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# OPTIMIZATION OF METHODS ORIENTED TO OMEGA EGGS CREATION IN THE SLOVAK REPUBLIC

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## ABSTRACT

The production of omega eggs was studied experimentally on the RIR layer hens after their feeding with addition of 0.5 % refined fish oil applied into feeding mixture HYD - 11. It was found that fish oil did not influence the production and quality of experimental eggs. The level of total poly-unsaturated fatty acids increased from 11.24 to 12.28 %, from total fat content egg yolk. The amount of omega-3 fatty acids (especially of docosahexaenoic acid - DHA) increased by 350 % in comparison to control eggs, the eicosapentanoic acid (EPA) increased from zero to 0.135 %. The ratio of omega-3 fatty acids improved from 10.08 to 3.26, that is, by 311 %. Using these techniques the omega-eggs are continually produced in large - scale farm for five years already.

**Key words:** Omega eggs, EPA, DHA, large - scale production

## INTRODUCTION

The importance of long-chain omega-3 fatty acids for growth and development, and their role in reducing the risk of cardiovascular and other diseases has been discussed by many investigators. The daily intake of omega-3 fatty acids is necessary for life. It is therefore important to eat foods that are rich in the two active omega fats – eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA).

EPA and DHA are known to reduce the risk of cardiovascular disease, by controlling blood lipid levels and by reducing platelet activity and aggregation, and they are essential for optimal functioning of nervous system. It is also important for the human and animal health to maintain a dietary balance between the ratio of n-6 to n-3 of poly-unsaturated fatty acids (PUFA) (Noble, 1996).

The findings that the consumption of omega-3 PUFA can have beneficial effects on human health have stimulated the possibility of increasing the levels of these fatty acids in foods by adding dietary fish oil. Blood pressure was found to reduce significantly in subjects who consumed the fatty acid modified eggs (Oh et al., 1994). Several studies have evaluated other blood characteristics as a result of consuming eggs containing an increased level of long chain omega-3 PUFA (Jiang and Sim, 1992). The enrichment of hen eggs by fish oil was intensively studied. Hargis et al. (1991) enriched the hen eggs by omega-3 fatty acids by adding 3 % menhaden oil into feeding. The results were compared with the amounts of omega-3 fatty acids in eggs from hens on an isocaloric control diet with no added fat. In response to added dietary menhaden oil, egg contained an average of 235 mg omega-3 fatty acid of which EPA and DHA represented approximately 89 %.

On the other hand, foodstuffs available on the market contain very little PUFA. The analysis of food value in Slovakia revealed their shortage, for this reason the Programme of Health Nutrition of the Slovak Republic

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which will continue till 2010 is expected to solve the incorporation of omega-3 fatty acids to human nutrition.

Our previous experiment studied the composition of omega eggs (Csuka et al., 2000). The continual actuality of the omega eggs is confirmed also by Vaško et al. (2005). The aim of the present experiment was to optimise the procedure of production of omega eggs which would be acceptable and suitable for human consumption and viable for large-scale farm production.

## MATERIAL AND METHODS

The experiment was realised in the Poultry Research Station in Ivanka pri Dunaji from July to October and started when the hens were in 6th month of laying period. The 48 layers of RIR breed were randomly divided into two groups and put into individual cages. The control group C was fed on standard layer mash HYD - 11 (160 N.kg $^{-1}$ of metabolizable energy and 8 % fat content). In the experimental group E the mash was fortified by 0.5 % fish oil Eifit-100 (Helm, AC Hamburg, Germany). Feeding was applied *ad libitum*. The doses of added oils were chosen on the basis of our experience with fish odour of eggs and results of Noble (1996) and Leskanich and Noble (1997).

During the experiments the egg laying intensity, egg weight and internal egg quality parameters such as index of egg shape, volume of outer thin white, volume of inner thin white, total volume of egg white, index of egg white, index of egg yolk, egg shell weight and yolk weight were measured. The egg weight was measured with egg-balance P-200 to a hundredth of milligram. The egg yolk weight and shell weight were individually weighed, while the weight of egg white was calculated.

