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Enrichment of table eggs with functional ingredients

Obogaćivanje konzumnih jaja funkcionalnim sastojcima

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Abstract

The research was conducted on 120 laying hens of Tetra SL provenience, which were divided into two experimental groups (K and P), with 60 laying hens in each group (12 replications, 5 hens per replication). The control group was fed standard diet, while laying hens of experimental groups were given modified diet supplemented with 5% of oil mixture, 0.5 mg/kg organic selenium, 200 mg/kg lutein and 200 mg/kg vitamin E. Portion of n-3 PUFA was significantly better in designed eggs than in conventional eggs (3.76% and 1.69%, P<0.001). Ratio of n-6/n-3 PUFA in experimental group was more than twice as favorable as in the control group (5.91 vs. 13.34; P<0.001). Content of selenium, lutein and vitamin E in designed eggs was statistically significantly higher than in conventional eggs (P<0.001). The content of selenium in egg yolks in conventional eggs was 0.053 µg/g and in designed eggs 0.143 µg/g, and in egg whites 0.387 µg/g and 0.662 µg/g, respectively (P<0.001). Lutein content increased from 12.44 µg/g inconventional eggs to 104.95 µg/g in designed eggs, while vitamin E increased from 12.5 µg/g to 19.82 µg/g egg yolk.

Keywords: designed eggs, lutein, omega-3 fatty acids, selenium, vitamin E

Sažetak

U istraživanju je korišteno 120 nesilica provenijencije Tetra SL, podijeljenih u dvije pokusne skupine (K i P) sa 60 nesilica po skupini (12 ponavljanja po 5 komada). Kontrolna skupina konzumirala je standardnu smjesu dok su nesilice pokusne skupine dobivale modificiranu smjesu u koju je dodano 5% mješavine ulja, 0,5 mg/kg organskog selena, 200 mg/kg luteina i 200 mg/kg vitamina E. Udio n-3 PUFA bio je značajno bolji kod dizajniranih jaja u odnosu na konvencionalna jaja (3,76% i 1,69%, P<0,001). Omjer n-6/n-3 PUFA u pokusnoj skupini bio je za više od dva puta povoljniji nego u kontrolnoj skupini (5.91 vs. 13,34; P<0.001). Sadržaj selena u

žumanjku i bjelanjku kod konvencionalnih jaja bio je 0.053 µg/g, a dizajniranih 0.143 µg/g, odnosno 0.387 µg/g i 0.662 µg/g (P<0.001). Sadržaj luteina povećan je od 12.44 µg/g kod konvencionalnih jaja na 104.95 µg/g dizajniranih jaja, a vitamina E također je povećan od 12.5 µg/g na 19.82 µg/g žumanjka.

Ključne riječi: dizajnirano jaje, lutein, omega-3 masne kiseline, selen, vitamin E

Introduction

Eggs are frequently used in human nutrition and represent one of the best and the cheapest sources of high quality protein. Eggs are also low in calories, but rich in vitamins and minerals. Eggs are attractive for scientists because they can be enriched with different nutricines. According to consumers' demands, it is possible to produce "designed eggs" enriched with one or more functional ingredients. On the world market, the most represented are eggs enriched with omega-3 fatty acids known as „Greek eggs" (Simopoulos and Salem, 1992), eggs enriched with several nutritive ingredients (omega-3, selenium, vitamins D, E, B_{12} and folic acid), known as VITA Eggs by Freshly Foods (Surai and Sparks, 2001), then eggs enriched with vitamin E and omega-3 fatty acids „Omega Plus" (Singh et al., 2012), as well as eggs enriched with selenium (first produced in 1998 at the Scottish Agriculture College), the biggest producer of which is nowadays Russia (Surai, 2000). Eggs enriched with vitamin E need to have higher concentration of that vitamin in their yolks when compared with conventionally produced eggs (Shahriar et al., 2008). Fats are the main content of yolk (Ahn et al., 1997), which stability and content of lipid fraction affect the quality and sensory traits of eggs (Franchini et al., 2002). Supplementation of Se can reduce the need for vitamin E in feed. If supplementation of Se ranges between 0.1-0.3 mg/kg (Pappas et al., 2005) or more (Haug et al., 2007; Mohiti-Asli et al., 2010) then vitamin E can be supplemented in the amount of 10-60 mg/kg of feed. The increased Se in feed resulted in the increased concentration of Se in egg yolks. Lutein, a fat soluble plant pigment, is added to laying hens' diet because of its antioxidative traits (Sindhu et al., 2010), its ability to accumulate in yolks and to increase yolk color intensity (Leeson et al., 2007; Skřivan et al., 2015).

