Croat. J. Food Sci. Technol. (2017) 9 (2) 158 - 167

The influence of hen age on fatty acid composition of commercial eggs

Tina Lešić[1], Greta Krešić[2], L. Cvetnić[3], M. Petrović[4], Jelka Pleadin[1*]

[1]*Croatian Veterinary Institute, Laboratory for Analytical Chemistry, Savska Cesta 143, 10000 Zagreb, Croatia*
[2]*Faculty of Tourism and Hospitality Management, University of Rijeka, Department of Food and Nutrition, Primorska 42, 51410 Opatija, Croatia*
[3]*Croatian Veterinary Institute, Laboratory for Mastitis and Raw Milk Quality, Savska Cesta 143, 10000 Zagreb, Croatia*
[4]*Andrija Štampar Teaching Institute of Public Health, Department of Environmental Protection and Health Ecology, Mirogojska Cesta 16, 10000 Zagreb, Croatia*

original scientific paper
DOI: 10.17508/CJFST.2017.9.2.12

Summary

The aim of this study was to investigate the nutritional composition of commercial eggs from Lohman Brown hens through fat and fatty acid content analysis, as well as to evaluate the effect of hen age on the above parameters. Egg samples (n=108) were collected every two weeks from 21- to 55- week old hens during the 2015/2016 autumn/winter period. The results revealed significant differences in fatty acid composition dependent on hen age ($p < 0.05$). When comparing eggs from the youngest, against those from the oldest hens, total saturated fatty acids (SFA) were found to be statistically significantly higher in the youngest hens ($p < 0.05$), whereas individual SFA shares did not statistically significantly differ between the two ($p > 0.05$). The total polyunsaturated fatty acid (PUFA) content was statistically significantly higher in eggs laid by 55- week old hens as compared to those laid by 21- week old hens. The n-6/n-3 and PUFA/SFA ratios were more favourable in the elder hens. In general, the results revealed hen ageing-based variations in fatty acid composition of their eggs, in particular in the representation of linoleic (LA), alpha-linolenic (ALA) and arachidonic acid (AA), for which statistically significant hen age-based differences were found.

Keywords: diet, eggs, lipids, fatty acid composition, hen age

Introduction

Due to the presence of essential amino acids as one of their constituents, eggs are a valuable source of high-value proteins, as well as fats, minerals, vitamins (A, D, E, K and B-complex), and macro- and microelements (Kralik and Kralik, 2017). Since eggs are one of the main dietary lipid sources, lipid composition of eggs has become an area of current scientific interest (Campos et al., 2016). The egg yolk consists of 30% of lipids whose composition varies depending on the hen strain and age, as well as egg weight (Roberts, 2004; Sahan et al., 2014). A strong negative publicity advertising harmful effects of cholesterol and its role in the development of cardiovascular diseases has led to the decrease in the egg consumption (Simopoulos, 2000). Until the year 2010, data gathered in Croatia had shown an increasing egg consumption trend, but ever since then, a slight decrease in the latter consumption has been witnessed. According to the latest data, the average consumption of eggs in Croatia equals to 152 pieces per capita (Statistical Yearbook, 2016). However, the recent research has proven cholesterol intake not to be the only cause of high blood cholesterol (Farrell, 1998; Nakamura et al., 2004; Petrović et al., 2012). Namely, an unfavourable ratio of n-6/n-3 polyunsaturated fatty acids, saturated fatty acids and trans-fatty acids is to be blamed for an increased blood cholesterol as well (Nakamura et al., 2004). In their 2003 study Milinsk et al. stated that the nutritional quality of lipids present in eggs should be evaluated in such a manner that not only the cholesterol level, but also the fatty acid composition should be taken into account.

One of the main roles of fatty acids in the body is to constitute cell membranes. The structure of fatty acids influences the penetration of nutrients into a cell, but also regulates biochemical and physiological processes in the organism. It has been proven that membranes containing more n-3 fatty acids are more permeable, the latter fact being of significance in the prevention of diseases, such as cardiovascular diseases, cancer, obesity, inflammatory diseases and diabetes (Gogus and Smith, 2010). The majority of health benefits are related to n-3 fatty acids, in particular to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), rather than alpha-linolenic acid (ALA). It has been suggested that the principal biological role of ALA is to serve as a substrate for the synthesis of

[*]Corresponding author: pleadin@veinst.hr

long- chain n-3 EPA and DHA (Trautwein, 2001; Fraeye et al., 2012).

About 60-70 % of fatty acids present in eggs are unsaturated, which goes in favour of the nutritional value of eggs. The ratio of polyunsaturated n-6/n-3 fatty acids found in eggs is 12:1, or even higher (Petrović et al., 2012), i.e. definitely higher than recommended by the EFSA (3-5:1; EFSA 2009). It has been shown that hens are able to deposit dietary lipids into the egg yolk, so that it is possible to modify the fatty acid composition of an egg by modifying diet. Given the beneficial effects of n-3 polyunsaturated fatty acids (PUFA) on human health, by virtue of diet modification, hens have been directed to produce eggs with a higher n-3 PUFA and a lower saturated fatty acid (SFA) content (Ajuyah et al., 2003; Woods and Fearon, 2009; Petrović et al., 2012; Fraeye et al., 2012; Imran et al., 2015).

Egg quality depends on numerous factors, such as hen breed, gender, and age, and technical factors, such as the raising environment, housing system, and diet (Nielsen, 1998; Millet et al., 2006; Pavlovski et al., 2011; Campos et al., 2016). Bearing in mind that the production of high-quality eggs is affected by different factors, the aim of this study was to investigate the nutritional value of commercial eggs from Lohman Brown hens through fat and fatty acid content analysis and to evaluate the effect of hen age on the aforementioned parameters.

