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Increase in lutein and zeaxanthin content in the eggs of hens fed marigold flower extract

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ABSTRACT: Marigold flower extract (MFE), a natural source of xanthophylls, was added to the diets of hens at different concentrations (0, 150, 250, and 350 mg/kg of diet) to determine the effects of MFE on hen performance, physical characteristics of egg quality, and carotenoid content of the egg yolk of hens housed in enriched cages. Additionally, the highest dose of MFE (350 mg/kg) was tested under commercial poultry conditions and compared with a feed mixture with added synthetic carotenoids and a control diet without synthetic carotenoids. The highest hen-day egg production ($P = 0.005$) and egg mass production ($P = 0.010$) was found in hens fed a diet supplemented with MFE at 150 mg/kg. The performance characteristics, however, were not influenced by MFE under commercial conditions. When the dose of MFE was increased, increased values were observed for DSM Yolk Colour Fan ($P < 0.001$), redness ($P < 0.001$), yellowness ($P < 0.001$), ratio of redness and yellowness ($P < 0.001$), and decreased for lightness ($P = 0.036$). In the commercial study, the synthetic carotenoids decreased the value of yellowness ($P < 0.001$) compared with the control group. The lutein and zeaxanthin content in yolk increased by approximately 11.5 and 5.9 mg/kg dry matter, respectively, after the MFE addition of 350 mg/kg. Supplementation with synthetic carotenoids significantly ($P < 0.001$) decreased the α-tocopherol content in egg yolk. In conclusion, MFE is a suitable natural alternative for increasing the xanthophyll contents in eggs compared with the commercially used synthetic carotenoids.

Keywords: carotenoids; *Tagetes erecta* L.; egg quality; yolk colour; hen performance

INTRODUCTION

From the viewpoint of consumers, darker egg yolks are required. Hens, like all animals, cannot synthesize their own pigments. However, they can store the pigments obtained from their diet. Carotenoids are a source of red and yellow pigments that alter egg yolk colour. In particular, synthetic carotenoids, such as ethyl ester of β-apo-8'-carotenoic acid and canthaxanthin known as Carophyll®Yellow and Carophyll® Red, respectively, are used for the colouring of egg yolk. However, several countries, such as Sweden, do not allow the use of synthetic pigments in hens' diets (Roberts 2004). Additionally, when feeding laying hens, canthaxanthin should not exceed 8 mg/kg (European Union Council Directive 1970) because at extremely high dosages minute crystals may form in the retina by a reversible deposition process (Breithaupt 2007). The corresponding upper concentration of the ethyl ester of β-apo-8'-carotenoic acid (Carophyll® Yellow) is 80 mg/kg. Therefore, interest in natural alternatives has increased.

The effect of three coloured carrot varieties on egg yolk colour and deposition of carotenoids in the yolk was evaluated by Hammershoj et al. (2010). Supplementing the feed of egg-laying hens with coloured carrots at 70 g per hen/day efficiently increased yolk colour parameters and total carotenoid content, especially lutein, α-carotene and β-carotene contents. Englmaierova et al. (2013) found that the addition of lutein at 250 mg/kg and the alga *Chlorella* at 12.5 g/kg significantly increased yolk colour compared with the control

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diet. Lutein increased the yolk redness whereas *Chlorella* increased the value of yellowness. Additionally, supplementation of feed with lutein and *Chlorella* significantly increased the concentration of lutein (from 12.8 to 133.9 and 49.0 mg/kg dry matter, respectively) and zeaxanthin (from 9.2 to 123.9 and 40.1 mg/kg dry matter, respectively) in the yolks. Kotrbacek et al. (2013) found that diets supplemented with *Chlorella* biomass at 10 and 20 g/kg significantly increased egg yolk concentration of total carotenoids by 46 and 119%, respectively. The maximum concentrations were achieved after a four-week diet supplementation and exceeded 25 and 40 mg per g of yolk for 10 and 20 g/kg of *Chlorella*, respectively. The effects of sea buckthorn berry flour on carotenoid accumulation in egg yolk and on egg production were examined by Dumbrava et al. (2006). Strong absorption was observed for the dihydroxy xanthophylls (lutein, zeaxanthin), and comparatively weak absorption was observed for the carotenoidic hydrocarbons (α-carotene, β-carotene). Moreover, sea buckthorn berry flour carotenoids contributed to increased egg production. These findings provide opportunities to improve the nutritional value of eggs using carotenoids from natural sources.

