

A Fatty acid profile and THE Health Lipid Indices in Table Eggs

Profil masnih kiselina i zdravstveni indeksi lipida u jajima na tržištu

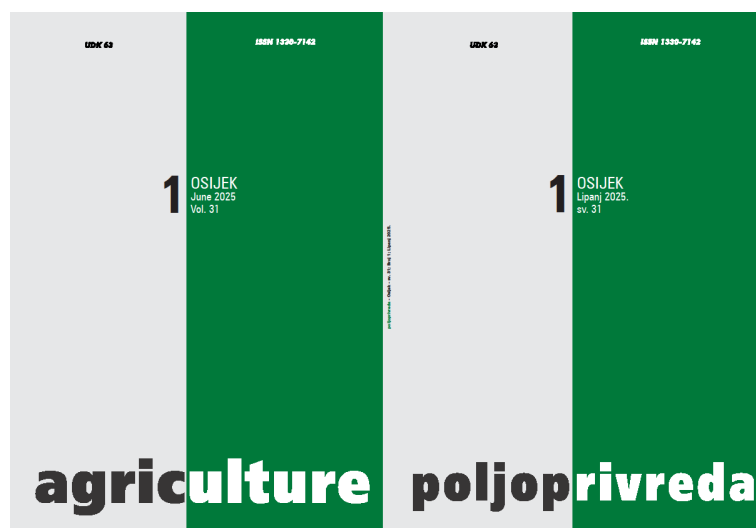
Kralik, Z., Kralik, G., Košević, M., Kralik, I.

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Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

A FATTY ACID PROFILE AND THE HEALTH LIPID INDICES IN TABLE EGGS

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SUMMARY

This paper investigates the profile of fatty acids, as well as qualitative (Σ PUFA/ Σ SFA, Σ n-6 PUFA/ Σ n-3 PUFA, LA/ALA, EPA+DHA), nutritional (NVI, AI, TI, SI, hHI, HPI), and metabolic (EI, THI, Δ 9-desaturase, Δ 5+ Δ 6 desaturase, KAI) indices in the table eggs obtained from four different producers (A, B, C, and D). For the purpose of this research, forty L-class eggs were bought in a grocery store. The eggs were produced on four farms in Croatia. The health-lipid indices were calculated on the basis of a fatty-acid profile in egg yolks. The most favorable qualitative indices were determined in the lipids of D eggs, with an exception of the EPA+DHA indices. The most nutritional indices were more favorable in the eggs of group A than in the eggs of groups B, C, and D. The metabolic indices THI and EI were more favorable in the eggs of the A and B group, respectively, while the Δ 9-desaturase and the Δ 5+ Δ 6 desaturase indices were more favorable in the eggs of group D. A correlation between the selected health-lipid indices was also established. A very strong positive correlation ($r = 0.97$) was determined between the quantitative lipid indices of the LA/ALA and the ratio of Σ n-6 PUFA/ Σ n-3 PUFA, while a very strong negative correlation was determined between the quantitative indices of Σ PUFA/ Σ SFA, Σ n-6 PUFA/ Σ n-3 PUFA, and LA/ALA and the metabolic indices of the Δ 5+ Δ 6 desaturase. An egg-group ranking based on all health indices resulted in the following value order: A = 42, B and D = 38, and C = 32.

Keywords: table eggs, fatty acids, health-lipid indices

INTRODUCTION

Eggs are a valuable source of essential nutrients, since they contain the proteins, lipids, minerals, and vitamins, as well as the bioactive substances beneficial for human health (Attia et al., 2014, 2015). Among many factors that affect the egg quality and a fatty-acid profile, special attention is devoted to the fat (oil) content in the laying hens' diet (Radanović et al., 2023; Z. Kralik et al., 2024) and to the influence of laying hens' genetic potential and age (Kucukyilmaz et al., 2012). The eggs' fatty acid content depends on their origin in the feed and, consequently, has different effects on human health status (Dal Bosco et al., 2022). To ensure the incorporation of desired fatty acids into table eggs, hens must be fed with a diet rich in polyunsaturated fatty acids. Vegetable oils, such as rapeseed and linseed, are rich in α -linolenic acid (α -LNA), the most abundant omega-3 fatty acid found in animal feed, while sunflower oil is rich in linoleic and oleic acid (Konuskan et al., 2019). In our country, sunflower oil is most commonly used

in the feed factories for the preparation of poultry-feed mixtures. Therefore, if an improvement of the eggs' fatty-acid profile is desired, it is necessary to include rapeseed and linseed oils in the feed, as they are rich in α -LNA. Modern diet usually contains excessive levels of omega-6 PUFA and the very low levels of omega-3 PUFA, which causes a high ratio of Σ n-6 PUFA / Σ n-3 PUFA and negative effects on human health (Omid et al., 2015). Simopoulos (2016) reported that the ratio of Σ n-6 PUFA / Σ n-3 PUFA in the Western diet was from 15:1 to 16.7:1. He also argued that such a ratio should be much closer, around 1:4, reaching an ideal of 1:1. In order to assess a table eggs' health benefit, it is necessary to calculate their health-lipid indices. Considering the importance of an individual fatty-acid content (SFA, MUFA, and PUFA) in the metabolic processes, these data are used when

