Effects of various n-3 lipid sources on the quality characteristics and fatty acids composition of chicken egg

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The present study was undertaken at Centre of Advanced Studies in Poultry Science, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India. 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 fatty acids in chicken egg from 20 – 70 weeks of age. The inclusion of n-3 lipid sources (at one, two and three per cent levels) independently and simultaneously in feed had no adverse effect on body weight, feed consumption, feed efficiency, egg production, livability, egg quality characteristics and sensory evaluation of eggs in layers. The supplementation of n-3 lipid sources in layer ration had significant ($P<0.01$) increase of n-3 fatty acids composition such as 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 ($p<0.01$) 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. Feeding layers with combination of fish + linseed + rapeseed oil at one per cent level gave the higher profit margin.

Key Words: n-3 lipid sources; production performances; fatty acids composition of chicken egg.

Introduction

Eggs are one of the highest nutrient dense foods containing high quality protein and balanced distribution of minerals and vitamins except vitamin C. Egg is a cheapest source of protein and easily available all over the world. Due to consumer awareness of the relationship between dietary lipid and the incidence of Coronary Heart Disease their attitude has changed towards egg consumption because of their fear that egg 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 Omega-3-fatty acids have provoked considerable effort to enrich animal products using various sources of Omega-3-fatty acids. These Omega-3-fatty acids are essential for proper brain development in infants and proper cardiac health in adults. However, the present day food consumption pattern in India causes acute shortage of these health-promoting nutrients, leading to growing neurological and cardiac problems.

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 Omega-3-fatty acids in chicken egg to improve human health significantly.

Materials and Methods

The layer biological experiment was started using two hundred and seventy three 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. n-3 lipid sources such as fish, linseed and rapeseed oils were

Table 1.

Treatment groups and experimental diets of layers

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

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 formed thirteen treatment groups (Table 1). All the diets were made isonitrogenous and isocaloric by adjusting the 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 libitum* up to 70 weeks of age.

Initial individual body weight of the pullets in all the treatment groups and subsequent body weight, feed consumption and egg quality parameters such as egg weight, shape index, albumen index, yolk index, Haugh unit score, yolk colour and shell thickness were recorded once in 28 days up to 70 weeks of age. During the experimental period, the egg production was recorded daily. Based on the data, egg production was calculated. Organoleptic properties of chicken eggs were recorded on a seven 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.

Eggs were collected once in every 10 weeks period to study the fatty acids composition of egg yolk. The egg yolks were used to extract the 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 two grams 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. Twenty-five micrograms of butylated hydroxyanisole (10 per cent) dissolved in 98 per cent 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 per cent 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^oC under nitrogen.

Thin layer chromatography was carried out as per the method of Du *et al.* (2000) to check completeness of the transmethylation process. The extracted and dried lipids were dissolved in chloroform to set the final concentration of lipid at 0.2 g per ml. The lipid – chloroform solution (150 µl) was loaded on to an activated (120^oC for 2 h) silica gel plate (20 x 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. Then 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 per cent 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 (Supelco 2380) of 30 m x 0.25 mm i.d, 0.25 μ film thickness. Ramped oven temperature conditions (180^0C for 5 min increased to 220^0C and held for 5 min) were used. Temperature of both injector and detector were 250 and 260^0C respectively.

The relative economics of producing n-3 fatty acids enriched chicken eggs were calculated on the actual prevailing cost of the feed during the study period. 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 wherever needed. Data on sensory evaluation of eggs were subjected to Kruskal - Wallis K - sample non - parametric test (Sokal and Rohlf, 1995).

Results and discussion

The mean body weight (g), feed consumption (g), feed efficiency, egg production (%), egg weight (g), overall acceptability and net profit per egg of all the treatment groups are given in Table 2. The data reveals a non significant difference in body weight, feed consumption (Zhi-Bin Huang *et al.,* 1990; Herber and Van Elswyk, 1996; Meluzzi *et al.,* 2000 ; Grobas *et al.*,2001 and Halle, 2001), feed efficiency (Zhi-Bin Huang *et al.*,1990 ;Meluzzi *et al.,*2000 ; Li *et al.*,1989 and Baucells *et al.,*2000), egg production (Hargis *et al.,*1992 ; Meluzzi *et al.,*2000; Herber and Van Elswyk,1996; Li *et al.,*1989 and Halle 2001), egg quality characteristics such as egg weight, shape index, albumen index, yolk index, Haugh unit score, yolk colour and shell thickness (Hargis *et al.,*1991 ; Van Elswyk *et al.,*1992 ; Baucells *et al.,*2000,Meluzzi *et al.*,2000 ;Grobas *et al.,*2001 ;Tallarico *et al.,*2002; Galobart *et al.,* 2001[a] and Galobart *et al.,* 2001[b]) and sensory quality characteristics such as colour, texture, flavour and overall acceptability (Zhi- Bin Huang *et al.,* 1990;Damiani *et al.*, 1994;Gonzalez-Esquerra and Leeson 2000; Huyghebaert, 1995 and Tallarico *et al.,* 2002)

