The effect of different oil supplementations on laying performance and fatty acid composition of egg yolk

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Paper received June 5, 2007; accepted January 12, 2008

ABSTRACT

The aim of the research was to determine effects of different combinations of rapeseed and fish oil - instead of soybean oil - on the performance of laying hens and on the profile of fatty acids in egg yolks, especially on n-3 polyunsaturated fatty acid (PUFA), when such oils are added to the diet of laying hens. The research was carried out on 90 laying hens of the Hy-Line hybrid ranging in age from 32 to 36 weeks old. Hens were divided into three groups (30 laying hens per groups) and fed with a commercial mixture that contained 17% of crude protein and 11.6 MJ ME. The research lasted for 28 days. The control group (C) was given diets with soybean oil supplemented in the amount of 5%, and experimental groups (E1 and E2) were fed diets that contained a combination of fish and rapeseed oils in different amounts. Diets given to the E1 group contained 3.5% of fish oil and 1.5% of rapeseed oil, while the E2 group was fed diets with 1.5% of fish oil and 3.5% of rapeseed oil. Production characteristics of hens were monitored during the whole experiment. Portion of saturated (SFA) and unsaturated fatty acids (MUFA and PUFA), as well as α-linolenic (αLNA, C:18:3n-3) eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic (DHA, C22:6n-3) acid were shown as a percentage of total fatty acids contained in yolk. There were statistically significant differences (P<0.05) only in the hens’ end weights and laying intensity between the C and E1 group, as well as between the C and E2 group. The portion of SFA in total fatty acids contained in yolk was not statistically significant (P>0.05) among investigated groups. Higher content of MUFA was noticed in both experimental groups, if compared to the control (E1 41.37%: E2 40.72%, C 36.95%, P<0.05). Content of αLNA differed significantly (P<0.001) between E1 and the control group, and the content of DHA differed significantly (P<0.001) between E1 and the control group, as well as between E2 and the control group. Total n-3 PUFA was increased in the E1 group for 2.10, and in the E2 group for 1.41 times than in the control group. The ratio of n-6/n-3 PUFA was the lowest (P<0.05) in egg yolks of the E1 group (4.01), followed by that of the E2 group (6.56), and finally that of the control group (11.08).

Key words: Laying hens, Egg yolk, n-3 PUFA, Fish oil, Rapeseed oil.

RIASSUNTO

EFFETTI DI DIFFERENTI OLI ADDIZIONATI ALLA DIETA DI OVAIOLE SULLE PERFORMANCE DI DEPOSIZIONE E SULLA COMPOSIZIONE IN ACIDI GRASSI DEL TUORLO

Lo scopo di questa ricerca è stato quello di determinare gli effetti di differenti combinazioni di olio di colza e di pesce addizionati alla dieta, alternativi all'olio di soia, sulle performance di galline ovaiole e sul profilo
degli acidi grassi contenuti nel tuorlo, con particolare riferimento al contenuto di acidi grassi polinsaturi n-3 (PUFA). La ricerca è stata condotta su 90 galline ovaiole ibride Hy-Line di 32-36 settimane di età. Gli animali sono stati divisi in tre gruppi (30 per gruppo) ed alimentati con un mangime composto del commercio contenente il 17% di proteine grezze e 11,6 MJ di EM, per una durata di 28 giorni. Al gruppo di controllo (C) è stata somministrata una dieta contenente olio di soia in quantità pari al 5%, mentre i gruppi sperimentali (E1 e E2) sono stati alimentati con diete contenenti olio di colza e di pesce in percentuali diverse. La dieta somministrata al gruppo E1 conteneva il 3,5% di olio di pesce e 1,5% di olio di colza, nel gruppo E2 le percentuali sono state invertite. Le prestazioni produttive delle galline sono state monitorate durante l’intero periodo sperimentale (peso vivo iniziale e finale, quantità di alimento assunto, capacità di ovodeposizione). Sono state rilevate le differenti percentuali di acidi grassi contenuti nel tuorlo: acidi grassi saturi (SFA) ed insaturi (MUFA e PUFA), α-linoleico (αLNA, C:18:3n-3), eicosapentaenoico (EPA, C20:5n-3) e docosaesaenoico (DHA, C22:6n-3). Sono emerse differenze statisticamente significative (P<0,05) per quanto concerne il peso finale e l’intensità di ovodeposizione tra il gruppo C e il gruppo E1, come tra il gruppo C ed il gruppo E2. La differenza di SFA nel contenuto di acidi grassi del tuorlo non è stata significativa tra i gruppi (P>0,05). Un contenuto più alto di MUFA è stato rilevato nei due gruppi sperimentali rispetto a quello di controllo (E1 41,37%, E2 40,72%, C 36,95%, P< 0,05). La quantità di αLNA è stata differente in modo altamente significativo (P<0,001) tra il gruppo E1 e il gruppo di controllo C. Il contenuto in DHA ha evidenziato differenze altezzamente significative (P<0,001) tra i gruppi sperimentali (E1 e E2) ed il gruppo di controllo (C). La quantità totale di n-3 PUFA è aumentata di 2,10 (E1) e 1,41 (E2) volte rispetto al gruppo di controllo C. Il rapporto n-6/n-3 PUFA più basso (P<0,05) si è riscontrato nei tuorli delle uova del gruppo E1 (4,01), seguito dal gruppo E2 (6,56) e infine dal gruppo di controllo (11,08).

