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Obogaćivanje konzumnih jaja luteinom

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Poljoprivreda/Agriculture

ISSN: 1848-8080 (Online)
ISSN: 1330-7142 (Print)

http://dx.doi.org/10.18047/poljo.26.1.8

Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

ISSN 1330-7142
UDK=637.482
DOI: 10.18047/poljo.26.1.8

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Kralik, G.[1,2], Kralik, Z.[1,3], Grčević, M.[1,3], Hanžek, D.[1,3], Margeta, P.[1,3], Galović, O.[1,4]

Original scientific paper
Izvorni znanstveni članak

SUMMARY

For the enrichment of eggs with lutein, a marigold flower extract – MFE (*Tagetes erecta *L.) was used. The two groups of laying hens were involved in the study – the control group (C) and the experimental group (E), respectively. The laying hens of the control group were fed by the lutein-free mixture, while the laying hens' E group consumed the mixture with the 3 g/kg of lutein. The laying hens' feeding lasted for 31 days, after which the quality of eggs (i.e., the shape index, egg weight and the egg main parts, shell strength and thickness, Haugh units – HU, egg white height, egg yolk color, egg white pH, and the egg yolk pH), as well as the lutein content and Thiobarbituric Acid Reactive Substances (TBARS) values in egg yolks, were determined. The study results have demonstrated that the added MFE exerted an effect of thickness, weight, and eggshell proportion reduction (P<0.001), as well as of the shell strength reduction (P=0.014). It has also reduced the HU value (P=0.039) and has increased the egg white content, egg yolk color, and the egg yolks' lutein content (P<0.001). A statistically significant difference in TBARS values was found between the fresh and the stored eggs in group C, as well as between the C and E groups in the fresh eggs (P<0.05). Our results indicate that MFE is suitable for the enrichment of table eggs with lutein.

Keywords: marigold flower extract, lutein, egg yolk color, egg quality, TBARS values

INTRODUCTION

Lutein is a pigment that can be accumulated in egg yolks. Lutein has a significant role in the maintenance of normal eye function, and it acts as an antioxidant, absorbs blue light, and protects the eyes from macular degeneration. Lutein is a yellow pigment from the carotenoids (xanthophylls) group. As the people cannot synthesize lutein, it has been consumed by diet and various supplements. Marigold flower (*Tagetes erecta* L.) is a plant rich in lutein that neutralizes free radicals in the body and protects the eyes from ultraviolet radiation. According to Šivel et al. (2014), this preparation comes to the market in the form of a concentrate (50-265 mg/g of dry matter) or powder (54.2-802.3 mg/g DM), while Lokaewmanee et al. (2011) state that a more intense pigmentation of egg yolks is achieved by the usage of marigold flower extract (MFE) than marigold flower meal (MFM). For a more intense yolk color, 30-40 mg/kg should be added to the feed, regardless of the fact whether the eggs are fresh or cooked. Niu et al. (2008) report an effective usage of peppers in the feeding of laying hens and in the deposition of pigments in egg yolks. A dilemma of using the pepper or the MFM in an egg enrichment with lutein was experienced by Oliveira et al. (2017). The authors concluded that a pepper extract intensifies the egg yolk color and improves the egg quality, whereas the MFE reduces the eggshell quality. The consumers prefer to use the eggs with the natural rather than with the artificial pigments (Dufosse 2006). Skřivan et al. (2016) found that the usage of MFE at a concentration of 550 mg/kg feed had a positive effect on the egg yolk color intensity, on the enrichment of eggs with carotenoids, and on the increase

