Yolk fatty acid composition of ω-3 eggs during the laying period

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Abstract

This study was carried out to investigate the effects of a special type of diet and hen age on yolk fatty acid composition of ω-3 eggs during the laying period.

Totally, 7,500 *Hy-Line* Brown Leghorn layers aged 24 wks at the start, were used in this study. These hens were caged in an environmentally control house and fed on a diet containing 10 % flaxseed meal plus a herbal mix of Mediterranean plants.

Results showed an 88 % laying egg production, a mean egg weight of 60.6 g and a 2.5 % mortality for the total laying period. In addition, the average daily feed consumption of hens was calculated to 110.4 g/hen. Concerning the fatty acid composition of egg yolks, the Linolenic acid (ω-3), EPA and DHA percentage was increased from the 22^{nd} to the 32^{nd} week of age (4.87 %; 1.30 % and 9.07 %; 1.90 %, for Linolenic acid, EPA and DHA, respectively) and remained constant until the 55^{th} week. Also, the Linoleic acid / Linolenic acid ratio was affected by the hen age.

It was concluded, that the enriched diet acting synergistically with herbal mix resulted to the further increase of the n-3 fatty acids in egg yolk. In addition, the level of omega-3 in egg yolk had a tendency to rise with the hen age, until 32 wks, and remained stable at the highest rate during the rest of the laying period.

Introduction

General composition of a hen's egg, reported decades ago, consisted of 58% white, 30-31 % yolk and 11% shell (Stadelman and Cotterill, 1977). However, when calculated on the basis of interior contents, 65% of egg contents is white and 35 % is yolk. In the yolk the total solids are generally 50 %, and major constituents of yolk are 16 % protein and 32 % lipid. Long–chain polyunsaturated fatty acids of n-6 and n-3 families constitute to about 3 % and 2 %, respectively, of the total fatty acids in this lipid (Ahn *et al.*, 1997; Nielsen, 1998). This makes egg yolk lipid an important potential source of these fatty acids in the production of certain products (e.g. infants formula for premature babies).Omega-3 fatty acids or n-3 family used by the human body to make anti-inflammation and anti-thrombotic substances, while omega-6 or n-6 family are made into substances that promote inflammation and thrombosis (Salem *et al.*,1996; Simopulos, 2003b).Therefore, not only is the presence of n-6 and n-3 in the diet necessary for health, but the ratio of n-6/n-3 is critical in achieving an appropriate balance of highly unsaturated fatty acids derived functions in the human body. The current recommended ratio of omega-6 to omega-3 intake is 4 to 1. Typically the western diet provides a ratio approximately 20 to 1, while in Greece prior to 1960 1/1-2/1 (Simopoulos, 2003b). On the other hand, animal by-products do not have enough linolenic acid (LNA) because of these products were used in patient nutrition diets, the need of linolenic acid did not sufficient; so that omega-3 enriched eggs are too important in such situations. Therefore, Ω-3 fatty acids, are considered beneficial and researches have been conducted trying to increase their quantity in food, specially eicosapentaenoic (EPA), and docosahexaenoic (DHA) (Stadelman and Pratt, 1989). Ferrier *et al.* (1995) mentioned that eating eggs enriched with ω-3 fatty acids produced significant increase in docosahexaenoic acid (DHA) concentration and other polyunsaturated ω-3 fatty acids in phospholipid fraction of human blood. From a nutritional point of view, eggs have always been one of the most complete foods available for man. Besides vitamins and mineral elements, eggs can provide three essential elements for a good diet: proteins, lipids and carbohydrates.

Flaxseed contains omega-3 fatty acids, which appear to benefit the human cardiovascular system by reducing low-density lipids, cholesterol, and blood platelet stickiness. Poultry rations containing flaxseed can increase levels of omega-3 fatty acids in egg yolks, and improve the omega-6/omega-3 fatty acid ratio.

It is well known, that yolk weight is correlated and increased linearly with egg weight. Yannakopulos *et al.* (1998a) reported that egg weight and yolk weight increased significantly with age of hens. However, little information is available on the effect of the age of hens on eggs yolk fatty acid composition.