Parole chiave: Galline ovaiole, Tuorlo, n-3 PUFA, Olio di pesce, Oli di colza.

Introduction

Over the last two decades consumers’ demands regarding food of dietetic quality have been constantly increasing. Such food also includes eggs. Despite their nutritional value and low price, eggs have a bad reputation due to high cholesterol levels (ca. 250 mg contained in yolk), as well as high content of fat. Scientific studies are aimed at changing fat content by introducing the n-3 fatty acids in egg yolk. Polyunsaturated n-3 fatty acids have beneficial effects on the lowering of plasmatic triglycerides and blood pressure, on healing tumours, thrombosis, as well as on the enhancement of the immune system (Simopoulos, 2000; Calvani and Benatti, 2003). Favourable effects on human health can be achieved by consuming only 0.5 g of n-3 PUFA daily (Mantzioris, 2000). Scientific efforts have been made worldwide to produce foodstuffs of animal origin that have the n-6/n-3 PUFA ratio as close as 1:1, and that are of desirable n-3 PUFA profile (Wijnia, 2005). Alteration of content and ratios of n-6/n-3 PUFA in egg production is achieved mostly through supplementation of linseed and rapeseed oils in the diets of laying hens (Simopoulos, 2000). Supplementation of vegetable oils results in the lowering of the n-6/n-3 PUFA ratio mostly through affecting ratios of linoleic (C18:2n-6, LA) vs α-linolenic (C18:3n-3, α-LNA) acid. The increase in the n-3 PUFA content (eicosapentaenoic, C20:5n-3, EPA, docosapentaenoic C22:5n-3, DPA and especially docosahexaenoic C22:6n-3, DHA) in poultry products is achieved by the supplementation of fish oil and other oils originating from sea organisms (Baucells et al., 2000; Sari et al., 2002; Husveth et al., 2003; Škrtrić et al., 2006; Huyghebaert et al., 2007). The aim of this research was to determine the effect of supplemented soybean oil (control), and a combination of rapeseed and fish oils (experimental groups) in diets on the content of α-LNA, EPA and DHA in egg yolk lipids. In Croatia, soybean oil is used in...
farming. Combining rapeseed oil and fish oil as supplements in the diet of laying hens is interesting because it affects the change in the n-6/n-3 PUFA ratio, as well as the interaction of particular fatty acids during the hens' metabolic processes, which consequently affects their deposition in egg yolks.

