Influence of Natural Pigments on Egg Quality and Yolk Colour Intensity - A Review

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Abstract
Eggs are a great source of animal protein that are rich in fatty acids, selenium, B vitamins, provitamin A, amino acids, and folic acid. Egg quality and yolk colour are important attributes for consumers. They associate a higher intensity colour of yolk with a healthy egg which its rich in nutrients. Because hens are unable to synthesise pigments, they must come from feed mixtures. Furthermore, the synthetic colourants are added to laying hens diets to improve the yolk colour intensity. However, human health received more attention in the last time, so, the consumers are attracted by natural-coloured eggs. Carotenoids are the most numerous and widespread group of pigments in nature. They are a class of widely distributed pigments that include red, yellow, and orange colour. Numerous studies have examined the use of carotenoids, particularly yellow carotenoids, in animal feed, which aid to make egg yolks more orange in colour. They are found in different plants, microalgae, bacteria, fungi and have various biological functions, including increasing production, antioxidant, anti-inflammatory, antibacterial and immunomodulator role. For practical purposes, the cost of carotenoids from natural sources needs to be considered. The objective of this review was to summarise the recent findings in available literature data on the supplementation with natural pigments based on marigold and calendula flowers and paprika extracts for egg quality and yolk colour pigmentation which are preferred over pigments of synthesis, due to their costs and biodisponibility.

Keywords: carotenoids, laying hens, marigold flowers, paprika powder extract, yolk colour.

1. Introduction

In recent years, the poultry production has grown in popularity. Eggs are an excellent source of animal protein (220-240 mg) [1], rich in fatty acids, selenium, B vitamins, provitamin A, amino acids, and folic acid [2]. These are a low-calories source (about 150 kcal/100 g) of high-quality proteins, providing 18 vitamins and minerals [3; 4]. The colour of food is a frequently observed sensory attribute that may not always correlate with the nutritional content of the food.

Frequently, consumers associate a vibrant yellow or golden-orange hue with a healthy egg suitable for various culinary uses when judging solely based on yolk colour intensity. Therefore, the preferences of European nations about the intensity of yolk colour may be different [5]. More yellow yolks are preferred in the north (units 9 – 11 of 'Roche yolk colour fan' – RYCF), while more intensely coloured eggs are chosen (units 12–14 RYCF) in the south. Egg yolk pigmentation is a result of carotenoids that are consumed by laying hens along with their nutrition. Carotenoids cannot be synthesized by hens [6]. Typically, only carotenoids that are soluble in lipids can be transported into the yolk [7]. The quantity of each carotenoid and the relationship between yellow

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and red dietary carotenoids have a direct impact on the yolk colour. Plant-based extracts are used to make natural pigments for poultry industry [5]. Several natural pigment extracts that we will discuss in greater detail include: tomatoes, marigold flowers, spirulina, chlorella, and red pepper, but there are others such as: carrots, red beets, red corn, lucerne, etc. These feed ingredients contain mainly lutein, zeaxanthin, β-carotene, and lycopene. The highest amount of a single carotenoid supplementation is 80 mg/kg diet, and the overall amount of dietary carotenoids cannot be more than 80 mg/kg diet. Lower supplementation levels for canthaxanthin were established in 2003 (Commission Directive 2003/7/EC) as a result of the discovery of colour crystal deposition in the retinas of both people and experimental animals [8]. The mixture of yellow and red carotenoids as well as the individual carotenoid supplementation amounts are essential for achieving a specific colour tone in egg yolks [9]. In essence, the global increased demand of eggs, due to their rich nutritional content determine a preference for increasing yolk colour intensity, using natural sources in a sustainable way, and achieving the desired yolk colour involves by a careful natural pigment's supplementation and diet balance.

