Kafkas Univ Vet Fak Derg
24 (3): 349-356, 2018
DOI: 10.9775/kvfd.2017.18961

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

The Effects of the Usage of Solvent Extracted Safflower Meal with Soybean Oil in the Laying Hen Diets on the Performance, Egg Quality and Egg Yolk Fatty Acid Composition [1]

Engin YENİCE [1] Meltem GÜLTEKİN [1] Züleyha KAHRAMAN [1] Barış ERTEKİN [1]

[1] *This study was supported by General Directorate of Agricultural Research and Policy (Project number: TAGEM/HAYSUD/14/06/02/01)*

[1] Poultry Research Institute, TR-06042 Yenimahalle, Ankara - TURKEY

Article Code: KVFD-2017-18961 Received: 02.11.2017 Accepted: 01.02.2018 Published Online: 01.02.2018

How to Cite This Article

Yenice E, Gültekin M, Kahraman Z, Ertekin B: The effects of the usage of solvent extracted safflower meal with soybean oil in the laying hen diets on the performance, egg quality and egg yolk fatty acid composition. *Kafkas Univ Vet Fak Derg,* 24 (3): 349-356, 2018. DOI: 10.9775/kvfd.2017.18961

Abstract

This study examined the effects of the usage of solvent extracted safflower meal (SM) with soybean oil (SO) in laying hen diets. The experimental diets were prepared with 0 (control), 4, 8, and 12% SM, with the SO content being 0.3, 0.62, 1.9 and 3.17%, respectively. A total of 216 ATAK-S hybrid laying hens were tested at between 22 and 52 weeks of age. No differences in liveability, body weight gain, egg production, egg weight and mass, feed intake, feed conversion ratio, and shape index were observed among the groups. SM at 4 and 12% increased eggshell breaking strength, SM at 8% increased eggshell thickness, and SM at 8 and 12% increased egg yolk colour, compared to the control. The addition of 8% SM decreased albumen height, and the addition of 4 and 8% SM decreased the Haugh unit, compared to the control and SM at 12%. SM supplementation at 12% increased vitamin E content of the egg yolk compared to other groups. As the SM and SO content of the diet increased, the amount of palmitic acid in the egg yolk decreased, while linoleic, linolenic and docosahexaenoic acids increased. We conclude that SM could be used to supplement the laying hen diets at a rate of up to 12%, together with SO, with no negative effects while simultaneously having a positive impact on egg quality.

Keywords: *Safflower meal, Soybean oil, Egg production and quality, Fatty acids, Vitamin E*

Yumurta Tavuğu Yemlerinde Solvent Ekstrakte Aspir Küspesinin Soya Yağı İle Birlikte Kullanımının Performans, Yumurta Kalitesi ve Yumurta Sarısı Yağ Asitleri Kompozisyonu Üzerine Etkileri

Öz

Bu araştırma yumurta tavuğu yemlerinde solvent ekstrakte aspir küspesinin (SM) soya yağı (SO) ile birlikte kullanımının etkilerini incelemiştir. Deneme yemleri %0 (kontrol), 4, 8 ve 12 SM olacak şekilde hazırlanmış ve yemlerin SO içeriği, sırasıyla, %0.3, 0.62, 1.9 ve 3.17 olmuştur. Toplam 216 adet ATAK-S hibrit yumurtacı tavuk, 22-52 haftalık yaşlar arasında test edilmiştir. Gruplar arasında yaşama gücü, canlı ağırlık kazancı, yumurta verimi, yumurta ağırlığı ve kütlesi, yem tüketimi, yem değerlendirme oranı ve şekil indeksi bakımından önemli farklılıklar gözlenmemiştir. SM'nin %4 ve 12 seviyeleri kabuk kırılma mukavemetini, SM'nin %8 seviyesi kabuk kalınlığını, SM'nin %8 ve 12 seviyeleri yumurta sarısı rengini kontrole göre artırmıştır. SM'nin %8 ilavesi ak yüksekliğini ve SM'nin %4 ve 8 ilavesi Haugh birimini kontrol ve %12 SM ye göre azaltmıştır. SM'nin %12 ilavesi yumurta sarısı E vitamini içeriğini diğer gruplara göre arttırmıştır. Yemdeki SM ve SO miktarı arttıkça, yumurta sarısındaki linoleik, linolenik ve dokosaheksaenoik asit miktarları artarken, palmitik asit miktarı azalmıştır. SM'nin, SO ile birlikte olumsuz etkileri olmaksızın yumurta tavuğu yemlerine %12 ye kadar ilave edilebileceği ve aynı zamanda yumurta kalitesi üzerine olumlu bir etkisinin olduğu sonucuna varılmıştır.

