Quality and oxidative stability of eggs laid by hens fed marigold extract supplemented diet

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ABSTRACT The research objective was to determine the influence of marigold extract and storage duration on the indicators of egg quality and on the oxidative stability of eggs. The 5-wk long research was carried out on 300 laying hens. Laying hens were divided into control group (C—without marigold extract supplementation) and 2 experimental groups (E1–supplemented 1 g/kg of marigold extract and E2–supplemented 2 g/kg of marigold extract to diet). The eggs were analyzed on 2 occasions, as fresh eggs (1 D after collecting in the facility) and after 28 D of storage in a refrigerator on +4°C. On both occasions, there were 30 eggs analyzed per each group. Statistical analysis of the research results proved influence of marigold extract supplementation and storage duration on the weight of eggs ($P < 0.05$) and on the eggshell thickness ($P < 0.001$), but they did not have any influence on other indicators of external egg quality. Storage duration affected ($P < 0.001$) all indicators of internal egg quality, whereas supplementation of marigold extract influenced ($P < 0.001$) only the pH value of yolk. Supplementation of marigold extract influenced ($P < 0.001$) the yolk color intensity. There was a significant increase in the value of yolk color, starting with 9.63 in the C group and raising to 12.77 (E1) and 13.50 (E2) in fresh eggs. Yolk color of stored eggs was more intensive than the yolk color of fresh eggs in all 3 groups ($P < 0.001$). Supplemented marigold extract did not influence the obtained results of lipid oxidation in yolks, whereas storage duration had influence ($P < 0.05$) on oxidation intensity. The most favorable value of oxidation in fresh eggs was determined in the E2 group (0.545 μg MDA/g), and in stored eggs in the E1 group (0.615 μg MDA/g). Based on the research results, it was concluded that the supplementation of marigold extract had favorable influence on the yolk color intensity and on the oxidative stability of eggs without having negative influence on other egg quality indicators.

Key words: marigold extract, storage duration, egg quality, yolk color, lipid oxidation

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INTRODUCTION

The quality and chemical composition of table eggs depend on many factors. Feeding of laying hens, feed supplements, duration and conditions of egg storage are very important for egg quality and its sustainability. Through laying hens' metabolic actions, different feed supplements get incorporated into eggs. Antioxidants, fat-soluble vitamins, pigments, and omega-3 fatty acids are especially important. These ingredients improve the nutritional composition of eggs, and their antioxidative properties (selenium, vitamin E, lutein) influence better sustainability of eggs (Surai et al., 2000). Lutein is a plant pigment which, thanks to its polarity and the conjugated double bond system, has antioxidative properties, and especially pronounced free radical scavenging ability (Sindhu et al., 2010). Lutein is the most prevalent carotenoid in marigold extracts (Sowbhagya et al., 2006; Tinoi et al., 2006). In the poultry industry, lutein is used as a supplement for broiler and laying hens' diet in order to increase the skin and yolk color intensity (Hamelin and Altemueller, 2012). In our previous research, it was shown that higher levels of lutein can be safely incorporated in layer hens' diet without adverse effect on hens' performance and health status (Grčević et al., 2016). Since it is soluble in fat, it is deposited in egg yolk. By supplementing increased amounts of lutein to the laying hens' diet, producers can increase the lutein concentration and color intensity in egg yolk. Eggs need to be stored in a refrigerator to maintain their quality. Depending on storage conditions, internal egg structure can degrade thus causing worse quality. As lutein is an antioxidant and natural pigment, it was used as a laying hens' diet supplement in this research

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in a form of marigold extract to determine its influence, as well as the influence of storage duration, on the indicators of external and internal egg quality and lipid oxidation in egg yolks.