A rich source of xanthophylls, particularly lutein (80–90%), is marigold (*Tagetes erecta* L.) (Quackenbush and Miller 1972). Lokaewmanee et al. (2011) found that lutein from marigolds improved egg yolk colour when supplemented at levels of approximately 30–40 mg/kg, and saponified lutein from marigold flower extract (MFE) appeared to be more effective in improving egg yolk colour than non-saponified lutein from marigold flower meal. Additionally, Karadas et al. (2006) compared the effects of different sources of natural carotenoids, such as alfalfa concentrate, tomato powder or marigold extract, in the diets of quails. In the diets supplemented with these natural carotenoids, the concentrations of lutein, zeaxanthin, lycopene, and β-carotene increased in egg yolks.

The carotenoids, lutein and zeaxanthin, are important for protection against age-related macular degeneration in humans (Granado et al. 2003). Because lutein-enriched eggs have greater lutein bioavailability for humans (Chung et al. 2004), increased consumption of lutein-enriched eggs is advantageous for human health compared with other supplements.

The natural carotenoids currently being tested are too expensive; therefore, they are less useful for practical applications. Thus, an affordable source of carotenoids extracted from marigold flowers (*Tagetes erecta* L.) was examined. The objective of this study was to evaluate the effects of MFE (0, 150, 250, and 350 mg/kg) in hen diet on the concentration of carotenoids in egg yolks and on yolk colouration. Moreover, the effects of MFE were determined for hen performance and egg quality. The highest dose was examined under commercial poultry conditions.

MATERIAL AND METHODS

Experiment 1. This experiment was started with hens at the age of 66 weeks and lasted for 14 weeks (including a 2-week preliminary period). One hundred and sixty ISA Brown hens were housed in enriched cages, with 10 hens per cage in an air-conditioned facility. The cage was 7560 cm^2 in area. Each cage contained the feeder (120 cm) and 3 nipple drinkers. The cages were equipped with a nest box, perch (150 cm), dust bath, and equipment for abrasion of the claws, which conformed to the European Union Council Directive (1999). Room temperature was maintained at 20–22°C, and the light cycle was 16 h of light and 8 h of darkness. Light intensity was approximately 10 lx in the central storey.

The hens were randomly distributed into 16 cages with four dietary treatments and four replicates of each. The hens were fed diets that lacked synthetic carotenoids. The ingredients and nutrient composition of the control diet are shown in Table 1. The three experimental diets were supplemented with 150, 250, or 350 mg/kg of Avizant® Yellow 20 HS (Lohmann Animal Health GmbH, Cuxhaven, Germany). Avizant® Yellow 20 HS is an extract from marigold flowers (*Tagetes erecta* L.) and contains natural xanthophylls (20 g/kg). The Avizant® Yellow 20 HS contained 980 g of dry matter, 21.263 mg/kg of lutein, and 9.649 mg/kg of zeaxanthin. Calcium carbonate was the major component of Avizant® Yellow 20 HS. Therefore, Avizant® Yellow 20 HS was dosed at the expense of limestone. All diets were stored in a dark, air-conditioned room at a temperature of 18–20°C and a relative humidity that ranged between 50 to 60% for 12 weeks. Feed and fresh water were supplied to the animals *ad libitum*. Feed intake was registered weekly per cage. The egg production and the health of hens were monitored daily. Every week, the eggs were weighed on three consecutive days, and the average values were recorded (Table 3).

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Table 1. Ingredients and chemical composition of the basic diet in Experiment 1[1]

Item	%
Wheat	26.5
Maize	35.0
Soybean meal	21.15
Rapeseed oil	3
Lucerne meal	2
Wheat bran	1.5
Dicalcium phosphate	1.3
Sodium chloride	0.2
Crushed limestone (1–2 mm)	8.6
L-Lysine hydrochloride	0.1
DL-Methionine	0.15
Vitamin-mineral premix[2]	0.5
Analyzed nutrient content (%)	
Dry matter	90.12
AME_N (by calculation; MJ/kg)	11.57
Crude protein	17.17
Crude fat	4.94
Crude fibre	3.42
Ash	11.85
Methionine (by calculation)	0.478
Calcium	3.73
Total phosphorus	0.62