Anahtar sözcükler: *Aspir küspesi, Soya yağı, Yumurta üretimi ve kalitesi, Yağ asitleri, E vitamini*

INTRODUCTION

Safflower *(Carthamus tinctorius* L.), in contrast to other seed oil plants, is tolerant of drought, cold and saline soils can be grown in a variety of climates in different seasons, and has a high fat content in its seeds. Safflower adapts to arid soils better than other seed oil plants and can be grown without any special conditions. These factors make it a crop

İletişim (Correspondence)
+90 312 6515171; Fax: +90 312 6515079
engin.yenice@tarim.gov.tr

with great potential in arid and semi-arid regions such as Turkey [1,2]. The oil content of the safflower seed is 20-40%. Safflower oil is rich in unsaturated fatty acids, especially linoleic acid (70-78%) which regulates triglyceride and cholesterol levels. In addition, the oil is rich in the important antioxidant α-tocopherol, making it important in human nutrition [3-5]. After oil extraction, the meal residue can be used as an alternative protein source in animal nutrition [6]; meal quality is dependent on the proportion of hulls and the amount of oil remaining after the extraction process. Today, the oil extraction process is conducted with solvent extraction after pressing. Undecorticated solvent extracted safflower meal contains 20-25% crude protein, 30-40% crude fibre and 0.5-1% oil [1,7,8]. While the crude fibre content decreases by up to 2% in extruded safflower meal obtained from seeds whose hull has been completely decorticated, the crude protein content increases to 50-55% [8]. However, it has been reported that removing the hull when making safflower meal is not economical [1]. Safflower meal is a good source of iron, zinc, and phosphorus [9] as well as the vitamins biotin, niacin, and riboflavin [10]. It's major limitations are a high fibre content, and being, low in lysine and methionine [11,12]. It has been reported, for the feeding of monogastric animals, that the lysine, methionine and isoleucine content and availability are lower in safflower meal compared to soybean meal [10]. Previous studies have reported that safflower meal (made from seeds decorticated or not) could be used at 10-15% in poultry diets without any negative impact on digestion, and performance, or meat and egg quality [7,13-17]. However; some studies have reported that safflower meal at levels above mentioned or at higher levels cause a decline in the body weight gain [18], egg weight [15,16,19], feed conversion [19] and egg quality [20].

When oils are added to the diet of poultry, they increase its energy density and metabolic energy efficiency, improve palatability and feed conversion, in addition to modifying the fatty acid profile of the egg yolk [21]. Soybean oil (SO), frequently preferred in poultry diets due to its beneficial properties and price, consists of 15% saturated, 25% monounsaturated and 60% polyunsaturated fatty acids. On average, SO contains 55% linoleic (omega 6), 25% oleic (omega 9), 10% palmitic, 5% linolenic (omega 3) and 5% stearic acids; its richness in terms of omega fatty acids increases its nutritional value [22].

In the present study, considering the positive effects of SO, solvent extracted safflower meal (SM) was added with SO to the laying hen diets in order to prevent possible decrease in the performance by increasing SM content of the diet and also to improve egg quality. There is also a lack of information on the effects of the addition of SM to the diet together with the SO. In addition, in countries such as Turkey, where locally-produced safflower meal is cheaper than imported soybean meal, it may be used in partial substitution of soybean meal as an alternative protein source. Therefore, this study was conducted to examine the effects of different levels of SM with SO in laying hen diets on performance parameters, egg quality, egg yolk fatty acid composition and vitamin E content.

MATERIAL and METHODS

Ethics Statement

The animal care protocol used in this study was reviewed and approved by the Ethics Committee of the Poultry Research Institute, Ankara, Turkey (04.09.2013-2013/04).

Experimental Design and Diets

In this study, SM was added to laying hen diets at the rate of 0 (control), 4, 8 and, 12%. The four experimental diets were prepared so as to be isocaloric and isonitrogenic: SO was added to compensate for the decrease in the metabolic energy that occurred as the amount of SM increased. The rate at which SO was added to the diets was 0.3, 0.62, 1.9, and 3.17%, respectively. The SM ratios in the diets were determined by taking into account the experiences gathered in the previous studies [13-20]. In the week prior to the start of the experiment, the hens were fed a commercial layers' diet. During this period, egg production and egg weight was monitored and hens with similar body weight and egg production were selected. A total of 216 brown, ATAK-S hybrid laying hens [23] were randomly distributed to individual cages (25×47×55 cm) in four groups of six replicates (9 hens in each replicate). The hens were tested at between 22 and 52 weeks of age. Feed and water were supplied ad libitum. The environment of the hen house was fully controlled, with a 16 h light period. Solvent extracted safflower meal manufactured in Turkey was obtained from Bunge Turkey (İzmir, Turkey). Safflower seeds were extracted by pressing with a screw press followed by extensive extraction of the oil using hexane as solvent. The solvent was then eliminated from the meal and the crude oil [24]. After SM was weighed and ground, it was placed in a mixing machine with 500 kg capacity along with the other feed ingredients, and the experimental feeds were prepared in the form of a mash. Crude nutrient, starch, and sugar analyses of feed ingredients were performed according to AOAC procedures [25] and the metabolic energy was calculated using the equations of Janssen [26] *(Table 1)*. The resulting values were used in the calculation of diet composition and chemical components. The experimental diets were formulated according to NRC requirements [27] *(Table 2)*. The amino acid composition of SM was analysed by the acid hydrolysis method using Zorbax Eclipse-AAA Columns and an Agilent 1100 HPLC system (Agilent Technologies, Wilmington, USA) [28-30]. First, the oils of the experimental diets and SM were extracted to determine the fatty acid composition. Then, methyl esters were formed from the extracted oils and the soybean oil and these were analysed with an Agilent HP GC 6890 Gas Chromatograph (Agilent Technologies, Wilmington, USA) [31].