The aim of presented research was to produce "designed egg" enriched with n-3 PUFA, selenium, lutein and vitamin E. By increasing the content of n-3 PUFA and other nutricines in eggs, the foodstuff was produced to represent significant contribution to the intake of functional ingredients in human nutrition.

Materials and methods

The research was performed on 120 laying hens of the Tetra SL hybrid. Experiment lasted for 5 weeks. The experiment started with formation of control (K–conventional eggs) and experimental (P–designed eggs) groups. Each group consisted of 12 cages with 5 laying hens per cage, i.e. 60 laying hens per group. The control mixture contained standard feedstocks used in mixtures for laying hens, while in the experimental mixture the triticale fraction was replaced by 5% oil mixture (0.75% fish oil, 1% flaxseed oil, 2% reptile oil, 1.25% soy oil), 0.5 mg/kg of organic selenium, 200

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mg/kg of lutein and 200 mg/kg of vitamin E. The control mixture contained 19% of crude protein and 11.15 MJ ME/kg, and modified mixture 20.3% of crude protein and 11.45 MJ ME/kg. Both groups of laying hens were kept in the same facility, and microclimatic conditions were corresponding to the requirements for this sort of poultry. Eggs were collected manually every day. Feeding and watering was automatic.

Fatty acids (FA) were determined on a total of 12 egg yolks, i.e. on 6 yolks per each experimental group. Homogenized samples' fat content was extracted with the method of Folch et al. (1957). All solvents used were ultrapure-grade by Sigma-Aldrich (Schnelldorf, Germany), and 100 mg/L butylated hydroxitoluene was added to the extraction mixture (chloroform/methanol 2/1 vol/vol) as antioxidant. After this, fatty acid containing lipids were transmethylated by the base-catalyzed sodium-methoxide method of Christie (1982). Gas liquid chromatography was performed on a Shimadzu 2010 apparatus (Kyoto, Japan), equipped with a SP-2380 (Supelco, Bellefonte, USA) type capillary column (30 m x 0.25 mm internal diameter, 0.2 μm film) and flame ionization detector. Characteristic operating conditions were: injector temperature: 270 °C, detector temperature: 300 °C, helium flow: 28 cm/sec. The oven temperature was graded: from 80 °C to 205 °C: 2.5 °C/min; 5 min at 205 °C; from 205 °C to 250 °C: 10 °C/min; 5 min at 250 °C. To identify individual FA in the chromatogram, a FA standard mixture (Supelco 37 Component FAME Mix, CRM 47885) was used. Portions of SFA and MUFA, as well as n-6 PUFA and n-3 PUFA acids were shown as a percentage of total fatty acids in the lipids of yolks.

Content of Se in eggs was determined on a total of 40 samples (20 yolks and 20 albumen), i.e. on 10 yolks and 10 albumens per one group. Laboratory equipment and glassware used for the analysis were immerged in a 10% solution of HNO_3 for 24 hours. As a preparation, there was on average 1 g of sample weighed. Then the sample was transferred into a Teflon cuvette for destruction. The sample was poured with 8 ml HNO_3 and 4 ml H_2O_2. After 15 minutes, cuvettes were closed and heated in a microwave oven (CEM, model Mars 5). After digestion in duration of 25 min., content of cuvette was transferred into a 50 ml flask and poured over with distilled water up to the mark. Then 20 ml of sample was taken out from the flask and transferred into another flask of 50 ml volume to be mixed with 20 ml of 36% HCl. Prepared samples were placed into a dryer and kept there for one hour at a temperature of 90 °C. After drying, flask was filled with distilled water up to the mark. When the samples cooled down to a room temperature, the content of selenium was read on a device Perkin Elmer Optima 2100 DV (Davidowski, 1993).

