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SUPER EGGS PRODUCTION – THE INFLUENCE OF FEED MODIFICATION ON DESIGNER EGG COMPOSITION – A REVIEW*

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Abstract

Chicken eggs are considered a superfood due to their rich nutritional value, including amino acids, essential fats, vitamins, and minerals, while also being affordable and versatile in cooking. Designer eggs, enhanced further through nutrient improvements, become "super eggs." Recent studies highlight feed modification, which is more complex than feed supplementation, as the most effective method for enriching eggs; apart from supplementation also includes the use of blends of seeds and grains, post-extraction meals, natural extracts, and oils. This review explores trends in modifying egg nutrient content by adjusting the chicken's diet to improve fatty acids, vitamins, proteins, minerals, antioxidants, and pigments important for human health. It is challenging to achieve a sufficiently significant level of modification and produce eggs that can be marked as a source of a given nutrient (polyunsaturated fatty acids, vitamins) or as having a reduced content of undesirable ingredients (such as cholesterol). Important aspects of egg modulation include feed costs, consumer acceptance of the product, and sustainability. Although there has been extensive research into egg fortification over the years, most studies have focused on single nutrients, and there is limited research on the bioavailability of nutrients from these designer eggs.

Key words: designer eggs, eggs composition, hen diet, nutrient content modification, nutrition value

Eggs are widely regarded as one of the most perfect food products. Their consumption is notably lower in countries where access to animal products is limited, such as many African nations, or where dietary restrictions exist due to religious beliefs, as seen in India. In contrast, egg consumption is highest in Mexico (Réhault-Godbert et al., 2019). The nutritional value of eggs primarily comes from the content of valuable proteins, as well as from fat-soluble vitamins and B vitamins, some trace elements, and fatty acids found in the yolk. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recognize hen egg proteins as the international standard (FAO, 2013; Leser, 2013). The quality of eggs is influenced by various factors, including genetics, the physiology of the hens, environmental conditions, and the quality of their feed (Wang et al., 2017; Goto et al., 2021; Hejdysz et al., 2024).

A typical hen egg consists of 58–63% of albumen, 27.5–31% of yolk, and 9.5–11% of shell, and membranes. At the molecular level, the main components of the egg as a whole are water (65.5–75%), proteins (11.8–12%), lipids (11–12%), and minerals, which are most concentrated in the shell (Lesnierowski and Stangierski, 2018; Réhault-Godbert et al., 2019).

The composition of egg white (albumen) and yolk varies significantly. Egg white consists mainly of water and is almost fat-free. In turn, the yolk consists of triglycerides (65%), phospholipids (28–30%), and cholesterol (4–5%), which constitute as much as 1/3 of the yolk's mass. The content of protein is higher in egg yolk, which is also about twice as rich in minerals, mainly potassium, sodium, calcium, magnesium, iron, zinc, selenium, and iodine (Lesnierowski and Stangierski, 2017; Réhault-Godbert et al., 2019). The chemical composition, detailing the components of egg white and yolk, is presented in Table 1 (Lesnierowski and Stangierski, 2018; Réhault-Godbert et al., 2019). Moreover, eggs have important economic value and due to their low cost, they are an important product for the consumer. Currently, eggs are also considered to be a source of interesting bioactive substances, acting as anti-bacterial, anti-carcinogenic, and immunostimulatory agents, including IgY, lysozyme, lecithin, avidin, and bioactive peptides (Lesnierowski and Stangierski, 2017; Zhang et al., 2021).

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Table 1. Hen egg composition (Lesnierowski and Stangierski, 2017; Réhault-Godbert et al., 2019)

	Egg white (%)	Egg yolk (%)
Water	~87	48–50
Proteins	~11	16–17.5
Lipids	0.02	32–34
Carbohydrates	~1	1
Minerals	~1	1–2

This review aims to collect, analyze, and evaluate scientific studies on feed modification to change the nutritional composition of eggs. Specifically, the review covers research on improving the content of fatty acids, proteins, vitamins, minerals, and antioxidants, pigments, as well as reducing unfavorable components such as cholesterol.

Methods

The review is a result of systematic analyses and evaluation of 127 scientific publications regarding the influence of feed modification on egg nutrient composition. The selected literature included articles published in peer-reviewed journals. Main studies were conducted within the last 20 years unless they had a significant impact on the knowledge about egg composition. The literature was searched in databases such as PubMed, Scopus, Web of Science and Google Scholar, Taylor & Francis, Wiley, and MDPI, with an application of the following keywords: designer eggs, egg fortification, feed modification, functional eggs, eggs composition, layer hen feed modification, egg lipid modification, egg protein modification, egg antioxidant modification, egg pigment modification, modification of vitamins/minerals in eggs. Each theme was studied and discussed in detail, comparing the results of different studies.

Modification of lipid composition of eggs

Due to the lipid content in egg white (Table 1), its importance in this egg part is practically ignored. Even though albumen lipids are present in trace amounts, they constitute a complex mixture of triglyceride, diglyceride, free fatty acid, cholesterol ester and cholesterol in acetone-soluble materials, and phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), sphingomyelin (SPM) and unknown material with choline base in acetone-insoluble materials. Most of them are bound with proteins (Sato et al., 1973).

Lipids are the richest nutritional component of egg yolk (Table 1). 702 lipids have been identified in egg yolk, including triglycerides, phosphatidylcholines, phosphatidylethanolamines, lysophosphatidylcholines, diglycerides, and others (He et al., 2023). Currently, every conscious consumer knows that it is not only the total fat amount supplied by a given food product that is important but also the fat composition. Most studies on egg designing are focused on lipid (fatty acids) composition in yolk. The total content of fat in egg yolk cannot be changed (Singh et al., 2012), but it has been proven that the fatty acid profile of chicken eggs can be significantly influenced by hens' diet supplementation, even resulting in the production of fortified eggs classified as functional food in the prevention of cardiovascular diseases. The lipid content in the diet of laying hens generally ranges from 5% to 7.5% and to achieve the recommended level of at least 5%, feed is usually supplemented by the addition 2–3% of oil (usually rape, soybean or corn) or fat (Lohmann, 2020).

The most beneficial for human health is an increase in egg content of polyunsaturated fatty acids (PUFA), mainly ω-3 fatty acids. These acids are essential for the proper development of the nervous system and protect against the development of metabolic syndrome and cancer (Karageorgou et al., 2023). To obtain the goal, the diet of laying hens was supplemented with additives such as fish oil (rich in long-chain eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), many raw materials and by-products of oil industry such as linseed and linseed oil (rich in α-linolenic acid), rapeseed or its oil, algae oil and other PUFA sources (Basmacıoğlu et al., 2003; Kopacz et al., 2021; Kralik et al., 2021; Saleh et al., 2019).

This type of egg modification is approved by consumers and is industrially used. Some effects of these actions are presented in Table 2. However, the issues that need to be taken into account here are costs, sustainability, and product acceptance.

The addition of oil to the hen diet should be prudently controlled, because a small amount may not affect egg quality. At the same time, excessively high doses can negatively impact both egg quality (mainly flavor, and egg weight) and production performance. Beneficial for humans seems to be the addition of e.g. 5% rapeseed oil (increases the ω-3 fatty acids content of yolk, including DHA), 1.5–4.5% of sesame oil (increases the UFAs (unsaturated fatty acids) content) 1–3% of palm oil (reduces triglyceride content and increases MUFAs (monounsaturated fatty acids) content, linseed oil in doses lower than 5% (increases the oleic acid, linolenic acid, EPA, and DHA content), <1.5% of fish oil (increases deposition of DHA and EPA), and <2.5% of microalgae oil (increases the ratio of n-3/n-6 PUFAs) (Gao et al., 2021).

