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Original Article

Quality of Eggs from Layers Reared under Alternative and Conventional System

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ABSTRACT

The study aimed to compare and assess some of the quality characteristics (morphology, colour of the yolk, total carotenoid content and fatty acid profile) of eggs obtained from hens reared conventionally or in alternative system having access to pasture. Twenty eggs of each rearing system were analysed for the above characteristics. The eggs obtained from the layer reared under the alternative system were significantly heavier than the conventional ones, displayed higher diameters of the albumen and albumen index, as well as increased diameter and weight of the yolk. On the other hand, their shell thickness and Haugh units were lower when compared to the eggs from conventionally reared hens. The colour of the eggs from the alternative system was darker and they had lower values of L* and higher of a* in the yolk, corresponding to the significantly higher content of the total carotenoids in comparison to the eggs obtained from the hens reared conventionally. Furthermore, the eggs from the hens having access to pasture showed clear advantage in the fatty acid profile and the related lipid nutritional indices. In general, the rearing in alternative system reduced considerably the content of C14:0, C16:0, C18:0, C16:1n-7 and C18:1n-9, as well as the saturated (SFA) and monounsaturated fatty acids (MUFA). On the other hand, with the exception of C20:3n-6, the contents of both individual and total polyunsaturated fatty acids (PUFA) were dramatically increased in the eggs from the hens reared in the alternative system. This led to favourably decreased values of n-6/n-3 ratio as well as values of both atherogenic (AI) and thrombogenic (TI) indices but augmented ratio between hypo- and hypercholesterolemic fatty acids.

INTRODUCTION

The 1968 the American Heart Association recommended decrease in the dietary daily intake of cholesterol (not more than 300 mg/d) for all the individuals and not more than 3 whole eggs per week, thus significantly impacting the diet of the population and drastically limiting a highly nutritious and affordable source of high quality nutrients, including choline (McNamara, 2015). However, recent studies have shown that moderate egg consumption does not increase the risk of heart disease for healthy adults, but even supplies valuable nutrients, such as iron, vitamin A, D, E, B_{12}, folate, selenium, lutein and zeaxanthin, as well as fatty acids, that are important for the human health.

The composition and quality characteristics of the eggs might be affected by various factors such as hen genotype, diet composition (Hammershøj & Steenfeldt, 2015), rearing systems. In recent years, the consumers' awareness towards healthier diet, has drawn the attention towards alternative rearing systems such as those offering access to pasture to the birds. Such kind of systems affect favourably the welfare of the birds as well as improve substantially the quality of the products (Sossidou *et al.,* 2015).

Since the alternative rearing systems are important in terms of both variation and animal welfare in egg production, it is required to study and put forward the effects of these production systems on productivity and quality of eggs in detail. Although various studies exist comparing the effect of the conventional and alternative systems on the quality of poultry products and in particular on the quality of the eggs, still the data is not highly conclusive.

Therefore, the aim of this work was to compare eggs obtained from hens reared conventionally and in alternative system with pasture access in regard to their morphological characteristics, colour, carotenoid content and fatty acid profile.

MATERIAL AND METHODS

Experimental birds and rearing systems

For the aim of the research a total of 350 Lohmann-Brown Classic layer hens at the age of 34 weeks were used. The experiment was designed as two trials carried out simultaneously in March–April 2019 at the experimental poultry farm of the Institute of Animal Science-Kostinbrod, Bulgaria (conventional system with 150 layers) and the symbiotic Livadi farm, situated in Damyanitsa village, Sandanski region, Bulgaria (alternative system with access to pasture with 200 layers).

Alternative system with pasture access

After hatching, the Lohman-Brown classic chickens were housed in conventional system until 18 weeks of age in the Institute of Animal Science – Kostinbrod. At the age of 19 weeks the pullets (n=200) were transferred to Livadi farm and reared according to the method of Salatin (2010) in alternative system with pasture access. This system uses mobile facilities of semi-open type surrounded by electric fence thus forming a paddock that are moved when the territory is grazed up. Each facility was equipped with feeders, drinkers, nests and roosts, as well as a paddock (800 m^2) ensuring minimum density of 4 m^2/ bird).

The rotation of the paddocks was done after at least 70% of the pasture is grazed. The grass in the pasture consisted mainly of alfalfa. Since the mobile facility is of semi-open type, the layers had full access to pasture. Additionally, the layers received concentrate feed prepared in the farm. That was based on wheat, corn and sunflower meal (ME 2876 kcal/kg; CP 21.19%) The trial was carried out at the time of the year when the natural climatic conditions in the region and the daytime coincide with the standards of the conventional rearing (14 h light and 10 h dark at average daily temperature of 24 ºC).

