IJAS Iranian Journal of Applied Animal Science

Effects of Rosehip Feed Supplementation on Egg Quality Parameters, Yolk Lipid Oxidation, and Blood Parameters of Laying Hens

Research Article

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Received on: 10 Sep 2020
Revised on: 12 Apr 2021
Accepted on: 1 May 2021
Online Published on: Dec 2021

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Online version is available on: www.ijas.ir

ABSTRACT

The current study was conducted to evaluate the influence of rosehip supplementation on egg production, egg morphological characteristics, yolk lipid oxidation, and some blood parameters of laying hens. The experiment was carried out with a total of forty laying hens Lohmann classic brown breed, which randomly divided in two groups-control and experimental (20 hens in each group). The diet of experimental hens was supplemented with 0.5% dried and milled fruits of rosehip. The trial lasted 50 days. Rosehip fruits supplementation to the layers diet for 40 days had no significant effect on their final live body weight, egg productivity, and egg morphological characteristics during the experimental period. However, the triglycerides level in the blood serum as well as yolk malondialdehyde (MDA) level significantly decreased during egg storage 30 days in the fridge ($P<0.05$), and during 30 days on room temperature ($P<0.01$). The use of 0.5% rosehip fruits led to a significant increase in egg yolk pigmentation ($P<0.001$).

KEY WORDS MDA level, poultry, *Rosa canina*, yolk pigmentation.

INTRODUCTION

Rosehip (*Rosa canina*, fam. Rosaceae) fruits are an important source of several biologically active substances such as vitamins C, B1, B2, PP, K, and E (in seeds); ß carotene (the yellow/orange pigment, which is a precursor of vitamin A); lycopene (the red pigment); pectin; flavonoids (campferol, quercetin, rutin); potassium, calcium, phosphorus salts (Demir and Özcan, 2001; Böhm *et al.* 2003; Ercisli, 2007; Guimaraes *et al.* 2010; Murathan *et al.* 2016). Rosehips are well known for their efficiency in strengthening the body's defense against infection, and particularly the common cold (Demir and Özcan, 2001). Currently, the rosehip is widely used as an aromatic and medicinal plant with high antioxidant activity (Yesilbag *et al.* 2011; Jakubcová *et al.* 2015). An experiment was performed with fattening lambs that received rosehip seeds in their diet at different levels (Esenbuga *et al.* 2011). The authors established a statistically significant effect of supplementation of 15% *Rosa canina* seed on the carcass weight and dressing percentages of the lambs. Loetscher *et al.* (2013) reported that the rosehip fruits supplemented to the diet influenced positively the carcass weight of broilers. Tekeli (2014) showed that rosehip fruits supplements decreased thiobarbituric acid reactive substances (TBARS) number in the broiler meat and increased carcass weight, compared to the broilers treated with rosemary. The studies about the use of plant supplements as a source of bioactive compounds in livestock nutrition have been in scientific focus last decade, because of their impact on the animal performances. The investigations of Baghban-Kanani *et al.* (2018), Baghban-Kanani *et al.* (2019a), Baghban-Kanani *et al.* (2019b), shows that supplementation of layers diet with different kinds of plants (sunflower meal, leaves of *Artemisia annua*, sesame seeds)

have no adverse effect over layers production performances, have a positive influence on some egg quality characteristics and egg oxidative stability.

The study of Kaya *et al.* (2019) shows that a commercial layer diet enriched with 10% and 15% rosehip seeds have positive effects on some egg quality parameters such as increasing yolk color, shell thickness, and shell weight and also increased some parameters in serum such as albumin, total cholesterol, very-low-density lipoprotein (VLDL), triglycerides and alanine aminotransferease (ALT) value. The tomato peels and rosehip meal can be a good source of natural antioxidants (Varzaru *et al.* 2020). The authors have found that transfer of phytochemicals from layers feed supplemented with rosehip meal to the eggs can have a significant influence over the lipid peroxidation during their storage. Data concerning the effect of rosehip fruits in layers are scarce.

The aim of the current research was to investigate the influence of dry rosehip fruits, supplemented to laying hens diet, on egg production, egg quality, yolk lipid oxidation, and some blood parameters.

