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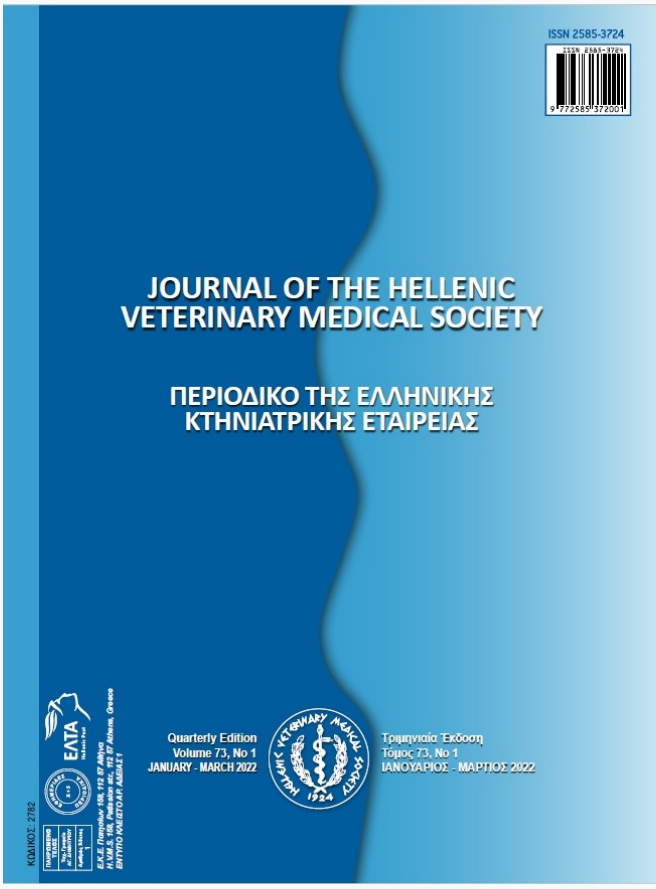

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Flaxseed and sunflower oil affect egg production and quality in hens exposed to stress

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ABSTRACT: In the study, effects of dietary supplementations with flaxseed oil and sunflower oil on production performance, egg quality, and the eggs' sensory attributes in laying hens exposed to high stocking density stress were investigated. A total of one hundred and forty-four 38-week-old "Atak-S" breed laying hens were used. The hens were divided into two main groups as stress group and non-stress group, which both were further divided into three subgroups: basal diet, 2% flaxseed oil diet, and 2% sunflower oil diet groups. High stocking density stress was induced with a space allowance of 357 cm^2 per hen. All hens were weighed initially and just before the study has been completed, and the body weight gain was calculated. Egg production per hen was daily recorded, and production performance, mean egg weight, and egg mass were estimated. Moreover, eggshell weight, thickness, strength, albumen height, and egg yolk color were measured. Trained panelists evaluated egg samples collected from each subgroup regarding sensory attributes such as taste, flavor, color, and texture. Flaxseed oil decreased egg production and egg mass in the non-stress group while increasing the stress group's same parameters. Sunflower oil increased average egg weight in all hens and paled the egg yolk's yellow color in the stressed hens. Moreover, the non-stress group's eggs were more appealing in taste than those of the stress group. Flaxseed-supplemented diet enhanced the sensory attributes in the eggs of both stress and non-stress groups. Furthermore, neither of the oil supplementations generated a strange or repulsive odor in the eggs. In conclusion, dietary flaxseed oil supplementation might be recommended to improve egg production and egg sensory attributes in stress-exposed laying hens. Sunflower oil supplementation might be offered for increasing egg production and some sensory parameters in both stressed and unstressed hens.

Keywords: hens, flaxseed oil, sunflower oil, high stocking density, stress, egg quality

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INTRODUCTION

Stress is a crucial environmental factor that adversely affects production performance, immunity, and welfare, increases the mortality rate, creating severe economic damage in poultry. Chickens used in commercial production can be exposed to many stressors, such as chick placement, beak trimming, transfer of chickens to breeder houses, heat-cold, high humidity, poor ventilation, insufficient lighting, vaccination, and microbial or viral challenges (Surai et al., 2019). High stocking density is one of the major stress factors in extensive farming, yet the breeders tend to house more hens per pen due to economic concerns (Carey et al., 1995), which reduces the space allowance per hen, causes rivalry due to inadequacy of drinkers and feeders, consequently leading to stress (Puron et al., 1995).

High stocking density was shown to have reduced feed intake, egg production, egg mass (Jahanian and Mirfendereski, 2015), and increased floor and broken egg ratios in floor-type pens (Kang et al., 2016). Roberts, (2004) thoroughly documented that several stress factors reduced eggshell quality. On the other hand, high stocking density effects were investigated only in a few studies, creating conflicting results. Jahanian and Mirfendereski (2015) indicated that eggshell thickness was increased in hens exposed to high stocking density, whereas Kang et al. (2018) pointed out a reduction in eggshell strength. Egg quality declined in Japanese quails (El-Tarabany et al., 2016) and layer ducks (Xiong et al., 2020) due to high stocking density. On the other hand, egg quality was reported to have remained unchanged in some other studies (Geng et al., 2020).

It is unlikely to avoid stress factors in poultry farming, and thus, several studies have been conducted regarding the potential solutions, including dietary supplements to alleviate stress response in poultry exposed to various stressors (Clavijo and Flórez, 2018). In the presented study, flaxseed and sunflower oils were administered as dietary supplements to minimize the potential adverse effects of stress in hens.