Materials and methods

Eggs production

The research was done on a family farm located in the central Croatia (Turopolje) and comprised a total of 500 laying hens. Lohmann Brown hybrid hens were housed in cages and fed on 150 g of complete laying hens-appropriate feed, containing maize, soybean meal, sunflower meal, alfalfa, wheat bream, bran, calcium carbonate, fosfonal, salt and 0.5% of the premix. Laying hens were housed in cages when 18 weeks old and started laying eggs when 19 weeks old. The study commenced at the week 21, when the laying rate reached 90% and lasted until the hens turned 55 weeks.

Sampling and sample preparation

Egg samples were collected every two weeks during the 2015/2016 autumn/winter season, the total number of sampling sessions finally amounting to 18. During each sampling session, a total of six eggs was sampled (total n=108).

The preparation of samples for the analysis included the refracting of eggshells and the homogenization of eggs. From six eggs collected during each sampling session, three homogeneous samples (n=3) were made using a laboratory homogenizer (Grindomix GM 200, Retsch, Haam, Germany). Immediately after sample preparation, samples were analysed for their total fat content. The extracted fat was stored in a refrigerator at -4 °C (for 72 h at the maximum) pending fatty acid composition analysis.

Lipid analysis

The total fat content was determined using the Soxhlet method (HRN ISO 1443:1999), which involves the digestion of samples by virtue of acid hydrolysis followed by the extraction of fats using petrol ether and a Soxtherm 2000 automated device (Gerhardt, Konigswinter, Germany). The results were expressed as the percentage (%) of weight, with the accuracy of 0.01%.

Fatty acid methyl esters were prepared from the extracted fat according to the ISO 12966-2:2011, which includes dissolution of glycerides in isooctane and trans-esterification using methanolic potassium hydroxide solution.

Fatty acid methyl esters were then analysed using gas chromatography (GC) according to the ISO 12966-4:2015; the analysis made use of a 7890B gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a flame ionization detector (FID) and a DB-23 capillary column measuring 60 m in length and having the internal capillary diameter of 0.25 mm and a 0.25 µm- thick stationary phase. At the split/splitless injector temperature was 270 °C, the split ratio was 1:50; at that point, 1 µL of the prepared methyl ester solution was injected. The carrier gas was helium, flowing at the constant linear velocity of 43 cm/s. The initial column temperature of 130 °C was gradually increased by 6.5 °C/min until the temperature of 170 °C was reached. The temperature was then increased at the rate of 2.75 °C/min until the temperature of 215 °C was attained; the latter temperature was maintained for 12 min and then increased again by 40 °C/min until the final column temperature of 230 °C was achieved, the latter temperature being maintained for 3 min. The components were detected by a FID at the temperature of 280 °C, hydrogen thereby flowing at the rate of 40 mL/min, the air flowing at the rate of 450 mL/min, and nitrogen, serving as the make-up gas, flowing at the rate of 25 mL/min. Fatty acid methyl esters were identified by virtue of comparison with the retention time of fatty acid methyl esters present in the standard Supelco™ 37 Component FAME Mix mixture

(Bellefonte, Pennsylvania, SAD) analysed under the same conditions. The results were expressed as the percentage (%) of total fatty acids, with the accuracy of 0.01%.

Basic chemical and mineral analysis of hen diet

The water content was determined using the gravimetric method (HRN ISO 6496:2001), which made use of an Epsa 2000 thermostat (BaRi, Velika Gorica, Croatia). The total protein content was determined by virtue of the Kjeldahl method (HRN ISO 5983-1:2008; HRN ISO 5983-2:2010) using a Digestion Unit 8 - Basic (Foss, Höganäs, Sweden), and a Kjeltec 8400 automated distillation & titration device (Foss, Höganäs, Sweden). The ash was determined according to the HRN ISO 5984:2004, while the calcium content was determined using the titrimetric method (HRN ISO 6490-1:1999), both of the above techniques thereby utilising a Nobertherm LV9/11/P320 furnace (Lilienthal, Germany). The crude fibre content was determined according to the HRN ISO 6865:2001 using FibreBag systems (Gerhardt, Bonn, Germany). The phosphorus content was determined according to the HRN ISO 6491:2001; the technique made use of a DR6000 spectrophotometer (Hach Lange, Germany). For the determination of the sodium content, the in-house validated potentiometric method was implemented, making use of an Easy Na Analyser (Mettler Toledo, Germany). The results were expressed as the percentage (%) of weight, with the accuracy of 0.01%.

Analytical methods' quality control

Standard analytical methods commonly used for fat and fatty acid content analysis were validated based on the determination of trueness. The values obtained for fat and seven individual CRM fatty acids (T0149 FAPAS, York, England; BCR 163 Institute for Reference Materials and Measurements, Belgium) were compared to the criteria laid down by the manufacturer and the criteria of repeatability defined under the ISO standards observed.

Statistical analysis

Statistical analysis was performed using the SPSS Statistics Software 22.0 (SPSS Statistics, NY IBM, 2013). In order to determine the differences between the sample groups, the independent sample t-test, One-way ANOVA and the robust Brown-Forsythe test were used. The decisions on the statistical significance were made at the significance level of $p < 0.05$.