The present study was carried out to investigate the effects of a special diet of flaxseed and herbal mix on egg yolk fatty acids composition, in relation to hen age, when egg weight remains constant, during the laying period under practical conditions

Materials and methods

Totally, 7500 *Hy-line* Brown Leghorn layers during one production cycle from 24 to 65 weeks of age, were used in this study. The hens were housed in cages and fed ad libitum a diet containing 10 % flaxseed meal plus a herbal mix from Mediterranean plants (enriched diet - Patent Gr 1002989).They were situated in a room and maintained at 21 ^{0}C through, while artificial light was provided a 16 h light:8 h dark daily cycle. During the study feed consumption, egg production and mortality was recorded. Also, in 24th, 28th, 32nd, 45th, 55th and 60th weeks of age, eggs were collected using the method of random sample, 20 eggs from every set were used for analysis. Eggs in all sets had about the same weight. The egg weight was recorded individually and lipid extraction was performed according to the method of Folch *et al.* (1957) and Bligh and Dyer (1959). Analysis of the fatty acid composition of the total lipid was carried out by gas chromatography (Hargis and van Elswyk, 1991).

It is noticed that since 1997 these eggs are established in the Greek market with trade mark *Ωmegga 3 eggs.*

All data were subjected to statistical analysis according to STATISTIX program.

Results and discussion

Results of performance in the total period showed an 88 % laying egg production, a mean egg weight value of 60.6 g and a 2.0 % mortality. In addition, the average daily feed consumption of hens was calculated to 110.4 g/hen. The egg production was not influenced by the enriched diet. The data obtained agree with the standards of *Hy-line* brown layers. Yannakopoulos *et al.* (1998b) reported that dietary flaxseed increased egg weight compared to the control diets

Age effects on egg yolk fatty acid composition during the laying period are shown in Table 1.

Table 1 Fatty acid composition of egg yolk lipids from hens in different ages during the laying period.

Egg weight(g)*	24thwk[1]	28thwk	32nd wk	45thwk	55thwk	60thwk
	49.50	48.43	47.79	49.50	50.93	52.89
Fat %	8.52^{a}	6.92^{b}	5.74^{b}	6.60^{b}	9.5^{a}	8.6^{a}
Fatty acid ,%						
C18:2n-6	17.32	18.61	18.01	17.70	15.04	18.61
C18:3n-3	0.44	4.82	9.07	7.10	6.83	6.45
C20:5n-3	0.23	0.89	0.69	0.48	0.43	0.21
C22:6n-3	0.32^{a}	1.03^{b}	1.25^{b}	1.10^{b}	1.33^{b}	0.85^{b}
Saturated (S),%	32.98	30.19	28.82	27.9	30.27	28.75
Monounsaturated (MUFA),%	47.46	44.03	41.63	45.53	45.32	44.60
Polyunsaturated (PUFA-P),%	19.56	25.78	29.55	26.57	24.41	26.65
Total n-3,%	1.92^{a}	7.01^{b}	11.33^{d}	9.8^{c}	8.94^{b}	7.83^{b}
Total n-6,%	17.67	18.77	18.22	17.6	15.47	18.82
Fatty acids ratio						
P/S	0.59	0.85	1.02	0.95	0.80	0.93
ω-6 (n-6)/(n-3)ω-3	9.20	2.67	1.60	1.79	1.73	2.40

^{a,b,c,d}Mean values in the same row with no common superscript are significantly different ($p<0.05$)

*edible portion (yolk+white)

[1]Start of the study

Although, there is no difference reported in egg weight among the set of eggs, there are differences in percentages of yolk fat. Older hens lay eggs, with more ($p<0.05$) yolk fat compared to that of younger ones (9.5% *vs.* 6.92%), when egg weight remains almost constant.

