Use of Grape Marc Flour Supplementation in Laying Hens' Diet on Laying Productivity, Egg Quality and Biochemical Parameters

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Abstract*: The present study was carried out to evaluate the effect of grape marc flour addition to the laying hens' diet on their egg production, egg quality, total yolk lipids and yolk total fatty acid composition, cholesterol content in the yolk and blood serum as well as on the yolk lipid oxidation. An experiment was conducted with a total of 90 laying hens (at 40 weeks old) from Lohmann Classic Brown breed, randomly divided into three groups, 30 hens in each (3 replications x 10 layers per group). The diet of the experimental hens was supplemented with 1% and 3% of grape marc flour. The trial duration was 48 days. Grape marc flour addition to the laying hens' compound feed did not significantly (P > 0.05) affect their live body weight, egg production, egg morphological properties as well as total yolk lipids content, total cholesterol content in the yolk and blood serum and yolk fatty acids composition. However, the egg yolk malondialdehyde (MDA) level during egg storage for 30 days at room temperature significantly decreased (P < 0.01) in comparison with control eggs. The addition of grape marc flour has the potential to extend the shelf life of eggs.*

Introduction

Grape (*Vitis vinifera* L.) is one of the richest sources of phenolic and other antioxidant compounds among fruits (Kupe et al., 2021). A large part of grape production is intended for the preparation of wines, juices, distillates and other products, generating by-products that could be used as ingredients for the development of new products as well as components in animal and poultry diets (Shirahigue et al., 2010). In this way, environmental pollution due to the accumulation of these residues is also prevented (Devesa-Rey et al., 2011; Christ and Burrit, 2013; Fontana et al., 2013). The wine industry generates substantial quantities of waste, such as grape marc, discarded clusters, seeds and sediments. In fact, pomace represents about 20–30% of the original grape weight (Dwyer et al., 2014). In the Balkan countries, there is a long-standing tradition for production of grape marc distillates after the winemaking process (Lukic et al., 2011). After the distillation process, the solid residue from grape obtained is called spent grape marc (Graça et al., 2018). Globally, the annual production of grape marc (GM), the residue of skins, seeds and stems remaining after making wine, has been estimated to be approximately nine million tons (Moate et al., 2020). A number of authors have reported the positive effect of the addition of grape pomace in the diet of ruminants (Babău et al., 2019), equine (Kollathova et al., 2021), rabbits (Bonzaida et al., 2021), laying hens (Kara et al., 2016; Mirghelenj et al., 2017; Olteanu et al., 2019), and quails (Froes et al., 2018). Sahin et al. (2008), Jung et al. (2011), and Zang and Kim (2014) explain this fact by the antioxidant action of polyphenols (catechin, epicatechin, procyanidin and anthocyanidin) which are contained in grape pomace. Malosini et al. (1993) conducted an experiment with heavy lambs receiving 30% and 60% of grape marc in their diet. The authors recommended that this by-product be added in limited quantities, because its higher intake leads to a decrease in digestibility. In fact, according to Wu et al. (2022), grape marc can replace 20% of the control ration to maintain sheep productivity, health, and environmental sustainability. Moate et al. (2020) observe a reduction of methane emissions but at the cost of decreased milk production when dairy cows are fed grape marc. There are no documented studies on the effect of using grape marc in the laying hens' diet on their egg productivity, egg quality and egg fatty acid profile. The aim of the current scientific work is to determine how the addition of a grape marc meal to the laying hens compound feed can affect egg performance, egg morphological properties, total yolk lipids, total yolk fatty acid profile, blood serum and yolk cholesterol contents, as well as yolk lipid oxidation.

Materials and methods

This experiment complies with Directive 2010/63/EU on the protection of animals used for scientific purposes, and the experimental procedures have been approved by the Bulgarian Animal Ethics Committee in accordance with Bulgarian Veterinary Law (2011) on the protection of animals used for experimental and other scientific purposes and relevant provisions

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Council Directive 86/609/EEC (Permission for using agricultural animals for scientific purpose, N177 obtained on the base of Protocol N33/18.06.2015).

