Enrichment of chicken table eggs with lycopene and astaxanthin

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Introduction

The colour of the egg yolk is an important factor that determines its attractiveness to the consumer (Honda et al., 2020). It is known that yolks are coloured by carotenoids, which are not able to be synthesized in the body of chickens and must come with feed (Nimalaratne & Wu, 2015; Nabi et al., 2020). In recent years, most food egg producers have abandoned synthetic dyes and preferred natural ones, including those of plant origin: products of processing tomatoes as a source of lycopene and various microscopic algae as a source of astaxanthin (Magnuson et al., 2018), and added vitamins to the diet supplements (Danchuk et al., 2019). Astaxanthin effectively pigments the yolk (Heng et al., 2020) and exhibits strong antioxidant activity, which is 10 times higher than β-carotene and 550 times higher than vitamin E. The intake of astaxanthin with food, including eggs, in the body of consumers, reduces the risk of cardiovascular disease, some cancers and eye diseases (Fakhri et al., 2018), increases the activity of antioxidant enzymes (Dose et al., 2016; Li et al., 2018) and suppresses the risk of diseases of the immune system (Nimalaratne & Wu, 2015, 2016). Enriching eggs with lycopene enhances the body’s immune response (Farruggia et al., 2018). Lycopene is found in significant concentrations in tomatoes and processed products and is one of 20 carotenoids found in human blood and tissues (Boyarcioglu et al., 2016). Several in vivo and in vitro studies have shown that lycopene is a potent antioxidant (Bacanli et al., 2017) that is able to absorb free radicals, inhibit signaling pathways and activate antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. Enrichment of edible eggs with lycopene or astaxanthin allows one not only to obtain an attractive colour of egg yolks, but also to improve their antioxidant composition and functional properties. However, studies of the effectiveness of different sources of lycopene and astaxanthin in laying hens contain differences in the optimal dose of carotenoids depending on the basic diet, combination with different oils, composition and form of supplements (dry powder, paste, oil extract) in the diet. Therefore, the aim of our study was to determine the effect of different doses of natural oil extracts of lycopene and astaxanthin on the quality of eggs and carotenoid composition of yolks.

Materials and methods

All experiments were performed in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Scientific Experiments or Other Scientific Purposes of 1986, as well as the Law of Ukraine “On Protection of Animals from Cruelty” of 21.02.2006, 3447-IV.

The study was conducted on the basis of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine. For this purpose, 45 laying hens of the High-line W36 cross-country aged 23 weeks were used, which were divided into three groups according to the principle of analogous groups: 15 heads in each. Chickens were kept in cage batteries for 5 heads each for 90 days (Table 1). Laying hens were fed lycopene supplements in the form of 6% oil extract obtained from tomatoes (LycoRed, Israel), as well as astaxanthin supplements – 10% oil extract obtained from the biomass of algae Haematococcus pluvialis (ALGAE Technologies, Israel). Throughout the experiment, chickens were fed complete feed, which consisted of the following components (g/kg): corn – 500.85, wheat – 90.00, soybean meal – 179.00, sunflower meal – 96.00, limestone – 114.00, monocalcium phosphate – 10.00, table salt, intox (sorbent) – 1.00, methionine – 1.30, Proactive (probiotic) – 1.00, mineral complex Rovimix – 1.00, lysine – 1.60, Millerzeim III 150 (multienzyme additive) – 0.15, sodium sulfate – 1.30, choline chloride – 0.30, vitamin complex – 0.20. 100 g of compound feed contains: moisture – 10.40 g, crude protein – 16.22 g, calcium – 4.76 g, total...
phosphorus – 0.77 g, sodium – 0.20 g, metabolic energy – 291.2 kcal. From 1 to 30 days of the experiment, laying hens were fed an average 91 g, and from 31 to 90 days – 97 g of feed per head per day. Experimental diets were prepared for 4 days, the feed mixture was mixed and stored in airtight food plastic containers. Watering of chickens was carried out at will with the use of cup drinkers. Daylight was 16 hours (illumination – 30 lux), darkness – 8 hours. The indoor air temperature was maintained at 21–23 °C, and the relative humidity was 60–62%.

Table 1

| Group                              | Diet                                                                 |
|------------------------------------|---------------------------------------------------------------------|
|                                    | 1–30th day   | 31–60th day | 61–90th day   |
| Control                            | Basic diet + 0.33 g/kg  | Basic diet + 0.66 g/kg  | Basic diet + 1.0 g/kg  |
| Lycopene                           | Basic diet + 20 mg/kg  | Basic diet + 40 mg/kg  | Basic diet + 60 mg/kg  |
| Astaxanthin                        | Basic diet + 10 mg/kg  | Basic diet + 20 mg/kg  | Basic diet + 30 mg/kg  |

Note: basic diet, the same supplements 1, 2, 3 show the same content of refined sunflower oil in the diet.

