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REGULAR ARTICLES

A comparative study on the effects of hemp seed oil versus four different UFA-rich seed oils' dietary supplementation on egg production performance, egg quality, and yolk fatty acids in laying hens

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Abstract
This study compared the effects of hemp seed oil versus four different UFA-rich seed oils in the diet of laying hens on egg production, egg quality, and fatty acid profile of the yolk. Soybean oil (SBO), sunflower oil (SFO), corn oil (CO), canola oil (CAO), and hemp seed oil (HSO) were included in the hens' diets in equal proportions. A total of one hundred and twenty White Leghorn hens were allocated into five groups with 8 replicates, each with 3 hens. The trial lasted 84 days and data were collected on egg production, quality, and fatty acid profile of the yolk. The results showed that none of the incorporated seed oils affected egg production parameters and eggshell quality. However, hemp seed oil altered yolk colour values similarly to canola oil by increasing the L* value of the yolk whilst decreasing the a* value ($P < 0.05$). Hemp oil increased the PUFA content in the yolk, similar to soybean, corn, and sunflower oil, but unlike the latter, it also enriched the n-3 fatty acids in the yolk ($P < 0.05$). In conclusion, hemp seed oil can be safely used in the diet of chickens without negative effects on egg production and egg quality like other seed oils. Furthermore, hemp seed oil can improve the desirable fatty acid content in the yolk and has the potential to produce n-3-enriched eggs.

Keywords Canola oil · Corn oil · Soybean oil · Sunflower oil

Introduction

Lipids are concentrated sources of energy and are used in poultry nutrition to improve energy stability, palatability, and physical quality of the feed. During the peak phase of laying hens, the intensity of lipid metabolism in the body increases, and the oils in the feed enter the body of the eggs via the ovaries (Gao et al. 2021). The vegetable oils commonly used in the feed are rich in PUFAs (polyunsaturated fatty acids) and the lipids in the egg yolk are mainly originated from the oils in the feed. Therefore, oils play a crucial role in the production performance and egg quality of laying hens (Gao et al. 2021). In addition, fats and oils provide significant amounts of linoleic acid, which is an essential nutrient for chickens, since it has been suggested that diets should contain at least 1% linoleic acid (Leeson and Summers 2009). It has been reported that high levels of linoleic acid in feed can improve egg production performance (Bohnsack et al. 2002). Another purpose of using lipid sources in egg production is to modulate the fatty acid profile (FA) of the yolk or generate functional eggs. Therefore, vegetable lipids, including oils from seeds rich in UFA, n-3, and n-6 (omega 3 and omega 6, respectively) FA, are preferred in the diet of hens (Simopoulos 2001). It has also been reported that fish oil, which is rich in n-3 PUFA, can increase the n-3 PUFA content of egg yolk in chicken diets (Ebeid et al. 2008; Saleh 2013). Manipulation of the fatty acid content of egg yolk can improve the health image of eggs and increase their value as a food supplement (Reda et al. 2020). In particular, eggs enriched with n-3 FA are becoming increasingly popular following the discovery that n-3 is an important micronutrient for normal growth and has been linked to the treatment and prevention of various diseases, including cancer (Yang et al. 2006, 2018; Leeson and Summers 2009; Reda et al. 2020). Clinical studies have shown that not only n-3 but