(1) Prof. Emer., Ph. D., Dr. h. c Gordana Kralik – Josip Juraj Strossmayer University of Osijek, Scientific Center of Excellence for Personalized Health Care, Trg sv. Trojstva 3, 31000 Osijek, Croatia (2) Nutricin j. d. o .o. Darda, Đure Đakovića 6, 31326 Darda, Croatia, (3) Assoc. Prof. Zlata Kralik (zlata.kralik@fazos.hr), Manuela Grčević, Ph. D., Danica Hanžek, M. Eng. Agr., Polonca Margeta, Ph. D. – Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia, (4) Assist. Prof. Olivera Galović – Josip Juraj Strossmayer University of Osijek, Department of Chemistry, Ulica cara Hadrijana 8/A, 31000 Osijek, Croatia

of an oxidative stability of the lipids during the egg storage. The authors noted that MFE is a more appropriate alternative supplement than the synthetic xantophylls. These statements are confirmed by the results of another research (Nain, 2011; Kralik et al., 2014, Englmaierová et al., 2018). Grčević et al. (2019) also state that a marigold flower extract is suitable for the improvement of oxidative stability of lipids (0, 1 and 2 g/kg food), a lutein content increase, and the achievement of a more intense egg yolk color. The aim of the study was to enrich the eggs with lutein by using a marigold flower extract in the feeding of laying hens. At the same time, the physicochemical properties of the lutein-enriched eggs were investigated, as well as the oxidative processes of lipids during the egg storage, in relation to the conventional eggs.

MATERIAL AND METHODS

Laying hens and feed

In the study, 72 laying hens of Tetra SL hybrids were used, which were 37 weeks old at the start of the experiment. Laying hens were divided into two groups (C–control group and the E–experimental group) and kept in the enriched cages (36 laying hens per group; three repetitions). Group C consumed the feed mixture without the addition of marigold flower extract, while 3 g/kg marigold flower extract was added to the group E mixture. The mixtures were balanced on the basis of a 17-percent crude protein and 11.5 MJ/kg ME (Table 1).

Table 1. The composition of laying hens' feed mixtures

Tablica 1. Sastav smjesa za nesilice

Ingredient - *Sastojak* (%)	C group – *Skupina C*	E group – *Skupina E*
Corn – *Kukuruz*	51.53	51.23
Soybean meal – *Sojino brašno*	23.23	23.23
Sunflower meal – *Suncokretovo brašno*	5.00	5.00
Lucerne – *Lucerka*	1.03	1.03
Fodder yeast – *Stočni kvasac*	0.50	0.50
Limestone – *Vapnenac*	10.70	10.70
Monocalcium phosphate – *Monokalcijev fosfat*	1.33	1.33
Salt – *Sol*	0.33	0.33
Methionine – *Metionin*	0.15	0.15
Soybean oil – *Sojino ulje*	5.00	5.00
Premix* – *Premiks*	1.20	1.20
Marigold flower extract – *Ekstrakt cvijeta kadife*	0.00	0.30
Total	100.00	100.00
Chemical analyses – *Kemijske analize*** (%)		
Crude protein – *Sirove bjelančevine*	17.07	
Crude fat – *Sirove masti*	7.47	
Crude fibers – *Sirova vlaknina*	4.83	
Crude ash – *Sirovi pepeo*	9.70	
ME, MJ/kg (calculated - *izračunana*)	11.50	

C group = without MFE; E group = 3 g kg^{-1} MFE; *Premix: calcium 33%, vit. A 833,340 I.U., vit. D_3 208,340 I.U., vit. E 8,350 mg, vit. K_3 170 mg, vit. B_1 150 mg, vit. B_2 375 mg, pantothenic acid 590 mg, niacin 2,100 mg, choline chloride 33,340 mg. vit. B_6 200 mg, vit. B_{12} 960 mcg, biotin 7,100 mcg, folic acid 70.5 mg. vit. C 1,900 mg, iron 2,500 mg, copper 415 mg, zinc 5,200 mg, manganese 5,835 mg, iodine 75 mg, selenium-yeast 35 mg, antioxidant (Apo-ester 85 mg, canthaxanthin 250 mg)

**Reference methods applied to the chemical analysis of feed: HRN ISO 6496: 200; HRN EN ISO 5983-2: 2010; HRN EN ISO 6865: 2001. Changed according to the instructions from the manual FOSS Fiber Cap; HRN ISO 5984: 2004; HRN ISO 6492: 2001. Modified according to the instructions of the extraction system ANKOM XT15; RU-5.4.2-11 (internal method).

