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## Enhancement of Nutraceutical Value of Table Eggs Through Poultry Feeding Strategies

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#### ABSTRACT

A significant focus in the nutrition era has been oriented to nutraceutical and functional foods. The health promoting potential of such provisions is ascribed to nutrition and these constituents have useful properties playing important role for egg producers. The biological and nutraceutical importance of egg is often due to the active compounds. It holds various bioactive compounds having significant nutraceutical properties like antioxidant, antiallergenic, antiartherogenic, antimicrobial and cardioprotective. Antioxidants are important against many disorders like hypercholesterolemia and cardiovascular diseases. The growing attention on natural antioxidants has promoted researches in investigating new antioxidant sources having profitable prospective. Confirmation for the effectiveness of the enrichment of eggs with bioactive compounds by inclusion of plant-derived materials in the diet of egg-producing poultry has been reviewed. This review study aims to discuss the antioxidant potential of natural substances used to enrich the eggs and highlight the importance of eggs as potential nutraceutical food. The evidence for health-promoting effects of enriched eggs has also been included.

**Key words:** Eggs, nutraceutical value, enriched eggs, designer eggs, antioxidants, feeding, poultry, health

#### INTRODUCTION

The description of active compounds in food is an indispensable step in the validation process of such nutritional strategies and it is essential for the exact definition of "functional food" (Jezewska-Zychowicz, 2009). The discovery of active components contained in food daily consumed, is an essential step in the realization of a nutraceutical food having the capacity to prevent chronic disorders (Leoncini *et al.*,

2012). The term nutraceutical (a combination of nutrition and pharmaceutical) indicates a food component that provides health benefits, including the prevention of diseases. Consumers are progressively more interested in functional foods, therefore in present scenario they are extremely careful on their wellbeing as a result demands of beneficial foods worldwide growing. In spite of slight variations in overall nutritional content of eggs from country to country (mostly related to the variations in diet fed to the hens), eggs are

widely recognized as naturally rich in proteins and a number of vitamins and minerals. The egg is often referred to as one of the original and natural functional foods. Nutritional claims allowed for regular eggs (standard diet fed to the hens) vary from country to country mostly due to regulations (Guyonnet, 2011).

Eggs provide a completely packaged, nutritious food including essential nutritional components (Laudadio et al., 2014). However, the uncertainties are related to the cholesterol level in eggs. It was reported by Gilbert (2000) that eggs result more healthier and valuable food when cholesterol content is reduced. Nevertheless, the cholesterol level derived from egg is much lesser than the cholesterol concentration synthesized by humans (Singh and Sachan, 2010). The interior components of egg can easily be transformed by poultry feeding practices (Laudadio and Tufarelli, 2011; Khan et al., 2012a). The eggs can also be improved thanks to additional nutrients such as selenium, vitamins B-group, vitamin E and particularly antioxidant compounds. Nevertheless, the fortification of different nutrients in egg is exclusively dependent on nutritional changes of laying hen diet (Ahmed and Abdelati, 2009; Singh and Sachan, 2010). This egg is moreover able to attract the market attention by varying their health status and interesting to a kind of the consumer who are available to spend for these changes in egg. Therefore, as need and production of enriched eggs increases, research is needed to establish their solidity and adequacy. Egg is a nutritive food rich in proteins and it is a cheap source of protein (Hasler, 2000). Altering the nutritive contents of the egg can thus help alleviating the problem of nutritional deficiencies in target population (Kassis et al., 2010). World bodies recommend for the intake of 3 servings of vegetables and 2 servings of fruits a day but this is not practically feasible in many countries which are developing. So there is a definite need for an alternative that can sort these problems (Dike, 2014). One should have a food item that is accepted by most of the people in the community, egg is one such food item that can be fortified with various nutrients. Egg is superior to animal meat in case of nutrients because animal meat contains more fat that can cause various health problems to human which include heart attack (Simopoulos, 1991).

#### EGG AND ITS BENEFITS

Egg is considered as the balanced diet that contains all nutrition of which major is contributed by protein. It also contains vitamins and minerals and can be considered as a low calorie diet. Very few sources supply vitamins like K and D to the human through diet and egg is one among them (Kassis *et al.*, 2010). Egg also supplies a factor that is very crucial for the development of human brain which is choline (Herron and Fernandez, 2004). Production of egg Is easy now-a-days as poultry production has become an industry. Hence egg offers good quality nutrients at low cost and it is used in most household for baking, cooking etc. (Stadelman *et al.*, 1995; Stadelman, 1999). Though it provides all nutrients that have a positive impact over human health egg

yolk also contains cholesterol that can harmfully affect human health. Cholesterol content of egg is approximately 200 mg which as per standards laid by America is just short by a hundred to meet the daily needs of a person (Weggemans *et al.*, 2001). So, once a person crosses the limit of cholesterol intake, diseases affecting the heart haunt him (Howell *et al.*, 1997).