The study was carried out in the Poultry Experimental Farm of the Institute of Animal Science – Kostinbrod, Bulgaria. A total of 90 Lohman Brown laying hens at the age of 40 weeks were randomly distributed into 3 groups (n = 30 hens/group): one control and two experimental (3 replications per group, 10 poultry in each replication). The layers from each replication were raised in separate boxes on a deep litter pen on a 16-hour lighting schedule. Water was supplied using nipple drinkers. The experiment duration was 48 days (14-day preparatory and 34-day experimental periods). During the preparatory period, the poultry from all the groups received compound feed for laying hens in the amount of 130 g/day/hen in order to eliminate the influence of the previous diet. During the experimental period, the hens received the same amount of this compound feed, whereas the diet of the experimental hens was supplemented with 1% (experimental group 1) and 3% (experimental group 2) of dried grape marc flour.

The ingredients and chemical composition of laying hens' diets are pointed in Table 1.

The total chemical composition of the diets and of the grape marc flour was determined as follows: moisture, crude protein, crude fat, and crude fibres by the conventional Weende analysis; the content of both Ca and P by AOAS (2007); ß carotene and licopene of the tested product by a method described by George et al. (2011); grape marc total polyphenol content after preliminary esterification by Folin-Ciocalteu method described by Blainski et al. (2013); fatty acids composition of grape marc lipid using HP 5890 II gas chromatograph equipped with flame ionization detector and type capillary column "Supelco" SP™-2390.

The pH values were measured using a pH meter Stirrer, type OP-951. The total antioxidant activity of grape marc was determined using the 2,2-diphenyl1-picrylhydrazyl (DPPH) method described by Petrova et al. (2016). The metabolizable energy of the diets was calculated according to Todorov et al. (2021). At the beginning and at the end of the trial, the live body weight of the poultry from the control and the experimental groups was measured. Daily laying intensity (in percent) was controlled throughout the trial. 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 taken: the weight of the egg, yolk, albumen and eggshell with shell membrane (by balance with a precision of 0.001 g); egg shell thickness (mm) without the shell membrane (measured at three locations by a micrometer Ames 25EE with a precision of 0.0001 mm); Haugh units (by index meter); shape index (by index meter Van Dorn De Bilt N 72205-1); albumen index (determined by measuring the large and small egg diameters and albumen height

Table 1. Composition and nutritive value of laying hens' diets

Ingredients, %	Control	Experimental group 1	Experimental group 2
Wheat	65.04	64.54	63.04
Soybean meal	10.50	10.50	10.50
Sunflower meal	12.0	11.50	11.0
Sunflower oil	2.0	2.0	2.0
Spent grape marc	0.0	1.0	3.0
Limestone	4.30	4.30	4.30
Limestone (little rocks)	4.30	4.30	4.30
Mono calcium phosphate	0.6	0.6	0.6
Complex premix 6015*	1.25	1.25	1.25
Nutritive value			
Metabolizable energy, kcal kg^{-1}	2720	2720	2720
Crude proteins, %	16.72	16.72	16.72
Crude fat, %	3.31	3.31	3.31
Crude fibre, %	4.23	4.50	5.07
Ca, %	3.6	3.6	3.6
P, %	0.51	0.51	0.51
pH	6.11	6.34	6.33

* 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.

using a caliper and calculated by the formula: I_{al} (%) = (h/[D+d]/2x100) where h is the height of the thick albumen (in mm); D is a big albumen diameter; and d is a small albumen diameter; yolk index (determined by measuring the yolk diameter and its height using a caliper and calculated by the formula: YI (%) = (h/d) x100) where h is height of the yolk and d is diameter of the yolk; egg yolk color (visually according to the Roche Color Fan). The content of Ca and P in the eggshell was determined according to AOAS (2007).

At the end of the treatment, 10 hens from each group were chosen randomly and blood samples were taken from *Vena cutanea ulnaris*. The content of total cholesterol in the blood serum was measured by commercial kits using biochemical analyser BioSystems (S. A. Costa Brava, Spain). At the end of the experiment, some lipid fractions of egg yolks of 10 eggs from each group were analysed. The total lipids were evaluated 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). The fatty acid composition of egg yolk lipids was estimated by HP 5890 II gas chromatograph equipped with flame ionization detector and type capillary column "Supelco" SP™-2390 with a length of 60 m and an inner diameter of 0.25 mm after preliminary esterification. At the end of the trial, the lipid oxidation of egg yolk was examined by analyses of 6 eggs from each group as TBARS according to the method of Castellini et al. (2006). Oxidation products were quantified as malondialdehyde equivalents (mg MDA 100 g^{-1}). The results obtained in this study were statistically processed by EXCEL 2007, single factor, ANOVA program. All dates are presented as means with their standard errors (X ± SE).

