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Enriching chicken eggs with omega-3 fatty acids for improving human health

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ABSTRACT

Layer chickens (180), 18-week-old were divided into 6 groups of 30 birds with 3 replicates and maintained up to 40 weeks of age. To study the effect of different n-3 fatty acids (n-3 FA) rich feed ingredients on n-3 FA content of the yolk, diets (diet- 2 to 4) with n-6: n-3 fatty acid ratio of 5: 1 and diet-5 with the ratio of 2.5: 1 were formulated with bajra (*Pennisetum glaucum*), linseed, rapeseed and fishmeal, and commercial layer diet (7: 1) as control (diet-1) were fed to birds. Inclusion of n-3 FA rich ingredients at different levels enhanced the n-3 FA content of the layer feeds by 29.83, 95.70, 111.89, 99.09 and 88.62% and decreased the n6: n3 ratio by 42.29, 51.17, 48.55, 60.40 and 58.26% in diets 2 to 6 respectively over the control. The n-3 FA rich diets significantly reduced the serum total cholesterol of layers in diet-6 fed group by 36.01% followed by diet 3 (29.95%), diet 4 (25.94%), diet 5 (24.30%) and diet 2 (14.22%) groups when compared to control. Egg yolk total cholesterol was significantly lowered by 27.04% in the diet-6 fed group. The n-3 fatty acids of the egg yolk were increased by 104.40, 80.70, 79.79, 45.25 and 39.99% for the diets- 6, 5, 2, 4 and 3 respectively.

The EPA content of egg yolk was not altered to any great extent, however, the DHA content of the egg yolk was significantly altered by the feeding of n-3 FA rich diets especially by diets 5, 6 and 2. The n-3 FA rich diets had little influence on linoleic acid content of egg yolk. The r^2 values were 0.897, 0.960, 0.572, 0.972, 0.877 and 0.852 for the diets 1 to 6 respectively, indicating that birds have better transfer efficiency of n-3 FA from feed to eggs. It can be concluded that addition of n-3 FA rich feed ingredients in diet would enrich the n-3 FA in egg yolk.

Key words: Chicken eggs, Human health, Omega-3 fatty acids

The omega-3 (n-3) fatty acids are considered to be nutritionally essential and synthesized only by plants as alpha linolenic acid (ALA, C18: 3_{n-3}). ALA can be elongated to eicosapentaenoic acid (EPA, C20: 5_{n-3}) and docosahexaenoic acid (DHA, C22: 6_{n-3}) in animals and human body, and the ability to elongate ALA to EPA and DHA is limited both in animals and humans (Stark *et al.* 2008). However, the EPA and DHA are abundant in fishes which derive these fatty acids from marine algae and lower plants. Due to increase in consumption of vegetable oils high in omega-6 fatty acids, the n3: n6 ratio of the human diet has become skewed. Diets should provide an adequate intake of polyunsaturated fatty acids (PUFA) with optimal balance between intake of n-6 PUFAs and n3 PUFAs (FAO 2003). Omega-3 fatty acids possess beneficial effects like anti-atherogenic, anti-thrombotic, anti-inflammatory, cardio-protective (Das *et al.* 2012) and are highly required for brain development. Hathwar *et al*. (2011) also emphasized in their review that chicken eggs and meat enriched with n-3 FA as functional foods could be a new approach to achieve healthier status thus reducing the risk of diseases. Considering the role of omega-3 fatty acids in human diet, Food and Drug Administration, USA (FDA 2004) has permitted that cardio-protective role can be claimed for products containing the omega-3 fatty acids. Excess intake of dietary cholesterol has been ascribed to cardiovascular diseases in human beings and this notion leads to a decline in egg consumption. Further, the position paper of American Dietetic Association and Dieticians of Canada (Kris Etherton 2007) recommended infant formula should contain DHA for aleast first year of age in infants. Previous studies showed that n3 FA could be enriched in poultry products by directly incorporating oils rich in n3 FA to the poultry diet (Moghadasian 2008). Inclusion of n3 FA rich oil sources in the poultry diet is practically not possible due to poor keeping quality of the feed. However, addition of n3 FA rich feed ingredients to poultry ration is feasible and the desirable dietary ratio of n3: n6 FA can be adjusted through software during feed formulation and the poultry farmers can use this software for producing designer eggs. Hence the present study was undertaken to evaluate locally available n3 FA rich feed

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ingredients to the poultry diet instead of oils and also to evaluate the influence of dietary n3 FA on egg cholesterol content.