Table 1. *Nutrient content of feed ingredients*

Nutrient Content	Yellow Corn	Full-fat Soybean	Soybean Meal	Solvent Extracted Safflower Meal
Crude protein (%)	7.3	35.5	45.6	23.0
Metabolic energy (kcal/kg)	3364	3477	2307	1176
Dry matter (%)	87.1	93.0	92.0	92.4
Ether extract (%)	3.4	19.5	1.2	0.6
Crude fibre (%)	2.1	5.1	6.2	37.0
Ash (%)	1.0	5.0	6.6	4.1
Sugar (%)	2.3	7.2	9.2	3.6
Starch (%)	63.0	7.0	8.1	1.3
Nitrogen-free substances (%)	75.4	23.0	36.8	64.7

Table 2. *Composition and chemical component of the experimental diets*

Feed Ingredients	Experimental Diets (%)			
	0% SM [1]	4% SM	8% SM	12% SM
Yellow corn	62.37	58.57	54.72	50.88
Full-fat soybean	2.0	6.0	6.0	6.0
Soybean meal	24.27	19.80	18.42	17.03
Solvent extracted safflower meal	0.0	4.0	8.0	12.0
Soybean oil	0.30	0.62	1.90	3.17
Ground limestone	8.44	8.43	8.42	8.41
Di calcium phosphate	1.65	1.63	1.60	1.58
Salt (NaCl)	0.35	0.35	0.35	0.35
DL-Methionine	0.25	0.23	0.22	0.21
Vitamin-mineral premix [2]	0.17	0.17	0.17	0.17
Salmonella inhibitor	0.20	0.20	0.20	0.20
Nutrient Content				
Crude protein (%)	16.50	16.50	16.50	16.50
Metabolic energy (kcal/kg)	2750	2750	2750	2750
Dry matter (%)	89.61	89.94	90.22	90.49
Ash (%)	12.13	12.13	12.13	12.14
Ether extract (%)	2.84	4.04	5.19	6.34
Crude fibre (%)	2.92	4.24	5.56	6.87
Calcium (%) [3]	3.50	3.50	3.50	3.50
Available phosphorus (%) [3]	0.40	0.40	0.40	0.40
Methionine (%) [3]	0.50	0.49	0.49	0.49
Methionine + Cystine (%) [3]	0.78	0.78	0.78	0.78
Lysine (%) [3]	0.88	0.87	0.86	0.85
Tryptophan (%) [3]	0.19	0.19	0.19	0.20
Palmitic acid (%)	0.33	0.46	0.54	0.59
Oleic acid (%)	0.77	1.08	1.39	1.68
Linoleic acid (%)	1.49	2.12	2.74	3.39
α-Linolenic acid (%)	0.11	0.17	0.24	0.31

[1] Solvent extracted safflower meal; [2] Vitamin-mineral premix provided per kilogram of diet; vit. A, 15.000 IU; vit. D_3, 5 000 IU; vit. E, 50 mg; vit. K_3, 10 mg; thiamine, 4 mg; riboflavin, 8 mg; pyridoxine, 5 mg; vit. B_{12}, 0.025 mg; niacin, 50 mg; Ca-pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.25 mg; ascorbic acid, 75 mg; choline, 175 mg; Mg, 35 mg; Mn, 56 mg; Zn, 140 mg; Fe, 56 mg; Cu, 10.5 mg; I, 1 mg; Co, 0.28 mg; Se, 0.28 mg; Mo, 0.7 mg; [3] Calculated values from NRC [29] tables

Production and Egg Quality Parameters

The body weight of the hens was individually measured at the beginning and at the end of the experiment and body weight gain was calculated. Egg production and hen deaths were recorded daily. Feed intake and egg weight were determined every 2 weeks. Egg mass was calculated from laying rate and egg weight [egg production (hen-day, %) × egg weight (g)/100], and feed conversion ratio was determined from feed intake and egg mass [feed intake (g/hen/day)/egg mass (g/hen/day)]. Thirty randomly selected eggs from each group were collected every 2 weeks, and egg quality characteristics were determined 24 h after collection of the eggs. Egg shape index was determined by the equipment that measures the width:length ratio as a percentage. Eggshell thickness was measured after peeling off the membrane under the shell with Mitutoyo digital micro meter (digital 395 series with 0.001 mm sensitivity, Kawasaki, Japan) on three points at the equatorial region of the egg, and expressed as an average value. Eggshell breaking strength, albumen height and Haugh unit were measured by using Futura 3/A egg quality measuring system (Futura, Lohne, Germany). Egg yolk colour (Roche Score, 1-15) was determined by CR-10 Konica Minolta Color Reader (Osaka, Japan).

Collection and Analysis of Samples

At the end of the experiment, 24 eggs were randomly taken from each group. Four of these eggs were combined (in 6 replicates), and egg yolk fatty acid composition and vitamin E content were measured. In the samples prepared with Folch method [32], the fatty acid composition was determined with an Agilent HP GC 6890 instrument [31], and vitamin E was determined with an Agilent HP HPLC 1100 instrument with the method specified in AOCS [33].