Feeding and watering of hens were *ad libitum*, and both groups of hens were in the same facility under the same microclimatic conditions during 16 daylight hours. The experimental part related to the feeding of laying hens with different feed mixtures lasted for 31 days. The laying hens' weights were determined at the beginning and at end of the experiment. The quantities of feed consumed were also measured, and egg production was determined. Subsequent to the feeding period with the mixtures of modified composition, the eggs were sampled to analyze their physicochemical properties, intensity of lipid oxidation, and the lutein content of the yolks.

Egg quality indicators

A total of 100 eggs, or 50 eggs per group, were used for a physicochemical property analysis. The eggs were those of the class L (egg weight 63-73 g). The eggs were analyzed a day subsequent to the collection (n=25 per group). The following quality indicators were determined: shape index, the weight of the egg and its basic parts, its strength, and eggshell thickness, Haugh unit, egg white height, egg yolk color, egg white pH and the egg yolk ph. On the basis of weights of the basic egg parts, their proportions (%) were calculated. The shape index was calculated from the measures of egg length and width according to the

following formula: IO = egg length/egg width * 100. The egg length and width were measured using a digital thickness gauge, with a measuring range amounting to 0-300 mm/0–12" (Insize, USA). The egg weight was measured using a laboratory scale (Mettler Toledo, BBK 422-6 DXS). The shell thickness was measured using an electronic micrometer with a measuring range from 0.25 mm to 0.001 mm in the middle of the eggshell, and an average of two measurements was used (Insize, USA). The Eggshell Force Gauge Model-II automatic device (Robotmation Co., Ltd., Japan) was used for the eggshell strength measurement. The yolk color, Haugh units (HJ), and egg white height were determined by an automatic device Egg Multi-Tester EMT-5200 (Robotmation Co., Ltd., Japan). The egg white and egg yolk pH values were measured by a pH meter MP 120 (model SevenEasy).

Lutein content analysis

For the analysis of lutein in egg yolks, 12 eggs, or six per group, were used. The yolk samples were prepared according to the Leeson and Caston's method (2004). Lutein in egg yolks was determined using the Shimadzu HPLC system by weighing 0.5 g of egg yolk in a test tube, overflowing it with 5 ml of acetone and vigorously stirring on a vortex mixer for 30 seconds. The samples were left to stand in the dark for one hour. Subsequent to a rest and filtration through a 0.45 μm membrane filter CHROMAFIL® Xtra CA-45/25 (MACHEREY-NAGEL GmbH&Co. KG, Düren, Germany), 1 ml of acetone extract was transferred to the HPLC vials and gently evaporated by heating. The residue in the vial was dissolved by the addition of 1 ml of hexane / ethyl acetate solution (65:35, v/v) and mixed on the vortex. The sample thus prepared was analyzed on a Viva C18 column (5 μm, 250x4.6 mm; RESTEK Corporation, Bellefonte, PA, USA). The mobile phase consisted of a mixture of methanol and tetrahydrofuran (THF) 9:1 (v/v). The flow rate was 1 ml/min, the analysis time was 20 minutes, and the measurement wavelength was 450 nm. The injection volume was 20 μl. The standard lutein curve was prepared using the lutein standard purchased from ChromaDex (Irvine, CA, USA).

