A comparison of lutein, spray-dried *Chlorella*, and synthetic carotenoids effects on yolk colour, oxidative stability, and reproductive performance of laying hens

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ABSTRACT: ISA Brown hens were fed diets supplemented with the synthetic carotenoids Carophyll Red and Carophyll Yellow at 20 and 15 mg/kg, respectively, lutein at 250 mg/kg, and the algae *Chlorella* at 12.5 g/kg. The synthetic carotenoids, lutein, and *Chlorella* significantly increased egg weight ($P < 0.001$), shell weight ($P < 0.001$), and thickness ($P = 0.017$) and decreased the yolk/albumen ratio ($P = 0.035$) of the eggs. Lutein but not the Carophylls or *Chlorella* significantly increased the shell breaking strength ($P = 0.032$). Furthermore, the carotenoids and *Chlorella* significantly ($P < 0.001$) increased yolk colour, and the yolk redness increased significantly ($P < 0.001$) in the following order: control < *Chlorella* < Carophyll < lutein. Lutein and *Chlorella* increased the yellowness of the yolks, and boiling the eggs for 5 min increased the redness of the yolks, while boiling them for 10 min increased the lightness and reduced the colour of the yolks. Supplementation of feed with lutein and *Chlorella* significantly ($P < 0.001$) increased the concentration of lutein (from 12.8 to 133.9 and 49.0 mg/kg dry matter) and zeaxanthin (from 9.2 to 123.9 and 40.1 mg/kg dry matter) in the yolks, and all carotenoids and *Chlorella* significantly ($P < 0.001$) increased the oxidative stability of the lipids of fresh eggs and eggs that had been stored at 18°C for 28 days.

Keywords: carophyll; alga; egg quality; yolk colour; cooking length

Carotenoids are routinely fed to chickens in the commercial poultry industry to provide pigmentation for egg yolks and, in some areas, to modify the pigmentation of chicken skin. Apart from their colouring effect, these pigments are important for their antioxidant and immunomodulatory functions (Goodwin, 1986). Colour is an important characteristic and selection criterion for food choice by consumers. To meet market needs, the feed industry frequently adds the synthetic carotenoids Carophyll® Red (canthaxanthin) and Carophyll® Yellow (ethyl ester of β-apo-8'-carotenoic acid) or the related carotenoids Lucantin® Red and Lucantin® Yellow to the diets of laying hens.

Eggs are good vehicles for the carry-over of carotenoids in the human food chain. Over the last few decades, an awareness of the role of lutein and zeaxanthin in the prevention of certain eye disorders has increased. These carotenoids accumulate in the macular region of the retina and protect against the development of cataracta and macular degeneration. Moreover, several studies have shown that egg yolk can provide a highly available source of lutein and zeaxanthin. Handelman et al. (1999) observed that diets containing cooked chicken egg yolks (1.3 egg yolk/day) increased plasma levels of lutein by 28% and that of zeaxanthin by 142% in adults aging 62 years on average. Furthermore, Goodrow et al. (2006) reported that serum levels of lutein were elevated by 26% and levels of zeaxanthin by 38% in older adults (> 60 years of age) who had consumed 1 egg/day for 5 weeks.

The enrichment of egg yolks with lutein by feeding hens diets containing commercially available

