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Study on the influence of dietary sea buckthorn meal on nutritional properties of laying hen eggs

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Abstract: The aim of the study was to evaluate the transfer of bioactive compounds from sea buckthorn meal (SBM) supplement to eggs and the effect on lipid quality and peroxidation process. Thirty-two weeks old TETRA SL laying hens were allocated to two dietary treatments, 30 birds each (15 replicate pens). The control diet (C) contained a maize-soybean diet and the experimental diet contained the previous C diet with 2% of maize replaced with SBM. Thirty-six eggs from each group were collected in order to determine the quality parameters at the end of experiment. The antioxidant profile of SBM showed that the phytoadditive is a valuable source of vitamin E, xanthophylls and polyphenols. The inclusion of 2% of SBM in the experimental diet led to a more than 25% increase of vitamin E and an almost 50% increase of xanthophylls compared to the control. The markers specific to the coronary risk decreased significantly in the experimental group compared to the control, showing a beneficial effect of dietary SBM on the quality of yolk lipids. The bioactive compounds detected in egg yolk showed a significant ($P < 0.05$) improvement of the antioxidant profile, the rate of vitamin (A and E), carotenoid (lutein and zeaxanthin) and mineral (iron and zinc) deposition increased after dietary SBM inclusion. Regarding the lipid peroxidation parameters, the dietary SBM inclusion acted in the first phase of the lipid peroxidation process by inhibiting the formation of primary oxidation products (hydroperoxides and conjugated dienes). In conclusion, the sea buckthorn supplementation of laying hen diet improved the bioactive compound concentrations in eggs and delayed the oxidation process of yolk during storage.

Keywords: yolk; bioactive compounds; lipid peroxidation

Eggs are an inexpensive food used in human nutrition all over the world and are considered an important source of high-quality protein, minerals and vitamins (Kishimoto et al. 2016). The nutritional value of eggs is partially attributed to polyunsaturated fatty acid (PUFA) content in yolk (Xie et al. 2020) but also to other bioactive lipophilic compounds such as vitamins or carotenoids, the yolk being a lipidic carrier of antioxidants. Value-added eggs enriched with PUFA or antioxidants are available in markets due to their preventive health benefits (Lee et al. 2011). Some authors (Poureslami et al. 2010) showed that the bioconversion capability of linolenic acid (LNA) to long-chain PUFA (LC PUFA) decreases with progression on the phylogenetic scale (rainbow trout; broiler chicken; human). So a nutritional strategy to improve the human diets in those nutrients is

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the consumption of value-added animal products. The problem of transfer of bioactive compounds from feeds to animal products is crucial because the oxidation susceptibility of eggs depends on fortified antioxidants. Synthetic antioxidants added to feeds are doubtable to consumers due to their possible toxicity (Orczewska-Dudek et al. 2018) and the natural antioxidants derived from plant components are more effectively incorporated into the lipids of tissues and transferred to the eggs.

The sea buckthorn is a shrub with yellow-orange small fruits, widely found throughout the world. It has been well established in the literature that sea buckthorn berries are rich in bioactive compounds such as carotenoids, flavonoids, fatty acids and vitamins, with potentially beneficial effects on human health (Suryakumar and Gupta 2011). Recently, co- or by-products resulting from the sea buckthorn processing as a health supplement for humans can be used as feed additive in animal nutrition. For example, in poultry nutrition, the sea buckthorn can be used as an alternative feed to maintain high-quality production, performance and yield (Momani Shaker et al. 2018). The biological action of minerals and vitamins, together with fatty acids, it was assumed that it contributes to different beneficial physiological effects associated with the ingestion of sea buckthorn pulp oil and fruit juice (Eccleston et al. 2002).

The objective of the study was to analyse the transfer of bioactive compounds from sea buckthorn meal dietary supplement to eggs and the effect on lipid quality and peroxidation process.

MATERIAL AND METHODS

The experiment complied with Directive 2010/63/EU on the protection of animals used for scientific purposes and the experimental procedures were approved by the Ethical Commission of National Research and Development Institute for Biology and Animal Nutrition.

