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Review Article

Production of heart-healthy eggs through dietary manipulations of hens

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Abstract

Changes in life style of people have led to increase in incidence of diseases such as heart failure, hypertension and diabetes. As a result, today's consumer is becoming aware of usefulness of functional foods. Eggs are considered to be complete food, but clinicians and dieticians are concerned about their cholesterol content. Hence, a large population of the world is unable to have the benefits of eggs. This paper reviews the feeding strategies by which egg can be converted into healthy product for heart. Fatty acid composition of egg yolk can be altered, by feeding chromium, marine algae, rapeseed and flax seed or their corresponding oils. While altering polyunsaturated fatty acids of yolk, antioxidants such as vitamin E are also supplemented. This not only adds to the nutritional value of eggs, but also improves the shelf-life of eggs and egg products. Chromium can be used for production of low cholesterol eggs and using such eggs can also avoid/delay/decrease the severity of diabetes. Low cholesterol eggs and conjugated linoleic acid rich eggs have also been patented by scientists. Most of the polyunsaturated fatty acid modifiers (except some herbs) reduce the sensory acceptability of eggs and performance of hens. So, more research is required to be done to establish the use as polyunsaturated fatty acid modifiers. The use of marine algae for producing docosahexaenoic acid rich eggs is promising, but it is also required to be thoroughly researched because some algal species accumulate certain heavy metals in higher concentration than surroundings. Although low cholesterol, high vitamin and high docosahexaenoic acid eggs are available in some metro cities of India, at relatively very high cost, the producers of these eggs have their 'trade secrets'. These eggs are not available to the average population of the country. Therefore, more research should be conducted on the use of locally available materials, combined with robust technology so that the poultry farmers can adopt the method of producing designer eggs so that these eggs are available to common man at reasonable cost. This would also improve economic status of poultry farmers.

Key words: Cholesterol manipulation, heart-healthy eggs, dietary manipulation, PUFA modification.

Because of a change in life-style of the urban population, the incidence of certain diseases such as congestive heart failure, hypertension and diabetes etc. has drastically increased. As a result, there is an increase in the awareness, regarding the usefulness of functional foods, among today's consumer. Eggs are considered as complete food with most of the nutrients available in required proportion. However, the clinicians and dieticians are always worried about cholesterol content of eggs. Because of it, a large population of the world cannot have the benefits of eggs. If this cholesterol scare is eliminated, almost whole of the non-vegetarian population (except those who are allergic to egg protein) can use eggs. The internal components of eggs can be altered by nutritional manipulations (Singh *et al.* 2010). The eggs so produced are called 'designer eggs'. Eggs having low cholesterol and enriched with vitamins and omega-3 fatty acids are available from 'Kansal Agro' and 'Suguna Foods' in some of the metro cities of India (thehindubusinessline.com). About 100 ml of the egg liquid form Kansal Agro contains three times more vitamin A, eight times more vitamin D3, ten times more vitamin E and five times more folic acid and the cholesterol content is 179 mg compared to 420 mg in regular egg. Similarly, Suguna specialized egg contains 370 mg docosahexaenoic acid (DHA)-sufficient to meet daily requirement. These eggs also fully meet the vitamin E requirement and upto 83% of selenium requirement (thehindubusinessline.com). However, regarding how to get these eggs, is a trade secret of these producers. This paper reviews the various aspects of nutritional manipulations of hens for production of heart- healthy eggs, so that the poultry farmers can adopt the technology and improve their returns as well as these eggs become available to average Indian population at reasonable price.