Prof. Dr. Zlata Kralik (zkralik@fazos.hr), Prof. Emer. Dr. Dr. h. c. Gordana Kralik, Manuela Košević, PhD, Prof. Dr. Igor Kralik — Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia

evaluating the qualitative, nutritional, and metabolic lipids in the egg yolks. Thus, n-6 and n-3 PUFAs are required for growth and cell renewal, blood-pressure regulation, and the proper functioning of the kidneys and immune system (Panaite et al., 2020). In the literature dealing with the table eggs' quality, a qualitative index ($\sum n-6$ PUFA / $\sum n-3$ PUFA) is most frequently quoted. The eggs with a favorable fatty-acid profile should have a ratio of $\sum n-6$ PUFA / $\sum n-3$ PUFA as narrow as possible. The eggs with a lower ratio of SFA/UFA have the lower values of atherogenic (AI), thrombogenic (TI), and hypo/hypercholesterolemic (hHI) indices, so they are recommended as a healthy foodstuff (Laudadio & Tufarelli, 2011; Grela et al., 2014). It is confirmed that myristic and palmitic fatty acids have atherogenic effects, while stearic acid exhibits a thrombogenic effect (Popa et al., 2012; Laudadio et al., 2015). Consuming food with a lower AI can contribute to the reduction of LDL and total cholesterol (Yurchenko et al., 2018). The thrombogenic index indicates the fatty acids' thrombogenic potential. Both indices are associated with a risk of cardiovascular disease, so their value should be as low as possible (Ulbricht & Southgate, 1991). The value of hHI is suitable when determining the fatty acids' effect on cholesterol metabolism. From a nutritional point of view, the high hHI index values are desirable. Dal Bosco et al. (2022) stated that the elongase (EI) and thioesterase (THI) indices, which evaluate the metabolism of myristic, palmitic, and stearic fatty acids, were suitable in the assessment of lipid metabolism. Vessby et al. (2002) pointed out that the $\Delta 5 + \Delta 6$ -desaturase indices were important in metabolizing n-6 and n-3 PUFAs from their precursors, linoleic (LA) and alpha-linolenic (ALA) acids, while Zhang et al. (2007) recommended the $\Delta 9$ -desaturase index. For a healthy human diet, the foods with a low hHI, AI, and TI are recommended. HPI is the opposite of AI. The HPI value in the foods should be higher, as a very high degree of protection against atherogenic diseases is indicated in that case (Dal Bosco et al., 2022; Qaid et al., 2023).

This research's objective was to determine a fatty acid profile in the egg yolks and to calculate the qualitative, nutritional, and metabolic indices from these values, which are important for human health. In addition, the aim of the study was to analyze the data obtained and to rank the producers in terms of the table eggs' lipid quality and to indicate which one among them fed the laying hens on the farm with the highest-ranked feed and for which the feeding could still be corrected.

MATERIAL AND METHODS

An Analysis of Fatty Acid-Profiles in the Egg Yolks

For the purpose of conducting this experiment, forty L-class table eggs were bought in a grocery store. The eggs were produced on four different farms in Croatia. The laying hens were raised in a cage system. The fatty-acid profile was analyzed using forty yolks - that is, using ten of them per each egg group. In order to determine the fatty-acid profile, the samples were processed in a Mars 6 microwave device (CEM Corporation, Matthews, NC, USA) by applying microwave radiation at 1200 W.

At the end of the process, the samples were extracted in pentane, transferred to vials, and stored in a freezer until an analysis was conducted using a gas chromatograph. Chromatographic analysis was conducted on a Scion 436-GC gas chromatograph (SCION Instruments, Goes, the Netherlands), equipped with a flame-ionization detector. The separation of fatty acids was performed on a Fawcax capillary column (30 m x 0.32 mm [inner diameter] x 0.25 μ m [film thickness]; Restek Corporation, Bellefonte, PA, USA). The injected sample volume amounted to 1 μ L, and the operating conditions were as follows: an injector temperature of 230°C, a detector temperature of 230°C, and a carrier-gas flow rate (hydrogen) at 2.5 mL/min. The oven-temperature program was set as follows: from 50 to 160°C with a heating rate of 20°C/min and from 160 to 225°C with a heating rate of 10°C/min, with a retention time at 225°C for 9 min. The analysis lasted for 21 min in total. A standard mixture of thirty-seven fatty acids (Food Industry FAME Mix, Restek Corporation, Bellefonte, PA, USA) was used in the chromatogram to identify the individual fatty acids. The portions of individual fatty acids are shown as a percentage of total fatty acids in lipids. Upon examining the chromatogram, the portions of individual fatty acids within total fat were calculated from the ratio of the area of a relevant peak to the total area of all fatty acid peaks.

Calculation of Indices

The health lipid indices were calculated using the results of an analysis of the egg yolks' fatty acid profile. As described by Simopoulos (2008) and have excessive amounts of omega-6 fatty acids compared with the diet on which human beings evolved and their genetic patterns were established. Excessive amounts of omega-6 polyunsaturated fatty acids (PUFA, one of the most represented lipid indices refers to the calculation of a ratio of polyunsaturated and saturated fatty acids, $\sum$ PUFA / $\sum$ SFA. Another index, described by Timmis et al. (2022) or the latest available year, are presented. Data sources include the World Health Organization, the Institute for Health Metrics and Evaluation, the World Bank, and novel ESC sponsored data on human and capital infrastructure and cardiovascular healthcare delivery. New material in this report includes sociodemographic and environmental determinants of CVD, rheumatic heart disease, out-of-hospital cardiac arrest, left-sided valvular heart disease, the advocacy potential of these CVD statistics, and progress towards World Health Organization (WHO, refers to a ratio of $\sum n-6$ PUFA / $\sum n-3$ PUFA. The third lipid index refers to a ratio of linoleic (LA; C18:2n-6) / α -linolenic (ALA; C18:3n-3) fatty acid (Ryman et al., 2017; Draycott et al., 2019).