Flax (*Linum usitatissimum* L.) is a plant grown since ancient times for its seeds and fibers (Carraro et al., 2012). Flaxseed contains a high amount of nutrients, such as proteins, vitamins, minerals, and antioxidants, and provides a rich energy source. It contains 35-40% vegetable oil, which is composed of 70% polyunsaturated (PUFA) (mainly alpha-linolenic acid, ALA), 20% monounsaturated (mainly oleic acid), and approximately 10% saturated fatty acids (palmitic and stearic acids) (Martinchik et al., 2012). It is also a good plant source of omega-3 fatty acid. It is used in poultry to enhance omega-3 levels in the carcass meat and eggs (Ahmad, 2017). However, it was reported that when the diet's flaxseed ratio exceeded 8%, cyanogenic glycoside and phytic acid contained in the flaxseed adversely affected feed digestibility resulting in reduced egg production (Beheshti Moghadam and Cherian, 2017). On the other hand, it is not the matter in question in terms of flaxseed oil, safely used at high concentrations in poultry diets. Flaxseed oil promoted growth in chicks, increased omega-3 level and oxidative stability of carcass, and improved carcass quality (Abbasi et al., 2019). Egg production was unchanged with flaxseed oil in some studies (Costa et al., 2008; Petrovic et al., 2012; Herkel et al., 2014). Nevertheless, studies demonstrated both an increase (Celebi and Utlu, 2006) and a decrease (Jia et al., 2008) in egg production. Likewise, an increase (Yi et al. 2014), a decrease (Raes et al., 2002) were also reported concerning egg weight, while some studies demonstrated no difference (Steinhilber, 2005). The studies investigating the effects of dietary flaxseed oil supplementation on egg quality also revealed conflicting results. Although several authors showed no difference in extrinsic egg quality parameters (Mazalli et al., 2004; Costa et al., 2008; Raes et al., 2002), eggshell weight and thickness were reported to have decreased (Grobas et al., 2001), and albumen weight was increased (Schumann et al. 2000). Moreover, the fatty acids such as ALA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) contained in the flaxseed oil were transferred into the egg at high concentrations (Ehr et al., 2017), prolonging the freshness of eggs (Lee et al., 2016).

Sunflower (*Helianthus annuus* L.) is an oilseed plant widely grown in the world. The seeds contain high amounts of vegetable oil, protein, non-digestible crude fiber, vitamins, and minerals (Le Clef and Kemper, 2015). Its oil content was elevated to 60% by genetic improvement studies (Vilvert, 2018). It also contains various fatty acids, including linoleic acid (55-75%), oleic acid (15-25%), palmitic acid (6%), and stearic acid (2%) (Lacombe and Berville 2001). Sunflower meal (SFM) is used in poultry feeding as a source of energy and protein. However, its high fiber yet low lysine content restrains the diet's digestibility (Baghban-Kanani et al., 2018). Therefore, various enzymes, essentially lysine, are added to the diet that includes SFM; hence, it was reported that SFM could be used in the diet of both broilers and laying hens at a 30% concentration, provided that the diet also contained enzymes (Seidavi et al., 2018). Once the diet included SFM, egg production, and quality were adversely affected, while -in contrast- concurrent niacin administration enhanced the relevant parameters (Baghban-Kanani et al., 2019). On the other hand, dietary sunflower oil supplementation showed no negative impact on egg production (Sangkaew et al., 2017), egg weight (Ceylan et al., 2011), and egg mass (Karunajeewa et al., 1989). Likewise, the intrinsic and extrinsic egg quality parameters were not negatively affected (Küçükersan et al., 2010; Dong et al., 2018).

The fatty acids in vegetable oils are accumulated in the egg yolk, causing lipid oxidation, hydrolysis, and polymerization (Wang et al., 2017), which -as a result- affected sensory attributes such as taste, flavor, color, and texture (Brelaz et al., 2019). On the other hand, stress further induced lipid oxidation (Eid et al., 2008), which might also alter sensory features. However, to the best of our knowledge, no data is available indicating the adverse effects of stocking density stress on the egg's sensory attributes.

The contradictory results concerning the effects of dietary flaxseed oil and sunflower oil supplementations despite the studies indicating their potential benefits in egg production and quality necessitates further studies. Furthermore, the potential positive effects of oil supplements on the relevant parameters and eggs' sensory attributes were not determined in stress-exposed hens.

The aim of the study was to investigate the effects of dietary flaxseed oil and sunflower oil supplementations on production performance, egg quality, and sensory characteristics in laying hens.

MATERIALS AND METHODS

Hens, study groups, and housing conditions

All experimental procedures were approved by the Animal Experiment Ethics Committee of the Istanbul University (Approval number 191-2018). A total of one hundred and forty-four 38-week-old Atak-S laying hens were included in the study. The hens were randomly divided into two main groups as the stress (n: 84) and the non-stress (n: 60) groups, which were further allocated into three subgroups: Basal diet, flaxseed oil diet, and sunflower oil diet groups, each containing 20 hens. Each sub-group was replicated four times. The stress group cages contained seven hens with a space allowance of 357cm$^{2/}$per hen, whilefive-hens were housed in each cage of the non-stress groupwith a space allowance of 500 cm^2 per hen. All hens were housed in stainless steel battery-type cages (width = 50 cm, length = 50 cm, height = 60 cm) which were equipped with linear feeders and nipple drinkers. The temperature, humidity, and ventilation were maintained by an automatic system according to the manufacturer's instructions in a 16/8 light-dark cycle.