1. Cholesterol manipulation

Average cholesterol content of a generic egg is 222 mg (Meluzzi *et al.* 1993). Many attempts have been made for lowering the egg cholesterol most of which have resulted in little practical application (Hargis 1988). Use of soybean, flaxseed, groundnut cake (Beynene 2004), ascorbic acid @ 1.3 g/kg (Nockles 1973), garlic juice (mixed in water in the ratio of 1: 1 W/W) @ 0.25, 0.50 and 1% of body weight of laying hens (Mahmoud *et al.* 2010), oil of rapeseed, sunflower, fish and linseed @ 1.5 and 3% for 12 weeks, (Ceylan *et al.* 2011), sunflower oil @ 8% (Sim and Bragg 1977) had no effect on the egg cholesterol contents while addition of sodium-potassium sulphate @ 1%, (Nockles 1973) *Lactobacillus acidophilus* @ 4.0 x 10^6 cfu/g of diet (Abdulrahim *et al.* 1996), *Bacillus subtillis* culture @ 500 mg/kg (Xu *et al.* 2006), Bioplus 2B (commercial probiotic) @ 1.28 x 10^6, 3.2 x 10^6 and 4.6 x 10^6 cfu/g feed (Mahdavi *et al.* 2005) and @ 250, 500 and 750 mg/kg (Kurtoglu *et al.* 2004), *Lactobacillus sporogenese* @ 100 and 150 mg/kg feed (Panda *et al.* 2008), *Trichoderma viride* culture and barley feeding (Qureshi *et al.* 1984), red palm oil and red palm oil + popped amaranth (Punita and Chaturvedi 2000), aqueous solution of pressed garlic oil through drinking water (Poltowicz and Wezyk 2013), onion and garlic supplementation (Siam and El-Dein 2010), tulsi supplementation @ 1% (Deshpande *et al.* 2009), beta cyclodextrin @ 6% in feed, statin, chromium, nicotinic acid, garlic, basil, plant sterol and omega-3 PUFA (Habibullah *et al.* 2007; Parker *et al.* 1999), copper @ 150mg/kg (Balevi and Coskun 2004) spirulina + basil (Johri 2016) and organic chromium (Evans 1989; Lien *et al.* 1993; Lien *et al.* 1996; Jensen and Maurice 1980) resulted in reduction in egg cholesterol. While dietary tamarind (Chowdhury *et al.* 2005) caused decrease in serum cholesterol only.

2. Manipulation of polyunsaturated fatty acids

It is important to know that cholesterol by itself is not harmful; it becomes harmful when it is oxidized. Cholesterol lowering effect of polyunsaturated fatty acids (PUFA) is well established (Kinsella *et al.* 1990; Demeyer and Doreau 1999). So, cholesterol in egg can be reduced by altering yolk fatty acid composition. Feeding PUFA rich feeds to layers resulted in a large increase in the relative and absolute concentration of PUFA in total lipids of yolk (Caston and Leeson 1990; Farrel and Gibson 1991; Hargis *et al.* 1991). Vitamin E is enhanced from 100 to 300 % in omega enriched eggs. These omega-3 designer eggs have high levels of vitamin E, which is an antioxidant which prevents oxidation of cholesterol and therefore, its ill effects. In order to improve their quality, these eggs are enriched with not only vitamin E, but also with selenium and carotenoides, which prevent oxidation of cholesterol.

a) Enrichment of eggs with omega fatty acids

Omega-3 fatty acid have been reported to protect against cardiovascular and inflammatory disease as well as certain types of cancers (Simopoulos 1991; Kinsella *et al.* 1990) and it has also been shown that it is an essential nutrient for adults and children (Bjerv 1991; Holman *et al.* 1982). The benefits of dietary omega 3-PUFA includes reduction in plasma triglycerides, blood pressure, platelet aggregation, thrombosis and atherosclerosis particularly in diabetes, tumour growth, skin diseases and enhanced immunity. Ferrier *et al.* (1992) reported that human consumption of linoleinic acid (LNA) enriched eggs decreased serum triglycerides and increased omega-3 fatty acids particularly docosahexenoic acid (DHA). When the diet of infants does not contain eicosapentanoic acid (EPA) or DHA then LNA should be supplied as 1% of energy intake. The vegetarian population and North Indians are at a risk of dietary deficiency of DHA because of no or negligible consumption of fish, respectively.

The egg industry can help to resolve this problem. In 1973, Sim and his team developed a designer egg rich in omega-3 as well as antioxidants. These eggs were got patented by the name of 'Professor Sim's designer eggs'. The egg which has been considered as an atherogenic food, now can be consumed safely if enriched with omega-3 fatty acids. The generic egg has only 60 mg omega-3 fatty acid as compared to an omega-3 enriched egg which may have upto 600 mg (Shane 2014), although in certain cases taste is adversely affected by high levels of fish oil or flax seed.

Omega-3 enrichment can be achieved by feeding flax seed (Caston and Leeson 1990; Jiang *et al.* 1992), marine algae (Mary and Elswyk 1997), fish oil (Yu and Sim 1987; Hargis *et al.* 1991), and rapeseed oil (Ceylan *et al.* 2011) to laying hens. Using spirulina and *tulsi* in designer egg feed containing 10% flax seed, 10% full fat sardine fish having 22% oil and 200mg vitamin E per kg diet, resulted in significant increase in EPA, DHA and vitamin E (Johri 2016). Designer eggs have higher PUFA specially LNA, EPA, DPA and DHA compared to regular eggs and they have almost comparable levels of DHA to that of fish oil. Fatty acid composition of oils rich in omega-3 PUFA, regular egg and omega-3 PUFA rich eggs is given in table 1.