Ochi and Tsuchiya (2018) described another lipid index, which represents a sum of two important n-3 fatty acid—namely, the eicosapentaenoic (EPA; C20:5 n-3) and docosahexaenoic (DHA; C22:6 n-3) fatty acids.

Chen et al. (2016) developed a formula for the calculation of nutritional-value index (NVI), by which they only assessed the content of stearic, oleic, and palmitic

fatty acids, as those fatty acids are dominant in the food of animal origin: $NVI = (C18:0 + C18:1n9) / (C16:0)$.

Ulbricht and Southgate (1991) published the following formulas for the calculation of atherogenic, thrombogenic, and saturation indices (AI, TI, and SI):

$$AI = (C12:0 + 4 * C14:0 + C16:0 + C18:0) / (\sum MUFA + \sum n-6 PUFA + \sum n-3 PUFA)$$

$$TI = (C14:0 + C16:0 + C18:0) / [(0,5 * \sum MUFA) + (0,5 * \sum n-6 PUFA) + (3 * \sum n-3 PUFA) + (\sum n-3 PUFA / \sum n-6 PUFA)]$$

$$SI = (C14:0 + C16:0 + C18:0) / (\sum MUFA + \sum PUFA).$$

The AI indicates a relation between the main saturated and unsaturated fatty acids, while the TI is used to further describe the fatty acids' thrombogenic potential, separating them into the prothrombogenic (C12:0, C14:0 and C16:0) and antithrombogenic fatty acids (MUFA, n-3 and n-6 PUFAs). The lower values of these indices suggest better nutritional properties of eggs in terms of the egg lipid quality.

The hypo/hypercholesterolemic index (hHI) and the health-promoting index (HPI) were described by Dal Bosco et al. (2022).

The hypo/hypercholesterolemic index (hHI) is calculated according to the following formula:

$$hHI = (C18:1n-9 + \sum n-6 PUFA + \sum n-3 PUFA) / (C12:0 + C14:0 + C16:0).$$

For the calculation of the health-promoting index (HPI), the following formula is applied:

$$HPI = (\sum MUFA + \sum n-6 PUFA + \sum n-3 PUFA) / [C12:0 + (4 * C14:0) + C16:0].$$

Dal Bosco et al. (2022) calculated the indices of elongase and thioesterase (EI and THI) by applying the following formulas:

$$EI = (C18:0 / C16:0) * 100$$

$$THI = (C16:0 / C14:0) * 100$$

The metabolic index of $\Delta 9$ -desaturase is calculated according to Zhang et al. (2007), as follows:

$$\Delta 9\text{-desaturase} = (C16:1 + C18:1n9) / (C16:0 + C18:0 + C16:1 + C18:1n9).$$

In order to evaluate the activity of the $\Delta 5$ - and $\Delta 6$ -desaturase—that is, the enzymes that catalyze the production of n-6 and n-3 PUFAs from the precursors of linoleic acid and α -linolenic acid—Vessby et al. (2002) applied the following formula:

$$\Delta 5 + \Delta 6\text{-desaturase} = (C20:2n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3).$$

Failla et al. (2021) calculated the KAI, the kinetic activity index, of the n-3 β -oxidation in myocytes by using the following formulaic expression

$$KAI = \sum n-3 PUFA / C18:3n-3.$$

Evaluation of Health-Lipid Indices

The values of the health lipid indices were determined according to their effect on human health and distributed into four categories with a specific number of points assigned to each category: Category 1 with four points (the most favorable one), Category 2 with three points, Category 3 with two points, and Category 4 with one point (the least favorable category). The sum of a multiple of points and a category frequency represent the total value of weights for the ranking of the A, B, C, and D groups of eggs.

Statistical Data Analysis

The results obtained in this research were processed in *TIBCO Statistica*® 14.0.0. (TIBCO Software Inc., 2020) and presented in tables as the means and standard deviation. The differences between the groups of eggs were determined by the Fisher's LSD test at the significance level of $P < 0.05$, $P < 0.01$, and $P < 0.001$. *Microsoft 365 Excel* was used for the calculation of correlation coefficient (r). A correlation between the individual health indices was determined as follows: a very weak when $r \leq 0.30$, medium when $r = 0.31$ to 0.50 , strong when $r = 0.51$ to 0.80 , and very strong when $r = 0.81$ to 1.00 .

