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Effect of increasing doses of marigold (*Tagetes erecta*) flower extract on eggs carotenoids content, colour and oxidative stability

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ABSTRACT. Two hundred and forty hens were assigned to six dietary treatments and fed a maize-wheat-soyabean diet supplemented per kg with 0, 150, 350, 550, 750 and 950 mg of marigold flower extract (MFE) containing lutein and zeaxanthin in the amount of 21.26 and 9.65 mg · kg^{-1}, respectively. There was observed no MFE addition effect on hens body weight and feed conversion ratio. The higher hen-day egg production was stated for group fed diet supplemented with 550 and 950 mg of MFE per kg of diet, whereas egg weight was increased in groups fed 550 and 750 mg MFE per kg of diet. The treatment effects on the albumen parameters, and yolk and shell percentages were not statistically significant. Dietary MFE addition increased the yolk colour score (DSM Yolk Colour Fan), and redness and yellowness of the yolks but decreased their lightness. Supplementation of MFE increased the lutein and zeaxanthin concentration in the egg yolks in a dose-dependent manner, from 12.34 and 5.92 mg · kg^{-1} dry matter (control) to 36.33 and 25.59 mg · kg^{-1} dry matter (group fed diet with 950 mg MFE per kg), respectively. No treatment effect on the concentrations of retinol and α-tocopherol in the yolk was observed. Dietary MFE significantly increased the oxidative stability of eggs lipids stored at 18 °C for 28 days. It can be concluded that 1. hen diet supplementation with MFE provides the yolk pigmentation required by consumers, and 2. MFE (in the amount of 550 mg · kg diet) is a suitable alternative to commercial synthetic xanthophylls.

Introduction

Xanthophylls belong to a sub-class of carotenoids and contain at least one oxygen atom in their molecular structure. Currently, there is an increasing interest in xanthophylls use in human food and animal feed applications. In poultry farming, synthetic xanthophylls are used as feed supplements to obtain optimum colouring of the broiler skin and, especially, of the egg yolk. The preferred synthetic red xanthophyll used in poultry farming is cantha-xanthin, and the preferred yellow one is β-apo-8′-carotenoic acid ethyl ester. The intensity as well as the colour of the yolk can be controlled by the type

and concentration of dietary xanthophylls. Both xanthophylls are available commercially on the market as Carophyll® Red and Carophyll® Yellow (DSM Nutritional Products, Basel, Switzerland) and Lucantin® Red and Lucantin® Yellow (BASF, Ludwigshafen, Germany). The carotenoids present in plants are promising alternative to synthetic xanthophylls.

Karadas et al. (2006) investigated the effects of lucerne concentrate, tomato powder and marigold extract as feed additives for quails on yolk pigmentation and the deposition of carotenoids in eggs. The concentrations of lutein, zeaxanthin, lycopene and β-carotene were increased in the eggs of quails fed diets supplemented with natural carotenoids compared with the control group. Lokaewmanee et al. (2009) reported higher pigmentation scores for yolk colour in the eggs of hens fed diet supplemented with mulberry leaves. Hammershøj et al. (2010) examined the effects of orange, yellow and purple carrots on the deposition of carotenoids in hens. Supplementing the feed of layers with coloured carrots significantly increased the total carotenoid content in the egg yolk; in particular, purple carrots increased the lutein content in the yolk. The yolk colour and carotenoid content were also positively correlated. Several researchers supplemented the laying hens diets with freshwater or marine algae. Mader et al. (1984) reported that the fortification of a commercial feed mixture with disintegrated and spray-dried algae *Scenedesmus obliquus* at a dose from 10 to 60 g · kg^{-1} resulted in several-fold increase in carotenoid levels both in the feed and in the yolk. At the same level of carotenoids in the feed, the yolk pigmentation effect of algae was higher than that of lucerne. Marine algae *Spirulina platensis* was used in the experiment of Zahroojian et al. (2011) who concluded that a diet containing 2.5% *S. platensis* could be as effective as a diet with the synthetic pigments Lucantin® Red and Lucantin® Yellow at dose of 35 and 30 mg · kg^{-1}, respectively. Kotrbáček et al. (2013) who studied the effect of supplementation of the commercial hens diets with heterotrophic *Chlorella*, observed that total carotenoids deposition increased by 46 and 119% when diets were supplemented with 10 and 20 g · kg^{-1}, respectively. Lutein and zeaxanthin represented more than 90% of the total carotenoids in the yolk. The deposition of carotenoids increased the pigmentation score of the yolk. Englmaierová et al. (2013) compared the effects of lutein, spray-dried *Chlorella* (cultivated autotrophically), and the synthetic xanthophylls Carophyll® Red and Carophyll® Yellow on the laying hen performance, yolk colour and oxidative stability of yolk lipids. Supplementation of the feed with lutein (250 mg · kg^{-1}) and *Chlorella* (12.5 g · kg^{-1}) significantly increased the concentration of lutein and zeaxanthin in yolks, increased the yolk colour, and improved the oxidative stability of the lipids of fresh eggs and eggs stored for 28 days. The contents of carotenoids and yolk pigmentation were increased also in the eggs of hens fed diet supplemented with marine algae *Nannochloropsis oculata* (Fredriksson et al., 2006) and *Sargassum dentifebium* (Al-Harthi and El-Deek, 2012).