The modification of the fat composition of yolk strongly depends on the fat sources in the diet. The goal is to reduce the content of saturated fatty acids, increase the content of ω-3 fatty acids, and decrease the $\sum$ ω-6 PUFA/$\sum$ ω-3 PUFA ratio. An observed increase in $\sum$ ω-3 PUFA is related not only to the increasing content of ALA (alpha-linolenic acid) but also to both EPA and DHA (Table 2). This is the consequence of *in vivo* metabolism, with ALA serving as a precursor for the synthesis of EPA and DHA through the desaturation-chain elongation pathway within the liver (Calder, 2012). The conversion of ALA to EPA competitively inhibits the conversion of linoleic acid (C18:2n-6) to arachidonic acid (C20:4n-6) due to the exploitation of the same enzyme (Δ6-desaturase) to polyunsaturated 20-carbon derivatives (Jia et al., 2008) in the first step of conversions.

In the case of olive pulp application, however, even if the content of PUFA was increased, the content of acids from the group of ω-6 increased significantly and it should be explained by the high supply of ω-6 acids from this additional feed ingredient (Dedousi et al., 2022). Due to the confirmed effects of CLA (conjugated linoleic acid) which works as an anti-cancer agent and reduces metabolic syndrome compounds, synthetic CLA was used as a feed component. The enrichment was dose-dependent, with the maximum content (plateau) achieved after 5% supplementation of feed (Shang, 2004). Moreover, an increase in CLA content caused a simultaneous increase in saturated fatty acids content and a decrease in MUFAs and other PUFAs content (Qi et al., 2011).

It may be interesting to mention the application of some surprising agro-industrial by-products. For example, as a source of ω-3 PUFAs, non-fermented and fermented peony seed dregs were used as an alternative feed component. Peony seeds are a source of oil widely consumed in China as a functional food. The addition of peony seed dregs (5%) and fermented dregs into the feed doubled the content of ω-3 PUFA and reduced the ratio of ω-6, it reduced the amount of ω-3 fatty acids by about 30% (Wan et al., 2022). Sergin et al. (2022) conducted research which even included using animal by-products of the meat industry (beef suet and liver) for supplementing hen diets

However, the idea of sustainable development and using by-products as a component of the diet is not the only way of hen feed modification aimed at improving eggs' lipid composition. Different legumes (usually peas, beans, lupines, and peanuts) are introduced to the feed due to the planned restrictions on the use of genetically modified raw materials in animal nutrition. They replace soy as a source of protein, but at the same time, they deliver lipids to the hen diet. Application of lupine meal (Timová et al., 2020) or a mixture of lupine and pea (Kowalska et al., 2021) as a protein source in the feed has also changed the fatty acid profile of egg yolk (Table 2).

It is not easy to modify the yolk fatty acids composition to obtain the threshold of 300 mg/egg of ω-3 PUFA, i.e. the level required for eggs to be labeled as the ω-3 PUFA sources (Branch, 2023). Usually, this required the use of feed supplementation higher than 5% by flaxseed or fish oil (Wang et al., 2017). Such a big increase in the content of ω-3 sources in the feed may cause difficulties in balancing feed composition properly. Moreover, a decrease in the transfer efficiency of the desired ω-3 fatty acids from feed to egg yolk is observed after exceeding certain additive values (Nain et al., 2012), which may significantly influence the cost of the modified product. Additionally, supplementing feed with fish oil, which is the best dietary source of DHA, may cause undesirable off-flavors of enriched eggs (Gonzalez-Esquerra and Leeson, 2000). Many sources of lipids are introduced into feed but the usage of some materials, even rapeseed oil delivers more ω-6 than ω-3 fatty acids (Zhang et al., 2023). It should also be remembered that the fatty acids profile may vary during storage (depending on time and conditions), and this applies mainly to PUFA (Drabik et al., 2021). This profile depends also on the hen's age and time of supplementation. Moreover, the enrichment of the content of unsaturated fatty acids in eggs may decrease their shelf-life and sensory attractiveness (Vlaicu et al., 2022).

The nutritional quality of eggs may also be improved by reducing the content of undesirable lipid substances that are considered hazardous to human health. Information about high cholesterol content in eggs (on average 195–230 mg) (Ceylan et al., 2011) strongly reduced their consumption. Currently, it is suggested that dietary cholesterol does not increase the concentration of unfavorable cholesterol fractions in the blood (Sunwoo and Gujral, 2015), but ways to decrease the cholesterol content in eggs are still being sought. Reduction of cholesterol content in eggs is difficult and does not exceed ~10%, with the application of different nutrients, nonnutritive factors, natural products, and pharmacological agents (Elkin, 2006). Thus, the cholesterol content in eggs is still high and examples of feed modifications that were aimed at lowering cholesterol in eggs are presented in Table 3. There is no unambiguous data on the relationship between yolk/egg mass and cholesterol content in it – some authors indicate a positive, others a negative correlation or no effect. The influence of breed and age of the laying hen is clearly emphasized (Dikmen and Sahan, 2007; Calik, 2016; Yenilmez and Atay, 2023).

Maximizing egg protein benefits

In yolk, low-density and high-density lipoproteins are present (68% and 16%, respectively), livetins with other soluble protein (10%) and phosvitins (4%) (Réhault-Godbert et al., 2019). 80% of yolk proteins consist of apovitellenin-1, apolipoprotein B, vitellogenins, serum albumin and immunoglobulins, ovalbumin, ovotransferrin, and the protein form non-soluble protein aggregates and a clear yellow fluid or plasma.

In gel-like albumen, on the other hand, 150 separate proteins are recognized. Among them, fibrous structural proteins (ovomucins), glycoproteins (ovalbumin – which consists of 50% of white protein; protease inhibitors), antibacterial proteins (lysozyme), and peptides are present (Réhault-Godbert et al., 2019).

The protein level in feed for laying hens should range between 15 and 20% and it is not possible to provide this level only with cereal ingredients (Alagawany and Mahrose, 2014; Wasielewska, 2021); soybean and canola meal are the most common source of protein in the hen diet (Iji et al., 2017). There are few studies on possible modifications of the protein composition of chicken eggs, even if eggs are considered a source of model protein (Leser, 2013).

Table 2. Effect of diet modification on egg fat composition (compared to control diet)