Results

The chemical composition, antioxidant properties and pH value of dried grape marc flour used in our study are shown in Table 2.

Table 3 presents a fatty acid composition of the tested by-product.

Table 4 presents the data of live body weight, laying intensity and mortality of the hens from the control and the experimental groups.

The results on the content of calcium and phosphorus in the eggshell are presented in Table 6.

The profile of egg yolk fatty acids is presented in Table 7. The dietary supplementation included 3% of grape marc.

Discussion

In the scientific literature, the data about crude protein, crude fiber and crude fat of grape marc and grape pomace vary significantly according to the reports provided by different authors (Malossini et al., 1993; Mihna & Muhammad, 2017; Moate et al., 2020; Kolláthová et al., 2021). In general, the results presented here are within the range of values obtained by these authors. The differences in the chemical composition are probably due to many factors, including the variety and the color of grape and the different pressing processes associated with making red and white wines (Spanghero et al., 2009). As seen from Table 2, the grape marc used in our scientific work did not contain carotenoids – lycopene and ß carotene. The tested by-product had lower total content of polyphenols than the grape pomace described by Olteanu et al. (2019), and its total antioxidant activity was lower. The pH value of the tested supplement was 4.89, while the pH values of the feed at the beginning and the end of the experiment were within close range of 6.11, 6.34, and 6.33 for the control group, and experimental groups 1 and 2, respectively.

Grape marc flour is rich in oleic acid (20.3%) and linoleic acid (49.9%) (Table 3). Palmitic acid is the most common saturated fatty acid accounting for 16% of total fatty acids in grape marc. These findings are

Table 2. Chemical composition, total antioxidant capacity and the pH value of dried grape marc flour

Parameters	Grape marc flour
Moisture, %	6.42
Crude protein, %	12.64
Crude fat, %	8.04
Crude fibre, %	39.15
Ca, %	0.515
P, %	0.175
Total phenolic content, mg GAE/100 g	4.00
Total antioxidant activity, mmol TE/100 g	52.80
ß- carotene, µg/g	0.00
Licopene, µg/g	0.00
pH	4.89

GAE – galic acid equilent; TE – Trolox equivalent

Table 3. Fatty acid content of grape marc flour (given in percentage of the total amount of fatty acids)

Fatty acid	%	Fatty acid	%
Lauric ($C_{12:0}$)	0.1	Linoleic acid (n-6) ($C_{18:2}$)	49.9
Myristic ($C_{14:0}$)	0.5	α-Linoleic ($C_{18:3}$)	0.7
Pentadecanoic ($C_{15:0}$)	0.1	Arachidic ($C_{20:0}$)	0.7
Ginkgolic acid ($C_{15:1}$)	0.1	Eicosenoic ($C_{20:1}$)	0.3
Palmitic ($C_{16:0}$)	16.1	Heneicosanoic ($C_{21:0}$)	0.6
Palmitoleic ($C_{16:1}$)	0.7	Eicosadienoic ($C_{20:2}$)	0.1
Heptadecanoic ($C_{17:0}$)	0.1	Eicosatrienoic ($C_{20:3}$)	1.0
Stearic ($C_{18:0}$)	8.4	Arachidonic ($C_{20:4}$)	0.1
Oleic ($C_{18:1}$)	20.3	-	-
Monounsaturated fatty acids (MUFA)	21.4	Polyunsaturated fatty acids (PUFA)	52.0
Unsaturated fatty acids	73.4	Saturated fatty acids	26.6

Table 4. Live body weight (g), laying intensity (%) and mortality of laying hens (X ± SE)

Parameters \ Groups	Control	Grape marc flour (%)	
		1.0	3.0
Initial body weight (g)	1832 ± 31.90	1768 ± 31.64	1875 ± 29.70
Final body weight (g)	1930 ± 35.61	1902 ± 32.11	1925 ±3 0.84
Laying intensity (%), start of experiment	88.75 ± 3.26	87.33 ± 3.33	86.89 ± 4.15
Laying intensity (%), end of experiment	90.43 ± 1.35	90.57 ± 1.34	92.86 ± 1.01
Mortality (%)	0.00	0.00	0.00

Table 5. Egg morphological parameters of the hens from the control and the experimental groups (X ± SE)