MATERIALS AND METHODS

The experiment was conducted in the Poultry Experimental Base of the Institute of Animal Science-Kostinbrod, Bulgaria, with a total of forty laying hens at the initial age of 42 weeks from lohman classic brown breed. The laying hens were randomly divided in two groups: in control (n=20) and experimental (n=20), kept in separate pens. Layers were raised on a deep litter pen on a 16-hour lighting schedule, 70-85% relative air humidity, and 21-24 ˚C air temperature. Water was supplied via nipple drinkers. The experiment lasted 50 days-10 days preparatory and 40 days experimental period. During the preparatory period, the hens were received diet for layers in the amount of 130 g/day/hen, with the goal of eliminating the influence of the previous diet. During the experimental period, the hens were received 130 g/day/hen of this compound feed, whereas the diet of experimental hens was supplemented 0.5% dried and milled fruits of rosehip. The ingredients and chemical composition of the diets are presented in Table 1. The compound feed nutritive value was determined by the conventional Weende analysis. The metabolizable energy was calculated according to WPSA (1989). The following analysis of the tested in this study product was made: crude proteins, crude fat, and crude fibers (by Weende analysis); the contents of both Ca (BSS 11 374-86, 1990) and P (BSS 4336-73, 1990); ß carotene and licopene (by the method described by George *et al.* 2011); total polyphenols content (using the Folin-Ciocalteu reagent according to Stintzing *et al.* 2005) and total antioxidant activity (by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described by Petrova *et al.* 2016).

Also, the pH value of the examined product was determined, using a pH meter Stirrer, type OP-951.

At the beginning and at the end of the trial, the live bodyweight of the hens from the control and experimental groups was measured.

The egg production (in percent) for each group was controlled every day. There is no any mortality in the experimental groups during the experiment.

Thirty eggs from each group, laid within two consecutive days, were taken at the beginning and at the end of the experiment and the following measurements were made:

– The weight of the egg, egg shell with shell membrane, egg yolk, and albumen was measured with electronic scale BOECO within 0.001 g.

– The shape index was measured by index meter.

– The height of albumen and egg yolk as well as the egg yolk width were measured with a caliper (in mm).

– Haugh unit was calculated by the formula:

$$HU = 100 \log (h + 7.17 - 1.7\ W^{0.37})$$

Where:

h: height of the thick albumen (in mm).

W: egg weight.

– The shell thickness (mm) without the shell membrane was measured at three locations (at both poles and in the middle) by a micrometer Amer 25EE with the precision of 0.0001 mm.

– The egg yolk colour was determined visually by the 15 roche colour fan having 15 degrees scale.

At the end of the treatment, 10 hens from each group were chosen at random and blood samples were taken from *Vena cutanea ulnaris.* The serum levels of total cholesterol, glucose, and triglycerides were measured by commercial kits using biochemical analyzer BioSystems (S.A. Costa Brava, Spain).

At the end of the experimental period, some lipid fractions of egg yolks of 10 eggs from each group were determined. The total lipids were determined by the method of Bligh and Dyer (1959). The total cholesterol content in the yolk was determined by the method of Schoenheimer-Sperry modified by Sperry and Webb (1950).

At the end of the trial, the lipid oxidation of egg yolk, of 6 eggs from each group was evaluated as TBARS according to the method of Castellini *et al.* (2006). Oxidation products were quantified as malondialdehyde equivalents (mg MDA 100 g^{-1}).

The statistical analysis of results obtained was performed using the computer program excel 2007.