RESULTS AND DISCUSSION

Table 1 contains an overview of a fatty-acid profile in yolks of the A, B, C, and D eggs. The most important saturated fatty acids (SFAs), such as myristic, palmitic, and stearic, differed statistically between the egg groups ($P < 0.05$). An SFA sum was the highest in the lipids of C eggs and the lowest in the lipids of A eggs ($P < 0.01$). There was a statistically significant difference determined for the content of palmitoleic, heptadecanoic, oleic, and eicosenoic fatty acids between the egg groups, as well as for the content of $\sum MUFA$ ($P < 0.001$). The lipids of D eggs contained the highest and the lipids of A eggs the lowest percentage of $\sum MUFA$ (50.66%:42.49%, respectively). As a precursor of n-6 PUFA, linoleic fatty acid was the most prevalent in the A eggs, while the lowest amount was present in the D eggs (21.58%:12.56%; $P < 0.001$, respectively). The $\sum n-6$ PUFA was the highest also in the A eggs and the lowest in the D eggs ($P < 0.001$). The highest percentage of ALA, as a precursor of n-3 PUFA, was determined in the yolk lipids of group D, while the least percentage was determined in group B ($P < 0.001$). The $\sum n-3$ PUFA differed significantly among the egg groups, as the lowest percentage was detected in group B, and the highest percentage in group C (0.61%: 1.61%; $P < 0.001$, respectively). Many authors emphasized a significance of the ratio of n-6 / n-3 PUFAs in the egg yolks (Simopoulos, 2010; Omidi et al., 2015; Kralik Z. et al., 2023; Radanović et al., 2023) for the preservation of human cardiovascular system by specifying that an

optimal ratio should be 1:4. The European Food Safety Authority (EFSA, 2009), however, does not recommend a favorable ratio of n-6 /n-3 PUFAs. Based on an assessment of cardiovascular health of the EU population, they propose a reference value of 2 g for a daily intake of ALA, the most common n-3 PUFA; 250 mg for a daily intake of EPA + DHA; and 10 g for a daily intake of LA, the most common n-6 PUFA. The polyunsaturated fatty acids, n-6 and n-3 PUFAs, are essential because the human body cannot synthesize them, so they have to be taken in through food. LA is the most represented fatty acid among the n-6 PUFAs, and ALA is the most common n-3 PUFA. With an enzymatic activity, they are metabolized into the long-chain arachidonic (AA), eicosapentaenoic (EPA), and docosahexaenoic (DHA) fatty acids (Simopoulos, 2010). The LA and ALA, as well as their derivatives, are important components of cell membranes. ALA is found in triglycerides and in cholesterol esters. In small amounts, it is also present in phospholipids. EPA is present in triglycerides and phospholipids, and DHA is found mainly in phospholipids. The human body cannot convert n-6 PUFA into n-3 PUFA due to a lack of the Δ^3 -desaturase enzyme (Kang, 2003; Simopoulos, 2010; Attia et al., 2024). An increased content of n-3 PUFA in the eggs laid by the hens fed with linseed oil was reported by Cherian (2008), as well as Batkowska et al. (2021) their fatty acid (FA). The same was established by Raza et al. (2016) for the eggs obtained from the hens fed with hemp oil. In many countries, the ratio of / n-3PUFAs is in disparity when compared to the desired 1:1 ratio (Simopoulos, 2010, 2016). The results of our research show that the

producers, especially those of the eggs of the A and B groups, did not consider the source of oil in the hens' diet for the purpose of enriching their eggs with n-3 PUFA but were probably more motivated by the cost of feed as a production input. Omri et al. (2019), Batkowska et al. (2021) their fatty acid (FA, Untea et al. (2020), and Kralik G. et al. (2024) discussed the importance of the incorporation of oils in the laying hens' diet from the aspect of influence on a fatty-acid egg profile. These authors confirmed that the portions of different ingredients in feeding mixtures affect the fatty acid profile in hens' feed and influence their deposition in eggs. In this research, the most favorable ratio of n-6 /n-3 PUFAs was confirmed for the eggs in group D (9.76) and the worst one for group B (35.55). Kralik G. et al. (2024) reported that the eggs laid by the hens fed with a diet containing 5% of soybean oil had a very wide ratio of n-6 /n-3 PUFAs (10.66), which is in accordance with the results obtained in this research for the eggs of groups C and D. A supplementation of vegetable oils and microalgae in the hens' diets resulted in narrowing that ratio to 4.31 and 4.15, respectively. In a research into the influence of composition of laying hens' feed on the fatty-acid content in table eggs, Krawczyk et al. (2015) established a ratio of n-6 /n-3 PUFAs for the control group, which was very similar to the ratio established in this research for the eggs in group D (9.14). A competition between the LA and ALA affected the synthesis of PUFAs. The LA gets quickly incorporated into the tissues, while the ALA inhibits the conversion of linoleic to arachidonic fatty acid during the elongation and saturation processes (Coetzee & Hoffman, 2002).

Table 1. A fatty-acid profile in the egg yolks (% in total fatty acids)

Tablica 1. Profil masnih kiselina u žumanjcima jaja (% u sumi masnih kiselina)