AME_N = apparent metabolizable energy
[1]experimental diets were supplemented with 150, 250 or 350 mg/kg of marigold flower extract
[2]vitamin-mineral premix provided per kg of diet: 3.0 mg retinylacetate, 3000 IU vitamin D_3, 30 mg vitamin E, 25 mg niacin, 8 mg Ca pantothenate, 2.0 mg thiamine, 5 mg riboflavin, 4 mg pyridoxine, 0.5 mg folic acid, 0.075 mg biotin, 0.01 mg cobalamin, 250 mg choline Cl, 2.0 mg menadione, 100 mg betaine, 7.5 mg butylated hydroxytoluene, 5.6 mg ethoxyquin, 1 mg butylhydroxyanisole, 0.7 g DL-methionine, 70 mg Mn, 50 mg Zn, 40 mg Fe, 6 mg Cu, 1 mg I, 0.3 mg Co, 0.2 mg Se

For determination of the physical characteristics, eggs were collected during the weeks 73 and 76. All laid eggs were examined. A total of 273 eggs were analyzed. The Haugh units (HU) were calculated according to the method of Haugh (1937). The shell breaking strength and the shell deformation were determined on the vertical axis using the Instron 3360 apparatus (Instron, Canton, USA), and the shell thickness (i.e. the values of the sharp and blunt ends and equator and the average of these 3 values), after removing the shell membranes, was

Table 2. Nutrient content of the soybean-corn-wheat commercial mixed feed[1] in Experiment 2[2]

Item	%
Dry matter	89.17
AME_N (by calculation; MJ/kg)	11.03
Crude protein	16.25
Crude fat	4.91
Crude fibre	5.83
Ash	11.65
Methionine[1]	0.39
Calcium	3.70
Total phosphorus[1]	0.45

AME_N = apparent metabolizable energy
[1]produced by De Heus, a.s., Bučovice, Czech Republic
[2]experimental diets were supplemented with 20 mg/kg of Carophyll® Red in combination with 15 mg/kg of Carophyll® Yellow or 350 mg/kg of marigold flower extract

measured using a micrometre. The colour of the yolk was measured using the DSM Yolk Colour Fan (DSM Nutritional Products, Basel, Switzerland). Other colour parameters of the yolk (L^*, a^*, b^*) were measured using a Minolta CR-300 colorimeter (Minolta, Osaka, Japan). The L^*, a^*, and b^* values reflect lightness (0 = black, 100 = white), redness (–100 = green, 100 = red), and yellowness (–100 = blue, 100 = yellow), respectively.

Ninety-six eggs were used to determine the α-tocopherol and carotenoid content in the egg yolks once during the experiment (70th week; 3 eggs per sample; n = 8). The α-tocopherol contents of the yolks were determined in accordance with EN 12822 (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 yolks was measured by HPLC, using the method of Froescheis et al. (2000), except that the extracts were evaporated at 50°C and methanol/tetrahydrofurane instead of hexan; dichloromethane was used to dissolve residues in a vacuum evaporator. An aliquot of 60 µl was subjected to HPLC (VP series) analysis. A Kinetex C18 column (100 × 4.6 mm; 2.6 µm) (Phenomenex, Torrance, USA) was used. A gradient system was established with acetonitrile : water : ethylacetate (88 : 10 : 2) as eluent A and acetonitrile : water : ethylacetate (88 : 0 : 15) as eluent B.

The mixed feed and MFE dry matter was determined with oven drying at 105°C to a con-

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Table 3. Performance characteristics of laying hens in Experiment 1

Characteristics	Marigold flower extract (mg/kg diet)				SEM	Probability
	0	150	250	350		
Hen-day egg production (%)	82.6^{bc}	89.4^{a}	81.3^{c}	86.5^{ab}	0.87	0.005
Egg weight (g)	67.5^{a}	66.5^{b}	66.2^{b}	66.7^{ab}	0.16	0.009
Egg mass (g/hen/day)	55.8^{bc}	59.5^{a}	53.8^{c}	57.7^{ab}	0.61	0.010
Feed intake (g/day/hen)	118.7	120.2	118.5	121.4	0.65	ns
Feed intake (g/egg)	145.7	135.2	148.9	142.9	1.82	ns
FCR (g/g)	2.30	2.14	2.37	2.26	0.030	ns

FCR = feed conversion ratio, ns = not significant

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

stant weight, and the crude protein content of the feed was measured using a Kjeltec Auto 1030 instrument (Tecator, Höganäs, Sweden). The fat content in the diet was determined by extraction with petroleum ether using a Tecator Extraction System 1045 Soxtec (Tecator). 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 (AOAC 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). The concentrations of vitamins in the feed and the content of carotenoids in the feed and Avizant were determined using the methods described previously.