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lutein (Leeson and Caston, 2004; Leeson et al., 2007; Golzar Adabi et al., 2010), algae (Fredriksson et al., 2006), and carrots (Hammershøj et al., 2010) has been reported. Dietary lutein supplementation at 250 mg/kg increased the lutein concentration in egg yolks from 0.12 to 1.35 mg/57 g of egg mass (Golzar Adabi et al., 2010). Additionally, plasma lutein was significantly increased in men around 60 years and who consumed one lutein-enriched egg per day. Leeson and Caston (2004) showed that adding lutein at a concentration of 375 mg/kg to the diet of hens increased the lutein content of their yolks from 0.3 to 1.5 mg/60 g of egg. However, when the hens were fed a diet supplemented with above 375 mg/kg, no further increase in yolk lutein content was observed. In the subsequent experiment, the authors showed that the lutein content in egg yolks plateaued at 125 mg/kg diet, or 1.67 mg/egg (Leeson et al., 2007). Fredriksson et al. (2006) tested the addition of the marine microalgae *Nannochloropsis oculata* at 20% of the dry matter (DM) diet of hens. The lutein and zeaxanthin content in the eggs after 4 weeks of feeding was 22 mg/kg, i.e. 1.32 mg/60 g of egg. Hammershøj et al. (2010) examined the effect of three carrot varieties on the deposition of carotenoids in the yolk. Hens were fed a standard feed that had been supplemented with orange, yellow or purple carrots at 70 g per hen per day. Carrot supplementation increased the lutein concentration in yolks from 7.5 to 8.2–12.2 mg/kg, and the concentration of total carotenoids was increased from 10.8 to 14.0–20.2 mg/kg. The deposition efficiency of lutein and zeaxanthin from feed to egg yolk was approximately 25%, and the deposition efficiency of β-carotene was only 0.5%.

The objective of the present study was to compare the effect of synthetic carotenoids (Carophylls), lutein, and spray-dried algae *Chlorella* on the performance of laying hens, colour of yolks, deposition of lutein, content of zeaxanthin and β-carotene in yolks, and oxidative stability of yolk lipids.

MATERIAL AND METHODS

Hens, diets, and husbandry

Two hundred and forty ISA Brown hens aged 25–39 weeks (including 2 weeks of preliminary period) were housed in three-floor, enriched cages, and 10 hens per cage were placed in the same air-conditioned facility. The cage provided 7560 cm^2 of floor area, which did not include the nest, 120 cm for the feeder, and 3 nipple water dispensers. The cages were equipped with a nest box, perch (150 cm), dust bath, and equipment for the abrasion of claws, which conforms to the European Union Council Directive 1999/74/EC (1999). The room temperature was maintained at 20–22°C, and the light cycle consisted of 16 h of light and 8 h of darkness. The light intensity was approximately 10 lx in the central storey.

Hens were randomly assigned to 1 of 4 dietary treatments, each with 6 replicate cages. The control group was fed a diet that lacked carotenoids. The hens of the second group (Carophyll) were fed a combination of Carophyll® Red and Carophyll® Yellow (DSM Nutritional Products, Basel, Switzerland, local supplier Trouw Nutrition Biofak-

Table 1. Ingredients and chemical composition of the basal diet[a]

Ingredient	g/kg
Maize	355.0
Wheat	243.2
Soybean meal	215
Rapeseed oil	30
Wheat bran	15
Lucerne meal	20
Fish meal	15
Dicalcium phosphate	18
Sodium chloride	2
Limestone	81
DL-Methionine	0.8
Vitamin-mineral premix[b]	5
Analyzed nutrient content	g/kg
Dry matter	885
AME_N by calculation (MJ/kg)	11.3
Crude protein	176.9
Calcium	37.4
Phosphorus	5.6

[a]other experimental diets were supplemented with 20 mg/kg Carophyll Red in combination with 15 mg/kg Carophyll Yellow, 250 mg/kg lutein, and 12.5 g/kg alga *Chlorella*

[b]vitamin-mineral premix provided per kg diet: retinylacetate 3.0 mg, vitamin D_3 3000 IU, vitamin E 30 mg, niacin 25 mg, Ca pantothenate 8 mg, thiamine 2.0 mg, riboflavin 5 mg, pyridoxine 4 mg, folic acid 0.5 mg, biotin 0.075 mg, cobalamin 0.01 mg, choline Cl 250 mg, menadione 2.0 mg, betain 100 mg, butylated hydroxytoluene 7.5 mg, ethoxyquin 5.6 mg, butylhydroxyanisole 1 mg, DL-methionine 0.7 g, Mn 70 mg, Zn 50 mg, Fe 40 mg, Cu 6 mg, I 1 mg, Co 0.3 mg, Se 0.2 mg