Feed modification/ addition	Effect on the content of												
	Σ saturated FA	Σ omega–3	Σ omega–6	Σ omega–6 /omega–3 (effect=value)	EPA	DPA	DHA	Linoleic acid	Linolenic acid	MUFA	Unit	Duration of the experiment	Reference
1	2	3	4	5	6	7	8	9	10	11	12	13	14
OILS													
1.5% fish oil	–1.14	+2.99	–2.10	–11.81=3.83	+0.18	+0.12	+2.64	–0.98	+0.09	ND	% of total FA	28 days	Basmacıoğlu et al., 2003
4.32% flax seed	–2.23	+4.04	–0.07 (NS)	–9.14=3.50	+0.06	+0.10	+1.05	+0.80	+2.87	ND			
1.5% fish oil + 4.32% flax seed	–1.11	+5.96	–0.82	–10.15=2.49	+0.26	+0.14	+2.25	+0.52	+3.38	ND			
8.64% flax seed	–1.69	+6.82	–0.46	–10.37=2.27	+0.12	+0.15	+1.33	+0.43	+5.27	ND			
0.3% fish oil + 4.7% soybean oil	–809.57	+53.85	+99.14	–1.36=7.33	+10.27	ND	+45.75	+86.61	–2.18	+656.59	mg fatty acids/100 g eggs	35 days	Kralik et al., 2021
0.6% fish oil + 4.4% soybean oil	–710.72	+67.53	+23.61	–2.06=6.63	+11.17	ND	+68	+27.45	–11.64	+619.59			
0.9% fish oil + 4.1% soybean oil	–708.03	+89.9	+32.57	–2.53=6.16	+12.07	ND	+83.15	+33.62	–5.32	+657.58			
1.2% fish oil + 3.8% soybean oil	–1010.02	+114.83	–19.8	–3.11=5.58	+16.64	ND	+110.13	+2.56	–11.95	+915			
1.5% fish oil + 3.5% soybean oil	–491.97	+122.76	–292.69	–4.15=4.54	+20.10	ND	+131.43	–248.81	–28.78	+661.91			
0.25% OmegaPro algal oil	ND	+0.11	–0.15	–6.7=6.6	ND	ND	+0.11	ND	–0.01	ND	g/100 g egg	12 weeks	Maina et al., 2023
0.76% OmegaPro algal oil	ND	+0.19	–0.34	–8.86=4.44	ND	ND	+0.20	ND	–0.01	ND			
0.23% crude algal oil	ND	+0.11	–0.20	–6.15=7.15	ND	ND	+0.10	ND	–0.01	ND			
0.69% crude algal oil	ND	+0.21	–0.34	–9.03=4.27	ND	ND	+0.21	ND	–0.01	ND			
Regular menhaden oil 2%	ND	+81	ND	ND	+16	+7	+59	ND	0	ND	mg/50 g egg	9 periods of 28 days each	Gonzalez-Esquerra and Leeson, 2000
Regular menhaden oil 4%	ND	+135	ND	ND	+30	+8	+97	ND	+12	ND			
Regular menhaden oil 6%	ND	+193	ND	ND	+45	+27	+170	ND	+32	ND			
Deodorized menhaden oil 2%	ND	+78	ND	ND	+13	+8	+61	ND	–3	ND			
Deodorized menhaden oil 4%	ND	+154	ND	ND	+31	+14	+88	ND	+4	ND			
Deodorized menhaden oil 6%	ND	+290	ND	ND	+61	+20	+188	ND	+11	ND			

Table 2 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.5% krill oil	ND	+6.04	–3.53	–12=2.2	+0.69	ND	+5.53	–1.23	–0.18	ND	% fatty acid	31 days	Zao et al.,
1.5% fish oil	ND	+7.12	–3.63	–17.31=1.89	+0.82	ND	+6.46	–1.12	–0.16	ND			
3% krill oil	ND	+7.22	–3.4	17.3=1.9	+1.2	ND	+6.13	–0.75	–0.11	ND			
DHA-rich microalgal oil 10%	ND	+104.2	ND	ND	+1.96	+0.3	+101.5	–53	+1	ND	mg/yolk	28 days	Elkin et al., 2023
DHA-rich microalgal oil 20%	ND	+209.2	ND	ND	+7.36	+4.01	+193.5	–13	+5.2	ND			
DHA-rich microalgal oil 30%	ND	+233.2	ND	ND	+8.89	+4.87	+218.5	–45	+2.2	ND			
DHA-rich microalgal oil 40%	ND	+239	ND	ND	+11.36	+5.75	+223.5	–80	–0.1	ND			
DHA-rich microalgae oil 40%+ High-oleic sunflower oil 20%	ND	+248.2	ND	ND	+8.18	+3.32	+236.5	–22	+1.2	ND			
DHA-rich microalgal oil 40%+ High-oleic sunflower oil 40%	ND	+209.2	ND	ND	+6.63	+1.75	+204.5	–40	–2.1	ND			
1.5% fish oil + 1.5% rapeseed oil + 2.0% linseed oil, 0.47 mg/kg Se, 125.2 mg/kg vit. E and 120.5 mg/kg lutein	–169.42	+276.07	–723.14	–6.5=2.19	+26.04	ND	+83.89	–651.98	+166.14	+616.48	mg FA /100 g of eggs	5 weeks	Kralik et al., 2023
Linseed oil 1% Linseed oil 2% Linseed oil 3% Linseed oil 4%	+0.39 –0.06 +0.07 –0.85	+1.25 +2.38 +2.8 +4.07	–0.65 –1.03 –0.7 –0.32	–6.9=6.38 –8.99=4.29 –9.53=3.75 –10.22=3.06	+0.01 +0.01 +0.04 +0.07	+0.14 +0.23 +0.22 +0.26	+0.51 +0.75 +0.81 +0.98	–0.72 –0.75 –1.05 +0.04	+0.59 +1.38 +1.71 +2.72	–0.91 –1.28 –1.42 –2.82	g/100 g of yolk	13 weeks	Petrović et al., 2012
Linseed oil 2.5%	–1.77	+1.4	–0.12	–7.45=7.37	ND	+0.07	+0.33	+0.51	+1.01	+0.51	%	4 weeks	Batkowska et al., 2021
Soybean oil 2.5%	–1.11	+0.56	+2.52	–2.95=11.87	ND	+0.04	+0.16	+2.69	+0.36	–2.02			
3.00% fish oil	–0.63	+3.3	–2.6	–30.97=2.55	+0.18	+3.02	ND	ND	ND	–0.04	%	9 weeks	Omidi et al., 2015
3.00% olive oil	–0.03	+0.38	–0.64	–18.19=15.33	0	+0.18	ND	ND	ND	+0.29			
3.00% grape seed oil	+1.26	–0.06	+8.74	+35.09 =68.61	0	+0.01	ND	ND	ND	–9.94			
3.00% canola oil	–2.55	+0.45	+2.08	–16.07=17.45	0	–0.19	ND	ND	ND	+0.02			
3.00% soybean oil	–1.43	+0.19	+7.2	+1.42=34.94	0	–0.19	ND	ND	ND	–5.96			

Table 2 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Soluble flaxseed oil 0.2%	–0.3	+0.23	+0.2	–3.88=14.03	0	ND	+0.24	+0.19	–0.02	–0.18	% of FAME	4 weeks	Lee et al., 2021
Soluble flaxseed oil 0.4%	+0.16	+0.48	–0.2	–6.98=10.93	0	ND	+0.49	–0.21	–0.02	–0.47			
Soluble flaxseed oil 0.6%	–0.47	+0.6	–0.6	–8.22=9.69	0	ND	+0.61	–0.58	–0.02	+0.45			
Soluble flaxseed oil 0.8%	–0.22	+0.79	–0.18	–9.12=8.79	0	ND	+0.79	–0.18	–0.03	–0.45			
7.8% flaxseeds and 7% pomegranate seed oil compared to 10% extra virgin olive oil	+4.35	+11.73	+4.62	–9.15	ND	+0.94	+3.08	+8.03	+6.62	89.38	mg/g of yolk	26 weeks	Ngo Njembe et al., 2021
Meals													
50% soybean meal/50% white lupine meal	–1.401	+0.323	+1.849	–8.2=17.42	+0.002	+0.045	–0.003	+1.881	+0.279	–0.463	g FA/100 g of fat	2nd–5th month of laying	Timová et al., 2020
100% white lupine meal	–3.602	+0.699	+3.999	–12.19=13.43	+0.005	+0.05	–0.002	+4.38	+0.646	–1.856		6th–12th month of laying	
1.5% rosehip meal with 7% flaxseed meal	–1.775	+3.023	–1.309	–12.94=6.17	ND	+0.171	+1.764	+0.991	+1.072	–0.785	g/100 g FA	4 weeks	Vlaicu et al., 2022
3% rosehip meal with 7% flaxseed meal	–1.193	+3.217	–1.53	–13.3=5.81	ND	+0.204	+1.911	+0.372	+1.04	–1.015			
9% pumpkin seed meal	+0.03	+0.44	+2.12	–3.16=17.20	ND	+0.02	+0.28	+2.28	+0.12	–2.52	g/100 g total FA	6 weeks	Vlaicu and Panaite, 2022)
9% rapeseed meal + 3% grapeseed meal	–52.25	–0.39	+86.6	+56.86=528.25	ND	–0.257	+0.07	+76.52	–0.397	–130.1	mg fatty acids/yolk	42 days	Vlaicu et al., 2021
9% flaxseed meal and 3% sea buckthorn meal	–38.4	+94.127	–106.42	–386.138 =85.252		+3.845	+63.636	–55.32	+30.545	–9.12			
Seeds													
Hemp seed 10%	+0.237	+0.398	+7.83	+1.342=9.54	+0.018	ND	+0.008	+7.988	+0.34	–8.481	% FA	84 days	Taaifi et al., 2023
Hemp seed 20%	–0.269	+2.445	+8.92	–2.043=6.155	+0.044	ND	+0.051	+9.097	+2.312	–11.103			
Hemp seed 30%	+0.254	+4.038	+9.79	–3.314=4.884	+0.068	ND	+0.057	+9.862	+3.842	–14.095			
Camelina seed 10%	+0.44	+1.93	–0.28	–3.54=2.45	ND	+0.21	+0.59	+0.11	+1.12	–2.09	%	16 weeks	Cherian and Quezada, 2016
Flax seed 10%	–0.66	+1.9	+0.62	–3.19=2.8	ND	+0.13	+0.69	+1.08	+1.07	–1.86			