Conventional system

The layers were reared in the experimental poultry farm of the Institute of Animal Science – Kostinbrod. A total of 150 layers were kept free in a deep litter box with an area of 22 m^2, with a density of the birds 7 birds/m^2. The box was equipped with feeders, drinkers and roosts. The lighting regime was 14 h light period and 10h dark. The feed, provided from commercial manufacturer and water were provided *ad libitum.*

The feed for the conventional group was based on wheat, corn and sunflower meal (ME 2755 kcal/kg; CP-16.60%).

Both feeds contained no colorants. The fatty acid composition of the feeds and pasture was analysed in the Laboratory of Lipid Analysis in the Institute of Animal Science-Kostinbrod. Fatty acid composition of the feeds and pasture are presented in Table 1.

Table 1 – Fatty acid composition (%of the total FAME) of the feeds of both systems and pasture.

Item	Alternative system	Conventional system	Pasture
C14:0	0.10	0.10	0.41
C16:0	12.63	12.55	24.46
C16:1n-7	0.15	0.23	0.99
C18:0	3.27	2.62	7.09
C18:1n-9	29.87	29.77	29.52
C18:2n-6	53.07	53.00	16.42
C18:3n-3	0.91	1.73	21.11

Morphological analysis

For the analysis of the morphology of the eggs, at the end of 34 weeks of age of the birds from both trials, 20 eggs per group were collected. A total of 40 eggs were weighed and analyzed by the Haugh method (1939). The eggs collected in same day were broken carefully, separating the contents from the shell, and the albumen height was obtained by EGG-Tester. The same equipment also detected the Haugh unit and yolk height which were described as internal egg quality parameters. The egg shell parameters (thickness and weight) were measured through electronic micrometer measurement equipment. The albumen weight and egg index (derived from the egg height and egg diameter) was calculated by the formula as described by Roush (1981).

Colour measurements

The colour of the yolk was measured by the EGG -tester according to the DSM (formerly Roche) yolk colour fan ranging 1-15. Furthermore, the egg yolk colour was determined instrumentally by Colorimeter PCE-CSM 4 (PCE Instruments) in the CIE L* a* b* space, with L* value indicating the lightness, representing dark to light (0 – 100), a* redness and b* yellowness. Samples were in a 7-cm diameter glass cell with an optically clear bottom.

Total carotenoids

The amount of total carotenoids was determined as described by Steinberg *et al.* (2000). Approximately 10 g of homogenized yolk were mixed with 10 ml of distilled water using Utraturrax. Then 1g of the diluted yolk was combined with 1 ml distilled water and 2 ml ethanol in a test tube, and stirred on Vortex. 4 ml n-hexane were added, the test tube was sealed with a glass stopper and shaken for 5 minutes on a horizontal shaker. The mixture was centrifuged for 5 minutes at 2500 r/min. The supernatant was then transferred into 1ml glass cell and measured against n-hexane with spectrophotometer at 450 nm. The content of the total carotenoids was calculated according to the following equation:

$$\text{Total carotenoid content } (\mu g/g) = \frac{A \times V(ml) \times 10^4}{A_{1cm}^{1\%} \times P(g)}$$

where A = Absorbance; V = Total extract volume; P = sample weight; A1cm1%=2592 (β-carotene Extinction Coefficient in hexane).

Fatty acid analysis

The fatty acid profile of the feed, pasture and yolk was determined by gas chromatography analysis after lipid extraction according to Bligh & Dyer (1959). Methyl esters of the total lipids, isolated by preparative thin layer chromatography, were obtained using 0.01 % solution of sulfuric acid in dry methanol for 14 h, as described by Christie (1973). The fatty acid composition of total lipids was determined by gas-liquid chromatography (GLC) analysis using a chromatograph C Si 200 equipped with a capillary column (DM - 2330:30 m×0.25 mm×0.20 μm) and hydrogen as a carrier gas. The oven temperature was first set to 160°C for 0.2 min, then raised until 220°C at a rate of 5°C/min and then held for 5 min. The temperatures of the detector and injector were 230°C. Methyl esters were identified through comparison to the retention times of the standards. Fatty acids are presented as percentages of the total amount of the methyl esters (FAME) identified (Christie, 1973). The amount of each fatty acid was used to calculate the indices of atherogenicity (AI) and thrombogenicity (TI), as proposed by Ulbricht & Southgate (1991):

$$AI=(4\times C14:0+C16:0)/[MUFA+\Sigma(\omega\text{-}6)+\Sigma(\omega\text{-}3)]$$

$$TI=(C14:0+C16:0+C18:0)/[0.5\times MUFA+0.5\times(\omega\text{-}6)+3\times(\omega\text{-}3)+(\omega\text{-}3)/(\omega\text{-}6)]$$