Lipid oxidation

The oxidation of lipids in egg yolks of the fresh and stored eggs was determined using the TBARS value (μg MDA–malondialdehyde/g egg yolk). The samples were prepared as follows: 10% trichloroacetic acid was added to the weighed egg yolk, the mixture was homogenized and centrifuged at 5500 rpm, 4°C. Subsequent to the centrifugation, a solution of thiobarbituric acid (pH 2.5) was added to the supernatant, the tubes were closed and immersed in a water bath at 95°C for 30 minutes. Subsequent to the cooling, distilled water was added, and the mixture was centrifuged at 5500 rpm, 4°C. The content of the colored product formed by the reaction of lipid peroxidation products with thiobarbituric acid was measured spectrophotometrically at 534 nm. The obtained values were compared with the standard curve prepared using the standard malondialdehyde tetrabutylammonium salt (Sigma-Aldrich, Switzerland) and expressed in μg MDA/g of egg yolk. For the purpose of oxidation determination, 20 eggs were used (10 per group).

Statistical analisys

The research results were processed using the TIBC Statistica ™ version 13.4.0.14. (Soft Inc.,©1984–2018). Descriptive statistics and an analysis of variance were performed (ANOVA). If the P value for the analysis of variance was statistically significant, the differences between the groups were tested by the Fisher LSD test ($P<0.05$; $P<0.01$; $P<0.001$).

RESULTS AND DISCUSSION

The weights of laying hens, consumption, and conversion of feed as well as egg production are shown in Table 2. Group C during the 31 days of the study increased the average body weight by 38 g ($P>0.05$), while the E group of laying hens reduced the average weight by 61 g ($P<0.05$). The average daily consumption of feed mixture in group C laying hens was 116.00±3.09 g, while in group E was 121.17±3.10 g, respectively. Egg production in both groups of laying hens was equal (80.0 and 80.5%), and 145.33±3.76 g or 150.33±3.77 g of the feed mixture was consumed per egg ($P>0.05$).

Table 2. The laying hens' performances during the experiment

Tablica 2. Performance kokoši nesilica tijekom pokusa

Indicator *Pokazatelj*	C group (Mean±SE)	E group (Mean±SE)	P value *P vrijednost*
Weight of the laying hens at the beginning of the experiment (g) *Masa kokoši nesilica na početku pokusa (g)*	1948.94±104.97	1986.94±122.54	0.140
Weight of the laying hens at the end of the experiment (g) *Masa kokoši na kraju pokusa (g)*	1997.88±127.47[a]	1936.72±128.63[b]	0.046
Feed consumption (g/day) *Konzumacija hrane (g/dan)*	116.00 ± 3,09	121.17 ± 3.10	0.302
Feed conversion (g/egg) *Konverzija hrane (g/jaje)*	145.33 ± 3,76	150.33 ± 3.76	0.399
Egg production *Proizvodnja jaja* (%)	80.00 ± 2,95	80.50 ± 2.89	0.459

C group = without MFE; E group = added MFE 3 g/kg feed; [a,b]$P<0.05$

The results of our research are in agreement with those reported by Antunas and Aydin (2014) regarding daily consumption of the feed mixture and the performance of the laying hens.

Skřivan et al. (2016) examined the effect of MFE (0; 150; 350; 550; 750 and 950 mg/kg) in feed for laying hens on egg production and quality. They found that MFE had no effect on laying hens body weight, feed conversion, and egg quality. However, higher concentrations of MFE influenced increased egg production, egg weight ($P<0.01$) and the mixture intake ($P<0.05$). Antunas and Aydin (2014) found no effect of MFE on egg production and egg quality indicators.

The physicochemical characteristics of the C and E group eggs and the weight of the basic parts are figured in Table 3. There were no statistically significant differences in the shape index, egg white, and egg yolk weight ($P>0.05$). The research data demonstrate that the lutein added to the laying hen feed mixture affect the eggshell quality indicators. The laying hens of the experimental group produced the eggs with a thinner and lighter shell ($P<0.001$) and the eggs with a lower shell strength ($P=0.014$) compared to the control group of laying hens. Although a statistically significant difference was found between the control and experimental groups in shell thickness, the obtained values are in the optimal interval for egg handling and transport. Petričević et al. (2017) determined an eggshell thickness of 0.313 mm in laying hens of Tetra SL hybrid at the age of 45 weeks, which is less than our result. A higher egg white and the lower egg shell proportions ($P<0.001$) were found in the eggs of the laying hens' experimental group when compared to the control group, whereas there was no difference between groups in the egg yolks proportions ($P>0.05$). In the study of Oliveira et al. (2017), it was found that the MFE reduced the percentage of shell ($P<0.02$), as well as the shell thickness ($P<0.001$). The similar results were obtained by Roberts (2004). Sharoni et al. (2012), Veprik et al. (2012), and Zhang et al. (2012) found that lutein and zeaxanthin inhibit an estrogenic activity in some tissues, which was probably the case with the shell formation in our study.

