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Production of designer egg: Effects of various n-3 lipid sources on fatty acids composition and sensory characteristics of chicken egg

S EZHIL VALAVAN[1], B MOHAN[2], P SELVARAJ[3], R RAVI[4], K MANI[5], S C EDWIN[6] and A BHARATHIDHASAN[7]

Veterinary College and Research Institute, Namakkal 637 002 India

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ABSTRACT

Layer biological experiment was conducted to study the effect of various n-3 lipid sources such as fish, linseed and rapeseed oils to enrich n-3 or omega -3-fatty acids in chicken egg from 20–70 weeks of age. The supplementation of n-3 lipid sources in layer ration significantly increase linolenic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), total n-3 fatty acids, total n-6 fatty acids and total n-3/n-6 fatty acids ratio of egg yolk, and a significant reduction in palmitic and stearic acid concentrations. The total unsaturated fatty acids concentration in egg yolk showed an increase in all the treated groups due to incorporation of various n-3 lipid sources in feed.

The inclusion of n-3 lipid sources (at 1, 2 and 3% levels) independently and simultaneously in feed had no adverse effect on sensory quality of egg. Feeding layers with 3% fish oil showed significant increase in EPA, DHA and total n-3 fatty acids in egg yolk.

Key words: Chicken egg, Enriched with n-3, Enriched with omega -3- fatty acids, Sensory characteristics

Egg is the cheapest source of protein and easily available all over the world. Owing to consumers' awareness of the relationship between dietary lipid and incidence of coronary heart disease their attitude has changed towards egg consumption as they fear that its cholesterol will raise their blood cholesterol levels. Eggs have been singled out as a food to avoid, which has resulted in a significant reduction in egg consumption in many parts of the world. The health promoting effects of dietary n-3 or omega-3-fatty acids have provoked considerable effort to enrich animal products using various sources of omega-3-fatty acids. Dietary intake of omega-3 fatty acids decreases risk of heart disease (Temple 1996) and plays an important role in preventing cancer (Pandalai *et al*. 1996) as well as immunologic reactions (Fernandes 1995). Omega-3 fatty acids are also essential for normal foetal brain and visual development (Neuringer *et al*. 1998). Since, poultry is capable of accumulating nutrients and health promoting non-nutrients, based on their dietary levels; an attempt has been made here to incorporate various n-3 or omega-3-fatty acids in chicken egg to improve human health significantly. The objective of the present biological experiment was to investigate the effect of various n-3 lipid sources such as fish, linseed and rapeseed oils on fatty acids composition and sensory characteristics of chicken egg.

MATERIALS AND METHODS

The layer biological experiment was started using 273 ready to lay (18 weeks of age) pullets of a single hatch and strain obtained from a local commercial layer farm. The pullets were weighed, leg banded and randomly allotted to thirteen treatment groups with three replicates having seven pullets each. Omega-3-fatty acids or n-3 lipid sources such as fish, linseed and rapeseed oils were incorporated into layer basal diets formulated as per the standard prescribed by BIS (1992) at the graded levels either independently or simultaneously and thus the experimental diets were prepared and were formed 13 treatment groups (Table 1).

All the diets were made isonitrogenous and isocaloric by adjusting other ingredients. Pullets of all treatments were reared in cage system of management with standard managemental practices throughout the experimental period except for the variation of n-3 lipid sources used in feed. The birds were fed with experimental diet *ad lib.* up to 70 weeks of age.

Present address: [1]Assistant Professor, Directorate of Distance Education, Tamil Nadu Veterinary and Animal Sciences University, Chennai (vet_ezhil2001@yahoo.co.in). [2]Professor and Head, ADSL, [5]Professor and Head, Education Cell, [6]Professor and Head, Department of Poultry Science, VCRI, Namakkal. [4]Professor and Head, Department of Animal Nutrition, VCRI, Tirunelveli. [3]Professor and Head, Department of Veterinary Physiology and Bio-chemistry, VCRI, Orathnad. [7]Assistant Professor, Department of Animal Nutrition, MVC, Chennai.