MATERIALS AND METHODS

Commercial layers (180) at the age of 18 weeks with average body weight of 1.2 ±0.2 kg were divided randomly into 6 groups of 30 birds each with 3 replicates (10 birds each). The birds were kept in raised cages with automatic nipple drinker and manual feeder. Four diets (diets 2 to 5) were formulated (Table 1) to contain n3: n6 FA ratio of 1: 5 using bajra (Pennisetum glaucum), linseed, rapeseed and fish meal. Diet 6 was prepared with a lower n3: n6 ratio of 1: 2.5 using the above ingredients. Commercial layer diet was used as control (diet 1) which had the n3: n6 ratio of 1: 7. All the 6 diets were formulated to contain CP 17% and ME 2.57 Mcal/kg and almost similar cost of feed (Rs/kg) with decreased n3: n6 FA by employing least cost feed formulation software. The rationale for choosing the above ingredients for this trial is because of their higher n3 FA content, their availability at local market and low cost (Nanjappan *et al.* 2009). The birds were maintained up to 40 weeks of age.

Blood and egg samples were collected at random from 6 birds in each group at monthly interval. The total cholesterol content of eggs and blood samples was estimated using diagnostic kits (Wybenga and Pileggi 1970).

The FA content of egg yolk was analysed as per Palmquist and Jenkins (2003). The fatty acid methyl esters were separated and quantified by gas chromatograph. Fatty acid methyl esters were identified by comparison with retention times of standard fatty acids. The fatty acids content were expressed as per cent of total fatty acids (Palmquist and Jenkins 2003).

Table 1. Ingredients (kg/1000kg) and nutrients composition of layer feed (%DM) used to enrich n-3 fatty acids in egg yolk of commercial layer chickens

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Maize	530	100	565	442	525	374
Bajra	0.0	478	10	100	0.0	150
Deoiled rice bran	125	100	0.0	100	100	100
Sunflower oil cake	68	33	145	55	132	109
Soya meal	111	115	153	163	42	55
Fishmeal	65.0	65.0	0.0	9.0	111	100
Rape seed	0.0	0.0	0.0	15.0	0.0	0.0
Linseed	0.0	2.0	10	0.0	0.0	13
Calcite	90	97	97	97	84	92
DCP	7.0	7.0	18	14	1.0	3.0
Methionine	0.60	0.78	0.65	0.99	0.06	0.24
Lysine	0.00	0.00	0.00	0.00	0.00	0.00
Salt	3.62	3.30	1.55	3.57	3.50	3.45
Total (kg)	1000	1000	1000	1000	1000	1000
Crude protein*	17.0	17.1	17.0	17.0	17.0	17.0
ME (Mcal/kg)*	2.57	2.57	2.60	2.57	2.57	2.57
Cost-Rs./kg	5.80	5.80	6.26	5.95	5.85	5.94

The completely randomized design and regression analysis were followed by including all the replicates in the corresponding group (Snedecor and Cochran 1994) and the data collected were analysed using SPSS® 10.0 software package.

RESULTS AND DISCUSSION

Inclusion of n3 FA rich ingredients such as bajra, linseed, rapeseed, fishmeal at different levels (Table 1) enhanced the n3 FA (Table 2) content of the layer feeds by 29.83, 95.70, 111.89, 99.09 and 88.62% as well as decreased the n3: n6 ratio by 42.29, 51.17, 48.55, 60.40 and 58.26% in diets 2 to 6 respectively over the control (diet 1). The higher total n3 FA content in the diet-4 fed group was due to the inclusion of fish meal (0.9%) and *bajra* (10%) during least cost formulation by the computer software to maintain the desired n3: n6 ratio in the diet in addition to rapeseed (1.5%). The lower content of n3 FA in diet-2 was due to the higher inclusion of *bajra* (47.80%) which contained a comparatively lower level of n3 FA than other n3 FA rich ingredients of this trial (Collins *et al.* 1997).