Table 3. Eggshell quality and the weight of the basic fresh egg parts

Table 3. Kvaliteta ljuske i mase osnovnih dijelova svježih jaja

Indicators *Pokazatelji*	C group (n=25) Mean±SE	E group (n=25) Mean±SE	P value *P vrijednost*
Shape index, % *Indeks oblika, %*	76.44±3.48	75.16±3,04	0.173
Shell thickness, mm *Debljina ljuske, mm*	0.44 ± 0.03^{A}	$0.40\pm0,04^{B}$	0.001
Shell strength, kg/cm^2 Čvrstoća *ljuske, kg/cm^2*	3.09 ± 0.65^{a}	2.71 ± 0.36^{b}	0.014
Egg weight, g *Masa jajeta, g*	65.43±2.93	65.46±2.98	0.969
Egg white weight, g *Masa bjelanjka, g*	39.54±2.62	40.93±2,58	0.063
Egg white share, % *Udio bjelanjka, %*	60.39 ± 1.84^{B}	62.51 ± 2.03^{A}	0.001
Egg yolk weight, g *Masa* žumanjka*, g*	17.34±1.04	17.11±1,28	0.473
Egg yolk share, % *Udio* žumanjka*, %*	26.53±1.49	16.14±1.84	0.423
Shell weight, g *Masa ljuske, g*	8.54 ± 0.46^{A}	7.42 ± 0.57^{B}	0.001
Egg shell share, % *Udio ljuske, %*	13.07 ± 0.81^{A}	11.34 ± 0.88^{B}	0.001

C group = without MFE; E group = added MFE 3 g/kg feed; $^{a,b}P<0.05$; $^{A,B}P<0.001$

Table 4 demonstrates the results of an internal egg quality study and the lutein content of egg yolks. The eggs of the laying hens' control group had a higher egg white height and a higher egg yolk pH than the eggs of the E group, but the differences were not statistically significant ($P>0.05$). The higher Haugh units were found in the C group eggs ($P=0.039$), as was a more intense egg yolk color ($P<0.01$) in the eggs of the laying hens' E group. The difference in lutein content between the laying hens' C and E groups was also significant. A determined lutein content was 9.91 times higher in the egg yolks of the group E than in the egg yolks of the group C ($P<0.01$).

Table 4. Internal quality indicators and the lutein content in the fresh egg yolks

Tablica 4. Pokazatelji unutarnje kvalitete i sadržaj luteina u svježim jajima

Indicators *Pokazatelji*	C group (n=25) Mean±SE	E group (n=25) Mean±SE	P value *P vrijednost*
Egg white height, mm *Visina bjelanjka, mm*	6.46±1.04	6.18±0.81	0.287
HU	77.56±5.94^{a}	73.50±7.53^{b}	0.039
Egg yolk color *Boja* žumanjka	12.24±0.87^{B}	13.88±0.33^{A}	0.001
Egg white pH value *pH vrijednost bjelanjka*	8.49±0.21	8.54±0.27	0.508
Eqq yolk pH value *pH vrijednost žumanjka*	6.01±0.07	5.98±0.09	0.251
Lutein content, mg/100 g of yolk *Sadržaj luteina, mg/100 g žumanjka*	0.72±0.11^{B}	7.14±0.72^{A}	0.001