Table 2 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Black cumin seeds 20 g/kg	–1.13	–0.06	+6.88	+4.75=19.57	–0.028		–0.1	+5.73	+0.029	–5.89	%	15 weeks	Szczerbińska et al., 2021 a
7.5% LinPRO extruded flaxseeds	+0.085	+130.2	+49.9	–2.538=2.976	+3.67	+3.6	+36.2	+76.4	+86.6	+0.097	mg/egg	18 days	Nain et al., 2012
15% LinPRO extruded flaxseeds	–0.046	+209.1	+27.3	–3.261=2.253	+6.84	+4.57	+20.1	+82.1	+177.4	–0.155			
10% extruded flaxseed	–17.61	+3.25	–18.32	–8.51=2.11	+1	+0.83	+3.6	–8.98	+1.43	+19.63	mg/g yolk	23 weeks	Mattioli et al., 2023
Co-extruded full-fat flaxseed	ND	+34.3	+3.1	–12=2.1	ND	+1	+5	+5.7	+29.8	ND	mg/g of dried yolk	30 days	Thanabalan et al., 2020
Co-extruded full-fat flaxseed enzyme supplement	ND	+34.2	+2.5	–12=2.1	ND	+0.6	+4.4	+5.5	+29.8	ND			
10% flax seeds	ND	+205.3	ND	ND	ND	+5.6	+29.6	ND	+170.1	ND	mg/egg	35 days	Zhang et al., 2017
10% perilla seeds	ND	+215.7	ND	ND	ND	+6.5	+34	ND	+175.2	ND			
10% *E. ulmoides* seeds	ND	+194.7	ND	ND	ND	+4.5	+29.4	ND	+161.3	ND			
Extracts													
1 g marigold extract/kg of feed	+4.802	+0.093	–2.558	–0.37=5.8	–0.01	+0.103	–0.019	–2.161	+0.009	–2.337	mg FA/g yolk	5 weeks	Grčević et al., 2019
2 g marigold extract/kg of feed	+1.638	+1.267	–2.915	–1.13=5.04	–0.01	+0.214	+0.971	–3.224	+0.059	+0.011			
500 ppm marigold extract	ND	+45	–21	–0.4=2.7	ND	ND	ND	ND	ND	ND	mg/100 g yolk	30 days	Kavtarashvili et al., 2021
700 ppm marigold extract	ND	+65	+44	–0.4=2.7	ND	ND	ND	ND	ND	ND			
900 ppm marigold extract	ND	+69	+209	0=3.1	ND	ND	ND	ND	ND	ND			
Algae													
1.5% microalgae A	ND	+69.6	ND	ND	+10.8	+11.2	+44.7	ND	+2.9	ND	mg/egg	35 days	Zhang et al., 2017
10% flaxseeds +1.5% microalgal AF	ND	+311	ND	ND	+6	+6.2	+122.2	ND	+176.6	ND			
10% perilla seeds + 1.5% microalgal AP	ND	+328.4	ND	ND	+6.1	+7.3	+132.2	ND	+182.8	ND			
10% . *E. ulmoides* seeds + 1.5% microalgae	ND	+297.2	ND	ND	+6	+5.6	+117.8	ND	+167.8	ND			

Table 2 – contd.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
1% microalgae;	+0.18	+0.24	–0.09	–6.43=11.15	ND	ND	ND	ND	ND	+0.14	g/100 g dry yolk	28 days	Mens et al., 2022
2% microalgae	+0.25	+0.44	–0.43	–9.38=8.2	ND	ND	ND	ND	ND	–0.01			
3% microalgae	+0.22	+0.63	–0.47	–10.89=6.69	ND	ND	ND	ND	ND	+0.28			
By-products													
5% peony seed dreg	–1.83	+0.73	–0.2	–4.91=7.12	0	ND	+0.47	+0.09	+0.26	+1.75	mg/g egg yolk	8 weeks	Wan et al., 2022
5% fermented peony seed dreg	–1.5	+0.78	+4.01	–2.9=9.13	–0.01	ND	+0.43	+4.01	+0.36	–2.56			
7% fermented peony seed dreg	–1.77	+0.95	+4.77	–3.44=8.59	0	ND	+0.43	+4.93	+0.5	–3.53			
9% fermented peony seed dreg	–2.51	+1.26	+5.48	–4.27=7.76	0	ND	+0.6	+5.71	+0.93	–3.81			
20% raw rapeseed cake	–3.2	+1.0	+4.4	–3=8.52	ND	ND	+0.41	+4.30	+0.58	–2.2	% of total FA	12 weeks	Kopacz et al., 2021
20% hydrothermally-treated rapeseed cake	–4.1	+0.95	+4.9	–2.71=8.81	ND	ND	+0.25	+5.00	+0.71	–1.8			
20% fermented rapeseed cake	–4.4	+1.18	+5.2	–3.26=8.26	ND	ND	+0.50	+5.10	+0.68	–2			
Beef by-products and corn/soy free feed (pas-ture raised)	–1.67	+1.03	–15.12	–44.91=5.72	ND	0.33	+0.47	–14.68	+0.24	+14.92	% of total fatty acids	ND	Sergin et al., 2022
Corn/soy feed (pasture raised)	–1.14	+1.27	–5.73	–39.84=10.79	ND	0.19	+0.63	–5.57	+0.46	+5.5			
							Alternatives						
1% green tea powder	+0.7	ND	ND	ND	ND	ND	+0.28	ND	+0.79	+4.6	mg/g	12 weeks	Chen et al., 2021
6% linseed meal and 2% dried kapia pepper	–0.82	+1.78	–4.57	–3.53=4.26	ND	+0.12	+0.88	–3.36	+0.81	+3.57	g/100 g total FAME	4 weeks	Panaite et al., 2021
6% linseed meal and 2% dried sea buckthorn pomace	–1.58	+1.84	–5.49	–3.54=4.25	ND	+0.09	+0.91	–3.105	+0.83	+3.95			
6% linseed meal and 2% dried carrot	–1.17	+1.82	–3.84	–3.42=4.37	ND	+0.12	+0.94	–3.05	+0.72	+3.22			

