Effect of PUFAs enrichment of quail eggs on the profile of n-3 fatty acids and phospholipids contained in yolk

Z. DOBRZAŃSKI[2], Ł. BOBAK[1], H. BEŃ[1] and T. TRZISZKA[1,*]

Wroclaw University of Environmental and Life Sciences, 51-630 Wrocław, Poland
[1]Department of Animal Products Technology and Quality Management
[2]Department of Animal Hygiene and Animal Welfare
***Corresponding author: Tadeusz.Trziszka@up.wroc.pl**

The aim of this study was to produce Japanese quail eggs enriched with PUFAs including the incorporation of n-3 fatty acids to the phospholipids fraction, which can significantly increase the value of their bioavailability. Enriched quail eggs were used as a starting material for the extraction of phospholipids. The birds, Japanese quail, were kept under standard conditions in two groups, a control one (C) and an experimental one (E). In the E group, birds received the same compound in feed as in the control group, but with the addition of fish oil, algae, linseed, selenium yeast and Humic preparations. The analysis of fatty acids in the lipid fraction in raw material was performed by gas chromatography (GC/MS) and analysis of the phospholipids was carried out with the use of HPLC.
The composition of the lipid fraction in experimental eggs has demonstrated over a 7-fold increase in n-3 fatty acids in comparison to the control group. The value of the parameter n-6/n-3 was over 4-fold reduced in the experimental group.
Long chain fatty acids, particularly DHA, were incorporated in the sn2 position of phospholipids.
The profile of phospholipids showed the presence of phosphatidylcholines (PC - lecithin) at the level 90% and phosphatidylethanolamine (PE - cephaline) at the level 9% and ca. 1% of other phospholipids. Phospholipid preparations derived from fortified quail eggs provide an interesting offer for the production of nutraceuticals, biomedical preparations and cosmetics.

Keywords: Japanese quail, egg, phospholipid, long chain poly-unsaturated fatty acid,

Introduction

Egg yolk contain many nutrients among other things phospholipids, phosvitin and immunoglobulin Y (IgY). Phospholipids is a class of lipids that contain phosphorus in sn3 position of the glycerol chain esterificated with choline to form the phosphatidylcholine (lecithin) or with ethanolamine (cephaline) are increasingly used as emulsifying agents in the fields of nutrition, pharmacology, and cosmetics (Ternes 2003, Siepka *et al.* 2010). Phospholipids isolated from egg yolk characterized by a higher content of lecithin and PUFA in compared to phospholipids isolated from plants raw materials (usually from soy beans). Supplementation of the human diet with α-linolenic acid (ALA, $C_{18:3}$) can reduce the amount of heart attacks due to blood pressure reduction and regulation of atherosclerosis. Simultaneously, ALA is used in the treatment of such diseases as rheumatoid arthritis, lupus, diabetes, dermatitis, or Crohn's disease. ALA regulates the level of bad cholesterol (LDL) and triglycerides and apoproteins (marker of diabetes) in plasma. Eicosapentaenoic (EPA; $C_{20:5}$) and docosahexaenoic (DHA; $C_{22:6}$) acids prevent atherosclerosis, thrombosis and embolism, which have found use in the prevention of heart disease and also promote the growth of the brain (the brain gray matter contain up

to 40% of DHA) and normal visual acuity (up to 50% of the fatty acids are retinal DHA). It reduces the level of triglycerides in the blood and reduce the risk of cancer (Kidd 2007).

Fatty acids profile in yolk phospholipids can be modified while supplementing the feed given to hens. Most feed is enriched with oil and linseed (the content of over 50% alpha linolenic acid), cold-water fish oil and algae (source of acids $C_{20:5}$ and $C_{22:6}$). These fatty acids (in particular EPA and DHA) are incorporated into the structure of phospholipid in sn2 position. This is especially important from a nutritional point of view that the LC-PUFA incorporated in the structure of phospholipids possess a greater bioavailability than triglycerides or fatty acid esters.

The aim of this study was to point out the effect of the addition of selenium yeast and algae on the feed and determine fatty acids profile of lipid fractions of quail's whole egg.