Fatty acid / Masna kiselina	A	B	C	D	P value / P vrijednost
Myristic (C14:0) / <i>Miristinska</i>	0.19±0.03 ^d	0.21±0.02 ^{cd}	0.23±0.03 ^{bc}	0.24±0.02 ^{ab}	**
Pentadecanoic (C15:0) / <i>Pentadekanska</i>	0.04±0.01 ^b	0.04±0.01 ^b	0.05±0.01 ^b	0.06±0.01 ^a	**
Palmitic (C16:0) / <i>Palmitinska</i>	23.60±0.60 ^c	23.81±0.57	24.94±0.94	24.23±0.75	***
Heptadecanoic (C17:0) / <i>Heptadekanska</i>	0.18±0.02 ^a	0.14±0.01 ^b	0.15±0.02 ^b	0.13±0.01 ^c	***
Stearic (C18:0) / <i>Stearinska</i>	8.13±0.45 ^b	8.37±0.62 ^{ab}	8.69±0.43 ^a	8.03±0.41 ^b	*
ΣSFA	32.14±0.52 ^b	32.55±0.73 ^b	34.06±1.06 ^a	32.69±0.83 ^b	***
Palmitoleic (C16:1) / <i>Palmitoleinska</i>	2.23±0.38 ^b	2.31±0.32 ^b	3.13±0.36 ^a	3.07±0.31 ^a	***
Heptadecenoic (C17:1) / <i>Cis-10-heptadekanska</i>	0.22±0.01 ^a	0.19±0.01 ^{bc}	0.20±0.01 ^b	0.18±0.01 ^c	***
Oleic (C18:1n9) / <i>Oleinska</i>	39.78±1.82 ^c	42.34±1.25 ^b	42.53±1.40 ^b	47.08±1.66 ^a	***
Eicosenoic (C20:1) / <i>Cis-10-eikozenska</i>	0.26±0.02 ^b	0.26±0.02 ^b	0.35±0.06 ^a	0.33±0.04 ^a	***
ΣMUFA	42.49±1.86 ^c	45.10±1.24 ^b	46.22±1.16 ^b	50.66±1.57 ^a	***
Linoleic (C18:2n6) / <i>Linolna</i>	21.58±2.00 ^a	19.03±1.62 ^b	15.07±0.64 ^c	12.56±1.48 ^d	***
γ-Linolenic (C18:3n6) / <i>γ-linolenska</i>	0.11±0.01 ^a	0.12±0.02 ^a	0.10±0.01 ^{ab}	0.09±0.01 ^b	**
Eicosadienoic (C20:2n6) / <i>Eikozadienska</i>	0.34±0.04 ^b	0.24±0.02 ^c	0.38±0.06 ^a	0.20±0.03 ^d	***
Eicosatrienoic (C20:3n6) / <i>Eikozatrienska</i>	0.23±0.04 ^b	0.17±0.01 ^c	0.29±0.06 ^a	0.16±0.03 ^c	***
Arachidonic (C20:4n6) / <i>Arahidonska</i>	2.14±0.13 ^{ab}	2.13±0.20 ^b	2.27±0.11 ^a	2.03±0.15 ^b	*
Σn-6 PUFA	24.40±2.05 ^a	21.69±1.73 ^b	18.11±0.71 ^c	15.04±1.60 ^d	***
α-Linolenic (C18:3n3) / <i>α-linolenska</i>	0.39±0.05 ^b	0.21±0.03 ^c	0.57±0.08 ^a	0.60±0.11 ^a	***
Eicosapentaenoic (C20:5n3) / <i>Eikozapentaenska</i>	0.06±0.02 ^c	0.07±0.01 ^b	0.11±0.03 ^a	0.09±0.02 ^{ab}	***
Docosahexaenoic (C22:6n3) / <i>Dokozaheksaenska</i>	0.49±0.03 ^c	0.33±0.07 ^d	0.93±0.11 ^a	0.85±0.07 ^b	***
Σn-3 PUFA	0.94±0.08 ^b	0.61±0.09 ^c	1.61±0.17 ^a	1.54±1.18 ^a	***

*P < 0.05; ** P < 0.01; *** P < 0.001

Table 2 presents the values of health lipid indices in eggs. The values of qualitative indices refer to the following ratios: Σ PUFA/ Σ SFA, Σ n-6 PUFA/ Σ n-3 PUFA, LA/ALA, and EPA+DHA. The calculated nutritional indices are as follows: NVI, AI, TI, SI, and hHI. Lipid metabolism in eggs of different groups is shown by the indices of EI, THI, Δ 9-desaturase, Δ 5+ Δ 6-desaturase, and KAI.

Unsaturated fatty acids are considered antithrombogenic because they inhibit plaque accumulation and lower levels of phospholipids, cholesterol, and esterified fatty acids (Monteiro et al., 2018; Omri et al., 2019). Therefore, the consumption of products with a low AI can affect a reduction of total cholesterol and LDL cholesterol in human plasma (Yurchenko et al., 2018). In a research by Untea et al. (2020), the determined values of AI in the chicken eggs ranged from 0.58 to 0.61, which higher was than the values obtained in this research. The same authors also published the results for the TI ranging from 0.92 to 0.97, which corresponded to our research results. The TI reflects the thrombogenic potential of fatty acids, which defines the ratio of prothrombogenic fatty acids (myristic, palmitic, and stearic) and the antithrombogenic fatty acids (MUFA, n-6 and n-3 PUFA). Watson et al. (2009) pointed out that a lower TI could help in lowering the risk of atrial fibrillation and other heart diseases. The atherogenic and thrombogenic indices indicate the relations between the SFAs with a proatherogenic effect and the UFAs with an antiatherogenic effect (Attia et al., 2015; Khalili Tilami & Kouřimská, 2022) fats, minerals, vitamins and bioactive components. We studied the effects of source of eggs in the retail market on fatty acids, lipid profiles and antioxidant status in eggs. Methods: Eggs from four sources named A, B, C, and D in the retail market were collected to determine fatty acid, total lipid, and cholesterol profiles; hypocholesterolemic, atherogenic and thrombotic indices; antioxidant status (e.g., of malondialdehyde).