Material and methods

The research was carried out on 90 laying hens of the Hy-Line hybrid with ages ranging from 32 to 36 weeks old. Laying hens were divided into three groups. Each group consisted of five cages, and each cage housed six hens (3x5x6). Hens were given a commercial diet of isoprotein and isocaloric composition (Table 1). Hens in the control group (C) were given diets with soybean oil supplemented in the amount of 5%, and the experimental groups (E1 and E2) were fed diets that contained a combination of fish oil and rapeseed oil in different amounts (E1 3.5% of fish oil and 1.5% of rapeseed oil, and E2 1.5% of fish oil and 3.5% of rapeseed oil). Hens were fed and watered ad libitum. During the experiment a lighting regime of 16h light and 8h darkness was applied. The hens were weighed at the beginning and the end of experiment by the Mettler Toledo VIPER SW 15 scales. Eggs used in analysis of fatty acids were collected on the last day of experiment. One egg was taken from each cage. Eggs were weighed, broken and the yolk was homogenized and prepared for analysis of fatty acids. The lipid profile was determined at the Faculty of Animal Science, Department of Chemistry & Biochemistry of the University of Kaposvár by means of gas chromatography in a Chrompack CP-9000 (Csapo et al., 1986). The operating conditions of the gas chromatography were as follows: 0.35 grams of dried egg-yolk was weighed into a flask, 8 cm³ concentrated hydrochloric acid was added and it was boiled for 60 minutes. After cooling down 7 cm³ ethanol was added, then 15 cm³ diethyl-ether following one-minute of shaking. The next extraction was with 15 cm³ benzine (b.p.<60 °C). After phase separation, the organic phase that contains about 150-200 mg fat was separated and evaporated under vacuum on a rotadest. Then 4 cm³ 0.5 M sodium-hydroxide in methanol was added, and boiled in a water bath for 5 minutes. Next 4 cm³ 14% boron-trifluoride in methanol was added and boiled for 3 minutes following the addition of 4 cm³ n-hexane. It was boiled for one minute after which the level of the organic phase was brought to the neck of the flask with saturated sodium-chloride solution. When the phases were separated samples were taken for analysis from the organic phase, and it was dried on sodium sulfate. The fatty acid methyl esters (FAMEs) were separated on a 100 m x 0.25 mm wall coated open tubular (WCOT) column equipped with a CP-SIL 88 (FAME) stationary phase. The quantitation of FAMEs was obtained with a flame ionisation detector (FID) at 270 °C. The temperature of the splitter injector was 270 °C, the carrier gas was helium with the head pressure of 235 kPa. The oven was temperature programmed from 140 °C (10 min.) with 10 °C/min increase up to 235 °C (26 min). The injected volume varied between 0.5 and 2 μl. The instrument was a Chrompack CP 9000 gas chromatograph. Portions of SFA and MUFA, α-LNA, as well as EPA and DHA acids show as a percentage of total fatty acids contained in yolk lipids.

The influence of different feeding treatments (oils) was determined by means of one-way variance analysis (One-Way ANOVA). If there was statistically significant influence of treatments determined (P<0.05), differences between groups were tested by Fischer's LSD-test. Research results were processed by Statistica v.7.1 software (StatSoft, Inc., 2005).
### Results and discussion

Content of fatty acids in soybean oil, rapeseed oil and fish oil, as well as in diets (\%) in total FA was presented in Table 2. Soybean oil contains 55.13\% of n-6 PUFA, out of which 55.08\% LA and 6.29\% αLNA. Rapeseed oil is rich in monounsaturated fatty acids - MUFA (59.45\%), having oleic acid (\(C_{18:1}, OA\)) as the most represented one. Rapeseed oil also contains 24.79\% LA and 6.94\% αLNA. Soybean oil and rapeseed oil do not contain EPA and DHA. Rapeseed oil has been recognised as a rich plant source of LNA. LNA can be converted to longer chain n-3 PUFA, such as EPA, DPA and DHA in poultry through elongation and desaturation pathway, thus enriching the egg yolk with n-3 PUFA (Sim, 1990; Yang et al., 2000; Rowgham et al., 2007). Fish oil is rich in n-3 PUFA, containing 10.30\% EPA and 19.15\% DHA. The fatty acid composition of the diet depended on the type and concentration of oils.