The currently being tested natural carotenoids are too expensive; therefore, they are unprofitable in practical applications. In contrary, flowers of marigold (*Tagetes erecta*) are profitable in practice and are among the best known natural sources of pigmentation for feed additives. Thus, the purpose of the present study was to evaluate the effect of marigold extract on the performance of laying hens, the quality parameters of eggs, the yolk colour, the yolk content of lutein, zeaxanthin, vitamins A and E, and the oxidative stability of yolk lipids. The dose-response efficacy of the marigold flower extract supplementation was assessed.

Material and methods

Hens, diets and husbandry

The experiment was approved by the Ethical Committee of the Institute of Animal Science (the Czech Republic).

Two hundred and forty 30-week old Lohmann Brown hens were used in the experiment. The experiment lasted 12 weeks (including 2 weeks of a preliminary period). The hens were housed in three-floor enriched cages, with 10 hens per cage. The cage provided 0.76 m^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, dust bath and equipment for the abrasion of claws (EU Council Directive 1999/74/EC, 1999). The cages were placed in the same air-conditioned facility at 20 – 22 °C with 16 h of light and 8 h of darkness.

The hens were randomly assigned to one of the six dietary treatments, each with 4 replicate cages. The control group was fed a ground basal maize-wheat-soyabean diet (Table 1). The experimental groups received diets supplemented with 150, 350, 550, 750 and 950 mg of Avizant® Yellow 20 HS (Lohmann Animal Health, Cuxhaven, Germany) per kg of diet. Avizant® Yellow 20 HS is the extract of marigold flowers containing 21.26 g of lutein and 9.65 g of zeaxanthin per kg. Marigold flowers were shredded and dehydrated with a drum dryer and the

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

Indices	Basal diet
Ingredients, g · kg^{-1}	
maize	350
wheat	280
soyabean meal	210.5
rapeseed oil	30
lucerne meal	20
limestone (1 – 2 mm)	91
dicalcium phosphate	9
NaCl	2
DL-methionine	1.5
L-lysine	1
vitamin-mineral premix[2]	5
Analysed chemical composition, g · kg^{-1}	
dry matter	901.2
crude protein	171.7
calcium	37.3
phosphorus	5.6
Nitrogen-corrected apparent ME[3], MJ · kg^{-1}	11.4

[1] other experimental diets were supplemented with 150, 350, 550, 750 or 950 mg · kg^{-1} of Avizant® Yellow 20 HS, which was added at expense of limestone; [2] premix provided per kg of diet: IU: vit. D_3 3000; mg: retinyl acetate 3, vit. E 30, niacin 25, Ca pantothenate 8, thiamine 2, riboflavin 5, pyridoxine 4, folic acid 0.5, biotin 0.075, cobalamin 0.01, choline Cl 250, menadione 2, betaine 100, butylated hydroxytoluene 7.5, ethoxyquin 5.6, butylhydroxyanisole 1, DL-methionine 0.7, Mn 70, Zn 50, Fe 40, Cu 6, I 1, Co 0.3, Se 0.2; [3] ME – metabolizable energy

carotenoids were extracted using an organic solvent (hexane). Calcium carbonate was the major component of Avizant® Yellow 20 HS. Therefore, Avizant® Yellow 20 HS was dosed at the expense of limestone. Extract of marigold flowers was added into the vitamin-mineral premix. Feed and fresh water were supplied *ad libitum*.