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also the total amount of PUFA, when replacing saturated fatty acids (SFA) in the human diet, have beneficial effects on the cardiovascular system (Jakobsen et al. 2009) and the egg has become a common source to increase the dietary intake of PUFA in humans. Soybean oil (SBO) is widely used in commercial egg production due to its high content of polyunsaturated fatty acids (PUFA) and linolenic acid (Dei 2011) and has become almost a standard feed ingredient in poultry diets. Corn oil (CO) and sunflower oil (SFO) are commonly used in chicken diets due to their high content of n-6 fatty acids, especially linoleic acid (Reda et al. 2020). SBO, CO, and SFO appear to be suitable lipid sources for producing eggs with enriched PUFAs, but due to economic concerns and competition with human diets, the search for alternative lipids that could replace them in poultry diets continues. Canola oil (CAO) has become a popular lipid source for diets due to its high content of oleic acid, known as n-9 (omega-9), and studies on the effects of CAO in chicken diets on egg yolk fatty acids have increased (Gül et al. 2012). Hemp seed oil (HSO), a by-product of hemp plant (*Cannabis sativa*) seeds, has the potential to be used in poultry diets due to its excellent micronutrient composition, such as high levels of PUFA and n-3 FAs, and its antioxidant capacity (Oomah et al. 2002). However, due to the legal status of hemp in many countries (Silversides and Lefrancois 2005), HSO cannot be evaluated as a feed additive and further studies are needed to use hemp seed oil as a lipid source in chicken feed. The nutritional properties of the lipid sources used in this study have been well identified and a wide body of knowledge is available in the scientific literature. Plant-based lipids, including some seed oils, have been widely studied in poultry diets and their advantages over animal fat sources have been elaborated. However, comparative studies on lipids of the same origin are needed to determine the crucial usability of newer lipids such as HSO in poultry nutrition. The idea of this study was to distinguish the merits of five different lipid sources of the same origin for chicken nutrition and to present their implications and reveal the usability of hemp seed oil for commercial egg production. In this context, the current study investigated five seed-based lipid sources with high UFA content—soybean, corn, sunflower, canola, and hemp seed oils—and their effects on egg-laying performance, egg quality, and egg yolk fatty acid composition.

Materials and methods

The animal experiment was conducted in the Poultry Unit of Prof. Dr. Orhan Düzgüneş Application and Research Farm of the Faculty of Agriculture, Selcuk University.

Animal management procedures

A total of one hundred and twenty 42-week-old White Leghorn chickens were randomly allocated to five treatment groups. Each treatment group contained 24 birds, with 8 replicates and 3 chickens per compartment. The experimental diets were formulated to be isocaloric and isonitrogenic and to meet the nutrient requirements of the National Research Council (NRC 1994) for laying hens. The ingredients and nutrient composition of the experimental feeds are shown in Table 1 and the fatty acid composition of the oil sources in Table 2.

Crude oils from soybean, sunflower, corn, and canola oils were obtained from a commercial local feed factory (Çöğenler Feed Factory, Konya, Turkey). Crude hemp seed oil was extracted by cold pressing (at about 45–50 °C) from hemp seeds (*Cannabis sativa*) using an expeller (Karaerler Machine, model NF 100, Ankara). The metabolic energy value of the seed-based oil sources was ≅ 8800 kcal/Kg. The experiment lasted 84 days and a 16-h light and 8-h dark programme was applied; feed and water were provided ad libitum. The cage dimensions were $40 \times 50 \times 40$ cm and corresponded to a total floor area of 2000 cm^2.

Production performance and egg quality

At the beginning and the end of the experiment, the hens were weighed, and the weight gain (WG) was determined from the differences. Feed intake (FI) and egg weight (EW) were recorded fortnightly. Egg production (EP) was recorded daily, and egg mass was calculated using the formula egg production × egg weight / period (days). Feed conversion ratio (FCR) was calculated using the ratio of feed intake to egg mass. Egg quality analyses were performed on all eggs collected on the last 2 consecutive days of the three 28-day periods (32 eggs from each group, 160 for each day of analysis). The breaking strength of eggshells was measured with a cantilever system using a force reader instrument (Orka Food Technology Ltd., Ramat Hasharon, Israel). The broken eggshells were rinsed under running water and dried in an oven at 60 °C for 12 h. The eggshells were weighed with a 0.001 g precision balance. The thickness of the eggshells (with membrane) was measured at 3 points on the eggs (1 point on the air cell and 2 random points on the equator) using a digital micrometre (Mitutoyo Inc., Japan). The colour values of the yolk were determined with a portable colour metre (Minolta Chroma Metre CR 400; Minolta Co., Osaka, Japan) and the parameters L*, a*, and b* corresponded to brightness (−100 / +100, dark/white), redness (−100 / +100, green/red), and yellowness (−100 / +100, blue/yellow), respectively.