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Table 1. Ingredients and content of diets fed to laying hens.

| Ingredients (%) | Control group (C) | Experimental group (E₁) | Experimental group (E₂) |
|-----------------|-------------------|-------------------------|-------------------------|
| Corn            | 47.66             | 47.66                   | 47.66                   |
| Soybean cake    | 20.02             | 20.02                   | 20.02                   |
| Toasted soybean | 4                 | 4                       | 4                       |
| Sunflower cake  | 6.65              | 6.65                    | 6.65                    |
| Dehydrated alfalfa | 3         | 3                       | 3                       |
| Limestone       | 11.23             | 11.23                   | 11.23                   |
| Mono calcium phosphate | 1.45    | 1.45                    | 1.45                    |
| Animal salt     | 0.36              | 0.36                    | 0.36                    |
| Synthesized methionin | 0.13    | 0.13                    | 0.13                    |
| Soybean oil     | 5                 | -                       | -                       |
| Rapeseed oil    | -                 | 1.5                     | 3.5                     |
| Fish oil        | -                 | 3.5                     | 1.5                     |
| Premix¹         | 0.5               | 0.5                     | 0.5                     |

Calculated composition: crude protein 17\%, crude fat 8.61\%, crude fibres 4.97\%, ash 14.57\%, lysine 0.85\%, methionine 0.40\%, triptophan 0.20\%, arginine 1.12\%, Ca 4.39\%, P usable 0.38\% and ME 11.60 MJ/kg.

¹ Premix (1 kg) contains: vitamin A 2,200,000 U, vitamin D₃ 400,000 U, vitamin E 3000 mg/kg, vitamin K₃ 400 mg/kg, vitamin B₁ 400 mg/kg, vitamin B₂ 800 mg/kg, nicotinic acid 6000 mg/kg, calcium panthotenate 1600 mg/kg, vitamin B₆ 700 mg/kg, vitamin B₁₂ 4000 mcg/kg, folic acid 150 mg/kg, biotin 10 mg/kg, colin chloride 80,000 mg/kg, vitamin C 4000 mg/kg, methionin 40,000 mg/kg, iodine (I) 160 mg/kg, manganese (Mn) 13,600-18,400 mg/kg, zinc (Zn) 12,000 mg/kg, cobalt (Co) 48 mg/kg, iron 5000 mg/kg, copper 500 mg/kg, selenium 30 mg/kg, β-apo ester of carotene acid 200 mg/kg, canthaxanthin 600 mg/kg and a plant base up to 1 kg.

Dietary treatments: C=5\% of soybean oil; E₁=3.5\% of fish oil +1.5\% of rapeseed oil; E₂=1.5\% of fish oil +3.5\% of rapeseed oil.
Table 2. Fatty acid contents of oils and diets (% of total fatty acids).