tory s.r.o., Prague, Czech Republic) in the amount of 20 and 15 mg/kg diet, respectively. Carophyll® Red and Carophyll® Yellow added canthaxanthin (2.0 mg/kg) and ethyl ester of β-apo-8'-carotenoic acid (1.5 mg/kg), respectively, to the diet. Lutein was added to the diet of the third treatment group (Lutein) as a Lutein powder extract (90%) (Alchimica, Prague, Czech Republic) at 250 mg/kg. The fourth treatment group (*Chlorella*) was fed a diet supplemented with 12.5 g/kg of spray-dried alga *Chlorella* sp. (autotrophic cultivation) (Institute of Microbiology, Třeboň, Czech Republic). The ingredients and nutrient composition of the basal diet are shown in Table 1, and all diets were stored in a dark, air-conditioned room at a temperature of 18–20°C and a relative humidity ranging 50–60% for 12 weeks. Feed and fresh water were supplied to the animals *ad libitum*. This experiment was approved by the Ethical Committee of the Institute of Animal Science.

Sampling and analyses

Eggs were collected daily, and the performance parameters were calculated weekly. Each week, all eggs were weighed on three consecutive days. To determine the physical characteristics of the eggs (Tůmová and Gous, 2012), 624 eggs were analyzed. The eggs were collected during the weeks 29, 34, and 37 of the hens' age. The shell breaking strength and shell deformation were determined on the vertical axis using the Instron 3360 apparatus (Instron, Canton, USA), and the albumen and yolk height were measured using a tripod digital micrometer (Keener et al., 2006). The Haugh units (HU) were calculated as indicated by Haugh (1937), and the shell thickness (i.e. the average of 3 values: from the sharp and blunt ends and equator) after removing the shell membranes was measured using a micrometer. The albumen, yolk, and shell percentages were determined by considering the individual weight of each egg and weight of its components, and the shell weight with membranes was determined after drying at 105°C. The egg-shell index was calculated after Ahmed et al. (2005) as follows:

$$SI = (SW/S) \times 100$$

where:
SW = shell weight
S = shell surface calculated as $S = 4.68 \times EW^{0.75}$
EW = egg weight

The formula for the albumen index calculation was: AI = albumen height/(0.5 long diameter of albumen + 0.5 short diameter of albumen) × 100. The yolk index was calculated as YI = (yolk height/yolk diameter) × 100 and the yolk colour was measured using the DSM Yolk Colour Fan (shown as La Roche in Tables) (DSM Nutritional Products, Basel, Switzerland). Other yolk parameters (L^*, a^*, b^*) were measured using a Minolta CR-300 colorimeter (Konica Minolta, Osaka, Japan). The L^*, a^*, and b^* values reflected lightness (0 = black, 100 = white), redness (–100 = green, 100 = red), and yellowness (–100 = blue, 100 = yellow), respectively.

The colour of fresh and cooked (for 5, 7, and 10 min) yolks (n = 10) was evaluated in week 37 of the hens' age, and 160 eggs were analyzed by the same method as described above.

Analyses of the P and Ca content of the eggshells were conducted twice during the experiment (during weeks 27 and 39 of the hens' age), and 384 eggs were analyzed (4 eggs per sample). Dried homogenized eggshells were ashed in a muffle furnace at 500°C for 12 h, and the ash was dissolved in 3M hydrochloric acid and quantitatively transferred into a volumetric flask. The P in the solution was determined using vanadate-molybdate reagent (AOAC, 2005; method 965.17), and the Ca concentration in the hydrochloric acid extract was measured by atomic absorption spectrometry using the Solaar M6 instrument (TJA Solutions, Cambridge, UK).

Four hundred and thirty-two eggs were used to determine the vitamin and carotenoid content in the egg yolks twice during the experiment (weeks 28 and 39 of the hens' age; 3 eggs per sample). The α-tocopherol, retinol, and β-carotene contents of the yolks were determined in accordance with the European standards EN 12822 (2000), EN 12823-1 (2000), and EN 12823-2 (2000) for high-performance liquid chromatography (HPLC). The instrument was equipped with a diode-array detector (VP series; Shimadzu, Kyoto, Japan). The content of lutein and zeaxanthin in the yolk was measured by HPLC according to a modified method of Froescheis et al. (2000).

