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Value Added Eggs Seem a Good Option for Improving Blood Omega-6: Omega-3 Ratio and Heart Health

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ABSTRACT

Background: *Omega-3 type of fatty acids have been known to be essential for not only heart health but overall wellness. Value added eggs with higher concentrations of omega-3-fatty acids in eggs are not only a palatable and easily available source but also heart healthy. However, the high concentration of cholesterol in eggs has been restrictive towards its acceptance.* ***Aim:*** *To check the efficacy of enriched eggs containing omega-3 fatty acids on the lipid profile of healthy volunteers to assess and set the leads whether their high fat content would have any adverse effect.* ***Methods and results:*** *Omega-3 eggs specially developed by us were given to normal healthy volunteers for two weeks and lipid profiles analyzed. Lipid profile normalizing effect of triglycerides and cholesterol was pronounced in the subjects studied.* ***Conclusion:*** *Consumption of omega-3 egg despite its total high fat content has no adverse effect. On the contrary it is beneficial and heart healthy retaining its natural nutrient quality.*

Keywords: Omega-3 eggs, alpha-linolenic acid, docosahexaenoic acid

Abbreviations: CVD: Cardiovascular Disease; EDTA: Ethylenediaminetetraacetic Acid; RBC: Red Blood Corpuscles; WBC: White Blood Cells; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoproteins; VLDL: Very-Low-Density Lipoprotein; EPA: Eicosapentaenoic Acid; LDL-C: Low Density Lipoprotein Cholesterol; HDL-C: High-Density Lipoproteins Cholesterol; VLDL-C: Very-Low-Density Lipoprotein Cholesterol; Hb: Haemoglobin; DHA: Docosa Hexaenoic Acid; ALA: Alpha Linolenic Acid

INTRODUCTION

Chicken eggs have been taken as a nutrient by humans since ancient times. Eggs occupy a very important place in the food chain because of their high nutritive value [1]. During the past few decades, reducing fat ingestion has been the main emphasis of dietary endorsements all over, to cut the risk of cardiac illness. Results from epidemiological analyses and well organized clinical experiments have showed that substituting saturated fats with the healthy unsaturated fats is a very effective measure in lowering the risk of cardiovascular disease than just reducing the overall fat intake [2]. Realizing that, the country egg, did not pose any serious health problems due to its higher omega-3 (n-3) content than the present poultry egg, there are several successful attempts in the development of designer eggs with high n-3 content [3-5]. The yolk fatty acid composition could be altered on feeding layer birds with specific diets [6]. These n-3 enriched eggs contain almost four-fold Docosa hexaenoic acid (DHA) than the normal amount in commercially sold eggs [7,8]. It has been shown that these n-3 enriched eggs are much safer than the conventional poultry eggs [9]. Considering the beneficial evidence of long chain n-3 fatty acids towards CVD (Cardio Vascular Disease) risk reduction to be satisfactorily convincing, both the European Cardiology Society as well as the American Heart Association have recommended a greater intake of either fish oil or fish [10,11]. However, concerns about the possibility of heavy metal contamination in fishes (which are good sources of n-3 fatty acid) and long term ingestion of fish oil have raised an alarm. In case of poultry eggs this factor is under total control but eggs are accepted with certain caution by the consumer because of the higher fat and cholesterol content. The body requires cholesterol for performing several important functions. However, excessive intake or synthesis of cholesterol which is not metabolized is a crucial provider to the blockage of blood vessels and proves detrimental to heart health.

In this context, it was felt necessary to know the safety factor of n-3 enriched eggs. Hence a pilot study on a small number of subjects was undertaken to get the leads if there is any adverse effect on the lipid profile of the consumer. N-3 enriched eggs are not readily available in India. Hence in association with Venkateshwara Hatcheries (VH) group, n-3 enriched eggs were developed in Somnath poultry farm.

METHODS

Egg analysis

Manipulation of the feed was done by incorporating a fixed concentration of alpha linolenic acid (ALA) rich flax seeds to the poultry feed. The analysis of the egg is given in Table 1.