The mean percentage of palmitic acid, stearic acid, oleic acid, linoleic acid, 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 in egg yolk estimated by Gas chromatography are given in Table 3. From the results, it can be noted that incorporation of various n-3 lipid sources in layer feed had significant effect on palmitic, stearic and oleic acids content of egg yolk. Sim *et al*. (1973) observed a preferential deposition of linoleic acid in egg yolk from rapeseed oil when fed to laying hens, which is in accordance with the results of this study. Supplementation of linseed oil up to three per cent level in this experiment gradually increased linolenic acid content of egg yolk, which substantiated with the results of Suzuki *et al*. (1994) and Meluzzi *et al.* (2001). Feeding of linseed oil up to three per cent level had higher linolenic acid content of egg yolk when compared to group supplemented with fish oil at one, two and three per cent 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).

Table. 2
Mean (± S.E.) 70th week body weight (g), feed consumption (g), feed efficiency, egg production (%), egg weight (g), overall acceptability and net profit per egg as influenced by various n-3 lipid sources in feed

Treatment groups	70th week Body weight	Feed consumption	Feed efficiency	Egg production	Egg weight	Overall acceptability	Net profit per egg produced (Rs.)
T_1 – Control	1443.88 ± 05.74	39519.10 ± 635.21	1.48 ± 0.07	87.85 ± 2.31	55.52 ± 0.62	6.51 ± 0.07	0.17
T_2 – FO 1%	1482.95 ± 29.46	39006.93 ± 567.72	1.49 ± 0.03	85.97 ± 1.77	55.23 ± 0.27	6.49 ± 0.08	3.13
T_3 – LO 1%	1463.43 ± 25.76	39203.98 ± 565.58	1.53 ± 0.08	85.85 ± 4.79	56.13 ± 0.27	6.56 ± 0.07	3.11
T_4 – RO 1%	1478.94 ± 24.57	40447.90 ± 657.44	1.64 ± 0.10	81.10 ± 3.27	55.33 ± 0.44	6.49 ± 0.07	3.04
T_5 – FO 2 %	1477.81 ± 12.64	40389.81 ± 551.97	1.53 ± 0.03	86.45 ± 2.23	55.74 ± 0.31	6.36 ± 0.06	3.04
T_6 – LO 2%	1447.33 ± 23.10	39515.58 ± 729.94	1.50 ± 0.06	87.18 ± 2.43	55.76 ± 0.27	6.21 ± 0.08	3.12
T_7 – RO 2%	1453.31 ± 15.89	39637.44 ± 514.15	1.61 ± 0.04	81.09 ± 1.85	56.37 ± 0.32	6.35 ± 0.10	3.03
T_8 – FO 3 %	1395.10 ± 12.73	38961.04 ± 161.03	1.45 ± 0.04	87.95 ± 2.02	54.60 ± 0.24	6.21 ± 0.05	3.08
T_9 – LO 3%	1417.29 ± 25.18	39698.58 ± 276.76	1.54 ± 0.04	84.69 ± 2.26	55.79 ± 0.41	6.20 ± 0.08	3.12
T_{10} – RO 3%	1458.38 ± 08.41	40143.35 ± 517.80	1.56 ± 0.05	84.76 ± 3.09	55.56 ± 0.34	6.27 ± 0.05	3.05
T_{11}	1437.91 ± 07.74	39524.89 ± 239.10	1.43 ± 0.01	89.67 ± 2.49	55.45 ± 0.59	6.35 ± 0.07	3.18
T_{12}	1474.45 ± 26.21	40431.30 ± 260.95	1.53 ± 0.08	86.66 ± 2.57	56.10 ± 0.53	6.37 ± 0.06	3.08
T_{13}	1432.03 ± 15.37	40280.12 ± 575.98	1.50 ± 0.01	88.02 ± 2.83	55.95 ± 0.34	6.37 ± 0.07	3.08