Table 1 The nutrient composition of experimental diets

Ingredients (%)	Experimental diet groups				
	SBO	SFO	CO	CAO	HSO
Corn	53.80	53.80	53.80	53.80	53.80
Soybean meal (47% CP)	23.00	23.00	23.00	23.00	23.00
Sunflower meal (35% CP)	8.00	8.00	8.00	8.00	8.00
Seed oil (≧8800 kcal ME/kg)	3.70	3.70	3.70	3.70	3.70
Limestone	9.10	9.10	9.10	9.10	9.10
Di-calcium phosphate	1.70	1.70	1.70	1.70	1.70
Salt	0.25	0.25	0.25	0.25	0.25
Premix[1]	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.00	0.00	0.00	0.00	0.00
DL-Methionine	0.20	0.20	0.20	0.20	0.20
Calculated nutrients					
Crude protein (%)	17.04	17.04	17.04	17.04	17.04
Metabolic energy (kcal)	2801.70	2801.70	2801.70	2801.70	2801.70
Crude fat (%)	6.05	6.05	6.05	6.05	6.05
Calcium (%)	3.94	3.94	3.94	3.94	3.94
Available phosphorus. (%)	0.42	0.42	0.42	0.42	0.42
Lysine (%)	0.83	0.83	0.83	0.83	0.83
Methionine (%)	0.47	0.47	0.47	0.47	0.47
Cysteine (%)	0.25	0.25	0.25	0.25	0.25
Methionine-cysteine (%)	0.73	0.73	0.73	0.73	0.73

SBO, soybean oil; *SFO*, sunflower oil; *CO*, corn oil; *CAO*, canola oil; *HSO*, hemp seed oil; *CP*, crude protein

[1]Premix provided the following per kg of diet: vitamin A: 8.800 IU, vitamin D_3: 2.200 IU, vitamin E: 11 mg, nicotinic acid: 44 mg, Cal-D-pantothenate: 8.8 mg, riboflavin: 4.4 mg, thiamine: 2.5 mg, vitamin B_{12}: 6.6 mg, folic acid: 1 mg, D-biotin: 0.11 mg, choline: 220 mg, manganese: 80 mg, copper: 5 mg, iron: 60 mg, zinc: 60 mg, cobalt: 0.20 mg, iodine: 1 mg, selenium: 0.15 mg

Table 2 Fatty acid profile of dietary seed oils

Fatty acids (%)	SBO	SFO	CO	CAO	HSO
Palmitic *16:0*	7.47	7.09	10.56	5.21	7.70
Stearic *18:0*	3.00	3.98	2.72	1.29	3.55
Oleic *18:1*	17.09	25.80	31.53	68.89	17.14
Linoleic *18:2*	56.19	63.13	55.19	18.78	55.22
α-Linolenic *18:3*	15.79	nd	nd	5.36	15.89
Arachidic *20:0*	0.46	nd	nd	0.16	0.50
Gadoleic acid *20:1*	nd	nd	nd	0.31	nd
ΣSFA	10.93	11.07	13.28	6.66	11.75
ΣMUFA	17.09	25.80	31.53	69.20	17.14
ΣPUFA	71.98	63.13	55.19	24.14	71.11
Σn-3	15.79	nd	nd	5.36	15.89
Σn-6	56.19	63.13	55.19	18.78	55.22

SBO, soybean oil; *SFO*, sunflower oil; *CO*, corn oil; *CAO*, canola oil; *HSO*, hemp seed oil; *ΣSFA*, total saturated fatty acid; *ΣMUFA*, total monounsaturated fatty acid; *ΣPUFA*, total polyunsaturated fatty acid; *Σn-3*, total omega-3 fatty acid; *Σn-6*, total omega-6 fatty acid; *nd*, not detected

Determination of fatty acids

On the last 3 consecutive days of the experiment, daily one egg was taken from each replicate and 24 from each treatment group in 3 days, a total of 120 eggs were collected, and the yolk was separated. The yolk oil was extracted using the ethanol/chloroform (1:1) solution method (Kovalcuks and Duma 2014). The fatty acid methyl esters of the yolk and seed oils were prepared according to the method recommended in EU Regulation 2568/91. The oils were weighed into screw-capped glass tubes (0.10 g) and dissolved in 10.0 mL of hexane. Then, 100 µL of 2 N potassium hydroxide solution in methanol was added to the tubes and shaken vigorously for 30 s. The tubes were then shaken for 5 min. The tubes were centrifuged at 2500 × g for 5 min and the upper layer was withdrawn into a small vial and stored at 0 °C until the time of analysis (Ayyildiz et al. 2015). The fatty acid composition of the methyl esters was detected by gas chromatography (GC; Shimadzu GC-2010 Plus, 86 Japan) using an FID detector and an HP -88 column (100 m × 250 µm × 0.20 µm id). The temperature at the injection block was 250 °C and the heating programme of the column oven was set to 2 min at 50 °C,

4 min between 50 and 250 °C, and 10 min at 250 °C. The carrier gas used was helium with a flow rate of 1.3 mL/min. Fatty acids were detected based on the retention time (min) and area (%) of the identified peaks and classified with fatty acid standards (Supelco® 37 Component FAME Mix, Merck, Germany) and expressed as a percentage.