Indices \ Groups	Control	Grape marc flour supplementation (%)		Control	Grape marc flour \supplementation (%)	
		1.0	3.0		1.0	3.0
	Start of the experiment			End of the experiment		
Egg weight, g	60.50 ± 0.77	57.64 ± 0.75	59.73 ± 0.74	64.94 ± 1.14	62.77 ± 0.83	64.53 ± 0.90
Albumen weight, g	38.76 ± 0.65	36.99 ± 0.53	37.45 ± 0.63	41.61 ± 0.89	39.59 ± 0.62	40.80 ± 0.73
Yolk weight, g	15.27 ± 0.23	14.57 ± 0.26	15.44 ± 0.20	16.31 ± 0.30	15.93 ± 0.22	16.01 ± 0.21
Shell weight, g	6.44 ± 0.09	6.20 ± 0.12	6.60 ± 0.11	7.24 ± 0.14	7.24 ± 0.11	7.60 ± 0.11
Shell thickness, mm	0.40 ± 0.004	0.39 ± 0.004	0.40 ± 0.004	0.40 ± 0.005	0.41 ± 0.003	0.42 ± 0.003
Haugh units	85.00 ± 0.84	83.07 ± 1.03	81.20 ± 1.18	84.00 ± 1.20	84.03 ± 1.17	84.03 ± 1.10
Shape index %	79.29 ± 0.53	79.42 ± 0.37	79.22 ± 0.49	78.90 ± 0.42	78.32 ± 0.46	78.87 ± 0.36
Albumen index %	10.72 ± 0.34	10.20 ± 0.36	9.43 ± 0.30	9.84 ± 0.40	10.31 ± 0.40	10.27 ± 0.39
Yolk index %	45.93 ± 0.64	43.44 ± 0.76	43.56 ± 0.44	43.67 ± 0.76	43.51 ± 0.68	42.46 ± 0.61
Yolk color (Roche)	4.18 ± 0.22	4.03 ± 0.15	4.10 ± 0.10	4.46 ± 0.22	4.30 ± 0.24	4.20 ± 0.25

in accordance with those of Moate et al. (2020). The essential linoleic acid has the highest proportion of polyunsaturated fatty acids (PUFA) in dried grape marc flour. Similar values of linoleic acid in grape pomace are reported by Ribeiro et al. (2015). The tested grape marc contains 21.4% of monounsaturated fatty acids (MUFA), 52% of polyunsaturated fatty acids (PUFA) and 26.6% of saturated fatty acids (SFA).

Laying hens' productivity and morphological properties of eggs

The live body weight of layers did not change significantly ($P > 0.05$) (Table 4). This parameter increased with 98 g, 134 g and 50 g for the control group and experimental groups 1 and 2, respectively, at the

Table 6. The content of calcium and phosphorus in the eggshell of laying hens from control and experimental groups (X ± SE)

Groups \ Indices		Calcium content (%)		Phosphorus content (%)	
		Start of the experiment	End of the experiment	Start of the experiment	End of the experiment
Control		34.80 ± 0.090	35.80 ± 0.080	0.120 ± 0.001	0.123 ± 0.001
Grape marc flour supplementation (%)	1.0	34.16 ± 0.100	35.38 ± 0.090	0.115 ± 0.002	0.116 ± 0.001
	3.0	34.64 ± 0.090	36.10 ± 0.100	0.125 ± 0.003	0.119 ± 0.002

Table 7. Fatty acid profile of lipids extracted from egg yolk (n = 6/group), (X ± SE)