The refraction of total egg white and egg yolk was measured by Abbe type refractometer in accordance with the manual. Before refractometric measurement of yolk 0.3 ml of 28-percentual ammonium chloride solution was added and mixed into 10 ml of yolk. It was measured at 20 °C. The egg shape index, egg white index and egg yolk index were detected by earlier published methods (Damiani et al., 1994).

Before the analysis of yolk one gram of yolk was mixed with 7 ml of 0.9 - percentual natrium chloride. The content of total cholesterol was measured with a test pack (Human, Gesselschaft für Biochemica und Diagnostica, GmbH, Taunustein, Germany), the very low-density lipoprotein (VLDL) of yolk was detected by the method of Griffin and Whitehead (1982), thiobarbituric acid reaction (TBA) by the method of Asakawa and Matsushita (1997). Spectrophotometer Beckman DU-5 was used during the experiment.

Fifth week after the start of the experiment, four mixed analytical samples each were prepared from thirty-two individual yolk samples, from control group and from experimental group of yolks. The samples were analysed by gas-chromatography for the content of individual fatty acids in the Food Research Institute, Bratislava.

The fatty acids were extracted and transesterified. The fat from 5 g yolk was extracted with 25 ml mixture of chloroform/methanol (2:1 v/w) by the method of Folch et al.(1957). Extraction solution of 0.02 % BHT antioxidant was used for this purpose. The extracted lipids were converted by transesterification to methylesters (FAME) with 14 % BF-3 (MeOH) under nitrogen. The FAME was measured by gas-chromatography with Fused silica capillary column omegamax 250 type, 30 m x 0.25 mm i. e. 0.25 μm of firm thickness Supelco with the equipment CP-9001 with FID detector Chrompack.

The individual fatty acids were identified by comparing the retention time with the help of standards of fatty acids (Supelco Mafreys Inc., USA) by the chromatographic program Mosaic.

**Table 1: Egg production per layer during 35 days**

| Group | Parameter | | | |
|---|---|---|---|---|
| | number of eggs | weight of egg (g) | laying intensity (%) | egg mass (g) |
| E | 18.12 | 61.72 | 75.5 | 11.18 |
| C | 18.60 | 60.09 | 77.5 | 11.19 |
| $\bar{x}$ G | 18.36 | 60.91 | 76.5 | 11.18 |
| s | 1.98 | 3.84 | - | - |
| sG | 0.24 | 0.46 | - | - |
| v (%) | 10.78 | 6.31 | - | - |

E - experimental; C - control; $\bar{x}$G - average for groups; s - standard deviation; sG - standard error; v - coeficient of variation

## RESULTS AND DISCUSSION

During the 35 days of the experiment, the hens from the experimental group with 0.5 % supplement of fish oil in food produced 18.12 eggs per hen, which was less than the hens in the control group (18.60). The difference is statistically insignificant. The average egg weight from the experimental group was higher by 1.63 g. The difference is again insignificant. That means the feeding of fish oil diet did not influence the egg production (Tab.1). The production of egg mass is equal in both groups.

Our findings from fortification of eggs with fish oil show that the fish oil level in feeding did not change the egg laying intensity which supports the results

**Table 2: Physical parameters of egg quality**

| Parameter | Group | | | | Global | | S |
|---|---|---|---|---|---|---|---|
| | C (n = 20) | | E (n = 20) | | | | |
| | $\bar{x}$ | s | $\bar{x}$ | s | $\bar{x}$ | s | |
| index of eff shape | 75.3 | 2.79 | 75.9 | 2.97 | 75.6 | 2.86 | n.s. |
| volume of thin outer | 8.94 | 0.75 | 8.70 | 0.54 | 8.82 | 0.65 | n.s. |
| white (ml) inner | 6.22 | 1.10 | 6.02 | 1.20 | 6.12 | 1.14 | n.s. |
| total volume of e. w. (ml) | 36.8 | 2.12 | 35.5 | 2.88 | 36.1 | 2.58 | ++ |
| index of eggs white | 0.057 | 0.0084 | 0.050 | 0.0071 | 0.053 | 0.0084 | n.s. |
| index of yolk | 0.404 | 0.02 | 0.398 | 0.02 | 0.401 | 0.02 | n.s. |
| egg shell weight (g) | 5.66 | 0.28 | 5.66 | 0.37 | 5.66 | 0.32 | n.s. |

++P<0.01, n.s.

n - number of tested eggs; $\bar{x}$ – average; S - significance (differences between groups are significant at the level $\alpha$ = 0.01); . n.s. - non significant; e.w. - egg weight. Other symbols are identical with Table 1

of Noble (1996) and also the results of Sheideler and Fronig (1996).