A literature search was conducted in several scientific databases (Scopus, PubMed, ScienceDirect, Web of Science, and Directory of Open Access Journals) and 81 bibliographic references, using the keywords "laying hens", "marigold flowers", „paprika powder extract", „carotenoids", „yolk colour". The literature search had no publication year limit to identify all studies involving natural pigments administration to laying hens.

2. Consumer Perception About Pigments

When it comes to the appearance of food, colour plays a very important role. Also, the colour of the food is an aspect related to the quality of the food. Sensory analysis is the most used instrument for evaluation. Regarding the sensory aspects, studies conducted in several European nations over the past ten years have shown that consumers place a high value on the egg's physical qualities, particularly its yolk colour, albumen consistency, and shell firmness [10]. While consumers' perceptions of egg yolk colour are typically influenced by factors such as geography, culture, and customs, it is true that most consumers worldwide prefer yolks with rich colours [11].

In the production of table eggs, the coloration of the yolk holds significance for meeting consumer demand.

Consumers usually tend to choose the eggs with yolk colour from dark yellow to orange [12]. The composition and distribution of pigmenting carotenoids in laying hens' diet greatly impacts the colour of their yolks, which is easily adjustable by dietary feed additives manipulation [10]. Egg colour depends on the presence of carotenoids in the feed, as hens are not able to synthesize pigments [13]. The synthesis of pigments in the egg yolk begins with the processes of digestion and metabolism, which are similar to that of cholesterol in poultry. After they are consumed, they are digested in the form of fat droplets, which are emulsified by bile salts, and transformed into micelles [14]. They are transported through the lipoproteins in the cell membrane and accumulate in the fat-rich tissues, then are deposited in the egg yolk [15; 16; 17], interfering with its composition [7]. As the consumption of food rich in carotenoids increases, there is a proportional rise in their deposition in the yolk, resulting in an intensified coloration. This can reach a saturation point, where further dietary pigment addition no longer has an effect [18]. Carotene and xanthophylls (lutein, cryptoxanthin, and zeaxanthin) represent the majority of egg carotenoids, which make up less than 1% of yolk lipids [19; 20; 21]. The variable content of the two carotenoids (lutein and zeaxanthin) in non-enriched eggs has been recently reported, ranging from about 167–216 μg/yolk for lutein and about 85–185 μg/yolk for zeaxanthin [20; 22]. These carotenoids, together with their isomer mesozaexanthin, are referred to as macular pigments and are most well-known for their role in the neural retina, where they are present in great concentration [23]. Because lutein and zeaxanthin have been demonstrated to have antioxidant, anti-inflammatory, and light-absorbing and blue-filtering optical properties, it has been suggested that they may help prevent immune-mediated macular degeneration and the development of age-related cataracts [21; 22; 23].

Eggs are a functional food that should be included in patient diet plans due to their high nutritional value. As a purine-free source of protein, they are especially helpful for feeding people who have gout. Furthermore, egg proteins may have a significant impact on performance during training for athletes since they may improve skeletal muscle synthesis when consumed in the diet.

Over the last two decades, there has been a change of public opinion against the use of synthetic pigments to enhance the colour of egg yolks. Several countries have even prohibited the use of synthetic pigments in laying hens' diets [24]. For consumers, egg quality and yolk colour are the most important characteristics [25]. They associate a more intense yolk colour with a healthy, nutrient-rich egg. There are two types of pigments namely: natural pigments and synthetic (artificial) pigments. Increasing yolk colour will be advantageous to both public health and the poultry industry [26; 27]. It should be noted that there are no restrictions on consumption related to religion for eggs, and they can be consumed anywhere in the world [28].

3. Natural Sources of Pigments

Eggs enriched with natural pigments are desirable in the human food chain due to their numerous health benefits [24]. Two closely related xanthophyll carotenoids, lutein, and zeaxanthin, have particular health benefits for humans, one of them being the prevention of macular degeneration [29; 30], which protects against cardiovascular disease [31; 32], oxidative stress [33], neurodegenerative disorders [34; 35], and cancer [36].