Diets with higher n3 FA significantly reduced ($p<0.01$) the serum total cholesterol of layers in the present study (Table 3). The reduction in cholesterol content was greater in the diet-6 fed group (36.01%) followed by diet-3 (29.95%), diet-4 (25.94%), diet-5 (24.30%) and diet-2 (14.22%) groups when compared to control. This finding concurs with Celebi and Utlu (2006) and Svedova *et al.* (2008) who observed feeding of flaxseed oil and linseed + fish oil respectively reduced the serum cholesterol in layers.

Similarly, egg yolk total cholesterol level reflected the serum level and it was significantly ($P<0.01$) lower by 27.04% in the diet-6 fed group. Higher level of *bajra* in the diet (diet 2) was able to reduce the total egg yolk cholesterol by only 6.83% and the effect was lower when compared to other diets. Reduction in egg cholesterol was observed by feeding chia seed to layers (Ayerza and Coates 2000). On the contrary, Carrillo-Dominguez *et al.* (2005) found egg cholesterol level was not altered by feeding flaxseed and fish oil, and red crab meal respectively. It is evident from this study that n3 FA rich diet may be useful in reducing the egg yolk cholesterol to overcome the fear of negative impact of egg cholesterol on human health.

Incorporation of n3 FA rich diets to layers resulted in 104.40, 80.70, 79.79, 45.25 and 39.99% increase in total n3 FA in the egg yolk for the diets 6, 5, 2, 4 and 3; respectively. The increased level of n3 FA in eggs obtained in this study concurs with Sujatha and Narahari (2011) who enriched chicken eggs with n3 FA by feeding of 15% flaxseed. However, the diet-6 was able to double the n3 FA content in the egg yolk with a concomitant decrease (53.40%) in the n3: n6 ratio compared to diet-1 and this might be due to the contribution of all the n3 FA rich ingredients in the diet

Table 2. Per cent of fatty acids composition of diets and egg yolk of layer chicken fed diets enriched with different n-3 fatty acids

	Diet						Yolk					
Fatty acids	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Myristic	2.3±	3.3±	1.9±	2.3±	5.6±	6.4±	0.40±	0.4±	0.38±	0.42±	0.53±	0.45±
	0.37	0.37	0.17	0.36	0.34	0.56	0.02	0.03	0.02	0.05	0.02	0.02
Palmitic	21.5±	24.8±	19.5±	19.3±	23.4±	28.5±	32.2^{ab}±	31.4^{ab}±	30.8^{c}±	31.3^{b}±	32.8^{a}±	32.1^{ab}±
	0.68	0.78	0.68	0.51	1.16	0.82	0.42	0.78	0.46	0.32	0.21	0.24
Stearic	4.4±	5.0±	2.7±	2.5±	7.3±	4.0±	9.5±	9.2±	9.1±	9.2±	8.3±	8.9±
	0.44	0.52	0.29	0.22	0.52	0.68	0.48	0.43	0.41	0.55	0.20	0.31
Oleic	31.1±	33.5±	27.5±	28.1±	27.5±	25.1±	39.8±	39.2±	41.1±	40.6±	38.4±	39.8±
	1.04	1.31	1.8	0.98	1.52	0.63	1.29	1.05	0.70	1.24	0.23	0.64
Linoleic	37.2±	28.8±	40.5±	40.4±	29.2±	29.3±	14.1±	14.3±	15.0±	14.6±	14.1±	13.4±
	0.33	0.83	1.47	1.54	1.67	0.92	0.39	0.47	0.50	0.10	0.19	0.38
Linolenic	0.57±	0.79±	5.9±	6.6±	0.41±	1.4±	0.60^{B}±	0.78^{B}±	1.1^{A}±	0.72^{B}±	0.66^{B}±	1.3^{A}±
	0.24	0.11	0.57	0.52	0.13	0.21	0.06	0.10	0.05	0.13	0.04	0.12
EPA	1.3±	1.6±	0.30±	0.12±	2.5±	1.7±	0.52±	0.60±	0.29±	0.45±	0.42±	0.40±
	0.22	0.17	0.07	0.04	0.32	0.25	0.20	0.16	0.04	0.12	0.06	0.06
DHA	0.90±	1.5±	0.64±	0.70±	3.8±	3.2±	1.6^{C}±	3.5^{A}±	2.4^{B}±	2.7^{B}±	3.8^{A}±	3.8^{A}±
	0.08	0.20	0.34	0.10	0.15	0.17	0.16	0.38	0.13	0.51	0.17	0.20
n6	37.2	28.8	40.5	40.4	29.2	29.3	14.1	14.3	15.0	14.6	14.1	13.4
n3	3.5	4.6	6.9	7.5	7.1	6.7	2.7	4.9	3.8	3.9	4.9	5.5
n3: n6	1: 10.5	1: 6.3	1: 5.9	1: 5.4	1: 4.2	1: 4.4	1: 5.2	1: 3.0	1: 4.0	1: 3.7	1: 2.9	1: 2.4