Lipid peroxidation in the yolks of fresh eggs and eggs that had been stored for 28 days at 18°C was measured twice during the experiment (weeks 27 and 39 of the hens' age) using the thiobarbituric acid method described by Piette and Raymond (1999), and in total, 864 eggs were analyzed (3 eggs per sample). Thiobarbituric acid-reactive substances were expressed as mg of malondialdehyde per kg.

The feed dry matter was determined by oven drying at 105°C to a constant weight, and the crude protein content of the feed was measured using a Kjeltec Auto 1030 instrument (Tecator, Höganäs, Sweden). Determination of the concentrations of P, Ca, vitamins, and carotenoids in the feed was performed using the above-described methods.

Statistical analysis

The data from the experiment were analyzed using the Analysis of Variance (ANOVA) with the General Linear Models (GLM) Procedure of SAS (Statistical Analysis System, Version 9.2, 2003). One-Way Analysis of Variance, where the main effect was the source of carotenoids, was used to compare the performance, physical characteristics, vitamin and carotenoid contents, and oxidative stability of yolks. The data regarding yolk colour characteristics were statistically analyzed by the Two-Way Analysis of Variance (with the main effect of cooking length, source of carotenoids, and their interaction), and all of the differences were considered significant at $P < 0.05$. The results given in Tables are presented as the mean and standard error of the mean (SEM).

RESULTS

Performance of hens and physical characteristics of eggs

The synthetic and natural carotenoids significantly increased egg weight and improved feed conversion (Table 2). The egg weight of hens that were fed the Carophyll diet was significantly higher than that of hens fed other diets; however, the egg mass production in hens fed the Carophyll diet was not significantly different from that of hens fed lutein or *Chlorella* diets. The weights of the eggs that were collected for measuring egg and shell characteristics were significantly lower in the control group (Table 3), and the albumen weights were significantly lower and the yolk/albumen ratios and yolk percentages were significantly higher than in the eggs of hens that were fed diets containing carotenoids. All carotenoids significantly increased the shell weight, shell surface, shell index, and shell thickness. Lutein but not Carophylls or *Chlorella* significantly increased the shell breaking strength of the eggs, and the eggshells of hens that were fed the diet containing *Chlorella* contained significantly less Ca and more P than the eggshells of other hens. Carotenoids significantly increased yolk colour, and according to the DSM Yolk Colour Fan, the strongest effect in fresh eggs was from lutein, which increased the redness (a^*) of the yolks, whereas *Chlorella* increased the yellowness (b^*) of the eggs. Boiling the eggs significantly influenced the colour of their yolks (Table 4). According to the DSM Yolk Colour Fan, boiling for 5 min increased the redness of the yolks from all groups of hens, and boiling for 10 min increased the lightness (L^*) and decreased the colour of the yolks. In hens that were fed lutein, the boiling of eggs for 10 min increased the yellowness of the yolks whereas in other hens, the yellowness of the yolks decreased.

Vitamins, carotenoids, oxidative stability of yolks

The diets did not differ in their concentrations of α-tocopherol and retinol (Table 5); however,

Table 2. Mean performance characteristics of laying hens

Characteristics	Control	Carophyll	Lutein	*Chlorella*	SEM	Probability
Hen-day egg production (%)	92.8	93.4	93.9	93.8	0.29	ns
Egg weight (g)	61.1^{c}	62.9^{a}	62.1^{b}	62.3^{b}	0.09	< 0.001
Egg mass (g/hen/day)	55.5^{b}	58.6^{a}	56.1^{b}	57.0^{ab}	0.36	0.013
Feed intake (g/day/hen)	118	118	117	117	0.30	ns
Feed intake (g/egg)	129	127	127	127	0.60	ns
FCR (g/g)	2.14^{a}	2.05^{c}	2.1^{a}b	2.06^{bc}	0.01	0.002
Mortality (%)	1.7	0	0	0	0	0

ns = nonsignificant

$^{a–c}$means in the same row with different superscripts differ significantly

Table 3. Physical characteristics of eggs and calcium and phosphorus contents in eggshells

