipids supplied in the diet can have a marked effect on the composition of the lipids within the animal. If the proper fatty acids are not supplied within the diet, or if the proper ratio of various fatty acids is not provided, then problems such as impairments in growth and/or reproduction can occur (Dalton, 2000). The most common lipid supplements in commercial vegetable diets are soybean and corn oils, mostly for economical and nutritional reasons (Meluzzi et al., 2001). Although they are rich in n-6 PUFA (Simopoulos& Robinson, 1998), they are expensive and compete with human nutrition (Pardı'o et al., 2005). Usually vegetable oils are used and the most used oil is soybean oil in broiler diets but also to obtain more profit and to recycle some waste materials can used like glycerol from process of biodiesel the most common energy supplements in commercial. The use of glycerol in a diet for farm animals was first attempted and reported in the 1960s and 1970s. Some studies published in this period describe the use of glycerol in the nutrition of poultry and discuss the effect of glycerol on reproduction (Neville et al, 1970; Westfall & Howarth, 1976, Suchy et al., 2012)

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 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., 2008) investigated the effects of dietary glycerol on cholesterol and fatty acid profile of pork lipid and meat. In previous experiments, dietary supplementation of crude glycerol has been reported to improve Growth performance of broiler chickens (Mclea et al., 2011) and pigs (Shields et al., 2011; Zijlstra et al., 2009). However, the reason for this improvement is not clear (Kim et al., 2013).

A study on glycerol for hens (Yalçın et al., 2010) has shown glycerol obtained from biodiesel production from soybean at the level of 5 and 7.5% in diets had increased egg yolk cholesterol content but no adverse effects were seen in other parameters. Soap stock is a by-product of the vegetable oil refining process formed by treating crude vegetable oils with alkali to produce a sodium soap, which is then separated by centrifugation (Cmolı'k & Pokorny, 2000 ; Pardı'o et al., 2005)

Soap stocks are increasingly being used as an animal feed additive, particularly in pig and poultry diets. It has no harmful effects (beyond gossypol) and can be used in a similar way to fat since it contains a high concentration of fatty acids. When soapstock is added into meal, it can improve the palatability, increase the energy density of the diet, reduce dust, help heat stress conditions, and improve pelleting of feed products by reducing feed particle separation. It also minimizes the build-up of feed particles on equipment used for feed mixing at the mill. However, long chain fatty acids are difficult to digest in animals (Johnson & McClure 1973), therefore soap stock must be added in small amounts (approximately 3.5%)(Bock et al., 1991, Dumont&Narine, 2007).

Pardı'o et al., (2003) has studied the effect of different soap stocks (corn, sunflower, canola, and soybean) on production performance and broiler skin pigmentation. Among the soap stocks studied, addition of soybean soap-stock (SSS) improved ($P = 0.005$) live BW gain of birds and produced the highest percentage of broiler skin pigmentation. Because SSS is a source of fatty acid, its inclusion in laying diets was considered (Pardı'o et al., 2005).

The aim of this study was to evaluate the effects of different levels of glycerol and acidulated soap stock on performance, egg quality and yolk fatty acid composition in laying hens egg.

MATERIAL and METHODS

A total of 60 44-week old Hy-Line W36 hens were distributed according to a completely randomized experimental design into four treatments, consisting of four levels of dietary glycerol with five replicate cages of three hens each. Layers were fed with a complete feeding mixture in a mash form. The difference between experimental diets and the control diets (100 % sunflower acidulated soap stock) was that 25, 50 and 75 % of acidulated soap stock (ASS) was replaced with glycerol. Glycerol used in this experiment was containing 3800 kcal metabolizable energy/kg. Ingredient and nutrient composition of experimental diets are shown in Table 1. The control diet was formulated to meet or exceed nutrient recommendations (NRC, 1994). Cage dimensions were 40 × 50 cm equalling 2,000 cm2 of total floor space. The laying hens diet and water were offered for ad libitum consumption (16.5% crude protein, 2,750 kcal ME /kg, 3.60% Ca, and 0.42% available P) during the experimental period. The experiment lasted 84 days and was divided into three 28-day period.

