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PRODUCTION OF DESIGNER FOOD: EFFECT OF SUPPLEMENTATION OF OMEGA -3 - FATTY ACIDS ENRICHED SOURCES ON FATTY ACIDS COMPOSITION OF CHICKEN EGG AND MEAT

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Abstract

The present study was undertaken at Centre of Advanced Studies in Poultry Science, Veterinary College and Research Institute, Namakkal. Layer and broiler biological experiments were conducted to study the effect of various Omega-3- fatty acids sources such as fish, linseed and rapeseed oils (at one, two and three per cent levels) to enrich Omega-3- fatty acids in chicken egg and meat. The supplementation of n-3 lipid sources in layer and broiler ration had significant (P<0.01) increase of omega -3- fatty acids composition such as linolenic acid, Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), total n-3 fatty acids and a significant reduction (p<0.01) in palmitic and stearic acid concentrations. The total unsaturated fatty acids concentration in egg yolk and breast and thigh meat of broilers showed an increase in all the treated groups due to incorporation of various n-3 lipid sources in feed.

I. INTRODUCTION

Due to increased literacy levels, consumers have become health conscious and want to consume only healthy foods. Therefore in many countries manufacturers have started producing health-promoting foods. In this context, foods enriched with Omega-3- fatty acids are gaining popularity because, these fatty acids have been reported to protect against cardiovascular and inflammatory diseases, certain types of cancer (Kinsella *et al.,* 1990), decreases plasma triglycerides, blood pressure, platelet aggregation, thrombosis and atherosclerosis particularly in diabetics and they also provide essential nutrients required for brain and visual development in children and enhance immunity in adults. Though fish, linseed and algae are rich sources of omega -3 fatty acids, many people are not consuming them due to various reasons. Hence, it is right time to engineer commonly consumed foods with beneficial components especially Omega -3- fatty acids. It is needless to say, chicken egg and meat play an important role in day- to-day human diets. The poultry has a peculiar capability of incorporating not only these omega -3- fatty acids but also anti - oxidants and immuno-modulators. Hence, a maiden attempt was made to engineer designer chicken meat by manipulating the fatty acids composition of meat by altering their dietary rations.

II. MATERIALS AND METHODS

Experiment I

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. Omega-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 formed thirteen treatment groups (Table 1). 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.

Experiment II

The broiler biological experiment was started using two hundred and seventy three commercial day old broiler chicks. The chicks were obtained from a local commercial broiler hatchery. Broiler chicks were weighed, wing banded and randomly allotted to thirteen treatment groups with three replicates having seven broiler chicks each. Omega-3- lipid sources such as fish, linseed and rapeseed oils were incorporated into broiler (starter and finisher) 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. Broiler chicks of all treatments were reared in cage system of management with standard managemental practices throughout the experimental period (7 weeks) except for the variation of n-3 lipid sources used in feed. The birds were fed with experimental diet ad libitum up to 7 weeks of age.

Table 1. Treatment groups and experimental diets of layers and broilers

T_1 Basal feed			
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 2 %
T_4	Basal feed+ Rapeseed oil (RO) 1%	T_{10}	Basal feed + RO 2 %
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 %

Eggs were collected once in every 10 weeks period to study the fatty acids composition of egg yolk. The breast and thigh meat samples were collected at 7th week from each carcass and stored at -20^{o} C for the estimation of fatty acid composition. The egg yolks, breast and thigh meat 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, breast and thigh meat 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^{o} C 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^{o} C 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^{o} C for 5 min increased to 220^{o} C and held for 5 min) were used. Temperature of both injector and detector were 250 and 260^{o} C respectively. The data collected in this experiment were subjected to statistical analysis as per Snedecor and Cochran (1989).

III. RESULTS AND DISCUSSION

The mean percentage of palmitic acid, stearic acid, linoleic acid, linolenic acid, Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), total n-3 fatty acids in egg yolk estimated by Gas chromatography are given in Table 2.