MATERIALS AND METHODS

Animals and Diets

All of the procedures used in the experiment were conducted according to protocols approved by the Bioethics committee for research on animals, Faculty of Agrobiotechnical Sciences Osijek, University of Osijek. The research was carried out on a total of three hundred 31-wk-old laying hens of the Tetra SL hybrid. Hens were randomly assigned to 3 experimental groups and each group was further divided into 5 replicates of 20 hens each (5 hens per cage, 4 cages per replicate). Prior to starting the experiment with the addition of marigold extract to hens' mixtures, all 3 group of hens were fed control mixtures for 4 wk, in order to get used to feed and to eliminate differences between groups at the beginning of experiment with the addition of marigold extract to the mixtures. Feed and water were offered for ad libitum consumption. After the initial 4 wk, the marigold extract was supplemented to diet as follows: C—without marigold extract, E_1–supplemented 1 g of marigold extract/kg feed, and E_2–supplemented 2 g of marigold extract/kg feed. Experimental period lasted for 5 wk. Marigold flower extract (*Calendula officinalis*) was purchased from Phyto Nutraceutical Inc. (Changsha, Hunan, China) and contained lutein (20%) as active ingredient. Accordingly, the calculated amount of lutein in mixtures for hens was 0, 200, and 400 mg/kg. Composition of feeding mixtures is shown in Table 1.

Table 1. Composition of feeding mixtures for laying hens.

Ingredient	Composition (%)		
	C	E_1	E_2
Corn	40.64	40.54	40.44
Triticale	15.00	15.00	15.00
Soybean meal	18.40	18.40	18.40
Toasted soybean	8.40	8.40	8.40
Sunflower meal	1.66	1.66	1.66
Alfalfa	1.00	1.00	1.00
Calcium grains	9.32	9.32	9.32
Monocalcium	1.60	1.60	1.60
Yeast	0.5	0.5	0.5
Salt	0.33	0.33	0.33
[1]Sal CURB	0.33	0.33	0.33
[2]MINERAL DETOX	0.24	0.24	0.24
[3]Probiotic Probio P-500	0.05	0.05	0.05
[4]Lysoforte	0.03	0.03	0.03
Methionine	0.24	0.24	0.24
[5]Premix	0.58	0.58	0.58
Marigold extract	0	0.1	0.2
Soybean oil	1.68	1.68	1.68
Total	100.00	100.00	100.00
Crude protein, %		18.00	
ME, MJ/kg		11.40	

C = without marigold extract, E1 = 1 g marigold extract/kg diet, E2 = 2 g marigold extract/kg diet.

[1]Antimicrobial (KEMIN, Herentals, Belgium).

[2]Natural zeolite (Mineral Promet, Velika Gorica, Croatia).

[3]Probiotic (4b1841, *Enterococcus faecium* DSM 7134) 10×10^9 CFU (Lactosan, Kapfenberg, Austria).

[4]Absorption enhancer (KEMIN, Herentals, Belgium).

[5]Premix (Agrocroatia Nova d.o.o., Sisak, Croatia), content per kg of diet: vitamin A 11,600 IU, vitamin D_3 2,900 IU, vitamin E 58 mg, vitamin K_3 3.48 mg, vitamin B_1 2.32 mg, vitamin B_2 5.8 mg, vitamin B_6 5.8 mg, vitamin B_{12} 17.4 μg, vitamin C 23.2 mg, biotin 69.6 μg, niacin (nicotinic acid) 46.4 mg, pantothenic acid 13.92 mg, folic acid 0.87 mg, choline chloride 580 mg, iodine 1.16 mg, manganese 104.4 mg, zinc 81.2 mg, cobalt 0.174 mg, iron 69.6 mg, copper 9.28 mg, selenium inorganic 0.29 mg, calcium 1.38 g, phytase 580 FYT, canthaxanthin 2.9 mg, beta-apo-beta-carotenoic acid 1.74 mg, antioxidant (butylhydroxytoluene) 116 mg.