C group = without MFE; E group = added MFE 3 g/kg feed; $^{a,b}P<0.05$; $^{A,B}P<0.001$

Oliveira et al. (2017) used a MFE (1 g/kg food) and pepper extract (56 g/kg food) in the egg enrichment with lutein. The ingredients added did not affect the weight of the eggs, the Haugh units, or the amount of eggs produced. The pepper extract lowered the pH of the egg white. Marigold flower extract in the laying hens' feed influenced the reduction of proportion ($P<0.02$) and thickness ($P<0.01$) of the eggshell, which was also the case in our study. Kralik et al. (2017) determined a yolk color of 12.80 in the market eggs. Given that the authors measured the yolk color in the eggs stored for seven days at 4ºC, the values are slightly higher than the yolk color of our Control group (12.24). Yan et al. (2014) stated that the addition of low concentrations of lutein to the laying hens' feed (40 mg/kg) affected the yolk color ($P<0.05$). Lokaewmanee et al. (2011) found an increase in the CIE a* value ($P<0.05$) and a decrease in CIE L* value ($P>0.05$ in the assessment of the egg yolk color when lutein was added 10-40 g/kg food. Sirri et al. (2007) found that an increase in lutein of 80-100 mg/kg of the mixture only affects the increase of the a* value, and has no effect on the b* and L* values, which is in agreement with the results of Englmaierová et al. (2013), as well as with those by Grčević et al. (2018). Lokaewmanee et al. (2011) added the marigold flower meal lutein to the laying hens' mixtures and the MFE lutein in amounts of 10, 20, 30 and 40 mg/kg, and found, using the Roche scale, that the best pigmentation effect was achieved at 40 mg/kg MFM and 20, 30 and 40 mg/kg of MFE. They recommend the usage of 30-40 mg/kg of MFE because a saponified lutein is more effective in the coloring of egg yolks than the lutein from the MFM. The authors find that xanthophylls from the MFE, which are esterified with fatty acids, are more easily absorbed in the laying hens' digestive tract. The MFE lutein is transported more rapidly through the intestines and deposits faster in yolks than an unsaponified lutein (Galobart et al. 2004). Lokaewmanee et al. (2011) also agreed with this statement. Šivel et al. (2014) reported that there was a difference in the lutein content (mg 100/g DM) in the samples of marigold flower concentrates (56.8-272) and in the marigold flower powder (55-802.3), respectively, and therefore the different results were obtained in the egg enrichment with lutein. Our results are in agreement with those by Grčević et al. (2018), as well as with those by Lokaewmanee et al. (2011), circumstantiating that MFE can successfully enrich the eggs with lutein if added to the laying hens' diet.

Table 5 figures the TBARS values in egg yolks from the C and E groups ($P=0.014$) in the fresh eggs. The differences in the stored eggs between the C and E groups were not statistically significant (C=1.08 and E=0.94 μg MDA/g; $P=0.085$). When observing the lipid oxidation processes within the Group C, a statistically significant difference in the TBARS values was found between the fresh and the stored eggs (0.89 : 1.08 μg MDA/g; $P=0.026$). Although the value of TBARS in the E group in the stored eggs is lower (0.94 μg MDA/g) than the one in the fresh eggs (1.02 μg MDA/g), the difference is not statistically significant ($P=0.093$).

Table 5. TBARS values (μg MDA/g egg yolk)

Tablica 5. TBARS vrijednosti (μg MDA/g žumanjka)

Group/Analysis time *Skupina/Vrijeme analize*	C group (n=5) (mean±SE)	E group (n=5) (mean±SE)	P value *P vrijednost*
Fresh egg Svježa jaja	$0.89\pm0.03^{a,x}$	1.02 ± 0.10^{b}	0.014
Stored egg *Skladištena jaja*	1.08 ± 0.11^{y}	0.94±0.08	0.085
P value P *vrijednost*	0.026	0.093	-