Humans are always attracted towards nutrients that are available in less quantity in food items but that are very essential for healthy living. There are few in this list which includes vitamin E, selenium, n-3 fatty acids and carotenoids. These nutritive factors have important role in healthy living of humans and hence they are rightly considered by nutritionist to enhance these nutrients in various food stuffs (Surai and Sparks, 2001). Eggs usually contain higher level of saturated fatty acids that are harmful to human. Omega 3-polyunsaturated fatty acids (PUFA) are the good fats to human health, hence various attempts have been made to increase PUFA content in the egg volk simultaneously decreasing the bad cholesterol content (Juturu, 2008). By altering the ration feed to poultry, scientists have tried to alter the fat content of the egg yolk. Attempts have been made to increase the  $\alpha$ -linolenic acid content of the yolk which can be converted into PUFA in human body (Hu et al., 1999). To achieve this plant sources like flaxseeds and linseeds that are rich in α-linolenic acid are incorporated in poultry ration (De Lorgeril et al., 1994; Ferrier et al., 1995). A major drawback noted by this method is that the conversion of α-linolenic acid to PUFA is not so effective. To negotiate this problem the best way to add stuffs that contain preformed PUFA which include fish oil (Leskanich and Noble, 1997). The problem noticed by addition of these oils has led to the fishy odour of the egg which is not accepted by many people (Van Elswyk et al., 1992). Eggs that are enriched with various essential nutrients are called as super eggs.

### ROLE OF THE ANTIOXIDANTS IN HUMAN HEALTH

In last decade there is an increase of studies related to novel progresses in diseases prevention particularly the function of antioxidants and free radicals (ROS). Thus, it will be relevant to study the potential role of the ROS in health disorders and the antioxidants in its prevention (Pandithurai and Murugesan, 2014). It was reported a dangerous influence of ionizing radiation might be attributed to creation of ROS (Sohal and Weindruch, 1996; Devasagayam et al., 2004). Since then ROS and Reactive Nitrogen Species (RNS) have gained renown importance (Gilbert, 1981). In the scientific and medical literature the definition of 'free radical' is utilized in an ample sense and includes related reactive species such as 'excited states' leading to ROS production or those species resulting from free radical reactions. The ROS have been involved in many human disorders (Halliwell and Gutteridge, 1985).

The relation between ROS and disorders could be clarified using the Oxidative Stress (OS) concept (Celi, 2011). In

animal healthy body, the of prooxidants formation as ROS and RNS are efficiently kept in control by different antioxidant defense levels (Devasagayam et al., 2004). Nevertheless, when it gets exposed to unfavorable physicochemical and environmental agents (pollutants, cigarette smoking, UV rays, radiation, toxic elements, over-feeding and diabetes), this delicately maintained balance is shifted in favor of pro-oxidants resulting in OS (Kilgour and Roberts, 2014).

Antioxidants are defined as substances neutralizing the free radicals (Powers et al., 2011). Nature has provided cells with enough defensive instruments against any damaging properties of free radicals: superoxide dismutase, glutathione peroxidase, glutathione reductase, thioredoxin and disulfide bonding are buffering systems in cells (Devasagayam et al., 2004). Because of capable antioxidant defensive methods are vital to slow down the OS, investigating for other antioxidants in disorders reduction has received much interest in the last decades. Important health benefits of natural and synthetic antioxidants like many vitamins, phenols and carotenoids present anti-cancer and anti-inflammatory properties. The antioxidants are able to neutralize free radicals and related events, take action at diverse phases. In fact, antioxidants proceed at the stage of repair, interception as well as prevention (Wojcik et al., 2010; Khan et al., 2012b; Rahal et al., 2014).

The vitamin C is a water-soluble free radical scavenger. Animals are not capable to synthezise ascorbic acid, so this complex must be supplied by diet, in particular with vegetable sources. The main natural sources of ascorbic acid are found in brussels sprouts, broccoli, grapes, kiwi fruits, cantaloupes, fruit, green or red pepper, strawberries, cabbage, kale. Daily dietary allowance was recommended to be 60 mg (Jacob and Sotoudeh, 2002). It also has the ability to scavenge OH, H<sub>2</sub>O<sub>2</sub>, ROO and O<sub>2</sub> (Padayatty *et al.*, 2006; Chen *et al.*, 2008). Ascorbic acid revealed to act a key-role in the modulation of immunological processes, above all involving in the IL-18 production, a great inducer of the interferon-γ (Kim *et al.*, 2000).

