EFFECT OF SYNTHETIC CAROTENOIDS, LUTEIN, AND MUSTARD ON THE PERFORMANCE AND EGG QUALITY*

M. Englmaierová, M. Skřivan

Institute of Animal Science, Prague-Uhříněves, Department of Nutrition Physiology and Animal Product Quality, Prague, Czech Republic

The aim of the present study was to evaluate the effect of synthetic and natural sources of carotenoids on the laying performance of hens, their egg quality, and the content of vitamins and carotenoids in the egg yolks. The hens were fed a diet without added carotenoids and supplemented with a combination of the following: Carophyll® Yellow and Carophyll® Red at 15 and 20 mg kg^{-1}, respectively; lutein at 100 mg kg^{-1} as a lutein powder extract (90%); and meal consisting of *Brassica juncea* (L.) at 10 g kg^{-1}. Carophyll and lutein significantly increased albumen quality, which was evaluated on the basis of albumen height ($P = 0.002$), albumen index ($P < 0.001$), and Haugh units ($P = 0.002$). The yolk colour parameters significantly increased ($P < 0.001$) in the following order: control, mustard meal, lutein, and Carophyll. The addition of Carophyll increased ($P < 0.001$) the redness value of the yolks, whereas lutein increased ($P < 0.001$) the yellowness of the yolks. Compared with the control group, supplementing the diets with Carophyll and lutein significantly ($P < 0.001$) increased the concentrations of β-carotene (by 47 and 66%), lutein (by 17 and 97%), and zeaxanthin (by 42 and 94%) in the egg yolks, respectively.

carotenoids; yolk quality; vitamin; Carophyll; *Brassica juncea*; laying hen

INTRODUCTION

Carotenoids are yellow, orange, and red natural pigments that are synthesized by photosynthetic organisms (plants and microorganisms), and they are responsible for the colouring of various fruits, vegetables, and flowers. Carotenoids may serve as a precursor to vitamin A; they also have antioxidant properties, which protect against the damaging oxidizing effects of free radicals and have immunomodulatory functions (Goodwin, 1986). Animals cannot produce carotenoids, so they must obtain them through their diet and store them in their fatty tissues (e.g. in egg yolk).

Carotenoids, lutein, and zeaxanthin are important for human protection against age-related macular degeneration (Granado et al., 2003). Dried marigold and paprika, or extracts from these and other plants are used as a natural source of carotenoids (Lokaewmanee et al., 2011). Leeson et al. (2007) showed that eggs could be enriched with 1.6 mg of lutein per 60 g egg from a basal level of 0.10 mg per 60 g egg with the addition of natural lutein (250 mg kg^{-1}) to the diet. Supplementing the feed of egg-laying hens with carrots efficiently increased yolk colour parameters and carotenoid contents, which gives opportunities for improved nutritional value of eggs from forage material-supplemented hens (Hammershøj et al., 2010). Lokaewmanee et al. (2011) showed that dietary lutein from marigold enhanced egg yolk colour at levels of approximately 30–40 mg kg^{-1}. It should be noted that increased lutein supplementation was related to decreased efficiency of its transfer to the egg. The transfer efficiency of lutein from feed to eggs was around 10% with 125 mg kg^{-1} in the diet, declining to 2–3% with 500 mg kg^{-1} (Steinberg et al., 2000; Leeson, Caston, 2004). Lutein-enriched eggs have greater lutein bioavailability for humans than other supplements (Chung et al., 2004).

From a consumer perspective, egg yolk colour is one of the main characteristics of egg quality. In intensive farming, synthetically produced carbonyl derivatives of carotenes are used as a source of carotenoids. These derivatives include ethyl ester of β-apo-8´-carotenoic acid known as Carophyll Yellow, canthaxanthin known as Carophyll Red, and astaxanthin known as Carophyll Pink. If canthaxanthin is added to the feed of laying hens, a maximum amount of 8 mg kg^{-1} is allowed. This regimentation is due to an unwanted side effect of canthaxanthin application (Arden, Barker, 1991; Baker, 2001; Breithaupt, 2007). Therefore,

* Supported by the Ministry of Agriculture of the Czech Republic, Project No. QJ1310002 and Project No. MZE 0002701404.

synthetic canthaxanthin is classified in the European Union as a potentially hazardous substance for human health.