Carotenoids are the most numerous and widespread group of pigments in nature. They are a class of widely distributed pigments that include red, yellow, and orange. Numerous studies have examined the use of carotenoids, especially yellow carotenoids, in animal feed, which help produce more orange egg yolks. They are found in various plants, microalgae, bacteria, fungi and have various biological functions, including production enhancement, antioxidant, anti-inflammatory, antibacterial and immunomodulatory roles. For practical purposes, the cost of carotenoids from natural sources must be considered.

- **Tomato powder extract** it obtained by rejected tomatoes for consumption as a result of sorting, tomatoes that are discarded by farmers when overproduction, and tomatoes that are not harvested at the seventh and eighth harvests. The tomatoes are still in good condition and nutritional value. Tomato powder extract is a low-cost byproduct of tomato manufacturing. In many countries, the disposal of this by-product has become an environmental issue [37; 38]. Dried tomato pomace (DTP) has the following composition: 10% moisture, 20.77% crude protein, 1760 Kcal/kg ME, 7.3% ether extract (EE), 39.8% crude fibre (CF), 4.24% ash, 0.5% calcium, and 0.45% phosphorus [39]. Due to its high fiber content, DTP is used as an alternative to synthetic supplements [40], but the low energy and high fibre contents of dried tomatoes make them a limiting factor in poultry diets. However, the extract has impressive levels of lutein, β-carotene, lycopene, and α-tocopherol, which are utilized to mitigate the impacts of oxidative responses in poultry diets. Additionally, research has demonstrated that tomato by-products added to feed work as a valuable source of protein, equivalent to soybean meal for poultry nutrition [41]. Apart from their nutritional value, bioactive chemicals are crucial. For instance, one of the bioactive components of tomatoes, lycopene, has been shown to help prevent cancer, heart disease, gingivitis, osteoporosis, mental illnesses, asthma, and sunburn caused by UV rays in people [42].

Therefore, more detailed studies are needed regarding the antioxidant potential of tomato on oxidative processes in chickens.

- **Marigold (*Tagetes erecta L.*)** is an ornamental plant with yellow and orange flowers that contains 1079.50 $\mu g/g^{-1}$ carotenoids, 232.34 $\mu g/g^{-1}$ β-carotene and 652.34 $\mu g/g^{-1}$ xanthophyll [43]; it has anti-cancer and antioxidant properties. As a result of antioxidant activity, carotenoids have been demonstrated to improve the colour of the yolk and lower cholesterol in eggs. Despite the fact that eggs are an inexpensive and comprehensive source of animal protein, their high cholesterol content is generally seen negatively, particularly by those who are affected by hypercholesterolemia. Therefore, diets rich in carotenoids, especially β-carotene, have been promoted in an attempt to lower the cholesterol in eggs. Marigold flowers extract it’s an abundant source of carotenoid pigments that are helpful in eye health. Marigold flower extract is a rich source of carotenoids, mainly lutein (80-90%) and zeaxanthin and a natural pigment used often for poultry nutrition. The flowers of annual herb marigold (*Calendula officinalis L*., Asteraceae family) possess many active substances, such as saponins, flavonoids [44], polyphenols [45; 46], carotenoids – 4200 mg/kg [47; 48], which have antioxidant effect and are essential for the immune system [49]. Marigold flowers have been incorporated into hens' diet as a natural supply of pigments to improve the colour of their yolks [50].

• ***Spirulina Platensis*** is a naturally occurring blue-green spiral filamentous alga that has a high nutritional content and potential for increased antioxidant activity. Its use enhances egg production, yolk redness, and production efficiency. It also has good amino acid profiles and excellent digestibility. Spirulina is acknowledged as a low-impact, sustainable source of protein, while its exact environmental impact varies greatly based on factors like climate and production technique. Due to its unusually high protein content (50–70%) by dry weight is regarded as a valuable source of important amino acids. Furthermore, it was discovered that spirulina contains a variety of essential components, such as carotenoids, vitamins, minerals, and important fatty acids and polysaccharides [51; 52].