Table 1. Chemical and fatty acid composition of the diet the study hens were fed on

Parameters		Results[a]
Basic chemical composition (%)	Moisture	10.80
	Protein	17.51
	Fiber	4.59
	Ash	8.21
	Fat	4.90
Mineral composition (%)	Ca	2.32
	P	0.54
	Na	0.17
Fatty acid composition (%)	C14:0	0.10
	C16:0	10.28
	C17:0	0.07
	C18:0	2.93
	C20:0	0.35
	C22:0	0.39
	C24:0	0.28
	SFA	14.43
	C16:1	0.13
	C18:1n-9	29.15
	C18:1n-7	0.74
	C20:1n-9	0.25
	MUFA	30.28
	C18:2n-6	54.07
	C18:3n-3	1.23
	PUFA	55.30
	n-6/n-3 ratio	43.96

[a]The results pertaining to the basic chemical and mineral composition are expressed as the % of weight. The results pertaining to the fatty acid composition are expressed as the % of total fatty acid content

Results and discussion

Since the egg fatty acid profile depends on hen feed composition, chemical and fatty acid composition of the hen feed was determined (Table 1).

The feed in question is characterized by high linoleic fatty acid (C18:2n-6, LA) content (54.07%), as well as by high n-6/n-3 ratio (43.96). Earlier studies also showed that commercial poultry diets are generally characterized by high n-6/n-3 ratios due to the fact that the main FA component in these cereal-based diets is LA (Koppenol et al., 2014). The results obtained with the quality control of analytical methods employed within this study frame, revealed the latter methods to be suitable for the determination of fat and fatty acid content (Table 2).

The majority of egg lipids are harboured by the egg yolk, while the albumen contains lipids only in traces (Senčić and Samac, 2017). In this study, the fat content and fatty acid composition had been consecutively determined in eggs collected every two weeks, starting from the beginning of the stable laying period (which corresponds to 21 weeks of hen age) to the Week 55, i.e. the usual end of the laying period. The fat content (%), established in eggs of hens of different age, displayed per weeks of age, is shown in Fig. 1. The above content ranged from the minimal 6.1% (in 21-week old hens) to the maximum of 9.5% (in 45-week old hens). Statistical analysis showed a significant influence of hen age on the fat content of eggs ($p < 0.05$). When comparing the fat content of eggs originating from the youngest hens as opposed to the oldest ones, it can be seen that the oldest hens laid eggs having a fat content higher (8.8%) than that of eggs laid by the youngest hens (6.1%) ($p < 0.05$).

Table 2. The results of verification of suitability of the employed analytical methods for the determination of fat content and fatty acid composition

Parameter	Assigned value [a] (%)	Obtained value (%)
Total fat	2.12–2.87	2.44±0.07
C14:0	2.29±0.04	2.27±0.04
C16:0	25.96±0.30	26.49±0.31
C16:1	2.58±0.16	2.34±0.08
C18:0	18.29±0.17	19.22±0.21
C18:1n-9c	38.30±0.40	37.64±0.27
C18:2n-6c	7.05±0.17	7.12±0.09
C18:3n-3	0.86±0.14	0.75±0.09

[a]The CRM value assigned for fat is given in form of range, while that pertaining to the fatty acids is expressed as mean values ± standard deviation. The obtained values are given as mean values (n=6) ± standard deviation

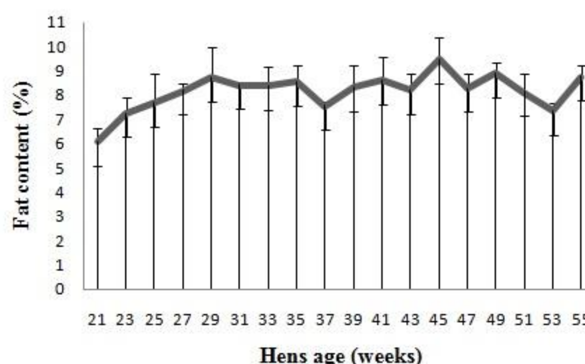

Fig. 1. Total fat content of eggs displayed based on hen age in weeks.
The results are expressed as mean values ± standard deviation.

A similar total fat content of eggs was determined in the research by Anderson (2011), in which the total fat content varied from roughly 6.2% to 9.2%. Cherian (2008) also showed a higher fat content in eggs from 42-week old hens as compared to that in eggs laid by hens of other age (26-, 34-, 50-, 58-, 62- week old). Yadgary et al. (2010) also reported a higher fat content in eggs from older hens as compared to eggs coming from younger hens (50 *vs.* 30 weeks of age). On the other hand, some researchers report no differences in the total fat content present in egg yolks from young *versus* old broiler breeder hens (28 *vs.* 58; 21 *vs.* 57 and 50 *vs.* 74 weeks of age) (Koppenol et al., 2014; Nielsen, 1998; Anderson, 2011). Higher fat content seen in eggs from older hens indicates that they deposit more yolk and fat in their eggs, which is associated with the changes in yolk size (Sahan et al., 2014). Changes in the total lipid content of the egg yolk achieved through different feeding practices have generally been reported as limited (Imran et al., 2015; Milinsk et al., 2003; Cobos et al., 1995).

The results of the fatty acid (FA) analysis obtained in this study were compared to the results of the analysis of standard mixture of fatty acid methyl esters containing 37 components, where 16 fatty acids were identified. Individual FAs determined by the analysis were grouped into saturated fatty acids - SFA (C14:0, C16:0, C17:0 and C18:0), monounsaturated fatty acids - MUFA (C14:1, C16:1, C17:1, C18:1n9, C18:1n7, C20:1) and polyunsaturated fatty acids - PUFA (C18:2n6, C18:3n3, C18:3n6, C20:2n6 and C20:4n6). The representation of the above fatty acid groups (SFA, MUFA and PUFA) relative of hen age is presented in Fig. 2, while the PUFA n-3 and n-6 content and the n-6/n-3 and PUFA/SFA ratios are given in Table 3.