Table 3. Influence of diet modification on egg cholesterol content

Diet modification/addition	Influence on cholesterol content	Unit	The duration of experiment	References
Oils				
1.5% fish oil	–1.15/–6.67	mg/g yolk / mg/egg	28 days	Basmacioğlu et al., 2003
4.32% flax seed	+0.22/+13.78			
1.5% fish oil + 4.32% flax seed	–0.2/+5.65			
8.64% flax seed	–0.92/–3.42			
Rubber seed oil 1% Rubber seed oil 2% Rubber seed oil 4% Rubber seed oil 6%	–1.25 –2.3 –1.27 –1.33	mg/g	8 weeks	Wen et al., 2019
Linseed oil 2.5% Soybean oil 2.5%	–0.75 +0.10	mg/g egg yolk	4 weeks	Batkowska et al., 2021
Meals				
9% pumpkin seed meal	–0.211/–0.027	g in 100 g dried yolk/g in 100 g egg	6 weeks	Vlaicu and Panaite, 2022
6% linseed meal and 2% dried kapia pepper	–0.17	g/100 g dried yolk	4 weeks	Panaite et al., 2021
6% linseed meal and 2% dried sea buckthorn pomace	–0.21			
6% linseed meal and 2% dried carrot	–0.22			
Seeds				
Hemp seed 10% Hemp seed 20% Hemp seed 30%	+1.686 –0.293 –2.455	mg/g yolk	84 days	Taaifi et al., 2023
10% extruded flaxseed	–0.12/+6.08	mg/g yolk/mg/egg	23 weeks	Mattioli et al., 2017
Black cumin (*Nigella sativa*) seeds 20 g/kg	–0.29	mg/g yolk	13 weeks	Szczerbińska et al., 2021 a
Black cumin (*Nigella sativa*) seeds 50 g/kg	–0.54			
By-products				
20% raw rapeseed cake	+0.4	% total FA	12 weeks	Kopacz et al., 2021
20% hydrobarothermally-treated rapeseed cake	–4			
20% fermented rapeseed cake	+0.8			
Alternatives				
1% green tea powder	–22.8	mg/egg	12 weeks	Chen et al., 2021

The addition of rosehip meal (1.5–3%) mixed with flaxseed meal influences the nutritional value of eggs, due to the increased content of essential amino acids at the expense of non-essential amino acids, the increased content of sulfur and aromatic amino acids, and those with antioxidant potential. This feed alteration did not change the content of crude protein in yolk (Vlaicu et al., 2022). Mori et al. (2020) suggested that both breed and feed affect the amino acids composition of egg yolk, especially fermented feed influenced cysteine content in yolk. However, in the studies of Kawamura et al. (2023) it was noted that the composition of egg amino acids (both in yolk and albumen) is dependent on the combined effect of feed (crude protein content in the diet) and housing system.

Research was also carried out on the possible influence of feed alteration on the activity of biologically active egg white proteins: lysozyme, cystatin, and serine protease inhibitors. The addition of mineral-humine preparations, components rich in ω-3 PUFA, and antioxidants (hibiscus, vitamins A+E) decreased cystatin, antitrypsin and antichymotrypsin inhibitory, and lysozyme activity. Only in fresh eggs obtained after feeding with the addition of rapeseed meal, the activity of cystatin was significantly increased (Kopeć et al., 2005). The activity of lysozyme in albumen decreased when soy protein in the feed was partially (10%) replaced with lupine protein, although the use of lupine protein as a source of protein in the concentration of 25% increased both lysozyme concentration and activity (Kowalska et al., 2020).

Eggs are also recognized as a good source of IgY (immunoglobulin Y) which acts as an anti-rotavirus and anti-bacterial agent. The amount of IgY may be further increased not only by hen immunization but by dietary modification by supplementation with ω-3 fatty acids, antioxidants, as well as some herbs such as basil leaves (Pulla and Sai Prasanna, 2019).

It has also been observed that diet composition may influence eggs' amino acid composition (Tomczak et al., 2024; Yu et al., 2024).

Currently, there are emerging reports related to the possible transfer of allergenic protein fragments from feed to eggs and poultry meat, which may significantly affect the nutritional value of those products (Tomczak et al., 2021; Saelens et al., 2023).

Increasing vitamin content

The content of vitamins in the diet of laying hens, whether derived from natural dietary components but much more often as concentrated supplements (Alagawany et al., 2020), directly affects the vitamin composition of eggs. This effect applies to both water-soluble and fat-soluble vitamins. Genetics and hen age are suggested as other factors influencing the vitamin content (Naber, 1993; Ko et al., 2021; Hejdysz et al., 2024). The time required for hens to respond to changes in vitamin content in their diet varies depending on the specific vitamin; for instance, response to vitamin A supplementation is slow (8–12 weeks), while the response to vitamin B_2 is rapid at a similar level of administration (Schiavone and Barroeta, 2011). Transfer efficiency is the highest for vitamin A, and high for pantothenic acid, riboflavin, biotin, and vitamin B_{12} as well; it is medium for vitamin E and D_3 and low for thiamine, vitamin K_1, folacin, niacin, and pyridoxine (Naber, 1993; Leeson and Caston, 2003; Schiavone and Barroeta, 2011). Since the most common modifications of egg nutrients concern fatty acids that occur in an egg after administration of various sources of lipids, the same studies also quite often control changes in the content of fat-soluble vitamins.

Vitamin E is an important antioxidant. A standard egg contains 1.9 mg of vitamin E/100 g and during the time of the egg enrichment this amount increases even more than 5-fold (Schiavone and Barroeta, 2011), providing even up to 80% of RDA (Recommended Dietary Allowance) per 100 g of egg. An increase in its content was noted when non-industrial hemp seeds (Taaifi et al., 2023) or palm oil were added to feed (Areerob et al., 2018). The vitamin concentration in the egg increased when hens grazed on grass rather than clover, and it was proved that the breeding system is also important (Karsten et al., 2010). Some research studied the combined effect of soy oil replacement in feed with a mixture of fish, linseed, and canola oil and supplementation of vitamin E (Kralik et al., 2023). However, in most studies on increasing the content of vitamins in eggs, vitamin preparations, not natural sources, were used (Park et al., 2005). Since vitamin supplementation did not affect hen weight or egg production (Park et al., 2005), even a high oversupply of vitamins was tested. Many studies confirmed linear relationships between concentration in fresh eggs and dietary concentration of alpha-tocopherol, up to doses of 20 g/kg feed. In higher doses, transfer efficiency diminishes. However, the data concerning the effectiveness of alpha-tocopherol are not compatible – for the same levels in the diet, the levels observed in eggs are different (Table 4). This results from many factors; apart from analytical problems, they also stem from interactions with other nutrients during absorption, e.g. interaction with vitamin A. It was also observed that at the time of egg forming, when a higher content of unsaturated fatty acids is present in the feed, the deposition of vitamin E decreases, due to its high utilization as a fatty acids antioxidant. The imbalance between the vitamin E and PUFA content was observed after adding up to 2.8% of fish oil, which increased the ω-3 PUFA content of yolks, leading simultaneously to increased levels of cytotoxic aldehydic lipid peroxidation products, such as malondialdehyde (MDA) (Grune et al., 2001).