The other significant scavenger of ROS in diet is greatly lipophilic vitamin E, present in nature in many compounds as  $\alpha$ ,  $\beta$ ,  $\gamma$  e  $\delta$ -tocopherols and  $\alpha$ ,  $\beta$ ,  $\gamma$  e  $\delta$ tocotrienols (Wojcik et al., 2010). Valuable source of tocopherols are nuts and the oil from vegetables (i.e., sunflower or wheat germ). The tocotrienols are all over in cereals and oils (wheat, barley, rice, barley, palm and bran oils). The alpha-tocopherol is the predominant form in diet that is known by hepatic alpha-tocopherol transfer protein and moved to blood and tissue (Traber, 2007). The α-tocopherol is considered to have significant function in atherosclerosis prevention. It was found that high doses of alpha-tocopherol inhibited the platelets adhesion and aggregation and also expression of molecules adhesion on monocytes. Based on the role of vitamin E as antioxidant and that OS resulted in cancer genesis, it was supposed that vitamin E could have a role against cancer development. It was supported by many animal studies (Zhang et al. 2004; Dong et al., 2007).

Over 600 fat-soluble plant pigments (carotenoids) are available in fruit and vegetables, such as α-carotene (vellow pepper, carrots, pumpkin, corn), β-carotene (alfalfa), lycopene, β-cryptoxanthin, lutein and zeaxanthin (spinach, broccoli, lettuce, corn) (Voutilainen et al., 2006). The β-carotene is the main vitamin A precursor (namely pro-vitamin A) and to date it represents the pigment widely studied. In plasma, carotenoids are associated either with low density lipoproteins (β-carotene, lycopene) or with high density lipoproteins (lutein and zeaxanthin). Since β-carotene is capable to stop cancer cells and apoptosis and in addition, successfully quench O<sub>2</sub> and in a straight line repress the oxidation of low density lipoproteins, significant consideration is focused on beta-carotene function in prevention of cancer as well as cardiovascular diseases (Krinsky and Johnson, 2005). Lately, more consideration has been paid to antioxidant role of lycopene, in fact it was found that lycopene may be involved in risk reduction of many health disorders (Wojcik et al., 2010). In contrast with plants and microorganisms, animals are unable to synthesize lycopene. Lycopene predominates in trans form, the most stable form, in tomato, however the cis form is considered the most bioavailable form in blood and tissue (Unlu et al., 2007). Lycopene has been evaluated as a chemopreventive compound in animal trials due to its function of inhibitor many diseases and disorders more than  $\alpha$  and β-carotene (Levy et al., 1995).

The identification of polyphenols, particularly flavonoids are classified into six subgroups such as flavones, isoflavones, flavanones, anthocyanidins and catechins (Pal and Verma, 2013). Given that polyphenols are rich dietary antioxidants, polyphenols have become indispensable for human and animal, principally as anti-cancer and anti-inflammatory agent. Many researches on the polyphenols anti-inflammatory and anticancer properties contained green and black teas have demonstrated the probable defensive act in diseases. The positive properties of polyphenols were demonstrated by studies showing that this compound reduce carcinogenesis in many tissues (Lambert et al., 2005). Moreover, it was reported that the major resource for anthocyanins is represented by red grape skin, elderberry, purple carrot, red cabbage and specially these molecules from purple carrot have valuable antioxidant property (Poudyal et al., 2010).

The most promising and the most frequently investigated polyphenol exhibiting beneficial role in health, is the resveratrol in trans form, polyphenol very present especially in red grape, peanuts and various berries (Smoliga *et al.*, 2011). During the last years, its overabundance of valuable health-related characteristics such as cardiovascular and protective properties were newly discovered as an important dietary supplement (Goswami and Das, 2009; Lin *et al.*, 2013). Given that OS is an important cause of increased risk of cardiovascular diseases, the treatment of many health disorders through the utilize of natural antioxidant sources, including ascorbic acid tocopherols, carotenoids and polyphenols, an

increase of attention in this era was received. In fact, it is well known that the vegetables and fruit intake daily and regularly consumed decrease and prevent significantly the development of health problems (Pham-Huy *et al.*, 2008).

#### ANTIOXIDANTS IN EGGS

Egg is considered a perfect natural food that has been consumed for centuries worldwide. Although egg contains all the indispensable nutrients for life, its consumption in several areas has reduces due to the public insight on its high cholesterol level (Nimalaratne et al., 2011). The antioxidant content of egg is almost nill and a fraction is found in egg yolk. So fortifying egg with antioxidant can enhance its quality which can be achieved by altering the ration in poultry feed (Carlsen et al., 2010). On the other hand, present data report that there was no full correlation between egg intake and plasma cholesterol concentrations as stated by Qureshi et al. (2007). Egg-yolk is a valuable source of many compounds significant for humans as well as animal wellbeing. It was well demonstrated that the diet supplied to laying hens has direct influence to yolk qualitative traits. By changes in diet, specific molecules having positive health effects can be incorporated in the egg-yolk (Kuhnle et al., 2008). To date, different investigations stated that active molecules in feed can be shifted from hens diet to egg-volk. Among compounds, both lutein and zeaxanthin resulted the most widely studied molecules in eggs. These help to reduce macular degeneration related to the age either filtering harmful light blue and/or as antioxidants (Chung et al., 2004). Isoflavones from soybean was found to contain phytoestrogens having possible health effects and they were detected in yolks (Kuhnle et al., 2008). Phenolic compounds usually contained in cereals, fruit and many vegetables are significant antioxidants, suggesting to occupy a defensive function in the various disorders of chronic nature (Liu, 2007). All the natural phenolic compounds derived from a common biosynthetic pathway, incorporating precursors from the shikimate and/or the acetate-malonate pathways (Colombo et al., 2014).