For the research, 5 eggs were selected from each group of chickens on the 30–31st, 60–61st and 90–91st days of the experiment. Determination of egg weight, albumen height, yolk colour (16-point YolkFartTM colour scale), Haugh unit, shell strength, and shell thickness was performed using a DET 6000 digital egg tester (NABEL Co., Ltd., Japan). The content of carotenoids in chicken egg yolks was determined by high performance liquid chromatography (HPLC). The research was performed in Research on Demand LAB, Zaporizhia (accreditation certificate No. GAGL 804.017). Before the study of egg yolk samples by HPLC, alkaline hydrolysis and extraction of carotenoids were performed (GOST EN 12823-2-2014 Products: 2014). Haugh unit, shell strength, and shell thickness was determined using a DET 6000 digital egg tester (NABEL Co., Ltd., Japan). The content of carotenoids in chicken egg yolks was determined by high performance liquid chromatography (HPLC). The research was performed in Research on Demand LAB, Zaporizhia (accreditation certificate No. GAGL 804.017). Before the study of egg yolk samples by HPLC, alkaline hydrolysis and extraction of carotenoids were performed (GOST EN 12823-2-2014 Products: 2014). After partial evaporation of the hexane extracts in a water bath under a stream of nitrogen they were dried under vacuum to a dry residue in a vacuum desiccator. The unsaponified residue was dissolved in 2 ml of mobile phase hexane: acetone in a volume ratio of 90:10 and filtered using a syringe with a PTFE filter 0.22 μm, 13 mm Luer Syringe Filter. The obtained extracts and standard solutions were used for chromatographic analysis on a HPLC system consisting of a Shimadzu LC-10 AD pump, a Kariba Instruments Model RC 3099 sampling device and a Shimadzu SPD-10 A spectrophotometric detector (Shimadzu, Japan).

Table 2

| Indicator                      | Control | LP20  | AST10 |
|-------------------------------|---------|-------|-------|
| Egg weight, g                 | 57.22 ± 0.18 | 56.32 ± 0.13 | 56.84 ± 0.06 |
| Albumen height, mm            | 7.34 ± 0.55  | 8.04 ± 0.41   | 7.48 ± 0.34   |
| Yolk colour, points           | 6.10 ± 0.11  | 7.20 ± 0.68   | 12.80 ± 0.22  |
| Haugh unit                    | 86.18 ± 3.34 | 90.62 ± 2.12  | 87.22 ± 1.95  |
| Eggshell strength, kgf        | 4.33 ± 0.109 | 4.96 ± 0.31   | 4.71 ± 0.06   |
| Shell thickness, mm           | 0.38 ± 0.01  | 0.39 ± 0.01   | 0.38 ± 0.01   |

Note: ND – not detected; different uppercase letters a, b indicate values that probably differed in one row of the Table (P < 0.05) by comparison using Tukey test with Bonferroni correction.

The addition of lycopene to the diet of chickens at a dose of 20 mg/kg of feed contributed to its deposition in the yolks, although it did not change the intensity of their colour compared to the control (Fig. 1a, b). Astaxanthin at a dose of 10 mg/kg of feed also accumulated in the yolks of chicken eggs and increased the intensity of their colour by 6.7 points compared to the control (P < 0.05, Fig. 1c, d) and 5.6 points compared to poultry feed mixture (P < 0.05, Table 2). Astaxanthin dietary supplement increased (P < 0.05) β-carotene deposition in chicken egg yolks compared to controls. Both LP20 and AST10 diets increased the content of unidentified carotenoids (P < 0.05) and the total content of carotenoids (P < 0.05) in chicken egg yolks compared to the control.

Table 3

| Indicator                      | Control | LP20  | AST20 |
|-------------------------------|---------|-------|-------|
| Egg weight, g                 | 59.74 ± 0.49 | 58.76 ± 0.52 | 58.50 ± 0.66 |
| Albumen height, mm            | 9.42 ± 0.48 | 9.60 ± 0.26  | 9.04 ± 0.26   |
| Yolk colour, points           | 5.60 ± 0.27 | 7.40 ± 0.27  | 14.80 ± 0.22  |
| Haugh unit                    | 96.70 ± 2.12 | 97.82 ± 1.24 | 94.98 ± 3.22  |
| Eggshell strength, kgf        | 4.47 ± 0.17  | 4.64 ± 0.27  | 4.45 ± 0.34   |
| Shell thickness, mm           | 0.39 ± 0.02  | 0.39 ± 0.01  | 0.40 ± 0.01   |

Note: ND – not detected; different lowercase letters a–c indicate values that probably differed in one row of the Table (P < 0.05) by comparison using Tukey test with Bonferroni correction.