Statistical Analysis

The results of experiment were analysed statistically using the analysis of variance procedures of the statistical programme MINITAB Release 14; means were compared for significance by Duncan's multiple range test [34] at $P<0.05$.

RESULTS

The amino acid composition of SM, and the fatty acid composition of SM and SO are given in *Table 3*. SO contained approximately 54% linoleic, 24% oleic, 10% palmitic, 8% linolenic and 3.2% stearic acid. The oil in the SM contained approximately 62% linoleic, 26% oleic, 6.4% palmitic and 2.4% stearic acid.

The effects of the usage of SM together with SO on performance parameters of the laying hens are shown in *Table 4*. No significant differences in liveability, body weight gain, egg production, egg weight, egg mass, feed intake and feed conversion ratio were observed among the experimental groups ($P>0.05$). The egg quality characteristics and egg yolk vitamin E content are

Table 3. *The amino acid composition of SM (%) and, fatty acid composition of SO and SM (% of total fatty acid)*

Amino Acid Composition	SM[1]	Fatty Acid Composition	SO[2]	SM
Aspartic acid	2.60	Myristic acid (C14:0)	0.06	0.13
Glutamic acid	5.80	Palmitic acid (C16:0)	10.43	6.44
Serine	1.39	Heptadecanoic acid (C17:0)	0.08	0.04
Histidine	0.65	Stearic acid (C18:0)	3.72	2.39
Glycine	1.53	Arachidic acid (C20:0)	0.31	0.40
Threonine	0.93	Behenic acid (C22:0)	0.31	0.32
Arginine	2.54	Lignoseric acid (C24:0)	0.09	0.16
Alanine	1.19	Palmitoleic acid (C16:1)	0.08	0.16
Tyrosine	0.62	Heptadecenoic acid (C17:1)	0.01	0.04
Cystine	<0.03*	Oleic acid (C18:1n9c)	23.5	26.34
Valine	1.30	Eicosenoic acid (C20:1)	0.17	0.42
Methionine	0.28	Nervonic acid (C24:1)	0.01	0.09
Tryptophan	<0.03*	Linoleic acid (C18:2n6c)	53.53	61.96
Isoleucine	0.96	Eicosadienoic acid (C20:2)	0.03	<0.01*
Leucine	1.84	Arachidonic acid (C20:4n6)	<0.01*	0.20
Lysine	1.18	α-Linolenic acid (C18:3n3)	7.46	0.69
Phenylalanine	1.24	Docosapentaenoic acid (C22:5n3)	0.02	0.02
Proline	1.63	Docosahexaenoic acid (C22:6n3)	0.04	0.19

*[1] Solvent extracted safflower meal; [2] Soybean oil; * Method detection limit*

Table 4. *The effects of the usage of SM with SO on the performance parameters of laying hens*

Treatment	Liveability (%)	Body Weight Gain (g/hen)	Egg Production (%/hen/day)	Egg Weight (g/egg)	Egg Mass (g/hen/day)	Feed Intake (g/hen/day)	Feed Conversion Ratio (g feed/g egg)
0% SM [1]	100	518	90.8	63.9	58.1	122.3	2.10
4% SM	100	525	92.0	63.4	58.3	126.2	2.16
8% SM	98.2	507	90.7	64.5	58.6	126.6	2.16
12% SM	100	540	91.5	64.6	59.1	126.0	2.13
SEM	0.46	12.0	0.36	0.25	0.27	0.84	0.02
P	0.413	0.815	0.604	0.285	0.589	0.236	0.596

[1] Solvent extracted safflower meal; SEM: Standard error of means

Table 5. *The effects of the usage of SM with SO on egg yolk vitamin E content and egg quality characteristics of laying hens*

Treatment	Shape Index (%)	Eggshell Breaking Strength (Newton)	Eggshell Thickness (10^{-2} mm)	Albumen Height (mm)	Haugh Unit	Egg Yolk Colour (Roche Score)	Vitamin E (mg/kg egg yolk)
0% SM[1]	78.3	37.8 ^{b}	32.3 ^{b}	7.1 ^{a}	91.2 ^{a}	12.5 ^{b}	95 ^{b}
4% SM	78.4	39.6 ^{a}	32.6 ^{ab}	6.9 ^{ab}	89.8 ^{b}	12.6 ^{b}	121 ^{b}
8% SM	78.2	39.3 ^{ab}	32.9 ^{a}	6.8 ^{b}	89.4 ^{b}	12.8 ^{a}	121 ^{b}
12% SM	78.0	39.9 ^{a}	32.7 ^{ab}	7.1 ^{a}	91.1 ^{a}	12.9 ^{a}	171 ^{a}
SEM	0.06	0.23	0.06	0.03	0.23	0.03	8.38
P	0.103	0.006	0.042	0.001	0.006	0.001	0.001

[a,b] Means within columns with no common superscripts are significantly different (P<0.05); [1] Solvent extracted safflower meal; SEM: standard error of means