Experimental design

A four-week experiment was performed with thirty-two weeks old TETRA SL laying hens. The birds were placed in digestibility pens (two chicks per replicate pen) and allocated to two dietary treatments, 30 birds each. The control diet (C) contained a maize-soybean diet with no added phytoadditive supplement and the experimental diet (SBM) diet contained the previous C diet with 2% maize replaced with sea buckthorn meal (Table 1). No mortality was recorded during the overall trial period.

The feed and water supply was administered *ad libitum*. The climate parameters (light, temperature and ventilation) were recorded throughout the experiment using a Big Dutchman Computer Viper Touch (Big Dutchman International GmbH, Vechta, Germany). The temperature was 23.08 ± 0.98 °C, relative humidity at 66.35 ± 5.68%, the light regime was adequate to the age of the hens (16 h light/8 h darkness).

Feed, leftovers and eggs were recorded and weighed daily in order to calculate the productive parameters (average daily feed intake, feed conversion ratio and laying percentage).

Eighteen eggs from each group were collected at the end of experiment in order to determine the quality parameters and nutrient composition. Another 18 eggs/group were collected for the shelf-life determination.

Dietary supplements

Sea buckthorn (*Hippophae rhamnoides* L., ssp. *carpatica*) fruits were harvested fully matured from an experimental field from Teleorman (2E PROD, Alexandria, Romania) and the meal is a co-product from cold pressed oil extraction.

Chemical analysis

Physical parameters of eggs. Physical parameters of egg quality were measured using an Egg Analyzer™, type 05-UM-001 (Orka Food Technology Ltd., Tel Aviv, Israel; weight, yolk colour intensity, Haugh unit and egg freshness), Eggshell Thickness Gauge (Orka Food Technology Ltd., Tel Aviv, Israel; eggshell thickness) and Egg Force Reader (Orka Food Technology Ltd., Tel Aviv, Israel; eggshell breaking strength).

Fatty acid determination. The sample used for fatty acid determination was prepared in accordance with the method described by Panaite et al. (2016). In order to determine the fatty acid

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Table 1. Composition and chemical analyses of basal diets

Ingredients	Control diet (%)	SBM (%)	Ingredients	Control diet (%)	SBM (%)
Corn	30.00	28.00	**Calculated analysis** (%)		
Wheat	31.46	31.46	Available phosphorous	0.42	0.42
Gluten	4.00	4.00	Lysine	0.87	0.87
Soybean meal	21.20	21.20	Methionine	0.44	0.44
Vegetable oil	1.46	1.46	Methionine + cysteine	0.78	0.78
Lysine	0.06	0.06	Threonine	0.58	0.58
Methionine	0.13	0.13	Tryptophan	0.18	0.18
Calcium carbonate	8.78	8.78	**Analysis of the bioactive components**		
Monocalcium phosphate	1.46	1.46	**Antioxidant profile**		
Salt	0.40	0.40	TAC (mM acid ascorbic)	36.37	37.38
Choline	0.05	0.05	TP (mg/g)	2.13	2.22
Sea buckthorn meal	0.00	2.00	Vitamin E (mg/kg)	38.73	49.07
Premix[1]	1.00	1.00	Lutein and zeaxanthin (mg/kg)	8.06	12.02
Total	100	100	**Fatty acids profile**		
Calculated analysis (%)			SFA (%)	19.05	19.83
Dry matter	88.06	88.11	MUFA (%)	27.97	30.69
ME poultry (kcal/kg)	2 800	2 783	PUFA (%)	52.71	49.31
Crude protein	17.80	17.96	n-3 (%)	4.18	4.65
Crude fat	2.94	3.22	n-6 (%)	48.52	44.66
Calcium	3.90	3.92	n-6/n-3	11.60	9.61

ME = metabolizable energy; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SBM = sea buckthorn meal; SFA = saturated fatty acids; TAC = total antioxidant capacity; TP = total polyphenols