The use of the LP40 diet increased (P < 0.05) the content of lycopene in chicken egg yolks and the intensity of their colour by 1.8 points (P < 0.05) compared with the control (Table 2, Fig. 1). The AST20 diet increased the deposition of astaxanthin in chicken egg yolks and improved the intensity of their colour by 9.2 points (P < 0.05) compared with the control (Fig. 1, a) and by 7.4 points (P < 0.05) compared with the LP40 diet (P < 0.05, Table 2). Lycopene supplementation at a dose of 20 mg/kg of feed did not affect, and astaxanthin supplement at a dose of 20 mg/kg of feed increased the content of unidentified carotenoids (P < 0.05) and the total content of carotenoids (P < 0.05) in chicken egg yolks compared to control (Table 3).
The effect of lycopene at a dose of 60 mg/kg and astaxanthin at a dose of 30 mg/kg of feed on the quality and carotenoid composition of chicken eggs (x ± SD, n = 5)

Table 4

| Indicator                      | Control     | LP40        | AST30       |
|-------------------------------|-------------|-------------|-------------|
| Egg weight, g                 | 60.44 ± 0.83 | 58.00 ± 2.56 | 59.54 ± 2.96 |
| Albumen height, mm            | 7.88 ± 0.23  | 8.70 ± 0.48  | 8.00 ± 0.35  |
| Yolk weight, g                | 9.60 ± 0.27  | 8.00 ± 0.00  | 14.40 ± 0.45 |
| Haugh unit                    | 88.72 ± 1.47 | 99.20 ± 1.86 | 84.54 ± 3.92 |
| Eggshell weight, kgf          | 4.80 ± 0.22  | 4.20 ± 0.33  | 4.60 ± 0.25  |
| Shell thickness, mm           | 0.39 ± 0.01  | 0.36 ± 0.02  | 0.36 ± 0.01  |
| Total carotenoids, mg/kg      |             |             |             |
| Astaxanthin, μg/kg            | ND          | ND          | 469.0 ± 80.3|
| Lycopene, μg/kg               | ND          | 283.7 ± 59.8| ND          |
| β-carotene, μg/kg             | ND          | 77.0 ± 16.3  | 83.3 ± 19.9 |
| Unidentified carotenoids in terms of β-carotene, mg/kg | 15.5 ± 0.26  | 16.5 ± 3.0   | 25.8 ± 3.2  |

Note: see Table 2.

Fig. 1. Colour of egg yolks of chickens of control group (a), LP20 (b), AST10 (c), LP40 (d), AST20 (e), LP60 (f), AST30 (g)

Further increase of lycopene content in the diet of laying hens to 60 mg/kg of feed did not affect the content of β-carotene, unidentified carotenoids and total carotenoids in egg yolks and increased the deposition of lycopene in egg yolks (P < 0.05) and the intensity of their colour by 2.4 points (P < 0.05) compared with the control (Table 4, Fig. 1a, f). The addition of astaxanthin to the feed of laying hens at a dose of 30 mg/kg of feed increased the content of astaxanthin (P < 0.05), unidentified carotenoids (P < 0.05), total carotenoids (P < 0.05) and the intensity of yolk colour by 8.8 points compared with the control (Table 4, Fig. 1a, g). However, it should be noted that the intensity of yolk colour and astaxanthin content in the yolks of AST30 hens did not increase compared to the dose of AST20 (Tables 3, 4).

Discussion

Only carotenoids that do not have provitamin activity, including lycopene (Imran et al., 2020) and astaxanthin (Davinelli et al., 2018), are able to accumulate in chicken egg yolks, while β-carotene is transformed into vitamin A, which although it enters the yolks of chicken eggs, does not affect their colour (Shevchenko et al., 2017a). Feeding laying hens with oil extracts of lycopene and astaxanthin in different doses for 90 days did not affect the indicators characterizing their quality, namely egg weight, albumen height, Haugh unit, strength and shell thickness, which is consistent with the results of studies by An et al. (2019). Previous studies have also shown that carotenoids on the example of β-carotene have a positive effect on the morphological parameters and chemical composition of quail eggs (Shevchenko et al., 2017b).

The lycopene diet, which was used in our experiment on laying hens, showed that lycopene from the oil extract of tomato products can accumulate in egg yolks. This fact is also confirmed in the studies of Habarabashaka et al. (2014), who showed that the inclusion of tomato flour in amounts of 3–9% in the diet of laying hens contributed to an increase in lycopene content and the intensity of the colour of chicken egg yolks. Despite the increase in the content of lycopene in the diet of chickens, the intensity of its deposition in the yolks did not increase significantly, which is consistent with the colour of the yolks of eggs of chickens in this group (Fig. 1b, d, f). The results of our studies are consistent with the data obtained by Kang et al. (2003) and Aldemir et al. (2012), which also indicate a certain limitation of the accumulation of lycopene in egg yolks. This may be due to the different ability of lycopene isomers to be absorbed in the digestive tract of birds (Honda et al., 2019). The results of the regression analysis of Olson et al. (2008) confirmed that for optimal inclusion of lycopene in egg yolk it is necessary to feed laying hens lycopene at a dose of 420 mg/kg of feed, however, saturation of the mechanisms of absorption in the intestine can reduce the intensity of its absorption by 4.5% at a dose of 65 mg/kg feed up to 0.06% for doses up to 650 mg/kg feed.