Table 6. *The effects of the usage of SM with SO on fatty acid composition of egg yolk (% of total fatty acid)*

Fatty Acids	Treatment				SEM	P
	0% SM [1]	4% SM	8% SM	12% SM		
Myristic acid (C14:0)	0.38 ^{a}	0.39 ^{a}	0.37 ^{ab}	0.32 ^{b}	0.009	0.004
Palmitic acid (C16:0)	25.30 ^{ab}	25.47 ^{a}	24.70 ^{b}	23.64 ^{c}	0.21	0.001
Heptadecanoic acid (C17:0)	0.15	0.17	0.17	0.18	0.005	0.129
Stearic acid (C18:0)	8.67	8.93	8.94	8.88	0.07	0.515
Arachidic acid (C20:0)	0.15 ^{a}	0.12 ^{b}	0.12 ^{b}	0.11 ^{b}	0.005	0.020
Behenic acid (C22:0)	0.14	0.15	0.15	0.15	0.006	0.969
Palmitoleic acid (C16:1)	3.25 ^{a}	3.29 ^{a}	2.78 ^{b}	2.40 ^{c}	0.09	0.001
Heptadecenoic acid (C17:1)	0.09	0.08	0.09	0.08	0.004	0.778
Oleic acid (C18:1n9c)	40.98 ^{a}	40.10 ^{a}	37.43 ^{b}	36.55 ^{b}	0.511	0.001
Eicosenoic acid (C20:1)	0.24 ^{a}	0.15 ^{b}	0.16 ^{b}	0.15 ^{b}	0.011	0.007
Nervonic acid (C24:1)	0.35 ^{a}	0.34 ^{a}	0.27 ^{b}	0.26 ^{b}	0.014	0.026
Linoleic acid (C18:2n6c)	16.78 ^{c}	17.32 ^{c}	20.83 ^{b}	23.14 ^{a}	0.702	0.001
Arachidonic acid (C20:4n6)	1.97	1.90	1.90	1.82	0.028	0.304
α-Linolenic acid (C18:3n3)	0.69 ^{c}	0.72 ^{c}	1.06 ^{b}	1.22 ^{a}	0.061	0.001
Docosahexaenoic acid (C22:6n3)	0.85 ^{b}	0.87 ^{b}	1.04 ^{a}	1.09 ^{a}	0.032	0.002

[a,c] Means within rows with no common superscripts are significantly different (P<0.05); [1] Solvent extracted safflower meal; SEM: standard error of means

presented in *Table 5*. No significant differences in shape index were observed among groups (P>0.05). SM at 4 and 12% increased eggshell breaking strength and SM at 8% increased eggshell thickness when compared to the control group (P<0.05). Moreover, SM supplementation at 8 and 12% increased the colour of the egg yolk when compared to the control and SM at 4% (P<0.05). However, SM at 8% decreased albumen height, and SM at 4 and 8% decreased Haugh unit, compared to the control and SM at 12% (P<0.05). The addition of SM at 12% to the diets together with SO increased the egg yolk vitamin E content (P<0.05) when compared to the control group.

In egg yolk, there were no significant differences among the experimental groups in terms of heptadecanoic, stearic and behenic acid from saturated fatty acids; heptadecenoic acid from monounsaturated fatty acids; arachidonic acid from polyunsaturated fatty acids (P>0.05). As the amounts of SM and SO increased in the diet, there were significant decreases in the amounts of myristic, palmitic and arachidic acid from saturated fatty acids; palmitoleic, oleic, eicosenoic and nervonic acid from monounsaturated fatty acids, and there were significant increases in the amounts of linoleic, α-linolenic and docosahexaenoic acid (DHA) from polyunsaturated fatty acids (P<0.05) *(Table 6)*.

DISCUSSION

SM had lower amino acid levels than did the soybean meal, in accordance with previous reports [10,11,27]. For instance, while the amounts of methionine, lysine, tryptophan and threonine from the limiting amino acids for poultry were respectively 0.28, 1.18, 0.03, 0.93% in SM, they were 0.62, 2.69, 0.74, 1.72% for the soybean meal specified in NRC [27]. As SO in the diet increased, the composition of fatty acids also changed depending on the fatty acid composition of SO.

In this study, the addition of SM to the diet up to 12% together with SO did not affect the performance parameters of the laying hens. Similarly, in other studies, the usage of safflower meal (from seeds decorticated or not) in different ratios (from 3 to 22%) in the laying hen diets did not affect liveability [15,19], body weight gain [13-17], egg production [13-17,19], egg weight and mass [14,17], feed consumption and feed conversion [13-17]. However, Ehsani et al.[19] reported that the addition of 7.5 and 10% safflower meal to the laying hen diets significantly decreased the feed conversion when compared to the control, 2.5 and 5% addition. The researchers attributed the decreases in performance parameters to the fact that crude fibre level is high [12] and amino acid digestibility is relatively low [10] in safflower meal, and phenolic glycosides in the safflower seed passing partially into the meal may decrease the availability of nutrients [35]. In the present study, it is thought that increasing SO together with SM in the diet prevented the negative effects on performance, possibly because the oils present have synergic impacts enhancing metabolic energy productivity and feed conversion. In addition, soybean oil is rich in linoleic acid, positively affecting egg weight [21].

