# Egg design – new formulation by enrichment of PUFAs using natural substances

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## Summary

784 Tetra SL brown hens in 7 feeding groups (6 experimental groups; different biologically active feed additives, including Humokarbowit + control group), 112 birds in each, were selected. The observations were performed for 12 weeks, from the 37$^{th}$ to 49$^{th}$ week of birds' life. After delivery to the laboratory, the eggs (120 from each groups) were broken, the yolk was separated and chemical analysis, including the analysis of dry matter and fat, was performed. In the fat fraction, fatty acids, including PUFA (with n-3 and n-6), were determined.

The aim of the experiment was to show a synergistic or inhibitory effect connected with the process of enrichment. It was observed that the use of flax seed and fish meal as carriers had a high, statistically significant influence on the increase of PUFA n-3 family content. The relevant decrease of n-6 fatty acids content in yolk in all experiment groups was a very important observation and may be explained by the use of Humokarbowit. The ratio was almost twice better (on average) in the enriched material than in the control group and reached the values from 6 to 7.

## Introduction

Functional food, nutraceuticals and natural (ecological) food are new areas of interest in research and food industry, including food products of animal origin. All these elements may be referred to as "designed food". In this category of food improvement, hen eggs are the best raw material. There is a growing interest among international teams of scientists in the methods of natural enrichment of eggs with biologically active substances which, for commercial reasons, implicates high interest of food and pharmaceutical industries, Ball (2004), Froning (2004), Juneja 2004, Trziszka *et al.*(2004), Watson (2004).

In case of the so called "designed eggs", eggs are naturally enriched through programmed feeding of layer hens with antioxidants, vitamins and polyunsaturated fatty acids (PUFA) which are transferred to egg content. The substances protect human organism from civilization diseases and, when contained in eggs, improve their nutraceutical, biomedical and functional qualities. This natural program of enrichment of eggs may be economically and socially beneficial for egg industry, Ball (2004), Drymel (2002), Juneja (2004).

As enrichment is performed based only on natural transformation of the substances from the feed to eggs through the hen, such eggs are positively accepted by consumers. It needs to be stressed that PUFA, so important for human life and health, are present in oils, fish and flax seed at the amount of 300 – 2500 mg/100g. However, their content in enriched eggs at the level as low as ca. 900 mg/100g is economically and, most important, nutritionally positive due to the presence of other compounds, such as phospholipids, vitamins, antioxidants etc. The pro-health qualities of eggs indicate the necessity of developing consumer awareness, including the so called "new formula of marketing strategy". In that aspect, the existing unsupported opinions of some medical societies should be revised and the nutritional, dietary and pro-health qualities of eggs should be promoted, Drymel (2004), Juneja (2004).

The aim of the present study was to attempt at modifying (improving) the chemical content of yolk by its enrichment with PUFAs through layer hens feeding.

The hens were fed with unconventional mineral-organic additives and herbs added to feed mixtures, Dobrzański, Tronina (1999), Drymel (2002), Trziszka *et al.* (2004).

## Experiment material

Eggs obtained from Tetra SL brown hens in 37 – 49 week of age were used as experiment material. The layer hens were kept in Specht cages.

From a group of 56 thousand layer hens, a total of 784 hens in 7 feeding groups, 112 birds in each, were selected. The observations were performed for 12 weeks. All mixtures were standard and balanced as far as energy (2700 Kcal) and protein content (16%) are concerned.

**Table 1 Presents the feeding model used in the experiment.**

| Group | Feed mixture and additives used |
|---|---|
| Group I | Standard feed mixture with an addition of Humokarbowit and enriched with fatty acids, incl. fishmeal and flax seeds |
| Group II | Feed as in Group I, with addition of Scullcap at the amount of 0.5% of the feed mass |
| Group III | Feed as in Group I, with addition of Herban herbal preparation at the amount of 0.5 kg/1t |
| Group IV | Feed as in Group I with 25% higher content of vitamin premix (vit. A,E,$D_3$, B-complex) |
| Group V | Feed as in Group I with 50% higher content of vitamin premix |
| Group VI | Feed as in Group IV with an addition of mineral chelats |
| Group VII | Standard feed mixture without additives – control group |

Humokarbowit is a humus-mineral preparation with biostimulating and prophylactic qualities. It is a mixture of a special variety of humine material (peat and brown coal) enriched with calcium and magnesium compounds. Moreover, it contains carboxylic groups, bitumens, hemicellulose, lignin, waxes, resins, plant hormones, proteins, amino acids and various macro elements. It is free from pathogenic microorganisms, bacteria and moulds, toxic pesticides and other harmful chemical substances, Dobrzański, Tronina (1999), Trziszka *et al.* (2004).