Egg Quality Indicators

Upon completion of the experimental period, there were 60 eggs of the weight class M (53 to 63 g) collected from each group and used in analysis to determine indicators of egg quality and freshness (shape index, weight of eggs and its main parts, strength and thickness of shell, pH of albumen and yolk, yolk color, height albumen, Haugh units [**HU**]). Indicators of egg quality were measured on 2 occasions: first on fresh eggs (1 D after collecting eggs in the facility) and after 28 D of storing eggs in a refrigerator at +4°C. On both occasions, there were 30 eggs analyzed from each group. Vernier scale was used to measure length and width of eggs, and the data were further used to calculate the shape index (**SI**) according to the formula: SI (%) = egg width (mm)/egg length (mm) x 100. Weights of eggs and of its main parts were measured by an electronic scale MT PB 1502-S (Mettler Toledo, Greifensee, Switzerland). Shell strength was measured by an automatic device Eggshell Force Gauge Model—II (Robotmation Co, Ltd., Tokyo, Japan), and the values were expressed in kg/cm^2. Shell thickness was measured on the equatorial part of the egg by using an electronic micrometer. Values of albumen and yolk pH were determined by digital pH-meter MT Seven Easy (Mettler Toledo, Greifensee, Switzerland). Albumen height, HU, and yolk color were determined by using the EggMulti-Tester EMT-5200 device (Robotmation Co, Ltd., Tokyo, Japan). CIE Lab indicators of yolk color were determined with the CR-300 Chroma Meter (Konica Minolta, Tokyo, Japan) on 10 samples per each group.

Lipid Oxidation

The oxidation of egg yolk lipids was determined in fresh eggs (n = 10 per group) and in eggs stored for 28 D in a refrigerator at +4°C (n = 10 per group), as follows: 4 g of yolk was weighed in a test tube, and then 12 mL of 10% trichloroacetic acid was added. The mixture was homogenized and centrifuged for 15 min at 5,500 × g at 4°C. After centrifugation, 2.5 mL of supernatant was collected and mixed with 1.5 mL of thiobarbituric acid (**TBA**) solution (pH 2.5). The test tubes were closed and immersed into water bath for 30 min at 90°C. After cooling, 1 mL of distilled water was added, and the mixture was centrifuged for 15 min at 5,500 × g at 4°C. The amount of colored

product, formed by the reaction of lipid peroxidation products with thiobarbituric acid, was measured spectrophotometrically at 532 nm. The obtained values were compared with standard curve prepared by using standard malondialdehyde (**MDA**) tetrabutylammonium salt (Sigma-Aldrich, Buchs, Switzerland), and expressed as μg MDA/g of yolk.

Statistical Analysis

Research results were processed by Statistica software (Tibco Software Inc., 2018). Presented statistical parameters were arithmetic mean ($\bar{x}$) and standard error of the mean. Testing of significance of differences within a group and between groups was done by using the GLM procedure of multivariate analysis of variance (3 × 2). The calculated F value was compared to the critical theoretic F value at a significance level of 5%. Significance of differences between mean values was determined by Fisher's LSD test.

RESULTS AND DISCUSSION

Results obtained by analyzing external egg quality indicators are shown in Table 2. Statistical analysis of research results proved that both marigold extract supplementation and storage duration had a significant influence on the egg weight and on the shell thickness. Egg length and width and the shape index were similar in all examined groups. The highest weight of eggs ($P = 0.028$) was determined in the E1 group, both in fresh and stored eggs. In their research, Jang et al. (2014) also reported higher weight of eggs in groups fed lutein supplement, although the difference was not significant ($P > 0.05$), whereas Skřivan et al. (2015) reported lower ($P < 0.05$) weight of eggs of group fed with marigold flower extract. Lokaewmanee et al. (2011) determined that lutein from marigold flower extract did not have influence of the egg weight.

During storage, eggs lose water through the shell pores, which causes a reduction in egg weight, as was also confirmed in this research ($P < 0.001$). Kralik et al. (2014) and Akter et al. (2014) confirmed a significant influence of storage duration on the reduction of egg weight.

Supplementation of lutein did not affect the differences in shell thickness of fresh eggs, whereas the thinnest shell was measured in stored eggs of the E1 group ($P = 0.001$). Lokaewmanee et al. (2011) did not determine the influence of lutein supplement (40 mg/kg) on the egg shell thickness. In our research, stored eggs had thicker shell than fresh eggs ($P < 0.001$). Our results are in accordance with the results of Batkowska et al. (2014), as they reported that eggs stored for 28 D had thicker shell ($P < 0.05$) than fresh eggs. On the contrary, Tabidi (2011), Akter et al. (2014), and Kralik et al (2014) did not determine the influence of storage duration on the egg shell thickness.