The egg lipid composition is the result of combination of *de novo* lipogenesis and incorporation of lipid components from the diet. Another factor regulating the quantity and type of fatty acid deposition is the feedback inhibition of dietary long chain PUFA (Reiser *et al.* 1963). The amount of omega-3 fatty acid is increased in eggs by two feeding methods (Leskanich and Noble 1997); one is by feeding linoleinic acid which is a precursor of DHA, for which the hens are fed flaxseed, rapeseed or their corresponding oils. Omega-3 enriched eggs "flax seed style" is widely accepted in the marketplace. The idea of using flax seed for omega-3 egg production began when sensory studies reported strong "fishy flavours" from fish oil based omega-3 enriched eggs, despite fish oil inclusion rates as low as 3% (Leskanich and Noble 1997). However, supplementing flax seed at 10% or higher usually decreases egg production and body weight of hens (VanElswyk 1997). Feeding fish oil primarily increases the yolk DHA and EPA levels which can become rancid quickly and produce off-flavours. Although flax seed also has high oil content, the type of PUFAs deposited into the egg are primarily alpha linoleic acid (ALA), known for having greater stability and a slower onset of rancidity (Gonzalez and Leeson 2001). Higher intake of ALA is protective against ischemic heart disease (Hu *et al.* 1999).

Sim *et al.* (1992), Jiang *et al.* (1992) and Sim (1993) carried out a series of studies to enhance the value of eggs enriched in omega-3 fatty acids with a significantly higher PUFA: SAFA ratio and lowering the omega-6: omega-3 fatty acid ratio. The hens fed flaxseed produced eggs enriched with omega-3 fatty acids in the following order: LNA>DHA>DPA>EPA. However, the feeds containing large amount of flaxseed develop a fishy odour or lower sensory quality of eggs during storage, due to combination of lipid rancidity in feed and lipid peroxidation in the eggs. The lipid rancidity in flaxseed is negligible in early storage period, but it increased markedly after 60 days storage. The results also showed that the whole flaxseed is well protected from peroxidation by the presence of its intrinsic tocopherol content. In case of broken flaxseed used for feed compounding and processing, tocopherol supplementation extends its stability (Gopalakrishnan *et al.* 1996). The 'fishy' odour which is due to rancidity of omega-3 fatty acids can be controlled by stabilizing dietary source of PUFA with a natural form of tocopherols as antioxidant before

Table 1. Fatty acid composition of oils rich in omega 3 PUFA, regular eggs and omega3 PUFA enriched eggs

Item	C16:0	C18:0	C18:1	C18:2	C18:3	C20:4	C20:5	C22:5	C22:6
Flaxseed	6.00	3.00	17.3	13.4	55.3	-	-	-	-
Fish oil	17.3	2.60	12.8	0.90	0.80	1.5	17.7	1.40	6.20
Canola oil	3.00	2.00	56.0	20.3	9.30	-	-	-	-
Lupin seed oil	6.10	1.50	50.0	17.4	10.6	-	-	-	-
Fenugreek oil	26.0	10.0	3.00	6.40	16.7	-	-	-	-
Soy bean oil	10.0	5.00	28.9	50.7	6.60	-	-	-	-
Regular egg	21.5	8.00	42.1	13.8	0.22	1.75	-	0.15	0.08
PUFA enriched egg	16.9	6.20	41.7	13.7	4.58	-	0.73	0.89	5.83

Source: Narahari *et al.* (2004)

incorporating in chicken feeds (Li *et al.* 1996; Qi and Sim 1998). Supplementing antioxidants in chicken feed not only effectively eliminates the off flavor problems, but also greatly improves stability of eggs. The tocopherol concentration of egg yolk increases (Li *et al.* 1996) linearly with increasing level of dietary tocopherols. Reduction of oxidation products and increasing the antioxidant concentration in the yolk were the major technological breakthrough eliminating the preceding sensory and off-flavour problems. Therefore designer eggs not only supply stable form of essential omega-3 PUFA, but also natural form of tocopherols including vitamin E to today's health-conscious consumer. Beynene (2004) reported that soybean feeding resulted in increase in linoleic acid and flaxseed feeding raised the level of ALA. Feeding flaxseed increased the EPA and DHA. However, PUFA modifying agents have some effects on sensory quality of eggs and performance parameters and, there are some conflicting reports also, which have been presented in table 2.