Table 1. Treatment groups and experimental diet of layers

Treatment groups	Experimental diet	Treatment groups	Experimental diet
T_1	Basal feed (control)		
T_2	Basal feed + Fish oil 1%	T_8	Basal feed + Fish oil 3%
T_3	Basal feed + Linseed oil 1%	T_9	Basal feed + Linseed oil 3%
T_4	Basal feed + Rapeseed oil 1%	T_{10}	Basal feed + Rapeseed oil 3%
T_5	Basal feed + Fish oil 2%	T_{11}	Basal feed + (Fish oil +Linseed oil +Rapeseed oil) 1%
T_6	Basal feed + Linseed oil 2%	T_{12}	Basal feed + (Fish oil +Linseed oil +Rapeseed oil) 2%
T_7	Basal feed + Rapeseed oil 2%	T_{13}	Basal feed + (Fish oil + Linseed oil+ Rapeseed oil) 3%

Eggs were collected once in every 10 weeks period to study fatty acids composition of egg yolk. The egg yolks were used to extract lipids and transmethylation was done using methylation procedure as described by Sukhija and Palmquist (1988). The thin layer chromatography (TLC) was performed for lipid class separation and to check the esterification process. From each group 2 g of egg yolk was weighed separately into test tubes, 10 volumes of Folch-I solution (containing chloroform methanol 2: 1vol/vol) (Folch *et al*. 1957) was added and homogenized for 10 seconds at high speed; 25 micrograms of butylated hydroxyanisole (10 per cent) dissolved in 98% ethanol was added to each sample prior to homogenization. The homogenate was filtered through Whatman No. 1 filter paper into 100 ml graduated cylinder and one-fourth volume (on the basis of Folch –1) of 0.88% sodium chloride solution was added and capped with glass stopper. The filtrate was mixed well. The cylinder was washed twice with 10 ml of Folch – II solution (3: 47: 48 of chloroform: methanol: water) and the contents were separated. The upper layer was siphoned off and the lower layer was taken into a glass scintillation vial and dried at 50°C under nitrogen. Thin layer chromatography was carried out as per the method of Du *et al*. (2000) to check completeness of transmethylation process.

The extracted and dried lipids were dissolved in chloroform to set the final concentration of lipid at 0.2 g/ml. The lipid – chloroform solution (150 microliter) was loaded on to an activated (120°C for 2 h) silica gel plate (20 cm × 20 cm). The plate was developed first in solvent – I, composed of chloroform : methanol : water (65 : 25 : 4, vol/vol/vol) until the solvent line reached the middle of the plate. The plate was air - dried and redeveloped in solvent – II, composed of hexane: diethyl ether (4 : 1 vol/vol) until the solvent front reached one inch below the top of the plate. After air-drying for 10 min at room temperature, the plates were sprayed with 0.1% of 2', 7' dichlorofluororescine in ethanol. Lipid classes were identified under UV light and methyl esters were scrapped into separate test tubes and dissolved in hexane and passed on to an anhydrous sodium sulphate column to remove any moisture before injecting into gas chromatography.

Fatty acids were identified with reference to the standards and they were quantified as per area normalization method. Then, they were expressed as percentage of total fatty acids. The fatty acid methyl esters were separated and quantified by gas chromatography using a fused silica capillary column of 30 m × 0.25 mm i.d, 0.25 microfilm thickness. Ramped oven temperature conditions (180°C for 5 min increased to 220°C and held for 5 min) were used. Temperature of both injector and detector were 250°C and 260°C respectively.

Organoleptic properties of chicken eggs were recorded on a 7- point Hedonic scale with ascending ratings for the desired attributes of colour, flavour, texture and overall acceptability (Panda *et al*. 1982) once in every 28 days. The data collected in this experiment were subjected to statistical analysis as per Snedecor and Cochran (1989). Angular transformation is applied to percentages before statistical analysis is done. Data on sensory evaluation of eggs were subjected to Kruskal - Wallis K - sample non - parametric test (Sokal and Rohlf 1995).

RESULTS AND DISCUSSION

The results of this study showed that incorporation of various n-3 lipid sources at graded level in layer feed had significant effect on palmitic, stearic and oleic acids content and (Table 2) of egg yolk. Linoleics and linolenic acid content were lower in control group and recorded higher in group T_9 respectively. Herstad *et al.* (2000) observed that in layer diet containing three per cent fish oil decreased the linoleic acid content of egg yolk, which is in agreement with the results of this study. Baucells *et al*. (2000) reported that hens fed on 4% fish-oil enriched diet which was replaced with linseed and rapeseed oils in various proportion and the results revealed that smaller proportion of fish oil in diets resulted in lower value of saturated fatty acids and higher values of n-6 fatty acids such as linoleic acid content regardless of the fat source used when replacing fish oil, which is partially agreeable with the results of this study. In general the n-6 fatty acids content of egg yolk increased mostly because of rise in linoleic acid among treatment groups, which is in agreement with the results of this study. The results showed a highly significant ($P<0.01$) difference due to dietary treatments on linolenic acid content of egg yolk.