Up till now, few data is existing on the possible polyphenols in poultry eggs. Hens are well known to be able to 'bioconvert' health-related molecules from diet to egg. Both wheat and maize are valuable food/feed ingredients in diet containing antioxidants. Since these are main feed components for poultry in Canada, the objectives of a study conducted by Nimalaratne et al. (2011) were to characterize the presence of novel phenolic compounds in egg yolk, total antioxidant capacity of yolk extracts and to evaluate the effect of cooking methods on their content and activity. The results of this study suggested that eggs contain important levels of antioxidants as well to its renowned nutrients. Thus, consuming cholesterolrich food such as eggs was once indicated as a potential causing factor for cardiovascular disorders and the findings might even give rise to a paradigm change in nutritional eggs evaluation.

#### ENRICHED EGGS

Food stuffs that provide nutrients above the basic requirement are called as functional foods (Bech-Larsen and Grunert, 2003). Eggs fortified with various nutrients are also classified in this category. A large number of scientific trials into the feeding of enriched diets to laying hens have been carried out, demonstrating the levels of feed supplementation required to give a significant increase in the eggs. Many trials aimed to demonstrate the helpfulness and possible effects of dietary enrichment on productive traits in layers (Kaminska et al., 2001; Bean and Leeson, 2003; Mazalli et al., 2004; Jia et al., 2008), eggs' qualitative parameters (Niemiec et al., 2001; Ajuyah et al., 2003; Laudadio et al., 2014), sensorial assessment (Nash et al., 1996; Rymer and Givens, 2005) and health related effects (Lewis et al., 2000; Pavet et al., 2004; Laudadio and Tufarelli, 2010). Moreover, with nutritional enrichment of laying hen diets it is not only consumers who benefit but also the producers in terms of bird health, performance and even egg quality.

A large number of scientific trials into the feeding of enriched diets to laying hens have been carried out, demonstrating the levels of feed supplementation required to give a significant increase in the eggs. Focus has been given mainly to improve the omega 3 fatty acid content of egg yolk and also to reduce the cholesterol level of the yolk. Researchers found that addition of copper sulphate pentahydrate to the poultry ration has reduced the cholesterol content (Liu et al., 2010). Addition of garlic paste to the poultry ration has also brought down the cholesterol content (Chowdhury et al., 2002).

Enriched eggs are defined as eggs having different characteristics compared to common eggs. So-named designer eggs represent a significant part of all shell eggs in many countries and these eggs are enriched in omega-3 fatty acids which benefit human nutrition (Leeson and Caston, 2003; Sawant et al., 2012). In fact, the most available enriched eggs are those with changed fat level. However, modified fat content are marketed as a low cholesterol content, few saturated fatty acids, high levels in omega-3 fatty acids, high vitamin B as well as iodine (Singh et al., 2012). During the past decades, Sim Jany and their colleagues in Canada have developed designer eggs high in omega-3 fatty acids and antioxidants and subsequently they patented these eggs as Sim's designer-eggs. Later, Farrell (1998) in Australia produced a different modified egg containing significant levels of folate and iron, very useful for people with anemia. Canadians Leeson and Caston (2004) developed eggs which was pretty high in lutein level as compared to the common eggs. These modified eggs operate as a tonic for retina helping to prevent ocular degenerations. Various companies also markets eggs that are fortified with omega 3 fatty acids. Nutriplus is the brand name of the egg with omega 3 fatty acid and the producer is also Nutriplus. This is available in Malaysia (Lau et al., 2013). Some workers have also used marine algae *Nannochloropsis oculata* to poultry diet to get altered fatty acid level in the egg yolk. By addition of algae there is PUFA production in yolk and also alteration in the carotenoid content in the egg yolk (Fredriksson *et al.*, 2006).