The h/H ratio was calculated, as suggested by Santos-Silva et al. (2002):

$$h/H=(C18:1+C18:2\omega\text{-}6+C20:4\omega\text{-}6+C18:3\omega\text{-}3+C20:5\omega\text{-}3+C22:5\omega\text{-}3+C22:6\omega\text{-}3)/(C14:0+C16:0)$$

Statistical analysis

Data were statistically evaluated applying General Linear Model (GLM) procedure in SPSS statistical software. Differences between the means were evaluated through t-test and considered significant at $p<0.05$. The results are presented as mean and standard error of the mean (SEM).

RESULTS

Morphology of the eggs

As presented in Table 2, the eggs obtained from the layers, reared in alternative system had higher weight when compared to the conventional ones ($p<0.05$). However, in regard to the shape of the eggs, both groups did not differ significantly. On the other hand, the dimensions and the derived index for the albumen were significantly affected by the rearing system, displaying higher values of the diameters and the index in the eggs from the pastured hens. Significant difference in the Haugh units ($p<0.01$) was observed showing slight disadvantage of the eggs from the alternative system, however the yolk of the pastured eggs had significantly higher diameter ($p<0.01$) and weight ($p<0.001$), while their shell thickness was lower ($p<0.05$).

Colour of the yolk and total carotenoid contents

The colour parameters of the yolk presented on Figure 1 show that the visual colour of the yolk was darker for the eggs from the alternative system (9.33 *vs*. 8.07). L * values observed in the eggs from pastured hens were significantly lower ($p<0.001$) when compared to the eggs from the conventionally reared hens. The values of this parameter were recorded 57.23 vs. 63.68, respectively for the alternative and regular eggs

Table 2 – Morphological traits of the eggs obtained from layers reared in alterative and conventional system.

Item	Production systems				Probability
	Alternative		Conventional		
	Mean	SEM	Mean	SEM	
Egg weight, g	61.11	1.42	63.75	0.57	0.0104
Egg shape					
d_1, mm[1]	58.07	0.70	56.67	0.33	0.0841
d_2, mm	45.26	0.37	44.67	0.27	0.2019
Index	78.06	0.84	78.87	0.73	0.4779
Albumen					
d_1, mm	87	2.34	79.20	1.31	0.0072
d_2, mm	75	1.64	68.13	1.08	0.0016
h, mm	6.3	0.34	7.51	0.18	0.0041
Index	79.14	5.34	102.51	3.36	0.0009
Weight, g	43.50	1.27	41.33	0.58	0.133
Yolk					
d, mm	37.06	0.36	35.13	0.36	0.0007
Weight, g	17.63	0.36	15.60	0.24	<0.0001
Shell					
Thickness, mm	0.41	0.006	0.43	0.01	0.0299
Weight, g	6.98	0.20	6.82	0.13	0.4881
Haugh units	75.11	2.93	85.59	1.06	0.0023

[1]d1- long diameters of the egg and albumen; d2- short diameters of the egg and albumen.

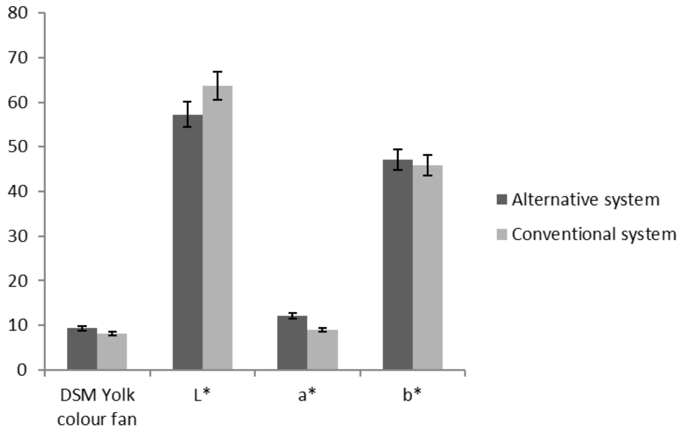

Figure 1 – Colour of the yolk in the eggs obtained from pastured and conventionally reared layers.