• Algae are polyphyletic, mostly aquatic organisms. This has attracted the attention of nutritionists due to their high content of carotenoids and unsaturated fatty acids. ***Chlorella vulgaris***, a unicellular freshwater alga, was grown heterotrophically. It's a naturally occurring single-celled green microalga that is being studied as a potential substitute for some of the soybean meal found in hens' diets [53]. Moreover, it decreases the levels of total cholesterol and triglycerides in serum and liver and encourages the growth of bacteria in the intestines that produce lactic acid [54]. According to earlier research, chlorella improves the quantity and quality of eggs by transferring carotenoid pigments (β-carotene and canthaxanthin), which intensifies the yolk's colour [55].

• **Red pepper (*Capsicum annuum L.*)** comes from fruits in the Capsicum family and are one of the most widely used foodstuffs in the world cuisine, traditional medicine, were assessed for their antioxidant potential. They have medicinal, colouring, flavouring, and preservation functions in addition to their use in food industry. It contains high levels of potassium, folic acid, vitamin C, and A. The carotenoid pigments that give red peppers their colour are capsanthin, capsorubin, and capsanthin 5,6-epoxid [56]. Because of these pigments, dried pepper powder is added to hens' feed to enhance the egg yolk's yellow shade [57; 58; 59]. Regarding consumer purchasing behaviour, egg yolk colour is a factor that is very important in the market [60, 61]. On the other hand, other reports suggest that peppers may have an impact on laying hens' capacity for production performance positively or negatively [62]. Despite the widespread knowledge of red pepper's antioxidant qualities, it's interesting to note that there are surprisingly few published studies on the impact of red pepper supplementation on birds' oxidative status [63].

4. The Applicability of Natural Pigments in The Feed of Laying Hens

In poultry farming, synthetic xanthophylls are used as feed supplements to achieve optimal colouring of egg yolk. Colour is an important characteristic and selection criterion for consumer food choice. Yolk colour intensity can be controlled by the type and concentration of dietary carotenoids: canthaxanthin, which is preferentially red, and β-apo-8'-carotenoic acid ethyl ester, which is preferentially yellow. Both carotenoids are commercially available. Canthaxanthin is available as Carophyll® Red (DSM Nutritional Products, Basel, Switzerland) and β-apo-8´-carotenoic acid ethyl ester as Carophyll®Yellow from the same company. The corresponding xanthophylls produced by BASF (Ludwigshafen, Germany) are Lucantin® Red and Lucantin® Yellow. In humans, some of the ingested canthaxanthin is absorbed (9 to 34%) and its elimination is characterized by a long half-life of about 5 days. The acute oral toxicity of canthaxanthin is very low. However, several animal studies indicated that daily oral administration of canthaxanthin was associated with crystalline deposits in the retina [64]. Based on this finding, canthaxanthin can be added at a maximum of 8 mg/kg and β-apo-8'-carotenoic acid ethyl ester at 80 mg/kg.

Nowadays, consumers have become more concerned about the use of synthetic additives in food and feed, so interest in natural alternatives has increased. Due to the ability to prevent diseases related to nutrition and the physical and mental well-being of consumers, eggs are included in the category of functional foods, especially when the diet of the laying hens has been enriched with natural extracts. Suitable alternatives to synthetic carotenoids are tomato powder, marigold flowers extract, red pepper extract, algae, and many others. Some of the natural sources are described in the table below:

