T.R.
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

EFFECTS OF ADDITION OF OMEGA-3 FATTY ACID RICH OIL SOURCES ON LAYING HEN PERFORMANCE, EGG QUALITY AND FATTY ACID COMPOSITION OF EGG YOLK

By
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Supervisor
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Master's Thesis

September 2024
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COMPLIANCE WITH SCIENTIFIC ETHICS

I certify that all information contained in this study has been obtained in accordance with academic and ethical standards. I also declare that consistent with these rules and behavior of conduct, I have fully cited and referenced all materials and results that are not part of this study.

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The Master's thesis entitled "**Effects of Addition of Omega-3 Fatty Acid Rich Oil Sources on Laying Hen Performance, Egg Quality and Fatty Acid Composition of Egg Yolk**" Was prepared by Erciyes University Graduate Thesis Proposal and Thesis Writing Directive.

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OMEGA-3 YAĞ ASIDINCE ZENGIN YAĞ KAYNAKLARI EKLENMESININ YUMURTA TAVUKLARINDA PERFORMANS, YUMURTA KALITESI VE YUMURTA SARISI YAĞ ASIDI KOMPOZISYONU ÜZERINE ETKILERI

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ÖZET

Bu çalışma, yumurta tavuğu yemlerine n-3 bakımından zengin yağların kuatılmaının performans, yumurta kalitesi ve yağ asitleri bileşimi üzerine etkilerini belirlemek amacıyla yapılmıştır. Çalışmada toplam 60 adet 54 haftalık yaşta Super Nick Chick beyaz yumurta tavuğu 5 muamele grubuna ayrılmıştır. Muamele grupları; 1) %3 ayçiçeği yağı (ATY) ilavesi (Kontrol, K), 2) %3 keten tohumu yağı ilavesi (KTY), 3) %3 kenevir tohumu yağı ilavesi (KEY), 4) %3 balık yağı ilavesi (BY) ve 5) %1,5 mikroalg+%1,5 ATY ilavesi (MALG) şeklinde düzenlenmiş olup, araştırma 14 hafta sürdürülmüştür. KTY ilaveli yemdeki n-3 FA oranı diğer gruplardan önemli derecede yüksek bulunmuştur (KEY'da 34.98'e karşı 11.85, BY'da 3.48, MALG'da 3.42 ve ATY'da 1.81). Ayrıca, BY katılan grupta Dokosaheksaenoik asit (DHA) ve eikosapentaenoik asit (EPA) oranı diğer gruplardan önemli derecede yüksek bulunmuştur ($P<0.01$). Muameleler canlı ağırlık, yem tüketimi, yem dönüşüm oranı, yumurta verimi, yumurta ağırlığı ve kitlesi, Haugh birimi, kabuk ağırlığı, kalınlığı, yüzey alanı ve albümin yüksekliğini genel olarak etkilememiştir. Yumurta kabuğu oranı, birim kabuk ağırlığı, yumurta sarısı Roche renk skalası, parlaklığı (L*), kırmızılık (a*) ve sarılık (b*) değerleri genel olarak muamelelerden etkilenmiştir ($P<0,05$). Yumurta sarısı n-3 FA oranı KTY eklenen grupta ve KEY eklenen grupta bazı dönemlerde artmıştır ($P<0,05$). MALG, BY ve KTY gruplarında DHA oranı 1. ve 2. dönemde K ve KEY gruplarından yüksek bulunmuştur. BY eklenen grupta 3. ve 7. dönemde DHA oranı ve K grubunun 5. dönemde DHA oranı diğer gruplardan düşük bulunmuştur ($P<0,05$). Sonuç olarak, yumurta tavuklarının n-3 yönünden zengin yağlarla beslenmesi, tavukların performans, yumurta kalitesi ve kan değerlerini etkilemeden yumurta sarısı n-3 yağ asidi ve DHA yönünden zenginleştirilebilir.

Anahtar Kelimeler: yumurta tavuğu, mikroalg, keten tohumu yağı, balık yağı, kenevir tohumu yağı, yağ asitleri, Dokosaheksaenoik asit

EFFECTS OF ADDITION OF OMEGA-3 FATTY ACID RICH OIL SOURCES ON LAYING HEN PERFORMANCE, EGG QUALITY AND FATTY ACID COMPOSITION OF EGG YOLK

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ABSTRACT

This study aimed to determine the effects of using n-3-rich fats in laying hen diets on performance, egg quality, and fatty acid composition. For this purpose, a total of 60 Super Nick Chick white laying hens at 54 weeks of age were divided into 5 different treatment groups. Treatment groups were arranged as: 1) 3% sunflower oil (SFO) addition (Control, C), 2) 3% flax oil addition (FLO), 3) 3% hempseed oil addition (HSO), 4) 3% fish oil addition (FO) and 5) 1.5% microalgae+ 1.5% SFO addition (MALG), and study lasted for 14 weeks. The n-3 FA ratio in the FLO-added feed was significantly higher than the other groups (34.98 vs. 11.85 in HSO, 3.48 in FO, 3.42 in MALG, and 1.81 in SFO). Also, the Docosahexaenoic acid (DHA) and eicosapentaenoic asit (EPA) ratio in FO feeds was significantly higher than in other groups ($P<0.01$). Fat sources did not significantly affect the live weight, feed consumption, feed conversion ratio, egg yield, weight, mass, Haugh unit, eggshell weight, thickness, surface area, albumin height and blood chemical composition values of the hens ($P>0.05$) in general. The eggshell ratio, eggshell weight per unit, egg yolk Roche color scale, brightness (L*), redness (a*), and yellowness (b*) values were affected significantly in most periods and overall period ($P<0.05$). The n-3 FA ratio of egg yolk in FLO-supplemented groups was higher than the other groups in periods 1, 2, and 7, and the n-3 FA ratio in HSO-supplemented group was higher than in the C and other groups in period 5 ($P<0.05$). The DHA ratio in the MALG, FO, and FLO groups was higher than in the C and HSO groups in periods 1 and 2. In periods 3 and 7, the DHA ratio in FO added group was higher than the other groups, and in period 5, the DHA ratio of the C group was lower than the other groups ($P<0.05$). As a result, feeding laying hens with n-3 rich oils can be enriched with egg yolk n-3 FA and DHA without affecting the performance, egg quality, and blood values of the hens.