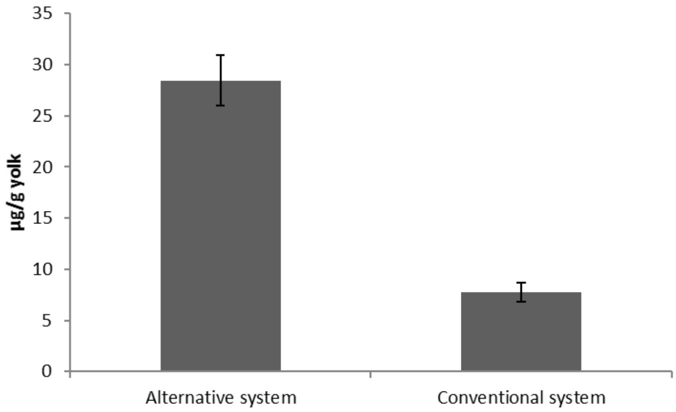

Figure 2 – Total carotenoid content of the yolk in the eggs obtained from pastured and conventionally reared layers.

meaning that the former had considerably darker yolk than the conventional ones. On the other hand, the a* values that we measured in the yolk were within the range of 9.03-12.10, respectively for the conventional and the eggs obtained from the hens having access to pasture. This corresponded to the lower L* observed in the pastured eggs. No significant difference was observed in regard to b* values presenting the yellowness. In line with the observed changes in the colour of the yolk, the content of the total carotenoids (Figure 2) differed significantly between both groups of eggs. The values recorded for the eggs from the alternatively reared are 4 times higher than the ones in the conventional eggs (7.72 *vs*. 28.45 μg/g).

Fatty acid composition and lipid nutritional indices

The fatty acid composition of the yolk was strongly influenced by the rearing of the layers. Significant differences were found in both individual fatty acids and their total amounts (Table 3). The eggs from the layers reared in alternative system with pasture access displayed significantly lower contents of C14:0 ($p<0.05$), C16:0 ($p<0.001$) and C18:0 ($p<0.001$), as well as significantly lower levels of C16:1n-7 and C18:1n-9 ($p<0.001$). On the other hand the eggs of the pastured hens had considerably higher contents of C18:2n-6 and C18:3n-3 ($p<0.001$), as well as C20:4n-6 ($p<0.05$).

Table 3 – Total lipid content, fatty acid profile (% of the FAME) and lipid nutritional indices of the eggs obtained from layers reared in alterative and conventional system.

Item	Production systems				Probability
	Alternative		Conventional		
	Mean	SEM	Mean	SEM	
Total lipids, %	29.35	0.63	28.65	0.74	0.474
Fatty acids, %					
C14:0	0.28	0.01	0.33	0.01	0.0165
C16:0	23.30	0.20	27.10	0.32	<0.0001
C16:1n-7	2.18	0.17	3.39	0.22	0.0001
C18:0	7.86	0.38	8.83	0.13	0.0199
C18:1n-9	40.53	0.74	43.51	0.36	0.0009
C18:2n-6	21.34	0.70	13.74	0.31	<0.0001
C18:3n-3	0.68	0.06	0.23	0.01	<0.0001
C20:2n-6	0.23	0.01	0.14	0.01	<0.0001
C20:3n-6	0.17	0.01	0.19	0.004	0.0825
C20:4n-6	2.44	0.13	2.09	0.06	0.0191
C22:5n-3	0.12	0.01	0.04	0.007	<0.0001
C22:6n-3	0.87	0.04	0.41	0.02	<0.0001
SFA[1]	31.44	0.38	36.26	0.29	<0.0001
MUFA	42.71	0.80	46.90	0.32	<0.0001
PUFA	25.87	0.76	16.84	0.35	<0.0001
Lipid nutritional indices					
n-6/n-3	14.48	0.92	23.76	0.95	<0.0001
P/S	0.82	0.02	0.46	0.03	<0.0001
AI	0.35	0.01	0.45	0,004	<0.0001
TI	0.82	0.01	1.08	0.01	<0.0001
h/H	2.80	0.04	2.20	0.04	<0.0001

SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polyunsaturated fatty acids; P/S- polyunsaturetaed/saturated fatty acids; AI- atherigenic index; TI- thrombogenic index; h/H- hypo/hypercholesterolemic fatty acids.