Table 1. Composition of experimental diets

	Experimental diets			
Ingredients (%)	**100 % ASS**	**75% ASS / 25 %Glycerol**	**50 % ASS / 50% Glycerol**	**25 % ASS / 75 %Glycerol**
Corn	51.20	51.50	51.50	51.00
Barley	11.00	10.00	8.60	6.00
Soybean meal	24.50	24.60	24.90	25.60
Sunflower acidulated soapstock	2.60	2.40	2.15	1.67
Glycerol	---	0.80	2.15	5.02
Limestone	8.25	8.30	8.30	8.30
Di-Calcium phosphate	1.75	1.70	1.70	1.70
Salt	0.35	0.35	0.35	0.35
Premix[1]	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10
TOTAL	100	100	100	100
Calculated nutrients				
Metabolizable Energy (Kcal/kg)	2754	2753	2753	2751
Crude protein (%)	16.56	16.52	16.51	16.51
Calcium (%)	3.60	3.61	3.61	3.61
Available phosphorus (%)	0.42	0.42	0.42	0.41
Lysine (%)	0.90	0.89	0.90	0.91
Methionine (%)	0.37	0.37	0.37	0.37
Methionine +Cystine, %	0.61	0.61	0.62	0.63

[1] Premix provided the following per kg of diet: vitamin A, 8.800 IU; vitamin D_3, 2.200 IU; vitamin E, 11 mg; nicotinic acid, 44 mg; Cal-D-Pantothenate, 8.8 mg; riboflavin 4.4 mg; thiamine 2.5 mg; vitamin B_{12}, 6.6 mg; folic acid, 1 mg; D-Biotin, 0.11 mg; choline, 220 mg; manganese, 80 mg; copper, 5 mg; iron, 60 mg; zinc, 60 mg; cobalt, 0.20 mg; iodine, 1 mg; selenium, 0.15 mg. ASS (Sunflower acidulated soapstock)

Feed intake (FI) and Egg weight (EW) were recorded biweekly. Egg production (EP) was recorded daily and Egg mass (EM) was calculated from collecting data of EP and EW at biweekly via: EM= (EP x EW) / Period (days). Feed conversion ratio (FCR; g of feed g of egg) was calculated via: FCR = FI (g of feed/hen/period) / EM (g of egg/hen/period).

The eggs were subjected to determine characteristics of eggshell quality parameters (shell breaking strength, shell weight and shell thickness) on all collected eggs produced at the last two days of each period during the experiment. Eggshell breaking strength was measured using a cantilever system by applying increased pressure to the broad pole of the shell using an instrument (Egg Force Reader, Orka Food Technology, Israel). Eggs were then broken, and eggshell, albumen, and yolk were separated and weighed. Eggshells were rinsed running water and dried in oven at 60 °C for 12 h, to determine eggshell thickness (including the membrane) in three points on the eggs (one point on air cell or the randomized two points of equator) using a micrometer (Mitutoyo, 0.01 mm, Japan). Eggshells were weighed using a 0.001g precision scale. Eggshell weight was calculated via: Eggshell weight (g/100 g egg) = [Eggshell weight (g) / Egg weight (g)]. A colorimeter (Minolta Chroma meter CR 400 (Minolta Co., Osaka, Japan) was used to assess the egg yolk color and the CIELAB colorimetric (Romero et al., 2002). At the end of the experiment, 15 eggs per group (3 eggs from each replicate) were randomly chosen to determine yolk fatty acid composition. Triacylglycerides were methylated using the ISO 5509 method (ISO, 1978) and fatty acid methyl esters were collected. The methyl esters of the fatty acids (1 µl) were analysed in a gas chromatography (Agilent 7890A), equipped with a flame ionising detector (FID) and a fused silica capillary column (100 m × 0.25 mm i.d; film thickness 0.20 micrometer). It was operated under the following conditions.

Oven temperature program: holded at 140 ^{0}C for 5 min. and raised to 240 ^{0}C at a rate 4^{0}C /min and then, kept at 240 ^{0}C for 15 min. Injector and detector temperatures were 260 and 280 ^{0}C, respectively. Carrier gas was helium, and the flow rate of helium was 1.51 ml/min. Split ratio was 30/1 µl/min.

Data were subjected to ANOVA by using MINITAB. Duncan's multiple range tests were applied to separate means. Statements of statistical significance are based on probability of $P<0.05$.

RESULTS and DISCUSSION

Dietary treatments had no significant effect on egg production (EP), feed intake (FI), feed conversion ratio (FCR), egg weight (EW) and egg mass (EM) of laying hens. (Table 2).