Experiment 2. The experiment was conducted under commercial conditions with 22 464 Hisex hens aged 31–38 weeks. The hens were housed in enriched cages as in Experiment 1. The poultry house was divided into 3 sections, and mixed feed was dosed separately for each group from 3 silos. The control group (11 232 hens) was fed a diet that lacked carotenoids. The nutrient composition of the control diet, which was based on a commercially mixed feed, is shown in Table 2. The hens of the second group (Carophylls; 5616 hens) were fed a combination of synthetic carotenoids Carophyll®

Table 4. Physical characteristics of eggs in Experiment 1

Characteristics	Marigold flower extract (mg/kg diet)				SEM	Probability
	0	150	250	350		
Haugh units	79.6	79.2	79.5	79.3	0.53	ns
Yolk colour						
DSM Yolk Colour Fan	7.7^{c}	8.3^{b}	8.9^{a}	8.9^{a}	0.06	< 0.001
Lightness (L^*)	60.1^{a}	59.9^{a}	59.9^{a}	58.8^{b}	0.19	0.036
Redness (a^*)	7.0^{c}	8.1^{b}	9.2^{a}	9.4^{a}	0.12	< 0.001
Yellowness (b^*)	50.0^{b}	53.1^{a}	53.5^{a}	54.1^{a}	0.31	< 0.001
Redness and yellowness ratio (a/b)	14^{c}	15^{a}	17^{a}	17^{a}	0.2	< 0.001
Shell thickness						
Blunt end (μm)	332	348	335	344	2.4	ns
Equator (μm)	345	357	349	352	2.2	ns
Sharp end (μm)	349^{b}	365^{a}	346^{b}	353^{ab}	2.3	0.029
Average (μm)	342	356	343	350	2.1	ns
Shell deformation (mm)	0.474	0.466	0.468	0.460	0.0030	ns
Shell breaking strength (N)	36.8	38.3	37.3	37.0	0.45	ns

ns = not significant

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

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Table 5. Physical characteristics of eggs in Experiment 2

Characteristics	Control	Carophylls	MFE	SEM	Probability
Haugh units	79.8[a]	76.0[b]	78.7[a]	0.56	0.017
Yolk colour					
DSM Yolk Colour Fan	6.3[c]	13.0[a]	8.7[b]	0.23	< 0.001
Lightness (L^*)	64.6[a]	56.6[c]	62.6[b]	0.34	< 0.001
Redness (a^*)	5.4[c]	21.0[a]	8.7[b]	0.56	< 0.001
Yellowness (b^*)	49.2[b]	45.7[c]	55.7[a]	0.41	< 0.001
Redness and yellowness ratio (a/b)	11[c]	46[a]	16[b]	1.3	< 0.001
Shell thickness					
Blunt end (μm)	365	361	353	3.1	ns
Equator (μm)	367	361	364	2.2	ns
Sharp end (μm)	372	383	377	2.4	ns
Average (μm)	368	368	365	2.2	ns
Shell deformation (mm)	0.493	0.481	0.489	0.0042	ns
Shell breaking strength (N)	44.0	43.6	43.6	0.56	ns

MFE = marigold flower extract (350 mg/kg diet), ns = not significant

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

Yellow and Carophyll® Red (DSM Nutritional Products) in amounts of 20 and 30 mg/kg, respectively. Carophyll® Yellow and Carophyll® Red added ethyl ester of β-apo-8'-carotenoic acid (2 mg/kg) and canthaxanthin (3 mg/kg), respectively, to the diet. Avizant® Yellow 20 HS was added to the diet of the third treatment group (5616 hens) at 350 mg/kg. Hen performance and egg quality characteristics, vitamin and carotenoid content and mixed feed analyses were determined as described in Experiment 1.

Statistical analyses. The data from the experiments were analyzed using the analysis of variance (ANOVA) with the General Linear Models (GLM) procedure of SAS software (Statistical Analysis Software, Version 9.2, 2003). One-way ANOVA was used to compare hen performance, physical characteristics of egg quality, and vitamin and carotenoid contents of the yolks. Differences were considered significant at $P < 0.05$. The results summarized in tables are presented as the mean and the standard error of the mean (SEM).