Characteristics	Control	Carophyll	Lutein	*Chlorella*	SEM	Probability
Egg weight (g)	61.0^{b}	63.0^{a}	62.3^{a}	62.6^{a}	0.21	0.005
Eggshell surface (cm^2)	72.3^{b}	73.9^{a}	73.3^{a}	73.5^{a}	0.16	0.006
Yolk and albumen ratio (%)	38.2^{a}	37.0^{b}	37.1^{b}	37.2^{b}	0.18	0.035
Albumen height (mm)	7.4	7.6	7.5	7.4	0.06	ns
Albumen index (%)	9.4	9.6	9.5	9.2	0.09	ns
Haugh Units	85.1	85.6	85.4	85.1	0.33	ns
Albumen weight (g)	40.0^{b}	41.6^{a}	41.1^{a}	41.2^{a}	0.17	0.006
Albumen percentage (%)	65.4	65.9	65.8	65.8	0.09	ns
Yolk height (mm)	18.0	18.1	17.9	17.9	0.04	ns
Yolk index (%)	44.3	44.5	44.7	44.3	0.11	ns
Yolk weight (g)	15.2	15.2	15.1	15.2	0.06	ns
Yolk percentage (%)	24.9^{a}	24.3^{b}	24.3^{b}	24.4^{b}	0.08	0.012
Yolk colour						
La Roche	6.4^{d}	10.7^{b}	13.1^{a}	8.9^{c}	0.12	< 0.001
Lightness (L^*)	64.2^{a}	60.3^{c}	57.5^{d}	61.4^{b}	0.17	< 0.001
Redness (a^*)	6.0^{d}	15.2^{b}	17.7^{a}	10.9^{c}	0.21	< 0.001
Yellowness (b^*)	48.8^{c}	47.6^{d}	55.1^{b}	57.2^{a}	0.24	< 0.001
Shell thickness (μm)	332^{b}	340^{a}	340^{a}	338^{ab}	1.0	0.017
Shell deformation (mm)	0.46	0.46	0.47	0.47	0.002	ns
Shell breaking strength (N)	39.0^{b}	39.4^{b}	41.1^{a}	40.1^{ab}	0.28	0.032
Shell index (g/100 cm^2)	8.1^{b}	8.4^{a}	8.4^{a}	8.3^{a}	0.03	0.005
Shell weight (g)	5.9^{b}	6.2^{a}	6.1^{a}	6.1^{a}	0.02	< 0.001
Shell percentage (%)	9.7	9.8	9.9	9.8	0.03	ns
Shell Ca content (g/kg ash)	394^{a}	393^{ab}	389^{b}	380^{c}	1.0	< 0.001
Shell P content (g/kg ash)	1.19^{c}	1.21^{c}	1.32^{b}	1.51^{a}	0.023	< 0.001

ns = nonsignificant

$^{a–d}$means in the same row with different superscripts differ significantly

supplementation of the feed with lutein increased its lutein and zeaxanthin concentrations. A large portion of lutein was lost during feed storage, presumably due to its low oxidative stability. Supplementation of the feed with spray-dried *Chlorella* increased its concentrations of β-carotene, lutein, and zeaxanthin, and concentrations of carotenoids in egg yolks paralleled those in the feed. The highest concentration of lutein and zeaxanthin was in the yolks of eggs from hens of the lutein group, and the highest concentration of β-carotene was in the eggs of hens that had been fed spray-dried *Chlorella*.

All carotenoids significantly increased the oxidative stability of yolk lipids, expressed as TBARS level (Table 6), and lutein and *Chlorella* supplements were more efficient in this respect than Carophylls.