Sampling and measurements

Eggs were collected daily, whereas the performance parameters were calculated weekly (feed intake and eggs weight per cage and group). The body weight was recorded in the 32 and 41 week of hen life. In the week 3, 6 and 9 of the study, the eggs were collected for analysis of the physical characteristics (Englmaierová et al., 2015). Once within each collection period, a whole-day egg production was analysed. All eggs laid in 24 h were collected at 7:00 and immediately analysed. In total 574 eggs were analysed. The technological parameters included: the eggs weight, the shell weight, thickness and breaking strength, the albumen index, the Haugh units and the yolk colour. The shell weight with membranes was determined after drying at 105 °C. The eggshell thickness (the average at both ends and at the middle, including shell membranes) was measured with a micrometer. The shell breaking strength and deformation were measured on the vertical axis using Instron 3360 equipment (Instron, Canton, MA, USA). The albumen height was measured using a tripod micrometer and Haugh units were calculated, as indicated by Haugh (1937). The albumen and yolk index were calculated as a ratio between the height and diameter of the albumen and the yolk, respectively. The yolk colour was determined using the DSM Yolk Colour Fan (DSM Nutritional Products, Basel, Switzerland) and Minolta CR-300 colorimeter (Konica Minolta, Osaka, Japan). The L*, a*, b* parameters correspond to the lightness, redness and yellowness, respectively.

Laboratory analyses

Two hundred and sixteen eggs were evaluated for the vitamin and carotenoid content in their yolks during the week 9 of the experiment [6 (treatments) × 12 (number of samples per treatment) × 3 (one sample consisted of a mixture of 3 egg yolks) = 216 eggs]. The content of lutein and zeaxanthin in the yolks was measured by high-performance liquid chromatography (HPLC) according to a modified method of Froescheis et al. (2000) with a use of HPLC instrument (VP series; Shimadzu, Kyoto, Japan) equipped with a diode-array detector. The α-tocopherol, retinol and β-carotene content in the yolks were determined in accordance with the European standards EN 12822 (2000), EN 12823-1 (2000) and EN 12823-2 (2000), respectively. The lipid peroxidation level in the yolks of fresh eggs (n = 12) and eggs stored for 28 days at 18 °C (n = 12) was assayed using the thiobarbituric acid method (Piette and Raymond, 1999).

The feed and yolk dry matter was determined by drying in oven (Memmert ULM 500; Memmert, Schwabach, Germany) at 105 °C to a constant weight, and the feed crude protein content was measured using Kjeltec Auto 1030 instrument (Tecator, Höganäs, Sweden). Analyses of the P and Ca content in the diets were conducted. Dry homogenized diets were ashed in a muffle furnace (LAC Ht40 AL; LAC, Ltd., Rajhrad, Czech Republic) at 550 °C, and the ash was dissolved in 3 M hydrochloric acid and quantitatively transferred into a volumetric flask. The total P in the solution was determined using a vanadate-molybdate reagent (AOAC International, 2005; method No. 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). The vitamins and carotenoids contents in the feed and Avizant were determined using the methods described above.

Statistical analysis

The data was analysed using analysis of variance (ANOVA) with the General Linear Models (GLM) procedure using SAS software (SAS v 9.2, 2003). A one-way analysis of variance was used. The main effect was the dose of MFE in diet. All differences were considered significant at $P < 0.05$. The results in the tables are presented as mean ± standard error of mean (SEM).