In our study, the experimental treatments had no effect on egg shape index, in accordance with the findings of previous studies [15,17,20]. The usage of SM together with SO enhanced eggshell thickness and breaking strength when compared to the control group. These results are in line with the study of Kim et al.[36] who reported that the addition of safflower seed powder increased eggshell breaking strength and tibial Ca and P content and positively affected the level of blood parathyroid hormone, as compared with control. Nevertheless, in some studies [15-17], the safflower meal substituted in different ratios instead of soybean meal did not affect eggshell thickness and breaking strength. In addition, in some studies [37,38], the supplementation of diets with different levels of soybean oil did not affect eggshell quality. In contrast to results in our study, Ehsani et al.[20] reported that there were significant decreases in the eggshell thickness and breaking strength when the diet contained 10% safflower meal. They specified that this situation might be dependent on the decrease of Ca and P absorption from the intestine due to the high cellulose content of the feed. In this study, it is thought that the enhancement in eggshell characteristics may be due to the positive effect of safflower on Ca metabolism [36] or vitamin E level in the diets; soybean oil and safflower seed have been reported as good sources of vitamin E [5,39] and vitamin E increased the eggshell thickness [40]. In our study, SM at 8% decreased albumen height, and SM at 4 and 8% decreased the Haugh unit, compared to the control and SM at 12%. Similarly, the supplementation of 10% safflower seed during the first 4-week period of the trial decreased Haugh unit when compared to the control and, 4 and 7% safflower seed groups [41]. However, in some previous studies, the inclusion in the diet of safflower meal in different ratios from 3 to 22% did not affect albumen height [13,14] and Haugh unit [16,17,20]. Grobas et al.[37] reported that the supplementation of 5 and 10% soybean oil to diets did not affect Haugh unit, but in another study [42], the supplementation of 2.3% soybean oil increased Haugh unit when compared to other oil supplements. In the current study, it is thought that the decline in the interior quality of egg may be due to the high crude fibre level [12] and low amino acid digestibility [10] of safflower meal. However, an increase in soybean oil in the diet had a positive effect on the egg interior quality.

In this study, SM at 8 and 12% significantly increased the egg yolk colour when compared to the control and SM at 4%. This mirrors previous studies in which, an increase in egg yolk colour occurred at 4% and above [15] and, at 13% and above [16], although, other studies have found egg yolk colour was not affected by the addition of safflower meal to the diet at 15-20% [13,14,17]. The improvement in egg yolk colour was believed to be caused by carotene and other colour substances present in the safflower seed [15,16]. In addition, in this study, it is possible that the increase in the oil content of the diets enhanced the absorption of pigmentation compounds absorbed together with the oils in the small intestine [43]. The addition of SM to the diet together with the SO increased the vitamin E content of the egg yolk in the present study. It is believed that this is a consequence of safflower seeds and soybean oil being good sources of vitamin E [5,39].

In this study, as the amount of SM and SO in the diet increased, significant decreases were observed in the amounts of some saturated and monounsaturated fatty

acids, and significant increases were observed in the amounts of some polyunsaturated fatty acids, in the egg yolk. The increases in linoleic acid from omega-6 fatty acids and linolenic acid from omega-3 fatty acids were particularly clear. It is considered that the usage of the SO in the experimental diets in increasing amounts together with the SM changed the composition of the egg yolk fatty acids. Our findings concur with another study [17] in which 0, 3, 6, 9, 12, and 15% safflower meal were used in the diets, where a significant decrease in total saturated fatty acids, and an increase in total unsaturated fatty acids, occurred with SM at 12 and 15%. In that study, researchers suggested that the increases and decreases occurring in the composition of the fatty acid may have been related to the soybean oil content of the diets. The lipids being the main component of the egg are easily affected by manipulation of the diet and diets rich in unsaturated fatty acids change the lipid profile of the egg yolk. Soybean, maize, peanut, cotton and safflower oils are rich in linoleic acid and these oils could significantly increase the amount of linoleic acid in the egg yolk [44,45].

In conclusion, the inclusion of up to 12% solvent extracted safflower meal in the laying hen diets, in combination with soybean oil, not only produced no negative impact on performance parameters but also led to improvements in egg quality by increasing yolk pigmentation, and vitamin E and unsaturated fatty acid content.

REFERENCES

1. Dajue L, Mündel HH: Safflower *(Carthamus tinctorius* L.) promoting the conservation and use of underutilized and neglected crops. 4th ed., International Plant Genetic Resources Institute (IPGRI), ISBN 92-9043-297-7, Via delle Sette Chiese, 142 Roma, Italy. 74 p., 1996.

2. Eryılmaz T, Cesur C, Yesilyurt MK, Aydın E: Aspir *(Carthamus tinctorius* L.), Remzibey-05 tohum yağı metil esteri: potansiyel dizel motor uygulamaları için yakıt özellikleri. *Türk Tar Doğa Bil Derg,* 1 (1): 85-90, 2014.