Composition of the diets

All hens received the same basal diet. The feed was provided by a commercial company. The supplementation groups further received flaxseed oil and sunflower oils at 2%. The amount of feed per day was estimated according to the manufacturer's instructions. All hens were fed for 15 weeks. Water was provided *ad libitum.* The diets' nutrient contents were analyzed in a commercial company's laboratory (BanvitAş, Bandırma, Balıkesir). The diet's formulation and chemical composition were shown in Table 1 and Table 2, respectively.

Table 1. Formulation of the basal diet

Ingredients	Basal diet (%)
Soybean meal	33, 00
Sunflower meal	27, 00
Full-fat soybeans	11, 00
Maize	15, 00
Soybean oil	0, 90
DCP	2, 00
DL-Metiyonin	0, 10
Limestone	9, 70
Vitamin + mineral premix[1]	1, 00
Salt	0, 30

[1]Supplied per kg of diet: vitamin A (retinyl palmitate), 15, 000 IU; vitamin D3, 2, 500 IU; vitamin E (DL-α-tocopheryl acetate), 20 mg; vitamin B1, 3 mg; vitamin B2, 7, 5 mg; D-pantothenic acid, 25 mg; vitamin B6, 5 mg; vitamin B12, 0.002 mg; biotin, 0.5 mg; niacin, 25 mg; vitamin K3, 1.25 mg; folic acid, 1.5 mg; choline chloride, 750 mg; cobalt, 1.2 mg; copper, 8.8 mg; zinc, 84 mg; manganese, 106 mg; iron, 44 mg; iodine, 1.2 mg; and selenium, 0.15 mg.

Oil supplements

Flaxseed and sunflower oils were purchased from a commercial company (AyhanSezer Co Ltd., Bandırma, Balıkesir) and stored at +4°C during the study. The composition of fatty acids in oils was presented in Table 3.

Table 2. Chemical composition (calculated) and energy levels of the experimental diets (calculated)

Faty acid name	Faty acid number	Flaxseed oil (ppm)	Sunflower oil (ppm)
Caprylic acid	C8:0	Nd	1.047
Lauric acid	C12:0	Nd	1.231
Myristic acid	C14:0	1.509	1.99
Palmitic acid	C16:0	2.027	40.13
Palmitoleic acid	C16:1	Nd	1.874
Ginkgolic acid	C17:1	1.49	1.646
Stearic acid	C18:0	2.239	17.504
Oleic acid	C18:1 (n-9)	4.535	197.074
Linoleic acid	C18:2 (n-6)	3.885	303.261
Arachidic acid	C20:0	1.828	2.878
Alpha-linolenic acid	C18:3 (n-3)	6.625	1.811
Eicosenoic acid	C21:1 (n-9)	Nd	2.17
Heneicosanoic acid	C21:0	Nd	4.581
Behenic acid	C22:0	Nd	4.705
Tricosylic acid	C23:0	Nd	1.662
Nervonic acid	24:1 (n-9)	Nd	2.521

Nd = Not determined

Table 3. Fat acid comosition of flaxseed oil and sunflower oil

	Basal diet	Flaxseed oil	Sunflower oil
Dry matter (%)	89, 37	89, 42	89, 22
Crude protein, %	17, 29	16, 8	16, 69
Crude fat, %	3, 85	5, 44	5, 43
Crude fibre, %	2, 7	2, 48	2, 52
Ash, %	15, 15	16, 62	16, 59
Nitr-free core matter, %*	50, 38	48, 08	47, 99
Starch, %	39, 02	38, 58	38, 72
Sugar, %	3, 41	3, 4	3, 41
Calcium, %	3, 69	3, 71	3, 65
Utilizable phophorus, %	0, 43	0, 41	0, 39
ME, MJ/kg feed**	10, 96	11, 35	11, 35

Nitrogen-free core matter*, % = Dry matter, % - (crude protein, % + crude fat, % + crude fiber, % + ash, %). **ME, MJ/kg food = (0, 03431 x crude fat, g/kg) + (0, 01551 x crude protein, g/kg) + (0, 01669 x starch, g/kg) + (0, 01301 x sugar, g/kg).

Fatty acid analysis

An oil sample of 100μl was dissolved in 10 ml hexane. 2N KOH dissolved in 100 ml methanol was added and centrifuged for 30 sec. The methyl ester layer formed on the top by centrifuging was gently removed and used for the analysis (David et al., 2003). The fatty acid analysis of methyl esters was carried out using a gas chromatograph (Shimadzu GC-MS QP 2010 ULTRA, Kyoto, Japan) equipped with a fused silica capillary column (RTX-2330, 60 m length × 0.25 mm i.d. with a 0.25 μm film thickness). The column temperature, injection temperature, interface temperature, and ion source temperature were 100, 250, 250, and 200 °C, respectively. The fatty acids were identified from their peak retention times compared to the standards. Helium was used as the carrier gas at 2 μL/ min (Omri et al., 2019).