RESULTS

The addition of MFE at 150 mg/kg significantly increased egg production ($P = 0.005$) and egg mass ($P = 0.010$) and decreased egg weight ($P = 0.009$) compared with the control group (Table 3). Feed intake and feed conversion ratios were not affected. In Experiment 2 under commercial conditions, where 350 mg/kg of MFE was used, hen performance characteristics were not affected.

Increasing doses of MFE increased the values of DSM Yolk Colour Fan ($P < 0.001$), redness ($P < 0.001$), yellowness ($P < 0.001$), and ratio of redness and yellowness ($P < 0.001$), and decreased lightness ($P = 0.036$) (Table 4). The shell thickness was only affected by MFE at the sharp end of the egg ($P = 0.029$). The eggs with the thickest shells were from hens fed 150 mg/kg of MFE. Also in Experiment 2, carotenoids affected yolk colour ($P < 0.001$) (Table 5). The eggs with the darkest yolks were from hens that were fed a combination of synthetic carotenoids (13.0), and lower values were observed in the MFE treatment (8.7) and the control group (6.3). The synthetic carotenoids increased the value of redness ($P < 0.001$), and MFE increased the value of yellowness ($P < 0.001$). The albumen quality was evaluated based on Haugh units ($P = 0.017$) and was the lowest with the synthetic carotenoids compared with the MFE additions and the control group.

The content of carotenoids such as lutein and zeaxanthin in the diet increased with dose increases of MFE from 2.2 to 6.2 and from 1.7 to 4.5 mg/kg dry matter of mixed feed, respectively (Table 6). As the MFE concentration in the diet increased, a simultaneous and significant increase ($P < 0.001$) was observed for lutein and zeaxanthin

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Table 6. Carotenoid and α-tocopherol content (mg/kg DM) in feed and egg yolks in Experiment 1

Characteristics	Marigold flower extract (mg/kg diet)				SEM	Probability
	0	150	250	350		
Mixed feed						
Lutein	2.2	3.9	4.7	6.2		
Zeaxanthin	1.7	3.0	3.8	4.5		
α-Tocopherol	25.0	25.3	23.6	24.1		
Egg yolk						
Lutein	18.1^{d}	22.6^{c}	26.2^{b}	29.8^{a}	0.96	< 0.001
Zeaxanthin	12.3^{d}	14.6^{c}	17.0^{b}	19.2^{a}	0.61	< 0.001
α-Tocopherol	131	125	131	121	1.9	ns

DM = dry matter, ns = not significant

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

Table 7. Carotenoid and α-tocopherol content (mg/kg DM) in feed and egg yolks in Experiment 2

Characteristics	Control	Carophylls	MFE	SEM	Probability
Mixed feed					
Lutein	1.4	1.1	6.0		
Zeaxanthin	1.0	0.8	4.2		
α-Tocopherol	11.9	11.5	13.4		
Egg yolk					
Lutein	8.8^{b}	9.4^{b}	20.3^{a}	0.83	< 0.001
Zeaxanthin	4.0^{b}	4.4^{b}	9.9^{a}	0.41	< 0.001
α-Tocopherol	80.6^{a}	72.6^{b}	80.5^{a}	1.05	< 0.001

DM = dry matter, MFE = marigold flower extract (350 mg/kg diet), ns = not significant

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

content in yolk. A similar increase in the content of the xanthophylls in mixed feed and yolk (P < 0.001) was also observed in Experiment 2 where 350 mg/kg of MFE was used (Table 7). Additionally, the presence of synthetic carotenoids in the diet significantly (P < 0.001) decreased α-tocopherol content in egg yolk in comparison with the control and the MFE treatments.