Statistical analysis

The experiment was designed as a complete randomised model and data were analysed using the one-way ANOVA procedure with Minitab software. Duncan's multiple range test was used to determine the differences amongst treatments that were found to be significantly different ($P < 0.05$).

Results

The effects of adding seed oils to the diet on egg production, egg quality, and yolk colour values are summarised in Table 3.

The data for weight gain, feed intake, feed conversion ratio, egg production, and egg mass were similar amongst the oil groups and the differences were insignificant. None of the seed oils changed egg quality parameters, including egg weight, eggshell breaking strength, egg weight, and eggshell thickness significantly. Egg yolk colour values ($P < 0.05$) L* and a* were affected by seed oils, whilst b* was not changed. Egg yolk brightness (L*) increased with the inclusion of HSO and CAO in the diet, but a* decreased significantly with the presence of HSO in the diet ($P < 0.05$). The results of the inclusion of seed oils in the diet of hens on the fatty acid content of egg yolk are shown in Table 4.

The different seed-based lipid sources did not influence the levels of palmitic acid, stearic acid, and eicosapentaenoic acid (EPA) in the egg yolk. The levels of myristic and palmitoleic acids in the yolk increased with the addition of SBO ($P < 0.05$). With the inclusion of CAO in the diet, the content of oleic acid in the yolk reached the highest value, whilst linoleic acid decreased ($P < 0.05$). Both HSO and CAO contributed into a higher content of linolenic acid in the yolk than other seed oils ($P < 0.05$). Arachidic acid content was increased in the SFO group but decreased in the HSO group ($P < 0.05$). SBO increased eicosadienoic acid, both SFO and CO increased erucic acid, and HSO enhanced docosahexaenoic acid (DHA) concentration in egg yolk ($P < 0.05$). The total SFA content of the egg yolk was not affected by the seed oils and the results were similar. However, the ΣMUFA and ΣPUFA content of the yolk was influenced by the oils in the diet, and with CAO in the diet, the ΣMUFA content of the yolk was significantly higher than in the other four groups ($P < 0.05$). SBO and HSO in the diet caused an increase of the ΣPUFA content of the egg yolk, whereas CAO in the diet reduced the ΣPUFA amount of the egg yolk ($P < 0.05$). The total amount of n-3 in the yolk was significantly changed by the dietary oils, and the highest Σn-3 content was found in the HSO group, whilst CAO in the diet resulted in a higher

Table 3 Effects of seed-based oils on laying performance, egg quality parameters, and yolk colour values

	SBO	SFO	CO	CAO	HSO	*P* value
Performance parameters						
Weight gain (g)	67.40 ± 32.31	40.20 ± 53.63	42.70 ± 64.06	54.60 ± 12.73	49.60 ± 39.15	0.99
Feed intake (g/d)	116.95 ± 1.79	113.61 ± 1.62	117.68 ± 1.81	114.02 ± 1.26	115.46 ± 1.48	0.32
Feed conversion ratio	1.98 ± 0.03	1.86 ± 0.04	1.89 ± 0.04	1.84 ± 0.04	1.83 ± 0.04	0.08
Egg production (%)	94.95 ± 1.33	96.73 ± 0.97	96.12 ± 0.79	95.71 ± 1.98	97.82 ± 0.30	0.54
Egg mass (g)	59.18 ± 0.88	61.18 ± 1.03	62.28 ± 1.10	62.15 ± 1.81	63.12 ± 0.80	0.20
Egg quality parameters						
Egg weight (g)	62.36 ± 0.92	63.28 ± 1.17	64.77 ± 0.85	64.89 ± 0.97	64.52 ± 0.84	0.29
Eggshell strength (kg/cm^2)	3.96 ± 0.12	4.51 ± 0.15	4.47 ± 0.16	4.41 ± 0.13	4.45 ± 0.17	0.16
Eggshell weight (g)	5.98 ± 0.09	6.31 ± 0.12	6.21 ± 0.09	6.26 ± 0.08	6.11 ± 017	0.32
Eggshell thickness (mm)	37.0 ± 0.42	39.0 ± 0.00	37.0 ± 0.04	38.0 ± 0.00	38.0 ± 0.1	0.22
Egg yolk colour values						
L*	58.23 ± 0.32^{b}	58.35 ± 0.42^{b}	58.27 ± 0.22^{b}	60.68 ± 0.44^{a}	60.31 ± 0.37^{a}	0.00
a*	4.90 ± 0.35^{a}	4.90 ± 0.30^{a}	5.18 ± 0.17^{a}	3.89 ± 0.58^{ab}	3.48 ± 053^{b}	0.03
b*	40.98 ± 0.41	39.17 ± 0.68	40.27 ± 0.56	39.23 ± 1.03	39.99 ± 0.92	0.43