Table 2. Mean % of Palmitic acid, Stearic acid, Linoleic acid, Linolenic acid, Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and total n-3 fatty acids in egg yolk of layers as influenced by various n-3 lipid sources in feed

Treatment Groups	Palmitic acid	Stearic acid	Linoleic acid	Linolenic acid	EPA	DHA	Total n-3 fatty acids
T_1 – Control	35.00^{e} ± 0.250	11.50^{f} ± 0.150	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
T_2 – Fish oil (FO) 1%	30.70^{c} ± 0.200	07.20^{ab} ± 0.070	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
T_3 – Linseed oil (LO) 1%	32.80^{d} ± 0.130	09.50^{e} ± 0.070	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
T_4 – Rapeseed oil (RO) %	30.40^{c} ± 0.390	10.70^{f} ± 0.190	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
T_5 – Fish oil 2 %	26.60^{a} ± 0.350	08.30^{c} ± 0.370	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
T_6 – Linseed oil 2%	30.70^{c} ± 0.213	08.70^{cd} ± 0.170	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
T_7 – Rapeseed oil 2%	30.60^{c} ± 0.160	08.60^{c} ± 0.600	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
T_8 – Fish oil 3 %	26.50^{a} ± 0.210	06.30^{a} ± 0.198	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
T_9 – Linseed oil 3%	29.40^{bc} ± 0.310	08.50^{c} ± 0.380	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
T_{10} – Rapeseed oil 3%	30.20^{c} ± 0.170	07.90^{bc} ± 0.160	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
T_{11} – (FO + LO + RO) 1 %	30.50^{c} ± 0.190	09.20^{de} ± 0.220	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
T_{12} – (FO + LO + RO) 2 %	30.10^{c} ± 0.190	07.50^{b} ± 0.050	15.40^{h} ± 0.17	0.79^{e} ± 0.05	1.50^{ef}± 0.14	3.00 ^{fg}± 0.21	4.40^{gh} ± 0.22
T_{13} – (FO + LO + RO) 3 %	29.60^{c} ± 0.150	07.20^{b} ± 0.100	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

Mean values not sharing a common superscript columnwise differ significantly. (P< 0.01)

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).

The mean percentage of myristic acid, 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 broiler breast and thigh meat estimated by Gas chromatography are given in Table 3 and 4.

Table 3. Mean (± S.E.) fatty acids composition (%) in breast muscle of broilers at seventh week of age as influenced by various n-3 lipid sources in feed