| Fatty acids         | Soybean oil | Rapeseed oil | Fish oil | C group | E₁ group | E₂ group |
|---------------------|-------------|--------------|----------|---------|----------|----------|
| Lauric              | 12:0        | 0.00         | 0.00     | 0.18    | 0.00     | 0.04     | 0.03     |
| Tridecanoic         | 13:0        | 0.00         | 0.00     | 0.07    | 0.00     | 0.00     | 0.00     |
| Myristic            | 14:0        | 0.05         | 0.07     | 6.11    | 0.35     | 1.16     | 0.68     |
| Pentadecanoic       | 15:0        | 0.01         | 0.03     | 1.07    | 0.04     | 0.20     | 0.15     |
| Palmitic            | 16:0        | 9.88         | 4.07     | 21.45   | 13.05    | 13.94    | 14.27    |
| Heptadecanoic       | 17:0        | 0.03         | 0.06     | 1.16    | 0.08     | 0.26     | 0.20     |
| Stearic             | 18:0        | 3.62         | 2.04     | 5.88    | 2.98     | 3.46     | 3.38     |
| Arachidic           | 20:0        | 0.08         | 0.63     | 0.73    | 0.41     | 0.78     | 0.00     |
| Behenic             | 22:0        | 0.07         | 0.37     | 0.11    | 0.33     | 0.46     | 0.44     |
| Tricosanoic         | 23:0        | 0.00         | 0.00     | 0.23    | 0.00     | 0.00     | 0.00     |
| Lignoceric          | 24:0        | 0.00         | 0.12     | 0.13    | 0.17     | 0.19     | 0.22     |
| Σ SFA               | 13.74       | 7.39         | 37.12    | 17.41   | 20.50    | 19.38    |
| Palmitoleic         | 16:1        | 0.04         | 0.23     | 5.09    | 0.05     | 0.83     | 0.51     |
| Heptadecenoic       | 17:1        | 0.02         | 0.06     | 0.36    | 0.34     | 0.32     | 0.31     |
| Elaidic             | 18:1t       | 0.00         | 0.00     | 0.23    | 0.14     | 0.21     | 0.23     |
| Oleic               | 18:1c       | 25.12        | 57.59    | 12.09   | 24.17    | 28.79    | 25.59    |
| Eicosaenoic         | 20:1        | 0.21         | 1.26     | 1.81    | 0.20     | 0.70     | 0.55     |
| Erucic              | 22:1        | 0.00         | 0.19     | 0.34    | 0.14     | 0.11     | 0.14     |
| Nervonic            | 24:1        | 0.00         | 0.12     | 1.11    | 0.00     | 0.17     | 0.09     |
| Σ MUFA              | 25.39       | 59.45        | 21.03    | 25.04   | 31.13    | 27.42    |
| Linoleic            | 18:2n6      | 55.08        | 24.79    | 2.60    | 53.41    | 39.10    | 45.29    |
| γ-linolenic         | 18:3n6      | 0.05         | 0.09     | 0.34    | 0.00     | 0.08     | 0.06     |
| Eicosadienoic       | 20:2n6      | 0.00         | 0.11     | 2.33    | 0.00     | 0.15     | 0.10     |
| Eicosatrienoic      | 20:3n6      | 0.00         | 0.00     | 0.52    | 0.05     | 0.16     | 0.05     |
| Arachidonic         | 20:4n6      | 0.00         | 0.00     | 1.64    | 0.00     | 0.19     | 0.11     |
| Docosadienoic       | 22:2n6      | 0.00         | 0.00     | 0.82    | 0.00     | 0.10     | 0.06     |
| Σ n-6 PUFA          | 55.13       | 25.02        | 8.25     | 53.46   | 39.79    | 45.67    |
| α-linolenic         | 18:3n3      | 6.29         | 6.94     | 1.87    | 4.08     | 4.06     | 4.85     |
| Eicosatrienoic      | 20:3n3      | 0.00         | 0.00     | 0.00    | 0.00     | 0.05     | 0.09     |
| Eicosapentaenoic    | 20:5n3      | 0.00         | 0.00     | 10.30   | 0.00     | 1.41     | 0.83     |
| Docosapentaenoic    | 22:5n3      | 0.00         | 0.00     | 1.51    | 0.00     | 0.30     | 0.08     |
| Docosahexaenoic     | 22:6n3      | 0.00         | 0.00     | 19.15   | 0.00     | 2.75     | 1.67     |
| Σ n-3 PUFA          | 6.29        | 6.94         | 32.83    | 4.08    | 8.58     | 7.53     |
| Σ n-6 PUFA/Σ n-3 PUFA| 8.76       | 3.61         | 0.25     | 13.10   | 4.64     | 6.07     |

*Dietary treatments: C=5% of soybean oil; E₁=3.5% of fish oil +1.5% of rapeseed oil; E₂=1.5% of fish oil +3.5% of rapeseed oil.*
Diets fed to hens in the control contained less saturated fatty acids (SFA) than diets of the experimental groups $E_2$ and $E_1$ (17.41%:19.38%:20.50%, respectively) and less MUFA (25.04%:27.42%:31.13%, respectively). In the control diet, there was no presence of EPA and DHA, unlike in the diets of the experimental groups. Content of EPA and DHA in the diets of the $E_1$ group was 1.41% and 2.75%, respectively, and of the $E_2$ 0.83% and 1.67%, respectively. Diets fed to the $E_1$ group contained more EPA and DHA than diets given to the $E_2$ group due to the higher portion of fish oil with respect to rapeseed oil (3.5%:1.5%). When compared to the C and $E_1$ groups (4.08% and 4.06%, respectively), a higher content of α-LNA in the hens’ diets was determined in the $E_2$ group (4.85%) because of the higher content of rapeseed oil with respect to fish oil (3.5%:1.5%). Stated facts point out that the $E_1$ group consumed a diet with a higher content of n-3 PUFA than did the $E_2$ and C groups (8.23%:7.35%:4.08%, respectively).

