COMPARISON OF NATURAL AND SYNTHETIC CAROTENOIDS: EFFECT ON YOLK COLOUR AND OXIDATIVE STABILITY OF YOLK LIPIDS

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Abstract- Two experiments were performed to evaluate the effect of synthetic and natural carotenoids on the daily output of eggs and some parameters of their quality. The hens were fed a control diet and diets supplemented with synthetic carotenoids Carophyll® Red and Carophyll® Yellow, lutein, algae *Chlorella*, and mustard meal. Carotenoids had no effect on hen-day egg production. Both synthetic and natural carotenoids significantly increased the intensity of yolk colour. Lutein increased the redness of yolks, whereas *Chlorella* increased the yellowness. Carophylls, lutein and *Chlorella* significantly improved the oxidative stability of yolk lipids. Feed supplementation with Carophylls and lutein significantly increased content of β-carotene, lutein and zeaxanthin in egg yolks. It can be concluded that (i) lutein and *Chlorella* are alternatives to synthetic carotenoids, and (ii) the use of *Chlorella* is more advantageous from an economical point of view than that of lutein.

Index Terms- Carotenoids, Yolk colour, Oxidative stability, Chlorella.

I. INTRODUCTION

Carotenoids are yellow, orange, and red pigments synthesized by plants and microorganisms. In the poultry industry carotenoids are routinely added to diets of laying hens to obtain optimum pigmentation of the egg yolk and increase oxidative stability of yolk lipids. Carotenoids may serve as a precursor of vitamin A and also have immune modulatory functions [1]. To overcome the shortage of natural carotenoids, synthetic carotenoids were prepared. Canthaxanthin is the preferred synthetic red xanthophyll in poultry farming available as Carophyll® Red (DSM Nutritional Products, Switzerland) or Lucantin® Red (BASF, Germany). The preferred yellow xanthophyll is β-apo-8´-carotenoic acid ethyl ester available as Carophyll® Yellow and Lucantin® Yellow. Carotens and β-cryptoxanthin are provitamins A. In the body can be converted to retinol. Lycopene, a bright red carotenoid found in tomatoes together with lutein and other carotenoids was tested in several studies. Ševčíková et al. [2] investigated the effect of lycopene supplementation on lipid profile and meat quality of broiler chickens.

Englmaierova et al. [3] reported the effect of lutein and synthetic carotenoids on performance of hens and quality of eggs. Lutein is an orange xanthophyll occurring in plants usually with carotens. Xue et al. [4] reported the effect of a tomato by-product on the yolk colour and lycopene content of eggs. Jang et al. [5] showed that both commercial lutein and lutein-containing extract of spinach significantly increased the egg yolk lutein content and yolk colour.
This paper summarizes results of two experiments aimed at improvement of oxidative stability and colouring of yolks using natural and synthetic carotenoids.

II. MATERIAL AND METHODS

1. Experiment I.
In this experiment lutein, dried *Chlorella* and synthetic carotenoids were compared. Two hundred and forty ISA Brown hens aged 25-39 weeks were housed in enriched cages, 10 hens per cage. There were four dietary treatments. The control diet contained maize, wheat and soybean meal as the main ingredients. The hens of the2nd group obtained a combination of Carophyll® Red and Carophyll® Yellow at 20 and 15 mg/kg, respectively. The hens of the 3rd group received diet supplemented with Lutein powder extract (Alchimica, Prague, Czech Republic) at 250 mg/kg. Diet of hens of the 4th group was supplemented with 12.5 g/kg spray-dried *Chlorella*, cultivated autotrophically in the Institute of Microbiology (Třeboň, Czech Republic).
Eggs were collected daily. Yolk colour was determined using Yolk Colour Fan (DSM Nutritional Products). Yolk colour parameters L* (lightness), a* (redness) and b* (yellowness) were determined using the Minolta colourimeter. Lipid peroxidation in yolks was determined in fresh eggs and eggs stored at 18°C for 4 weeks. The thiobarbituric acid method by Piette and Raymond [6] was used.Thiobarbituric acid-reactive substances (TBARS) were expressed as mg of malondialdehyde per kg.The data were analyzed by one-way ANOVA (SAS, version 9.2).

2. Experiment II
In the second experiment, synthetic carotenoids, lutein and mustard were compared. Mustard seed is a potential source of carotenoids[3]. One hundred and sixty ISA Brown hens, 20-34 weeks of age were

housed in enriched cages, conforming to the EU Directive 1999/74/EC. The hens were assigned to one of 4 dietary treatments. Control hens were fed a diet without carotenoids. Hens of the 2nd group were fed a combination of Carophyll® Red and Carophyll® Yellow as described previously, hens of the 3rd group were fed a diet supplemented with lutein at 100 mg/kg.