In this paper, the AI and TI differed significantly between the egg groups ($P < 0.001$). The lowest value

was obtained in group A and the highest value in group C (0.359:0.392, i.e., 0.918:1.004, respectively). Krawczyk et al. (2015) reported that the AI in eggs ranged from 0.37 to 0.41 and the TI from 0.86 to 0.91, depending on the laying hens' diet. In this research, considering the fact that the eggs were produced on four different farms, it could be assumed that the different feeding regimes, as well as other on-farm factors, influenced the nutritional indices. The values of AI and TI in the fresh egg yolks reported by Tadesse et al. (2023) were higher than the values obtained in this research.

In this research, the nutritional-value indices (NVI) were lower in group A than in the remaining egg groups. The hHI value was opposed to the values of AI and TI. Attia et al. (2024) confirmed that a linseed cake added to the laying hens' diet (in the amount of 0.5 and 1%) affected the content of specific fatty acids, as well as the values of the egg-related health-lipid indices. A greater amount of linseed cake in the hens' diet influenced a reduction of AI and TI values and an increase in the hHI values. The hHI values in the egg yolks reported by Tadesse et al. (2023) lipid health indices, and oxidative stability of eggs, requiring adequate research attention. This study investigated the effects of feeding a moderate level of flaxseed (FS ranged from 2.38 to 2.56, which was in accordance with our results. In a research by Kralik G. et al. (2024), the hHI in eggs ranged from 2.976 to 3.068, which was slightly higher than the values calculated in this research. The results published by Batkowska et al. (2021) their fatty acid (FA) proved that, unlike soybean oil, linseed oil supplemented to the hens' diet lowered the AI and TI and increased the hHI in eggs. These authors also pointed out that linseed oil increased the portion of UFA/SFA, which influenced the nutritional-value index. The hHI is suitable when considering the fatty acids' effect on cholesterol metabolism, and from a nutritional point of view, the high values of this index are desirable.

Table 2. Health lipid indices in egg yolks

Tablica 2. Zdravstveni lipidni indeksi u žumanjcima jaja

Health lipid indices / Zdravstveni lipidni indeksi	A	B	C	D	P value / P vrijednost
Qualitative lipid indices / Kvalitativni lipidni indeksi					
Σ PUFA/ Σ SFA	0.789 $\pm$ 0.07 ^a	0.685 $\pm$ 0.06 ^b	0.581 $\pm$ 0.03 ^c	0.507 $\pm$ 0.06 ^d	***
Σ n-6 PUFA/ Σ n-3 PUFA	25.95 $\pm$ 2.03 ^b	35.55 $\pm$ 5.55 ^a	11.23 $\pm$ 1.19 ^c	9.76 $\pm$ 0.57 ^d	***
LA/ALA	55.89 $\pm$ 4.75 ^b	90.59 $\pm$ 11.56 ^a	26.88 $\pm$ 4.21 ^c	21.17 $\pm$ 1.99 ^c	***
EPA+DHA	0.522 $\pm$ 0.04 ^c	0.399 $\pm$ 0.07 ^d	1.041 $\pm$ 0.11 ^a	0.944 $\pm$ 0.09 ^b	***
Nutritional lipid indices / Nutritivni lipidni indeksi					
Nutritional value index (NVI) / Indeks nutritivne vrijednosti	2.03 $\pm$ 0.11 ^b	2.13 $\pm$ 0.08 ^b	2.06 $\pm$ 0.13 ^b	2.27 $\pm$ 0.12 ^a	***
Atherogenic index (AI) / Aterogeni indeks	0.359 $\pm$ 0.01 ^c	0.366 $\pm$ 0.01 ^{bc}	0.392 $\pm$ 0.02 ^a	0.375 $\pm$ 0.01 ^b	***
Thrombogenic index (TI) / Trombogeni indeks	0.918 $\pm$ 0.02 ^c	0.948 $\pm$ 0.03 ^{bc}	1.004 $\pm$ 0.04 ^a	0.971 $\pm$ 0.03 ^b	***
Saturation index (SI) / Saturacijski indeks	0.470 $\pm$ 0.01 ^b	0.481 $\pm$ 0.01 ^b	0.513 $\pm$ 0.02 ^a	0.484 $\pm$ 0.01 ^b	***
Hypo/hypercholesterolemic index (hHI) / Hipo/hiperkolesterolni indeks	2.74 $\pm$ 0.10 ^a	2.69 $\pm$ 0.09 ^{ab}	2.47 $\pm$ 0.14 ^c	2.59 $\pm$ 0.12 ^b	***
Health-promoting index (HPI) / Indeks promicanja zdravlja	2.78 $\pm$ 0.09 ^a	2.73 $\pm$ 0.09 ^{ab}	2.55 $\pm$ 0.13 ^c	2.66 $\pm$ 0.12 ^b	***
Metabolic lipid indices / Nutritivni lipidni indeksi					
Elongase index (EI) / Indeks elongaze	34.49 $\pm$ 2.46	35.21 $\pm$ 2.94	34.88 $\pm$ 2.14	33.09 $\pm$ 2.07	n. s.
Thioesterase index (THI) / Indeks tioesteraze	12310.7 $\pm$ 1804.3 ^a	11213.5 $\pm$ 1077.2 ^{ab}	10876.6 $\pm$ 1431.1 ^b	10086.4 $\pm$ 988.4 ^b	**
Δ 9-desaturase Δ 9-desaturaza	0.56 $\pm$ 0.01 ^c	0.58 $\pm$ 0.01 ^{ab}	0.57 $\pm$ 0.01 ^b	0.61 $\pm$ 0.01 ^a	***
Δ 5 + Δ 6-desaturase Δ 5 + Δ 6-desaturaza	0.13 $\pm$ 0.01 ^b	0.14 $\pm$ 0.01 ^b	0.22 $\pm$ 0.01 ^a	0.23 $\pm$ 0.01 ^a	***
Kinetic activity index (KAI) / Indeks kinetičke aktivnosti	2.43 $\pm$ 0.18 ^b	2.88 $\pm$ 0.31 ^a	2.84 $\pm$ 0.24 ^a	2.59 $\pm$ 0.17 ^b	***