Treatment Groups	Palmitic acid	Stearic acid	Linoleic acid	Linolenic acid	EPA	DHA	Total n-3
T_1 – Control	32.70 ^{e} ± 0.64	19.50 ^{c} ± 0.60	13.00 ^{a} ± 0.36	00.76 ^{a} ± 0.22	00.60 ^{a} ± 0.20	00.65 ^{a} ± 0.35	02.00 ^{a} ± 0.20
T_2 – Fish oil (FO) 1%	22.10 ^{ab} ± 0.44	13.50 ^{ab} ± 0.54	14.60 ^{abc} ± 0.37	02.60 ^{b} ± 0.64	03.10 ^{d} ± 0.46	07.10 ^{d} ± 0.65	13.10 ^{e} ± 0.45
T_3 – Linseed oil (LO) 1%	23.10 ^{ab} ± 0.25	14.90 ^{b} ± 0.54	18.40 ^{def} ± 0.60	04.70 ^{c} ± 0.40	00.53 ^{a} ± 0.57	01.10 ^{ab} ± 0.45	06.40 ^{b} ± 0.31
T_4 – Rapeseed oil (RO) %	26.10 ^{bcd} ± 0.96	14.70 ^{b} ± 0.74	16.30 ^{bcde} ± 0.81	04.70 ^{c} ± 0.43	00.63 ^{a} ± 0.25	01.10 ^{ab} ± 0.24	06.50 ^{b} ± 0.35
T_5 – Fish oil 2 %	21.20 ^{ab} ± 0.15	11.90 ^{ab} ± 0.43	15.10 ^{abcd} ± 0.17	03.20 ^{b} ± 0.50	05.60 ^{e} ± 0.22	09.90 ^{e} ± 0.39	18.80 ^{g} ± 0.42
T_6 – Linseed oil 2%	21.11 ^{a} ± 0.35	12.10 ^{ab} ± 0.98	21.30 ^{f} ± 0.72	05.90 ^{cd} ± 0.37	00.71 ^{a} ± 0.14	01.30 ^{b} ± 0.22	07.90 ^{bc} ± 0.36
T_7 – Rapeseed oil 2%	23.30 ^{abc} ± 0.96	13.30 ^{ab} ± 0.85	19.10 ^{ef} ± 0.99	04.80 ^{c} ± 0.57	00.70 ^{a} ± 0.10	01.20 ^{b} ± 0.17	06.80 ^{b} ± 0.49
T_8 – Fish oil 3 %	23.20 ^{abc} ± 1.20	10.70 ^{a} ± 1.16	14.50 ^{ab} ± 0.93	02.70 ^{b} ± 0.41	07.50 ^{f} ± 0.40	12.60 ^{f} ± 0.29	22.90 ^{h} ± 0.45
T_9 – Linseed oil 3%	22.00 ^{ab} ± 1.83	10.80 ^{a} ± 0.79	19.30 ^{ef} ± 0.87	07.70 ^{d} ± 2.00	01.30 ^{bc} ± 0.16	01.30 ^{b} ± 0.22	10.20 ^{d} ± 1.40
T_{10} – Rapeseed oil 3%	29.60 ^{de} ± 0.85	13.80 ^{ab} ± 0.86	16.80 ^{cde} ± 0.94	05.90 ^{cd} ± 0.83	01.40 ^{c} ± 0.23	01.40 ^{b} ± 0.17	08.90 ^{cd} ± 0.69
T_{11} – (FO + LO + RO) 1 %	28.30 ^{cde} ± 0.57	13.30 ^{ab} ± 0.47	16.60 ^{cde} ± 0.26	02.70 ^{b} ± 0.46	01.10 ^{b} ± 0.17	05.40 ^{c} ± 0.55	09.40 ^{cd} ± 0.44
T_{12} – (FO + LO + RO) 2 %	25.60 ^{abcd} ± 0.67	12.40 ^{ab} ± 0.68	16.00 ^{abcde} ± 0.75	04.90 ^{c} ± 0.42	01.00 ^{b} ± 0.16	09.40 ^{e} ± 0.91	15.60 ^{ef} ± 0.71
T_{13} – (FO + LO + RO) 3 %	24.60 ^{abcd} ± 1.19	11.00 ^{a} ± 0.54	17.10 ^{cdef} ± 0.59	04.90 ^{c} ± 0.25	01.60 ^{c} ± 0.19	09.80 ^{e} ± 0.60	16.50 ^{fg} ± 0.56

Table 4. Mean (± S.E.) fatty acids composition (%) in thigh muscle of broilers at seventh week of age as influenced by various n - 3 lipid sources in feed