There is one report (Narahari *et al.* 2004) suggesting that the use of herbs modify PUFA of eggs and they do not have any bad effect on sensory characteristics of eggs as well as performance of laying hens. Siam and El-Dein (2010) showed that onion and garlic supplementation significantly decreased serum, yolk and liver cholesterol content and the effect was higher in ISA brown hens than the Fayoumi hens and also supplementation throughout the week showed more pronounced effects compared to supplementation of 3 days per week. The effect of different cholesterol lowering agents may also depend on the age and strain of the laying hens, and length of period of supplementation. Hence more research into the true effects of these materials on production parameters and egg characteristics is required.

Cost involved in feeding of fish and fish oil, would increase the cost of production, however, the other feeding materials such as algae, rapeseed and flax seed or their oils would relatively have less effect on the cost of feeding. While feeding whole flax seed, the use of grit, although a minor cost, is highly recommended, if feeding ground flax seed, the feed company will likely charge for the processing, Vitamin E supplementation may be an extra cost, but it is a reclaimable cost as it adds an extra value from consumers' view point.

Eggs can also be enriched with conjugated linoleic acid (CLA). CLA have been reported to have wide range of health promoting effects, such as anti-atherosclerotic, anti-atherogenic, anti-carcinogenic, anti-diabetic and immune stimulatory effects (IP *et al.* 1991; Pariza *et al.* 2000; Suksombat *et al.* 2006). A diet based approach offers great potential to reduce cardiovascular diseases, as about 80% heart attacks

Table 2. Effects of PUFA modifying agents on egg quality and performance of layers

S. No.	Ingredient	Effects other than omega fatty acids	Reference
1.	Flax seed	Fishy odour and poor sensory quality (on storage)	Sim *et al.* (1992); Jiang *et al.* (1992); Sim (1993)
		Decrease in gain in weight	Scheideler *et al.* (1997); Caston *et al.* (1994)
		Increase in egg production, decrease in egg weight, yolk weight and egg shell quality.	Scheideler and Froning (1996)
		Decrease in egg production.	Aymond and VanElswyk (1995)
		No effect on egg production.	Jiang *et al.* (1992)
2.	Fish oil	Fishy taste, fishy aroma and fishy aftertaste.	Leskanich and Noble (1997); Mary and Elswyk (1997)
3.	Marine algae	Decrease in egg production, decrease in yolk weight in hens of 56 weeks age, no effect on egg production, and decrease in yolk weight in hens of 24 weeks age.	Herber and Elswyk (1996)
4.	Menhaden oil	Decrease in yolk weight	Herber and Elswyk (1996)
5.	Herbs	No negative effects.	Narahari *et al.* (2004)

can be avoided by diet modifications. Conjugated linoleic acids are isomers of linoleic acid (a polyunsaturated omega 6 fatty acid). Dietary CLA is contributed by ruminant foods such as dairy and beef. Based on animal data it is estimated that approximately 3 g per day of CLA would be required to produce beneficial effects in humans (Pariza *et al.* 2000), but the current consumption is less than 600 mg per day in the U.S. where a large amount of beef is also consumed. In India certainly, there is much less intake of CLA because of no consumption of beef. This might also be one of the reasons that Indian population is more vulnerable to cardio-vascular disease (CVD). Chicken eggs, due to their high content of nutrients, low cost and versatility, are a popular food item. CLA enrichment of eggs can be done by feeding CLA to laying hens. Eggs from CLA fed hens are a good source of CLA in human diets (Du *et al.* 1999). It has been reported (Suksombat *et al.* 2006) that increasing the amount of CLA in hens' diet did not only increase the amount of CLA in egg yolk, but also resulted in reduction in the amount of yolk PUFA. Rahim *et al.* (1999) got a method of CLA enhancement of egg patented. They have disclosed poultry feed supplement in this patent. This supplement has to be fed to the laying hen for at least 1 day prior to laying. However, the success of such CLA modified eggs and egg products will depend on acceptable sensory characteristics and stability during cooking, storage and processing. These factors also need to be investigated in detail and then only a proper feeding strategy can be developed to have eggs enriched with CLA.

b) Direct enrichment with docoshexaenoic acid

EPA and DHA are the most bioactive omega-3 FA (Mary and Elswyk 1997) in human subjects. Moreover, it has been found that health benefits from omega-3 enriched eggs can be limited because the conversion of linoleinic acid into DHA in human body is not forever effective, particularly in children and in elderly persons, and almost all health promoting properties of omega-3 fatty acids are correlated with DHA (Lopez-Bote *et al.* 1998). For this reason, the addition in the diet of preformed DHA, in the form of fish oil is a more promising way (Van Elswyk *et al.* 1992). However, use of marine algae, which are rich in DHA, may represent a more efficient means of producing DHA enriched eggs. Supplying DHA directly in the form of marine algae, also provides naturally occurring carotenoides which enhance oxidative stability to both omega-3 enriched poultry rations and resulting poultry products and it is more efficient for DHA deposition than dietary menhaden oil. (Van Elswyk 1997). However, algae accumulate certain heavy metals in it therefore, its use should be done under strict quality control. More work is required to be done on addition of pre-formed DHA in eggs and research should be focused on removal of fishy odour, in order to have sure benefits of designer eggs by having higher levels of EPA and DHA.