Chemical analysis of vitamin E content in egg yolks was performed on 10 yolks, i.e. on 5 yolks of each experimental group. Firstly, 5 g of yolk was weighed and placed into a 50 ml Erlenmeyer flask. Then 15 ml of methanol was added. The content was left in a dark place at a room temperature for 16 hours and then it was decanted from the Erlenmeyer flask into a glass and left to precipitate. For further analysis, there was supernatant used, which was filtrated through microfilter PTFE 0.45 μm into a vial. The sample prepared in that way was analyzed with liquid chromatography (HPLC system Agilent Technologies 1200 Series with DAD detector). While measuring, it was important that liquid chromatography assured some specific conditions, such as: column - Agilent, Zorbax C18 (2.1 x 100), 1.8 μm, wave length

290 nm (UV area), mobile phase-100% methanol (HPLC cleanliness), retention time for tocopherol 2.707 minutes.

Content of lutein in egg yolks was determined on 20 yolks, i.e. on 10 yolks per each group. Determination of lutein content in yolks was performed according to the method of Leeson and Caston (2004), as follows: 0.5 g of yolk was weighed into a test tube, and then 5 ml acetone was added and strongly mixed on a vortex mixer for 30 seconds. Samples were left in a dark place for 1 hour. Afterwards they were filtered through 0.45 µm membrane filter and 1 ml of acetone extract was transferred into HPLC vial to evaporate slowly by heating. The rest was melted by addition of 1 ml of hexane/ethyl-acetate solution (65:35, v/v) and mixed on a vortex. Analysis of lutein content was performed by passing of prepared samples through RESTEK Viva C18 column (5 µm, 250 x 4.6 mm). Mobile phase was made of mixture of methanol and tetrahydrofuran (THF) 9:1 (v/v). Flow speed was 1 ml/min, duration of analysis was 20 minutes, and wavelength of measurement was 450 nm. Volume of injected sample was 20 µl. Standard curve of lutein was prepared by using standard lutein bought from a company ChromaDex (Irvine, CA, USA). All used chemicals were of HPLC quality.

Effects of dietary treatments on the weight of fresh eggs and their main parts, portions of main parts, indicators of external and internal quality of fresh eggs, profile of fatty acids, as well as on the content of selenium, lutein and vitamin E were determined by using the following model:

$$y_{ijklm} = \mu + T_i + e_{ij}$$

where: y_{ij} = estimated traits; µ = intercept; T_i = fixed effect of treatment i (i=T1; T2); e_{ij} = residual.

The significance of differences between the classes of treatment was tested by Scheffe's method of multiple comparisons using the ANOVA procedure of SAS (SAS Institute Inc., 2000).

Results and discussion

The Table 1 overviews the content of fatty acids in yolk lipids. Designed eggs contained significantly less (P<0.001) saturated fatty acids (SFA) than conventional eggs. Content of the most represented SFA, palmitic (C16:0) and stearic (C18:0), was lower for 20% and 10%, respectively in yolks of designed eggs than of conventional eggs (P<0.05).

Similar content of total n-6 PUFA was determined in both groups (21.75% and 22.13%, P=0.629). If compared to conventional eggs, designed eggs had for about 10% more arachidonic acid (C20:4n-6, P=0.12) in yolks. Yolks of designed eggs contained significantly more (P<0.001) α-linolenic (αLNA, C18:3n3), eicosatrienoic (C20:3n3), eicosapentaenoic (EPA, C20:5n3) and docosahexaenoic (DHA, C22:6n3) acid, but there was no statistically significant difference referring to portion of docosapentaenoic acid (DPA, C22:5n3) when compared to conventional eggs (P>0.05).

Designed eggs had for about two times more αLNA, seven times more EPA and three times more DHA than yolks of conventional eggs. It should be also emphasized that the ratio n-6/n-3 PUFA in the experimental group was more than twice better than in the control group (5.91:13.34; P<0.001). While discussing the research results, synergic activity of antioxidants shall be considered (Zduńczyk et al., 2013).

Table 1. Profile of fatty acids in yolk lipids (% in total fatty acids, $\overline{x}$)