#### NUTRITIONAL STRATEGIES FOR EGG ENRICHMENT USING ANTIOXIDANTS

The most well-known antioxidant utilized in animal nutrition is the alpha-tocopherol which is simply deposited in egg-yolk (Singh et al., 2012; Skrivan and Englmaierova, 2014). Nevertheless, other available compounds and molecules in eggs may also prevent oxidation, as Se and carotenoids. Antioxidants are deposited in yolks in function of dietary inclusion level. Enriching eggs with alpha-tocopherol or carotenoids may be obtained in an ample variety and moreover without negative effects on qualitative characteristics of eggs. Carotenoids of synthetic origins frequently supplemented to the poultry diet represented almost the total pigments in egg-yolk. Conversely, modifying egg using Se is quite more difficult because of high concentration of Se in food and feed can be toxic for both humans and animals (Pophaly et al., 2014). However, no harmful impact of antioxidants on egg quality parameters are to date reported.

Enriched vitamin E eggs can be obtained with high level of vitamin-E when compared to standard egg by supplying hens with rations containing high vitamin E level (Leeson and Caston, 2004). Higher levels of vitamin E may be obtained by supplementation of ingredients in form of natural sources. Supplementation with vitamin-E in diet for laying hens determine the following positive effects (Singh *et al.*, 2012): reduction of blood free radicals; decrease of cancer risk thanks to reduction of free radicals formation; reduction of the heart risk acting as antioxidant; delay of the development off-odors since vitamin E acts as high-quality antioxidant.

The most frequent methods to enrich eggs with both vitamins and minerals is to use high levels in the diet and it resulted a extremely useful practice to produce enriched eggs. Enriching eggs with vitamin E is a very accepted practice because of its antioxidative properties for all species (Mohiti-Asli et al., 2008). However, to date few trials aimed to evaluate the transfer of vitamin E from diet to eggs during the second laying production phase utilizing old molted laying hens are available. The researchers are imperative for producers because of significance of maintenance egg value and for improvement of nutraceutical properties of eggs. In a trial, Gjorgovska et al. (2012) used laying hens fed with basal diet supplemented with vitamin E and selenium as control group, while the birds from the experimental treatments were fed with higher dietary level of vitamin E in two different increasing concentrations. The vitamin E level in egg-yolk increased in laying hens when fed higher vitamin E level. The higher levels of vitamin E in diet determined an increase of egg production and laying duration. Moreover, a similar tendency was reported increasing Se level in the diet (Gjorgovska et al., 2012).

Another research was conducted to evaluate the effect of dietary vitamin E levels on metabolic traits in heat stressed hens (Yardebi and Turkay, 2008). The study used Leghorn laying hens which were assigned to two groups and initially subjected to the same environmental conditions. Diets were based on standard layer rations with different increasing levels of dietary vitamin E. Before, during and after heat stress exposure blood samples were collected from birds. Significant increases in plasma malondialdehyde, erythrocyte MDA, glutathione peroxidase, catalase, superoxide dismutase and MDA concentration in egg yolk and a reduction in plasma vitamin E were observed in groups during heat stress. Quality parameters in eggs were also reduced in the experimental group during heat stress. Dietary higher levels of vitamin E improved some of metabolic consequences determined by heat stress; moreover, there was no verification of advantageous influence on egg produced under heat stress within Se levels studied.

Currently, Se enriched eggs are existing worldwide. Among countries, Russia results the most advanced country for the Se enriched eggs production of (Fisinin et al., 2009). As expected, the cost of the Se enriched eggs is higher compared to a standard egg. Nowadays, the Se level in egg may be simply changed by supplementing high levels of Se feeds to poultry (Surai and Fisinin, 2014). The Se is one of the most essential nutrient because it is involved in very crucial roles in both humans and animals (Tufarelli and Laudadio, 2011). The Se enriched eggs were for first time obtained by the Scottish Agriculture College in 1998 (Surai, 2000) supplementing organic Se from yeast to diets fro laying hens. The Se occurs in the egg mostly in the form of selenomethionine. As well known, Se is a fundamental component of many bioactive Se-proteins important for wellbeing coming from various ingredients such as cereal grains, meat from poultry or fish (Hattingh et al., 2008). It was demonstrated that Se helps in: Arthritis reduction, cancer (Papp et al., 2007), cystic fibrosis, immunodeficiency, muscular dystrophy, diabetes (Surai, 2000); decreasing the risk of DNA damage associated with cancer; Se improve blood fluidity providing protective factor against cardiovascular diseases (Abdulah et al., 2006); Se may offer a valuable way through rising antioxidant protection (Venardos et al., 2007); Se enriched eggs can decrease the osteoporotic hip fracture risk (Zhang et al., 2006).

The effects of inclusion level of Se (as Se yeast or sodium selenite) in diet for hens, on egg performance, qualitative traits and yolk Se level was evaluated by Gjorgovska and Filev (2010). This trial was conducted using molted hens. The control group received a basal diet with Se from sodium selenite and the other groups with two different levels of Se from sodium selenite and Se yeast, respectively. From findings, the eggs produced were higher in hens fed Se yeast in diet. The egg qualitative traits were superior hens fed supplemental Se. The Se concentration in yolk was higher in laying hens fed with higher level of Se. Thus, it was concluded that Se level in diet may have an important effect on egg productive performance and some egg qualitative traits as well as on egg yolk Se concentration.