Results and discussion

The marigold flower extract (MFE; Avizant® Yellow 20 HS) used in this study contained 21.26 g of lutein and 9.65 g of zeaxanthin per kg (the results of the analyses carried out in the Institute of Animal Science, the Czech Republic). Whereas, the manufacturer declared total xanthophylls content as 20 g per kg mainly consisting of lutein (17 g per kg). The manufacturer showed lower values of these active compounds, especially at the end of the period of validity, as the carotenoids are very unstable. The hen diet supplementation with MFE increased the dietary content of lutein from 1.01 to 4.86 mg · kg^{-1} and that of zeaxanthin from 0.69 to 6.55 mg · kg^{-1} in a dose-dependent manner but did not increase the content of β-carotene, retinol or α-tocopherol (Table 2).

The MFE addition into diet did not influence hen body weight at the end of the experiment; however feed intake decreased in group fed diet supplemented with 150 mg of the MFE per kg of diet (Table 3). Also Chowdhury et al. (2008) showed that the use of 4% marigold in the diet of laying pullets had no effect on body weight and feed consumption. Similar results mentioned Altuntaş and Aydin (2014) in 80-week-old hens fed diet supplemented with 0, 10 or 20 g of marigold flour per kg of diet.

Both 550 and 950 mg of MFE per kg of diet additions increased hen-day egg production in comparison to control group. Also the egg weight was influenced by MFE supplementation and was increased in groups fed diet containing 150, 550 and 750 mg of MFE per kg of diet in comparison to control group. The feed conversion calculated as g of feed per egg did not differ between all groups. MFE addition did not influence albumen parameters (albumen percentage, height and index), but there were observed changes in some parameters describing yolk in experimental groups. The supplementation of MFE in the amount of 550 and 950 mg · kg^{-1} significantly decreased yolk height and yolk index. The shell thickness was increased in group fed diet supplemented with 550 mg of MFE per kg of diet. Also in this group the shell deformation was decreased. The shell breaking strength was decreased after MFE addition in the amount of 150 and 950 mg per kg of diet. The shell percentage did not differ between all groups. In contrast to these results, Chowdhury et al. (2008), Lokaewmanee et al. (2011) and Altuntaş and Aydin (2014) did not record the effect of marigold supplementation on the egg production and egg quality parameters.

The hen diet supplementation with MFE significantly increased the concentration of lutein and zeaxanthin in eggs in a dose-dependent manner (from 12.34 to 36.33 mg · kg^{-1} of dry matter, and from 5.92 to 25.59 mg · kg^{-1} of dry matter, respectively) (Table 5). In fresh eggs only the highest MFE addition decreased lipid peroxidation level in comparison to control group; however the lipid oxidative stability in eggs stored for 28 days at 18 ºC was significantly improved by all marigold flower extract additions. There was no treatment effect on

Table 2. Effect of marigold flower extract addition into hen diet on carotenoids, vitamin A and vitamin E content in diets, mg · kg^{-1} DM[1]

Indices	Marigold flower extract dose, mg · kg^{-1} diet					
	0	150	350	550	750	950
Lutein	1.01	2.36	3.85	4.86	9.01	9.52
Zeaxanthin	0.69	1.55	2.46	3.42	6.55	7.16
β-Carotene	0.31	0.55	0.28	0.49	0.38	0.42
Retinol	1.79	3.84	1.81	1.67	2.27	2.64
α-Tocopherol	24.6	29.5	25.4	23.9	25.3	28.7

[1]DM – dry matter

Table 3. Effect of marigold flower extract addition into hen diet on hen performance between 32 and 41 week of laying period

Indices	Marigold flower extract dose, mg · kg^{-1} diet						SEM	*P*
	0	150	350	550	750	950		
Body weight in 32 week, g	1863	1758	1815	1789	1849	1762	9.0	NS
Body weight in 41 week, g	1919	1868	1778	1897	1824	1979	22.3	NS
Hen-day egg production, %	85.3^{bc}	82.0^{c}	85.2^{bc}	91.1^{a}	85.8^{b}	90.1^{a}	0.62	<0.001
Egg weight, g	63.5^{c}	64.3^{ab}	63.8^{bc}	65.0^{a}	65.1^{a}	63.8^{bc}	0.11	<0.001
Total mass of all eggs laid during the experiment, kg · hen^{-1}	3.79	3.69	3.81	4.15	3.91	4.04	0.059	NS
Feed intake, g · day^{-1} per hen	115.8^{ab}	110.8^{c}	113.4^{bc}	118.6^{a}	117.3^{a}	116.8^{ab}	0.55	<0.001
Feed conversion, g feed · g^{-1} egg	2.18	2.15	2.14	2.05	2.14	2.08	0.02	NS