The egg yolk fat mainly consists of oleic (C18:1n-9, OA), palmitic (C16:0, PA) and linoleic (C18:2n-6, LA) fatty acid. These are followed by stearic (C18:0) and palmitoleic (C16:1) acid, while the representation of other fatty acids was below 1%. OA represented about 40-46 % of all FAs and 85- 87 % of all MUFAs. As for the SFAs, palmitic and stearic fatty acid represented about 98% of all SFAs, the palmitic acid content thereby ranging from 23.52 to 26.45 % and that of a stearic acid from 5.34 to 6.84 % *(data not shown).* A similar representation of SFAs (29.51 - 33.14 %), MUFAs (46.19 – 53.04 %) and PUFAs (15.04 - 22.75 %) was reported by Cherian et al. (2002).

This research revealed a significantly higher average proportion of n-6 fatty acids (22.38%) present in eggs as compared to n-3 fatty acids (0.50%) (Table 3). Kazmierska et al. (2005) reported the highest content of n-6 PUFA in hen eggs in comparison to other bird species. In another study of FA composition of eggs from Lohman hens fed on commercial mixtures, the n-6 and the n-3 content were more favourable (16.5% and 1.0%, respectively) (Milinsk et al, 2003). The predominance of n-6 fatty acids is primarily attributable to linoleic acid; in this study, the latter was proven to represent about 93- 98 % of the total n-6 fatty acid content, mostly due to the feed the hens were fed on (Table 1). LA enhances the synthesis of lipoproteins and increases yolk weight (March and MacMillan, 1990).

Table 3. Total n-3 and n-6 PUFA content and fatty acid ratios relative of hen age

Hens' age (weeks)	Total n-3	Total n-6	n-6/n-3	PUFA/SFA
21	0.26 ± 0.02	14.78 ± 0.71	56.61 ± 2.54	0.47 ± 0.02
23	0.25 ± 0.04	16.66 ± 2.74	66.29 ± 7.69	0.54 ± 0.09
25	0.29 ± 0.02	16.39 ± 0.95	56.64 ± 1.00	0.52 ± 0.05
27	0.39 ± 0.10	19.87 ± 4.23	51.43 ± 4.32	0.62 ± 0.16
29	0.29 ± 0.07	17.32 ± 2.47	60.31 ± 7.25	0.54 ± 0.11
31	0.36 ± 0.03	18.67 ± 0.98	51.45 ± 2.82	0.58 ± 0.03
33	0.33 ± 0.10	18.22 ± 3.46	55.91 ± 5.79	0.57 ± 0.10
35	0.42 ± 0.04	19.44 ± 1.53	46.08 ± 0.33	0.64 ± 0.05
37	0.39 ± 0.06	17.97 ± 0.87	46.58 ± 6.85	0.65 ± 0.04
39	0.48 ± 0.13	20.71 ± 4.53	44.03 ± 3.25	0.69 ± 0.13
41	0.40 ± 0.04	21.56 ± 0.90	54.46 ± 3.75	0.72 ± 0.05
43	0.36 ± 0.04	22.38 ± 2.23	62.01 ± 2.74	0.73 ± 0.07
45	0.32 ± 0.02	17.65 ± 1.26	55.32 ± 1.32	0.57 ± 0.05
47	0.34 ± 0.13	18.81 ± 3.61	58.49 ± 9.69	0.59 ± 0.12
49	0.26 ± 0.05	15.14 ± 1.28	58.51 ± 5.04	0.47 ± 0.06
51	0.26 ± 0.05	16.26 ± 2.07	63.04 ± 7.21	0.50 ± 0.09
53	0.27 ± 0.04	14.91 ± 2.14	55.84 ± 2.76	0.46 ± 0.06
55	0.50 ± 0.06	21.94 ± 2.21	43.85 ± 1.37	0.76 ± 0.05
Total	0.34 ± 0.06	18.26 ± 2.12	54.83 ± 4.21	0.59 ± 0.07

The results are expressed as mean values ± standard deviation (n=3). PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids

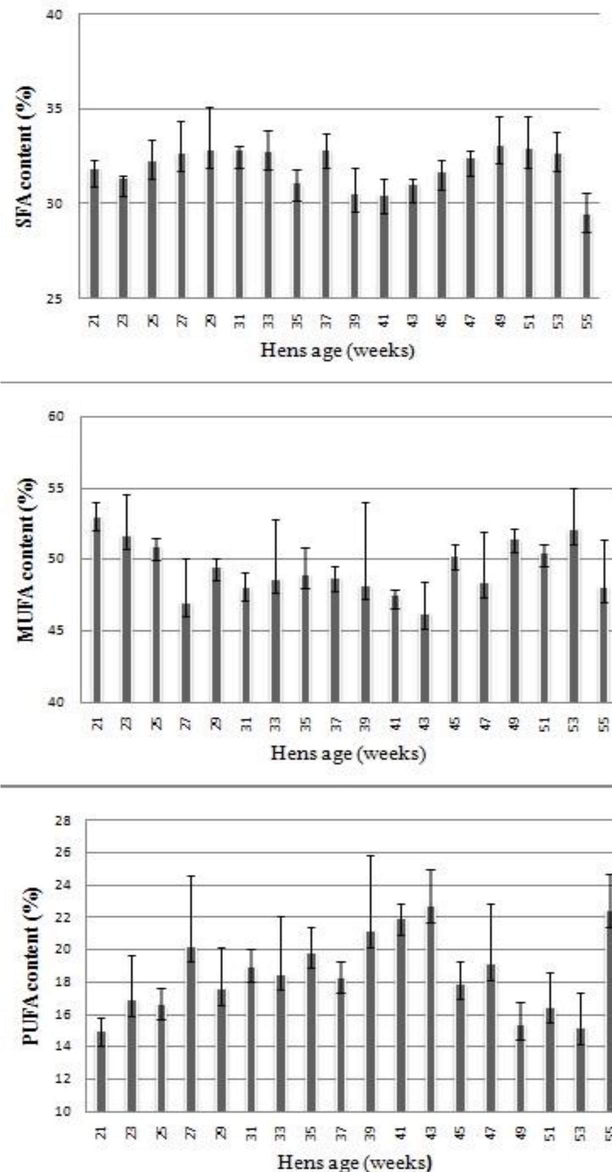

Fig. 2. SFA, MUFA and PUFA contents displayed based on hen age in weeks. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. The results are expressed as mean values ± standard deviation

Research shows that C18:1 and C18:2 fatty acids are most affected by feeding management, as opposed to SFAs (Cobos et al., 1995). In other studies of fatty acid composition of conventional eggs, the proportion of n-6 in eggs ranged from 15.0 to 23.8 %, while the proportion of n-3 ranged from 0.38 to 1.36 %, with the pertaining n-6/ n-3 ratio spanning from 11.03 to 62.63 (Petrović et al., 2012; Samman et al., 2009; Milinsk et al., 2003; Cherian et al., 2002; Škrtić et al., 2007; Woods and Fearon, 2009). The representation of n-6 determined in this study was similar to the n-6 representation reported by the abovementioned studies, while the proportion of n-3 was similar to the lower n-3 values reported by these studies. The n-6/n-3 ratios established in this research varied from 44.03 to 66.29 (Table 3), i.e. were much higher than recommended (3-5:1) (DOH, 1994; Gogus and Smith, 2010). Nowadays, industrial production of animal feed rich in grains and containing n-6 fatty acids, leads to the production of meat and eggs rich in n-6 and poor in n-3 fatty acids (Simopoulos, 2000). Apart from the n-6/n-3 ratio, the nutritional quality can also be assessed based on the PUFA/SFA ratio. In this study, the latter ranged from 0.46 to 0.76 (Table 3), which is in agreement with valid recommendations (≥ 0.45; DOH, 1994). PUFA/SFA ratios of the conventionally produced eggs were similar, as

reported by other researcher studies, and ranged from 0.45 to 0.68 (Cherian et al., 2002; Milinsk et al, 2003; Samman et al., 2009; Petrović et al., 2012; Škrtić et al., 2007).

Alpha-linolenic acid (C18:3n-3, ALA) was the only fatty acid determined in eggs that belongs to the n-3 PUFA group. It has been reported that hens have a limited ability to convert ALA to eicosapentaenoic (EPA) and docosahexaenic acid (DHA). However, it is believed that the conversion efficiency is affected by factors such as hen age and strain, as well as by high proportion of n-6 fatty acids, which adversely affects the conversion efficiency. At high LA concentrations, the conversion of ALA into EPA and DHA is inhibited. It has been postulated that older hens have a larger liver allowing for a more efficient conversion of ALA into DHA (Fraeye et al., 2012). However, it has also been suggested that hen aging diminishes desaturation and elongation of n-3 FAs (Cherian, 2008). Zero DHA in eggs primarily comes as a result of a lipid dietary source (Table 1). Koppenol et al. (2014) reported a strong dependency of EPA and DHA concentrations in eggs on dietary EPA and DHA concentrations. The levels of ALA obtained in this study (0.25 – 0.50 %) (Fig. 3) were similar to those obtained in other studies, which reported the ALA representation of 0.15 to 0.38 % (Milinsk et al., 2003; Sahan et al., 2014; Škrtić et al., 2007; Kazmierska et al., 2005). In earlier studies on the conventionally produced eggs, DHA was either not detected (Sahan et al., 2014; Škrtić et al., 2007; Woods and Fearon, 2009) or detected in the range of 0.10 – 0.85 % (Petrović et al., 2012; Samman et al., 2009; Imran et al., 2015; Milinsk et al., 2003; Cherian et al., 2002; Cherian, 2008).