Another experimental trial conducted by Invernizzi et al. (2013) evaluated the bioavailability of Se yeast in diet for laying hens and the effects on productive traits, egg qualitative parameters as well as tissue Se evaluation. Laying hens (ISA Brown) were divided into three dietary groups as follow: A control group fed a basal diet containing 0.11 mg Se kg<sup>-1</sup>; a basal diet plus inorganic Se; and a group fed a basal diet containing organic Se (yeast). Hen's productive traits were not influences by Se inclusion, in spite of the Se source. Egg qualitative parameters were higher in hens fed diets containing Se compared to control treatment. Breast, liver and skin Se concentrations were higher in birds fed organic Se while kidney Se level was higher in hens fed inorganic Se. Eggs from Se yeast had more Se contents than inorganic Se. Metabolites in blood were not influenced by Se typology. A high Se concentration was reported in egg and breast from hens fed organic Se. As result, Se enriched eggs and muscles from Se yeast in laying hens diet enhanced the antioxidant status.

Many studies have addressed the possibility of antioxidants supplemented in diet of laying hens, as vitamin E and Se, to reduce cholesterol levels and to increase the amount of polyunsaturated fatty acids. A study conducted by Sujatha and Narahari (2011) using laying hens evaluated the effects of designer diets enriched with omega-3 and antioxidants from natural ingredients on egg yolk quality. The birds fed diets as follow: Control (without enrichment); a diet containing flaxseeds, vitamin E and spirulina; a diet containing fish oil, organic Se and spirulina and a diet containing flaxseed, fish oil, vitamin E, organic Se and spirulina. All three enriched diets improved volk carotenoids and omega-3 concentrations with reduction in SFA concentrations with significant changes in oleic acid in yolk fat. The three diets also decreased the yolk cholesterol. Thus, adding Se and vitamin E in diet synergistically increased omega-3 deposition in yolk.

Selenium and vitamin E are important elements of the antioxidant system to reduce lipid oxidation. In omega-3 eggs, omega-3 PUFA are susceptible to oxidative damage during cooking and storage. A research from the University of Alberta (Canada), focused on the stability of n-3 PUFAs enriched eggs fortified with vitamin E and/or organic selenium following cooking and storage (Ren et al., 2013). Both vitamin E and organic selenium offer protective effects by reducing oxidation in raw eggs, allowing a better  $\omega$ -3 enriched egg to be marketed. A mix of antioxidants was beneficial for stabilizing long chain  $\omega$ -3 PUFAs and is something for the industry to consider as they improve this product.

Carotenoid enriched eggs: Eggs fortification with carotenoid is the novel idea in the area of the designer eggs. The pigment enriched eggs looking attractive to people due to its gorgeous nature of more deep yellow coloration. Among carotenoids, canthaxanthin typically used for pigment production of enriched yolk by feeding hens with diets high levels of this pigment (Grashorn and Steinberg, 2002). However, there are quantities of other pigments that can be utilized for egg

fortification (Singh et al., 2012). These carotenoid pigments are naturally obtained from many natural sources (Nezhad et al., 2008). For example, blue green algae is the another resource of carotenoids which is essentially high in protein but also supplies specific pigments such as spirulina and it can be used for improvement of the carotenoids level in yolk (Singh et al., 2012). Usually carotenoids in poultry eggs are of hydroxyl molecules named xanthophylls (Moeller et al., 2000), while lutein and zeaxanthin are the two most frequent xanthophylls reported in egg-yolk. The positive effect of pigment in the egg-yolk (Kang et al., 2003; Singh et al., 2012) including: the prevention of macular degeneration, the improvement of the yolk colour; the pigment operates as antioxidant compound.