C group = without MFE; E group = added MFE 3 g/kg of feed; $^{a,b,x,y}P<0.05$; ^{a,b} letters above numbers represent the difference between the values shown in the rows; ^{x,y} letters above numbers represent the difference between the values shown in the columns

Grčević et al. (2019), as well as Kralik et al. (2018), have reported that MFE has an effect on the oxidative stability of eggs during storage. Akter et al. (2014) found the TBARS values similar to our results subsequent to the 28 days of egg storage. Englmaierová et al. (2013) also state that lutein enhances an oxidative stability of eggs. The authors added 250 mg/kg of lutein to the mixtures and found 1.17 and 0.87 mg of MDA/kg in the fresh eggs from the control and experimental groups, respectively, and 1.28 and 1.04 mg of MDA/kg of egg yolk after 28 days of storage.

CONCLUSION

Based on our study results, it can be concluded that the addition of MFE 3 g/kg to the laying hens' feed significantly affects the increase in lutein content in the egg yolks (7.14±0.12 mg/100 g) when compared to the Control group (0.72±0.11 mg/100 g), which is an increase of 9.91 times ($P<0.01$). The added MFE in the mixture increases the egg white content of the egg and intensifies the yolk color, but it decreases the quality of shell. A statistically significant difference in the TBARS values was found between the fresh and the stored eggs (0.89: 1.08 μg MDA/g; $P=0.026$) in the C group and between the C and E groups in the fresh eggs (0.89 : 1.02 μg MDA/g; $P=0.014$). It also improves the oxidative stability of lipids in eggs during their storage. Using the MFE in the laying hens' feed, the eggs can be effectively enriched with lutein and declared a functional product.

ACKNOWLEDGEMENT

This study is supported by the European Structural and Investment Funds grant for the Croatian National Scientific Center of Excellence for Personalized Health Care (grant #KK.01.1.1.01.0010) and by Ministry of Science and Education of the Republic of Croatia.

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OBOGAĆIVANJE KONZUMNIH JAJA LUTEINOM

SAŽETAK

U svrhu obogaćivanja jaja luteinom, upotrijebljen je Marigold Flower Extract – MFE (ekstrakt cvijeta kadife – Tagetes erecta L.). U istraživanju su korištene dvije skupine nesilica – kontrolna (C) i pokusna (E). Kontrolna skupina nesilica hranjena je smjesom bez dodatka luteina, dok su nesilice E skupine konzumirale smjesu s 3 g/kg luteina. Hranidba nesilica trajala je 31 dan, nakon čega je obavljena analiza kvalitete jaja (indeks oblika, masa jaja i osnovnih dijelova u jajetu, čvrstoća i debljina ljuske, Haugh jedinice – HU, visina bjelanjka, boja žumanjka, pH bjelanjka i pH žumanjka), te je utvrđen sadržaj luteina i TBARS (Thiobarbituric Acid Reactive Substances) vrijednosti u žumanjcima. Rezultati istraživanja pokazali su da dodani MFE utječe na smanjenje debljine, težine i udjela ljuske (P<0,001), odnosno na smanjenje čvrstoće ljuske (P=0,014). Također, on utječe na smanjenje vrijednosti HU (P=0,039), a povećava udjele bjelanjka, boju žumanjka i sadržaj luteina u žumanjcima jaja (P<0,001). Statistički značajna razlika u vrijednostima TBARS-a utvrđena je između svježih i čuvanih jaja kod C skupine, kao i između C i E skupina kod svježih jaja (P<0,05). Naši rezultati ukazuju da je MFE pogodan za obogaćivanje konzumnih jaja luteinom.

Ključne riječi: ekstrakt cvijeta kadife, lutein, boja žumanjka, kvaliteta jaja, TBARS vrijednosti

(Received on January 21, 2020; accepted on May 13, 2020 - *Primljeno 21. siječnja 2020.; prihvaćeno 13. svibnja 2020.)*