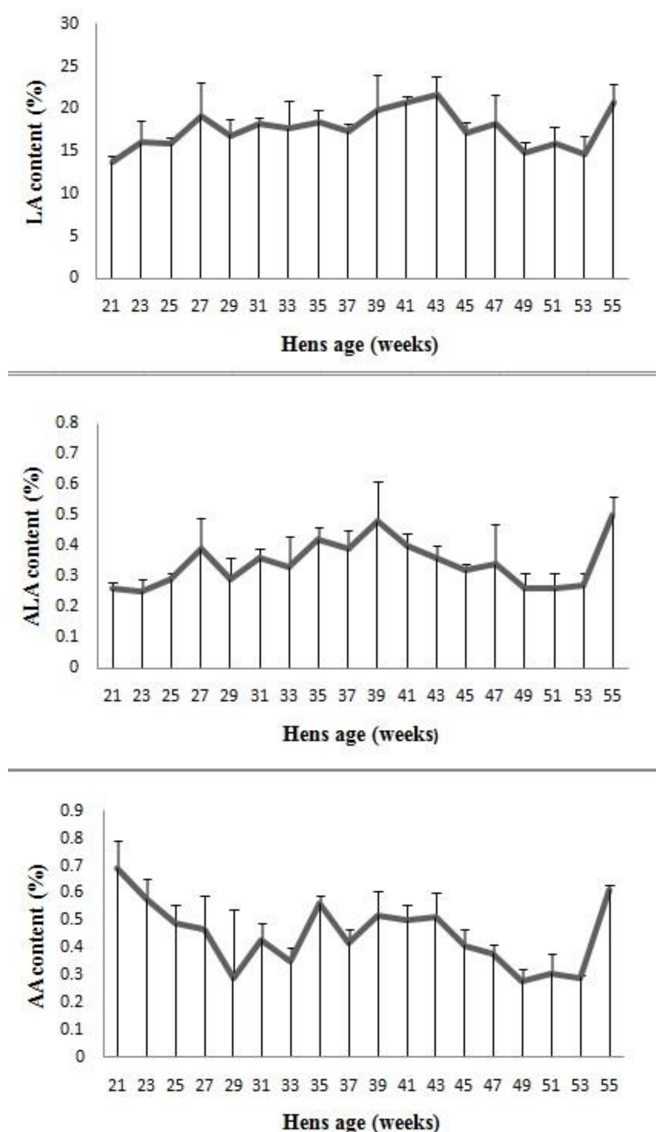

Fig. 3. Individual PUFA (LA, ALA, and AA) contents displayed based on hen age in weeks. PUFA, polyunsaturated fatty acids; LA, linoleic acid; ALA, alpha linolenic acid; AA, arachidonic acid. The results are expressed as mean values ± standard deviation

Arachidonic acid (C20:4n-6, AA) is essential for embryonic development and chicken growth (Sahan et al., 2014). Although AA was not detected in feed (Table 1), its presence in eggs (0.69%) indicates its synthesis from LA. In comparison to the results of this study, other researchers reported higher egg AA contents ranging from 1.54 to 2.63 % (Milinsk et al., 2003; Cherian et al., 2002; Škrtić et al., 2007; Kazmierska et al., 2005).

The metabolism of a breeder hen is in function of the animal age and influences changes in the yolk fatty acid composition (Latour et al., 1998). The results of this study showed significant differences in fatty acid composition depending on hen age, confirmed also by other studies (Cherian, 2008, Koppenol et al., 2014, Nielsen, 1988, Latour et al., 1998; Sahan et al., 2014; Yadgary et al., 2010). Other factors affecting the fatty acid composition, such as feeding regimens and raising environments, have also been studied (Milinsk et al., 2003; Cobos et al., 1995; Campos et al., 2016; Anderson 2011; Cherian et al., 2002; Pavlovski et al., 2011).

In this study, a statistically significant difference ($p < 0.05$) in SFA and PUFA representation (that is to say, the representation of all PUFAs, n-6 and n-3) and the n-6/n-3 and PUFA/SFA ratios established in hens of different ages was found. As for individual fatty acids, a statistically significant difference was found in the representation of three FAs, namely LA, ALA and AA, respective of hen age, as shown in Fig. 3.

When comparing eggs from the oldest and the youngest hens (i.e. the eggs collected in the last and the first week of this investigation), SFAs were found to be statistically significantly lower ($p < 0.05$), but individual SFA contents were not found to be significantly different ($p > 0.05$). The total PUFA content was statistically significantly higher ($p < 0.05$) in eggs from 55- week old hens as compared to eggs coming from 21-week old ones (Fig. 2). The nutritional n-6/n-3 and PUFA/SFA ratios were statistically significantly ($p < 0.05$) more favourable in the oldest hens (Table 3). When it comes to the individual fatty acid contents established in the first, as compared to the last week of this investigation, a statistically significant difference was found only for LA and ALA ($p < 0.05$) (Fig. 3). In general, the results of this study pointed towards variations in FA contents in hens of a different age.

Similar to our results, Koppenol et al. (2014) showed the concentration of yolk SFA to be lower in 58-week old (36.16%) as compared to younger, 28-week old hens (37.32%). Contrary to the above, Latour et al. (1998) showed the hen age to have influence on the most represented SFAs, that is to say, palmitic and stearic acid, the ratio of these fatty acids being higher in eggs from older than in eggs from younger hens. Sahan et al. (2014) reported a lower yolk palmitic acid content in older as compared to younger hens, while for stearic acid a higher content was reported in older hens.

Opposite to the results obtained in this research, other researchers reported generally higher PUFA contents in eggs of younger hens (Nielsen 1998, Koppenol et al., 2014) and no hen age-related differences in the n-6/n-3 ratio (Koppenol et al., 2014). The results of this investigation are in agreement with the results of Latour et al. (1998), where LA content in fresh eggs was also significantly higher in older (64-week old; 20.30%) than in younger hens (36- and 51- week old; 12.98 and 13.58%, respectively). A different trend was reported by Sahan et al. (2014) and Koppenol et al. (2014), who claimed to have found higher LA and ALA contents in younger hens (36 *vs.* 52; 28 *vs.* 43 and 58). In this study, the AA content was not found to be significantly different ($p > 0.05$) in the oldest as compared to the youngest hens, which is in agreement with the study by Koppenol et al. (2014), while some authors observed a positive correlation between hen age and egg AA content (Cherian et al., 2008; Latour et al., 1998; Sahan et al., 2014). However, in this study, a significant difference in the AA content determined in eggs coming from hens of different age was found.