The contents of the long chain polyunsaturated n-3 fatty acids – C22:5n-3 and C22:6n-3 were considerably higher in the eggs form the hens reared in alternative system. The changes in the individual fatty acids, induced by the rearing in alternative system with access to pasture or the conventional rearing were reflected in their total amounts and the lipid nutritional indices. Generally, the eggs from the hens having access to pasture, had lower total mounts of SFA ($p<0.001$) and MUFA ($p<0.001$) but higher of PUFA ($p<0.001$) when compared to the conventional ones. The considerably augmented content of n-3 PUFA led to lower n-6/n-3 ratio in the eggs ($p<0.001$) of the pastured hens, which displayed also lower values of the atherogenic and thrombogenic indices ($p<0.001$) and higher values of the ratio between hypo- and hypercholesterolemic fatty acids (h/H).

DISCUSSION

Morphology of the eggs

The comparisons between the conventional and alternative systems in regard to their effect on the morphological traits of the eggs have not been consistent in literature. Our results showed that the eggs of the layers reared in alternative system and having access to pasture were heavier that the ones obtained from the conventionally reared, corresponding to the higher value recorded for the weight of the yolk in the pastured eggs. Contrary to us, Krawczyk (2009) did not find significant difference in these parameters when comparing eggs from intensively reared layers and layers reared under organic conditions or backyard with free access to grass, however the latter showed lower weight of the egg and yolk when compared to the organic eggs. Furthermore, Krawczyk & Gornowicz (2010) observed lower weight of yolk and eggs from hens that were reared with access to vegetation in comparison to eggs from intensively reared layers but only when the hens were at the age of 32-36 weeks. This was confirmed also by Turker & Alkan (2017) who did not find difference in the average weight of the eggs form hens reared in deep litter and with access to pasture.

On the other hand, our results are in line with those of Kralik *et al.* (2013) and Chen *et al.* (2018) who found that the weight of the eggs produced from free range layers was significantly higher that the weight of the eggs from layers raised in cages, mainly due to the increased weight of the yolk. Also, Dalle Zotte *et al.* (2013) observed significantly higher weight of the egg but lower shell thickness in organic vs conventionally reared layers which they attributed to the later age of the hens. As in a previous study (Petkov *et al.,* in press), the eggs shape index did not differ between the conventional and alternative systems, indicating no negative effect of the production system on this trait, however, the Haugh units were lower in the eggs of the layers that had access to pasture. Our results are in agreement with Sekeroğlu & Sarica (2005) and Samiullah *et al.* (2014) who reported lower Haugh units in the eggs produced from free range layers when compared to layers reared litter or in commercial cages and are contrary to Yilmaz Dikmen *et al.* (2017). The latter stated the Haugh units in the eggs of free range layers are increased in comparison to the layers reared in conventional and enriched cages. The lower Haugh units of the eggs produced by the pastured layers observed in the study might be due to the the high temperatures outdoors and the environmental conditions that could not be strictly controlled as in the conventional trial. Despite the differences in the Haugh units that we observed, the egg albumen was classified predominantly as firm (AA) in both conventional and alternative system.

Colour and carotenoids

Yolk colour is important commercial characteristic. Our study indicates more intensive yolk colour in the eggs of the hens reared in alternative system when compared to the conventional ones when measured on a scale 1-15 (9.33 *vs*. 8.07), which we attribute to the access to green vegetation that is rich in carotenoids. Various results about the effect of the rearing systems on the yolk colour have been reported in literature. Similar to us, darker yolk have been reported by Sokolovicz *et al.* (2018 a, b) when comparing free range and organic eggs to eggs obtained from layers reared in litter. However earlier, Krawczyk & Gornowicz (2010) observed paler colour of the yolks in free –range layers at two ages having access to vegetation. Also, Rossi (2007) found most intensive yolk colour in the eggs of caged layers, and significantly lower values for eggs from organic and free-range hens, attributing this to the feed colouring addtives used in the conventional system. On the other hand, Yilmaz Dikmen *et al.* (2017) reported lack of influence of the rearing system on the colour of the yolk. The darker visual colour of the yolk that we registered in this study corresponded to the lower values of the L*parameter, presenting the lightness, and the higher values of the a* presenting the redness of the yolk. Similarly, Gałązka-Czarnecka *et al.* (2019) reported higher a* values in the eggs of free ranged hens that those of the hens reared in cages, however they did not observe any difference in the L* values between the two housing systems.