Ratios of n-6/n -3 PUFA were closer in the $E_1$ and $E_2$ groups than in the C group (4.64 and 6.07:13.1). Production parameters of hens were shown in Table 3. At the end of experiment, the weight of hens in the control was statistically significantly lower ($P<0.05$) than the weight of hens in the experimental groups. Hens of C and $E_1$ groups consumed 120 g of food daily, while the $E_2$ group had 119 g of food daily. Laying intensity was higher in the C group (93.10%) than in the $E_1$ and $E_2$ (89.52% and 88.21%, respectively; $P<0.05$). Cherian and Sim (1997) and Meluzzi et al. (2000) did not determine the effect of oil sources and different amounts of vitamin E on laying hens’ performances, such as body weight, food intake and laying percentage. Filardi et al. (2005) stated that soybean oil and rapeseed oil did not affect egg production and food conversion. On the contrary, Celebi and Utlu (2006) pointed out that dietary fat supplementation affected food intake, egg production and food efficiency ($P<0.05$). Brake (1990) determined that ani-

### Table 3. Production parameters.

| Parameter                  | C group $\bar{x}$±SD | $E_1$ group $\bar{x}$±SD | $E_2$ group $\bar{x}$±SD | $P$ value |
|----------------------------|----------------------|--------------------------|--------------------------|-----------|
| Hens’ weight at the beginning of experiment (g) (n=30) | 1964.90 ± 109.19  | 2044.00 ± 164.64         | 2044.03 ± 153.29         | 0.054     |
| Hens’ weight at the end of experiment (g) (n=30)    | 1971.93b ± 122.17    | 2092.96a ± 144.00        | 2073.03a ± 198.08        | 0.008     |
| Eggs per hen (n.)          | 26.06 ± 3.25         | 25.06 ± 4.02             | 24.70 ± 3.55             | 0.286     |
| Laying intensity (%)       | 93.10 ± 2.43a        | 89.52 ± 2.92b            | 88.21 ± 1.99b            | 0.005     |
| Food consumption (g/day)   | 120 ± 10.15          | 120 ± 12.10              | 119 ± 11.50              | 0.357     |

$a$, $b$: Means with different letters indicate significant differences between groups ($P<0.05$).

Dietary treatments: $C=5\%$ of soybean oil; $E_1=3.5\%$ of fish oil $+1.5\%$ of rapeseed oil; $E_2=1.5\%$ of fish oil $+3.5\%$ of rapeseed oil.
Table 4. Fatty acid contents of egg yolk (% of total fatty acids).

| Fatty acids        | Control group (C) | Experimental group (E1) | Experimental group (E2) |
|--------------------|------------------|-------------------------|-------------------------|
| Myristic 14:0      | 0.21             | 0.32                    | 0.31                    |
| Pentadecanoic 15:0 | 0.07             | 0.18                    | 0.11                    |
| Palmitic 16:0      | 22.09            | 22.04                   | 22.52                   |
| Heptadecanoic 17:0 | 0.26             | 0.43                    | 0.34                    |
| Stearic 18:0       | 8.95             | 8.10                    | 8.18                    |
| Behenic 22:0       | 0.04             | 0.11                    | 0.00                    |
| Tricosanoic 23:0   | 0.03             | 0.00                    | 0.00                    |
| Σ SFA              | 31.65            | 31.18                   | 31.46                   |
| Palmitoleic 16:1   | 1.29             | 1.84                    | 1.71                    |
| Heptadecenoic 17:1 | 0.23             | 0.41                    | 0.27                    |
| Elaidic 18:1t      | 0.18             | 0.43                    | 0.34                    |
| Oleic 18:1c        | 35.03            | 38.33                   | 38.16                   |
| Eicosaenoic 20:1   | 0.22             | 0.29                    | 0.24                    |
| Nervonic 24:1      | 0.00             | 0.07                    | 0.00                    |
| Σ MUFA             | 36.95            | 42.37                   | 40.72                   |
| Linoleic 18:2n6    | 26.35            | 20.13                   | 22.36                   |
| γ-linolenic 18:3n6 | 0.13             | 0.16                    | 0.12                    |
| Eicosadienoic 20:2n6 | 0.29            | 0.23                    | 0.19                    |
| Eicosatrienoic 20:3n6 | 0.19          | 0.23                    | 0.18                    |
| Arachidonic 20:4n6 | 1.84             | 1.21                    | 1.36                    |
| Σ n-6 PUFA         | 28.80            | 21.96                   | 24.21                   |
| α-linolenic 18:3n3 | 1.17             | 2.31                    | 1.21                    |
| Eicosatrienoic 20:3n3 | 0.01           | 0.10                    | 0.01                    |
| Eicosapentaenoic 20:5n3 | tr*            | 0.22                    | 0.10                    |
| Docosapentaenoic 22:5n3 | 0.16        | 0.21                    | 0.14                    |
| Docosahexaenoic 22:6n3 | 1.26            | 2.64                    | 2.23                    |
| Σ n-3 PUFA         | 2.60             | 5.48                    | 3.69                    |
| Σ n-6 PUFA/Σ n-3 PUFA | 11.08        | 4.01                    | 6.56                    |