Conclusions

The results of this study revealed variations in fat content of eggs and fatty acid composition throughout the laying period. The fat content found in younger hens' eggs was lower than that in eggs of the oldest hens. When comparing eggs from the oldest and the youngest hens, it was found that the SFA content was statistically lower ($p < 0.05$) in the oldest hens, whereas individual SFA contents did not differ across various hen age groups in a statistically significant manner ($p > 0.05$). The nutritional n-6/n-3 and PUFA/SFA ratios were more favourable in the oldest hens. The fact that commercial eggs have a high n-6/n-3 ratio suggests the need for feeding practice improvement. The effect of breeder age is in interaction with breeder diet, as well as with the feed composition, environmental conditions and breed itself.

References

Ajuyah, A. O., Cherian, G., Wang, Y., Sunwoo, H., Sim, J. S. (2003): Maternal dietary FA modulate the long-chain n-3 PUFA status of chick cardiac tissue. *Lipids* 38, 1257-1261. https://doi.org/10.1007/s11745-003-1186-6.

Anderson, K. E. (2011): Comparison of fatty acid, cholesterol, and vitamin A and E composition in eggs from hens housed in conventional cage and range production facilities. *Poult. Sci.* 90, 1600-1608. https://doi.org/0.3382/ps.2010-01289.

Campos, A. M., Ricardo, F., Alves, E., Reis, A., Couto, D., Domingues, P., Domingues, M. R. M. (2016): Lipidomic investigation of eggs'yolk: Changes in lipid profile of eggs from different conditions. *Food Res. Int.* 89, 177-185. https://doi.org/10.1016/j.foodres.2016.07.006.

Cherian, G., Holsonbake, T. B., Goeger, M. P. (2002): Fatty acid composition and egg components of specialty eggs. *Poult. Sci*. 81 (1), 30-33. https://doi.org/10.1093/ps/81.1.30.

Cherian, G. (2008): Egg quality and yolk polyunsaturated fatty acids status in relation to broiler breeder hen age and dietary n-3 oils. *Poult. Sci.* 87 (6), 1131-1137. https://doi.org/10.3382/ps.2007-00333.

Cobos, A., Hoz, L., Cambero, M. I., Ordonez, J. A. (1995): Dietary modification and hen strain dependence of egg yolk lipids. *Food Res. Int.* 28 (1), 71-76. https://doi.org/10.1016/0963-9969(95)93333-P.

Department of health (DOH) (1994): Nutritional aspects of cardiovascular disease. Reports on health and social subjects No.46, London, HMSO.

EFSA (2009): Scientific Opinion of the Panel on Dietetic products, Nutrition and Allergies on a request from European Commission related to labeling reference intake values for n-3 and n-6 polyunsaturated fatty acids. *The EFSA Journal* 1176, 1-11. https://doi.org/10.2903/j.efsa.2009.1176.

Farrell, D. J. (1998): Enrichment of hen eggs with n-3 long-chain fatty acids and evaluation of enriched eggs in humans. *Am. J. Clin. Nutr.* 68 (3), 538-544.

Fraeye, I., Bruneel, C., Lemahieu, C., Buyse, J., Muylaert, K., Foubert, I. (2012): Dietary enrichment of eggs with imega-3 fatty acids: A review.*Food Res. Int.* 48, 961-969. https://doi.org/10.1016/j.foodres.2012.03.014.

Gogus, U., Smith, C. (2010): N-3 omega fatty acids: A review of current knowledge. *Int. J. Food Sci. Tech.* 45 (3), 417–436. https://doi.org/10.1111/j.1365-2621.2009.02151.

ISO Standard ISO 12966-2:2011 (2011): Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters- Part 2: Preparation of methyl esters of fatty acids, Brussels, Belgium.

ISO Standard ISO 12966-4:2015 (2015): Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters- Part 4: Determination by capillary gas chromatography, Brussels, Belgium.

ISO Standard HRN ISO 1443:1999 (1999): Meat and meat products - Determination of total fat content, Brussels, Belgium.

ISO Standard HRN ISO 6496: 2001 (2001): Animal feeding stuffs- Determination of moisture and other volatile matter content, Brussels, Belgium.

ISO Standard HRN ISO 6865: 2001 (2001): Animal feeding stuffs- Determination of crude fibre content- Method with intermediate filtration, Brussels, Belgium.

ISO Standard HRN ISO 5984: 2004 (2004): Animal feeding stuffs- Determination of crude ash, Brussels, Belgium.

ISO Standard HRN ISO 6490-1: 1999 (1999). Animal feeding stuffs- Determination of calcium content- Part 1: Titrimetric method, Brussels, Belgium.

ISO Standard HRN ISO 6491: 2001 (2001): Animal feeding stuffs- Determination of phosphorus content- Spectrometric method, Brussels, Belgium.

ISO Standard HRN ISO 5983-1:2008 (2008): Animal feeding stuffs- Determination of nitrogen content and calculation of crude protein content – Part 1: Kjeldahl method, Brussels, Belgium.

ISO Standard HRN ISO 5983-2:2010 (2010): Animal feeding stuffs- Determination of nitrogen content and calculation of crude protein content – Part 2: Block digestion and steam distillation method, Brussels, Belgium.

Kazmierska, M., Jarosz, B., Korzeniowska, M., Trziszka, T., Dobrzanski, Z. (2005): Comparative analysis of fatty acid profile and cholesterol content of egg yolks of different bird species. *Pol. J. Food Nutr. Sci.* 14 (55), 69-73.

Koppenol, A., Delezie, E., Aerts, J., Willems, E., Wanf, Y., Franssens, L., Everaert, N., Buyse, J. (2014): Effect of the ratio of dietary n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid on broiler breeder performance, egg quality, and yolk fatty acid composition at different breeder ages. *Poult. Sci.* 93 (3), 564-573. https://doi.org/10.3382/ps.2013-03320.