Supplementation of linseed oil up to 3% level in this experiment gradually increased linolenic acid content of egg

Table 2. Mean (± SE) palmitic, stearic, oleic, linoleic, linolenic, EPA, DHA, total n-3 fatty acids, total n-6 fatty acids (%) and total n-3/n-6 fatty acids ratio in egg yolk of layers as influenced by various n-3 lipid sources in feed

Treatment Groups	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	EPA	DHA	total n-3 fatty acids	total n-6 fatty acids	total n-3/ n-6 fatty acids ratio
T_1 – Control	35.00^{e}	11.50^{f}	33.70^{a}	11.50^{a}	0.62^{a}	0.67 ^{a}	1.10 ^{a}	2.10^{a}	12.40 ^{a}	0.23^{a}
	±0.250	±0.150	±0.190	±0.30	±0.01	±0.05	±0.01	±0.04	±0.19	±0.03
T_2 – FO 1%	30.70^{c}	07.20^{ab}	36.30^{ef}	12.80^{cde}	0.68^{b}	1.60 ^{f}	4.30 ^{h}	4.80^{h}	13.50^{cd}	0.54 ^{f}
	±0.200	±0.070	±0.200	±0.11	±0.02	±0.10	±0.08	±0.25	±0.19	±0.05
T_3 – LO 1%	32.80^{d}	09.50^{e}	36.50^{fg}	13.20^{de}	0.83^{ef}	0.81^{ab}	1.30^{ab}	2.50^{bc}	13.40^{c}	0.24 ^{a}
	±0.130	±0.070	±0.270	±0.24	±0.06	±0.04	±0.08	±0.04	±0.16	±0.05
T_4 – RO 1%	30.40^{c}	10.70^{f}	36.40^{f}	13.30^{de}	0.79^{e}	0.83^{bc}	2.10^{cd}	3.20^{d}	13.30 ^{ab}	0.31^{b}
	±0.390	±0.190	±0.170	±0.22	±0.04	±0.11	±0.04	±0.05	±0.12	±0.06
T_5 – FO 2%	26.60^{a}	08.30^{c}	36.20^{de}	12.40^{bc}	0.71^{c}	3.60^{g}	5.50 ^{i}	7.80 ^{j}	14.60 ^{ef}	0.79 ^{g}
	±0.350	±0.370	±0.320	±0.10	±0.03	±0.22	±0.13	±0.10	±0.16	±0.04
T_6 – LO 2%	30.70^{c}	08.70^{cd}	36.70^{g}	15.70^{h}	0.93^{gh}	0.80^{ab}	1.30^{b}	2.70^{c}	15.90 ^{h}	0.24 ^{a}
	±0.213	±0.170	±0.127	±0.15	±0.09	±0.09	±0.09	±0.09	±0.09	±0.08
T_7 – RO 2%	30.60^{c}	08.60^{c}	35.10^{c}	15.40^{gh}	0.70^{f}	1.10^{d}	2.70 ^{ef}	3.70 ^{f}	14.00^{de}	0.31^{b}
	±0.160	±0.600	±0.170	±0.14	±0.04	±0.05	±0.12	±0.07	±0.09	±0.14
T_8 – FO 3%	26.50^{a}	06.30^{a}	35.00^{b}	12.7 0^{cd}	0.68^{c}	4.90^{h}	6.60 ^{j}	9.70 ^{k}	15.30^{gh}	0.98 ^{h}
	±0.210	±0.198	±0.200	±0.10	±0.03	±0.42	±0.36	±0.17	±0.11	±0.03
T_9 – LO 3%	29.40^{bc}	08.50^{c}	35.30^{c}	17.20^{i}	1.00^{h}	0.97^{cd}	2.20^{d}	3.70 ^{f}	15.90^{h}	0.29^{b}
	±0.310	±0.380	±0.210	±0.06	±0.07	±0.05	±0.08	±0.25	±0.11	±0.13
T_{10} – RO 3%	30.20^{c}	07.90^{bc}	35.40^{c}	14.80^{f}	0.84^{f}	1.10 ^{d}	3.80 ^{gh}	4.50^{h}	14.80 ^{g}	0.42 ^{de}
	±0.170	±0.160	±0.100	±0.09	±0.08	±0.18	±0.28	±0.25	±0.08	±0.04
T_{11}	30.50^{c}	09.20^{de}	36.00^{cd}	14.50^{ef}	0.78^{de}	1.20^{d}	2.30 ^{de}	3.60^{ef}	14.70 ^{fg}	0.32 ^{bc}
	±0.190	±0.220	±0.070	±0.11	±0.03	±0.06	±0.12	±0.09	±0.10	±0.04
T_{12}	30.10^{c}	07.50^{b}	35.40^{c}	15.40^{h}	0.79^{e}	1.50^{ef}	3.00 ^{f}	4.40^{gh}	15.90^{h}	0.37^{cd}
	±0.190	±0.050	±0.090	±0.17	±0.05	±0.14	±0.21	±0.22	±0.20	±0.06
T_{13}	29.60^{c}	07.20^{b}	35.00^{bc}	15.50^{h}	0.81^{e}	1.60^{f}	4.30 ^{h}	5.50^{I}	13.50^{d}	0.45^{e}
	±0.150	±0.100	±0.060	±0.13	±0.06	±0.13	±0.25	±0.19	±0.13	±0.04