A study from Japan was conducted to investigate the effects of two dietary lutein sources, such as the commercial lutein and the emulsified crude extract of spinach containing lutein, on the lutein transfer into egg yolk as well as the antioxidant defense system in the liver of laying hens (Jang et al., 2014). White Leghorn hens were assigned to a basal diet (CON) and that supplemented with a commercial lutein (LUT, 40 mg lutein kg<sup>-1</sup> of diet) and the crude extract of spinach dissolved into oils with lecithin (ECE, 40 mg lutein kg<sup>-1</sup> of diet) for five weeks. From findings, there was no difference in body weight and relative live weight among dietary groups. The concentration of egg yolk lutein and color increased in the LUT and ECE groups compared with the CON group. The LUT group showed a higher yolk lutein and much a lower variability of average yolk lutein content. Moreover, the dietary supplementation with lutein and the emulsified crude extract of spinach to laying hens resulted in significant increase in the content of egg yolk lutein and yolk color, indicating that both supplements may potentially be applicable for the production of egg-enriched lutein in laying hens. Recently, Benakmoum et al. (2013) aimed to evaluate the extent of enrichment of egg yolk with carotenoids and phenols through feeding sun dried tomato peels. Tomatoes are usually associated to the Mediterranean diet, linked with low risk of coronary diseases. Lycopene represents the main carotenoid in tomatoes and their byproducts, considered as its significant dietary source in human diet (Shi and le Maguer, 2000). Lycopene (all trans form) is attached to tomatoes membranes and has no provitamin A properties, in fact the long conjugated double chain renders its antioxidative activity of importance to human health (Unlu et al., 2007). However, heating and cooking can transform the trans double bonds to cis, leading to a more absorbed form. Tomato byproducts and particularly peels, have attracted large interest in nutritional aspects of both human and animal since they are valuable sources of natural antioxidants mostly in form of carotenoids as well as phenols. In a study conducted by Benakmoum et al. (2013), Dried Tomato Peels (DTP) were tested in hens at levels of 0, 40, 70, 100 and 130 g  $kg^{-1}$  diet, for 26 days. Egg yolk from DTP-enriched diets, had lower amounts of cholesterol  $(14-17 \text{ mg g}^{-1})$  and triglyceride  $(188 \text{ mg g}^{-1})$  as with that of control group. Following DTP-enriched diets, total phenols was 2.0-3.6-fold higher,  $\beta$ -carotene 1.7-2.7-fold higher and lycopene increased between 26.5 and 42.8  $\mu g$  g<sup>-1</sup>, thus the optimal supplementation level resulted DTP at 7%.

The poultry eggs are considered a valuable source of nutrients, in particular protein, lipid, vitamins and minerals and many other bioactive compounds having antioxidant properties (Kovacs-Nolan et al., 2000; Nimalaratne et al., 2011). However, there are few available information on the consequence of cooking methods and digestion on antioxidant activities of egg. As a high-protein food product, liberation of many peptides in human gastrointestinal tract might further improve the antioxidant activities and consequently determine positive influences to health (Remanan and Wu, 2014). The objective of a recent study was to evaluate the influence of cooking methods and on antioxidant activities in egg (Remanan and Wu, 2014). The results suggested that fresh egg yolk has higher antioxidant activity than fresh egg white and whole eggs and compared to boiled or frying eggs. Thus, the Authors highlighted the importance of egg as dietary antioxidants.

An overview of the strategies for enriching the nutraceutical value of the eggs with emphasis on the use of antioxidants and natural substances including herbs for production of designer and functional eggs as well as their health-promoting effects highlighting the importance of eggs as potential food are presented in Fig. 1.

## HERBAL ENRICHED DESIGNER OR FUNCTIONAL EGGS

As previously mentioned, the egg is often referred to as one of the original and natural functional foods. Numerous studies have demonstrated that variations in rations fed laying hens can greatly impact the level of nutrients such as vitamins, minerals, omega-3 polyunsaturated fatty acids (PUFA) and lutein (Schiavone and Barroeta, 2011). Some studies indicate that organic eggs do not have much difference in this cholesterol content compared with conventional egg. Omega 3 fortified eggs by feeding oils have shown higher PUFA (Samman et al., 2009). Multiple nutritional enhancements of eggs can easily be achieved commercially (Bourre, 2005),

using the most natural way (feeding the hens) as the means to enrich eggs in nutrients. Egg is the most effective vehicle for different health-promoting incorporating molecules (Leeson and Caston, 2004). Herbs enriched egg can be produced by the incorporation of active compounds such as lutein, allicin, taurine, betaine, lumiflavin, eugenol and other molecules, according to the ingredients fed to poultry (Table 1). Furthermore, this egg had lower cholesterol in yolk (-25%) when compared to standard egg. Consuming these eggs by humans resulted a considerable decrease in blood triglycerides concentrations, increased in high density lipoproteins, enhanced immunological parameters and improved haematocrit. The bioactive compounds in these nutraceutical eggs and the role in both humans and animals wellbeing are summarized in Table 1. These type of herbal enriched designer eggs were build up by Narahari et al. (2004) in India. Recently, Raj et al. (2013) investigated the value addition of eggs with use of natural supplements in poultry feed such as garlic, fenugreek and bay to validate the reduction in cholesterol in egg-yolk. It was found that supplementing of diet with garlic resulted in high egg-yolk hypolipidemic activities. Conversely, supplementing diet with fenugreek and bay determined a production of eggs with significant content of vitamin E and Se (Raj et al., 2013). Thus, the tested herb supplements produced enriched eggs in omega-3 PUFA having positive effects to wellbeing. Consequently, these studies demonstrated that egg having enriched nutritional characteristics may be produced thanks to changes in laying hens' diet. Similarly, another study was conducted in white leghorn layers by feeding with holy basil, pearl millet, flaxseed and sardines. Oils from all these herbal leaves were fed to different group of chicken and their effect on various parameters in egg production was noted (Kirubakaran et al., 2011). At the end of the trail it was found that chicken fed with flaxseed and sardine oils produced eggs with higher level of polyunsaturated fatty acids in their yolk. This increase in polyunsaturated fatty acid is attributed to oils that are fed to them as they are usually rich in PUFA. Flaxseed and holy basil feeding increased α-linolenic acid in the egg yolk (Kirubakaran et al., 2011). Supplementation of thyme has been found to improve the antioxidant property of egg yolk (Gopi et al., 2014).