Kralik, G., Kralik, Z. (2017): Poultry products enriched with nutricines have beneficial effects on human health. *Med. Glas.* 14 (1), 1-7. https://doi.org/10.17392/879-16.

Latour, M. A., Peebles, E. D., Doyle, S. M., Pansky, T., Smith, T. W., Boyle, C. R. (1998): Broiler breeder age and dietary fat influence the yolk fatty acid profiles of fresh eggs and newly hatched chicks. *Poult. Sci.* 77, 47-53.

Imran, M., Anjum, F. M., Nadeem, M., Ahmad, N., Khan, M. K., Mushtaq, Z., Hussain, S. (2015): Production of Bio-omega-3 eggs through the supplementation of extruded flaxseed meal in hen diet. *Lipids Health Dis.* 14, 126. https://doi.org/10.1186/s12944-015-0127-x.

March, B. E., MacMillan, C. (1990): Linoleic acid as a mediator of egg size. *Poult. Sci.* 69, 634-639. https://doi.org/10.3382/ps.0690634.

Milinsk, M. S., Murakami, A. E., Gomes, S. T. M., Matshuta, M., De Souza, N. E. (2003): Fatty acid profile of egg yolk lipids hens fed diet rich in n-3 fatty acids. *Food Chem.* 83, 287-292. https://doi.org/10.1016/S0308-8146(03)00094-3.

Millet, S., De Ceulaer, K., Van Paemel M., Raes, K., De Smet, S., Janssens, G. P. J. (2006): Lipid profile in eggs of Araucana hens compared with Lohmann selected Leghorn and ISA Brown hens given diets with different fat sources. *Br. Poult. Sci.* 47 (3), 294-300. https://doi.org/10.1080/00071660600741818.

Nakamura, Y., Okamura, T., Tamaki, S., Kadowaki, T., Hayakawa, T., Kita, Y., Okayama, A., Ueshima, H. (2004): Egg consumption, serum cholesterol, and cause-specific and all-cause mortality: The National Integrated Project for Prospective Observation of Non-communicable Disease and Its Trends in the Aged, 1980 (NIPPON DATA80). *Am. J. Clin. Nutr*. 80, 58-63.

Nielsen, H. (1998): Hen age and fatty acid composition of egg yolk lipid. *Br. Poul. Sci.* 39 (1), 53-56. https://doi.org/10.1080/00071669889394.

Pavlovski, Z., Škrbić, Z., Lukić, M., Lilić, S., Krnjaja, V., Stanišić, N., Petričević, V. (2011): Comparative analysis of fatty acid profile and cholesterol content in table eggs from different genotype hens. *Biotech. Anim. Husbandry* 27 (3), 669-677. https://doi.org/10.2298/BAH1103669P.

Petrović, M., Gačić, M., Karačić, V., Gottstein, Ž., Mazija, H., Medić, H. (2012): Enrichment of eggs in n-3 polyunsaturated fatty acids by feeding hens with different amount of linseed oil in diet. *Food Chem*. 135, 1563-1568. https://doi.org/10.1016/j.foodchem.2012.06.020.

Roberts, J. R. (2004): Factors affecting egg internal quality and egg shell quality in laying hens. *J. Poultry Sci.* 41, 161-177. https://doi.org/10.2141/jpsa.41.161.

Samman, S., F., Kung, P., Carter, L. M., Foster, M. J., Ahmad, Z. I., Phuyal, J. L., Petocz, P. (2009): Fatty acid composition of certified organic, conventional and omega-3 eggs. *Food Chem*. 116, 911-914. https://doi.org/10.1016/ j.foodchem.2009.03.046.

Senčić, Đ., Samac, D. (2017): Nutritional value of eggs in human nutrition. *Meso* 1, 68-72.

Simopoulos, A. P. (2000): Human requirement for n-3 polyunsaturated fatty acids. *Poult. Sci.* 79, 961-970.

Sahan, U., Ipe, A., Sozcu, A. (2014): Yolk sac fatty acid composition, yolk absorption, embryo development, and chick quality during incubation in eggs from young and old broiler breeders. *Poult. Sci*. 93, 2069-2077. https://doi.org/10.3382/ps.2013-03850.

Škrtić, Z., Kralik, G., Gajčević, Z., Bogut, I., Hanžek, D. (2007): The increase of the n-3 PUFA content in eggs. *Poljoprivreda* 13, 47-52.

Croatian Bureau of Statistics. 2016. Statistical Yearbook. http://www.dzs.hr/Eng/Publication/stat_year.htm. Accessed March 6, 2017.

Trautwein, E. A. (2001): N-3 fatty acids- Physiological and technical aspects for their use in food. *Eur. J. Lipid Sci. Technol.* 103, 45-55. https://doi.org/10.1002/1438-9312(200101)103:1<45::AID-EJLT45>3.0.CO;2-9.

Woods, V. B., Fearon, A. M. (2009): Dietary sources of unsaturated fatty acids for animals and their transfer into meat, milk and eggs: A review. *Livest. Sci.* 126, 1-20. https://doi.org/10.1016/j.livsci.2009.07.002.

Yadgary, L., Cahaner, A., Kedar, O., Uni, Z. (2010): Yolk sac nutrient composition and fat uptake in late-term embryos in eggs from young and old broiler breeder hens. *Poult. Sci.* 89, 2441-2452. https://doi.org/10.3382/ps.2010-00681.

Received: May 10, 2017
Accepted: September 25, 2017