Table 1 Comparison of commercial egg with omega-3 enriched egg

Parameters	Omega-3 egg	Commercial egg
Weight	51 g	50.5 g
Total fat	6600 mg	6450 g
Cholesterol	189.26 mg	270.66 mg
Vitamin E	1.8 mg	1.2 mg
Omega-3 fatty acid content		
ALA	220 mg	0.8 mg
EPA	6 mg	1 mg
DHA	100 mg	10 mg

The analysis was verified independently by a National Accreditation Board for Testing and Calibration Laboratory (NABL) approved by the Government of India. Nutritional analysis showed that both eggs were similar in all aspects except for the concentrations of n-3 fatty acids and vitamin E which were found elevated in the fortified eggs Table 2.

Demographic data

Table 2 Demographic data of volunteers prior to consuming omega-3 eggs

Sr. No.	Parameter	Observation
1	No. of volunteers	31
2	Mean age	38.6
3	Male	18
4	Female	13
5	Vegetarian (n)	-
6	Non-vegetarian (n)	31 (26-consumed boiled eggs 5-consumed omelettes)
7	Triglyceride A<150	20
	(n) B >150	11
8	Cholesterol A<200	13
	(n) B>200	18
9	Age (n)<30	12 Chol high-2 TG high-2
	>30	19 Chol high-16 TG high-6

Subjects

A total of 31 subjects who did not have any apparent ailment, and willingly participated in the study were enrolled. Their personal data sheets and sensory response data of the subjects were also collected prior to the dietary intervention. The subjects consuming n-3 enriched eggs were mainly non-vegetarians (Table 2).

Study design

This is a randomized control trial in which healthy normal volunteers consumed two eggs a day for four days in a week (i.e. a total of 8 eggs per week) for two weeks.

Ethical consideration

Ethical clearance from the concerned institutional ethical committee was obtained for this study.

Egg consumption

Eggs were consumed either as boiled eggs or omelettes. Twenty-six volunteers consumed boiled eggs and five

consumed them in the form of omelettes, where additional fats would have to be added during its preparation thus raising the total fat consumption.

Blood collection

A total of ten millilitres of blood sample was collected from the volunteers before and after two-week consumption of n-3enriched eggs. Five millilitres of blood was collected in EDTA bulbs and separated into RBC, WBC, and plasma for analysis of fatty acid; two millilitres of blood was taken for estimating haemoglobin levels; and remaining three millilitres was collected in heparin bulbs for analysis of cholesterol and triglycerides.

Fatty acid analysis

Methyl esters of the plasma and RBC fraction were prepared in accordance with the procedure of Manku, et al. [12]. Herein plasma and RBC fatty acids were methylated and then analyzed by Gas chromatography as described by Arvindakshan, et al. and compared with standards [13].

Estimation of haemoglobin, cholesterol and triglyceride level in blood

Estimation of haemoglobin (Hb) was carried out by fully automated cell counter haematology Sysmex KX-21 (Sysmex Corporation, Kobe Hyogo, and Japan).

Estimation of triglyceride was carried out by Enzokit Triglyceride kit (RFCL Limited, Haridwar, India). Estimation of cholesterol, LDL-C, HDL-C and VLDL-C was carried out by Atozyme cholesterol Enzymatic kit (Accurex biomedical PVT. LTD. Mumbai, India)

Statistical analysis

Categorical variables were expressed as means ± standard deviations. Statistical analysis was done using the t-test with p values of <0.05 considered statistically significant.

RESULTS

All participants completed the study. Compliance was extremely good. None of the volunteers exhibited any adverse reaction.

Haemoglobin levels

There was a statistically significant increase in the Hb levels of volunteers (From a mean 13.4 g/dl to 13.7 g/dl). Experiments on long term are necessary to ascertain this (Table 3).

Table 3 Haemoglobin levels before and after supplementation

Egg group	Pre-supplementation (N=31)	Post-supplementation (N=31)	p-value
Haemoglobin	13.477 ± 0.33	13.735 ± 0.339	p=0.0307

Fatty acid profile in RBC and plasma

Average values of fatty acids in the plasma and RBC of the volunteers at the beginning and at the end of study is presented in Table 4.