[1]Content per kg of diet: vitamin A: 13 500 IU; vitamin D_3: 3 000 IU; vitamin E: 27 mg; vitamin K_3: 2 mg; vitamin B_1: 2 mg; vitamin B_2: 4.8 mg; pantothenic acid: 14.85 mg; nicotinic acid: 27 mg; vitamin B_6: 3 mg; vitamin B_7: 0.04 mg; vitamin B_9: 1 mg; vitamin B_{12}: 0.018 mg; vitamin C: 25 mg; manganese: 71.9 mg; iron: 60 mg; copper: 6 mg; zinc: 60 mg; cobalt: 0.5 mg; iodine: 1.14 mg; selenium: 0.18 mg

profile, a Clarus® 500 (Perkin-Elmer Inc., Waltham, MA, USA) gas chromatograph was used: FID detector and TRACE™ TR-FAME (Thermo Electron Corp., Waltham, MA, USA), 60 m × 0.25 mm × 0.25 µm capillary separation column. Atherogenic (AI) and thrombogenic (TI) indices were calculated according to (Mattioli et al. 2018) and peroxidability index was calculated as described by Galobart et al. (2002):

$$PI = (\%\ \text{monoenoic FA} \times 0.025) + (\%\ \text{dienoic FA} \times 1) + (\%\ \text{trienoic FA} \times 2) + (\%\ \text{tetraenoic FA} \times 4) + (\%\ \text{pentaenoic FA} \times 6) + (\%\ \text{hexaenoic FA} \times 8) \quad (1)$$

$$AI = (C12{:}0 + 4 \times C14{:}0 + C16{:}0)/(\Sigma MUFA + \Sigma n6 + \Sigma n3) \quad (2)$$

$$TI = (C14{:}0 + C16{:}0 + C18{:}0)/(0.5 \times \Sigma MUFA + 0.5 \times \Sigma n6 + 3 \times \Sigma n3 + \Sigma n3/\Sigma n6) \quad (3)$$

where:

PI – peroxidability index;
FA – fatty acid;
AI – atherogenic index;
MUFA – monounsaturated fatty acid;
TI – thrombogenic index.

Trace element determinations. Flame atomic absorption spectrometry (SOLAAR M6 Dual Zeeman Comfort; Thermo Electron Corp., Waltham, MA, USA) was used to determine the trace mineral concentrations in plant and yolk tissue samples according to the method described by Untea et al. (2012).

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Content of total polyphenols and total antioxidant capacity. The total phenol content of vegetal extracts (1 g of dried vegetal material extracted in 10 ml methanol, in the dark for 24 h) and yolk extracts (yolk samples homogenized in 0.05 M phosphate buffer) was measured by the Folin-Ciocalteu method. The calculation of the polyphenol concentration was done using the calibration curve of gallic acid and the results were reported as mg gallic acid equivalents per gram of dried sample.

The total antioxidant capacity was performed by phosphomolybdenum method as described by Untea et al. (2020). The antioxidant activity was expressed as ascorbic acid equivalents (mM ascorbic acid).

Lutein and zeaxanthin determination. Lutein and zeaxanthin were determined by reversed-phase high-performance liquid chromatography (HPLC) (Series 200 HPLC; Perkin-Elmer Inc., Waltham, MA, USA) with a UV detector (445 nm) after an extraction step performed according to the method previously described by Varzaru et al. (2015). The HPLC separation was carried out on a 5 µm C18 reversed-phase column (250 × 4.60 mm inner diameter) (Nucleodur, Macherey-Nagel, Germany) with a mobile phase of 13% water and 87% acetone, isocratic conditions at a flow rate of 1.0 ml/minute.

Vitamin E and A determination. Vitamin E determination was performed according to the method described in EC Regulation No. 152/2009, using a high-performance liquid chromatograph and a PDA-UV detector (HPLC Finningan Surveyor Plus; Thermo-Electron Corp., Waltham, MA, USA) at the wavelength of 292 nm for vitamin E and 325 nm for vitamin A. The HPLC separation was carried out on a HyperSil BDS C18 column, with silica gel (250 × 4.6 mm), particle size 5 µm (Thermo Electron Corp., Waltham, MA, USA), with a mobile phase of 4% water and 96% methanol, isocratic conditions at a flow rate of 1.5 ml/minute.