Materials and methods

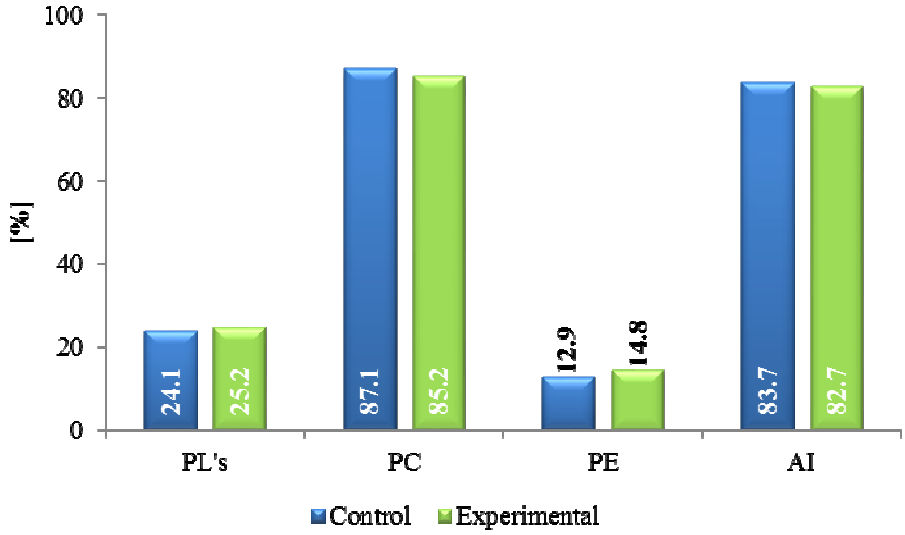
Eggs were collected from Japanese quails (Coturnix coturnix japonica J-11) kept in the battery cages in groups of 40 per square meter. Birds from the age of 16 up to 24 weeks were fed ad libitum; with a standard mixture Farm line G-090 for quails in the control group (C). For birds in the experimental group fodder was enriched with the Saccharomyces cerevisiae yeast with a concentration of 500 mg selenium Se/kg (0,3 g/kg), preparation of DHA Gold (5 g/kg, Novus International, Inc., MO, USA) and flaxseed (40 g/kg). Collected eggs were higienized and then automatically broke using egg shell breaker UDTJ-150 (OVO TECH; Starachowice; Poland). The homogenous whole mass of the quail eggs was dehydrated using the Mobile Minor spray drier (A/S; Gladsaxevej; Denmark) with disk atomization system. The parameters of the drying process has been maintained at the level of 185±5°C for inlet air and 90±2°C for outlet air.

The total lipid content in raw material was determined by Folch method (1957), phospholipids extracted using the modified method described by Palacios and Wang (2005). The fatty acid profile was analyzed after methylation (14% BF_3 in methanol). The analysis was performed in a gas chromatograph with a spectroscopy mass detector (GC6890/5973 MSD, column – HP88). HPLC analysis of PLs was done using HP 1200 system equipped with Diode Array Detector (DAD G1315B) on the LiChrospher® 100 with Diol function on C-chains column (150 × 4.6 mm). The HPLC condition flow rate 2.5 mL/min, phase A - acetonitrile, phase B – methyl alcohol, phase C – isopropyl alcohol in the ratio of 100:10:1.8 (v/v/v). GC/MS and HPLC/DAD were purchased from Agilent Technologies, Inc. CA, USA.

Results and discussion

In the phospholipid fractions in our study were determined for experimental and control groups, respectively 0.53 and 1.22% of EPA (tab. 1). This value is significantly greater to that reported by Genchev (2012) marked only 0.33% of eicosapentaenoic acid in the PL's fraction extracted from the Pharaoh quails eggs. A similar relationship is observed in the content of DHA, in the present study indicates approximately 3 times more of this acid in the control group and 9.5 times more in the experimental group in the phospholipids this is as opposed to testing Genchev (2012) who obtained only 1.04% of docosahexaenoic acid in the PL's fraction. In tests carried out by a team Arantes da Silva (2009) was added to the fodder of 5% linseed, led it to 8 fold increase in ALA content (from 0.25 to 2%). In our study, more than 5% of ALA was determined in the TAG fraction which shows that this acid is not incorporated into PL's fraction as EPA and DHA. Kaźmierska (2007) by adding to the fodder of 1% linseed and 0.5% fish oil obtained about 4 fold increase in the n3 fatty acid group to 2.88% in the experimental group. It is less 2.5 times of the growth acid group compared to our study which was obtained n3 PUFA content in egg yolk lipids at level 7.62% including 4.75% ALA. The phospholipid content in the tested raw materials amounted average to 25% (fig. 1) its a value of approximately 8% lower than in the study Genchev (2012).