Performance parameters

Eggs were collected daily at the same hour, weighed on a precision scale with 0.1g accuracy, and the measurements were recorded. Egg production, mean egg weight, and egg mass were calculated (Egg production = the number of produced egg/number of hens x 100. Egg mass = egg production (%) x mean egg weight (g) (Araújo et al., 2015).

Quality parameters

The sensory attributes were evaluated in eggs collected at the last week of the study. Twenty eggs were randomly selected from each subgroup. (A total of 60 eggs from the stressed group 3x20 and 60 eggs from the unstressed group 3x20). External quality parameters such as eggshell weight, thickness, and strength and internal quality parameters such as Haugh unit, albumen height, and yolk color were estimated. Eggshell weight was measured by a precision scale with 0.01g accuracy (Precisa XT 6200C, Dietikon, Switzerland). Other quality parameters were analyzed by a digital egg tester (DET6000, Nabel Co., Ltd, Kyoto, Japan), and eggshell thickness was measured with the equipment's accessory digital caliper rule.

Sensory attributes of eggs

Seven trained female panelists performed the sensory analysis. All panelists were professionals at the research/development laboratory of a commercial poultry company. A total of 72 eggs (12 eggs from each subgroup) collected on the same day were used to evaluate sensory attributes. The eggs were boiled for 9 min, cooled under tap water for 10 min, peeled, and then cut into four pieces along the longitudinal axis. The pieces were labeled according to the subgroup they belonged to and then offered to the panelists. Cold drinking water was provided to clear the mouth between tastings. The panelists scored the eggs on a scale of 1-5 concerning the parameters such as taste, flavor, color, and texture. The sum of the scores was obtained to rank the groups. The panelists were also asked to evaluate the eggs' odor, whether they smelled strange such as fish oil.

Statistical analysis

SPSS software (SPSS, Inc., Chicago, USA) was used for the statistical analysis. Initially, the Shapiro-Wilk test was applied to determine the distribution of the data. The normally distributed data were analyzed by a factorial ANOVA in a 2x3 factorial design. Stress and feed were held as constant factors, whereas the measured parameters were considered the dependent variables. The presence and absence of stress or three different diet types (basal, flaxseed oil-supplemented, and sunflower oil-supplemented) served as factors. Therefore, the main effects of stress and diet and stress-diet interaction were analyzed. When the interactive effect or the main effect of diet was significant, the source of difference was determined by the Post hoc tests, before which Levene's test was applied to check whether the data were homogeneously distributed. The Tukey test was performed for the Post hoc analysis of the homogenously distributed data (significant at $P>0.005$ level). The Games-Howel test was applied for the non-homogenous data (significant at $P<0.005$ level). Mean values and standard errors of the mean were used in tables and graphics. Results were determined significant if the P-value was 0.05 or lower.

RESULTS

The fatty acid contents of the flaxseed oil and sunflower oil were shown in Table 3. The flaxseed oil contained eight different fatty acids, the majority of which was composed of alpha-linolenic acid (n:3; omega-3) with a 27.45% concentration. The n:6/n:3 ratio was calculated as 0.58. Sunflower oil was rich in fatty acids with sixteen different components. Linoleic acid (n:6, omega-6) was the highest fatty acid content with a 51.74% concentration. The n:6/n:3 for the sunflower oil was calculated as 167.

Table 4. Effects of dietary flaxseed oil and sunflower oil supplementation and on body weight gain stressed and unstressed in laying hens

		Initial body weight (gr)			Final Body weight (gr)			Body weight gain (gr)		
Stressed hens	Basal diet	1759.2	±	17.1	1920.5	±	31	161.2	±	16.8
	Flaxseed oil	1763.3	±	18.2	2032.3	±	38	268.9	±	40.3
	Sunflower oil	1773	±	17.8	2048.6	±	47	289.6	±	52.3
Unstressed hens	Basal diet	1794.5	±	20.8	2037.5	±	44	264.5	±	39.9
	Flaxseed oil	1804.2	±	20.7	2055.2	±	49	260.7	±	49.5
	Sunflower oil	1773.1	±	21.8	2046.3	±	52	242.5	±	46.8
		P= 0.180								
					P values					
		Stress			0.895			0.193		
		Diet			0.132			0.648		
		Stress x Diet			0.734			0.368		

Body weights of the hens were presented in Table 4. The induced stress (P = 0.265) or dietary flaxseed oil and sunflower oil supplements (P = 0.227) had no significant impact on body weights. Besides, stress-diet interaction was also found insignificant (P = 0.288).

The effects on egg production, egg weight, and egg mass were shown in Figure 1. Based on the data, the stress-diet interaction affected egg production (P = 0.001). Flaxseed oil increased egg production in the stress-exposed hens, whereas -in contrast- egg production was reduced in the non-stress group. Sunflower oil showed no impact on egg production.