*a, b, c: Means with different letters indicate significant differences between groups (P<0.05).
Z, x: Means with different letters indicate significant differences between groups (P<0.01).
A, B, C: Means with different letters indicate significant differences between groups (P<0.001).
Dietary treatments: C=5% of soybean oil, E₁= 3.5% of fish oil +1.5% of rapeseed oil, E₂= 1.5% of fish oil +3.5% of rapeseed oil.
*tr= in traces.
mal fat (5%) had a positive effect on egg production and on lowering food intake. Grobas et al. (2001) stated that fat supplementation (linseed and soybean oil) produced egg mass output (P<0.05) and food efficiency of hens (P<0.001). Although results of studies into the effects of oils on hens’ performances are contradictory, they all confirm that the lipid profile of egg yolk changed as a function of dietary fat sources.

Table 4 presents the content of fatty acids in egg yolks (as % of total fatty acids). Experimental groups E1 and E2 had slightly less SFA in yolk lipids than the control group (31.18%, 31.46% and 31.65%, respectively). Noticed differences in the content of SFA were not statistically significant (P>0.05). Similar results were obtained by Škrtić et al. (2006). They determined significantly lower (P<0.001) content of SFA in yolks of hens in the control group being fed diets that contained 4% of rapeseed oil and 2% of fish oil with respect to yolks of hens being fed with 6% of sunflower oil. A very highly significant difference (P<0.001) was confirmed regarding the content of MUFA in egg yolks between the control and the E1 and E2 groups. Eggs obtained from the E1 and E2 groups contained higher amounts of palmitic acid (C16:0, PA, P<0.001) and OA (P<0.05). The content of n-6 PUFA in egg yolks demonstrated a statistically significant difference (P<0.05) between the C, E1 and E2 groups. The most represented was LA, however, its content was significantly (P<0.001) lower in the E1 and E2 groups than in the control group. If compared to the control, feeding of hens in the E1 and E2 group with diets which contained a combination of fish oil and rapeseed oil, resulted in reduced deposition of LA in egg yolk. It is important to point out the significantly (P<0.001) reduced deposition of arachidonic acid (C20:4n-6, AA) in egg yolks of the E1 and E2 groups with respect to the C group.
DHA was very low unlike in the group being fed with menhaden oil. Kralik et al. (2005) pointed out significantly lower (P<0.001) content of EPA + DHA in yolks of the control group (vegetable oil) in comparison to those of the experimental group of hens which consumed diets supplemented with oil originating from sea organisms (3.33%). The content of EPA and DHA in egg yolks can be increased in two ways. The first way is to increase dietary content of αLNA, which is then converted into EPA and DHA in the hen’s organism, and newly synthesized n-3 polyunsaturated fatty acids are deposited in eggs (Cherian and Sim, 1991). The content of EPA and DHA can also be increased by increasing the intake of these fatty acids by feeding fish products (Husveth et al., 2003; Huyghebaert et al., 2007). Bavelaar and Beynen (2004) determined that the maximum concentration of DHA, around 1.5% of total fatty acids, was reached if the diet contained more than 7% of αLNA. The content of egg yolk was not clearly influenced by the αLNA intake, but there was a linear relationship with the EPA intake, although the efficiency of incorporation was very low. Dietary DHA was found to be efficiently incorporated into egg yolk.