$^{a,b,c}P < 0.05$

Data are presented as means ± SEM

SBO, soybean oil; *SFO*, sunflower oil; *CO*, corn oil; *CAO*, canola oil; *HSO*, hemp seed oil

Table 4 Effects of seed-based oils on egg yolk fatty acid profile

Fatty acids (%)	SBO	SFO	CO	CAO	HSO	*P* value
Myristic *14:0*	0.288 ± 0.012^{a}	0.238 ± 0.009^{b}	0.218 ± 0.001^{b}	0.216 ± 0.010^{b}	0.222 ± 0.009^{b}	*0.00*
Palmitic *16:0*	25.14 ± 0.033	24.54 ± 0.266	24.19 ± 0.405	24.12 ± 0.559	24.30 ± 0.199	0.29
Palmitoleic *16:1*	3.56 ± 0.198^{a}	2.81 ± 0.137^{b}	2.42 ± 0.066^{b}	2.63 ± 0.177^{b}	2.64 ± 0.115^{b}	*0.00*
Stearic *18:0*	8.59 ± 0.582	8.68 ± 0.106	9.25 ± 0.561	8.52 ± 1.701	10.18 ± 0.244	0.17
Oleic *18:1*	39.56 ± 0.901^{b}	39.92 ± 0.651^{b}	40.15 ± 1.090^{b}	46.06 ± 0.077^{a}	38.76 ± 0.017^{b}	*0.00*
Linoleic *18:2*	20.04 ± 1.13^{a}	19.46 ± 0.745^{a}	19.59 ± 1.29^{a}	14.34 ± 1.01^{b}	19.18 ± 0.433^{a}	*0.01*
α-Linolenic *18:3*	0.321 ± 0.03^{c}	0.277 ± 0.02^{c}	0.325 ± 0.03^{c}	1.211 ± 0.202^{b}	2.096 ± 0.212^{a}	*0.00*
Arachidic *20:0*	0.321 ± 0.038^{ab}	0.399 ± 0.004^{a}	0.333 ± 0.105^{ab}	0.309 ± 0.009^{ab}	0.250 ± 0.041^{b}	*0.03*
Eicosadienoic *20:2*	0.2150 ± 0.014^{a}	0.152 ± 0.090^{ab}	0.166 ± 0.026^{ab}	0.107 ± 0.002^{b}	0.189 ± 0.031^{ab}	*0.03*
Eicosapentaenoic *20:5*	0.013 ± 0.003	0.016 ± 0.001	0.021 ± 0.003	0.057 ± 0.037	0.0246 ± 0.006	0.41
Erucic *22:1*	1.169 ± 0.083^{b}	2.087 ± 0.027^{a}	1.883 ± 0.014^{a}	1.418 ± 0.101^{b}	1.492 ± 0.119^{b}	*0.00*
Docosahexaenoic *22:6*	0.178 ± 0.043^{ab}	0.079 ± 0.001^{b}	0.083 ± 0.005^{b}	0.165 ± 0.023^{ab}	0.224 ± 0.040^{a}	*0.02*
ΣSFA	34.34 ± 058	33.86 ± 0.202	34.00 ± 0.48	33.17 ± 1.24	34.95 ± 0.109	0.44
ΣMUFA	44.29 ± 0.95^{b}	44.82 ± 0.78^{b}	44.47 ± 1.09^{b}	50.12 ± 0.182^{a}	42.90 ± 0.161^{b}	*0.00*
ΣPUFA	20.7 ± 1.08^{a}	19.99 ± 0.781^{ab}	20.19 ± 1.34^{ab}	15.88 ± 1.14^{b}	21.71 ± 0.506^{a}	*0.01*
Σn-3	0.498 ± 0.04^{c}	0.356 ± 0.02^{c}	0.409 ± 0.03^{c}	1.37 ± 0.18^{b}	2.32 ± 0.22^{a}	*0.00*
Σn-6	20.25 ± 1.12^{a}	19.618 ± 0.754^{a}	19.76 ± 1.31^{a}	14.44 ± 1.01^{b}	19.374 ± 0.438^{b}	*0.01*