Mustard seed is a potential source of carotenoids. Mustard seed meal has limited uses as animal feed because of its high content of erucic acid and other anti-nutritional substances. Therefore, information in the literature regarding mustard seed colouration of egg yolks is not sufficient. This study's objective was to compare the effect of synthetic carotenoids (Carophyll), lutein, and mustard meal on the laying performance of hens, their egg quality, deposition of vitamins and carotenoids in the yolks, and oxidative stability of yolk lipids.

MATERIAL AND METHODS

Hens, diet, and husbandry

One hundred and sixty ISA Brown hens, aged 20–34 weeks (including 2 weeks of preliminary period), were housed in three-storey enriched cages, and 10 hens per cage were placed in the same air-conditioned facility. The cages provided 7560 cm^2 of floor area, which did not include the nest, 120 cm^2 for the feeder and 3 nipple water dispensers. The cages were equipped with a nest box, perch (150 cm), dust bath, and equipment for claw abrasion, 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 light and 8 h darkness. The light intensity was approximately 10 lx on the central storey.

The hens were randomly assigned to 1 of 4 dietary treatments, each with 4 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® Yellow and Carophyll® Red (DSM Nutritional Products, Basel, Switzerland, local supplier Trouw Nutrition Biofaktory s.r.o., Prague, Czech Republic) in the amount of 15 and 20 mg kg^{-1}, respectively. Carophyll® Yellow and Carophyll® Red added ethyl ester of β-apo-8´-carotenoic acid (1.5 mg kg^{-1}) and canthaxanthin (2.0 mg kg^{-1}), 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 100 mg kg^{-1}. The fourth treatment group (Mustard meal) was fed a diet supplemented with 10 g kg^{-1} of meal from *Brassica juncea* (L.) Czern. (Opaleska variety) (OSEVA PRO s.r.o., Opava, Czech Republic). The mustard meal contained 923 g of dry matter, 282 g of crude protein, 307 g of fat, 49 g of ash, 15.9 MJ of apparent metabolizable energy (AME_N), 11.9 mg of lutein, and 5.2 mg of zeaxanthin per kg and 20.3% (i.e. % of total determined fatty acids) of erucic acid. The ingredients and nutrient composition of the basal diet are shown in Table 1. The control diet contained (per kg dry matter (DM)): 26.9 mg of α-tocopherol, 1.9 mg of retinol, 0.7 mg of β-carotene, 1.5 mg of lutein, and 1.1 mg of zeaxanthin. All feed was stored in a dark, air-conditioned room at a temperature of 18–20°C and at a relative humidity that ranging 50–60%. Feed and fresh water were supplied to the animals *ad libitum*.

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

Ingredient (g kg^{-1})	
Maize	355.0
Wheat	246.2
Soybean meal	215
Rapeseed oil	30
Wheat bran	15
Lucerne meal	20
Fish meal	15
Dicalcium phosphate	11
Sodium chloride	2
Limestone	85
DL-Methionine	0.8
Vitamin-mineral premix[2]	5
Analyzed nutrient content (g kg^{-1})	
Dry matter	890.0
AME_N by calculation (MJ kg^{-1})	11.4
Crude protein	166.5
Calcium	37.1
Non phytate phosphorus	3.1

[1]other experimental diets were supplemented with 20 mg kg^{-1} of Carophyll Red in combination with 15 mg kg^{-1} of Carophyll Yellow, 100 mg kg^{-1} of lutein, and 10 g kg^{-1} of mustard meal

[2]Vitamin-mineral premix provided per kg of 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, betaine 100 mg, butylated hydroxytoluene 7.5 mg, ethoxychin 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

Sampling and analyses

The eggs were collected every day, and the laying performance parameters were calculated weekly. Each week, the eggs were weighed on three consecutive days, the average values are shown in Table 2. The eggs for the physical characteristics determination were collected during the weeks 23, 27, and 31 of the hens' age. All laid eggs were examined. A total of 415 eggs were analyzed. Egg weight was measured on a labora-