* Means of twelve observations for feed and six observations for yolk. Means within the same row bearing different upper case (P<0.01) and lowercase (P<0.05) superscripts differ significantly.

Table 3. Serum (mg/dl) and egg yolk total cholesterol (mg/g yolk) content of hens consumed different diets enriched with n-3 fatty acids

Treatment	Serum cholesterol	% reduction over control	Egg yolk cholesterol	% reduction over control
Diet -1	190.5^{a} ± 3.80	–	11.4^{a} ± 0.45	–
Diet -2	163.4^{b} ± 2.26	14.2	10.6^{ab} ± 0.43	6.8
Diet -3	133.4^{bc} ± 2.49	30.0	9.5^{b} ± 0.54	16.9
Diet -4	141.1^{bc} ± 9.39	25.9	8.8^{b} ± 0.31	23.1
Diet -5	144.2^{bc} ±12.07	24.3	9.0^{b} ± 0.15	20.8
Diet -6	121.9^{c} ± 5.95	36.0	8.3^{b} ± 0.30	27.0

* Means of 6 observations. Means within a column bearing different superscripts differ significantly (P<0.01)

(Table 1). Our observations were similar to the reports of Collins *et al.* (1997), Bean and Leeson (2003) and Amini and Ruiz-Feria (2007) in layer chicken, who also reported reduction in n3: n6 ration by feeding n3 FA rich diets.

The EPA content of egg yolk was not altered to any great extent due to feeding of n-3 FA rich diets to the birds. However, the DHA content of the egg yolk was significantly (P<0.01) altered by the feeding of n-3 FA rich diets especially by diets 5, 6 and 2. The enhanced level of DHA in eggs over EPA found in our study is in line with Carrillo-Domýnguez *et al*. (2005). This author reported that DHA was preferentially accumulated in the yolk compared to EPA in spite of higher level in diet. Linoleic acid content of yolk was maintained fairly at very narrow range of 13.42 to 14.98% in all the groups of this study. Similarly, Schreiner *et al.* (2004) observed no changes in the linoleic acid content after feeding seal blubber oil at different level in layers. It is evident from the present study that linoleic acid content of the egg was maintained irrespective of n3 FA source in the diet. In other words, n3 FA content of the diet had little influence on n6 fatty acids in the eggs.

Enrichment of n3 FA in the egg yolk had not altered the composition of other major fatty acids. This observation of the present study agrees with the Baucells *et al.* (2000) who reported that substitution of fish oil with other fat sources hardly affected the saturated fatty acids content of the eggs. On the contrary, Yalcyn *et al.* (2007) found feeding of fish oil and flaxseed reduced the total saturated fatty acids content in chicken eggs. When the n3 FA content of diets were compared with egg yolk n3 FA, the r^2 values were 0.897, 0.960, 0.572, 0.972, 0.877 and 0.852 for the diets 1 to 6 respectively indicating that birds have better transfer efficiency of n3 FA from feed to eggs.

It can be concluded that inclusion of n3 FA rich feed ingredients in diet which are available in local market would be able to produce desired ratio of n3: n6 FA enriched eggs with lesser cholesterol content.

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