Oxidative stability parameters

Primary oxidation products. The peroxide value was determined spectrophotometrically using a V-530 Jasco spectrophotometer (Japan Servo Co., Ltd., Tokyo, Japan) by ferric thiocyanate method and it was expressed as milliequivalents of oxygen molecule per kg lipids (Pegg 2005). The yolk lipids reacted with chloroform/methanol solution (7 : 3, vol/vol) and 50 µl of xylenol orange (10 mM) and 50 µl of $FeCl_2$ (1000 mg/kg) were added. After 5 min incubation at room temperature, the spectrum was recorded at 560 nm. The conjugated dienes (CD) (primary products of peroxidation) and trienes (CT) (secondary products of peroxidation) were measured applying a spectrophotometric procedure (Pegg 2005). The yolk extract was dissolved in 2,2,4-trimethylpentane and the absorbances were measured at 233 nm (CD) and 268 nm (CT).

Secondary oxidation products. The *p*-anisidine value was measured by the reaction of yolk lipid extract dissolved in iso-octane with *p*-anisidine in acidic conditions (Pegg 2005).

The thiobarbituric acid reactive substances values were determined by third derivative spectrophotometry (Botsoglou et al. 1994) and were expressed as milligrams of malondialdehyde per kg tissue. The fresh yolk sample (5 g) was mixed with 10 ml trichloroacetic acid (7.5%) and 5 ml butyrate hydroxytoluene in ethanol (0.8%). An aliquot volume (2.5 ml) was mixed with 1.5 ml of 0.8% thiobarbituric acid solution and incubated at 80 °C for 50 min. Following the incubation, the absorbance was read at 532 nm (spectrum 0) and 540 nm (3^{rd} spectrum) using a spectrophotometer (Jasco V-530; Japan Servo Co., Ltd., Tokyo, Japan).

Statistics

The data obtained was analysed by one-way analysis of variance (ANOVA). Differences between groups were detected by Tukey multiple range test, using the XLSTAT software 2020 (Addinsoft, Paris, France). Probability less than 0.05 was considered significant and variability in the data was expressed as the standard error of the mean (SEM)

RESULTS

Nutrient composition of dietary supplement

Table 2 documents the nutrient composition of sea buckthorn meal used in the experiment. The antioxidant profile showed that the phytoadditive chosen for the experiment proved to be a valuable source of vitamin E, xanthophylls (lutein and zeaxanthin) and polyphenols. The inclusion of 2% of sea buckthorn meal in the experimental diet led to a more than 25% increase of vitamin E (38.73 mg/

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Table 2. Chemical composition of dietary supplement (sea buckthorn meal) (n = 3)

Analysed parameters	Sea buckthorn meal
Proximate composition	
Crude protein (%)	15.32
Crude fat (%)	16.67
Crude fibre (%)	22.96
Crude ash (%)	1.86
ME (kcal/kg)	2 447.00
Antioxidant profile	
TAC (mM acid ascorbic)	113.37
TP (mg/g)	21.65
Vitamin E (mg/kg)	295.31
Lutein and zeaxanthin (mg/kg)	168.75
Mineral profile	
Zinc (mg/kg)	25.20
Iron (mg/kg)	57.51
Copper (mg/kg)	8.96
Fatty acids profile	
SFA (%)	24.37
MUFA (%) of which:	50.30
Oleic acid (C18:1)	36.85
PUFA (%) of which:	25.33
n-3 (%) of which:	3.70
Alpha linolenic acid (C18:3n3)	2.34
n-6 (%) of which:	21.63
Linoleic acid (C18:2n6)	21.14
n-6/n-3	5.85

ME = metabolizable energy; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; TAC = total antioxidant capacity; TP = total polyphenols

kg vitamin E in C diet and 49.07 mg/kg vitamin E in SBM diet) and an almost 50% increase of lutein and zeaxanthin compared to the control diet (8.06 mg/kg in C diet and 12.02 mg/kg in SBM diet). The concentrations of fatty acids in sea buckthorn meal did not change the main fatty acid profile of compound feeds used in the experiment.