Mean values not sharing a common superscript column wise differ significantly (P< 0.01). FO, Fish oil; LO, Linseed oil; RO, Rapeseed oil. T_{11} – (FO+LO+RO) 1%, T_{12} – (FO+LO+RO) 2%, T_{13} (FO+LO+RO) 3%;

yolk, which substantiated with the results of Meluzzi *et al.* (2001). Antruejo *et al*. (2011) supplemented LNA rich ingredient in layer diet and noted increased omega -3 fatty acids in egg yolk. In this trial, supplementation of linseed oil up to 3% level had higher linolenic acid content of egg yolk when compared to group supplemented with fish-oil at 1, 2 and 3% levels and control groups. Similarly, Baucells *et al*. (2000) observed increased total n-3 fatty acid in the form of linolenic acid when replacing fish oil with linseed oil. It is nothing new that increasing levels of linolenic acid from vegetable sources result in its increased concentration in the yolk lipids (Leskanich and Noble 1997). The level of linolenic acid is higher in linseed oil than the rest of the n-3 lipid sources used in this study. These facts reinforce once again the theory of slight *de novo* synthesis of these long chain polyunsaturated fatty acids from their precursors (Baucells *et al*. 2000).

The eggs collected from the birds in group T_8 had the highest value of EPA, DHA, total n-3 fatty acids content and higher n-3/n-6 fatty acids ratio whereas the lowest values were recorded in control group. The comparison of means suggested that the eggs collected from the birds in groups T_6, T_9 and T_{12} recorded the highest concentration of total n-6 fatty acids in egg yolk over the eggs collected from the birds in control group. The study revealed a four fold increase in EPA content of egg yolk due to n-3 lipid source supplementation, which is highly significant (P<0.01). The above observation is in agreement with the earlier reports of Chen Ijen *et al*. (2000) and Meluzzi *et al*. (2000).

Due to n-3 lipids source supplementation at graded level to layers in this experiment, highly significant (P<0.01) 5.5 fold increase in DHA content of egg yolk was observed, which is in agreement with the earlier reports of Chen Ijen *et al*. (2000) and Meluzzi *et al*.(2001).

According to Brenner (1989), alpha linolenic acid is an essential fatty acid, which through successive metabolic desaturation and elongation reaction gives rise to EPA and DHA. As in this study, the main phospholipid classes of egg yolk from hens fed fish oil contained significantly higher levels of DHA than EPA indicating that DHA rather than EPA may be preferentially incorporated into membranes (Cossignani *et al*. 1994). On the other hand, it is interesting

Table 3. Mean (± SE) sensory evaluation values on yolk colour, texture, flavour and overall acceptability of eggs as influenced by various n-3 lipid sources in feed

Treatment groups	Colour	Texture	Flavour	Overall acceptability
T_1 – Control	3.77±0.04	3.71±0.07	3.82±0.04	6.51±0.07
T_2 – FO 1%	3.73±0.04	3.58±0.09	3.73±0.03	6.49±0.08
T_3 – LO 1%	3.69±0.04	3.57±0.09	3.87±0.05	6.56±0.07
T_4 – RO 1%	3.69±0.03	3.54±0.06	3.74±0.05	6.49±0.07
T_5 – FO 2%	3.63±0.03	3.47±0.06	3.48±0.02	6.36±0.06
T_6 – LO 2%	3.42±0.05	3.23±0.05	3.47±0.05	6.21±0.08
T_7 – RO 2%	3.53±0.05	3.32±0.03	3.45±0.07	6.35±0.10
T_8 – FO 3%	3.43±0.06	3.21±0.05	3.35±0.04	6.21±0.05
T_9 – LO 3%	3.44±0.05	3.25±0.07	3.37±0.03	6.20±0.08
T_{10} – RO 3%	3.46±0.04	3.32±0.05	3.41±0.04	6.27±0.05
T_{11}	3.45±0.04	3.36±0.05	3.54±0.0 4	6.35±0.07
T_{12}	3.49±0.07	3.41±0.04	3.56±0.07	6.37±0.06
T_{13}	3.52±0.07	3.41±0.06	3.57±0.06	6.37±0.07