Referring to the results presented in Table 3, it is clear that supplementation of marigold extract had influence only on the pH value of yolk ($P < 0.001$), whereas storage duration influenced all indicators of internal egg quality ($P < 0.001$). Albumen weight reduced and yolk weight increased over storage duration. Akter et al. (2014) and Batkowska et al. (2014) pointed out lower ($P < 0.05$) albumen weight and higher yolk weight of eggs stored for 28 D, which is in accordance with our results.

Albumen height and HU are indicators of egg freshness. In this research, it was determined that albumen height and HU were influenced by storage duration ($P < 0.001$). Values of albumen height reduced in stored eggs if compared to the fresh eggs. Values of HU were also following the values of albumen height. Although there was no influence of marigold extract confirmed, it was noted that the greatest values of albumen height and HU were measured in the E2 group, both in fresh and in stored eggs. Samli et al. (2005) reported a reduction ($P < 0.001$) of albumen height and of HU already after 10 D of storing eggs at 5°C. Kralik et al. (2014) determined a reduction ($P < 0.05$) of albumen height and of HU after 28 D of storing eggs in a refrigerator, which corresponds to our results. Storage of eggs affected the increase of pH values of albumen and yolk, which was also confirmed in our research ($P < 0.001$). Both examined factors and their interaction influenced the pH value of yolk ($P < 0.001$). Fresh eggs had lower values of yolk pH than stored eggs. Stored eggs of the E1 group exhibited lower value ($P < 0.001$) of yolk pH than other groups, whereas pH values of fresh eggs were similar in all applied treatments. Jin et al. (2011) reported an increase in pH values of albumen ($P < 0.001$) and of yolk ($P = 0.011$) already after 10 D of storing eggs at 5°C. Kralik et al. (2014) and Akter et al. (2014) determined an increase ($P < 0.05$) of pH values in albumen and yolk of eggs stored for 28 D in a refrigerator, which is in accordance with the results obtained in this research.

Figure 1 presents the values of egg yolk color of experimental groups measured on fresh and stored eggs. Statistical analysis of obtained results proved that marigold extract supplementation and storage duration had influence ($P < 0.001$) on egg yolk color.

Leeson and Caston (2004) stated that yolk color increased ($P < 0.01$) by supplementation of lutein to hens' diet already after 7 D of the feeding treatment. Yolk color stabilized at 13 to 14 by supplementation of 250 mg/kg of lutein, whereas the color value in group with no supplemented lutein was 6 to 7. Supplementation of more than 250 mg/kg of lutein did not affect further increase of yolk color intensity. Similar results were published by Leeson et al. (2007) as they achieved increase of yolk color ($P < 0.01$), from 6.6 in the control group to 13.9 and 13.7 by adding 125 and 250 mg/kg of lutein, respectively. Jang et al. (2014) supplemented only 40 mg/kg of lutein and increased ($P < 0.05$) yolk color from 7.7 in the control to 9.45 in experimental

Table 2. Indicators of external egg quality ($\bar{x}$; n = 30 per group).

Group	Storage duration (days)	Egg length (mm)	Egg width (mm)	Shape index (%)	Egg weight (g)	Shell strength (kg/cm^2)	Shell thickness (mm)
C	1	56.40	43.30	76.79	59.56^{a,b}	3.205	0.365^{c}
	28	56.73	43.47	76.65	58.43^{c}	3.343	0.380^{a,b}
E_1	1	56.53	43.43	76.85	60.35^{a}	3.239	0.362^{c}
	28	56.70	43.63	76.96	59.58^{a,b}	3.211	0.369^{c}
E_2	1	56.77	43.27	76.27	60.05^{a}	3.119	0.371^{b,c}
	28	56.93	43.50	76.44	59.02^{b,c}	3.343	0.387^{a}
SEM		0.219	0.143	0.351	0.360	0.088	0.004
				Sources of variation			
Group (G)		0.387	0.484	0.284	0.028	0.831	0.001
Storage duration (SD)		0.215	0.090	0.856	0.001	0.121	<0.001
G x SD		0.908	0.973	0.896	0.875	0.349	0.425

$\bar{x}$ = arithmetic mean; SEM = standard error of the mean.
[a–c]Values within a column with different letters differ significantly at $P < 0.05$.
C = without marigold extract, E1 = 1 g marigold extract/kg diet, E2 = 2 g marigold extract/kg diet.