The parameters of other quality characteristics were studied on 20 eggs from the experimental group and 20 eggs from the control group. They are presented in Tab. 2. The egg weight from the control group was moderately higher but the increase is not significant. Some small and insignificant differences were also noted in the index of egg shape and index of yolk. In the index of egg white there were significant differences. In the control group the index was higher by 0.057. The volume of thin external white and thin internal white and total egg white was significantly higher in the control group. The shell weight of the two groups was equal. In general, we can say that the omega eggs and the control group eggs have very similar parameters in most of the external egg quality parameters.

The calculated index of egg white, index of egg

**Table 3: Phenotypic correlation between egg quality parameters**

| Parameter of egg morphology | | Parameter | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | B | C | D | E | F | G | H | I | J | K |
| A | egg weight | -0.119 | 0.738+++ | 0.694+++ | 0.824+++ | 0.845+++ | 0.958+++ | 0.090 | 0.148 | 0.724+++ | 0.820+++ |
| B | index of egg shape | | 0.014 | -0.083 | -0.056 | -0.251 | -0.086 | 0.030 | 0.026 | -0.185 | -0.020 |
| | vol. of thin albumen | | | | | | | | | | |
| C | out | | | 0.436++ | 0.747+++ | 0.480++ | 0.752+++ | 0.124 | -0.073 | 0.371+ | 0.621+++ |
| D | inn | | | | 0.924+++ | 0.594+++ | 0.630+++ | 0.108 | 0.110 | 0.575+++ | 0.596+++ |
| E | tot. | | | | | 0.643+++ | 0.784+++ | 0.132 | 0.051 | 0.583+++ | 0.704+++ |
| F | vol. of thick albumen | | | | | | 0.828+++ | 0.080 | 0.080 | 0.615+++ | 0.614+++ |
| G | vol. of total albumen | | | | | | | -0.011 | 0.142 | 0.508+++ | 0.737+++ |
| H | index of albumen | | | | | | | | -0.265 | 0.312 | -0.008 |
| I | index of yolk | | | | | | | | | 0.152 | -0.078 |
| J | weight of yolk | | | | | | | | | | 0.622+++ |
| K | weight of shell | | | | | | | | | | |

+ P<0.05,++P<0.01, +++P<0.001, n.s.
n = 40

**Table 4: Parameters of internal quality of eggs**

| Parameters of nutritional quality | | | Group | | S | $\bar{x}G$ | | |
|---|---|---|---|---|---|---|---|---|
| | | | C | E | | $\bar{x}$ | s | v (%) |
| yolk | cholesterol | mg.100 g $^{-1}$ | 1520.9 | 1463.4 | n.s. | 1481.8 | 81.0 | 5.47 |
| | protein | g.100 g $^{-1}$ | 13.81 | 13.67 | n.s. | 13.7 | 1.02 | 8.49 |
| | albumin | g.100 g $^{-1}$ | 5.56 | 5.50 | n.s. | 5.5 | 0.46 | 8.49 |
| | lipids | g.100 g $^{-1}$ | 36.37 | 34.95 | n.s. | 35.6 | 2.34 | 6.55 |
| | triglycerides | g.100 g $^{-1}$ | 0.393 | 0.584 | +++ | 0.488 | 0.13 | 25.69 |
| | VLDL | S-H u. | 10.2885 | 10.4675 | n.s. | 10.3779 | 1.2808 | 12.34 |
| | TBA | ext. U | 0.393 | 0.584 | +++ | 0.488 | 0.125 | 25.69 |
| | weight | g | 19.59 | 19.11 | n.s. | 19.32 | 1.26 | 6.53 |
| | refraction | index | 1.41869 | 1.41682 | ++ | 1.4174 | 0.0014 | 0.101 |
| thin albumen | volume | ml | 13.75 | 14.07 | n.s. | 13.91 | 3.36 | 24.13 |
| | refraction | index | 1.35649 | 1.35565 | n.s. | 1.3561 | 0.0013 | 0.084 |
| thick albumen | volume | ml | 18.20 | 19.18 | n.s. | 18.69 | 2.42 | 12.96 |
| | refraction | index | 1.35625 | 1.35562 | n.s. | 1.3559 | 0.0011 | 0.082 |