Fatty acid	Conventional eggs	Designed eggs	P value
Myristic (C14:0)	0.25	0.2	0.057
Pentadecanoic (C15:0)	0.06	0.06	0.382
Palmitic (C16:0)	26.55^{a}	21.49^{b}	<0.001
Heptadecanoic (C17:0)	0.18	0.18	0.788
Stearic (C18:0)	8.22	7.58	0.052
Arachidic (C20:0)	0.02	0.02	0.751
Heneicoanoic (C21:0)	0.01	0.01	0.083
Behenic (C22:0)	0.01	-	-
Tricosanoic (C23:0)	0.01^{b}	0.04^{a}	<0.001
Lignoceric (C24:0)	0.01	-	-
SFA	35.34^{a}	29.59^{b}	<0.001
Myristoleic (C14:1)	0.04	0.03	0.219
Palmitoleic (C16:1)	2.31^{a}	1.86^{b}	0.043
Elaidic (18:1n9t)	0.16^{a}	0.07^{b}	<0.001
Oleic (18:1n9c)	37.14^{b}	40.33^{a}	0.005
Octadecenoic isomer A (18:1)	1.11^{b}	2.05^{a}	<0.001
Octadecenoic isomer B (18:1)	0.31	-	-
Eicosenoic (20:1n9)	0.13^{b}	0.19^{a}	0.001
MUFA	41.21^{b}	44.52^{a}	0.004
Linoleic (C18:2n6)	19.69	20.21	0.499
Octadecadienoic isomer A (18:2)	0.07	-	-
Octadecadienoic isomer B (18:2)	0.06	-	-
Octadecadienoic isomer C (18:2)	0.04	-	-
Octadecadienoic isomer D (18:2)	0.03	-	-
γ-linolenic (C18:3n6)	0.11^{a}	0.08^{b}	0.012
c9,t11-CLA	0.02	0.02	0.732
Eicosadienoic (C20:2)	0.13	0.14	0.26
Eicosatrienoic (C20:3n6)	0.15	0.13	0.158
Arachidonic (C20:4n6)	1.34	1.44	0.122
Docosatetraenoic (C22:4n6)	0.10	0.08	0.249
n-6 PUFA	21.75	22.13	0.629
α-linolenic (18:3n3)	0.89^{b}	1.72^{a}	<0.001
Eicosatrienoic (C20:3n3)	0.01^{b}	0.03^{a}	<0.001
Eicosapentaenoic (C20:5n3)	0.01^{b}	0.07^{a}	<0.001
Docosapentaenoic (C22:5n3)	0.09	0.13	0.052
Docosahexaenoic (C22:6n3)	0.68^{b}	1.81^{a}	<0.001
n-3 PUFA	1.69^{b}	3.76^{a}	<0.001
n-6/n-3 PUFA	13.34^{a}	5.91^{b}	<0.001

^{a,b}P<0.05

The Table 2 presents the content of n-3 PUFA in mg/yolk, i.e. in mg/100 g of yolk of conventional eggs and of designed eggs. In 100 g of egg yolk, 1,009 µg/g of n-3 PUFA is introduced into the organism, which is 2.4 times more than that of conventional eggs.

Table 2. Content of fatty acids in egg yolk

	Conventional eggs			Designed eggs		
FA	%* in fatty acids	mg/ yolk	mg/100 g yolk	%* in fatty acids	mg/ yolk	mg/100 g yolk
αLNA	0.89	38.36	222	1.72	78.09	462
EPA	0.01	0.43	2.5	0.07	3.18	18.8
DHA	0.68	29.31	170	1.81	82.17	486
n-3 PUFA	1.69	72.84	422	3.76	170.7	1,009

*Presented on 95.5% of fat content (95.5% are fatty acids), and the rest (4.5%) is cholesterol.

αLNA is the most represented n-3 PUFA in human nutrition and it is also a precursor of long-chain PUFA. In a human organism, possibility of αLNA conversion into EPA and DHA is less than 5% and it depends on concentrations of n-6 PUFA and long-chain PUFA in food (Brenna, 2002). In order to provide for the intake of EPA and DHA, which are important for preservation of blood vessels, digestion and immune system, as well as brain, it is necessary to use other sources of the stated FA through food (Marik and Varon, 2009). Cardiovascular diseases (CVD) are the leading cause of death in many countries. Omega-3 polyunsaturated acids (n-3 PUFA) have a protective role among people whose daily intake higher than 30% of total energy comes from fish and plant oils, so the mortality caused by CVD is lower among that population (Psota et al., 2006). Singh et al. (2012) pointed out the advantages of omega-3 enriched eggs, as follows: reduced risk of cardiac diseases for 50-70%, favorable effect on brain development and function in children and pregnant women, improved supply of cells with oxygen, healing of rheumatoid arthritis and inflammatory damages and improvement of immune responses. Scheideler and Lewis (1997) stated that „regular“ eggs contained 60 mg of n-3 PUFA, and omega-3 enriched eggs had a concentration of up to 350 mg of n-3 PUFA. While considering all the above stated results, it is important to point out that the results of presented research also proved significant increase of total n-3 PUFA in designed eggs.