c) Chromium

As mentioned previously, the use of chromium resulted in reduction of egg cholesterol, there is a direct relationship between dietary concentration and yolk chromium accumulation (Anderson 1987). Chromium is an indispensable component of glucose tolerance factor. Many plant products contain only low levels of chromium and grinding removes about 83% of a grain's original content of chromium (Gibson 1989). Most of Indian population use chapatti, as staple food which obviously has very low levels of chromium. So, under Indian conditions, lowering of egg cholesterol by using chromium would provide double benefit; good for heart as well as for avoiding/delaying/reducing the severity of diabetes (which is the mother of a number of diseases). Nakaue and Hu (1997) reported that supplementation of chromium @ 0, 200, or 800 ppb, in hens of 22 and 75 weeks age resulted in significantly less cholesterol content in 22 weeks old hens than the control group, whereas, 75 week old hens showed no decrease in cholesterol.

d) Drugs

Egg cholesterol is reduced by at least 50% through one of the two mechanisms (Elkin *et al.* 1997):1) reduction of VLDL synthesis, VLDL secretion and/or cholesterol richness of VLDL particles, 2) interference with the uptake of VLDL at the level of oocyte plasma membrane (the oocyte vitellogenesis receptor). The reason for this is that almost all of the cholesterol and most of the lipid destined for oocytes (future egg yolk) uptake is synthesized in the hens' liver and packaged in the form of VLDL and transported to plasma. Thus anything that alters VLDL synthesis, composition or secretion by liver will be expected to alter egg cholesterol. Plasma VLDL and vitellogenin which account for approximately 60 and 24 % of egg yolk dry matter, respectively, are taken up into growing oocytes by process of endocytosis. Therefore, anything which could selectively block VLDL uptake by oocyte would be expected to markedly alter egg yolk cholesterol and fat content. Elkin and his team (1997) reported that egg cholesterol can be reduced by 45 to 50 % by a drug atrovastatin without impairing egg production. They also reported that a decrease in cholesterol level is usually associated with production of infertile eggs. Moreover, having low levels of cholesterol in eggs by genetic selection is extremely difficult which is due to physiological control mechanism which result in decrease in egg production (Hargis 1988) and ultimately cessation of egg production, when cholesterol accumulation in egg is inadequate for the survival of embryo (Villa 1999). However, Mariey *et al.* (2012) reported that the use of spirulina decreased the cholesterol content of eggs and it also increased the fertility and hatchability of eggs significantly. Meier and Wilson (1997) got a US patent for method of altering the cholesterol content of eggs. They found that when n-dihydroxyphenyalanine (L-DOPA) is elevated in the blood stream of hens, they produce eggs with less cholesterol content, a lower ratio of saturated to unsaturated fatty acids and higher total protein. The L-DOPA is used as a dietary supplement. However, the use of various pharmacological agents for decreasing egg cholesterol will be limited if such agents or their metabolites are excreted into the eggs (Hargis 1988).

Conclusions

Despite frequent claims to the contrary, it has not been possible to reduce the cholesterol content of eggs under commercial conditions for extended periods. Under experimental conditions cholesterol in eggs can be lowered by administration of statin drug but egg production is markedly reduced and this approach would be prohibitively expensive. There are conflicting reports regarding the effect of chromium on organoleptic and production parameters, but it reduces the egg cholesterol. So more work is needed to be done to establish the level, form of chromium and its effect on non-diabetic and diabetic persons, as it can have added advantage of avoiding/delaying or decreasing the severity of diabetes. Marine algae can be used to have eggs with high omega-3 and low omega-6 fatty acids. However, some algal species are known to accumulate heavy metals at concentration higher than their surroundings, while others generate pathological metabolites that cause neurodegenerative disorders. Such species require establishment and evaluation before recommendation as feed supplements. Similarly supplementing flax seed at 10% or higher usually decrease egg production and the body weight of hens. Therefore, drawing conclusion regarding type, level and form of various supplements (processing); their effects on overall production performance, egg quality, economics, and the strain and age of the hens, is difficult. Therefore, different promising materials especially chromium, algae, flax seed and herbs are required to be thoroughly examined for production of heart-healthy eggs, especially for commercial production.

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