DISCUSSION

In previous studies, dietary antioxidants did not affect the performance of laying hens. Leeson and Caston (2004) did not observe an effect by lutein that was supplemented at 125 to 1000 mg/kg of a corn-soybean diet on egg production, egg weight, feed intake, or shell quality. Furthermore, lutein at 125 or 250 mg/kg of diet had no effect on feed intake, egg weight or eggshell deformation (Leeson et al., 2007). Canthaxanthin supplementing the diet at a dose of 6 mg/kg improved the hatchability rate but did not influence the laying rate of broiler breeders (Rosa et al., 2012). In our experiment, Carophylls, lutein, and spray-dried *Chlorella* significantly increased egg weight by 2.9, 1.6, and 2.0%, respectively, and significantly increased albumen weight but not yolk weight. The design of

Table 4. Effect of cooking length on yolk colour

Cooking length (min)	Carotenoid	La Roche	Lightness (L^*)	Redness (a^*)	Yellowness (b^*)
0	Control	5.6^{f}	64.6^{c}	4.7^{gh}	48.8^{efg}
	Carophyll	10.9^{c}	60.6^{e}	14.7^{d}	47.5^{fgh}
	Lutein	13.1^{b}	56.9^{fg}	18.3^{c}	55.9^{c}
	Chlorella	7.6^{e}	62.2^{d}	8.4^{f}	55.8^{c}
5	Control	4.9^{g}	60.8^{de}	8.3^{f}	51.8^{de}
	Carophyll	9.9^{d}	56.0^{gh}	26.9^{a}	50.2^{defg}
	Lutein	13.9^{a}	47.4^{i}	20.7^{b}	48.8^{defgh}
	Chlorella	9.4^{d}	53.8^{h}	14.1^{d}	51.4^{def}
7	Control	4.4^{gh}	65.5^{c}	7.6^{f}	46.8^{fgh}
	Carophyll	11.0^{c}	57.2^{fg}	17.4^{c}	44.9^{h}
	Lutein	12.8^{b}	59.1^{ef}	18.8^{bc}	60.2^{b}
	Chlorella	3.4^{i}	78.4^{b}	7.3^{f}	53.5^{cd}
10	Control	1.0^{k}	87.4^{a}	3.5^{h}	37.4^{i}
	Carophyll	3.5^{hi}	81.1^{b}	11.3^{e}	37.7^{i}
	Lutein	7.1^{e}	79.9^{b}	14.3^{d}	73.3^{a}
	Chlorella	2.3^{j}	80.0^{b}	6.5^{fg}	39.7^{i}
SEM		0.20	0.47	0.36	0.45
Probability	C	< 0.001	< 0.001	< 0.001	< 0.001
	CL	< 0.001	< 0.001	< 0.001	< 0.001
	C × CL	< 0.001	< 0.001	< 0.001	< 0.001

CL = cooking length, C = carotenoid

$^{a–i}$means in the same column with different superscripts differ significantly

the present experiment precluded the identification of the reasons for the improvements in performance; however, a beneficial effect of carotenoids on the health of the hens may be involved. The highest shell breaking strength was observed in eggs of hens that were fed lutein, but no relationship of this parameter to shell thickness, shell weight or Ca and P content of the shells was apparent. A greenish hue of the

Table 5. Vitamin and carotenoid content in feed and egg yolks

Characteristics	Control	Carophyll	Lutein	*Chlorella*	SEM	Probability
Mixed feed (mg/kg DM)						
α-Tocopherol	29.90	28.30	30.60	25.40		
Retinol	2.91	2.93	2.78	2.62		
β-carotene	0.57	0.53	0.63	4.35		
Lutein	1.20	1.40	67.20	21.40		
Zeaxanthin	1.00	0.70	63.70	18.70		
Egg yolk (mg/kg DM)						
α-Tocopherol	143^{ab}	147^{a}	130^{c}	136^{bc}	1.50	0.002
Retinol	10.10^{c}	10.70^{b}	11.20^{a}	10.60^{b}	0.09	0.002
Beta-carotene	0.05^{b}	0.04^{b}	0.10^{b}	0.36^{a}	0.016	< 0.001
Lutein	12.80^{c}	11.50^{c}	133.90^{a}	49.0^{b}	5.07	< 0.001
Zeaxanthin	9.20^{c}	8.70^{c}	123.90^{a}	40.1^{b}	4.84	< 0.001