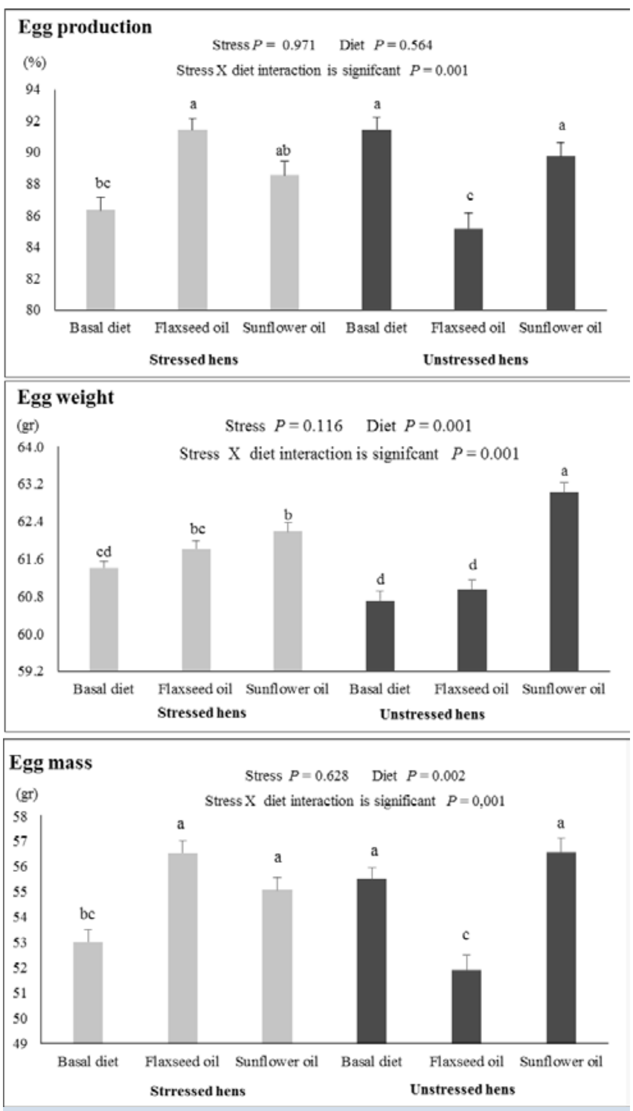

Figure 1. The effect of flaxseed oil and Sunflower oil on production performance in stressed and unstressed laying hens

Stress-diet interaction significantly affected egg weight (P = 0.001). Flaxseed oil generated a limited-level increase in egg weight in the stressed hens. No significant difference was noted in the non-stress group. Sunflower oil significantly increased egg weight regardless of absence or presence of stress.

The data revealed a significant stress-diet interaction effect on egg mass (P = 0.001). Flaxseed oil significantly increased egg mass in high stocking density stress-exposed hens while decreasing the unstressed birds' relevant parameter. In contrast, sunflower oil showed no significant impact on egg mass.

The data regarding eggs' external quality parameters were shown in Figure 2. The results revealed the noninfluence of stress, diet, and stress-diet interaction on eggshell weight (P = 0.491, P = 0.256, and P = 0.488, respectively), eggshell thickness (P = 0.720, P = 0.301, and P = 0.999, respectively), and eggshell strength (P = 0.232, P = 0.078, and P = 0.953, respectively).

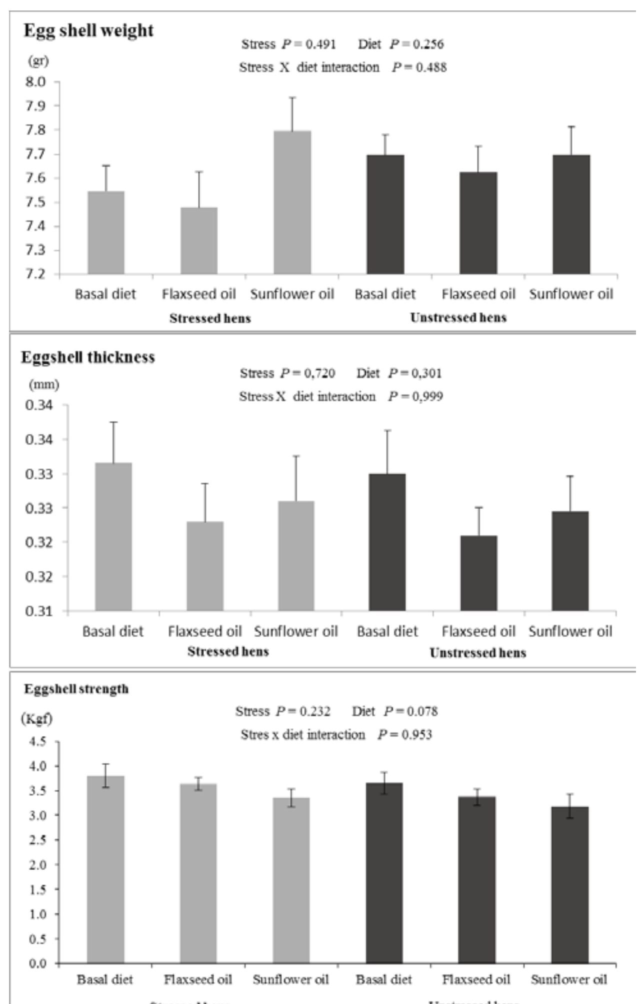

Figure 2. The effect of flaxseed oil and Sunflower oil on external egg quality parameters in stressed and unstressed laying hens

The data regarding eggs' internal quality parameters were shown in Figure 3. Based on the data, stress, diet, and stress-diet interaction did not affect Haugh unit (P = 0.736, P = 0.749, and P = 0.687, respectively) and albumen height (P = 0.493, P = 0.889, and P = 0.859, respectively). Nevertheless, the stress-diet interaction has a significant effect on egg yolk color (P = 0.029). Dietary sunflower oil supplement significantly reduced egg yolk yellow color intensity in the stressed hens while exerting no significant impact in the unstressed birds. Flaxseed oil did not generate a significant difference in hens' yolk color regardless of absence or presence of stress.