3. Weiss EA: Oilseed Crops. 2nd edn., Blackwell Scientific, Oxford, UK, pp.93-129, 2000.

4. Grundy SM: Comparison of monounsaturated fatty acids and carbohydrates for owering plasma cholesterol. *N Engl J Med,* 314, 745-748, 1986. DOI: 10.1056/NEJM198603203141204

5. Bozan B, Temelli F: Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. *Bioresource Technol,* 99 (14): 6354-6359, 2008. DOI: 10.1016/j.biortech.2007.12.009

6. Kohler GO, Kuzmicky DD, Palter R, Guggolz J, Herring VV: Safflower meal. *J Amer Oil Chem Soc,* 43, 413-415, 1966. DOI: 10.1007/BF02646802

7. Thomas VM, Katz RJ, Auld DA, Petersen CF, Sauter EA, Steele EE: Nutritional value of expeller extracted rape and safflower oilseed meals for poultry. *Poult Sci,* 62, 882-886, 1983. DOI: 10.3382/ps.0620882

8. Farran MT, Barbour GW, Usayran NN, Kayouli C: Metabolizable energy and amino acid digestibility of decorticated extruded safflower meal. *Poult Sci,* 89, 1962-1966, 2010. DOI: 10.3382/ps.2009-00559

9. Gowda NKS, Ramana JV, Prasad CS, Singh K: Micronutrient content of certain tropical conventional and unconventional feed resources of Southern India. *Trop Anim Health Prod,* 36 (1): 77-94, 2004. DOI: 10.1023/B:TROP.0000009522.30949.1d

10. Darroch CS: Safflower meal. **In,** Thacker PA, Kirkwood RN (Eds): Nontraditional Feed Sources for Use in Swine Production. Butterworths, Stoneham, Massachusetts, 373-382, 1990.

11. Valadez S, Featherston WR, Pickett RA: Utilization of safflower meal by the chick and its effect on plasma lysine and methionine concentrations. *Poult Sci,* 44, 909-915, 1965. DOI: 10.3382/ps.0440909

12. Kratzer FH, Williams DE: Safflower oil meal in rations for chicks. *Poult Sci,* 30 (3): 417-421, 1951. DOI: 10.3382/ps.0300417

13. Grau CR, Zweigart PA: Safflower seed meal. *Calif Agr,* 7 (12): 8, 1953.

14. Petersen CF, Wiese AC, Anderson GJ, Lampman CE: The use of safflower oil meal in poultry rations. *Poult Sci,* 36, 3-8, 1957. DOI: 10.3382/ps.0360003

15. Farran MT, Barbour GW, Usayran NN, Yau SK, Salameh G: Production and egg quality parameters of laying hens fed de-hulled safflower meal in partial or total substitution of soybean meal. *Proceedings of XIII European Poultry Conference,* 23-27 August, Abstract's Book, 380, Tour, France, 2010.

16. Barbour GW, Usayran NN, Yau SK, Murr SK, Shaib HA, Abi Nader NN, Salameh GM, Farran MT: The effect of safflower meal substitution in a lysine fortified corn-soybean meal diet on performance, egg quality, and yolk fat profile of laying hens. *J Appl Poult Res,* 25, 256-265, 2016. DOI: 10.3382/japr/pfw008

17. Çalışlar S, Kuştimur H: The effects of safflower meal on the performance, egg quality traits, yolk fatty acids and cholesterol levels in laying hens. *Anadolu J Agr Sci,* 32, 269-278, 2017. DOI: 10.7161/omuanajas.321121

18. Kuzmicky DD, Kohler GO: Safflower meal-utilization as a protein source for broiler rations. *Poult Sci,* 47, 1266-1270, 1968. DOI: 10.3382/ps.0471266

19. Ehsani A, Mahdavi AH, Samie AH, Dolatkhah B: Effects of dietary administration of multi-enzyme on productive performance of laying hens fed different levels of safflower meal. *J Anim Poult Sci,* 2, 108-119, 2013.

20. Ehsani A, Mahdavi AH, Dolatkhah B, Samie AH: Exogenous enzyme improves immunocompetence in laying hens fed diets containing safflower meal. *J Anim Poult Sci,* 3 (2): 57-65, 2014.

21. Oliveira DD, Baiao NC, Cançado SV, Oliveira BL, Lana AMQ, Figueiredo TC: Effects of the use of soybean oil and animal fat in the diet of laying hens on production performance and egg quality. *Cienc Agrotec,* 35 (5): 995-1001, 2011. DOI: 10.1590/S1413-70542011000500018

22. Eren A, Kocatürk M, Hoşgün EZ, Azcan N: Bazı soya hat ve çeşitlerinde tohum verimi, yağ-protein ve yağ asitleri içerikleri ve aralarındaki ilişkilerinin belirlenmesi. *SDÜ Zir Fak Derg,* 7 (1): 1-9, 2012.

23. Tavukçuluk Araştırma Enstitüsü Müdürlüğü: Hibrit Kataloğu. http://arastirma.tarim.gov.tr/tavukculuk/Belgeler/Hibrit%20Katalog%202015/Hibrit%20Katalog%202015%203lu.pdf, *Erişim tarihi:* 16.10.2017.