Scullcap is a perennial root plant and its most biologically active chemical components are flavonolids with triple and quadruple hydroxylation and strong biostimulating qualities.

Before the first group of eggs was collected, the layer hens were fed with experiment feed for 3 weeks. The experiment material was divided into two categories, i.e. A) fresh, immediately after laying and B) stored at room temperature for 28 days.

## Methods

After delivery to the laboratory, the eggs were broken, the yolk was separated and chemical analysis, including the analysis of dry mater and fat, was performed. In the fat fraction, fatty acids, including PUFA (with n-3 and n-6), were determined.

## PUFA determination

The fat sample was saponified with sodium methanol and then estrified with 14% solution of $BF_3$ in methanol.

The methyl esters of fatty acids were analyzed using gas chromatography. A Philips gas chromatograph equipped with a flame ionization detector was used.

Conditions of the chromatography analysis:
Rtx 2330 column;105 m, feeder temp. 230°C, detector temp. 240°C,
column temp. 160°C /30 min.(3°C/min);180°C/17 min. (5°C/min); 210°C/45min/.
The results were statistically analysed

## Results and discussion

The results are presented in diagrams (Figures 1-6).

The data presented indicate statistically significant differences between fresh and stored material. Dry mass content (Figure 1) in fresh material was at the level of 48.1 – 49.0% and 47.0 – 48.1% in stored material.

The fat content in yolk (Figure 2) ranged from 31.6 to 32.3% in fresh material and from 31.0 to 31.5% in stored material and the differences were statistically significant. Similarly as in case of percentage content of dry mass, the highest fat content was observed in eggs from Group VII (control group).

The main objective of the study was to analyse fatty acids content.

The saturated fatty acids (Figure 3) were at different levels within the experiment groups. The lowest content of saturated acids was observed in both fresh and stored material in Group VII and Group I. The differences between these groups were not statistically significant. The highest content of saturated fatty acids was observed in group II, IV and III and the differences between them were statistically significant.

The highest content of unsaturated acids n-6 was observed in Group VII and Group I in both fresh and stored material and the differences between groups were not statistically significant. The lowest content in fresh material was observed in Group IV (9.6%), in Group II (9.9%), Group III and IV (10.3%) and in Group V (10.7%) and the differences between them were not statistically significant.

The content of n-3 family polyenic acid remained at unfavourably low level in Group VII (control), i.e. 0.9% in fresh eggs and 1.14% in stored eggs. The highest levels of n-3 acids were observed in fresh material in Group I (1.72%), in Group II (1.67%), VI (1.61%), III (1.55%) and 1.53% in Group IV and V.

In stored material, the lowest values were observed in Group VII (1.14%) and the highest in Group III (1.73%) and Group IV (1.68%). Statistically significant differences were observed only between Group VII and other groups, which indicates an important influence of feed enrichment. The ratio between n-6 and n-3 families is the most important in the analysis of polyenic fatty acids. The lower the ratio, the higher quality the fat. The lowest n-6/n-3 ratio was observed in fresh material in Group II (5.93), in Group IV (6.25) and Group VI (6.36). In the stored material, the worst ratio was observed in Group VII (11.27). In Group III, Group IV and Group V, the ratio was the best and was 5.96, 6.05 and 6.11, respectively.

The results of the present study indicate big influence of feed additives, constituting only a small part of the feed mass, on modification of fatty acids content of feed administered to layer hens.

In the process of modification of chemical composition of eggs, apart from enrichment with vitamins, enrichment with polyunsaturated fatty acids (PUFA) is also very important, Ahn *et al.*(1995), Baucells *et al.* (2000), Codony *et al.* (1995), Lewis e al. (2000), Pisulewski (2000), Van Elswyk (1997). This method of modification was the key problem in the present study, especially as, apart from PUFA carriers, other substances with high biological activity were used. The aim of the experiment was to show a synergistic or inhibitory effect. It was observed that the use of flax seed and fish meal as carriers had a high, statistically relevant influence on the increase of PUFA n-3 family content. Moreover, the enrichment of feed mixture with Humokarbowit and Scullcap had a relevant influence on the increased level of n-3 fatty acids. The significant decrease of n-6 fatty acids content in yolk in all experiment groups was a very important observation and may be explained by the use of Humokarbowit.