++P<0.01, +++P<0.001, n.s. Other symbols are identical with Tables 1 and 2

yolk and index of egg shape are similar to the those reporter earlier by Csuka and Baumgartner (1981). Results on higher volume of thick white compared to thin white are in accordance with the results of Monsey et al. (1977).

From the documented parameters phenotypic correlations were calculated which are mostly positive and significant. The correlation between the egg weight and volume of total egg albumen is the strongest ($r$ = 0.958 $^{+++}$).

Besides the classical internal egg quality parameters other parameters of eggs were also analysed. The omega-eggs contained significantly less total cholesterol. The correlation between VLDL and cholesterol in yolk is not significant. Our experimental results are demonstrated in detail in Tables 4 and 5.

The level of total yolk cholesterol detected in our experiments (1220.9 mg in 100 gram of yolk) is nearly identical with the results of Miyoshi and Mitsumoto (1994) as well as Csuka and Baumgartner (1981). Detected total yolk protein content (13.81 g per 100 gram) is comparable with the results of Rotenberg and Sorensen (1978). Yolk albumin level could be compared to our earlier results found in the Japanese quails' eggs (Csuka and Baumgartner, 1998). Detected findings about feeding of fish oil not affecting the level of yolk lipid content are in agreement with the results of Noble (1996) and Hammershoj (1995). A significant increase of TBA-reaction in fish oil fed hens' yolk was also found by Marshall et al. (1994).

The egg yolks and albumen refraction parameters are comparable with our early results (Csuka et al., 2000, 2002; Csuka and Baumgartner, 1998, 1981). The level of yolk refraction in omega eggs could be the consequence of lower level of fats and proteins in these yolks. The low and negative correlation between egg weight and yolk cholesterol ($r$ = – 0.075) is comparable with the results of Menge et al. (1974) ($r$ = – 0.062) in hens and Baumgartner and Simeonova (1992) ($r$ = – 0.083 to – 0.111) in the quail. The correlation between VLDL and cholesterol ($r$ = 0.076) is comparable with our earlier results (Csuka et al., 2000, 2002; Csuka and Baumgartner 1998, 1981). The higher correlation between the refraction on thin and thick egg whites $r$ = 0.884 makes it possible to detect egg white protein. It would be enough just to measure the thin egg white refraction. The result of fatty acid composition in the control group and the omega egg group are comparable with the results of Noble (1996), Hammershoj (1995), Aymond and van Elswyk (1995) and Sheideler and Frohing (1996).

The chromatographic analysis of egg fats showed minimal differences between the volume of saturated fatty acids in both the control and omega eggs (Tab. 6). The largest differences were found in heptadecane acid (C 17:0). The differences between the total volume of SFA in the two groups are minimal. The content of palmitic acid (C 16:0) is the highest.

Out of monosaturated fatty acids (MUFA) the eggs contained oleic acid in high quantity (C 18:1) while arachic (C 20:1) and myristoleic acid (C 14:1) were