n. s. P > 0.05; *P < 0.05; ** P < 0.01; *** P < 0.001

The metabolic indices differ between the egg groups (the highest THI and EI values were calculated in the eggs of group A and B, respectively, while the lowest values were confirmed for the eggs in group D). The enzymatic activities of Δ 9-desaturase and Δ 5+ Δ 6-desaturase differed with respect to the egg origin. The highest values were determined in the D eggs and the lowest values in the A eggs (0.61: 0.56 and 0.23: 0.13, respectively). The kinetic activity index (KIA)

reached its highest value in the B eggs and the lowest value in the A eggs (2.88:2.43, respectively).

The weighted value of health-lipid indices (15 different indicators) was as follows: A = 42, B = 38, C = 32, and D = 38. The ranking of health-lipid indices according to a weight size established a qualitative egg-group order of A, B = D, and C.

Table 3 illustrates a calculated correlation coefficient (r) between the health-lipid indices.

Table 3. A correlation coefficient (r) between the health-lipid indices

Tablica 3. Koeficijent korelacije (r) između zdravstvenih lipidnih indeksa

	$\Sigma n-6 / \Sigma n-3$ PUFA	Σ PUFA/ Σ SFA	LA/ ALA	EPA+ DHA	NVI	AI	TI	SI	hHI	HPI	EI	THI	$\Delta 9$ -desaturase	$\Delta 5 + \Delta 6$ -desaturase	KAI
$\Sigma n-6 / \Sigma n-3$ PUFA	1														
Σ PUFA/ Σ SFA	0.63	1													
LA/ALA	0.98	0.55	1												
EPA+DHA	-0.94	-0.58	-0.91	1											
NVI	-0.24	-0.52	-0.15	0.17	1										
AI	-0.41	-0.57	-0.40	0.42	-0.40	1									
TI	-0.42	-0.75	-0.36	0.42	-0.08	0.91	1								
SI	-0.34	-0.57	-0.30	0.37	-0.29	0.93	0.97	1							
hHI	0.45	0.61	0.45	-0.46	0.37	-0.99	-0.90	-0.91	1						
HPI	0.40	0.58	0.39	-0.41	0.39	-1.00	-0.91	-0.92	1.00	1					
EI	0.19	0.02	0.29	-0.15	0.40	-0.31	0.04	0.06	0.35	0.32	1				
THI	0.36	0.53	0.32	-0.30	-0.07	-0.52	-0.45	-0.37	0.51	0.53	0.30	1			
$\Delta 9$ -desaturase	-0.42	-0.65	-0.36	0.34	0.88	-0.19	-0.02	-0.25	0.13	0.17	-0.07	-0.29	1		
$\Delta 5 + \Delta 6$ -desaturase	-0.88	-0.86	-0.81	0.87	0.42	0.49	0.60	0.47	-0.53	-0.49	-0.07	-0.42	0.57	1	
KAI	0.03	-0.29	0.22	0.08	0.28	0.11	0.30	0.28	-0.08	-0.10	0.46	-0.07	0.11	0.23	1

An analysis of correlation coefficients between the selected health indices from Table 3 is presented in Table 4. Since they were connected, the evaluation of correlation coefficients pointed out a need for comparison of qualitative indices such as the ratios Σ PUFA/ Σ SFA,

$\Sigma n-6$ PUFA/ $\Sigma n-3$ PUFA, and LA/ALA alongside other investigated health-lipid indices. An overview of the direction and the strength of correlations (Table 4) can be useful for the researchers when comparing their own results.