Table 1 Composition and nutritive value of the compound feed for laying hens

Ingredients, %	Control group	Experimental group
Wheat	64.34	63.84
Sunflower meal	14.00	14.00
Soybean meal	9.00	9.00
Sunflower oil	2.00	2.00
Rosehip fruits	–	0.50
Limestone	4.50	4.50
Monocalcium phosphate	0.40	0.40
Complex premix 6015*	1.25	1.25
Antioxidant (paradigmoks)	0.01	0.01
Nutritive value		
Metabolizable energy, kcal kg^{-1}	2710	2710
Crude proteins, %	16.40	16.31
Crude fat, %	3.19	3.17
Crude fibers, %	4.58	4.62
Lysine, %	0.79	0.79
Methionine, %	0.43	0.43
Ca, %	3.73	3.73
P, %	0.49	0.49

* Complex premix contains: Mn (MnO): 120 mg/kg; Zn (ZnO): 110 mg/kg; Fe ($FeSO_4$): 140 mg/kg; Cu($CuSO_4$): 18 mg/kg; I ($Ca(IO_3)_2$: 1.80 mg/kg; Se (Na_2SeO_3): 0.35 mg/kg; vitamin A (retinyl acetate): 9900 UI; vitamin D_3 (cholecalciferol): 3000 UI; vitamin E (DL-alpha-tocopherol): 30 mg/kg. It does not contain nutritive antibiotics, synthetic dyes and carotenoids or other stimulants.

The statistical significance between the control and experimental group was determined by one-way analysis of variance (ANOVA). The internal and external egg traits, blood biochemical traits (glucose, triglycerides, and cholesterol), and lipid egg yolk traits (total lipids, cholesterol and MDA) are compared within the control and experimental group. The values are presented as mean(s) ± statistical error for each variable.

RESULTS AND DISCUSSION

The chemical composition, antioxidant activity as well as pH values of the used in our investigation products are presented in Table 2. The analyzed rosehip fruits are a rich source of Ca (0.5%), P (0.174%), crude fibers (32.24%), total polyphenols (1652.60 mg GAE $100g^{-1}$), ß carotene (95.34 µg g^{-1}), lycopene (125.80 µg g^{-1}). This chemical composition of the dry rosehip fruits is in accordance with the results reported by Demir and Özcan (2001) and Yildiz and Alpaslan (2012).

According to Anderson (2009), the carotenoid content in rosehips is highly dependent on variety, habitat, year and varies widely – from 21 to 472 µg g^{-1}. This product has high antioxidant activity – 9436.30 µmol TE (Trolox equivalents) 100 g^{-1}.

The live body weight of laying hens is an indicator of their health status. This parameter did not change significantly during the experimental period. At the beginning of the experiment the body weight 1846 g ±27.65 and 1804 g ±29.29, and at the end 1936 g ±28.34 and 1857 g ±25.27 for the control group and experimental group respectively.

The live body weight increased by 90g and 53 g for both the control and experimental groups, respectively, at the end of the treatment. There was no significant difference in egg production between the control and experimental groups. The values were 86.67% ±1.44 and 85.00% ±2.50 and at the end for the control group and experimental group respectively ± for the control group and experimental group respectively and 90.00 ± 1.29 for the control group and experimental group respectively. The experiment shows that daily consumption of supplemented feed with rosehip fruit (130 g) is the same for both groups of layers, control and experimental. Kaya *et al.* (2019) reported increased feed consumption in experimental groups of chickens because of the low energy content of feed supplemented with rosehip seed. But the study of Varzaru *et al.* (2020) shows that daily intake of feed supplemented with rosehip was not increased significantly by the laying hens and the weight was also not significantly affected.

The results of this study are in agreement with the findings of Loetscher *et al.* (2014) who found that the supplementation of 2.5% rosehip to laying hens diet did not affect hen's body weight and laying performance.

The influence of rosehip fruits supplementation on internal and external egg quality parameters is presented in Table 3. The values of egg weight and albumen weight in hens from the experimental group were significantly lower ($P<0.05$) compared to the control group, both at the start and at the end of the experimental period. The use of rosehip fruits did not also change significantly the yolk weight, egg shell weight, egg shell thickness, Haugh unit, shape index.