Table 2. Effect of different levels of glycerol addition to diets on performance in laying hens from 44 to 56 weeks of age. Results are expressed as mean ± standard error.

Performance Parameters	Diets			
	100 ASS %	**75 ASS %/ 25 G%**	**50 ASS % / 50 G%**	**25 ASS % / 75 G%**
Egg production (%)	94.19± 1.61	96.06±2.24	93.60±1.09	93.50±0.89
Feed intake (g/hen/day)	125.96±2.32	126.44±2.85	125.74±2.17	129.50±1.94
Feed conversion ratio (g feed/g egg)	2.10±0.06	2.00±0.03	2.05±0.04	2.07±0.02
Egg weight (g)	63.77±0.75	65.66±1.44	65.46±0.34	66.94±0.87
Egg mass (g/hen/day)	60.06±1.12	63.00±1.38	61.26±0.52	62.59±1.03

ASS :Sunflower acidulated soapstock , G: Glycerol

İn terms of performance parameters of this study are consistent with the findings of researches involving acidulated soap stock and glycerol in laying hens (Pardı'o et al., 2005, Yalçın et al., 2010). The results of the present study are similar to those reported by Bregendahl et al., (2008), in experiment with Single Comb White Leghorn hens, found that EP, EW, EM and FI were not affected when 5, 10, or 15% crude glycerol was incorporated into the diet. 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 FI of laying hens. Yalçın et al., (2010) reported that the study hens fed diets with 7.5% glycerol consumed significantly less feed than those of other groups but the inclusion of 2.5 and 5% glycerol in diets had no significant effects on feed intake during the 16 week experimental period. Swiatkiewicz and Koreleski (2009), reported that the inclusion of 2, 4, or 6% crude glycerine had no significant effect on laying performance parameters as compared with the control group. Duarte et al., (2014), reported that egg production and feed conversion were not affected by mixed crude glycerine inclusion. The results of this study consistent previous research that used acidulated soap stock in laying hens. Mızrak et al., (1999), reported sunflower oil as an energy source was replaced by different levels (0, 25, 50, 75, and 100 %) of sunflower soap-stock in the experiment) and egg weight, feed consumption, feed efficiency and body weight gain were not significantly affected by the treatments. The inclusion of glycerol in the diet of laying hens had no significant effect on egg specific gravity, egg shell breaking strength, egg shell weight and egg shell thickness, egg shape index, albumen index, and haugh unit, albumen pH, yolk pH value. L and b values of egg yolk had no significant affected by the glycerol levels in diet. The highest egg yolk a value was determined in 75 % ASS/ 25 % G of group, the lowest value determined in 25 % ASS / 75 % G of group (Table 3).

Table 3. Effect of different levels of glycerol addition to diets on egg quality parameters in laying hens from 44 to 56 weeks of age. Results are expressed as mean ± standard error.

Egg Quality Parameters	Diets			
	100 ASS%	75 ASS%/ 25 G%	50 ASS% / 50 G%	25 ASS% / 75 G%
Egg specific gravity (g/cm^3)	1.0809±0.00	1.0811±0.00	1.0800±0.00	1.0803±0.00
Eggshell breaking strength (kg)	3.78±0.07	3.91±0.04	3.80±0.08	3.67±0.04
Eggshell weight (g/100 g egg)	5.83±0.08	5.86±0.10	5.86±0.07	5.97±0.15
Eggshell thickness (mm)	0.3534±0.05	0.3520±0.00	0.3484±0.00	0.3490±0.00
Egg shape index (%)	73.08±0.93	73.34±0.44	73.86±0.18	73.58±0.41
Albumen index (%)	5.28±0.08	5.27±0.24	4.95±0.04	5.09±0.14
Haugh unit	89.96±0.46	90.17±1.44	87.92±0.43	88.60±0.95
Yolk pH	5.33±0.10	5.36±0.07	5.35±0.09	5.35±0.05
Albumen pH	7.08±0.17	7.07±0.13	7.12±0.14	7.05±0.11
Egg yolk color value				
L	54.74±0.29	55.17±0.26	55.11±0.68	54.72±0.37
a	3.35^{bc}±0.20	4.15^{a}±0.18	3.56^{b}±0.13	2.93^{c}±0.14
b	42.00±0.33	42.59±0.48	42.18±0.17	41.75±0.28

ASS :Sunflower acidulated soapstock , G: Glycerol $^{a, b, c}$: Values in rows are statistically different; P<0.05

Mızrak et al., (1999), reported sunflower soap-stock replaced soybean oil in the diet improved egg yolk pigmentation. In terms of other data similar results were reported by Swiatkiewicz and Koreleski (2009), 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 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. Duarte et al., (2014) reported that egg quality parameters were not affected by mixed crude glycerine at any of the inclusion levels.