DISCUSSION

The addition of MFE affected some hen performance characteristics, such as egg production, egg mass, and egg weight, but only in Experiment 1 (Table 3). Other performance characteristics were not affected, even when the experiment was conducted under commercial conditions. Moreover, in previous studies, pigment supplementation was not associated with changes in the performance of laying hens. Lokaewmanee et al. (2011) found that lutein from marigold at dietary levels of 10, 20, 30, and 40 mg per kg of diet had no significant effects on production performance, including feed consumption, body weight, hen-day egg production, and egg mass. Similar conclusions were found by Hasin et al. (2006). Lu et al. (2013) also found similar results in a study that used 40 g per kg of marigold meal or 3 levels of marigold extract (the lutein contents were 12.5, 25.0, and 50.0 mg/kg) in the diet of laying hens. In the present study, supplementation with 150, 250 or 350 mg of MFE increased the content of lutein in the diet by 1.7, 2.5, and 4.0 mg/kg, respectively, compared with the control group (Table 6).

The carotenoids are usually lipophilic substances, and thus, they are stored well in fats. Bartov and Bornstein (1967) showed that hens have the ability to transport 20–60% of the pigment in their diets into their yolks. Therefore, in both Experiment 1 (Table 4) and Experiment 2 under commercial

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conditions (Table 5), MFE addition significantly affected yolk colour. These data are in agreement with other studies in which marigold added to hen diets resulted in increased egg yolk colour darkness (Hasin et al. 2006; Rowghni et al. 2006; Lokaewmanee et al. 2011). Other natural sources of carotenoids that are effective in the colouring of egg yolks include red peppers (Lokaewmanee et al. 2011), tomatoes (Akdemir et al. 2012), carrots (Hammershoj et al. 2010), and algae (Fredriksson et al. 2006; Englmaierova et al. 2013; Kotrbacek et al. 2013). The pigmenting efficiency of a source depends on absorption, transport, excretion, rate of deposition in various tissues and of conversion of the carotenoids (Nys 2000). In Experiment 2, synthetic carotenoids decreased Haugh units. However, Krinsky (1993) stated that the addition of synthetic carotenoids and lutein significantly increased the albumen index, most likely because of their antioxidant properties. Additionally, Zhang et al. (2011) found significant improvement in the antioxidant status of the egg yolk after supplementation with canthaxanthin.

In poultry, the pigmentation of eggs is not affected by β-carotene in the diet. The birds mainly accumulate oxycarotenoids in their body or eggs (Goodwin 1986; Hencken 1992). This is consistent with findings of Hammershoj et al. (2010), who found that the deposition efficiency of lutein and zeaxanthin from feed to egg yolk was approximately 25%, while the deposition efficiency of β-carotene was only 0.5%. In another study, Hencken (1992) showed that carotenoid deposition in yolk varied from 14% for astaxanthin to 30–40% for canthaxanthin, with zeaxanthin being intermediary at 25%. In the present studies, the addition of 350 mg/kg MFE increased both the lutein content (by 11.7 and 11.5 mg/kg dry matter) and the zeaxanthin content (by 6.9 and 5.9 mg/kg dry matter) in yolks of eggs from the first and second experiments, respectively, compared with the control group (Table 6 and 7). Leeson et al. (2007) stated that eggs could be enriched to 1.6 mg of lutein/60 g egg from a base level of 0.10 mg/60 g egg with the addition of natural lutein (250 mg/kg) to the diet. The transfer efficiency of lutein from feed to eggs was approximately 10% with 125 mg/kg in the diet, declining to 2–3% with 500 mg/kg (Steinberg et al. 2000; Leeson and Caston 2004). From Chung et al. (2004) it is evident that lutein-enriched eggs have greater lutein bioavailability for humans than other supplements.

In both experiments, the content of α-tocopherol remained unchanged in response to dietary marigold addition. This result is consistent with Akdemir et al. (2012) who tested tomato powder as source of carotenoids, and observed an increase in carotenoid and vitamin A content in egg yolk, although vitamin E content in yolk was not affected. Lutein at 250 mg/kg significantly increased retinol content and decreased α-tocopherol content in a study by Englmaierova et al. (2013). Neither Karadas et al. (2006) nor Englmaierova and Skrivan (2013) could find a natural or synthetic carotenoid that affected the deposition of retinol and α-tocopherol in the yolk.

CONCLUSION

Marigold flower extract in the diet of hens did not cause deterioration in the quality of eggs but did increase the yolk colour darkness and carotenoid content of the yolk. Thus, marigold flower extract is a suitable alternative to commercial synthetic carotenoids. For optimum yolk colour and content of carotenoids, a dose of 250 mg/kg MFE in the diet is sufficient. However, this level of MFE decreased performance of laying hens, so from the economic viewpoint the results are worse than in the case of the lower dose (150 mg/kg).

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