^{abc} means with different superscipts within a row are significantly different, NS – not significant

Table 4. Effect of marigold flower extract addition into hen diet on eggs quality

Indices	Marigold flower extract dose, mg · kg^{-1} diet						SEM	*P*
	0 (n = 89)	150 (n = 99)	350 (n = 100)	550 (n = 90)	750 (n = 100)	950 (n = 96)		
Albumen percentage, %	65.9	66.1	65.7	66.1	65.8	65.6	0.08	NS
Albumen height, mm	7.53	7.76	7.65	8.01	7.93	7.60	0.14	NS
Albumen index, %	9.48	9.65	9.51	9.53	9.78	9.34	0.17	NS
Haugh units	85.1	86.7	85.9	83.6	87.2	85.5	0.37	NS
Yolk/albumen ratio, %	36.4	36.5	37.2	36.0	36.6	37.1	0.16	NS
Yolk percentage, %	24.0	24.0	24.4	23.7	24.0	24.3	0.08	NS
Yolk height, mm	18.6^{a}	18.5^{ab}	18.6^{a}	18.2^{c}	18.7^{a}	18.3^{bc}	0.04	<0.001
Yolk index, %	45.6^{a}	45.1^{ab}	44.8^{bc}	44.3^{c}	45.2^{ab}	44.4^{c}	0.11	0.002
Yolk colour[1]								
DSM Yolk Colour Fan	5.67^{f}	7.31^{e}	8.52^{d}	8.93^{c}	9.51^{b}	10.55^{a}	0.071	<0.001
L*	64.29^{a}	62.38^{b}	62.09^{b}	61.78^{bc}	60.55^{cd}	59.27^{d}	0.202	<0.001
a*	4.94^{f}	6.17^{e}	7.77^{d}	9.04^{c}	10.54^{b}	11.51^{a}	0.108	<0.001
b*	46.98^{d}	50.79^{c}	53.78^{b}	55.59^{a}	56.30^{a}	56.49^{a}	0.204	<0.001
Shell percentage, %	10.1	9.9	10.0	10.1	10.1	10.1	0.03	NS
Shell thickness, µm	351^{b}	343^{b}	349^{b}	369^{a}	355^{ab}	350^{b}	2.32	0.040
Shell deformation, mm	0.50^{ab}	0.49^{bc}	0.51^{a}	0.48^{c}	0.49^{bc}	0.48^{bc}	0.01	0.002
Shell breaking strength, g · cm^{-2}	4431^{a}	4197^{c}	4448^{a}	4256^{abc}	4415^{ab}	4205^{bc}	31.9	0.040

[1]DSM Yolk Colour Fan – egg yolk colour measured with DSM Yolk Color Fan, L* – lightness, a* – redness, b* – yellowness; $^{a–f}$means with different superscipts within a row are significantly different; NS – not significant

Table 5. Effect of marigold flower extract addition into hen diet on content of carotenoids, vitamin A and vitamin E in egg yolks and lipid peroxidation level[1] in fresh (MDA_0) and stored for 28 days (MDA_{28}) eggs

Indices	Marigold flower extract dose, mg · kg^{-1} diet						SEM	*P*
	0	150	350	550	750	950		
	n = 12							
Lutein, mg · kg^{-1} DM[2]	12.34^{e}	18.56^{d}	29.11^{c}	30.27^{bc}	30.80^{b}	36.33^{a}	1.09	<0.001
Zeaxanthin, mg · kg^{-1} DM	5.92^{f}	10.27^{e}	14.93^{d}	18.92^{c}	20.81^{b}	25.59^{a}	0.87	<0.001
Retinol, mg · kg^{-1} DM	10.40	10.17	10.79	11.00	10.45	10.98	0.10	NS
α-Tocopherol, mg · kg^{-1} DM	166.0	160.0	174.1	171.9	170.6	174.6	1.75	NS
MDA_0, mg · kg^{-1}	0.384^{abc}	0.358^{c}	0.405^{a}	0.393^{ab}	0.359^{bc}	0.321^{d}	0.006	<0.001
MDA_{28}, mg · kg^{-1}	0.819^{a}	0.749^{b}	0.726^{b}	0.736^{b}	0.616^{c}	0.533^{d}	0.014	<0.001