Content of selenium, lutein and vitamin E in eggs are overviewed in the Table 3. Concentration of selenium in edible parts of eggs was statistically very highly significantly ($P<0.001$) influenced by feeding treatments and egg part. Eggs of experimental group contained more selenium ($P<0.001$) than eggs of the control

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group. Furthermore, there was statistically very highly significantly higher concentration of selenium (P<0.001) in yolks than in albumens. The experimental group had almost two times more selenium in yolks and almost three times more selenium in albumen than the control group.

Table 3. Concentration of selenium, lutein and vitamin E in eggs of control and experimental group ($\overline{x}$)

	Conventional eggs	Designed eggs	P value
Selenium - albumen (µg/g)	0.053^{b}	0.143^{a}	<0.001
Selenium - yolk (µg/g)	0.387^{b}	0.662^{a}	<0.001
Lutein (µg/g)	12.44^{b}	104.95^{a}	<0.001
Vitamin E - yolk (µg/g)	12.5^{b}	19.82^{a}	<0.001

^{a,b}P<0.001

Bourre and Galea (2006) stated that concentration of selenium per 100 g of standard eggs was 7 µg, and of “multi-enriched” eggs was 28 µg. Trend of selenium increase in eggs was the same as in this research (conventional < designed), however, if considering the content of selenium, the stated authors reported lower value of selenium per 100 g of eggs than the value in this research. Attia et al. (2010) recommended 0.4 ppm of organic selenium in feed for laying hens to achieve selenium enriched egg yolks. At that concentration, selenium in feed affected increase of Se in egg yolks for 70.3%. Organic selenium proved more efficient than inorganic, which was also confirmed by researches of Skřivan et al. (2006), Leeson et al. (2008) and Gajčević et al. (2009). Content of lutein in egg yolks of experimental group of laying hens that consumed 200 mg/kg lutein and other functional ingredients was 8.4 times higher than in conventional eggs. According to Landrum and Bone (2001) average daily intake of lutein in adults was less than 1 mg/day, so one egg enriched with average 1.6 mg lutein represents significant contribution to daily intake of lutein. Surai et al. (2000) supplemented feed for laying hens with increased amounts of vitamin E, lutein, selenium and DHA and produced eggs with 7.7 times more selenium, 26.8 times more vitamin E, 15.9 times more lutein and 6.4 times more DHA in comparison to commercial eggs. Level of lutein in eggs in their experiment was 1.9 mg, which was slightly more than in this research. Gjorgovska et al. (2012) supplemented laying hens’ feed with 0.38 mg/kg Se and 0.46 mg/kg Se, and basic feed contained 0.3 mg/kg Se. Average concentration of Se in egg yolks of the control group was 0.7 µg, and in the experimental groups it was 2.92 µg and 4.7 µg.

The Table 3 also presents concentration of vitamin E in egg yolks (µg/g). The control group contained statistically very highly significantly less (P<0.001) vitamin E in egg yolks than the experimental group. Content of vitamin E in the control group was for 1.59 times lower than in the experimental group. Gjorgovska et al. (2011) pointed out

that statistically significantly lower content of vitamin E was determined in yolks of the control than in yolks of the experimental group (supplementation of 100 and 230 mg vitamin E/kg of laying hens' diet, respectively). Grobas et al. (2002), as well as Sujatra and Narahari (2011), reported that concentration of vitamin E in diet followed the trends of increase of the concentration of vitamin E in yolks, which is corresponding to results obtained in this experiement.

Conclusions

This paper investigates the possibility of enriching table eggs with n-3 PUFA, selenium, lutein and vitamin E. For that purpose, laying hens' diets were prepared as of conventional and modified composition. Tested laying hens of Tetra SL provenience were divided into two groups (K and P). Research results proved successful for enriching eggs of the experimental group with the nutricines, as of the following values: omega-3 polyunsaturated fatty acids (% in total fatty acids) in yolk were 2.24 times higher (αLNA 1.93 times, EPA 7 times, DHA 2.66 times), selenium (µg/g) in yolk was 7.3 times higher, in albumen 4.63 times, lutein in yolk (µg/g) was 8.36 times higher and vitamin E (µg/g) in yolk was 1.58 times higher than in the control group. Enriched eggs represent a significant contribution to the intake of nutricines in the human body.

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