Effect of SBM dietary supplement on bioproductive parameters

The bioproductive parameters recorded during the experimental period are presented in Table 3. The feed conversion ratio calculated for the dietary SBM supplemented group had a higher value compared to the control group, but not statistically supported. No significant differences were observed between groups for any of the studied parameters.

Table 3. Effect of sea buckthorn dietary supplement (SBM) on bioproductive parameters (n = 30)

Parameters	Control	SBM	SEM	P-value
Average daily feed intake (g/hen)	108.29	108.34	1.167 6	0.980 0
Feed conversion ratio (kg feed/kg egg)	1.83	1.91	0.027 6	0.065 6
Laying percentage	98.22	97.74	0.735	0.649 1

Effect of SBM dietary supplement on quality parameters of eggs

The qualitative evaluation of eggs after four experimental weeks (Table 4) showed no significant differences between groups, except the yolk colour and eggshell thickness. The yolk colour was significantly (P < 0.000 1) improved under the influence of sea buckthorn meal but the eggshell thickness was negatively affected (P < 0.000 1) by the dietary treatment.

Effect of SBM dietary supplement on fatty acid profile of eggs

Dietary SBM was not an important contributor to the fatty acid profile of the experimental compound feed, but significant influences were noticed on fatty acid concentrations of eggs yolk (Table 5) as follows: saturated fatty acids (SFA) decreased

Table 4. Effect of sea buckthorn dietary supplement (SBM) on quality parameters of eggs (n = 18)

Parameters	Control	SBM	SEM	P-value
Egg weight (g)	60.68	60.31	0.439	0.680 9
Albumen weight (g)	45.44	44.75	0.356	0.342 3
Yolk weight (g)	15.24	15.56	0.254	0.540 1
Colour of egg yolk	5.61[a]	7.06[b]	0.154	< 0.000 1
Haugh unit	74.96	77.02	1.719	0.555 4
Eggshell thickness (mm)	0.377[b]	0.363[a]	0.002	< 0.000 1
Eggshell strength (kgF)	3.933	4.07	0.075	0.366 2

[a,b]Means within a row with no common superscript differ (P < 0.05)

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in SBM group compared to C group, while monounsaturated fatty acids (MUFA) and n-3 polyunsaturated fatty acids recorded a significant increase in SBM yolk samples ($P < 0.05$).

Effect of SBM dietary supplement on lipid quality indices of egg yolk

Table 6 presents the parameters of lipid quality while a significant increase ($P < 0.05$) of n-3 LC PUFA can be observed in the experimental group (SBM) compared to the control group. Also, the indices specific to the coronary risk (AI and TI) decreased significantly showing a beneficial effect of dietary SBM on lipid quality.

Table 5. Effect of sea buckthorn dietary supplement (SBM) on the fatty acid profile of eggs ($n = 6$)