[1]lipid peroxidation level – thiobarbituric acid reactive substances (TBARS) content expressed as mg of malondialdehyde (MDA) per egg kg; [2]DM – dry matter; $^{a–f}$ see Table 4; NS – not significant

the concentration of retinol or α-tocopherol in the eggs observed (Table 5).

Currently, there is a tendency to replace synthetic feed additives with natural substances derived from plants, which are free from undesirable side effects. It was shown in our previous study that spray-dried *Chlorella* is a suitable source of carotenoids that quadrupled the concentration of lutein and zeaxanthin in yolks and increased the oxidative stability of yolk lipids when compared with the control group and group with addition of Carophyll® Red (Englmaierová et al., 2013). Positive effect of carotenoids on oxidative stability was observed also in the case of grazing (Skřivan et al., 2014, 2015b). In the present study, hens fed diet with 950 mg of MFE per kg (which is about 9.5 mg lutein per kg) deposited lutein and zeaxanthin in the amount of 7.5 and 5.3 mg per 100 g of egg yolk, respectively. MFE at dose of 150 mg · kg^{-1} increased the lutein and zeaxanthin in yolks by 62.9 and 73.3%, respectively. In group fed diet with 950 mg of MFE per kg of diet, the concentration of both carotenoids tripled in comparison with the control group. Lokaewmanee et al. (2011) reported that hens fed MFE in the amount of 443 mg · kg^{-1} diet containing 10 mg of lutein per kg deposited lutein and zeaxanthin in the amount of 1.8 and 0.7 mg per 100 g of egg yolk, respectively. In Japanese quails fed diet with addition of 2 g of MFE per kg of diet (10.6 mg of lutein per kg), the egg yolks contained lutein and zeaxanthin in the amount of 3.11 and 0.30 mg per 100 g, respectively (Karadas et al., 2006). The deposition of lutein and zeaxanthin in egg yolk may be influenced by the composition of the basal diet.

For example, adding linseed to diets supplemented with lutein depressed the yolk lutein content (Leeson and Caston, 2004).

Green leafy vegetables and eggs are the major food sources of lutein and zeaxanthin in most human diets (Johnson et al., 2010). The presence of both xanthophylls in food is beneficial for human health. Lutein and zeaxanthin are deposited in the macular region of the retina and reduce the risk of age-related macular degeneration (Landrum et al., 1999). At present, there is no recommended daily intake for lutein and zeaxanthin. However, a recent dose-response meta-analysis showed that every 0.3 mg per day increment in dietary lutein and zeaxanthin intake was associated with a 3% reduction of the nuclear cataract risk, which is the most common type of cataract (Ma et al., 2014). In the present study, the contents of lutein and zeaxanthin in a 60 g egg from the control hens were 0.37 and 0.18 mg, respectively, whereas in hens fed diet with 950 mg of MFE per kg of diet the corresponding lutein and zeaxanthin contents were 1.09 and 0.77 mg, respectively. The daily intake of lutein and zeaxanthin in humans is variable (Bone et al., 2000). In people consuming less than 1 mg lutein and zeaxanthin daily, one lutein-enriched egg per day provides protection to the eye against oxidative stress and the development of age-related macular degeneration.

Dietary MFE increased in a dose-dependent manner the yolk colour score assessed using the DSM Yolk Colour Fan from 5.67 to 10.55 as well as redness of the yolk (from 4.94 to 11.51; Table 4). Also yellowness of the yolk increased but from the amount of 550 mg of MFE per kg of diet this parameter was kept on stable level. The decrease in yolk lightness was observed for all experimental groups. In the previous experiment performed by Skřivan et al. (2015a), MFE supplementation at a dose of 250 and 350 mg · kg^{-1} increased the yolk colour score measured with the use of DSM Yolk Colour Fan identically to 8.9. It is well recognized that yolk colour is important for consumers. It was estimated that deeply hued yolks are the most popular all over the world (Beardsworth and Hernandez, 2004).