Table 2. Performance characteristics of laying hens

Characteristics	Control	Carophyll	Lutein	Mustard meal	SEM	Probability
Hen-day egg production (%)	89.0	90.0	88.5	91.8	0.67	ns
Egg weight (g)	59.8	59.3	59.6	59.4	0.24	ns
Egg mass (g per hen per day)	53.2	53.4	52.7	54.5	0.52	ns
Feed intake (g per day per bird)	114.2	114.0	115.4	113.6	0.46	ns
FCR (g feed) (g egg)$^{-1}$	2.15	2.13	2.19	2.08	0.019	ns

FCR = feed conversion ratio, ns = nonsignificant

Table 3. Physical characteristics of eggs

Characteristics	Control	Carophyll	Lutein	Mustard meal	SEM	Probability
Egg weight (g)	58.9	58.3	58.8	58.7	0.27	ns
Eggshell surface (cm^2)	70.6	70.2	70.5	70.5	0.21	ns
Yolk and albumen ratio (%)	36.3	36.4	35.5	35.9	0.20	ns
Albumen height (mm)	8.1^{ab}	8.4^{a}	8.4^{a}	7.7^{b}	0.07	0.002
Albumen index (%)	10.7^{b}	11.6^{a}	11.9^{a}	10.2^{b}	0.13	< 0.001
Haugh units	89.6^{ab}	91.4^{a}	91.3^{a}	87.9^{b}	0.38	0.002
Albumen weight (g)	38.7	38.6	38.9	38.9	0.19	ns
Albumen percentage (%)	65.8	95.7	66.3	66.1	0.10	ns
Yolk height (mm)	18.5	18.6	18.6	18.5	0.04	ns
Yolk index (%)	47.5	47.8	48.5	48.2	0.15	ns
Yolk weight (g)	14.0	13.9	13.8	13.9	0.09	ns
Yolk percentage (%)	23.8	23.8	23.5	23.6	0.09	ns
Yolk colour						
La Roche	7.7^{c}	11.8^{a}	8.4^{b}	8.3^{b}	0.09	< 0.001
Lightness (L*)	63.1^{a}	58.0^{c}	61.7^{b}	62.3^{b}	0.17	< 0.001
Redness (a*)	7.1^{c}	17.8^{a}	9.1^{b}	7.4^{c}	0.23	< 0.001
Yellowness (b*)	52.5^{b}	49.0^{c}	54.6^{a}	52.9^{b}	0.19	< 0.001
Shell thickness						
Blunt end (mm)	0.336	0.336	0.331	0.327	0.0015	ns
Equator (mm)	0.338	0.338	0.335	0.333	0.0013	ns
Sharp end (mm)	0.349	0.346	0.344	0.341	0.0014	ns
Average (mm)	0.342	0.341	0.338	0.335	0.0013	ns
Shell deformation (mm)	0.488	0.486	0.491	0.484	0.0021	ns
Shell breaking strength (N)	43.60	44.53	43.79	43.08	0.321	ns
Shell index (g 100 cm^{-2})	8.6	8.6	8.5	8.5	0.03	ns
Shell weight (g)	6.1	6.0	6.0	6.0	0.03	ns
Shell percentage (%)	10.3	10.4	10.2	10.2	0.04	ns

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

Table 4. Vitamin and carotenoid content (mg kg^{-1} dry matter) in egg yolks

Characteristics	Control	Carophyll	Lutein	Mustard meal	SEM	Probability
α-Tocopherol	152.0	154.9	160.3	155.3	1.51	ns
Retinol	8.98	9.36	9.18	9.41	0.075	ns
β-Carotene	0.053^{c}	0.078^{b}	0.088^{a}	0.055^{c}	0.0026	< 0.001
Lutein	16.09^{c}	18.74^{b}	31.68^{a}	15.64^{c}	1.085	< 0.001
Zeaxanthin	10.48^{c}	14.84^{b}	20.28^{a}	10.95^{c}	0.645	< 0.001

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

tory scale, the average values are shown in Table 3. 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 (K e e n e r et al., 2006). The Haugh units (HU) were calculated according to the methods of H a u g h (1937), and the shell thickness (i.e. the average thickness of the sharp end, the blunt end, and the 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 the weight of its components, and the shell weight with membranes was determined after drying at 105°C. The egg-shell index was calculated after A h m e d 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).