The results clearly indicate synergism in the transmission of fatty acids from feed to egg matter with parallel increase of the content of other biologically active substances [Drymel (2002), Trziszka *et al.* (2004).

Moreover, the ratio between polyenic acids n-6 and n-3 in the enriched eggs is extremely important from medical point of view. The ratio was almost twice better (on average) in the enriched material than in the control group and reached the values from 6 to 7.

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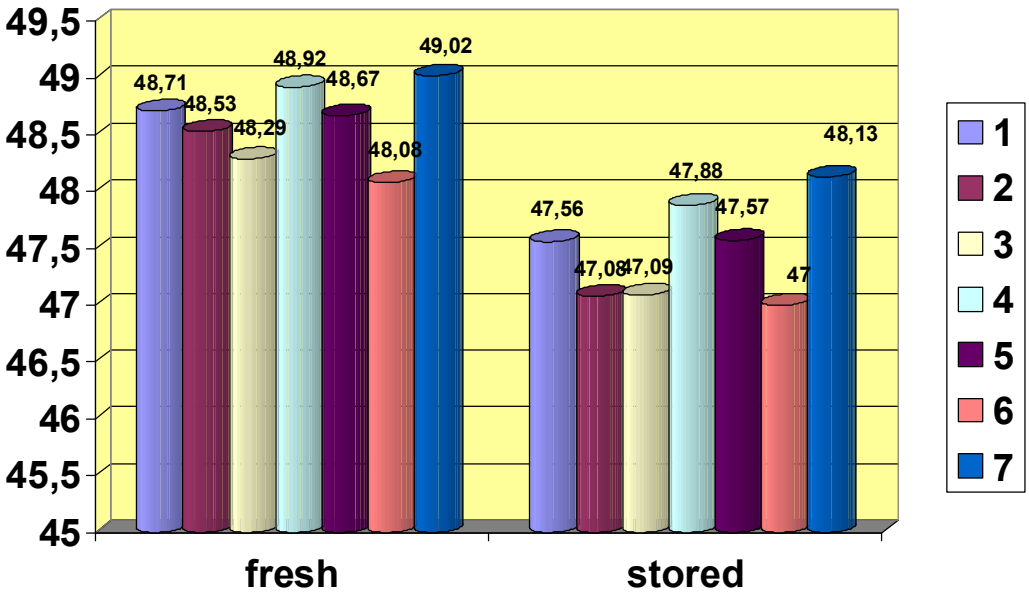