Treatment Groups	Palmitic acid	Stearic acid	Linoleic acid	Linolenic acid	EPA	DHA	Total n-3
T_1 – Control	29.50 ^{f} ±0.85	17.80 ^{ab} ±0.57	13.10 ^{ab} ±0.16	00.65 ^{a} ±0.44	00.26 ^{ab}	00.53 ^{a} ±0.27	01.40 ^{a} ±0.53
T_2 – Fish oil (FO) 1%	23.50 ^{de} ±0.87	12.70 ^{defg} ±0.52	13.90 ^{abc} ±1.09	02.00 ^{b} ±0.27	03.70 ^{d} ±0.20	02.70 ^{d} ±0.37	08.50 ^{de} ±0.21
T_3 – Linseed oil (LO) 1%	23.60 ^{de} ±0.97	12.90 ^{efg} ±0.17	15.50 ^{bcd} ±1.13	03.70 ^{de} ±0.28	00.23 ^{a} ±0.77	00.91 ^{ab} ±0.41	05.60 ^{bc} ±0.25
T_4 – Rapeseed oil (RO) %	24.40 ^{de} ±0.71	13.90 ^{fg} ±0.79	15.10 ^{bcd} ±1.11	02.20 ^{bc} ±0.72	00.40 ^{ab}	01.50 ^{bc} ±0.73	04.80 ^{b} ±0.80
T_5 – Fish oil 2 %	14.80 ^{b} ±0.60	09.50 ^{ab} ±0.38	14.40 ^{abcd} ±0.74	04.20 ^{ef} ±0.18	00.60 ^{a} ±0.26	05.50 ^{fg} ±0.57	15.80 ^{f} ±0.48
T_6 – Linseed oil 2%	19.40 ^{bc} ±0.30	10.40 ^{bc} ±0.19	17.00 ^{de} ±0.40	06.14 ^{g} ±0.17	01.70 ^{c} ±1.67	01.00 ^{ab} ±0.16	09.40 ^{de} ±0.71
T_7 – Rapeseed oil 2%	22.40 ^{cde} ±0.49	11.90 ^{cde} ±0.44	14.20 ^{abcd} ±0.57	04.80 ^{ef} ±0.12	01.40 ^{c} ±1.92	02.70 ^{d} ±0.91	08.80 ^{de} ±0.35
T_8 – Fish oil 3 %	19.70 ^{bc} ±0.74	11.20 ^{cd} ±0.33	11.90 ^{a} ±0.32	03.00 ^{cd} ±0.38	09.10 ^{f} ±0.27	05.90 ^{g} ±0.70	18.10 ^{f} ±0.49
T_9 – Linseed oil 3%	14.60 ^{a} ±0.45	08.50 ^{a} ±0.24	22.40 ^{f} 0.35	07.85 ^{h} ±0.68	01.50 ^{c} ±0.32	01.60 ^{bc} ±0.38	09.30 ^{de} ±0.54
T_{10} – Rapeseed oil 3%	19.50 ^{bcd} ±0.43	12.00 ^{cde} ±0.40	17.40 ^{de} ±0.26	05.10 ^{fg} ±0.46	01.70 ^{c} ±0.09	02.50 ^{cd} ±0.50	09.00 ^{de} ±0.18
T_{11} – (FO + LO + RO) 1 %	24.60 ^{e} ±0.41	14.20 ^{g} ±0.12	17.10 ^{de} ±0.26	02.10 ^{bc} ±0.10	01.30 ^{c} ±0.27	03.60 ^{de} ±0.11	07.20 ^{cd} ±0.18
T_{12} – (FO + LO + RO) 2 %	22.10 ^{cde} ±0.47	12.30 ^{defg} ±0.33	19.80 ^{ef} ±0.56	02.30 ^{bc} ±0.35	01.00 ^{bc}	03.70 ^{ef} ±0.52	07.50 ^{d} ±0.51
T_{13} – (FO + LO + RO) 3 %	23.60 ^{de} ±0.50	12.70 ^{defg} ±0.49	16.80 ^{cde} ±0.39	03.90 ^{de} ±0.69	01.50 ^{c} ±0.23	04.40 ^{efg} ±0.56	10.00 ^{e} ±0.73

Mean values not sharing a common superscript columnwise differ significantly. (P< 0.01)

From the table, it was observed that there was significant decrease in values of palmitic and stearic acids in all treated groups. However in control group, the breast meat showed the higher values of the above acids when compared to thigh meat. Oleic, linoleic, linolenic acids, EPA, DHA and total n-3 fatty acids in breast and thigh meat of broilers fed n-3 lipid sources were highly significant ($P<0.01$).

Similar to these results, Miller and Robisch (1969) reported that the fish oils at 1.5 and 2.5 per cent level fed to broilers had influenced the fatty acid patterns of the tissue lipids. Crespo and Esteve - Garcia (2001) observed that birds fed with linseed oil presented the highest values of linolenic acid in all tissues, which is in agreement with the results of this study. Chanmugam *et al.* (1992) found that levels of EPA were increased ($P<0.05$) in all the groups fed with fish oil than other groups which is in agreement with the results of this study. He further observed that birds supplemented with diet rich in linolenic acid content had significantly higher levels of n-3 fatty acids and high n-3 : n-6 ratio than those supplemented with same level of fish oil which is not in agreement with the results of this study.

It is concluded that the total unsaturated fatty acids concentration in egg yolk, breast and thigh meat of broilers showed an increase in all the treated groups due to incorporation of various Omega-3-fatty acids sources in feed.

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