Fatty acids	Control	SBM	SEM	*P*-value
C14:0	0.36	0.31	0.028 5	0.288 4
C15:0	0.07	0.06	0.005 9	0.392 9
C16:0	25.98[a]	24.86[b]	0.127 7	0.000 1
C17:0	0.11	0.14	0.016 9	0.298 5
C18:0	10.83	10.75	0.263 4	0.283 0
Total SFA	37.34[a]	36.12[b]	0.181 3	0.000 8
C14:1	0.08	0.08	0.007 5	0.945 9
C15:1	0.09	0.11	0.014 4	0.424 0
C16:1	3.71	3.83	0.084 8	0.355 3
C17:1	0.10	0.07	0.011 0	0.072 3
C18:1	33.59	34.54	0.363 6	0.405 6
C22:1n9	0.11	0.11	0.009 7	0.741 9
C24:1n9	0.24	0.28	0.012 8	0.077 3
Total MUFA	37.94[b]	39.02[a]	0.321 8	0.039 3
C18:2n6	16.39	16.26	0.054 3	0.116 8
C18:3n6	0.13	0.13	0.004 4	0.683 7
C20:2n6	0.16	0.19	0.009 2	0.051 1
C20:3n6	0.26	0.28	0.013 2	0.315 5
C20:4n6	3.88	4.02	0.144 0	0.517 1
C22:4n6	0.96	0.94	0.038 2	0.760 6
Total n-6	21.78	21.82	0.157 7	0.863 7
C18:3n3	0.57	0.57	0.022 3	0.903 2
C20:3n3	0.31	0.33	0.025 5	0.725 3
C22:5n3	0.14	0.15	0.006 1	0.154 0
C22:6n3	1.69[b]	1.94[a]	0.064 8	0.020 7
Total n-3	2.71[b]	2.99[a]	0.060 4	0.008 1
Total PUFA	24.49	24.81	0.196 9	0.276 1
Other fatty acids	0.23[a]	0.05[b]	0.027 0	0.000 8

MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids

[a,b]Means within a row with no common superscript differ ($P < 0.05$)

Table 6. Indices of the biological value of yolk lipids ($n = 6$)

Indices	Control	SBM	SEM	*P*-value
n-6/n-3	8.05	7.29	0.148 1	0.004 8
n-3 LC PUFA	2.14[b]	2.42[a]	0.067 0	0.015 1
n-6 LC PUFA	5.26	5.43	0.178 1	0.519 0
AA/LA	0.24	0.25	0.009 3	0.450 7
DHA/LNA	3.02	3.40	0.196 7	0.196 6
AI	0.61[a]	0.58[b]	0.005 6	0.006 5
TI	0.97[a]	0.92[b]	0.006 1	0.000 1
PI	52.83	55.37	1.153 8	0.149 9

AA = arachidonic acid; AI = atherogenic index; DHA = docosahexaenoic acid; LA = linoleic acid; LNA = linolenic acid; PI = peroxidability index; LC PUFA = long-chain polyunsaturated fatty acid; SBM = sea buckthorn meal; SFA = saturated fatty acid; TI = thrombogenic index

[a,b]Means within a row with no common superscript differ ($P < 0.05$)

Effect of SBM dietary supplement on bioactive nutrient content

The bioactive compounds detected in egg yolk (Table 7) showed a significant ($P < 0.05$) improvement in the antioxidant profile, the rate of vitamin and carotenoid deposition being increased under

Table 7. Bioactive nutrient content of egg yolk at the end of experiment ($n = 6$)

Bioactive nutrients	Control	SBM	SEM	*P*-value
Antioxidants				
TP (mg/g)	0.86	0.88	0.016 8	0.276 7
Vitamin E (mg/kg)	105.12[a]	148.36[b]	6.741 0	0.001 9
Vitamin A (mg/kg)	10.60[a]	39.47[b]	1.467 2	< 0.000 1
Lutein and zeaxanthin (mg/kg)	9.54[a]	13.86[b]	0.520 3	0.000 2
Minerals				
Copper (mg/kg)	2.40[b]	1.76[a]	0.080 7	0.000 2
Iron (mg/kg)	154.85[a]	161.44[b]	1.441 5	0.013 1
Zinc (mg/kg)	79.09[a]	81.51[b]	0.424 0	0.002 4

SBM = sea buckthorn meal; TP = total polyphenols

[a,b]Means within a row with no common superscript differ ($P < 0.05$)

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dietary SBM influence. Also, iron and zinc concentrations increased ($P < 0.05$) in yolk samples collected from the experimental group compared to the control.

Effect of SBM dietary supplement on lipid peroxidation parameters

The data presented in Table 8 show significantly ($P < 0.05$) decreased concentrations of peroxide value and conjugated dienes, proving that in the first phase of the lipid peroxidation process the dietary supplement (SBM) acted by inhibiting the formation of primary oxidation products.