The effects of dietary supplementation of glycerol on yolk fatty acids composition are shown in Table 4.

Table 4. Effect of different levels of glycerol addition to diets on egg yolk fatty acid compositions in laying hens from 44 to 56 weeks of age. Results are expressed as mean ± standard error.

Fatty acids profile (%)	Diets			
	100 ASS%	75 ASS%/ 25 G%	50 ASS% / 50 G%	25 ASS% / 75 G%
Palmitic acid (16:0)	21.49^{a}±0.69	21.58^{a}±0.32	20.20^{b}±0.35	19.94^{b}±0.34
Palmitoleic acid (16:1)	2.21^{A}±0.40	2.62^{B}±0.28	3.45^{AB}±0.12	4.40^{A}±0.49
Stearic acid (18:0)	7.17±0.88	5.53±0.58	6.19±0.74	6.51±0.58
Oleic acid (18:1)	48.67±1.03	50.90±0.63	50.23±0.84	50.26±0.34
Linoleic acid (18:2)	20.46±0.96	19.34±0.55	19.93±0.60	18.70±0.69
Linolenic acid (18:3)	Nd	0.157±0.00	nd	0.377±0.17
ΣSFA	28.66±3.38	27.11±1.47	26.39±2.10	26.45±0.73
ΣMUFA	50.88^{b}±2.32	53.52^{ab}±1.64	53.67^{ab}±2.01	54.66^{a}±1.52
ΣPUFA	20.46±2.15	19.36±1.23	19.93±1.33	18.88±1.73

ASS :Sunflower acidulated soapstock , G: Glycerol nd:not detected
$^{a, b, c}$: Values in rows are statistically different; P<0.05
^{A,B,C}: Values in rows are statistically different; P<0.01

The inclusion of glycerol in the diet of laying hens had no significant effect on stearic, oleic, linoleic and linolenic acid contents of egg yolk. Palmitic acid content of egg yolk was significantly decreased with fed diet containing levels of 50 and 75 % glycerol.

The highest values of palmitic acid was determined in the groups fed diets containing levels of 0 and 25 % glycerol (P<0.05).

Palmitoleic acid content of egg yolk was higher in the group fed diet containing levels of 50, and % glycerol (P<0.01). Glycerol supplementation had no significant effect on total saturated fatty acid (SFA) and polounsaturated fatty acid (PUFA) content of egg yolk. Total monounsaturated fatty acid (MUFA) content of egg yolk fed with 100 % acidulated sunflower soap stock of group was significantly increased than fed with the other groups (P<0.05).

The fatty acid profile of eggs is highly dependent on the diet of laying hens (Yannakopoulos et al., 2005, Sosin et al., 2006). Mizrak et al., (1999) reported that replacement of sunflower oil by its acidulated soap stock increased oleic acid content of the egg yolk, however, decreased the linoleic acid.

Some researchers (Kijora et al., 1995; Mourot et al., 1994) reported that crude glycerol supplementation has slightly increased the oleic acid content and decreased the proportions of the linoleic and linolenic acids. Boso et al., (2013) reported that the percentage of PUFAs and omega-6 fatty acids in egg from laying hens fed with glycerol increased as the levels of inclusion. Duarte et al., (2014) reported that alpha linoleic acid and polyunsaturated fatty acid (PUFAs), percentages, as well as ω-6/ω-3 ratio, linearly increased with increasing glycerine levels in laying hens. 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 crude oil or soap stock due to the addition of glycerol.

The reason of increase in palmitic acid and decrease in palmitoleic acid in the egg yolk may be attributed to acidulated sunflower soap stock contains higher palmitic acid and lower palmitoleic acid than glycerol.

CONCLUSION

In conclusion, the results of this study show that it is possible to replace 75 % of glycerol with acidulated sunflower soap stock (4,5 % in diet) that serves as the major energy source of feeding mixtures intended for utility layers, without any significant effects on egg production and egg quality. And also, by the increase of the glycerol level in diet of hens, a slight decrease in palmitic acid content and increased monounsaturated fatty acids (MUFA) percentage was observed.

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