Bavelaar and Beynen (2004) determined that the content of EPA in egg yolk could be modified through a diet that contained EPA, while DHA could be increased if the diet was rich in LNA or DHA. Content of DHA in egg yolks differed significantly (P<0.01) among the groups. The highest content of DHA was determined in the E1 group (2.62%), followed by the E2 group (2.23%), while the C group contained the least amount of DHA (1.26%). Sari et al. (2002), Husveth et al. (2003), as well as Huyghebaert et al. (2007) also reported on the increased content of DHA in egg yolk as a consequence of supplementing fish oil to the diet of laying hens. The Authors pointed out that the content of DHA was in positive correlation with the content of αLNA, EPA + DHA in diet, and in negative correlation with the content of LA. The conversion of EPA to DHA was relatively limited at higher dietary DHA concentration. Content of total n-3 PUFA (Table 4) in egg yolk lipids was the highest in the E1 group (3.5% of fish oil and 1.5% of rapeseed oil), followed by the E2 group (1.5% of fish oil and 3.5% of rapeseed oil) and the least in the C group (5% of soybean oil). Enrichment of egg yolk with n-3 PUFA depended on the increased content of n-3 PUFA in the hens’ diet, and it was in relation to the lowering of n-6 PUFA in egg yolks, which was also stated by Herbert and Van Elswyk (1996) and Meluzzi et al. (2000). According to Simopoulos (2000), LA and αLNA are very important as they are precursors of n-6 PUFA and n-3 PUFA. LA is metabolised in other n-6 acids, including AA, and αLNA is metabolised in n-3 acids, such as EPA and DHA. Because of that fact, if LA is present in greater amounts it will inhibit transformation of LNA into EPA and DHA and vice versa, and if there is not enough LA, AA will be formed in lower amounts. Our research results supported those of Simopoulos (2000), as the lowering of the content of LA in egg yolks was followed by reduced content of AA. There was a desirable ratio of n-6/n-3 PUFA (4 to 5) determined in egg yolk lipids of the E1 group, which is in line with up-to-date nutritional trends (Lewis et al., 2000). This ratio was 6.56 in the E2 group, which was still more favourable than the ratio of 11.08 established in the C group. Ferrier et al. (1995), Scheidler and Froning (1996), and Beynen (2005) succeeded in lowering the ratio of n-6/n-3 PUFA by increasing the content of n-3 PUFA in the hens’ diet. Our research showed that the combination of 3.5% fish oil and 1.5% rapeseed oil (E1) in the hens’ diets was more efficient in enriching egg yolk with n-3 PUFA than the combination...
of 1.5% fish oil +3.5% rapeseed oil (E2). The research also confirmed the ability of laying hens to synthesize EPA and DHA through metabolic processes if they have sufficient amounts of αLNA at their disposal. This was also found out by Cherian and Sim (1991). Bavelaar and Beynen (2004) pointed out that dietary fatty acids substantially diluted de novo synthesized fatty acids when they were incorporated in egg yolk.

Conclusions

Based on the completed research into the effect of fish and rapeseed oils (E1 and E2 groups) and soybean oil (C group) supplements in the diet of laying hens and their influence on production parameters, as well as on the content of fatty acids in egg yolk lipids, the following conclusions were drawn:

Laying hens of the E1 and E2 group had significantly higher (P<0.05) live weights at the end of the experiment and weaker laying intensity than hens in the control group.

Modification of fatty acid content in the laying hens’ diet affected fatty acid deposition in egg yolk. An increase in the content of αLNA, EPA and DHA in egg yolks resulted in lower deposition of AA.

It was confirmed that laying hens can synthesize EPA and DHA in their organisms in limited amounts if they receive sufficient amounts of αLNA through diets.

Supplementing hens’ diets with fish and rapeseed oils instead of soybean oil resulted in improved content of n-3 PUFA.

This study is part of a research project financed by the Ministry of Science and Technology of the Republic of Croatia.

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