Table 2 Chemical composition, total polyphenols, and pH value of the used rosehip fruits

Parameters	
Moisture, %	7.94
Dry matter, %	92.06
Crude proteins, %	5.10
Crude fat, %	3.50
Crude fibers, %	32.24
Ca, %	0.50
P, %	0.174
ß carotene, µg g^{-1}	95.34
Licopene, µg g^{-1}	125.80
Total polyphenols, mg GAE (equivalents of gallic acid) $100g^{-1}$	1652.60
Antioxidant activity, µmol TE (trolox equivalents) $100g^{-1}$	9436.30
pH	4.68

Table 3 Effects of supplemented diet with rosehip on internal and external egg quality

Items	At the beginning of the experiment		P-value	At the end of the experiment		P-value
	Control group	Experimental group		Control group	Experimental group	
Egg weight, g	65.57±5.97	63.57±4.75*	< 0.05	65.49±5.13	63.39±4.65*	< 0.05
Albumen weight, g	42.15±4.49	40.68±3.45*	< 0.05	42.10±3.96	40.51±3.22*	< 0.05
Yolk weight, g	16.31±1.52	15.92±1.37	NS	16.24±1.13	15.95±1.50	NS
Egg shell weight, g	7.37±0.92	7.09±0.85	NS	7.07±0.85	6.88±0.90	NS
Albumen, %	64.30±6.57	63.84±1.90	NS	64.61±6.37	63.77±2.46	NS
Yolk, %	24.91±2.76	25.03±1.71	NS	24.92±1.90	25.18±1.63	NS
Egg shell, %	11.23±0.88	11.13±0.78	NS	10.78±0.85	10.82±0.90	NS
Egg shell thickness, mm	0.39±0.03	0.40±0.02	NS	0.40±0.03	0.39±0.02	NS
Sharp	0.39±0.03	0.40±0.03	NS	0.40±0.03	0.40±0.02	NS
Middle	0.39±0.03	0.40±0.02	NS	0.40±0.03	0.39±0.02	NS
Bottom	0.39±0.03	0.40±0.02	NS	0.39±0.03	0.39±0.03	NS
Haugh unit (HU)	72.37±9.09	72.20±9.73	NS	69.53±9.29	71.03±8.08	NS
Yolk colour	2.89±0.18	2.93±0.16	NS	2.94±0.17	4.23±0.28	< 0.001
Albumen height, mm	7.25±1.20	6.42±1.93	NS	6.14±1.29	6.37±1.03	NS
Shape index, %	78.80±2.19	79.08±3.32	NS	78.35±2.44	79.30±2.18	NS
Yolk width, mm	39.06±2.43	38.84±1.51	NS	38.69±1.61	39.77±2.49	NS
Yolk height, mm	15.55±1.23	15.02±1.20	NS	15.70±1.17	16.67±1.80	NS
Edible portion	58.20±5.28	56.48±4.06	NS	58.40±4.53	56.51±3.95	NS
Albumen:yolk ratio	38.90±3.76	39.31±3.71	NS	38.82±3.62	39.51±3.98	NS

NS: non significant.

According to Kaya *et al*. (2019) layers nourished with feed with 15% rosehip seed produced eggs with better characteristics such as shell thickness, shell weight and yolk color as a result of increased content of Ca and carotenoids in rosehip seeds at the beginning of the experimental period the yolk colour intensity into groups varied in close range – from 2.89 (in control) to 2.93 (in experimental group) points on the roche colour fan. At the end of the trial, a significant increase of yolk colour pigmentation by 1.29 points for the treated group (P<0.001) compared with the control was registered. Based on the fact supported by various scientific investigations (Kang *et al*. 2003; Karadas *et al*. 2006; Olson *et al*. 2008; Akdemir *et al*. 2012) that the carotenoids from the diet passed unchanged in egg yolk, can be concluded that rosehip fruits used in our study as a natural source of ß carotene and lycopene are suitable additive for egg yolk pigmentation.

A strategy has been proposed for partial replacement of yellow pigments by red pigments over the recent years. According to Mascarell *et al*. (2012), the use of natural red pigment ensures also a percentage close to 15% of yellow xanthophylls.

The supplementation of laying hens diet with different kinds of plant meals can positively influence the layers production capacity as well as eggs quality characteristics. According to the Baghban-Kanani *et al*. (2018) dietary supplementation with sunflower meal and multi-enzyme complex in layers diet have a positive effect in developing low cholesterol table eggs. The dietary treatments did not significantly affect egg-specific gravity, egg weight, shell strength and thickness, Haugh unit, and shape index. These results are similar to the results of this survey (Table 4).