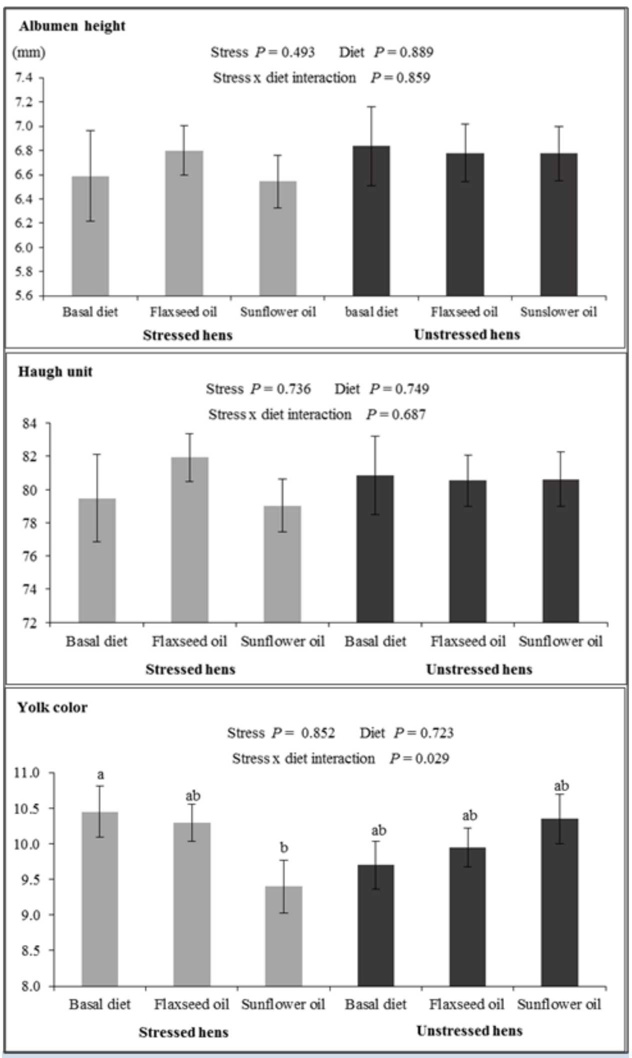

Figure 3. The effect of flaxseed oil and Sunflower oil on internal egg quality parameters in stressed and unstressed laying hens

Sensory quality tests revealed no repulsive odor in eggs. The unstressed hens' eggs undoubtedly tasted much better with a more desirable flavor than those of the stressed hens. When the diet groups of the stressed hens were compared, flaxseed oil supplementation elicited a more appealing taste in eggs than in other subgroups. No significant difference was noted in terms of flavor, yolk color, and texture. Likewise, in the non-stress group, eggs of hens fed with flaxseed oil-supplemented diet ranked first in terms of taste, followed by basal diet and sunflower oil received hens, respectively. Oil supplementations also improved the eggs' texture of the unstressed hens.

DISCUSSION

Fatty acid composition of dietary vegetable oil supplements -in particular- is of great importance while evaluating their potential benefits on production performance; hence, studies revealed divergent results concerning the effects of oil-supplemented diets in chickens (Ahmad, 2017). In the presented study, flaxseed oil contained approximately 37.6% saturated fatty acids (SFA) (myristic, palmitic, stearic, ginkgolic, and arachidic acid) and 62.3% unsaturated fatty acids (UFA) (alpha-linolenic acid, linoleic acid, and oleic acid) with an SFA/UFA ratio of 0.60. The fatty acid content of vegetable oils differs depending on environmental factors, plant variety (cultivar), and genotype (Wang et al., 2017). For instance, a study comparatively investigating the fatty acids compositions of four different flaxseeds revealed divergent results than the presented study, indicating that the SFA, UFA, and SFA/UFA ratios were 10-16%, 84-89%, and 0.12-0.19, respectively (Qiu et al., 2020). The SFA/UFA or n-6/n-3 PUFA ratios play a critical role in the manifestation of oils' biological effects in hens. Therefore, the fatty acids composition of vegetable oils should be analyzed before being administered as dietary supplements.

In the study, neither the stress model induced by high stocking density nor the dietary oil supplements impacted bodyweights. Bodyweight is known to have changed due to the type, duration, and severity of stressors. Altan et al. (2000) reported that body weight was decreased in heat stress-exposed broiler chicks, whereas Plavnik and Yahav (1998) obtained the exact opposite results. On the other hand, Nelson et al. (2018) showed no influence of stress on body weight, which is compatible with our findings. The presented study revealed consistent findings with previous studies concerning dietary oil supplementations' effects in terms of body weight. Likewise, flaxseed oil and sunflower oil supplements were reported not to have exerted a significant difference in body weights of laying hens (Eseceli and Kahraman 2003). Similarly, sunflower oil-supplemented diet did not affect body weight in broiler chicks (Fébel et al., 2008). Constant body weight is desirable in laying hens after maturity, which reveals that energy balance is maintained. When body weight increases, feed intake should also be increased to compensate for the metabolic rate (Du Plessis and Erasmus, 1972). On the contrary, when body weight is reduced, dietary energy will be targeted to restore weight loss instead of enhancing production capacity. Economic loss is bound to be an inevitable outcome in either case. Therefore, the fact that the bodyweight remained unchanged either by stress or dietary applications was considered a positive output of the study.