Table 3. Indicators of internal egg quality ($\bar{x}$; n = 30 per group).

Group	Storage duration (days)	Albumen weight (g)	Yolk weight (g)	Albumen height (mm)	HU	pH of albumen	pH of yolk
C	1	36.84^{a}	15.36^{d}	6.87^{a}	82.04^{a}	8.68^{c}	5.99^{c}
	28	35.36^{b}	16.03^{b,c}	5.88^{b}	75.85^{b}	9.25^{a}	6.23^{a}
E_1	1	36.95^{a}	15.74^{b,c,d}	6.87^{a}	82.15^{a}	8.73^{b,c}	6.01^{c}
	28	34.92^{b}	16.70^{a}	5.81^{b}	74.88^{b}	9.26^{a}	6.10^{b}
E_2	1	36.73^{a}	15.47^{c,d}	7.11^{a}	83.80^{a}	8.75^{b}	6.01^{c}
	28	35.09^{b}	16.22^{a,b}	5.95^{b}	76.38^{b}	9.22^{a}	6.22^{a}
SEM		0.298	0.231	0.195	1.347	0.023	0.016
				Sources of variation			
Group (G)		0.785	0.068	0.601	0.486	0.337	<0.001
Storage duration (SD)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
G x SD		0.634	0.807	0.909	0.883	0.101	<0.001

$\bar{x}$ = arithmetic mean; SEM = standard error of the mean.
[a–d]Values within a column with different letters differ significantly at $P < 0.05$.
C = without marigold extract, E1 = 1 g marigold extract/kg diet, E2 = 2 g marigold extract/kg diet.

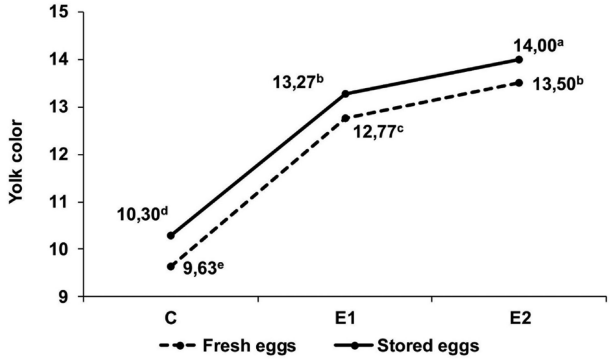

Figure 1. Egg yolk color. C = without marigold extract, E1 = 1 g marigold extract/kg diet, E2 = 2 g marigold extract/kg diet; [a,b,c,d,e]values with different letters differ significantly at $P < 0.05$; each group consisted of one hundred 31-wk-old Tetra SL hybrid laying hens which were fed experimental diets for 5 wk; n = 30 eggs per group; eggs were analyzed as fresh (1 D after collection on the farm) and stored (after 28 D of storage in a refrigerator at 4°C).

group. Our results are in accordance with results of the mentioned authors that confirm the increased intensity of yolk color after supplementing lutein to laying hens' diet.

In our research, there was higher ($P < 0.001$) intensity of yolk color determined in stored eggs than in fresh eggs in all 3 applied treatments. Similar results were obtained in research by Jin et al. (2011), where yolk color increased ($P < 0.001$) in eggs stored for 10 D at 5°C. However, storing of eggs at higher temperatures (21 and 29°C) exhibits the reverse trend, i.e., a reduction of yolk color intensity with storage time. Carranco-Jáuregui et al. (2006) stated that storage of eggs at 4°C during 30 D did not affect the yolk color intensity, whereas storage of eggs at 20°C caused a reduction ($P < 0.05$) of yolk color intensity. Batkowska et al. (2014) did not determine the influence of storage duration on the yolk color intensity. On the other hand, Hagan et al. (2013) and Kralik et al. (2014) determined a reduction ($P < 0.05$) of yolk color intensity by storing eggs at a room temperature and in refrigerator. In the available literature, there is not many data referring to the increase of yolk color intensity in dependence on duration and temperature of storage.