Fatty acid	Control %	Grape marc flour supplementation 3%	Fatty acid	Control %	Grape marc flour supplementation 3%
$C_{6:0}$ Caproic acid	0.125 ± 0.25	-	$C_{18:2}$ (ω-6) Linoleic acid	8.05 ± 0.68	8.07 ± 0.97
$C_{8:0}$ Caprylic acid	0.1 ± 0.00	-	$C_{18:3}$ (ω-3) ϒ-Linoleic acid	0.22 ± 0.03	0.2 ± 0.025
$C_{10:0}$ Capric acid	0.1 ± 0.00	0.15 ± 0.05	$C_{20:0}$ Arachidic acid	0.1 ±0 .00	0.1 ± 0.00
$C_{12:0}$ Lauric acid	0.1 ± 0.00	0.15 ± 0.05	$C_{20:1}$ Eicosenoic acid	0.35 ± 0.022	0.37 ± 0.07
$C_{14:0}$ Myristic acid	0.46 ± 0.024	0.54 ± 0.04	$C_{21:0}$ Heneicosylic acid	0.13 ± 0.02	0.28 ± 0.087
$C_{14:1}$ Myristoleic acid	0.13± 0.33	0,125 ± 0.025	$C_{20:2}$ (ω-6) Eeicosadienoic acid	0.1 ± 0.00	0.12 ± 0.016
$C_{15:0}$ Pentadecylic acid	0.12 ± 0.02	0.12 ± 0.02	$C_{20:3}$ (ω-3) Eicosatrienoic acid	0.1 ± 0.00	0.1 ± 0.00
$C_{15:1}$ Ginkgolic acid	0.17 ± 0.05	0.15 ± 0.03	$C_{20:4}$(ω-6) Arachidonic acid	0.55 ± 0.11	0.50 ± 0.07
$C_{16:0}$ Palmitic acid	31.88 ± 0.72*	33.03 ± 0.70	$C_{22:0}$ Behenic acid	0.1 ± 0.00	0.16 ± 0.06
$C_{16:1}$ Palmitoleic acid	2.4 ± 0.15	3.12 ± 0.40	$C_{23:0}$ Tricosylic acid	0.13 ± 0.02	0.12 ± 0.02
$C_{17:0}$ Margaric acid	0.2 ± 0.00	0.18 ± 0.016	$C_{22:2}$(ω-6) Docosadienoic acid	0.25 ± 0.07*	0.43 ± 0.15
$C_{17:1}$ Heptaeceonic acid	0.125 ± 0.025	0.16 ± 0.024	$C_{20:5}$(ω-3) Eicosapentaenoic acid	0.43 ± 0.05	0.52 ± 0.12
$C_{18:0}$ Stearic acid	10.72 ± 0.75	10.00 ± 0.81	$C_{24:0}$ Lignoceric acid	0.18 ± 0.03	0.14 ± 0.02
$C_{18:1}$ Oleic acid	43.12 ± 1.21*	41.58 ± 1.035	$C_{22:6}$(ω-3) Docosahexaenoic acid	0.1 ± 0.00	0.13 ± 0.025
Saturated fatty acids	44.13 ± 1.45	44.6 ± 1.56	Monounsaturated fatty acids	46.2 ± 1.23*	45.4 ± 1.03
Unsaturated fatty acids	55.87 ± 1.44	55.4 ± 1.56	Polyunsaturated fatty acids	9.67 ± 0.83	10.9 ± 1.05*

Significance * $P \leqslant 0.05$

end of the trial. At the beginning of the experiment, the hens' laying intensity was as follows: 88.75% for the control group, 87.33% for experimental group 1, and 86.89% for experimental group 2, while at the end of the treatment, it slightly increased and the measured values reached 90.43%, 90.57%, 92.86% for the control group, and experimental groups 1 and 2, respectively. The differences between the groups were not significant ($P > 0.05$). At the end of the experiment, an increase in laying intensity was observed by 1.68%, 3.24%, 5.97% for the control group and experimental groups 1 and 2, respectively. Kara et al. (2016) obtained similar results when feeding a supplemented diet with 4% and 6% grape pomace for 12 weeks. Alm El Dein et al. (2017) established a significant increase of the laying intensity ($P < 0.05$) without a significant effect on the body weight by adding 1%, 2%, 3% and 4% of grape pomace (except for the level of 1%) to laying hens' diet. There was no mortality observed in all the groups during the treatment. Throughout the experiment, the poultry of all three groups consumed the diets with appetite and were in good health, lively, with good exterior and plumage.

The results reporting egg morphological properties of laying hens from the control and the experimental groups are presented in Table 5. The grape marc addition in doses of 1% and 3% did not affect significantly the weight of the egg, albumen, yolk and eggshell, as well as the shell thickness, Haugh units, shape albumen and yolk indexes. As far as we know, few studies are currently available about the dietary supplementation of grape pomace in layers and its impact on egg morphological parameters (Romero et al., 2022; Kara et al., 2016; Ozgan, 2008). Kara et al. (2016) included 4% and 6% of grape pomace in layers' compound feed, but here no significant effects on egg quality were observed either. Romero et al. (2022) reported increasing the egg yolk color and Haugh units in the groups with the intake of grape pomace and extract. In contrast to findings, Ozgan (2008) reported an increase in the albumen index with the addition of 2% of grape pomace to laying hens' diets.