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Herbs*	Active ingredient in herbs	Benefits in relation to human health
Turmeric powder	Flavonoids compounds	Antimicrobial, antioxidant
Garlic, onion and their leaves	Allicin, Allyl sulfide	Reduce LDL cholesterol, anticarcinogenic properties
Sugar beer, grape pulp	Betaine	Decrease plasma homocysteine which ruptures arterial walls
Alfalfa, marigold petals, red pepper, spirulina	Carotenoid pigments	Antioxidant, anticarcinogenic
Basil leaves	Eugenic acid, Eugenol	Immunomodulatory properties
Marigold petals, bay	Lutein	Antioxidants, improves vision
Tomato pomace, grape pulp	Lycopene	Decrease LDL cholesterol, antioxidant, anticarcinogenic
Citrus pulp	Nirangenin	Reduce LDL cholesterol
Flaxseed, canola, fish, oils, insects, worms	n-3 PUFA	Decrease LDL, hypertension, angina, atherosclerosis
Seeds, legumes, weeds, fenugreek	Phytosterols	Increase HDL, decrease blood sugar
Fenugreek, spices	Quercitin, Lutein, Citogenin	Induce insulin secretion, antimicrobial and tonic activity
Brewery waste, yeast, fermented products	Statin	Reduce LDL cholesterol
Broccoli, cauliflower, cabbage, radish leaves, waste	Sulphoraphane	Anticarcinogenic and antioxidant properties
Bran	Tocotrienols	Decrease LDL cholesterol
Milk, eggs, meat products	Taurine	Prevent atherosclerotic

<sup>\*</sup>Adapted from Narahari et al. (2004) and Singh et al. (2012)

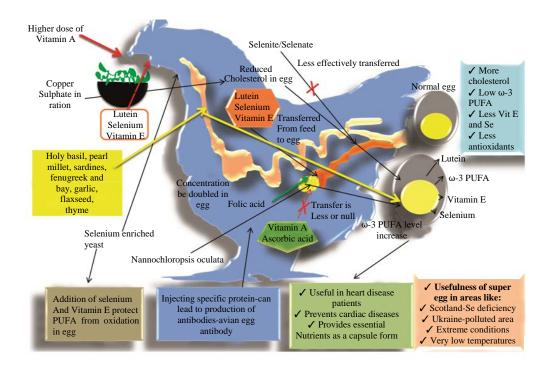


Fig. 1: An overview of enriched nutraceutical eggs and their health-promoting effects

#### CONCLUSIONS

The global egg production over the past decade has grown at fast pace in parallel with the demand for more eggs and animal proteins. People recognized more and more the health positive effects given by the level and value of proteins and other nutrients in eggs. The concern about cholesterol in spite of the lack of scientific validity continues to restrict consumption of eggs for some consumers. Several countries that are in developing stage and face crisis for supplying good nutritious food for the humans need some functional foods. Fortified or designed eggs with various nutrients can serve the purpose to support their such needs. Much work has to be carried out in order to help the people in need of this useful technology. Specialty eggs and egg products should develop more and more share of the global market as these products address some specific needs expressed by consumers. Experience and evidence showed that it is possible to create functional, enriched eggs to help correct dietary deficiencies. For example, one selenium-enriched egg can deliver approximately 50% of the recommended daily allowance and omega-3 eggs can deliver DHA and EPA in place of oily fish. Enriched eggs offer a way to increase the levels of health-promoting micronutrients, without significantly changing eating habits. Consumers buy, cook and eat eggs in the same way, just simply having to choose an enriched brand. Eggs can be marketed well as a functional food as they are increasingly seen to be part of a healthy diet. Consumer awareness of the health benefits of DHA and selenium, in particular, means that they are ideal candidates for enriching

eggs. Working with several micronutrients may increase their marketability, as well as providing effective tools for brand promotion. By giving injections of protein of interest it is possible to design eggs with protective antibodies (avian egg antibodies) produced against a particular pathogen. This technology of designer eggs can be used with greater potential once it is studied well.

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