[a,b,c]$P < 0.05$

Data are presented as means ± SEM

SBO, soybean oil; *SFO*, sunflower oil; *CO*, corn oil; *CAO*, canola oil; *HSO*, hemp seed oil; *ΣSFA*, total saturated fatty acid; *ΣMUFA*, total monounsaturated fatty acid; *ΣPUFA*, total polyunsaturated fatty acid; *Σn-3*, total omega-3 fatty acid; *Σn-6*, total omega-6 fatty acid

Σn-3 compared to SBO, CO, and SFO ($P < 0.05$). The total amount of n-6 decreased with the inclusion of CAO and HSO in the diet ($P < 0.05$).

Discussion

Previous studies have confirmed that dietary lipid types do not affect egg production and egg weight (Ceylan et al. 2011; Abbasi et al. 2019). It was reported that egg yield, egg weight, and feed intake did not change with the inclusion of different lipid sources (cotton, corn, flax, soybean, olive, sunflower, fish, tallow, and rendering) in the diet (Balevi and Coskun 2000). Omidi et al. (2015) used five different lipids rich in UFAs (fish, olive, grapeseed, canola, and soybean oils) in the diet and found that performance parameters during the laying period did not differ significantly amongst treatments. It has been reported that no change was found in egg production data in laying hens fed with different levels of fish oil (Ebeid et al. 2008). It has long been known that vegetable oils in the diet of chickens can increase egg weight, whilst fats from animal sources have less effect on it (Jensen et al. 1958; Shutze et al. 1958; Treat et al. 1960). To test this hypothesis, Whitehead et al. (1993) reported that corn oil in the diet of hens increases egg weight compared to fish oil, coconut oil, and tallow. Moreover, egg size may increase when hens are fed diets high in oil and rich in unsaturated fatty acids (Galea 2011) which affects shell quality. It has been reported that feeding canola oil, which is high in linoleic acid, results in higher egg weight than feeding poultry fat (Bohnsack et al. 2002). The results of some studies comparing the effects of different vegetable oils in the diet of chickens showed that egg quality parameters were not influenced by the oil sources. For example, Batkowska et al. (2020) showed that the inclusion of 2.5% linseed or soybean oil in the diet of laying hens had no negative effect on egg quality. Similarly, Küçükersan and Küçükersan (2010) found no differences in egg quality when high UFA oils such as sunflower, fish, soybean, and hazelnut oil were included in the feed at a concentration of 3%. A recent study proved that linseed and soybean oil have no effect on quality traits, but increase the weight of chicken eggs (Batkowska et al. 2021). However, some studies reported that olive and sunflower oil in the diet increased the weight of eggs compared to sesame, cottonseed, hazelnut, maize, soybean, and fish oil, but the thickness of the eggshell was not changed by the lipid sources in the diet (Güçlü et al. 2008). Depending on the amount of added oil in the diet, the amounts of other feeds may change so that the fatty acid profile and nutrient composition of the diet may vary, and these changes may affect egg quality parameters. In our study, the experimental diets were formulated with the same amount of feed ingredients and with the

same content of seed-based oils (3.7% of the diet), so that the FA changes in the experimental diets were caused by the added oils and the FAs of the experimental diets in this study clearly had no effect on the egg quality parameters.