**Table 5: Phenotypic correlation between internal parameters of egg quality**

| Parameter of egg quality | | Parameter | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | B | C | D | E | F | G | H | I | J | K | L | M |
| A | yolk cholesterol | 0.037 | 0.064 | 0.393 | -0.035 | 0.076 | -0.086 | -0.075 | 0.100 | -0.192 | 0.274 | 0.094 | 0.155 |
| B | protein | -0.071 | -0.071 | -0.164 | 0.046 | -0.111 | -0.172 | 0.215 | 0.058 | -0.209 | 0.169 | 0.102 | 0.309 |
| C | albumin | | | -0.194 | -0.196 | 0.092 | -0.154 | -0.393 | -0.021 | -0.274 | 0.086 | 0.185 | -0.010 |
| D | lipids | | | | -0.311 | -0.072 | -0.114 | 0.328 | 0.087 | -0.072 | 0.007 | 0.003 | -0.097 |
| E | triglycerides | | | | | 0.118 | -0.032 | -0.240 | -0.342 | -0.062 | -0.329 | 0.240 | -0.272 |
| F | VLDL | | | | | | 0.140 | -0.331 | 0.182 | -0.074 | 0.326 | 0.062 | 0.183 |
| G | TBA | | | | | | | 0.211 | 0.241 | -0.148 | 0.057 | 0.164 | 0.026 |
| H | weight | | | | | | | | 0.246 | -0.016 | 0.016 | 0.075 | 0.009 |
| I | refraction | | | | | | | | | -0.169 | 0.299 | -0.088 | 0.284 |
| J | thin albumen volume | | | | | | | | | | 0.257 | -0.451 | -0.320 |
| K | refraction | | | | | | | | | | | -0.071 | 0.884+++ |
| L | thick albumen volume | | | | | | | | | | | | -0.043 |
| M | refraction | | | | | | | | | | | | - |

+++P<0.001 Symbols are identical with those of Tables 1, 2 and 4

present in less quantity. The differences in MUFA between the two groups of eggs were small.

The main aim of the experiments was to increase the poly-unsaturated omega-3 fatty acids (PUFA). The feeding enriched with 0.5 % of fish oil moderately increased the total volume of PUFA (11.24 and 12.28 %) but the volume of targeted omega-3 fatty acids in the eggs, namely DHA (C 22:6, n-3) increased by 350 %. The volume of EPA (C 20:5, n-3) increased from zero to 0.135 %. The ratio of omega-6 to omega-3 fatty acids improved from 10.14 (C group) to 3.26 (group E), that means by 311 %.

The significant increase of EPA and DHA was also reported by Noble (1996), Hargis et al. (1991) and Damiani et al. (1994).

## CONCLUSION

In this experiment the content of EPA and DHA was found to increase significantly, while the omega-6 to omega-3 ratio decreased and also the yolk fat refraction. Each experimentally produced egg contained more than the daily recommended dose of EPA and DHA. The application of fish oil also moderately decreased the total yolk cholesterol content, yolk lipids, yolk triglycerides and did not change the other egg parameters significantly, with acceptable taste and odour of eggs. No unusual taste was observed. The increase of TBA reaction is a logical consequence of higher PUFA content of eggs. Therefore the omega-3 enriched eggs could be produced and marketed in our conditions.