Table 4 presents the yolk color values determined by the colorimeter Minolta CR 300 (CIE Lab values).

Applied treatments and storage duration have influenced CIE Lab color indicators. As of the data

Table 4. Egg yolk color depending on supplemented marigold extract and storage duration ($\bar{x}$; n = 10 per treatment).

Group	Storage duration (days)	Yolk color		
		CIE L*	CIE a*	CIEb*
C	1	62.92^{a,b}	2.48^{e}	46.63^{c}
	28	64.65^{a}	4.08^{d}	54.58^{a,b}
E_1	1	59.71^{c,d}	9.51^{c}	52.77^{b}
	28	61.06^{b,c}	11.61^{b}	58.85^{a}
E_2	1	59.03^{e}	11.18^{b}	51.95^{b}
	28	60.82^{c,d}	13.54^{a}	56.10^{a,b}
SEM		0.686	0.407	1.701
Sources of variation				
Group (S)		<0.001	<0.001	0.012
Storage duration (VS)		0.005	<0.001	<0.001
S x VS		0.940	0.637	0.541

$\bar{x}$ = arithmetic mean; SEM = standard error of the mean.
$^{a-e}$Values within a column with different letters differ significantly at $P < 0.05$.
C = without marigold extract, E1 = 1 g marigold extract/kg diet, E2 = 2 g marigold extract/kg diet.

presented in Table 4, the degree of yolk lightness (CIE L*) decreased ($P < 0.001$) along with the increase of marigold extract content in diet, whereas the degree of redness (CIE a*) ($P < 0.001$) and yellowness (CIE b*) ($P = 0.012$) of yolk increased. On the other hand, storage duration affected the increase of CIE L* values ($P = 0.005$), as well as the increase ($P < 0.001$) of CIE a* and CIE b* values of color. Englmaierová et al. (2013) studied the influence of lutein supplementation (250 mg/kg) on CIE Lab indicators of yolk color. They determined influence ($P < 0.001$) of lutein on all indicators of color. The CIE L* value decreased, and CIE a* and CIE b* values increased by supplementation of lutein. Supplementation of 100 mg/kg of lutein to laying hens' diet in the research of Englmaierová and Skřivan (2013) affected the same trend of changes in color ($P < 0.001$), by decreasing values of CIE L* and increasing values of CIE a* and CIE b*. Results of the mentioned researches are in accordance with the results obtained of this research. Lokaewmanee et al. (2011) and Sirri et al. (2007) confirmed a significant influence of lutein supplementation only on the CIE a* color value. In their research, there were lower amounts of lutein, i.e., total xanthophyll, supplemented to feed than in our research; therefore, the values of CIE L*, CIE a*, and CIE b* were lower than those values obtained in our research.

Results of lipid oxidation in egg yolks are presented in μg MDA/g yolk (Figure 2). Supplemented marigold extract did not influence the obtained results, whereas storage duration had influence ($P < 0.05$) on oxidation intensity. The most favorable (the least) value of oxidation in fresh eggs was determined in the E2 group (0.545 μg MDA/g), and in stored eggs in the E1 group (0.615 μg MDA/g). Values of oxidation increased over egg storage duration, and the values of egg yolks of experimental groups were slightly lower after 28 D of storage when compared to the control group, but those differences were not statistically significant.