Concerning the fatty acids composition of egg yolks the Linolenic acid (LNA), EPA and DHA differences in yolk were higher after 32 weeks of hen age and remained about the same until the age of 55 weeks. These changes of fatty acid percentages in response to age of hens and to flaxseed diet have been hypothesized to be related to the influence of n-3 PUFA on hepatic lipid metabolism (Van Elswyk *et al.*,1994). Sell *et al.* (1968) using rape seed oil reported that consistent yolk LNA was obtained between 14 and 90 days of feeding. Thus, in the present study the highest linolenic acid concentration in yolk up to 32 weeks (eight weeks after feeding the enriched diet) may be explained by the length of the feeding time.

Sim and Cherian (1994) reported that a plateau of ω-3 PUFA incorporation in the egg yolk can be reached within 9-12 days; 18:3ω3 was the major ω-3 PUFA deposited into egg yolks, although considerable amounts of EPA, DPA and DHA, were also deposited. Also, Van Elswyk (1997) reported that yolk LNA, EPA and DHA contents stabilize after four weeks of feeding. Hargis and Van Elswyk (1991) reported that yolk n-3 fatty acid deposition plateaus reached after four weeks feeding.

Yolk Linolenic acid deposition in response to the feeding flax seed type (ground or whole) appears unchanged. In the current study 10 % flaxseed yields over 400 mg/egg. This result is greater than that of 16.3 mg/g Linolenic acid of yolk reported by Caston and Leeson, 1990; Aymond and Van Elswyk; 1995; Scheideler and Froning, 1996. Also, yolk DHA concentration is not provided directly by dietary flax seed. The concentration of DHA in enriched eggs ranges between 5-7 mg/g of yolk (Van Elswyk, 1997). In the present study, the herbal mix in the diet, seems to have the promote effect to increase the linolenic acid and DHA into yolk of *Ωmegga 3 eggs*. As previously noted, these omega-3 enriched eggs contain DHA, but they also contain LNA, as well as linoleic acid (omega 6) from the flaxseed and other nutrients in the hen's diet. This value, as well as LNA, is higher after 32 weeks of age of birds. Thus, the incorporation of LNA and DHA into egg yolk appears to be a gradual process over a period of several weeks. Chen *et al.* (1965) indicated that yolk LNA changes had been stabilized after two weeks of feeding a 10 % flax seed oil diet. Yolk LNA, EPA and DHA contents were reported to stabilize after four weeks of feeding. While marked increases in yolk n-3 PUFA are observed after one week, yolk fatty acid stabilization appears to require more time. Hepatic lipid enzyme systems apparently require more than nine days to respond to supplemental n-3 PUFA. Also, the DHA is not provided directly by dietary flaxseed. The production of yolk DHA from dietary LNA relies upon in vivo production from elongation and desaturation of LNA. The mobilization of the enzymes required by the liver to complete these reactions may need to take several weeks to stabilize. It is noted, that given the enriched diet, egg-laying hens will convert LNA to DHA and deposit both omega-3 fats into yolk, replacing less healthy saturated fats in the process. In addition the data from the present study shows that the saturated fatty acids decrease as polyunsaturated fatty acids increases.

In addition, the linoleic to linolenic acid ratio was affected by hen age. Based on present results, eggs from hens after 32 weeks (older hens) had better ratio than those from younger ones. The ratio at the start of the study (24 weeks of age) was 9.20 compared to 1.60 at 32 weeks of age. Simopoulos (2003a) reported that the eggs of chickens found on the Peloponnese in Greece that graze on wild plants and purslane were shown to contain twenty times more omega-3 fatty acids than standard supermarket eggs. They had a ratio of omega -6 to omega-3 fatty acids of 1 to 1, whilst the supermarket eggs had a lopsided ratio of 20 to 1.Therefore, not only is the presence of omega 6 and omega 3 in the human diet necessary for health, but the ratio of omega-6 to omega-3 is critical to achieving an appropriate balance of these fatty acids (*National Institutes of Health. EFA Edition. http://efaeducation.nih.gov/ Accessed 22 Nov 04*)

It was concluded that the eggs from younger hens would be a better source of omega-3 fatty acids and enriched diet has acted synergistically with the herbal mix to further increase of n-3 fatty acids in egg yolks. In addition, the level of omega-3 in egg yolk (*Ωmegga 3 eg*gs) had a tendency to rise by aged of hens, until 32 wks.