The colour of the egg yolk is another effective factor that can influence consumer choice as much as the size of the eggs and can be easily influenced by feed ingredients. For this reason, feed premixes containing carotenoids, which provide the preferred dark yellow yolk colour, are often used in the diets of laying hens. It is reported that unrefined seed oils can contain a detectable amount of carotenoids (Franke et al. 2010), but this amount depends on the carotenoid content of the seed. In addition, the literature mentions that different vegetable oils, depending on their content or mixed oil sources in the diet, can alter the pigmentation of the egg yolk (Faitarone et al. 2016). In this study, a* value, indicating redness, decreased significantly with the intake of HSO and decreased slightly with the intake of CAO, compared to CO, SBO, and SFO. The L* value, indicating the brightness of the yolk, increased in parallel with the decrease in a* value in the CAO and HSO groups. The results of the current study have shown that HSO and CAO can cause pale yellow yolk colour due to their various pigment contents such as chlorophyll or carotenoids, which can affect yolk colour, and this fact should be considered when selecting seed-based oils for laying hen diets. The fatty acid composition of egg yolk has become an important issue today as there is evidence of a link between the fatty acid profile of the human diet and cardiovascular disease. Since it has been understood that the issue of dietary oil is important in designing a PUFA- and Σn-3-enriched egg, studies have been presented that used lipids high in UFA or PUFA such as vegetable, nut, and fish oils in the diet of chickens (Alagawany et al. 2019). Apart from vegetable oils, fish oil, which is rich in PUFA, was used to improve the FA profile of egg yolk, and Ebeid et al. (2008) reported that with the increase of fish oil in the diet, the n-3 total PUFA content increased, but the n-6 PUFA content in yolk lipid decreased.

The results of our study showed that dietary seed oils altered the DHA, ΣPUFA, and Σn-3 content of egg yolk ($P < 0.05$), and this change was directly related to the FA composition of the oil in the diet. Previous studies have also shown that SO, CO, and SFO improved the n-6 content of egg yolk (Kang et al. 2006; Güçlü et al. 2008; Oliveira et al. 2010). CAO, which has the highest oleic acid content, increased the ΣMUFA content but decreased the ΣPUFA content of egg yolk. Similarly, CAO was reported to enrich the oleic acid content of the yolk instead of SO in the hens' feed (Gül et al. 2012). In addition, feeding laying hens with diets high in MUFA has been found to increase the oleic acid content in egg yolks (Milinsk et al. 2003). Oleic acid is not essential for humans and to produce ΣPUFA and Σn-3 design eggs CAO may not be the best choice. On the other hand, Σn-9 has been defined as a beneficial fatty acid for human health (Galán-Arriero et al. 2017) and CAO can be used in the diet of laying hens to improve the MUFA content in the egg yolk. The enrichment effect of dietary HSO on α-linolenic acid, DHA, and PUFA content in egg yolk was impressive. HSO caused the highest content of linolenic acid in the egg yolk. In humans, linolenic acid can be converted to a limited extent to EPA and DHA (Fraeye et al. 2012) and many studies have shown that higher intakes of omega-3 PUFAs, especially EPA and DHA, are linked to lower chronic disease incidence (Djuricic and Calder 2021). Our previous study also confirmed that HSO in the diet significantly increases ΣPUFA and Σn-3 content in quail egg yolk compared to SBO and SFO (Göçmen et al. 2021). In another study, HSO in the diet was reported to significantly increase n-3 PUFA content in egg yolk (Goldberg et al. 2012). A recent meta-analysis confirmed that table eggs from chickens fed whole hemp seeds or their by-products containing HSO in varying amounts had higher levels of long-chain fatty acids ALA (α-linolenic acid), DHA, and n-3 (Fabro et al. 2021). We concluded that it is possible to add HSO at 3.7% level in the diet of laying hens without differences or negative effects on performance and egg quality parameters. SBO, SFO, and CO helped to increase the ΣPUFA and Σn-6 content of the yolk fat and improve the pigmentation of the yolk. Dietary CAO may contribute to the ΣMUFA and Σn-9 content of the egg but may reduce the redness of the yolk. HSO is promising for the design of Σn-3-rich eggs, but changes in yolk colour should be considered when using HSO in the diet. On the other hand, HSO is a newer lipid source for animal nutrition, and further research on its use in laying poultry nutrition is needed.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Gülşah Kanbur, Rabia Göçmen, and Yusuf Cufadar. The first draft of the manuscript was written by Gülşah Kanbur and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The data that support the findings of this study are available from the corresponding author, Gülşah Kanbur, upon reasonable request.

Declarations

Statement of animal rights In this study, the animal experiment was conducted according to the guidelines of the local ethics committee of Selcuk University, which were prepared following the "Directive 2010/63/EU is the European Union (EU) legislation". All procedures in this study complied with the ethical principles of animal welfare.

Competing interests The authors declare no competing interests.

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