DISSCUSSION

The carotenoid concentrations in sea buckthorn residues were found to be 10.81 mg/100 g (Momani Shaker et al. 2018). In a study on six Romanian sea buckthorn varieties, Pop et al. (2014) found the total carotenoid content between 53 and 97 mg/100 g. The differences regarding the carotenoid content in sea buckthorn reported by different authors might be explained by geographic location, growing or storage conditions (Tudor et al. 2020) but also they depend on the vegetal part considered for analysis (fruit, leaves or co- or by-products resulted from oil extraction). A study on leaves of seven Russian varieties of sea buckthorn revealed the total phenol concentration between 2.20 to 3.62 g/100 g; fat-soluble vitamin E concentration ranged between 9.01 and 25.71 mg/100 g and the trace elements were found to be 52.01 mg/kg Mn; 42.30 mg/kg Fe; 4.51 mg/kg Cu; 9.84 mg/kg Zn (Gradt et al. 2017).

Some authors considered that the main limitations of the phytoadditive inclusion in monogastric feeds are the lignified cell wall fraction and tannins, because they can affect the digestibility of feed ingredients (Mabusela et al. 2018) and the inclusion levels in the diets have an important impact on egg weight. Contrary to our results, other authors (Momani Shaker et al. 2018) reported a significantly increased number of total laid eggs under the influence of 5% sea buckthorn fruit residues in diet. In a study where the diet of Rhode Island Red × Fayoumi laying hens contained 3 g/kg sea buckthorn seeds, egg weight and egg production were significantly improved but eggshell parameters were not influenced (Chand et al. 2018). In studies conducted on broilers, the authors reported no influence of sea buckthorn residues on production data of birds (Orczewska-Dudek et al. 2018).

The quality parameters of eggs did not differ significantly between groups in egg, albumen and yolk weights, shell strength or Haugh units, observations recorded also by other authors when the experimental laying hens were fed 5% and 6.5% dietary sea buckthorn, respectively (Momani Shaker et al. 2018). A 25% more intense colour was registered in the SBM supplemented group compared to the control, this observation being associated with the level of carotenoids in the diets by other authors (Momani Shaker et al. 2018). Dumbrava et al. (2006) considered that the presence of lutein and zeaxanthin from sea buckthorn is the main reason for carotenoid content in egg yolk and their pigmentation.

The results of the present study showed that no significant differences between groups were recorded in alpha linolenic acid (LNA) (C18:3n3) and linoleic acid (LA) (C18:2n-6) concentrations in egg yolk but the content of docosahexaenoic acid (DHA) (C22:6n-3) increased ($P < 0.05$) in SBM yolk samples. LNA and LA are considered as main fatty acids due to their importance as precursors of long-

Table 8. Effect of sea buckthorn dietary supplement (SBM) on lipid peroxidation parameters ($n = 6$)

		Control	SBM	SEM	*P*-value
Primary oxidation products	PV (meq active O_2/kg)	0.179[b]	0.143[a]	0.008 1	0.020 1
	CD (µmol/g)	7.544[b]	4.944[a]	0.502 8	0.011 3
Secondary oxidation products	CT (µmol/g)	2.293	2.149	0.262 2	0.693 5
	p-anisidine	6.418	5.744	0.636 8	0.482 4
	TBARS (mg/kg)	0.147 7	0.144 5	0.008 8	0.820 1

CD = conjugated dienes; CT = conjugated trienes; PV = peroxide value; TBARS = thiobarbituric acid reactive substances

[a,b]Means within a row with no common superscript differ ($P < 0.05$)

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chain n-6 and n-3 PUFA (LC PUFA). Dietary LNA and LA are incorporated into animal tissues and by desaturation and elongation they are converted into eicosapentaenoic acid (C20:5n-3), DHA and arachidonic acid (AA) (C20:4n-6), respectively (Coetzee and Hoffman 2002). These elongated metabolites (long-chain fatty acids) are deposited in the egg yolk. The reaction yield is different due to a competitive interaction between LNA and LA. If large amounts of LA are present, the conversion of LNA into eicosapentaenoic acid and DHA is inhibited (da Silva Filardi et al. 2005). The delta-6-desaturase is the rate limiting step in the synthesis of LC PUFA from their 18-carbon precursors. The higher values recorded for the ratios of n-3 LC PUFA and their precursors (DHA/LNA) and n-6 LC PUFA and their precursors (AA/LA) in SBM group compared to the control show an improved bioconversion of fatty acids stimulated by the dietary supplement.