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

A four-fold increase in Eicosapentaenoic acid (EPA) content of egg yolk was recorded due to n-3 lipid source supplementation, which is highly significant ($P<0.01$). Similar results were observed by Zhi - Bin Huang *et al*. (1990), Hargis *et al*. (1991), Van Elswyk *et al*. (1992), Suzuki *et al.* (1994), Cherian *et al*. (1996), Meluzzi *et al.* (1998), Chen Ijen *et al*. (2000), Meluzzi *et al*. (2000), Herstad *et al.* (2000) and Halle (2001). The Docosahexaenoic acid (DHA) content of egg yolk was increased 5.5 fold when compared to control group which is in agreement with the earlier reports of Zhi - Bin Huang *et al*. (1990), Hargis *et al*. (1991), Van Elswyk *et al*. (1992), Suzuki *et al.* (1994), Takita *et al.* (1995), Cherian *et al*.(1996), Meluzzi *et al.*(1998), Chen Ijen *et al*. (2000), Herstad *et al.* (2000), Meluzzi *et al*.(2000 and2001).

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 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

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

a,b,c,... Mean values not sharing a common superscript column wise differ significantly. (P< 0.01)

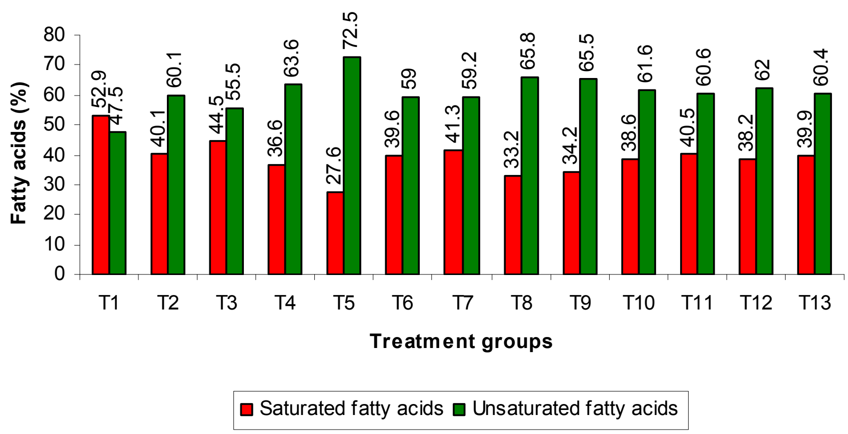
T_{11} – (FO+LO+RO) 1%; T_{12} – (FO+LO+RO) 2 %; T_{13} – (FO+LO+RO) 3 %

Fo – Fish oil; LO – Linseed oil; RO – Rapeseed oil

yolk was much higher than EPA content as reported previously by other researchers (Van Elswyk *et al*., 1992; Takita *et al*., 1995 and Chen Ijen *et al*., 2000). There was a significant increase in total n-3 fatty acids content in egg yolk in all the treatment groups, which is in agreement with the earlier reports of Damiani *et al.* (1994). The layers fed different n-3 lipid sources showed highly significant (P<0.01) difference on total n-6 fatty acids content in egg yolk. Layers fed with fish oil at 1 per cent level had the higher values of total n-6 fatty acid content when compared to eggs collected from the control and other treated groups. Similar observation was made by Baucells *et al.* (2000).

Highly significant (P<0.01) difference was observed on total n-3 / n-6 fatty acids ratio in egg yolk in the study. Inclusion of various n-3 lipid sources at graded level in layer diet had higher n-3 / n-6 fatty acids ratio was observed in treated group which is in agreement with the earlier reports of Hargis *et al.* (1991). Takita *et al.* (1995) concluded that the n-3 / n-6 fatty acids ratio of egg yolk increased significantly in fish oil fed group. However, Meluzzi *et al.* (2001) indicated that n-3 / n-6 fatty acids ratio in the egg yolk was directly related to feed intake. In general, the total unsaturated fatty acids concentration in egg yolk (Fig.1) showed an increase in all the treated groups due to incorporation of various n-3 lipid sources in feed.

Figure 1: Concentration of saturated and unsaturated fatty acids in egg yolk of layers at 70 weeks of age as influenced by various n-3 lipid sources in feed



The experiment proved that all the eggs collected from treated groups were sold at the rate of Rs 4.20 per egg in the market, which increased the profit margin of the eggs. The eggs collected from control group were sold at the rate of Rs 1.20 per egg. Hence from this study, it is concluded that the use of n-3 lipids viz. fish oil, linseed oil, rapeseed oil and their combinations could increase the returns to the farmers on the rupee spent.

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