**Figure 1 Yolk – dry mass [%].**

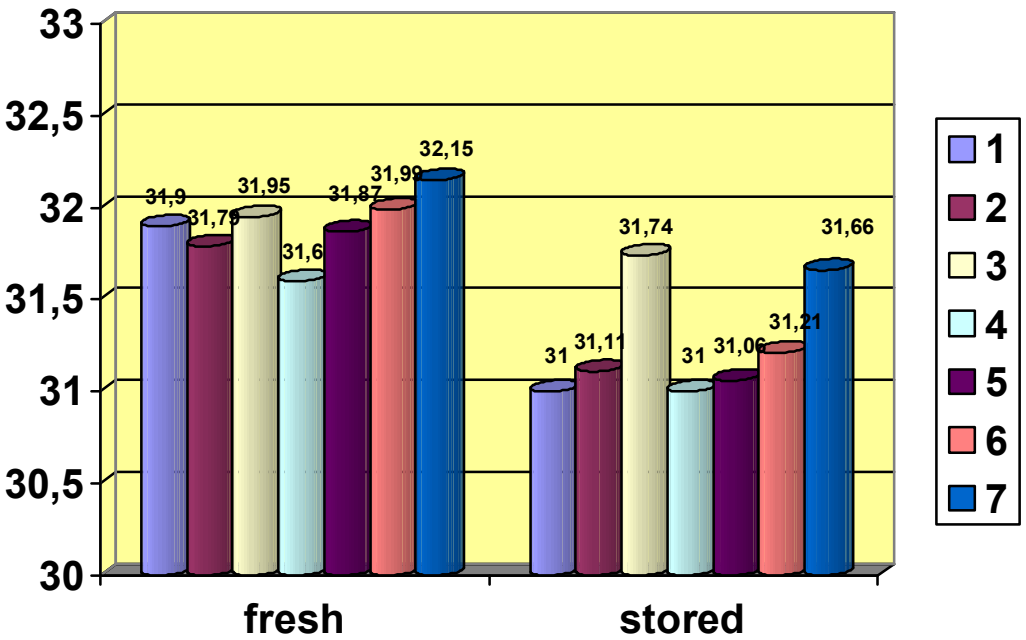


**Figure 2 Yolk – fat content [%].**

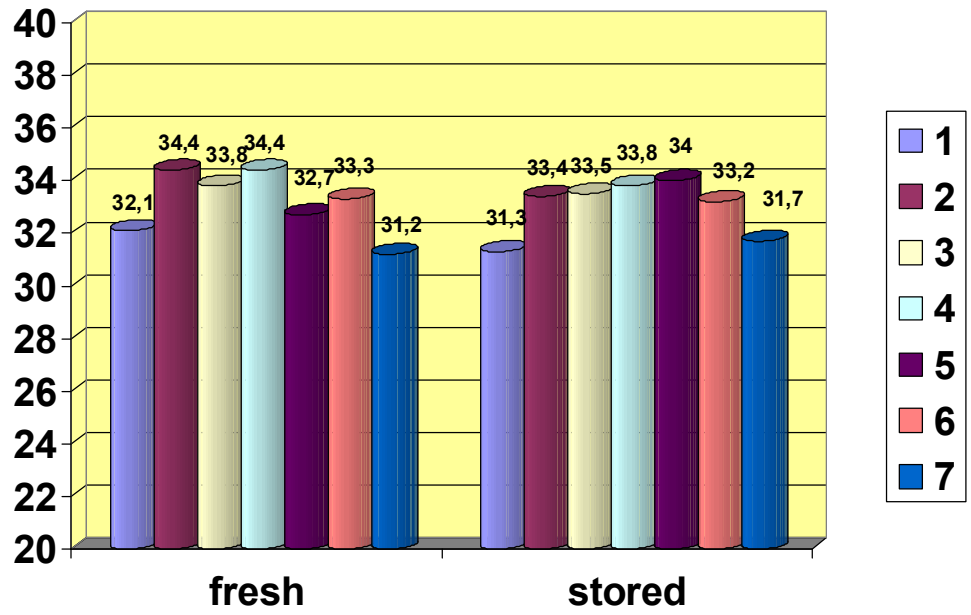


**Figure 3 Fatty acids – saturated [%].**

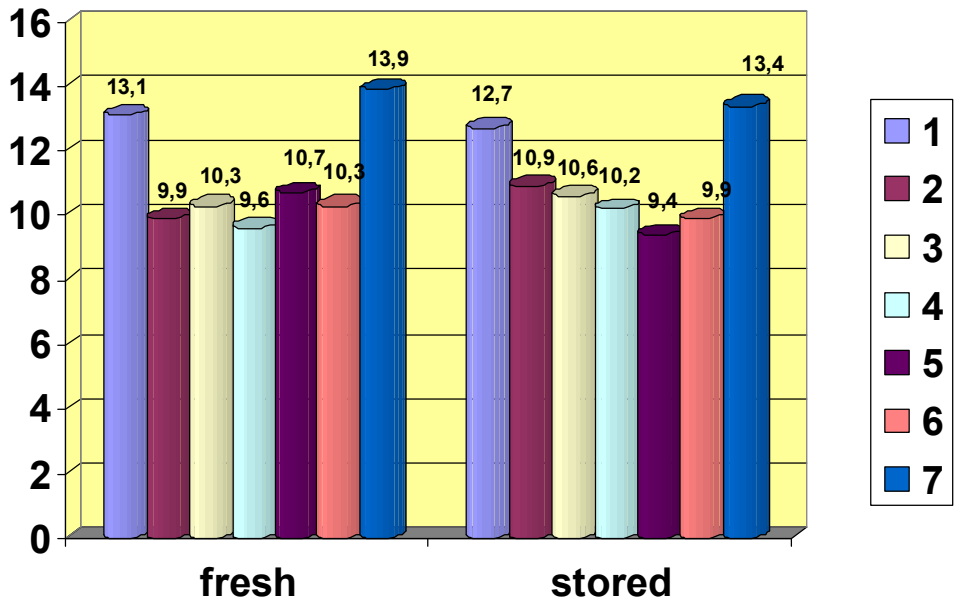