The inclusion of 1% and 3% of grape marc to the hens' diet did not have a negative effect on the content of calcium and phosphorus in the eggshell (Table 6).

In the commercial egg market, richer-colored yolks are in demand, and this characteristic depends exclusively on the compound feed. This is due to the fact that even though hens are not able to synthesize pigments, they are able to absorb between 20% and 60% of the diet pigments (Moura et al., 2011). In this study, the yolk color intensity into the groups varied in close range from 4.03 to 4.46 points on the Roche Color Fan both at the beginning and at the end of the trial ($P > 0.05$). This fact can be explained by the lack of carotenoids in grape marc used in our research. Froes et al. (2018) noticed an increase of yolk pigmentation density in quails' egg when feeding grape pomace supplemented diet (2%, 4%, 6%). According to the authors, this enhancement of yolk color is due to the anthocyanins' content in grape pomace.

Fatty acid composition of egg yolk

The dietary inclusion of 3% of grape marc reduced the proportion of MUFA in the yolk with respect to the control group (45.4% vs. 46.2%, $P = 0.05$) and increased the proportion of PUFA (10.9% vs. 9.67%, $P = 0.05$) (Table 7). The content of oleic acid decreased (41.58% vs. 43.12%, $P = 0.05$) as compared with the eggs of control hens. Romero et al. (2022) reported a reduction of SFA proportion in the yolk (31.9% vs. 32.9%, $P = 0.001$), a MUFA decrease (39.5% vs. 41.4%, $P < 0.001$), and a PUFA percentage decrease (28.9% vs. 25.7%, $P < 0.001$), as compared with the eggs of the control hens.

Yolk lipids and TBARS value

The content of total yolk lipids, total cholesterol in the yolk and blood serum as well as the lipid oxidation are presented in Table 8. There are no significant differences in regard to the total yolk lipids and the total cholesterol content in the yolk and blood serum between the groups ($P > 0.05$). The results obtained were in compliance with the experiment performed by Kara et al. (2016). Herber and Van Elswyk (1996) considered that the cholesterol in the egg yolk changed slightly or in many cases did not change at all under the influence of genetic, pharmacological or nutritive factors. As it can be seen from Table 8, dietary supplementation of grape marc in the doses of 1% and 3% significantly reduced MDA concentration in yolk after egg storage at room temperature ($P \leqslant 0.01$). This leads to an increase of eggs' shelf life. Similar results were found by other authors when adding grape pomace to the hens' diet (Brenes et al., 2010; Brannan, 2009; Banon et al., 2007; Lau and King, 2003; Pazos et al., 2004; Carpenter et al., 2007). These results can be explained by the antioxidant properties of the polyphenolic compounds contained in grape pomace and grape marc (Monteiro et al., 2021).

Conclusions

The dietary inclusion of grape marc flour in doses of 1% and 3% did not significantly change the body weight, laying intensity, egg morphological properties, the content of yolk lipids or the total cholesterol content in the blood serum and the egg yolk ($P > 0.05$). The addition of 3% of grape marc significantly decreased the content of oleic acid and significantly reduced the proportion of MUFA in the yolk. In addition, it significantly increased the proportion of PUFA as compared with the eggs of the control hens ($P < 0.05$). The use of grape marc in the hens' diet improved the shelf life of eggs. This is due to reduced concentration of MDA ($P < 0.05$) in egg yolk after storage of eggs at room temperature.

Table 8. The effect of dietary grape marc on yolk lipids, yolk cholesterol, and lipid oxidation (X ± SE)

Groups / Indices	Control	Grape marc flour supplementation (%)	
		1.0	3.0
Total lipids g/100 g yolk	34.89 ± 0.42	36.10 ± 0.44	35.74 ± 0.33
Total cholesterol, mg/100 g yolk	1475.86 ± 37.86	1430.63 ± 44.96	1442.64 ± 20.06
Total cholesterol in blood serum, mmol/L	4.25 ± 0.38	4.23 ± 0.27	4.11 ± 0.27
Malondialdehyde (MDA), μg/g			
At the end of the experiment	0.48 ± 0.02	0.58 ± 0.04	0.60 ± 0.04
Storage 30 days in a fridge	0.85 ± 0.05	0.80 ± 0.05	0.84 ± 0.04
Storage 30 days at room temperature	4.15 ± 0.67	1.19 ± 0.08**	1.25± 0.07**

Significance by: ** $P \leqslant 0.01$

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