Synthetic carotenoids are routinely used in poultry diets to increase yolk pigmentation and to improve the lipid oxidative stability. If canthaxanthin, present in Carophyll® Red and Lucantin® Red, is added to the laying hens feed, a maximum of 8 mg · kg^{-1} is allowed, supposing that the total concentration of canthaxanthin and other carotenoids does not exceed 80 mg · kg^{-1} (EU Commission Directive 2003/7/EC). The use of synthetic carotenoids is not allowed in organic farming. The acute oral toxicity of canthaxanthin is very low. Experiments conducted on monkeys revealed that the oral administration of canthaxanthin was associated with crystalline deposits in the retina. The grade of crystals in monkey retinas was dose dependent, with a threshold level at 0.6 mg of canthaxanthin per kg body weight per day (Goralczyk et al., 2000). A similar observation based on the biostatistical eva-luation of 411 cases was reported previously in humans (Köpcke et al., 1995). The EFSA Panel on Food Additives and Nutrient Sources added to Food (EFSA, 2010) thus allocated an acceptable daily intake of canthaxanthin at the amount of 0.03 mg · kg^{-1} body weight per day (using an uncertainty factor of 10).

Conclusions

It can be concluded that marigold flower extract (MFE) supplementation to laying hen diet influence egg production and its quality. The most recommended dose would be 550 mg of MFE per kg of diet. This amount is optimal in terms of increasing egg production and efficiency of yolk enrichment with carotenoids, which have positive influence on yolk colour and increase oxidative stability of lipids in stored eggs. So it can be stated that MFE is a suitable alternative to commercial synthetic xanthophylls.

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References

AOAC International, 2005. Official Methods of Analysis of AOAC International. 18th Edition. Gaithersburg, MD

Al-Harthi M.A., El-Deek A.A., 2012. Effect of different dietary concentrations of brown marine algae (*Sargassum dentifebium*) prepared by different methods on plasma and yolk lipid profiles, yolk total carotene and lutein plus zeaxanthin of laying hens. Ital. J. Anim. Sci. 11, 347–353

Altuntaş A., Aydin R., 2014. Fatty acid composition of egg yolk from chickens fed a diet including marigold (*Tagetes erecta* L.). J. Lipids 2014, ID 564851, doi: dx.doi.org/10.1155/2014/564851

Beardsworth P.M., Hernandez J.-M., 2004. Yolk colour – an important egg quality attribute. Int. Poultry Prod. 12, 17–18

Bone R.A., Landrum J.T., Dixon Z., Chen Y., Llerena C.M., 2000. Lutein and zeaxanthin in the eyes, serum and diet of human subjects. Exp. Eye Res. 71, 239–245

Chowdhury S.D., Hassin B.M., Das S.C., Rashid M.H., Ferdaus A.J.M., 2008. Evaluation of marigold flower and orange skin as sources of xanthophyll pigment for the improvement of egg yolk color. J. Poultry Sci. 45, 265–272

EFSA, 2010. Scientific opinion on the re-evaluation of canthaxanthin (E 161 g) as a food additive. EFSA J. 8, 1852

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 (Belgium)

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 (Belgium)

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 (Belgium)

Englmaierová M., Skřivan M., Bubancová I., 2013. A comparison of lutein, spray-dried Chlorella, and synthetic carotenoids effects on yolk colour, oxidative stability, and reproductive performance of laying hens. Czech J. Anim. Sci. 58, 412–419

Englmaierová M., Skřivan M., Skřivanová E., Bubancová I., Čermák L., Vlčková J., 2015. Effects of a low-phosphorus diet and exogenous phytase on performance, egg quality, and bacterial colonisation and digestibility of minerals in the digestive tract of laying hens. Czech J. Anim. Sci. 60, 542–549

EU, 2003. Commission Directive 2003/7/EC of 24th January 2003 amending the conditions for authorisation of canthaxanthin in feedingstuffs in accordance with Council Directive 70/524/EEC. Off. J. Eur. Commun. L 22, 28-30