Vitamin A is responsible for good vision, the immune system, reproduction, and growth and development (Schiavone and Barroeta, 2011). The main natural sources of this vitamin in the hen diet are carotenoids of corn, algae meal, and dehydrated alfalfa meal (Khan et al., 2023). Due to the efficient transfer of vitamin A from the diet into an egg, the production of eggs labeled as "rich in vitamin A" is possible (Naber, 1993; Schiavone and Barroeta, 2011). Its standard content in a chicken egg is 227 μg/100 g and after supplementation in the diet, its content increases by about 50%, which means that 100 g of egg provides ~40% RDA (Schiavone and Barroeta, 2011) (Table 4).

One of the functions of vitamin D in the human body is to regulate calcium and phosphorus metabolism. In hens, it additionally influences egg formation. The linear relationship between the content of vitamin D_3 in feed and egg (Barnkob et al., 2020), and its deposition in the yolk is around 2.2 times more efficient than that of D_2 (Mattila et al., 2004). Some authors suggest that supplementation of $25(OH)D_3$ is more efficient in poultry nutrition than the basic form of vitamin D_3 in egg (Świątkiewicz et al., 2016), and it is effectively transferred from hen diet to yolk, but it is still questionable if it is beneficial in case of bioavailability of the vitamin by human (Mattila et al., 2011).

Since egg is one of the few sources of this vitamin in the human diet (contains 1.8 μg/100 g of standard egg), and at the same time its deficiency is commonly reported, attempts have been made to increase the content of this vitamin. Eggs, the consumption of which provided more than 50% of the RDI after eating one piece, were successfully produced (Browning and Cowieson, 2014; Clark et al., 2021). The content of vitamin D in eggs remains quite stable over 8 days – 3 weeks supplementation period (Mattila et al., 2004; Yao et al., 2013) (Table 4). Interestingly, exposing laying hens and even liquid yolk to UVB radiation is a method used to stimulate the natural synthesis of vitamin D in eggs (Barnkob et al., 2020).

In humans, vitamin K is responsible for the synthesis of factors involved in blood clotting, but it also participates in bone formation processes. In hens, it also takes part in eggshell formation. A standard egg is not a good source of this vitamin, delivering ~12% of RDA/100 g of eggs. Few experiments on vitamin fortification were done, but only in some experiments, the product which delivered more than 100% of RDA in 100 g was obtained, as it is presented in Table 4 (Suzuki and Okamoto, 1997; Leeson and Caston, 2003).

Table 4. Influence of diet modification on vitamin content in eggs

Vitamins	Modification/addition level compared to control diet	Experiment duration	Increase by (%)	Reference
1	2	3	4	5
Vit. E		**Materials**		
	Nonindustrial hemp seed (30%)	4 months	16	Taaifi et al., 2023
	Crude palm oil (2%)	6 weeks	~10 (in total tocopherol and total tocotrienol)	Areerob et al., 2018
	18 ppm of marigold extract "Biofon Yellow"	60 days	13	Kavtarashvili et al., 2021
	5% of soybean oil was replaced by 1.5% fish oil + 1.5% rapeseed oil + 2.0% linseed oil +100 mg/kg vit. E	5 weeks	174	Kralik et al., 2023
		Supplements		
	200 IU/kg	90 days	670	Leeson and Caston, 2003
	Vitamin A at 15,000 IU/kg of diet Vitamin A 30,000 IU/kg of diet	11 weeks	24.9 44.0	Mori et al., 2003
Vit. D	Materials			
	Addition to the diet 0.1–0.4 % of irradiated shitake (5,000 IU/g D_2)	4 weeks	900	Kawazoe et al., 1994
		Supplements		
	0 IU D_3 & 34.5 IU 25(OH)D_3 (IU/kg feed) 0 IU D_3 & 69 IU 25(OH)D_3 (IU/kg feed) 2,500 IU D_3 & 0 IU 25(OH)D_3 (IU/kg feed) 2,500 IU D_3 & 34.5 IU 25(OH)D_3 (IU/kg feed) 2,500 IU D_3 & 69 IU 25(OH)D_3 (IU/kg feed) 7,500 IU D_3 & 0 IU 25(OH)D_3 (IU/kg feed) 7,500 IU D_3 & 34.5 IU 25(OH)D_3 (IU/kg feed) 7,500 IU D_3 & 69 IU 25(OH)D_3 (IU/kg feed)	9 weeks	55 88 43 107 156 184 191 391	Browning and Cowieson, 2014 a
	3,500 IU D_3 & 0 IU D_2 (IU/kg feed) 12,500 IU D_3 & 0 IU D_2 (IU/kg feed) 0 IU D_3 & 6000 IU D_2 (IU/kg feed) 0 IU D_3 & 15000 IU D_2 (IU/kg feed)	44 weeks	120 524 0 166	Mattila et al., 2004
	Up to 75 µg 25(OH)D_3 /kg feed	6 weeks	40	Clark et al., 2021
	9,700 IU D_3/kg feed 17,200 IU D_3/kg feed 4,700 IU D_3/kg feed 102,200 IU D_3/kg feed	10–35 weeks	720 1280 1900 29150	Yao et al., 2013
	5 000 IU/kg of feed	90 days	192	Leeson and Caston, 2003

Table 4 – contd.

1	2	3	4	5
Vit. K		**Supplements**		
	5 mg/kg feed	90 days	0	Leeson and Caston, 2003
	9.9 mg K_3 /kg feed	12 week	94	O'Sullivan et al., 2020
	20.7 mg K_3/kg feed		110	
	42.7 mg K_3 /kg feed		113	
	100 mg K_2/kg feed	31 days	~750	Suzuki and Okamoto, 1997
	200 mg K_2/kg feed		~750	
	500 mg K_2/kg feed		~1200	
	1,000 mg K_2/kg feed		~1500	
Vit. A		**Supplements**		
	30,000 IU/kg feed	90 days	27	Leeson and Caston, 2003
	15,000 IU retinyl acetate/kg feed	11 weeks	36.6	Mori et al., 2003
	30,000 IU retinyl acetate/kg feed		53.3	
B group vitamins		**Supplements**		
	Vitamin B_1 + 20 mg/kg	90 days	37	Leeson and Caston, 2003
	Vitamin B_2 + 25 mg/kg		12	
	Vitamin B_6 + 10 mg/kg		22	
	Biotin + 1 mg/kg		6	
	Folic acid + 2 mg/kg		11	
	Niacin + 400 mg/kg		64	
	Pantothenic acid + 10 mg/kg		58	
	Vitamin B_{12} + 100 µg/kg		285	
	10 mg folic acid/kg	6 weeks	100	Yu et al., 2024
	8 mg folic acid/kg	12 weeks	103	Hoey et al., 2009
	16 mg folic acid/kg		140	
	32 mg folic acid/kg		119	