Table 4 Biochemical parameters of layers from control and experimental groups

Items (mmol liter^{-1})	Control group n=10	Experimental group n=10	P-value
Glucose	9.22±0.44	8.92±0.55	NS
Triglycerides	11.95±1.54	7.30±1.16	< 0.05
Cholesterol	2.75±0.17	2.32±0.19	NS

NS: non significant.

Table 5 The contents of lipids, total cholesterol, and MDA in egg yolk

Item	Control group	Experimental group	P-value
Lipids, g 100 g^{-1}	36.00±0.59	35.71±0.47	NS
Cholesterol, g 100 g^{-1}	1536.78±39.90	1514.48±45.83	NS
Malondialdehyde (MDA), µg g^{-1}			
At the end of the experiment	0.32±0.08	0.54±0.11	NS
Storage 30 days in fridge	1.18±0.10	0.65±0.13	< 0.01
Storage 30 days at room temperature	5.53±0.34	1.13±0.21	< 0.01

NS: non significant.

The rosehip fruit supplementation causes a decrease in cholesterol levels in egg yolk but it was not significant as it was in the research of Baghban-Kanani *et al.* (2018). Unlike the results of the mention authors, the level of triglycerides in blood serum in the experimental group in our study was significantly lower and the level of cholesterol shows a downward trend.

The effects of supplemented diet with rosehip on some blood serum biochemical parameters are shown in Table 4. The content of the triglycerides in the blood serum of experimental hens was significantly lower compared to the control group (P<0.05). There was a tendency to lower glucose and total cholesterol levels in the blood serum of the experimental group (P>0.05).

According to Tekeli (2014) the reduction of triglycerides and cholesterol in the blood serum is caused by the high content of cellulose and flavonoids in rosehip fruits.

Nurulhuda *et al.* (2012) reported that phenolic compounds such as flavonoids, anthocyanidins, and anthocyanins reduced endogenous cholesterol absorption and synthesis.

Table 5 presents the data about the contents of total lipids, total cholesterol, and MDA in egg yolk. There were no significant differences concerning total lipid content and total cholesterol content in the yolk between the control and experimental groups (P>0.05). It was found that the yolk MDA level in the experimental group decreased significantly (P<0.01). The supplementation of rosehip as a supplement to laying hen diet significantly reduced the yolk MDA concentration of eggs analyzed after 30 days storage at +4 ˚C and room temperature (P<0.01) compared to eggs under the same conditions of the control group. This fact may be explained by the high lycopene content in the rosehip fruits.

Akdemir *et al.* (2012) reported that MDA concentrations decreased linearly in the blood serum and yolk as tomato powder concentration in the diet increased.

Sahin *et al.* (2008) performed the experiment with quails fed with diets containing 0, 100, and 200 mg lycopene per kilogram for 90 days and reported that correlation between egg yolk lycopene content was positive with egg yolk colour (r=0.85) and negatively correlated with yolk MDA content (r=0.76) (P<0.0001 for both).

CONCLUSION

According to the results of the present study, the implementation of rosehip fruits in a hen diet can have a positive influence over the hen blood and eggs quality parameters. It decreased significantly triglycerides in the blood serum (P<0.05) and yolk MDA level during 30 days of egg storage in fridge (P<0.01) and during 30 days at room temperature (P<0.01). The tested rose hip supplement with high antioxidant activity significantly increased egg yolk pigmentation (P<0.001) and did not have a negative influence over final live body weight and egg productivity as well as egg morphological characteristics. The supplementation of 0.5% dried and milled rosehip fruits to the layers diet did not influence negatively final live body weight and egg productivity as well as egg morphological characteristics. The tested supplement significantly increased egg yolk pigmentation (P<0.001). It decreased significantly triglycerides in the blood serum (P<0.05) and yolk MDA level during 30 days of egg storage in the fridge (P<0.01) and during 30 days at room temperature (P<0.01). Further studies are required to elaborate on the impacts of rosehip and similar products on animal health, digestive activities and immune system.

ACKNOWLEDGEMENT

This study was funded by the Scientific Research Projects Committee at University Ss Ciril and Methodius in Skopje, Republic of Macedonia (project number 326-20).

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