The last experimental group received a diet supplemented with 10 g/kg of meal from *Brassica juncea* (L.). The mustard meal was obtained from Oseva Pro, Ltd. (Opava, Czech Republic). The mustard meal contained lutein and zeaxanthin at 11.9 and 5.2 mg/kg, respectively. For sampling and yolk colour determination see Experiment I. The β-carotene contents of yolks were determined in accordance with the standard EN 12823-2 [7], other carotenoids as described previously [3].The HPLC instrument (VP series; Shimadzu, Kyoto, Japan) equipped with a diode-array detector was used. The data were analyzed statistically using one-way ANOVA.

III. RESULTS

Table 1 presents results of the first experiment. Neither synthetic nor natural carotenoids significantly influenced hen-day egg production. Carophylls, lutein and *Chlorella* significantly increased the yolk colour. The strongest effect was that of lutein (supplied at 250 mg/kg), which increased the redness, whereas *Chlorella* increased the yellowness. Carophylls were better colouring agents than dried *Chlorella*. All supplements significantly increased the oxidative stability of yolk lipids, expressed as malondialdehyde level.

Results of the second experiments are shown in Table 2. The source of carotenoids, either synthetic or natural, did not influence the hen-day egg production. Colour of yolks in hens fed the mustard meal was comparable to that of yolks in hens fed lutein at 100 mg/kg and significantly higher than in control hens.Feed supplementation with Carophylls and luteinsignificantly increased content of β-carotene, lutein and zeaxanthin in egg yolks. The contents of carotenoids in yolks of hens fed the mustard meal were not significantly different from the control.

Table 1:Daily output of eggs, yolk colour and oxidative stability of fresh eggs and eggs stored for 28 days. Results of the Experiment I.

	Control	Carophylls	Lutein	*Chlorella*
Hen-day egg production (%)	92.8	93.4	93.9	93.8
Yolk colour (DSM Fan)	6.4^{d}	10.7^{b}	13.1^{a}	8.9^{c}
Lightness (L*)	64.2^{a}	60.3^{c}	57.5^{d}	61.4^{b}
Redness (a*)	6.0^{d}	15.2^{b}	17.7^{a}	10.9^{c}
Yellowness (b*)	48.8^{c}	47.6^{d}	55.1^{b}	57.2^{a}
TBARS (mg MDA/kg)				
0 days	1.17^{a}	1.00^{b}	0.87^{c}	0.90^{c}
28 days	1.28^{a}	1.16^{b}	1.04^{c}	1.07^{c}

$^{a-d}P < 0.05$; MDA – malondialdehyde

Table 2: Daily output of eggs, yolk colour and contents of carotenoids in yolks. Results of the Experiment II.

	Control	Carophylls	Lutein	Mustard
Hen-day egg production (%)	89.0	90.0	85.5	91.8
Yolk colour (DSM Fan)	7.7^{c}	11.8^{a}	8.4^{b}	8.3^{b}
Carotenoids (mg/g DM)				
β-Carotene	0.053^{c}	0.078^{b}	0.088^{a}	0.055^{c}
Lutein	16.1^{c}	18.7^{b}	31.7^{a}	15.6^{c}
Zeaxanthin	10.5^{c}	14.8^{b}	20.3^{a}	10.9^{c}

$^{a-c}P < 0.05$; DM – dry matter

IV. DISCUSSION

All carotenoids are efficient antioxidants that alleviate the oxidative stress. Eggs are excellent vehicles for the carry-over of carotenoids in the human food chain [8]. Lutein and its isomer zeaxanthin accumulate in the macular region of the retina and protected eyes against the development of cataract and macular degeneration [9]. Lutein-enriched eggs have greater lutein bioavailability for humans than commercial supplements [10].

The egg yolk colour is one of the main characteristics of egg quality. To satisfy consumer's demand, synthetic carotenoids are used as colouring agents. These carbonyl derivatives include ethyl ester of β-apo-8´-carotenoic acid, canthaxanthin and astaxanthin. However, some harmful side effects of the canthaxanthin application have been described in the literature [11]-[12], thus the maximum amount of canthaxanthin should be 8 mg/kg feed. Lutein and spray-dried algae *Chlorella* thus represent a suitable alternative to synthetic carotenoids. From an economical point of view, the use of *Chlorella* is more advantageous than that of lutein, which is rather expensive. Our results are consistent with those of Kotrbáček et al. [8] who supplemented a diet of laying hens with 1 or 2% of dry disintegrated *Chlorella*. Egg yolk deposition of total carotenoids was significantly ($P< 0.01$) increased by 46 and 119%, also the yolk colour was increased. Another natural source of carotenoids, usable in the nutrition of layers, is the extract of flowers of marigold [13], available commercially as Avizant® Yellow 20 HS (Lohmann Animal Health, Cuxhaven, Germany).

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