Table 1. Natural sources of carotenoids in laying hens' diet

Country	Inclusion rate	Number of birds/ Hybrid	Age	Results	References
• **Tomato powder extract**					
Italy	15%	576/ Hy-Line & White Leghorn	57, respectively 36 weeks old	Feeding of 15% dried tomato pomace (DTP) improve significantly ($p<0.05$) egg traits, in particular egg-yolk colour	[65];
China	0.1%	90/ Hy-Line Variety White	30 weeks old	The 28-day treatment did not affect the egg performance. A significantly increase in yolk colour parameters and lycopene content was observed.	[66];
Turkey	5 or 10 g/kg	90/ Lohmann LSL	49 weeks old	Positive effects of the addition of tomato extract in the feed of laying hens were observed by increasing feed intake ($p<0.01$), egg production ($p<0.001$), egg weight ($p<0.01$), yolk weight ($p<0.01$) and yolk colour ($p<0.0001$). The dietary treatments did not affect shell weight, shell thickness or Haugh units. Tomato powder increased serum and yolk lycopene, β-carotene, lutein, and vitamin A in the experimental groups.	[67];
Iran	150; 170 and 190 g/kg	144/ Lohmann Brown LSL-LITE	65 weeks old	Significant effects ($p<0.01$) on egg yolk colour, especially at the inclusion level of 19%, without negative effects on the egg quality. Tomato by-products contain high amounts of fiber. Inclusion rate of 19% dried tomato pomace, its a good suited substitute for feedstuff in laying hen rations without any negative effect on the performance of laying hens, compared with hens fed the corn-soybean meal control diet.	[68];
• **Marigold powder extract**					
Brazil	2.10; 2.40; 2.70; 3.00 ppm	288/Hisex lineage laying hens	75 weeks old	The percentage of albumen showed a linear reduction when the extract of the marigold flower was included. The levels of marigold and canthaxanthin flower extract did not influence the pH of albumen and yolk during the experiment. The inclusion of marigold flower extract (2.60 ppm/kg) influenced the percentage of the yolk	[69];
Croatia	0.2 and 0.4%	300/Tetra SL	31 weeks old	Increased lutein concentration and colour intensity in yolks;	[70];
Czech Republic	150; 350; 550; 750; 950 mg · kg^{-1}	240/ Lohmann Brown	30 weeks old	Hen egg production and egg weight increased significantly in the experimental groups. Lutein and zeaxanthin content, yolk colour parameters and yolk lipids of oxidative stability were increased. The most recommended dose of marigold flower extract would be 550 mg per kg of diet. At this dose, egg yolks contained lutein and zeaxanthin at 30.3 and 18.9 mg/kg dry matter,	[71];

Country	Inclusion rate	Number of birds/ Hybrid	Age	Results	References
				respectively, which increased basal lutein and zeaxanthin concentrations by 45.3% and 119.6%, respectively.	
Turkey	0.1 and 0.2%	60/ Hyline-5	80 weeks old	Inclusion of marigold flower extract in the diet of laying hens did not affect final body weight, feed consumption, or egg production, compared to the control group. Inclusion of marigold in the diet had no effect on any egg parameters, except shell strength and yolk colour of the eggs. Shell strength of the eggs from hens fed 10 g kg−1 marigold flour is significantly greater than the control (*p*<0.05) and 20 g kg−1 marigold supplementation to diet showed a yolk colour score of 10.77, which was higher than the control score of 9.77 (*p*<0.05).	[72];
Japan	10-50 mg/kg	90/Boris Brown	25 weeks old	Lutein at 40 mg/kg diet provided as marigold flower extract increased yolk redness, but did not influence performance parameters	[73];
• ***Spirulina platensis* extract**					
Romania	2%	120/ Lohmann Brown	38 weeks old	The experimental group, showed a significantly higher percentage	[74];
Italy	1% and 2%	648/ Hy-Line	63 weeks old	Using Spirulina extract in the diet of laying hens did not have effects on productive parameters, except yolk colour and physical traits (eggshell thickness and strength), who increased significantly at level 2%.	[75];
Tunisia	1.5 and 2.5%	45/ Lohmann White	44 weeks old	The a* parameter (redness) of the egg yolk increased from 1.33 (control group) to 12.67 (1.5% spirulina) and 16.19 (2.5% spirulina) The b* parameter decreased in the experimental groups, while the control group recorded the highest value The inclusion of 2.5% spirulina in the diet significantly increased egg weight No significant effects were observed on other productive parameters;	[4];
Iran	1.5%; 2% and 2.5%	160/ Hy-line W36	63 weeks old	Spirulina platensis was as effective as the synthetic pigments Lucantin® Red and Lucantin® Yellow in producing a pleasing colour of the egg yolk.	[76];
• ***Chlorella vulgaris* extract**					
Korea	5%	144/ Hy-Line Brown	21 weeks old	The use of Chlorella extract has been successfully used in the feed of laying hens, without affecting laying performance and jejunal histopathology, but with improved results on the colour of the yolk	[77];
Romania	2%	120/ Lohmann Brown	38 weeks old	Concerning the initial or final body weight, there was no significant differences at experimental group vs control. Significant differences were observed by improving egg weight, size, yolk intensity, B-carotene content and antioxidant capacity. In addition, the content of omega3 fatty acids has increased significantly;	[74];
Iran	1-5%	378/ Lohmann	40 weeks old	At high levels of inclusion, Chlorella extract had	[78];