Table 5. Influence of diet modification on pigment content in eggs

Diet modification/addition	Analyzed compound	Duration of experiment	Increase by (%)	Reference
Materials				
1% of dried stevia leaves 2% of dried stevia leaves	Lutein/zeaxanthin/total carotenoids	4 weeks	34 / 36 / 35 57 / 59 / 59	Pirgozliev et al., 2022
0.5% of rosehip leaves 1.0 % of rosehip leaves	Color differences – ΔE calculated	6 weeks	-15 6	Vlaicu et al., 2024
1% of *Scenedesmus obliquus* (microalgae)	Lutein/cantaxanthin	30 days	48 / 18	Jiru et al., 2021
Coloured carrot (*Daucus carota*) Orange *Bolero* var. Yellow *Rainbow* var. Purple *Purple Haze* var.	Lutein/zeaxanthin/α-carotene/ β-carotene/total carotenoids	28 days	10 / -15/ 5700 / 6033 / 29 39 / 0 / 1500 / 1633 / 36 64 / -17 / 12800 / 11200/87	Hammershøj et al., 2010
30 g/kg of dried broccoli stems and leaves 60 g/kg of dried broccoli stems and leaves 90 g/kg of dried broccoli stems and leaves	Xanthophyll	60 days	8 19 25	Hu et al., 2011
10 mg/kg apoester** 10 mg/kg canthaxanthin 3% clover extract 10 mg/kg paprika oleoresin 10 mg/kg Aztec marigold extract	Total carotenoids	10 weeks	518 655 1066 172 359	Alay and Karadas, 2017
Saponified paprika 2–16 mg/kg Unsaponified oleoresin paprika 4–16 mg/kg	Total carotenoids	20 days	46–150 13–129	Lai et al., 1996
10 ppm of marigold extract 14 ppm of marigold extract 18 ppm of marigold extract	Total carotenoids	60 days	140 157 297	Kavtarashvili et al., 2021
Marigold flower extract – 3 g/kg of lutein	Lutein	32 days	890	Kralik et al., 2020
Genetically engineered maize enriched in carotenoids Genetically engineered maize enriched in ketocarotenoids Standard commercial yellow maize	Total carotenoids	32 days	3070 1346 537	Moreno et al., 2016
10 g/kg dietary tomato powder	Lutein	90 days	31.8	Akdemir et al., 2012
High β-cryptoxanthin maize High β-carotene maize Yellow maize	β-cryptoxanthin/provitamin A*	20 days	660 / 90 62 / 22 92 / 34	Liu et al., 2012
Supplements				
120.5 mg/kg lutein	Lutein	5 weeks	~800	Kralik et al., 2023

*Provitamin A equivalents are the combined concentrations of β-cryptoxanthin and twice the β-carotene.
**Studies on quail breeder.

In the case of water-soluble vitamins, vitamin C is not present in eggs, whereas others (B-group vitamins) are found both in albumen and yolk. Vitamins B_1, B_5, B_7, and B_9 are concentrated in the yolk, while B_{12}, in approximately equal amounts, is found in both the yolk and egg white (Schiavone and Barroeta, 2011).

A few attempts have been made to increase the content of homocysteine cofactors, folic acid, and cobalamin (Table 4). The amount of folacin in eggs (51.2 µg/100 g of the standard egg; 25% RDA) may be increased 2–3 times after hens' dietary supplementation, the deposition is based on a saturation model (Schiavone and Barroeta, 2011). The synergistic effect of vitamin C concentration in feed was observed on folate content in eggs (Gu et al., 2022). In the case of cobalamin, which is important for methylation processes in organisms (it acts as a regulator of erythrocyte production, a cofactor of homocysteine metabolism), some authors suggest fast and efficient transport of cobalamin from feed to eggs (from 2.1 up to 4.8 µg/100 g of egg) (Naber, 1993), while others did not observe any changes (Bunchasak and Kachana, 2009). However, when a premix of vitamins was used, an almost fourfold increase was noted (Leeson and Caston, 2003). Studies on feed enrichment with an additional amount of thiamine, riboflavin, pyridoxine, biotin, niacin, and pantothenic acid were presented by Leeson and Caston (2003) (Table 4); however, due to the rare studies on their insufficiency in humans (Onishi and Ishida, 2021), the interest in increasing their amount in eggs is small.

Improving antioxidant activity and content

Food that is rich in antioxidants neutralizes free radicals produced in the body. When increasing the content of antioxidants in eggs, the overarching goal is to prevent the oxidation of valuable egg components, mainly unsaturated fatty acids, during storage. It may be expected that the following compounds are responsible for the antioxidative activity of eggs: amino acids (mainly aromatic), proteins (ovalbumin, ovotransferrin, phosvitin, and some enzymes), phospholipids, phenolic compounds, vitamins (vitamin E), pigments (carotenoids), and some minerals (selenium and iodine). The total antioxidant activity of eggs could increase in both yolk and albumen through modification of poultry feed composition.

To increase the antioxidant content in eggs, waste products of the food industry were usually suggested as a feed component. This effect was observed after adding rosehip meal (Vlaicu et al., 2022), pumpkin meal (Vlaicu et al., 2022), green tea powder (Chen et al., 2021), grapeseed meal, sea buckthorn meal (Vlaicu et al., 2021), but also after adding alfalfa and flax freeze-dried sprouts (Mattioli et al., 2023). An increased content of antioxidants was often related to an increased content of PUFA (Gaffney et al., 2015; Vlaicu et al., 2022).

In some experiments, feeds were enriched with supplements which affected antioxidant activity. In the research by Al-Shammari et al. (2024), antioxidant activity in quail eggs increased after supplementing the feed with commercial preparation of creatine monohydrate and L-carnitine which were added to reduce stress induced by 2.5 ppm lead acetate.

The application of supplements containing selenium increased the activity of GPX (glutathione peroxidase), and the antioxidant profile of eggs (Pan et al., 2011; Muhammad et al., 2021). Moreover, a confirmed increased content of secoisolariciresinol and lignan metabolites with antioxidant and hormonal activity (enterodiol and enterolactone) was noted in eggs obtained after feeding hens with flaxseeds and flax sprouts addition (Mattioli et al., 2017, 2023).

The trend of application of plant by-products rich in polyphenols in the diet formulation is noted. However, despite consumer acceptance of such modification and a proven increase in antioxidant activity in the products, the problem is the lack of equalization of the content of these compounds in these by-products. The bioavailability of these ingredients for humans is also questionable, although they significantly affect the quality of the product (Vlaicu et al., 2023).

Modification of the content of some other antioxidants (such as e.g. selenium, amino acids, vitamins, and dyes) is discussed in the remaining parts of the article.

Increasing the content of minerals

Most minerals that are important for human health are deposited in yolk, whereas their concentrations in the albumen are limited. The consumed part of an egg is rich in calcium, potassium, and phosphorus, but also other essential elements, including iron, zinc, copper, magnesium, manganese, and selenium (Réhault-Godbert et al., 2019). Egg shells have a much higher ash content, and feed modifications are often carried out to improve the quality of the shell (usually its thickness and strength). In the case of shell quality, the importance of calcium and phosphorus is well known. During the mineralization of eggshell layers, amorphous calcium carbonate deposited in the outer shell membrane is transformed into calcite crystals, which is inhibited by the phosphorus. Simultaneously a calcium and phosphorus-rich hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ crystal layer, internal to the cuticle, is formed (Sinclair-Black et al., 2023). It is also proven, although not fully clarified, that dietary supplementation of Zn, Cu, and Mn improves the eggshell structure. Mn enhances uronic acid and glycosaminoglycans synthesis, which promotes eggshell calcification (Xiao et al., 2014), while Zn increases the expression of carbonic anhydrase and osteopontin and increases the activity of carbonic anhydrase, thus influencing the eggshell structure (Zhang, 2013). In designed, nutraceutical food, egg shells are also sources of elements that are important for humans (Kobus-Cisowska et al., 2020; Arnold et al., 2021). However, the presented review is focused on the modification of egg yolk and white – traditionally consumed egg parts.