The effect of dietary flaxseed oil on egg production differed depending on the absence and presence of stress. While it increased egg production and egg mass in the stressed hens, it generated an exact opposite effect in the unstressed birds. Flaxseed oil supplement was previously reported to have decreased egg production and egg mass. Petrovic et al. (2012) and Schumann et al. (2000) showed that diets containing 2% and 4% of flaxseed oil, respectively, reduced egg production in laying eggs. Shahid et al. (2020), consistently with our results, pointed out a decrease in egg mass in hens fed with a long-term (12 weeks) flaxseed oil-containing diet. It was previously stated that flaxseed diets adversely affected egg production by impairing the feed digestibility due to the anti-nutritional factors in flaxseed (Ahmad, 2017). However, why egg production was decreased by flaxseed oil supplementation decreased has not been clearly understood. Previous studies demonstrated that high energy level diets caused a decline in feed intake, leading to decreased egg production (Huang et al., 1989). In contrast, despite that sunflower oil is a high energy source, no adverse effect of sunflower oil-supplemented diet was noted on egg production in the study, which was thereby associated with an alternative underlying mechanism. When considering these oils' fatty acid compositions -unlike sunflower oil- flaxseed oil contains higher amounts of long-chain unsaturated fatty acids such as myristic acid, stearic acid, arachidic acid (Tablo 3), which were reported to have accelerated intestinal motility (Zhao et al., 2018). Furthermore, dietary oil supplementations were stated to have altered the transit of feed through the gastrointestinal tract and its absorption (Brelaz et al., 2019). Hence, flaxseed oil was shown to have increased intestinal motility due to its cholinergic and histaminergic properties (Parkman et al., 1999; Fabisiak et al., 2017). Therefore, it can be deduced that the decrease in egg production and egg mass in the unstressed hens that received flaxseed oil-supplemented diet was associated with increased intestinal motility and, accordingly, impaired absorption due to saturated fatty acids contained in flaxseed oil.

The fact that flaxseed oil increased egg production in the stressed hens was an intriguing finding. The egg-laying process is governed by the hypothalamic-pituitary-gonadal (HPG) axis in the hens, and the HPG axis is activated by gonadotropin-releasing hormone (GnRH) (Mishra et al., 2019). The high omega-3 content of flaxseed oil stimulates GnRH secretion (Tran et al., 2016), and one of the key stimulants of GnRH is catecholamines (Ottinger et al., 1995). Catecholamine release from the adrenal glands is elevated during stress response (Sabban, 2007). It can be deduced that GnRH production was stimulated both by catecholamine release, emerging as a stress response, and omega-3 contained in flaxseed oil in the stress-exposed hens fed with the flaxseed oil-supplemented diet. Therefore, it was considered that increased GnRH secretion might have resulted in an increase in egg production in the stressed hens that received flaxseed oil in their diet. Besides, corticotrophin-releasing factor (CRF) produced by the hypothalamus during stress is known to slow down intestinal motility, leading to prolonged transition of feed (Mertz, 2003), allowing improved feed absorption, which was considered to be associated with an increase in egg production and egg mass in the study.

Egg weight is a production parameter of great concern for breeders since consumers tend not to purchase small-sized eggs, which reduces their market value (Grobas et al., 1999). Physiological (hens' being at the initial phase of laying) and nutritional factors (a low-energy diet or low amino acid diet) influence the egg's size. Some stress factors are also known to reduce egg weight (Lara et al., 2013). Nevertheless, high stocking density did not affect egg weight in the presented study. Furthermore, flaxseed oil did not exert an adverse effect on egg weight. On the contrary, a slight increase was noted in egg weight in the stressed hens. On the other hand, sunflower oil substantially increased egg weight attributed to the sunflower oil's high linoleic acid (approximately 51.74%) content. It is considered that less effectiveness of flaxseed oil might have been associated with its lower linoleic acid (approximately 16.1%) content. Linoleic acid and other long-chain fatty acids within lipoprotein complexes provide the egg's development and play a significant role in egg weight gain (March and MacMillan, 1990). Hence, oil supplementations rich in linoleic acid were reported to have increased egg weight (Ribeiro et al., 2007). Moreover, their positive influence on egg weight was documented to have been more prominent in hens with insufficient linoleic acid storage. (Grobas et al., 1999). Therefore, the positive effect of sunflower oil on egg weight was considered explicit by the increase achieved in the stressed hens in the presented study, unlike previous reports.

Egg quality is defined by the egg's intrinsic and extrinsic properties. In the study, eggshell weight, eggshell thickness, and eggshell strength were analyzed as extrinsic quality parameters. Corticosterone hormone released during stress response in poultry was indicated to have played a significant role in eggshell formation (Klingensmith et al., 1984). It adversely affects the laying period and reduces calbindin and osteopontin expressions effective in eggshell formation (Kim et al., 2015), impairing the shell quality such as weight, thickness, and strength. Concordantly, it was previously reported that specific stressors reduced eggshell weight (Mack et al., 2013), eggshell thickness (Mahmoud et al., 1996), and eggshell strength (Lin et al., 2004). In the presented study, the induced stress model (high stocking density stress) had no adverse effect on eggs' external quality parameters, which was considered to be associated with long-term stress exposure, allowing stress coping mechanisms to elicit adaptive responses to in-time reduced corticosterone release. Likewise, chronic stocking density stress was reported to have no impact on eggshell strength in hens (Garcia-Rebollar et al., 2008). The available data revealed the inefficaciousness of oil supplements on eggshell quality. It was previously reported that dietary sunflower oil supplementation did not affect eggshell weight (Ceylan and Çufadar, 2018), thickness (Cachaldora et al., 2005), and strength (Dong et al., 2018) (Promila et al., 2017). The conflicting results were indicated to have been linked with the dietary oils' fatty acid contents (Ding et al., 2017). High eggshell quality emerges as a crucial parameter through all aspects of the egg industry from production to consumption to avoid significant economic losses. The fact that eggshell quality was not affected with oil-supplemented diets was considered a favorable outcome.