24. Tasan M, Gecgel U, Demirci M: Effects of storage and industrial oilseed extraction methods on the quality and stability characteristics of crude sunflower oil *(Helianthus annuus* L.). *Grasas Aceites,* 62 (4): 389-398, 2011. DOI: 10.3989/gya.126010

25. AOAC: Official Methods of Analysis. Association of Official Analytical Chemists. 15th ed., Arlington, Virginia, 1990.

26. Janssen WMMA: European Table of Energy Values for Poultry Feedstuffs. 3rd ed., Beekbergen, Netherlands: Spelderholt Center for Poultry Research and Information Services, 1989.

27. NRC: Nutrient Requirements of Poultry. 9th Revised ed., 19-34, National Academy Press, Washington, DC, USA, 1994.

28. Gratzfeld-Huesgen A: Sensitive and reliable amino acid analysis in protein hydrolysates using the Agilent 1100 series HPLC. Technical note, Publication Number 5968-5658E, 12 p. Agilent Technologies, Inc. Wilmington, USA, 1998.

29. Henderson JW, Ricker RD, Bidlingmeyer BA, Woodward C: Amino acid analysis using Zorbax Eclipse-AAA Columns and the Agilent 1100 HPLC, Part No. 5980-1193E, 10 p. Agilent Technologies, Inc. Wilmington, USA, 2000.

30. Leo M, Nollet L: Handbook of Food Analysis, Physical Characterization and Nutrient Analysis. Vol. 1, Revised and Expanded Second edn., Marcel Dekker Inc., USA, 88-96, 2004.

31. AOCS: Official Method Ce, Fatty acid composition in official methods and recommended practices of the American Oil Chemists' Society, 1-62,

Champaign, IL, Method, 1989.

32. Folch J, Lees M, Sloane Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem,* 226, 497-509, 1957.

33. AOCS: AOCS Official Method Ce 8-89. Determination of tocopherol and tocotrienols in vegetable oils and fats by HPLC. **In,** Official Methods and Recommended Practices of the American Oil Chemists' Society. 5th edn., AOCS Champaign, IL,USA, 1991.

34. Duncan DB: Multiple range and multiple F tests. *Biometrics,* 11, 1-42, 1955.

35. Palter R, Lundin RE, Haddon WF: A cathartic lignan glycoside isolated from Carthamus tinctorus. *Phytochemistry,* 11, 2871-2874, 1972. DOI: 10.1016/S0031-9422(00)86527-9

36. Kim EJ, Shin SC, Yon, SJ, Ahn BK, Kang CW: Effects of dietary safflower seed powder and chitosan on hatching egg production and eggshell quality in aged egg type breeder hens. *Korean J Poult Sci,* 33 (1): 15-24, 2006.

37. Grobas S, Mendez J, Lazaro R, de Blas C, Mateos GG: Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. *Poult Sci,* 80, 1171-1179, 2001. DOI: 10.1093/ps/80.8.1171

38. Güçlü BK, Uyanık F, İşcan KM: Effects of dietary oil sources on egg quality, fatty acid composition of eggs and blood lipids in laying quail. *S Afr J Anim Sci,* 38 (2): 91-100, 2008.

39. Herting DC, Drury EJE: Vitamin E content of vegetable oils and fats. *J Nutr,* 81, 335-342, 1963. DOI: 10.1093/jn/81.4.335

40. Önol AG, Daşkıran M, Cengiz Ö, Nazlıgül A, Sarı M: Effects of dietary vitamin E and lysine supplementation on performance and egg shell quality parameters of heat stressed laying hens in early laying period. *Kafkas Univ Vet Fak Derg,* 18 (1): 49-54, 2012. DOI: 10.9775/kvfd.2011.4740

41. Hosseini Vashan SJ, Afzali N, Mallekaneh M, Nasseri MA, Allahresani A: The effect of different concentrations of safflower seed on laying hen's performance, yolk and blood cholesterol and immune system. *Int J Poult Sci,* 7 (5): 470-473, 2008. DOI: 10.3923/ijps.2008.470.473

42. Göçmen R, Kanbur G, Cufadar Y: Yumurta tavuğu rasyonlarına soya yağı, ayçiçek asit yağı ve kombinasyonlarının ilavesinin performans, yumurta kalitesi ve yumurta sarısı yağ asidi kompozisyonuna etkileri. *Tavukçuluk Araş Derg,* 12 (1): 12-15, 2015.

43. Hencken H: Chemical and physiological behaviour of feed carotenoids and their effect on pigmentation. *Poult Sci,* 71, 711-720, 1992. DOI: 10.3382/ps.0710711

44. Milinsk MC, Murakami AE, Gomes STM, Matsushita M, De Souza NE: Fatty acid profile of egg yolk lipids from hens fed diets rich in n-3 fatty acids. *Food Chem,* 83, 287-292, 2003. DOI: 10.1016/S0308-8146(03)00094-3

45. Shafey TM, Dingle JG, Mc Donald MW: Comparison between wheat, triticale, rye, soybean oil and strain of laying bird on the production and cholesterol and fatty acid content of eggs. *Br Poult Sci,* 33 (2): 339-346, 1992. DOI: 10.1080/00071669208417472