Table 4 Plasma and RBC fatty acid analysis before and after supplementation

% Fatty acids	Plasma		p-value	RBC		p-value
	Before	After		Before	After	
SATD	29.567 ± 0.61	29.252 ± 2.01	0.34545	34.815 ± 0.32	35.948 ± 0.5	0.086
PUFA	46.453 ± 1.03	47.393 ± 0.89	0.2161	29.5187 ± 0.3	29.7354 ± 0.39	0.3257
LA	37.530 ± 1.22	37.945 ± 0.87	0.3658	12.271 ± 0.34	12.367 ± 0.37	0.4054
AA	6.994 ± 0.37	6.819 ± 0.26	0.2916	14.704 ± 0.25	14.240 ± 0.24	0.0304
n-6	44.8029 ± 1.1	45.3848 ± 0.89	0.1749	27.0522 ± 0.3	26.6671 ± 0.39	0.19285
ALA	0.467 ± 0.05	0.486 ± 0.05	0.376	0.1209 ± 0.02	0.1277 ± 0.02	0.3969
EPA	0.1854 ± 0.01	0.2164 ± 0.02	0.1107	0.138 ± 0.02	0.155 ± 0.02	0.0756
DHA	0.998 ± 0.11	1.307 ± 0.09	0.00198	2.2067 ± 0.24	2.7851 ± 0.16	0.00078
n-3	1.6509 ± 0.12	2.0103 ± 0.01	0.002597	2.369 ± 0.24	3.0683 ± 0.17	0.000514
n-6:n3	33.793 ± 3.31	25.277 ± 1.79	0.0016	13.490 ± 1.23	9.428 ± 0.47	0.000289

An enhancement in total concentration of n-3 fatty acids particularly DHA with a significant decline in n-6: n-3 ratio was noticed both in the plasma (p=0.0016) samples and RBC (p=0.000289). There was a significant increase in total n-3 levels in both plasma (p=0.002597) and RBC (p=0.000514). There was a significant decrease in Arachidonic acid (p=0.034) in the RBC.A slight though not significant decrease (p=0.086) in the saturated fatty acid levels and n-6 levels (p=0.192) in the RBC was also noted.

Cholesterol and triglyceride levels

A sub division into two groups A and B was done for the purpose of analysis.

A- those having initially triglyceride levels lower than the normal values of 150 mg/dl, and those having initially higher triglyceride levels; and A) those having cholesterol levels lesser than 200 mg/dl and B) those having cholesterol levels greater than 200 mg/dl. The data presented in Table 5 shows a significant decline in both triglyceride and cholesterol levels in the subjects included in B group. Triglyceride and cholesterol levels decreased significantly in the sub group which had higher initial levels whereas in the volunteers who had initially normal levels, there was a change within the normal range.

Table 5 Cholesterol and triglyceride levels in the serum of volunteers

Egg group	Triglyceride						Cholesterol					
	n=20 <150 (Group A)		p-value	n=11 >150 (Group B)		p-value	n=13 <200 (Group A)		p-value	n=18 >200 (Group B)		p-value
	Before	After		Before	After		Before	After		Before	After	
Mean	96.15 ± 5.41	93 ± 4.77	0.2923	189.72 ± 10.93	141.63 ± 11.41	0.000293	166.92 ± 6.86	183.23 ± 6.61	0.0465	237.94 ± 6.12	197.5 ± 4.97	0.00001

Table 6 provides details of HDL, LDL, VLDL cholesterol profiles after consumption of n-3 enriched eggs. The consumption of n-3-enriched eggs increases the cardio-protective good cholesterol HDL significantly (p=0.01) and decreases the bad cholesterol LDL (p=0.03) which is further corroborated by a significant decrease in LDL/HDL ratio (p=0.01), VLDL (p=0.002) and total cholesterol/HDL ratio (p=0.003).

Table 6 HDL, LDL, VLDL levels in the serum of volunteers

Groups	HDL		LDL		LDL/HDL		VLDL		Total Chol/HDL	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Mean	46 ± 0.57	47.45 ± 0.49	136.29 ± 6.74	122.07 ± 3.82	2.96 ± 0.15	2.58 ± 0.09	25.87 ± 1.92	22.05 ± 1.3	4.53 ± 0.17	4.05 ± 0.1
p-value	0.01		0.03		0.01		0.002		0.003	

DISCUSSION

Egg is an excellent source of proteins and many other important nutrients. The main inhibition in popularizing eggs has been its high content of total fats with cholesterol which is considered harmful for cardiac health. Even in n-3 enriched eggs, the basic question whether consuming them with or without high contents of other fats, structured the trial to answer current nutritional issues. The pilot study would give leads for undertaking long term studies if found that conducting such a study is not harmful to the volunteers on a long-term basis. The data analyzed brings out the following facts. Consumer response sensory studies showed better acceptability and no adverse reaction in all the subjects studied.