Country	Inclusion rate	Number of birds/ Hybrid	Age	Results	References
		LSL-Lite laying hens		positive effects on the serum contents of triglycerides, cholesterol, LDL, and the serum content of HDL. There are evidences showing a significant increase in lipid profile of birds reared under heat stress condition.	
Czech Republic	12.5 g/kg of spray-dried alga *Chlorella* sp.	240/ ISA Brown laying hens	25-39 weeks old	Chlorella significantly increased yolk colour compared with the control diet. Additionally, supplementation of feed with lutein and Chlorella significantly increased the concentration of lutein (from 12.8 to 133.9 and 49.0 mg/kg dry matter, respectively) and zeaxanthin (from 9.2 to 123.9 and 40.1 mg/kg dry matter, respectively) in the yolks.	[79];
• **Red pepper extract**					
Turkey	4%	90/ Lohmann Brown	30 weeks old	Egg quality parameters were not statistically affected, except for the yolk colour, which was significantly increased compared to Control group. Red pepper powder had a significant impact on pro- and anti-inflammatory processes. In terms of egg performance parameters and the palatability of the supplemented diet, did not reduce feed consumption for hens.	[80];
Turkey	0.5%; 1%; 1.5%	160/ Super Nick Chick	78 weeks old	The effects of supplementation with different levels of inclusion, brought benefits to egg production, egg mass and also feed conversion rate. Regarding the colour of the yolk, it increased with the rate of inclusion of red pepper extract in the diet.	[62];
Japan	0.5%	32/ Boris Brown	39 weeks old	Neither performance nor other egg quality parameters were influenced. Red pepper stimulated intestinal villi (villus height, villus area, cell area, and cell mitosis in all intestinal segments). Compared to the control group, Roche yolk colour values were increased in all treated hens.	[81];

5. Conclusions

The primary sensory characteristic of yolk colour is its intensity, which is subjectively perceived by the consumer.
The search for natural, healthier substitutes for synthetic pigments and medicinal treatments in chicken farms is becoming more and more popular worldwide. Many natural sources with high potential for animal feed additives have already been identified and studied, some of these being highlighted in this article. For this reason, finding widely available substitute feed additives is crucial to safeguarding the poultry industry, especially in developing nations. These natural pigments can potentially be used in layer farms as natural growth and egg production promoters and aid in the sustainability of the poultry.
Based on available data and previous investigations, it can be concluded that all these natural sources of pigments can be widely used in laying hens' nutrition.

Acknowledgements

This research work was carried out with the support of Ministry of Research, Innovation and Digitization, and was financed from Project PN 23-20.01.01.

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