The most common sources of minerals in the laying hen diet are inorganic sources (European Commission, 2013). The concentration of some elements in the edible part may be quite easily increased by the hen diet. The goal of many studies was to increase selenium content in eggs. Selenium is a constituent of selenoproteins, the best-known antioxidant and catalyst for the production of active thyroid hormone (Rayman, 2000). The average hen egg contains about 5 µg Se/egg and higher Se content ensures egg freshness, due to its antioxidative activity (Schiavone and Barroeta, 2011). Due to the high selenium toxicity, its dietary recommendations for selenium consumption range between 0.05–0.08 mg Se/kg diet (Dale, 1994), and usually its sources in the diet are sodium selenite or selenate, and selenomethionine (from natural sources, such as soy meal or oil seeds) (Surai et al., 2007). Adding 0.5% selenium-enriched *Chlorella vulgaris*, when selenium is bound in the form of selenium amino acids, increases the content of this element in eggs by 100–152% (Jiru et al., 2021). Adding organic selenium (Kralik et al., 2023) was a little bit less effective, but they still observed enrichment of its content in eggs by 1.81 times. A threefold increase in the content of selenium occurred after the application of selenium-enriched probiotics yeast (Pan et al., 2011). Fast transfer of nano-Se into eggs was confirmed by Radwan et al. (2015), who noted a fourfold increase in the concentration of nano-selenium in egg white, and a fivefold increase in egg yolk when adding 0.4 mg/kg to the diet.

Bennett and Cheng (2010) confirmed that egg Se content increased linearly as dietary levels of Se increased, up to the content of 5.1 µg/g of Se in the diet, and did not affect egg production and the laying hen welfare. An egg, fortified with selenium, after the diet supplementation with 0.3–0.5 mg/kg of organic selenium sources, delivers 20–40 µg of Se (Surai et al., 2007; Fisinin et al., 2009). Then, single pieces deliver up to 70% of human Se RDA, thus becoming a functional food.

Another micronutrient that is essential for humans, and whose content may be increased by feeds, is iodine. This element is responsible for the proper functioning and morphology of the thyroid gland. Usually, an egg contains 4–10 mg of iodine, mainly in the yolk (Schiavone and Barroeta, 2011). Dobrzański et al. (2020) studied the possible fortification of iodine in eggs and noted more than a 50% increase in its content after feeding an experimental group with feeds containing 300 mg/kg of iodine and increased content of selenium and vitamin E. The content of iodine in boiled egg after supplementing feeds with 13 mg/kg was ~1.17 µg/g of yolk and ~0.11 µg/g of albumen. However, the feeding cost which was comparable to the control group was incurred when supplementation was with 9.25 mg/kg, and then the iodine content was respectively ~0.71 µg/g of yolk and 0.08 µg/g of white.

Iron is another essential element for human health and its inadequate supply in the diet is the cause of the most common type of malnutrition in the world, affecting especially women and children. It causes delays in children's development and behavioral problems (Schiavone and Barroeta, 2011). Egg yolk is a good source of iron, and transferring iron from the diet into an egg was confirmed. Usually, iron is delivered to the diet in an inorganic form (e.g. $FeSO_4$) (Cao et al., 2023). The addition of iron complexed with amino acids influenced egg quality and laying rate (Cao et al., 2023). The addition of Fe-methionine (100 mg/kg) caused a 14% increase in its content after just ten days of supplementation (Park et al., 2005) while Fe-Lys-Glu was not so efficient (7% increase in the iron content of yolk after 12-week supplementation by addition of 60 mg of preparation per 1 kg of the feed) (Cao et al., 2023). On the other hand, enrichment of soy meal with some elements (Zn, Cu, Fe, Cr) proved iron (an increase by 274%), zinc (an increase by 32.3%), and copper (a 47.3% increase) accumulation in albumen (Witkowska et al., 2019).

During the studies on eggshell quality (Mabe et al., 2003) a small increase in some other minerals (Zn, Mn, and Cu) in edible parts of eggs was also confirmed. As a good source for the transfer of microelements [Cu(II), Zn(II), Co(II), Mn(II), Cr(III)] into an egg, enriched macroalgae (*Enteromorpha prolifera* and *Cladophora* sp.) were studied (Michalak et al., 2011). However, the transfer of elements is complicated, and during egg formation, an increase in the concentration of some elements may occur at the same time as the reduction of others (Dobrzański et al., 2020).

Pigment application to improve egg color

Consumers often expect the color of eggs to be stable (i.e. yellow-orange yolks, and transparent albumen), while in fact, it may be changed easily by the diet composition. Though egg color does not reflect the nutritional value of eggs, it must be remembered that carotenoids which give yolk the color influence human health, acting as antioxidants, which are important for the eyesight and circulatory system. The content of carotenoids increases when hens are raised in pastures, with access to fresh grass (Sergin et al., 2022). Moreover, laying hens react quite quickly to the presence of these dyes in feeds. Studies were done with the application of those pigments both as chemical preparations, as well as components of the plant or other raw materials. To increase the content of carotenoids in the yolk, the following sources of carotenoids in feed were used: commercial corn hybrids (Kljak et al., 2021) and corn, additionally fortified in those compounds (Liu et al., 2012; Moreno et al., 2016), marigold flower extract (Alay and Karadas, 2017; Grčević et al., 2019), stevia (*Stevia rebaudiana*) (Pirgozliev et al., 2022), rosehip (*Rosa canina* L.) leaves (Vlaicu et al., 2024), microalgae preparation (increase in lutein and canthaxanthin in eggs by 48% and 18%, respectively was noted) (Jiru et al., 2021), various colored carrot (*Daucus carota*) (Hammershøj et al., 2010), stems and leaves of broccoli (Hu et al., 2011), pepper extract (Alay and Karadas, 2017; Lai et al., 1996), tomato powder (Akdemir et al., 2012), alfalfa, lutein enriched food materials (Pitargue et al., 2019), as well as such exotic sources as golden snail (*Pomacea canaliculata*) eggs (Nusantoro et al., 2020). Pure lutein (Pitargue et al., 2019; Kralik et al., 2020), astaxanthin (Dansou et al., 2021) or canthaxanthin (Alay and Karadas, 2017) preparations have also been applied successfully (Table 5).

Conclusions

Nowadays, there is a growing awareness in society which is looking for functional foods and at the same time, foods that are cheap and produced in accordance with the principles of sustainable development. Eggs, classified as superfoods, may become a functional food, remaining part of the traditional human diet with low caloricity. Designer eggs are easily accepted by consumers, and their production is often based on the modification of laying hen feed.

It must be remembered that feed modification may lead to an undesirable effect, affecting both hen health and egg quality. This is why the modulation must be performed while maintaining appropriate proportions in the nutrients provided to animals and not to deliver components that are toxic to them. Currently, as we strive to use raw materials as efficiently as possible, we still should remember that some industrial by-products, incorporated into the hen diet, impair the quality of eggs and mortality of animals (e.g. coffee husk which is rich in fiber but also in alkaloid – caffeine) (Teixeira et al., 2020). Moreover, designer eggs cannot show any defects, such as changes in shape, undesirable color, odor or taste, as was mentioned above e.g. in the case of lipids modification.

Since the influence of interactions between individual feed ingredients on their deposition in eggs is still not fully understood, eggs are usually enriched with a single nutrient (group of nutrients) without examination of the impact on other groups of nutrients. Clinical studies on the bioavailability of nutrients are needed to monitor the bioavailability of nutrients from designer eggs (Vlaicu et al., 2023).

List of abbreviations

DHA – docosahexaenoic acid
DPA – docosapentaenoic acid
EPA – eicosapentaenoic acid
FA – fatty acids
FAME – total fatty acid methyl esters
IgY – immunoglobulin Y
LPC – lysophosphatidylcholine
MDA – malondialdehyde
MUFA – monounsaturated fatty acids
PC – phosphatidylcholine
PUFA – polyunsaturated fatty acids
RDA – Recommended Dietary Allowance
UFA – unsaturated fatty acids

Authors' contributions

M. Zielińska-Dawidziak, P. Klimowicz, A. Tomczak: conceptualization. M. Zielińska-Dawidziak, P. Klimowicz: data curation. A. Tomczak: funding acquisition. M. Zielińska-Dawidziak: supervision.

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