The recommended daily dose of lycopene for humans is 10–30 mg (Rao & Agarwal, 1999). When fed to laying hens on the LP90 diet, one egg weighing 62 g has a yolk weighing about 18 g, which will contain up to 5 μg of lycopene, which is 1/2000 of the recommended dose and does not pose a risk to the health of the consumer. Given the dietary characteristics of edible eggs, enrichment with lycopene will improve their antioxidant properties and biological integrity.

It should also be noted that in our experiment we obtained a significantly lower content of lycopene and β-carotene in chicken egg yolks using a diet with lycopene oil extract at doses of 20–60 mg/kg of feed than Omri et al. (2019) for the use of 1% dried tomato paste and 1% sweet red pepper. We were unable to obtain a colour scale of egg yolks depending on the dose of lycopene in the diet of chickens 20–60 mg/kg. However (Bernamoun et al., 2013), using in the diet of chickens dried tomato peel in doses of 4–13%, the yolk received 12.0–14.6 against 8.5 points in the control of the colour scale. Such differences in the results of the research are explained by the different basic diet for chickens used by the researchers, as well as the different composition of additives derived from tomato products: oil extract (in our case) and dried powders or paste (in other studies).

The use of astaxanthin oil extract in the diet of chickens in our experiment showed that it increased the deposition of astaxanthin and improved the intensity of yolk colour only at doses of 10 and 20 mg/kg of feed. Further increase of the dose of astaxanthin supplement to 30 mg/kg of compound feed was not expedient, as it did not increase the deposition of astaxanthin in the yolks and increase the intensity of their colour (Tables 3, 4). It should be noted that despite the lower content of astaxanthin in the diet of chickens, it stained egg yolks better than lycopene (Fig. 1b–g). This indicates a higher intensity of absorption of astaxanthin compared to lycopene in the digestive system of chickens. A similar conclusion was drawn for the uptake of lycopene and astaxanthin in rats.
in egg yolks and allowed them to obtain a colour scale 12.8–14.8 points of the colour spectrum of yolks desired for consumers. As shown in the studies of Spada et al. (2016) among the surveyed 240 consumers, 85% pay attention to the colour of the yolks when buying eggs, 66% prefer eggs with yolks enriched with carotenoids. This allows us to consider astaxanthin one of the promising dyes for egg yolks.

Astaxanthin derived from H. pluvialis microalgae has been approved as a colorant for use in salmon feed and as a dietary supplement for human consumption at doses up to 12 mg per day for over 20 years and up to 24 mg per day for up to 30 days in Europe, Japan and the United States (Visioli & Artaria, 2017). If we take into account that a chicken egg weighing 62 g has a yolk weighing about 18 g, the intake of astaxanthin in the human body for the consumption of one egg of chickens receiving diets AST10, AST20, AST30 (Tables 2–4) will be 6–9 μg. This is almost 11/1000 of the above recommended daily human dose and should not pose a health risk. Given the proven positive effect of astaxanthin on the human body (Fuldbjer et al., 2018), enrichment of egg yolks with this carotenoid will be an important factor in improving their quality and biological integrity (Langi et al., 2018).

**Conclusions**

Feeding laying hens with supplements of lycopene oil extracts at doses of 30, 40 and 60 mg/kg and astaxanthin at doses of 10, 20 and 30 mg/kg of feed for 30 days in increasing concentrations did not affect egg weight, albumen height, Haugh unit, strength and the thickness of the shell. Lycopene oil extract in doses of 20, 40 and 60 mg/kg increases the content of lycopene and provides the intensity of yolk colour at the level of 7.4–8.0 points. Additions to the diet of laying hens of astaxanthin oil extract in doses of 10, 20 and 30 mg/kg of feed increase the content of astaxanthin, β-carotene, the total content of carotenoids in the yolks and the intensity of their colour to 12.8, 14.8 and 14.4 on a 16-point colour scale YolkFanTM. To obtain an attractive colour of chicken egg yolks, it is advisable to use supplements of astaxanthin oil extract in doses of 10 and 20 mg/kg of feed for 30 days. The obtained research results can be the basis for the development of technology for the production of functional table eggs enriched with carotenoids. This allows us to consider astaxanthin one of the promising dyes for egg yolks.

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