Table 4. The evaluation of correlation coefficients

Tablica 4. Vrijednovanje koeficijenata korelacije

Correlation strength / jakost veze	Positive correlation / pozitivna korelacija	Negative correlation / negativna korelacija
Very weak / vrlo slaba $r \leq 0.30$	Σ PUFA/ Σ SFA: EI $\Sigma n-6$ PUFA/ $\Sigma n-3$ PUFA: EI, KAI LA/ALA: KAI, EI	Σ PUFA/ Σ SFA: KAI; $\Sigma n-6$ PUFA/ $\Sigma n-3$ PUFA: NVI; LA/ALA: NVI, SI
Medium / srednja $r = 0.31$ to 0.50	$\Sigma n-6$ PUFA/ $\Sigma n-3$ PUFA: hHI, HPI, THI LA/ALA: hHI, HPI, THI	$\Sigma n-6$ PUFA/ $\Sigma n-3$ PUFA: AI, TI, SI, $\Delta 9$ -desaturase LA/ALA: AI, TI, $\Delta 9$ -desaturase
Strong / jaka $r = 0.51$ to 0.80	Σ PUFA/ Σ SFA: $\Sigma n-6$ PUFA/ $\Sigma n-3$ PUFA, LA/ALA, hHI, HPI, THI	Σ PUFA/ Σ SFA: EPA+DHA, NVI, AI, TI, SI, $\Delta 9$ -desaturase
Very strong / vrlo jaka $r = 0.81$ to 1.00	LA/ALA: $\Sigma n-6$ PUFA/ $\Sigma n-3$ PUFA	Σ PUFA/ Σ SFA: $\Delta 5 + \Delta 6$ desaturase; $\Sigma n-6$ PUFA/ $\Sigma n-3$ PUFA: EPA+DHA, $\Delta 5 + \Delta 6$ desaturase; LA/ALA: EPA+DHA, $\Delta 5 + \Delta 6$ desaturase

CONCLUSION

This research, conducted on retail table eggs (the groups A, B, C, and D), confirmed the different fatty-acid profiles, as well as the different values of health-lipid indices. The highest-ranked qualitative indices (Σ PUFA/ Σ SFA, $\Sigma n-6 / \Sigma n-3$ PUFAs, and LA/ALA) were obtained in the egg lipids in group D, while EPA+DHA were the highest-ranked in the egg lipids in group C. The most nutritional indices (AI, TI, SI, and hHI) were the highest-ranked in the eggs of group A, except for the HPI,

which was the highest-ranked in the eggs of group D. The metabolic indices THI and EI were the most favorable in the eggs of group A and B, respectively, while the most favorable $\Delta 9$ -desaturase and $\Delta 5 + \Delta 6$ -desaturase were detected in the eggs of group D. The KAI was the highest-ranked in the eggs of group B. A very strong positive correlation ($r = 0.97$) between the selected health indices revealed that a reduction of the ratio of LA/ALA affected a reduction of the ratio $\Sigma n-6 / \Sigma n-3$ PUFAs. When compared to the metabolic indices of $\Delta 5 + \Delta 6$

desaturase, a very strong negative correlation was determined between the qualitative indices Σ PUFA/ Σ SFA, Σ n-6/ Σ n-3 PUFAs, and LA/ALA ($r = -0.85$; $r = -0.88$, and $r = -0.81$, respectively). The established correlation indicated that a reduction of the ratio of qualitative indices (Σ PUFA/ Σ SFA, Σ n-6/ Σ n-3 PUFAs, and LA/ALA) affected an increase in the values of metabolic health-lipid index ($\Delta 5 + \Delta 6$ desaturase). By ranking the values of qualitative, nutritional, and metabolic indices obtained in this research, the egg producers were ranked as A, B = D, and C. Accordingly, a recommendation for consumers would be to buy and consume the eggs from the producer A. That producer applied the most acceptable laying-hen feeding on his farm, which was confirmed by the most favorable lipid-index quality in the eggs.

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PROFIL MASNIH KISELINA I ZDRAVSTVENI INDEKSI LIPIDA U JAJIMA NA TRŽIŠTU

SAŽETAK

U radu se istražuju profil masnih kiselina te kvalitativni (Σ PUFA/ Σ SFA, Σ n-6 PUFA/ n-3 PUFA, LA/ALA, EPA+DHA), nutritivni (INV, AI, TI, SI, hHI, HPI) i metabolički (EI, THI, Δ 9-desaturaza, Δ 5+ Δ 6 desaturaze, KAI) indeksi u jajima podrijetlom od četiriju proizvođača (A, B, C i D). Za potrebe analize u trgovačkome je centru kupljeno četrdeset jaja L razreda od četiri proizvođača s područja Republike Hrvatske. Na osnovi rezultata profila masnih kiselina u žumanjcima jaja izračunani su zdravstveni lipidni indeksi. Najpovoljniji kvalitativni indeksi ustanovljeni su u lipidima jaja D razreda, s izuzetkom EPA+DHA indeksa. Većina nutritivnih indeksa bila je povoljnija u jajima A skupine u odnosu na jaja B, C i D skupina. Metabolički indeksi THI i EI bili su povoljniji u jajima A odnosno B skupine, dok su Δ 9-desaturaza, kao i indeksi Δ 5+ Δ 6 desaturaze, bili povoljniji u jajima D skupine. Utvrđena je i korelacija između odabranih zdravstvenih lipidnih indeksa. Pozitivna vrlo jaka ($r = 0,97$) povezanost utvrđena je između kvantitativnih lipidnih indeksa, i to LA/ALA u odnosu na omjer Σ n-6 PUFA/ Σ n-3 PUFA, dok je negativna vrlo jaka povezanost utvrđena između kvalitativnih indeksa Σ PUFA/ Σ SFA, Σ n-6 PUFA/ Σ n-3 PUFA i LA/ALA u odnosu na metaboličke indekse Δ 5+ Δ 6 desaturaze. Rangiranje skupina jaja na osnovi svih zdravstvenih indeksa pokazalo je kalkulatívne vrijednosti A = 42, B i D = 38 i C = 32.

Ključne riječi: konzumna jaja, masne kiseline, zdravstveni lipidni indeks

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