Figure 1 The composition of phospholipid fractions



PL's – phospholipids; PC- phosphatidylcholine; PE – phosphatidylethanolamine; AI - acetone-insoluble matter

Table 1 Content of fatty acids in total lipids, phospholipids and triglycerides fraction in the control (C) and experimental (E) group (%)

Fraction	Group	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$ (*n*-6)	**$C_{18:3}$ (*n*-3)**	$C_{20:4}$ (*n*-6)	**$C_{20:5}$ (*n*-3)**	**$C_{22:6}$ (*n*-3)**	Σ *n*-3	*Ratio n*-6/ *n*-3
Total lipids	C	27.66	0.58	10.20	46.23	11.71	0.49	2.17	0.00	0.96	1.45	9.57
	E	25.21	0.62	10.78	39.00	15.40	4.75	1.36	0.43	2.44	7.62	2.20
TAG	C	28.22	0.72	6.94	51.91	11.25	0.55	0.39	0.00	0.00	0.55	21.04
	E	27.38	0.80	8.03	44.12	13.46	5.16	0.27	0.18	0.61	5.95	2.31
PL's	C	24.18	2.50	21.18	26.47	15.20	0.07	6.67	0.53	3.19	3.79	5.77
	E	23.26	1.44	21.86	21.23	15.82	0.70	4.54	1.22	9.94	11.86	1.72

Conclusions

It has been shown a significant effect of egg enrichment in PUFA (EPA, DHA) by feeding of the quail, using oil and linseed, yeast and algae . Particularly important observation concerns the incorporation of PUFAs in sn2 position of phospholipids. Transesterification of the long chain fatty acids from natural raw materials into triglycerides form of quail egg phospholipids allows to increased their nutritional value and bioavailability.

Acknowledgements

The research was financed by the National Science Centre grants no. NN209 137840

References

Adlercreutz P., Virto C., Persson M., Vaz S., Adlercreutz D., Svensson I., Wehtje E. (2001) Enzymatic conversions of polar lipids. Principles, problems and solutions. *Journal of Molecular Catalysis B: Enzymatic* **11**, 173-178.

Arantes da Silva W.; Naiverti Elias A.H.; Aricetti J.A.; Sakamoto M.I., Murakami A.E.; Marques Gomes S.T., ; Visentainer J.V., Evelázio de Souza N., Nilson; Matsushita M. (2009) Quail egg yolk (*Coturnix coturnix japonica*) enriched with omega-3 fatty acids. *Food Science and Technology* **42**, 2, 660-663.

Genchev A. (2012) Quality and composition of japanese quail eggs (*Coturnix japonica*). *Trakia Journal of Sciences*, **10**, 2, 91-101.

Gładkowski W., Kiełbowicz G., Chojnacka A., Pisarski B., Wawrzeńczyk W. (2010) Izolowanie fosfolipidów z liofilizowanych żółtek jaj kurzych. *Przemysł chemiczny* **89/4**, 352-355.

Kaźmierska M., Korzeniowska M., Trziszka T., Jarosz B. (2007) Wpływ wzbogacania paszy w polienowe kwasy tłuszczowe na wybrane cechy jaj przepiórczych. *Polish Journal of Food Nutrition Science*, **57**, 4B, 281-284.

Kidd P.M. (2007) Omega-3 DHA end EPA for Cognition, Behavior, and Mood: Clinical Findings and Structural-Functional Synergies with Cell Membrane Phospholipids. *Alternative Medicine Review*, 12, 3, 207-227.

Palacios L.E., Wang T. (2005) Egg-Yolk Lipid Fractionation and Lecithin Characterization**.** *Journal of the American Oil Chemist's Society* **82,** 8, 565-569.

Siepka E., Bobak Ł., Trziszka T. (2010) Frakcjonowanie żółtka w celu pozyskiwania preparatów wzbogaconych w substancje biologicznie czynne. *Żywność. Nuka. Technologia. Jakość*. **6**, 73, 158 – 167.

Ternes W. (2003). Egg lipids. *In: Sikorski ZE, Kolakowska A, editors. Chemical and functional properties of food lipids. Boca Raton: CRC Press*.