One hundred and forty-four eggs were used to determine the vitamin and carotenoid content in the egg yolks twice during the experiment (during weeks 25 and 32 of age; 3 eggs per sample). The α-tocopherol, retinol, and β-carotene contents of the yolks were determined in accordance with the European standards E N 1 2 8 2 2 (2000), E N 1 2 8 2 3 - 1 (2000), and E N 1 2 8 2 3 - 2 (2000) for high-performance liquid chromatography (HPLC, instrument 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 F r o e s c h e i s et al. (2000).

The feed and mustard meal dry matter was determined by oven drying at 105°C to a constant weight, and the crude protein content of the feed and mustard meal was measured using a Kjeltec Auto 1030 instrument (Tecator, Höganäs, Sweden). Analyses of the P and Ca content of the diets were conducted. Dry homogenized diets were ashed in a muffle furnace at 550°C, and the ash was dissolved in 3M hydrochloric acid and quantitatively transferred into a volumetric flask. The total P in the solution was determined using a vanadate-molybdate reagent (A O A C , 2005; method 965.17), and the Ca concentration in the hydrochloric acid extract was measured by atomic absorption spectrometry using a Solaar M6 instrument (TJA Solutions, Cambridge, UK). Phytate P in the feed was determined by a capillary isotachophoretic method (D u š k o v á et al., 2001). The fat content in the mustard meal was determined by extraction with petroleum ether using a Tecator Extraction System 1045 Soxtec (Foss Tecator AB, Höganäs, Sweden). The concentration of erucic acid in mustard meal was determined by gas chromatographic analysis using a Hewlett-Packard 5890 gas chromatograph equipped with a programmed HP−Innova capillary column (180–240°C) and flame ionization detector. The concentrations of vitamins and carotenoids in the feed and mustard meal were determined using the above-described methods.

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 the yolks.

All of the differences were considered significant at $P < 0.05$. The results in the Tables are presented as the mean and standard error of the mean (SEM).

RESULTS

The source, either synthetic or natural, of carotenoids did not influence the laying performance characteristics of the hens (Table 2). As shown in Table 3, supplementing feed with carotenoids had a significant effect on the albumen quality and yolk colour. Higher albumen quality characterized by albumen height ($P = 0.002$), albumen index ($P < 0.001$), and Haugh units ($P = 0.002$) was found in the eggs of hens that were fed diets containing additions of Carophyll or lutein. According to the DSM Yolk Colour Fan, carotenoids increased ($P < 0.001$) the yolk colour. The highest efficiency was observed from synthetic Carophyll (11.8). Lutein (8.4) and mustard meal (8.3) also increased the colour of the yolks, in comparison with the control group (7.7). These results correspond with lightness values (L^*; $P < 0.001$). In addition, Carophyll increased ($P < 0.001$) the redness (a^*) of the yolks, whereas lutein increased ($P < 0.001$) the yellowness (b^*) of the yolks. The addition of carotenoids did not influence the parameters of yolk and shell quality.

Feed supplementation with lutein and Carophyll significantly ($P < 0.001$) increased the concentrations of β-carotene, lutein, and zeaxanthin in the egg yolks (Table 4). The content of α-tocopherol and retinol in the egg yolks was not influenced by the source of carotenoids.

DISCUSSION

The present study did not ascertain any change in the performance characteristics of laying hens which

corresponds with data given in the studies by Leeson, Caston (2004) and Leeson et al. (2007); it means that the dietary lutein level (from 125 to 1000 mg kg^{-1} and 125 or 250 mg kg^{-1} of diet) did not affect egg production, the feed intake or the egg weight. Furthermore, Lokaewmanee et al. (2011) showed that the production performance, including hen-day egg production, feed consumption, and egg mass, was not significantly influenced by lutein supplementation levels (10–40 mg kg^{-1} of diet). Contrary to our study, Marangos, Hill (1976) stated that mustard meal could be safely used in a layer diet at 12%, but caused a fishy flavour.