**Table 6:** **Fatty acids content in control and experimental eggs**

| Fatty acid | Control group | | | | | | Experimental group | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C1 | C2 | C3 | C4 | $\bar{x}C$ | s | E1 | E2 | E3 | E4 | $\bar{x}E$ | s |
| C 14 : 0 | 0.43 | 0.47 | 0.50 | 0.44 | 0.460 | 0.032 | 0.420 | 0.460 | 0.420 | 0.440 | 0.435 | 0.019 |
| C 14 : 1 | 0.05 | 0.07 | 0.06 | 0.07 | 0.063 | 0.010 | 0.080 | 0.080 | 0.090 | 0.090 | 0.085 | 0.006 |
| C 16 : 0 | 26.98 | 26.65 | 26.82 | 26.76 | 26.80 | 0.138 | 27.02 | 26.92 | 27.19 | 26.85 | 26.99 | 0.148 |
| C 16 : 1 | 4.35 | 4.94 | 4.82 | 4.62 | 4.68 | 0.258 | 4.63 | 4.65 | 4.84 | 4.48 | 4.65 | 0.148 |
| C 17 : 0 | 0.16 | 0.16 | 0.19 | 0.18 | 0.173 | 0.015 | 0.220 | 0.220 | 0.240 | 0.220 | 0.225 | 0.010 |
| C 18 : 0 | 8.50 | 8.20 | 8.50 | 8.28 | 8.37 | 0.154 | 7.84 | 7.72 | 7.71 | 8.20 | 7.87 | 0.229 |
| C 18 : 1 n 9 | 45.42 | 45.16 | 44.50 | 45.42 | 45.13 | 0.434 | 45.65 | 45.18 | 43.51 | 44.33 | 44.42 | 0.699 |
| C 18 : 1 n 7 | 2.48 | 3.00 | 2.83 | 2.66 | 2.74 | 0.223 | 2.65 | 2.85 | 2.95 | 2.68 | 2.78 | 0.142 |
| C 18 : 2 n 6 | 7.87 | 7.81 | 8.22 | 8.30 | 8.05 | 0.246 | 8.33 | 7.85 | 8.08 | 8.06 | 8.08 | 0.196 |
| C 18 : 3 n 6 | 0.140 | 0.100 | 0.100 | 0.080 | 0.105 | 0.025 | 0.70 | 0.060 | 0.70 | 0.080 | 0.070 | 0.008 |
| C 18 : 3 n 3 | 0.240 | 0.170 | 0.190 | 0.180 | 0.195 | 0.031 | 0.200 | 0.190 | 0.200 | 0.210 | 0.200 | 0.008 |
| C 20 : 1 | 0.470 | 0.280 | 0.310 | 0.320 | 0.345 | 0.085 | 0.230 | 0.280 | 0.280 | 0.250 | 0.260 | 0.024 |
| C 20 : 3 n 9 | 0.190 | 0.100 | 0.100 | 0.90 | 0.120 | 0.047 | 0.050 | 0.060 | 0.050 | 0.050 | 0.053 | 0.005 |
| C 20 : 3 n 6 | 0.220 | 0.120 | 0.170 | 0.120 | 0.158 | 0.048 | 0.060 | 0.060 | 0.100 | 0.110 | 0.083 | 0.026 |
| C 20 : 4 n 6 | 1.83 | 1.79 | 1.97 | 1.75 | 1.84 | 0.016 | 1.25 | 1.24 | 1.25 | 1.28 | 1.26 | 0.017 |
| C 20 : 5 n 3 | - | - | - | - | - | - | 0.130 | 0.140 | 0.150 | 0.120 | 0.135 | 0.021 |
| C 22 : 5 n 3 | tr. | 0.260 | 0.500 | 0.500 | 0.315 | 0.101 | tr. | tr. | 0.170 | 0.140 | 0.078 | 0.011 |
| C 22 : 6 n 3 | 0.670 | 0.720 | 0.670 | 0.680 | 0.685 | 0.121 | 2.30 | 2.18 | 2.70 | 2.41 | 2.40 | 0.222 |
| SFA | 36.06 | 35.48 | 36.00 | 35.64 | 35.80 | 0.280 | 35.50 | 35.32 | 35.56 | 35.71 | 35.52 | 0.161 |
| MUFA | 52.77 | 50.48 | 52.52 | 53.09 | 52.22 | 0.448 | 52.24 | 53.04 | 51.67 | 51.83 | 52.19 | 0.612 |
| PUFA | 11.16 | 11.07 | 11.47 | 11.25 | 11.24 | 0.166 | 12.26 | 11.64 | 12.77 | 12.46 | 12.28 | 0.477 |

$\bar{x}C$, $\bar{x}E$ - average for control and/or experimental group; s - standard deviation; tr. - trace; SFA - saturated fatty acids; MUFA - mono-unsaturated fatty acids; PUFA - poly-unsaturated fatty acids

Fatty acid (mastné kyseliny): C 14 : 0 myristic; C 14 : 1 myristicoleic; C 16 : 0 palmitic; C 16 : 1 palmitoleic; C 17 : 0 heptadecanic; C 18 : 0 stearic; C 18 : 1 n 9 oleic; C 18 : 2 n 6 linoleic; C 18 : 3 n 6 linolenic; C 18 : 3 n 3 linolenic; C 20 : 1 eicosenoic; C 20 : 3 n 9 eicosatrienoic; C 20 : 3 n 6 eicosatrienoic; C 20 : 4 n 6 rachidonic; C 20 : 5 n 3 eicosapentaenoic; C 22 : 5 n 3 docosapentaenoic; C 22 : 6 n 3 docosahexaenoic

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