**Figure 4 Fatty acids – unsaturated n-6 [%].**

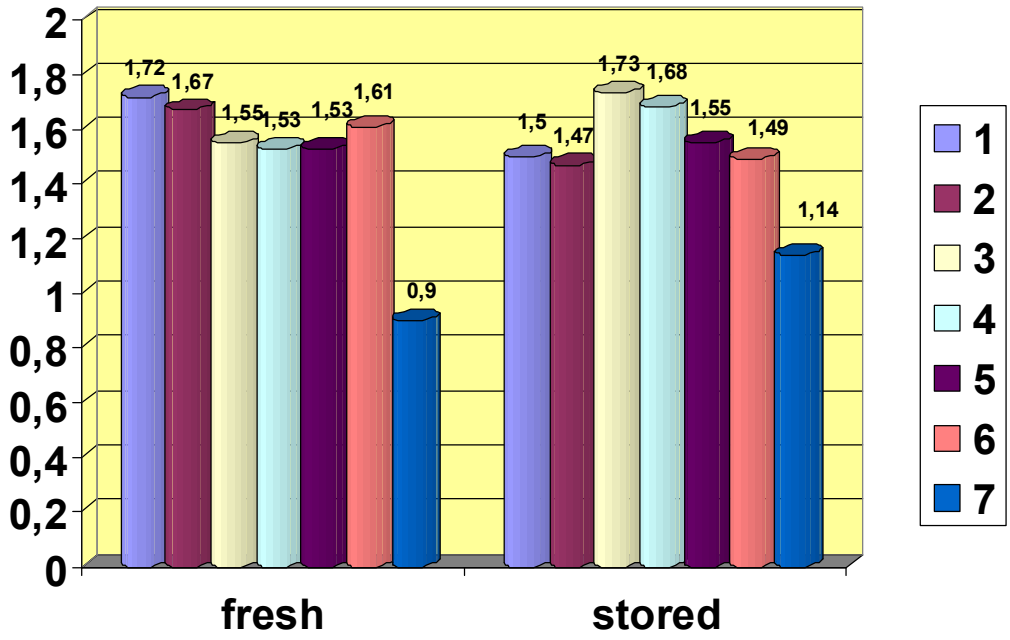


**Figure 5 Fatty acids - unsaturated n-3[%].**

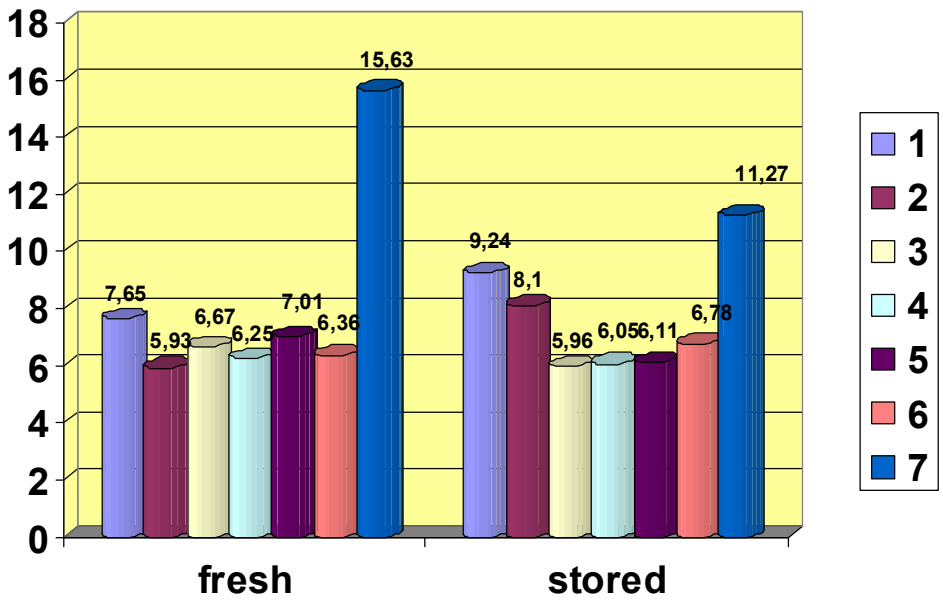


**Figure 6 n-6 / n-3 fatty acids ratio.**