EU, 1999. Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. Off. J. Eur. Commun. L 203, 53–57

Fredriksson S., Elwinger K., Pickova J., 2006. Fatty acid and carotenoid composition of egg yolk as an effect of microalgae addition to feed formula for laying hens. Food Chem. 99, 530–537

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. J. Chromatogr. B Biomed. Sci. Appl. 739, 291–299

Goralczyk R., Barker F.M., Buser S., Liechti H., Bausch J., 2000. Dose dependency of canthaxanthin crystals in monkey retina and spatial distribution of its metabolites. Invest Ophthalmol Vis Sci. 41, 1513–1522

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. J. Sci. Food Agric. 90, 1163–1171

Haugh R.R., 1937. The Haugh unit for measuring egg quality. US Egg Poultry Mag. 43, 552–555, 572–573

Johnson E.J., Maras J.E., Rasmussen H.M., Tucker K.L., 2010. Intake of lutein and zeaxanthin differ with age, sex, and ethnicity. J. Am. Diet. Assoc. 110, 1357–1362

Karadas F., Grammenidis E., Surai P.F., Acamovic T., Sparks N.H., 2006. Effect of carotenoids from lucerne, marigold and tomato on egg yolk pigmentation and carotenoid composition. Brit. Poultry Sci. 47, 561–566

Köpcke W., Barker F.M., Schalch W., 1995. Canthaxanthin deposition in the retina: a biostatistical evaluation of 411 patients. J. Toxicol. Cutan. Ocul .Toxicol. 14, 89–104

Kotrbáček V., Skřivan M., Kopecký J., Pěnkava O., Hudečková P., Uhríková I., Doubek J., 2013. Retention of carotenoids in egg yolks of laying hens supplemented with heterotrophic Chlorella. Czech J. Anim. Sci. 58, 193–200

Landrum J.T., Bone R.A., Chen Y., Herrero C., Llerena C.M., Twarowska E., 1999. Carotenoids in the human retina. Pure Appl. Chem. 71, 2237–2244

Leeson S., Caston L., 2004. Enrichment of eggs with lutein. Poultry Sci. 83, 1709–1712

Lokaewmanee K., Mompanuon S., Khumpeerawat P., Yamauchi K., 2009. Effects of dietary mulberry leaves (*Morus alba* L.) on egg yolk color. J. Poultry Sci. 46, 112–115

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. J. Poultry Sci. 48, 25–32

Ma L., Hao Z.X., Liu R.R., Yu R.B., Shi Q., Pan J.P., 2014. A dose–response meta-analysis of dietary lutein and zeaxanthin intake in relation to risk of age-related cataract. Graefes Arch Clin Exp Ophthalmol. 252, 63–70

Mader P., Mikolášek A., Lidická M., Nováková V., Hartlová L., Staněk J., 1984. Algae as a natural source of carotenoids in laying hen feed mixtures (in Czech). Živoč. Výr. 29, 557–567

Piette G., Raymond Y., 1999. Comparative evaluation of methods to determine rancidity in processed meat (in German). Fleischwirtschaft 7, 69–73

SAS, 2003. SAS/STAT User´s Guide (release 9.2). SAS Institute. Cary, NC

Skřivan M., Englmaierová M., 2014. The deposition of carotenoids and α-tocopherol in hen eggs produced under a combination of sequential feeding and grazing. Anim. Feed Sci. Tech. 190, 79–86

Skřivan M., Englmaierová M., Skřivanová E., Bubancová I., 2015a. Increase in lutein and zeaxanthin content in the eggs of hens fed marigold flower extract. Czech J. Anim. Sci. 60, 89–96

Skřivan M., Pickinpaugh S.H., Pavlů V., Skřivanová E., Englmaierová M., 2015b. A mobile system for rearing meat chickens on pasture. Czech J. Anim. Sci. 60, 52–59

Zahroojian N., Moravej H., Shivazad M., 2011. Comparison of marine algae (*Spirulina platensis*) and synthetic pigment in enhancing egg yolk colour of laying hens. Brit. Poultry Sci. 52, 584–588