DM = dry matter

$^{a–c}$means in the same row with different superscripts differ significantly

Table 6. Oxidative stability of yolks of fresh eggs (MDA 0) and eggs stored at 18°C for 28 days (MDA 28)

Characteristics	Control	Carophyll	Lutein	*Chlorella*	SEM	Probability
MDA 0	1.17[a]	1.00[b]	0.87[c]	0.90[c]	0.018	< 0.001
MDA 28	1.28[a]	1.16[b]	1.04[c]	1.07[c]	0.013	< 0.001

MDA = malondialdehyde content (mg/kg)

[a–c]means in the same row with different superscripts differ significantly

egg yolks of lutein-fed hens, which was mentioned by Breithaupt (2007), was observed neither in fresh nor in boiled eggs in our experiment.

The intensity and colour (yellow-red) of yolks can be controlled by the concentration and type of dietary carotenoids. The deposition of carotenoids in yolks depends on their polarity, which is lower in nonpolar carotenes than in xanthophylls that contain at least one atom of oxygen. Indeed, the deposition of β-carotene in egg yolks was low in the present study, whereas the deposition of lutein, zeaxanthin, and retinol was high. Kotrbáček et al. (2013) showed that egg yolk deposition of total carotenoids was significantly increased (by 46 and 119%) after addition of 10 and 20 g of heterotrophic *Chlorella* per kg diet, respectively. The oxidative stability of yolk lipids was higher in eggs of hens that were fed lutein and spray-dried *Chlorella* than in eggs of hens that were fed Carophylls; however, the concentration of antioxidants in the feed of the former hens was higher, mainly because the concentration of Carophylls in that feed has been limited by the EU legislation. When canthaxanthin, which is present in Carophyll® Red, is added to the feed of laying hens, a maximum amount of 8 mg/kg is allowed (European Union Council Directive 70/524/EEC, 1970). A reason for this limit is the formation of crystalline deposits in the retina of humans who take in high dietary amounts of canthaxanthin (Arden and Barker, 1991). The corresponding upper concentration of the ethyl ester of β-apo-8'-carotenoic acid (Carophyll® Yellow) is 80 mg/kg.

Canthaxanthin and its related yellow-pigmented ethyl ester of β-apo-8´-carotenoic acid are synthetic compounds that are not allowed in organic farming. Lutein is also forbidden in organic farming because this pigment is usually obtained by extracting plant material using organic solvents (Breithaupt, 2007). A more suitable source of carotenoids is spray-dried *Chlorella*, which quadrupled the concentration of lutein and zeaxanthin in yolks when compared with the control and increased the oxidative stability of yolk lipids. The positive effect of selenium-enriched *Scenedesmus* on oxidative stability of lipids was described by Skřivan et al. (2010). Zahroojian et al. (2011) concluded that the marine algae *Spirulina platensis*, when added at 2.5% to a wheat-soybean diet, was as effective in producing agreeable yolk colour as the synthetic pigments Lucantin® Yellow and Lucantin® Red (BASF). Furthermore, supplementation of laying hens' diets with the marine algae *Nanochloropsis oculate* at 10% (based on DM) significantly increased the redness and decreased the lightness of their yolks (Fredriksson et al., 2006). When 20% algae were included in the diet of hens, the content of total carotenoids in their eggs increased from 9.7 to 37.0 mg/kg of the eggs.

CONCLUSION

From the present experiment it can be concluded that lutein and spray-dried *Chlorella* increased the concentration of health-promoting carotenoids in yolks and increased egg production and the quality of the eggs. From an economical point of view, the use of *Chlorella* is more advantageous than that of lutein.

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