References

AHN,D.U., KIM, S.M. and SHU, H. (1997). Effect of egg size and strain and age of hens on the solids content of chicken eggs. *Poultry Science* **76**:914-919

AYMOND, W.M. and VAN ELSWYK, M.E. (1995). Yolk thiobarbituric acid reactive substances and n-3 fatty acids in response to whole and ground flaxseed. *Poultry Science* **74:**1388-1394

BLIGH, E.G. and DYER, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**:911-917

CASTON, L. and LEESON, S. (1990). Research note: dietary flax and egg composition. *Poultry Science* **69:**1617-1620

CHEN, P.H., COMMON, R.H., NIKOLAICZU K.N. and MACRAE, H. F. (1965). Some effects of added dietary fats on the lipid composition of hen's egg yolk. *Journal of Food Science* **29:**838-845

FERRIER, L.K., CASTON, L., LEESON, S., SQUIRES, J., WEAVER, B.J. and HOLUB, B. J. (1995). a-linolenic acid and docosahexaenoic acid-enriched eggs from hens fed flaxseed: influence on blood lipids and platelet phospholipids fatty acids in humans. *American Journal of Clinical Nutrition* **62**:81-86

FOLCH, K., LEES, M. and SLOANE STANLEY, G.H. (1957). A simple method for isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226:**497-509

HARGIS, P.S. and VAN ELSWYK, M.E. (1991). Modifying yolk fatty acid composition to improve health quality of egg. In: *Fat and Cholesterol Reduced Foods: Technologies and Strategies* (Haberstroh, C. and Morris, C.E., Eds), Huston, Texas, Gulf Publishing Company, pp 249-260

NIELSEN, H. (1998).Hen age and fatty acid composition of egg yolk lipid. *British Poultry Science* **39:**53-56

SALEM, N.J., SIMOPOULOS, A.P., GALLI, C., LAGARDE, M. and KNAPP, H.P. (1996). Fatty acids and lipids from cell biology to human disease. *Lipids, **31*** (Suppl):SI-S326

SCHEIDELER, S.E. and FRONING, G.W. (1996). The combined influence of dietary flax seed variety, level, form, and storage conditions on egg production and composition among vitamin E supplemented hens. *Poultry Science* **75**:1221-1226

SELL, J.L., CHOO, S.H. and KONDRA, P.A. (1968). Fatty acid composition of egg yolk and adipose tissue as influenced by dietary fat and strain of hen. *Poultry Science* **47:**1296-1302

SIM, J.S. and CHERIAN, G. (1994). a-Linolenic acid for designing poultry products for human consumption: Canadian designer eggs and nutritional significance. *Flax Council of Canada*

SIMOPOULOS, A.P. (2003a).Omega-6/omega-3 Essential Fatty Acid Ratio: The Scientific Evidence – Preface. *World's Review of Nutrition and Dietetics*. Ed. A.P. Simopoulos, Cleland LG **Vol 92**

SIMOPOULOS, A.P. (2003b). Importance of the ratio of omega-6/to omega-3 essential fatty acids: evolutionary aspects. *World's Review of Nutrition and Dietetics*. Ed. A.P. Simopoulos, Cleland LG **Vol 92**

STADELMAN, W.J. and COTTERILL, O.J. (1977). Egg Science and Technology. AVI, Westport, CT.