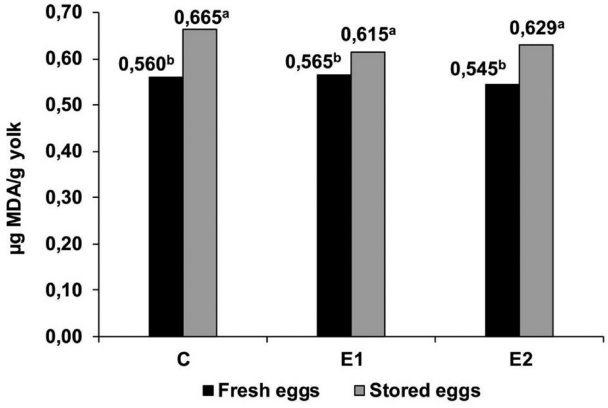

Figure 2. Lipid oxidation of egg yolks measured on fresh and stored eggs. C = without marigold extract, E1 = 1 g marigold extract/kg diet, E2 = 2 g marigold extract/kg diet; ^{a,b}values with different letters differ significantly at $P < 0.05$; each group consisted of one hundred 31-wk-old Tetra SL hybrid laying hens which were fed experimental diets for 5 wk; n = 30 eggs per group; eggs were analyzed as fresh (1 D after collection on the farm) and stored (after 28 D of storage in a refrigerator at 4°C)

Akter et al. (2014) determined influence ($P < 0.05$) of storage duration (28 D) on the increase of the TBARS values in egg yolks stored at 4°C, which is in accordance with our results. Englmaierová et al. (2013) determined influence ($P < 0.001$) of lutein supplementation (250 mg/kg) on the improvement of oxidative stability of yolk lipids during 28-D long storage at 18°C. So the fresh eggs of the control and of the experimental group had 1.17 and 0.87 mg MDA/kg, respectively. Values obtained after 28 D of storage were 1.28 and 1.04 mg MDA/kg. There was positive influence of lutein on the lowering of lipid oxidation. Results obtained by the mentioned authors confirmed the trend of lowering oxidation of egg yolks by supplementing lutein to diet, as defined in our research. Still, in our research, there was no statistically significant influence of the treatment. Nain (2011) studied the influence of lutein supplemented to laying hens' diet (500 mg/kg)

on the lipid oxidation of egg yolks of fresh eggs and of eggs stored for 30 D at 4°C. These results confirmed influence ($P < 0.001$) of storage duration and lutein supplementation on the TBARS values. Fresh eggs of the control group had 0.6142 mg MDA/kg yolk, and the experimental group had 0.4960 mg MDA/kg. Although the difference was not significant, the influence of lutein on the lowering of the oxidation intensity in yolks of experimental group is obvious. In our research, there was no significant difference between the values of lipid oxidation in fresh egg yolks, although the group with 400 mg/kg of marigold extract exhibited slightly lower value of oxidation than the control group. In eggs stored for 30 D at 4°C, Nain (2011) determined the TBARS values of 0.7523 mg MDA/kg yolk in the control and 0.5632 mg MDA/kg yolk in the experimental group. Determined value of oxidation in the control group was higher ($P < 0.001$) in comparison to fresh eggs, whereas the value of oxidation in the experimental group did not differ from the value determined for fresh eggs. Still, it was significantly lower than the value measured in stored eggs of the control group (protective action of lutein). In our research, marigold extract did not have a significant influence on the lowering of oxidation, either in fresh or stored eggs, but we suppose there is a positive influence since values of oxidation in experimental groups were lower than in the control group. Since egg is a closed system within which egg shell protects inner content from exposure to oxygen and oxidizing agents, the occurrence of oxidative reactions is minimal. The presence of natural antioxidant substances, such as lecithin, additionally enhances antioxidative protection. By keeping table eggs in refrigerator, the development of oxidative products is negligible, whereas storage at a room temperature causes the increase of TBARS and reduction of antioxidant content (e.g., vitamin E) in eggs (Franchini et al., 2002).

To summarize, supplementation of increased amounts of marigold extract to laying hens' diet influenced the increase of egg weight of experimental groups, but it did not influence other indicators of external egg quality. Indicators of internal egg quality, primarily albumen height and HU, were reduced with storage duration. It was noted that the groups with marigold extract supplementation had higher values of those indicators than the control group, but the differences were not statistically significant. Marigold extract supplementation had the strongest influence on the increase of egg yolk color intensity in experimental groups. Based on the results obtained in this research, it is concluded that marigold extract supplementation had a positive influence on yolk color intensity, and the storage duration significantly influenced the egg weight and internal egg quality. Supplemented marigold extract and proper storage conditions sustained egg quality during the experimental period of 28 D.

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