The contents of the carotenoids that we determined to be considerably higher in the yolk of the layers reared with access to pasture are in line with the results of other studies. Mugnai *et al.* (2009, 2014) also reported significant increase in the carotenoid content in the eggs of layers when they were reared organically with increased area of pasture. Furthermore, Skřivan & Englmaierová (2014) and Gałązka-Czarnecka *et al.* (2019) reported significantly higher content of carotenoids in grazing and free ranged hens respectively. Karsten *et al. (*2010) reported significant increase in vitamin A content in the yolk of the eggs from hens grazing various pastures including alfalfa, grass and clover in comparison to caged hens, while Sokołowicz *et al.* (2018) a reported increase in this compound in the egg of free ranged native Polish hens, depending on the breed. Our results are in line with Karadas *et al.* (2006) who presented considerable augmentation of the carotenoids in egg yolk when the hens were fed concentrate of lucerne.

Fatty acid composition

In this study we determined a total of 12 fatty acids in the yolk. The major saturated fatty acids are C16:0 followed by C18:0, while the monounsaturated and polyunsaturated fatty acid that are most abundant are C18:1n-9 and C18:2n-6. With the exception of C20:3n-6 all the individual as well as total amounts of the fatty acids were affected by the rearing system of hens. Generally, rearing in the alternative system with access to pasture affected positively the fatty acid profile of the yolk. The saturated fatty acids were considerably decreased in the yolk of the eggs from the pastured hens which as associated with the decrease of C14:0 and C16:0, as well as C18:0. The former two fatty acids are associated with increased hypecholesterolemia and hence their decrease is favourable for the dietetic qualities of the yolk.

Decreased total SFA content have been also reported by Mugnai *et al.* (2014), however they did not show effect of the rearing system in neither of the individual saturated fatty acids. On the other hand, the content of the MUFA was also decreased in the pastured eggs. This contradicts to the results of Mugnai *et al.* (2014) and Sokołowicz *et al.* (2018a) who showed increase in the total MUFA however, our results are in line with Karsten *et al.* (2010) who reported significant decrease in C18:1n-9 in the pastured hens compared to the caged. The PUFA was also significantly affected by the rearing system, leading to higher content in the yolk of the eggs from the alternative system. Significant increase was observed in the n-3 as well as the n-6 individual fatty acids, however the n-6/n-3 ratio was favourably decreased in the eggs form the grazing layers. Its values were 15.35 vs. 24.21, respectively for the eggs from the pastured and conventionally reared hens. Despite the decrease, the ratio remains above the value of 4 that is recommended for a healthy diet. Increase in the PUFA, especially in n-3 is due to the pasture, which contains higher amounts C18:3n-3 that is a precursor for the synthesis of the other long chain n-3 PUFA. Increased content of C18:3 n-3 and C22:6 n-3 was reported by Mugnai *et al.* (2014), however the authors showed significant decrease in C18:2n-6 and C20:4n-6. In our study the increase of these fatty acids might be attributed to the additional supplementation of the hens with feed which has high content of C18:2n-6, as seen from Table 2.

Karsten *et al.* (2010) showed considerable increase in C18:3n-3 and the total n-3 PUFA, while no effect on the n-6 PUFA. Contrary to us, Küçükyılmaz *et al.* (2012) reported augmented content of n-3 PUFA in the eggs of conventionally reared hens compared to organically reared ones, and subsequently lower values of n-6/n-3 in the conventional eggs. The changes in the fatty acid composition lead to favourable decrease in the values of the AI and TI. AI varied into the range of 0.35-0.44 while TI was within the range of 0.82-1.08, respectively for the eggs of the grazing and conventionally reared layers. Similar values were reported by Mugnai *et al.* (2014) who also indicated favourable decrease in these parameters in the yolk of eggs from organically reared hens. On the other hand, these authors did not report any significant effect of the organic system with pasture access on the h/H ratio in the eggs, which is not in agreement with our results. In this study we observed significant decrease in the values of this ratio in the eggs of the pastured hens, which corresponded to the decrease in the saturated fatty acids, namely C14:0 and C16:0.

To conclude, the eggs obtained from conventionally reared hens and hens reared in alternative system having access to pasture differed significantly in regard to their quality characteristics. The latter had higher weight corresponding to the higher diameter and weight of the yolk, as well as higher diameters and index of the albumen. However, they had thinner shell and lower Haugh units. The eggs obtained from the layers reared in alternative system had darker yolk, higher content of carotenoids and showed advantage of the fatty acid profile and the associated lipid nutritional indices. Thus, they might be considered healthier than the conventional eggs.

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