Contrary to the control diet, the addition of synthetic Carophyll and lutein significantly increased the albumen index, probably because of their antioxidant properties (Krinsky, 1993). Zhang et al. (2011) found significant improvement in the antioxidant status of the egg yolk after canthaxanthin supplementation. The lower quality of albumen detected in eggs from hens receiving mustard meal was presumably caused by the anti-nutritional substances contained in mustard. Shell and yolk quality, with the exception of yolk colour, were not influenced by the dietary treatments in the present study. These results are consistent with the observations of Leeson et al. (2007) and Lokaewmanee et al. (2011), who did not find any effect of lutein on egg quality.

From the viewpoint of consumers, the yolk colour is the first parameter of yolk quality, but it generally ranks third among egg quality parameters, after freshness and eggshell quality (Nys, 2000). Consistent with the results of the present study relating to yolk colour, Leeson, Caston (2004) and Leeson et al. (2007) reported that the addition of lutein to hen diets significantly affected the yolk colour; however, the amount of lutein addition influenced this effect minimally. A significant increase in the yolk colorimetric score of the Roche Yolk Colour Fan was evident after the diet was supplemented with 6 mg kg^{-1} of canthaxanthin (Zhang et al., 2011).

Consistent with our results, Karadas et al. (2006) did not find an effect of natural carotenoids on the deposition of retinol and α-tocopherol in the yolk. The lutein, zeaxanthin, and β-carotene content increased significantly in the treatments in the following order (from least to greatest): control, mustard meal, Carophyll, and lutein. Leeson et al. (2007) showed that the addition of 250 mg kg^{-1} of lutein increased its content to 1.6 mg per egg. In a previous study, Leeson, Caston (2004) evaluated the effect of maize-soy diets supplemented with lutein at 125–1000 mg kg^{-1}, which increased the lutein content from 0.3 to 1.5 mg per egg. In addition, Steinberg et al. (2000) and Karadas et al. (2006) noted that increased lutein supplementation is related to decreased efficiency of its transfer into the egg. The transfer efficiency of lutein from feed to eggs was about 10% with 125 mg kg^{-1} of feed, declining to 2–3% with 500 mg kg^{-1}.

CONCLUSION

Carophyll and lutein significantly increased the albumen quality, yolk colour, and carotenoid content. In the case of lutein, 100 g kg^{-1} was sufficient. The inclusion of 10 g kg^{-1} of mustard meal in the diet affected the yolk colour in a manner similar to lutein (evaluated according to the DSM Yolk Colour Fan). The performance was not affected by the source of carotenoids.

REFERENCES

Ahmed AMH, Rodriguez-Navarro AB, Vidal ML, Gautron J, García-Ruiz JM, Nys Y (2005): Changes in eggshell mechanical properties, crystallographic texture and in matrix proteins induced by moult in hens. British Poultry Science, 46, 268–279. doi: 10.1080/00071660500065425.

Arden GB, Barker FM (1991): Canthaxanthin and the eye – a critical ocular toxicologic assessment. Journal of Toxicology – Cutaneous and Ocular Toxicology, 10, 115–155.

AOAC (2005): Official Methods of Analysis. 18th Ed. Association of Official Analytical Chemists, Gaithersburg.

Baker RTM (2001): Canthaxanthin in aquafeed applications: Is there any risk? Trends in Food Science and Technology, 12, 240–243. doi: 10.1016/S0924-2244(01)00091-7.

Breithaupt DE (2007): Modern application of xanthophylls in animal feeding – a review. Trends in Food Science and Technology, 18, 501–506. doi: 10.1016/j.tifs.2007.04.009.

Chung HY, Rasmussen HM, Johnson EJ (2004): Lutein bioavailability is higher from lutein-enriched eggs than from supplements and spinach in men. Journal of Nutrition, 134, 1887–1893.

Dušková D, Marounek M, Březina P (2001): Determination of phytic acid in feeds and faeces of pigs and poultry by capillary isotachophoresis. Journal of the Science of Food and Agriculture, 81, 36–41.

EN 12822 (2000): Foodstuffs – Determination of vitamin E by high performance liquid chromatography – measurement of α-, β-, γ- and δ-tocopherols. European Committee for Standardization, Brussels.