Haugh unit and albumen height represent the eggs' protein content and freshness. High levels of these parameters are an indicator of good egg quality. Neither stress nor dietary oil supplementations affected Haugh unit and albumen height in the study, which is compatible with previous reports. It was shown that Haugh unit (Al-Saffar and Rose, 2002) and albumen height (El-Tarabany et al., 2016) were not influenced by stress. Likewise, dietary flaxseed oil and sunflower oil supplementations were reported to have no impact on the Haugh unit (Cherian et al., 2007; Midilli et al., 2009) and albumen height (Cedro et al., 2009). Egg yolk color is a crucial intrinsic egg quality parameter that determines consumer preferences. In the study, yolk color significantly faded in the stressed hens fed with a sunflower oil-supplemented diet while generating no significant impact in the unstressed birds (Figure 3). β-carotene is one of the main constituents of yolk coloration, and dietary intake of β-carotene is benefitted in metabolic regulation processes against stress exposure due to its good antioxidant properties. However, sunflower oil (Xixuan et al., 2000) and its high omega-6 content (Hollander et al., 1978) were shown to have decreased β-carotene absorption in the body. When the stress-exposed hens have received a sunflower oil-supplemented diet, β-carotene absorption is inevitably bound to have declined while a great deal of it is concurrently used up to compensate to the demands of the oxidative processes. Therefore, paling of yolk's yellow color in the stressed hens on sunflower oil diet was considered to be associated with reduced β-carotene levels. There is an existing false notion that an intense yellow color is an indicator of eggs' high nutritional value (Moreno et al., 2020) and thus affected consumer demand increasing the market value. However, no correlation was established between yolk color's intensity and the egg's nutritional value (Mızrak et al., 2012). On the other hand, yolk color should be taken into account while selecting eggs for their breeding merits since carotenoids facilitate antioxidant defense mechanisms in tissue responses to oxidative damage during the incubation period. (Blount et al., 2000). Even though this does not pose a problem concerning the eggs' nutritional value, a faded yellow yolk color in the stressed hens that received sunflower oil was considered an unfavorable result regarding both consumer demand and breeders concerning the selection of eggs to be kept for incubation.

Consumer demands are mostly directed by sensory properties of foods since the urge for purchase is essentially based on the color, odor, taste, and flavor of the food products (Ertaş and Doğruer, 2010), which also applies to consumers preferences for eggs. Various vegetable oils and fatty acids have been widely used in recent years to enhance eggs' nutritional quality (Keten, 2019). On the other hand, dietary oil supplements were also reported to have altered eggs' sensory characteristics (Rokka et al., 2002). In the presented study, oil supplements did not adversely affect the eggs' sensory attributes. The absence of a strange odor in eggs was associated with the optimum concentrations of oils in the diet since it was previously shown that higher concentrations of vegetable oils deteriorated the taste and flavor of the eggs, generating a repulsive odor. (Hayat et al., 2010). The collected data revealed that dietary flaxseed oil supplementation improved the eggs' taste both in the stressed and unstressed hens. It was also previously reported in similar studies that flaxseed adversely affected the eggs' taste (Leskanich and Noble 1997; Subhani et al., 2020), which was indicated to have varied depending on the preparation method of oils, concentrations of their volatile components, or trimethylamine content (Parpinello et al., 2001). In the study, stress adversely affected the eggs' taste. Poor palatability was previously associated with the substantial role of oxidative processes of lipids contained in eggs in the stress-exposed hens (Imran et al., 2015). Likewise, oxidative stress was reported to have induced lipid oxidation in the egg as in tissues. (Bölükbaşı et al., 2007). The improved taste in the stressed hens' eggs that received a flaxseed oil-supplemented diet was considered to have resulted from flaxseed oil's antioxidant properties (Hashim et al., 2019).

CONCLUSION

Flaxseed oil increased production performance in the hens exposed to high stocking density stress, improved eggs' taste and texture in both stressed and unstressed birds with no impact on intrinsic or extrinsic egg quality parameters. Therefore, it was deduced that a flaxseed oil-supplemented diet might have generated beneficial effects in the stress-exposed hens. However, its potential adverse effect on egg production and egg mass should be carefully assessed in the unstressed hens. Dietary sunflower oil supplementation proved efficient in improving production performance and egg texture in both stressed and unstressed birds without exerting a negative impact on eggs' internal and external quality parameters except for egg yolk's yellow color. Therefore, it can be concluded that sunflower oil is recommended to be included in both stressed and unstressed hens' diets due to its benefits.

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CONFLICT OF INTEREST

There is no conflict of interest in the present publication.

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