AI is the ratio between main saturated and main unsaturated fatty acids. The SFA are considered proatherogenic, meaning that they favour the adhesion of lipids to cells, while UFA are considered antiatherogenic because they prevent the coronary disease occurrence. TI shows the tendency to form clots in the blood vessels. The SFA are considered prothrombogenic, and UFA are considered antithrombogenic (Mattioli et al. 2018). Antioxidant properties of dietary supplement can stimulate the metabolic pathway of n-3 and n-6 fatty acids. Atherogenic and thrombogenic indices show the probability of occurrence of pathogenic phenomena like atheroma or thrombus formation (Criste et al. 2018). In the present study the AI and TI were significantly lower in the egg yolk of SBM supplemented group compared to C group. The obtained results show a potential beneficial effect of these eggs on human health by protection of the cardiovascular system.

When they studied the transfer of bioactive compounds from pasture to the meat of organic free-range chickens, Dal Bosco et al. (2016) observed that higher concentrations of PUFA are easily oxidized resulting in a higher value of peroxidability index. In our study, the differences between groups were not statistically significant proving that the antioxidant profile of SBM group egg yolk delayed the PUFA oxidation process.

Freshness, eggshell quality and yolk colour are considered the main egg quality parameters by consumers. Carotenoids are the main components that give colour to the egg yolk. According to the literature (Yang and Kallio 2005), the amounts of carotenoids in sea buckthorn fruits range between 27 to 105 mg/kg dry matter and are mainly represented by lutein and zeaxanthin, which represent 81–96% of the total carotenoids. The required time for stabilisation of xanthophyll deposition in egg yolk is up to 11 days (Karadas et al. 2006). The profile of carotenoids can be changed through feed manipulations. Beta-carotene is a widespread carotenoid but it is found only in small amounts in egg yolk due to its efficient bioconversion in vitamin A. In the present study, the concentrations of xanthophylls were by 45% higher than in the control group, while vitamin A was almost four times higher than in the reference group.

The results obtained in our study showed an improved lipid profile of egg yolk belonging to the SBM dietary supplemented group (Tables 5 and 6). An increasing n-3 fatty acid can lead to a higher susceptibility to lipid oxidation depending on the balance of anti- and prooxidant compounds (Criste et al. 2018). The inclusion of SBM in the diet of laying hens produced more than a 30% increase of lipophilic antioxidant compounds and their positive effect on the oxidative stability of eggs during storage can be observed. The presence of large amounts of lipophilic antioxidants in egg yolk of SBM group led to a significant reduction of primary peroxidation products, proving an efficient effect of dietary SBM antioxidants in the first phase of lipid degradation process. Retinol is a carotenoid produced in the liver from the breakdown of beta-carotene and it presents the ability to inhibit the peroxyl radical propagation, and vitamin E inhibits the lipid peroxidation by donating its phenolic hydrogen to peroxyl radicals, becoming unable to continue the chain reaction (Carocho and Ferreira 2013). Carotenoids are considered the most efficient molecules for singlet oxygen quenching, lutein being recognized for its ability to diminish the presence and formation of reactive oxygen species (Lee et al. 2004).

CONCLUSIONS

The dietary inclusion of sea buckthorn meal in laying hen diets has a beneficial effect on bioactive compound concentrations of eggs, by increasing the antioxidant profile of eggs and improving

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the fatty acid profile of egg yolk lipids. Also, sea buckthorn meal supplements influenced the oxidative stability of eggs during storage by delaying the lipid oxidation process.

Conflict of interest

The authors declare no conflict of interest.

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