EN 12823-1 (2000): Foodstuffs – Determination of vitamin A by high performance liquid chromatography – Part 1: Measurement of all-trans-retinol and 13-cis-retinol. European Committee for Standardization, Brussels.

EN 12823-2 (2000): Foodstuffs – Determination of vitamin A by high performance liquid chromatography – Part 2: Measurement of β-carotene. European Committee for Standardization, Brussels.

European Union Council Directive (1999): Council Directive 1999/74/EC of July 19th, 1999. Official Journal of the European Communities, L 203, 53–57.

Froescheis O, Moalli S, Liechti H, Bausch J (2000): Determination of lycopene in tissues and plasma of rats by normal-phase high-performance liquid chromatography with photometric detection. Journal of Chromatography B, 739, 291–299. doi: 10.1016/S0378-4347(99)00562-9.

Goodwin TW (1986): Metabolism, nutrition, and function of carotenoids. Annual Review of Nutrition, 6, 273–297. doi: 10.1146/annurev.nu.06.070186.001421.

Granado F, Olmedilla B, Blanco I (2003): Nutritional and clinical relevance of lutein in human health. British Journal of Nutrition, 90, 487–504. doi: 10.1079/BJN2003927.

Hammershøj M, Kidmose U, Steenfeldt S (2010): Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (*Daucus carota*) varieties as forage material for egg-laying hens. Journal of the Science of Food and Agriculture, 90, 1163–1171. doi: 10.1002/jsfa.3937.

Haugh R (1937): The Haugh unit for measuring egg quality. US Egg and Poultry Magazine, 43, 552–555, 572–573.

Karadas F, Grammenidis E, Surai PF, Acamovic T, Sparks C (2006): Effects of carotenoids from lucerne, marigold and tomato on egg yolk pigmentation and carotenoid composition. British Poultry Science, 47, 561–566. doi: 10.1080/00071660600962976.

Keener KM, McAvoy KC, Foegeding JB, Curtis PA, Anderson KE, Osborne JA, Bush DJ (2006): Effect of testing temperature on internal egg quality measurements. Poultry Science, 85, 550–555.

Krinsky NI (1993): Actions of carotenoids in biological systems. Annual Review of Nutrition, 13, 561–587. doi: 10.1146/annurev.nu.13.070193.003021.

Leeson S, Caston L (2004): Enrichment of eggs with lutein. Poultry Science, 83, 1709–1712.

Leeson S, Caston L, Namkung H (2007): Effect of dietary lutein and flax on performance, egg composition and liver status of laying hens. Canadian Journal of Animal Science, 87, 365–372. doi: 10.4141/A06-043.

Lokaewmanee K, Yamauchi K, Komori T, Saito K (2011): Enhancement of yolk color in raw and boiled egg yolk with lutein from marigold flower meal and marigold flower extract. Journal of Poultry Science, 48, 25–32. doi: 10.2141/jpsa.010059.

Marangos AG, Hill R (1976): Use of rapeseed meals and a mustard seed meal as a protein source in diets for laying pullets. British Poultry Science, 17, 643–653. doi: 10.1080/00071667608416321.

Nys Y (2000): Dietary carotenoids and egg yolk coloration – a review. Archiv für Geflügelkunde, 64, 45–54.

Steinberg W, Grashorn MA, Klunter AM, Schierle J (2000): Comparative pigmentation efficiency of two products containing either apo-ester or tagetes extracts in egg yolks and liquid eggs. Archiv für Geflügelkunde, 64, 180–187.

Zhang W, Zhang KY, Ding XM, Bai SP, Hernandez JM, Yao B, Zhu Q (2011): Influence of canthaxanthin on broiler breeder reproduction, chick quality, and performance. Poultry Science, 90, 1516–1522. doi: 10.3382/ps.2010-01126.

Received for publication on Februry 13, 2013
Accepted for publication on July 29, 2013

Corresponding Author:

Prof. Ing. Miloš S k ř i v a n , DrSc., Institute of Animal Science, Přátelství 815, 104 00 Prague 10-Uhříněves, Czech Republic, phone: +420 267 009 720, e-mail: skrivan.milos@vuzv.cz