T_{11}, (FO+LO+RO) 1%; T_{12}, (FO+LO+RO) 2%; T_{13}, (FO+LO+RO) 3%. FO, Fish oil; LO, linseed oil; RO, rapeseed oil.

to note that, although the fish oil used in this study contained a higher level of EPA than DHA, the resulting level of DHA in the egg yolk was much higher than EPA content as was previously found by other researchers (Chen Ijen *et al*. 2000). It must be recalled that DHA obtained in yolk was from a double pathway; one from the direct deposit of DHA from the diet and the other by *de novo* synthesis from its precursors (Linolenic acid and EPA) given in the diet (Leskanich and Noble 1997).

In this study, highly significant (P<0.01) difference among dietary treatments was noted on total n-3 fatty acids content of egg yolk. Comparison of means suggested that the total n-3 fatty acids content increased due to the incorporation of dietary n-3 lipid sources of layers which is in agreement with the earlier reports of (Laca *et al*. 2009).

The layers fed different n-3 lipid sources at graded level showed highly significant (P<0.01) difference on total n-6 fatty acids. Comparison of means indicated that the eggs collected from the birds supplemented with fish oil at 1 per cent level had higher values of total n-6 fatty acid content when compared to eggs collected from the control and other treated groups. Similarly, Baucells *et al.* (2000) indicated that smaller proportion of fish oil in layer diet resulted in higher values of n - 6 fatty acids content regardless of the fat source used when replacing fish oil.

The n-6 fatty acids content increased mostly because of the rise in linoleic acid.

Highly significant (P<0.01) difference due to dietary treatment was observed on total n-3/n-6 fatty acids ratio in egg yolk in this study. The comparison of means suggested that the inclusion of various n-3 lipid sources at graded level in layer diet had higher n-3/n-6 fatty acids ratio when compared to eggs collected from control group. Petrovic *et al*. (2012) observed that supplementation of linseed oil at 1 to 4 per cent decreased the ratio between n-6 and n-3 polyunsaturated fatty acids in eggs.

Meluzzi *et al.* (2001) indicated that n-3/n-6 fatty acids ratio in the egg yolk was directly related to feed intake which are in agreement with the results of this study.

In general, the total unsaturated fatty acids concentration in egg yolk showed an increase in all the treated groups due to incorporation of various n-3 lipid sources in feed. Increased concentration of PUFA rich oils in diets resulted in less saturated fatty acids and more n-3 PUFA in egg yolks (Kralik *et al*. 2008, King'ori A M 2012).

Sensory quality characteristics (Table 3) such as colour, texture, flavour and overall acceptability did not get altered significantly due to incorporation of n-3 lipid sources at graded level in layer diets. The eggs collected from the birds in group T_6 recorded the lowest egg colour score, when compared to eggs collected from the birds in control group. The egg texture score ranged between 3.21 (T_8) and 3.71 (control). The values of flavour due to various treatments ranged between 3.35 (T_8) and 3.87 (T_3). The eggs collected from the birds in group T_3 recorded higher overall acceptability scores over the eggs collected from the birds in other treated groups and the poor overall acceptability score was found in T_9. The organoleptic evaluation of eggs remained acceptable without causing fishy flavour by feeding up to 1.5% (Laca *et al*. 2009) fish oil to layers and taste of hard-boiled eggs was not influenced by flaxseed oil (Tallarico *et al.* 2002) which are almost agreeable to the results of this study. However, Meluzzi *et al.* (2000) reported that sensory analysis of boiled and scrambled eggs showed a substantial difference between two marine oils with EPA oil, and fishy flavour is easily identified, particularly in boiled eggs when oil supplementation exceeded two per cent, which is not in agreement with this study.

The supplementation of n-3 lipid sources independently and simultaneously in layer ration had significant (P<0.01) increase of oleic acid, linoleic acid, linolenic acid, EPA, DHA, total n-3 fatty acids, total n-6 fatty acids and total n-3/n-6 fatty acids ratio of egg yolk and a significant reduction (p<0.01) in palmitic acid and stearic acid concentrations. The total unsaturated fatty acids concentration in egg yolk showed an increase in all the treated groups due to incorporation of various n-3 lipid sources in feed. Sensory qualities of eggs were not affected by supplementing various n-3 lipid sources layer in feed.

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