STADELMAN, W.J. and PRATT, D.E. (1989). Factors influencing composition of the hen's egg. *World's Poultry Science Journal*, **45:**247-266

VAN ELSWYK, M.E. (1997). Nutritional and physiological effects of flaxseed in diets for laying fowl. *Worlds Poultry Science Journal*, **Vol.53:**253-264

VAN ELSWYK, M.E., HARGIS, B.M., WILLIAMS, J.D. and HARGIS, P.S. (1994). Dietary menhaden oil contributes to hepatic lipidosis in laying hens. *Poultry Science* **73:**653-662

YANNAKOPOULOS, A.L., TSERVENI-GOUSI, A.S. and CHRISTAKI, E. (1998a). Effect of natural zeolite on yolk:albumen ratio in hen eggs. *British Poultry Science* **39**:506-510

YANNAKOPOULOS, A.L., TSERVENI-GOUSI, A.S. and YANNAKAKIS, E. (1998b).Effect of feeding flaxseed to laying hens on the he performance and fatty acid composition of yolk eggs. The Return of ω3 fatty acids into the food supply. I. Land-based animal food products and their health effects. Simopoulos AP (ed).*World's Review of nutrition and dietetics*. **vol. 83**

The color of egg yolk is an important parameter for consumers and is produced by carotenoid pigments in the feed. The yolk color depends not only on the levels of pigmenting substances, namely, xanthophylls, present in the feed, but also on the type and the ratio of these compounds [1]. There are different sources of xanthophylls used for egg yolk pigmentation.Â Water and feed were provided ad libitum during the study. The photoperiod was maintained at 16L:8D throughout the study. Body weights of laying hens were determined at the beginning and end of the study.Â Table 4 shows the fatty acid composition of eggs from laying hens fed diets supplemented with 0, 10, or 20 g kgâˆ'1 marigold flour. Inclusion of marigold in the diet at either level significantly. The fatty acid compositions of the yolks (in mg/g of yolk) as a function of the two dietary treatments and over the entire feeding duration are presented in Table 3. Total saturated fatty acids (SFA) showed a highly significant (p Ë, 0.0001) time effect for both F-OL and FS-PSO diets. Palmitic and stearic acids decreased in the yolks in both groups all along the experiment, with the lowest levels seen at period 6. However, higher concentrations of these fatty acids were observed with the FS-PSO diet compared to F-OL. As compared with the FS-PSO group, feeding the F-OL diet to hens resulted in higher concentrations of hypogeic acid (C16:1c7), OA and cis-vaccenic acid (C18:1c11) in yolk lipids. Fatty acid composition of different dietary oils. The FA composition of the fish, olive, grape seed, canola and soybean oil is shown in Table 3. The fish oil of was a rich source of LC PUFA; n-3 and contained EPA (5.59 %) and DHA (14.20%). The fish oil had the highest amount of C14:0, C16:0, C18:0, EPA, DHA, SFA and total omega-3 FAs and the lowest amount of C18:2. Olive and canola oil were rich in C18:1 and had 69.00% and 58% of this FA, respectively. The grape seed and soybean oil were rich in C18:2 and had 68.00% and 55.50% of this FA, respectively. Performance.Â Based on the results, adding 3% fish oil to the laying hens diet, could increase DHA and EPA content of egg yolk with their consequent health benefits. References. Leeson S, Summers JD. systems 5.2 Fatty acid composition in egg yolk from three different studies. 6 Discussion 6.1 Conclusion. Acknowledgements.Â The fatty acid compositions were analyzed and the results were compared with other studies of hen systems. A literature review was done to try to find out what kind of diet the laying hens have had from the fatty acid compositions in the eggs.Â Barn-laid hens are similar to free range production sys-tems where hens are the hens are kept indoors (Samiullah et al., 2017). Organic and conventional eggs do not have enough difference in saturated fat that it would have a significant effect on the metabolism of the consumer according to Samman et al. Saturated fatty acids contain the maximum level of hydrogen atoms possible, while in unsaturated fatty acids, some of the hydrogen atoms are missing and have been replaced with double bonds between the carbon atoms. Figure 1. Structure of a triglyceride and saturated, monounsaturated and polyunsaturated fatty acids. The fat is termed â€œmonounsaturatedâ€ if there is one double bond, and â€œpolyunsaturatedâ€ if there are two or more double bonds. The omega-3 and omega-6 are fatty acids both types of polyunsaturated fat. The difference is in where the first of the double bonds occurs.