There was a minor rise in Hb levels in all the subjects (Table 3). It would be of interest to study the RBC count and Hb levels after 110 days of supplementation of n-3 enriched eggs to derive the exact picture Table 3.

Consumption of 8 n-3 eggs each week in either form (boiled or fried omelettes with less oil) did not exhibit any adverse effects on the lipid profile as well as the levels of saturated fat. On the contrary, an improvement in the levels of cholesterol and triglycerides was noted. Also, the levels of total omega-3 in the plasma and RBC (Table 4) indicated a significant increase showing that n-3 enriched eggs can be a good source to increase blood n-3 fatty acids in humans as shown by earlier researchers [7,14]. Very minor changes in the n-6 levels were noted probably due to the short period of study. The n-6 levels in RBC also show a slight decrease though not statistically significant except in the case of Arachidonic acid. The slight increased level of plasma n-6 fatty acids in the plasma is also insignificant. The n-6: n-3 ratios in both plasma as well as RBC decreased significantly after supplementation with n-3 enriched eggs. In spite of the continuous feeding of high fat content for two weeks, the levels did not show any significant

increase in the plasma. On the contrary, there was also a moderate depletion in the saturated fat levels indicating that excess fat (cholesterogenic) is being metabolized.

One of the major findings of the study is the normalizing effect of both triglyceride and cholesterol on consumption of n-3 eggs (Table 5). It is only when the levels of cholesterol and triglycerides are higher than normal (B group) that n-3 egg consumption brings it down. On the other hand, when the cholesterol and triglyceride levels are on the lower side of the normal, they either get slightly elevated or remain steady, yet remaining within normal limits. Suk showed that plasma triglycerides decreased on consumption of omega-3 eggs, though cholesterol levels remain unaffected making n-3 eggs more healthful than control commercial eggs [15]. In our study we find a significant decrease in the triglyceride as well as cholesterol levels as also observed by an earlier work [16]. Also, dietary benefits of eggs could be obtained without the undesirable effects of higher cholesterol ingestion on consuming 12 n-3 eggs per week as observed earlier by Young Lee [17].

Increase in the cholesterol levels in the volunteers having low levels initially (A group) is intriguing. Perhaps, the excellent nutritive supplement through eggs might be boosting the anabolic activity demanding higher synthesis of cholesterol by the body for optimal metabolism. It is this cholesterol synthesized by the body which is represented by the significant rise in the levels of cholesterol of the sub-group (A) that had initially low cholesterol levels. It is necessary to undertake studies on larger samples and in more details to understand and interpret this finding.

The improvement in lipid profile is significant in just a two-week period in all the subjects. Serum HDL, VLDL, LDL, LDL/HDL and total cholesterol/HDL levels showed a significant improvement (Table 6). Significant increase in cardio-protective HDL cholesterol, with a concomitant significant decrease in LDL, the so called bad cholesterol, and VLDL which are risk factors for atherosclerosis, is also noticed.

Earlier studies show that in vitro both eicosa pentaenoic acid (EPA) and DHA inhibit triglycerides synthesis and their secretion. In humans, a reduction in triglyceride concentrations has been attributed to omega-3 fatty acids [18,19], with more inconstant effects on other lipid parameters [19]. Our results are in accordance with the above work. ALA content per se in the eggs too appears to be contributory even though only a small portion of ALA is known to be metabolized to DHA [20,21]. However, in a healthy ageing population, consumption of ALA might favourably alter the concentrations of LDL cholesterol [22]. This could be the reason n-3 eggs containing ALA, apart from DHA exhibit positive effects even in the elderly population of the study who are more at risk for atherosclerosis. Earlier studies also show that in coronary artery disease valuable inclusion of dietary n-3 fatty acids could be used as a secondary preventive measure [23].

These leads on a small study suggest that despite an increase in total fat, n-3 eggs are not harmful to human health. On the contrary, a favourable improvement in lipid profile is observed on the intake of n-3 egg. N-3 enriched eggs being a more acceptable form of food could well be considered as a desired produce to enhance the dietary concentrations of n-3 fatty acids in humans. Benefits of all the nutrients in eggs could thus be obtained without any harmful effect of increased levels of cholesterol [17]. As n-3 fatty acids are considered healthy in all stages of life [24], consumption in the form of eggs would be an easier option. Eggs could thereby become an important part of a healthy diet globally with certain dietary manipulations in the poultry feed [25]. For a better understanding; larger studies with n-3 egg in different ailments, and different age groups are called for.

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