Keywords: Laying hen, Microalgae, Flaxseed oil, Fish oil, Hempseed oil, Docosahexaenoic acid

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ABBREVIATIONS

a*:	Egg yolk redness value
b*:	Egg yolk yellowness value
L*:	Egg yolk brightness value
HU:	Haugh unit
Hmm	Albumin height
CP:	Crude protein
SFO:	Sunflower oil
FLO:	Flaxseed oil
HSO:	Hempseed oil
FO:	Fish oil
MALG:	Microalgae
FI:	Feed intake
EP:	Egg production
FCR:	Feed conversion ratio
RRS:	Roche color scale values
EW:	Egg weight
EM:	Egg Mass
ESW:	Eggshell weight
EST:	Eggshell thickness
ESA:	Eggshell surface area
ES/U:	Eggshell per unit
FAs	Fatty acids
SE:	Standard error
P:	Level of significance
PUFA:	Polyunsaturated fatty acid
MUFA:	Monounsaturated fatty acid
n-3:	Omega-3
EPA:	Eicosapentaenoic acid
DHA:	Docosahexaenoic acid

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INTRODUCTION

In order to the body to remain healthy, it is necessary to take in the nutrients it needs with sufficient variety and amounts with food. While protein, energy, water, vitamins and minerals are the main components, small compounds such as a variety of fatty acids including omega-3 (n-3) fatty acids (FAs) have to be provided to the body according to requirements. The n-3 FAs are important for the body and must be received through diet because the body is unable to produce them. Dietary n-3 FAs are integrated into the cell membranes of all tissues. Different n-3 FAs are essential in a well-maintained ratio for a variety of developmental, physiological, biological, and health-promoting activities (Richardson, 2006). The n-3 FAs are important nutrients that support the growth and health and production of poultry (Simopoulos, 2011; Arias-Rico et al., 2018; Cherian, 2015; Lee et al., 2019).

Omega 3 fatty acids can be provided by the oils added to chicken rations. However, some oils are rich in n-3 FAs, but some oils are not sufficient in terms of n-3 FAs. For this reason, the composition of the oil sources added to the rations affects the composition of the fatty acids of the eggs and meat of the poultry. It has been shown that when oils rich in omega 3 FAs are given to laying hen feeds, the n-3 FAs ratio in the eggs of the hens increases. However, n-3 FAs sources are not sufficient in most seeds, and their usefulness in the animal also varies. Among the most used oil sources is fish oil, but since fish oil is high in unsaturated FAs, so, it can cause easy oxidation and it cannot be used in the desired proportions and it gives the products a fishy smell. For this reason, seed oils have become more preferred. This is one of the most used n-3 FAs rich seed oil is flaxseed oil (Neijat et al., 2016a). Recently industrial hempseed oil a new seed oil in the industry rich in n-3 FAs sources caused an increase in n-3 FAs in poultry meat (Yalcin et al., 2018) and eggs (Mierlita et al., 2024; Konca et al., 2019). Apart from this, one of the other sources is microalgae Microalgea's new sources for omega-3 FAs sources in poultry diets (Sefer et al., 2011; Thapa, 2020) and it has been transformed into various products and sources rich in DHA have been commercialized. Docosahexaenoic acid (DHA) is a necessary n-3 FA for brain development and other bodily processes. The DHA is concentrated in the brain membrane lipids and the retina's visual components (Herber and Van Elswyk, 1996; Innis, 2008).

Kalakuntla et al. (2017) reported that adding 2 and 3% mustard oil (MO), linseed oil (LO) and fish oil (FO) instead of soybean oil (SO) to broiler starter and finisher diets increased the omega-3 fatty acid ratio and decreased the omega-6 and omega-6:omega-3 fatty acid ratio in meat without changing the performance, carcass yield and sensory properties of meat. In the study, also, it was also determined that the thiobarbituric acid (TBA) ratio increased with LO and FO compared to soybean oil (Kalakuntla et al., 2017). In hens feeding with a high n-3 polyunsaturated fatty acids (PUFA) diets, n-3 PUFA concentration increases in eggs (Hargis et al., 1991).

Although the ω-6 and n-3 FAs are necessary FAs in human diets, significant variations in diets have led to changes in the intake of these FAs. This change may cause an imbalance in the ω-6 and n-3 rates. As a result, eggs may be an obvious option for increasing daily n-3 consumption to achieve the necessary dosages (Baião and Lara, 2005). The uptake of n-3 polyunsaturated FAs (PUFA) into the egg depends both on the efficiency of transfer of preformed n-3 FAs from the diet into the egg and on endogenous PUFA metabolism and deposition (Neijat et al., 2016a). Enrichment of eggs with n-3 FUFA may be achieved via rations rich in these FAs. As a result of enriched eggs with essential PUFAs in the human diet, particularly those linked to the prevention of chronic diseases associated with poor nutrition. Alpha-linolenic acid (ALA) from plant sources can be fed to chickens to increase the amount of n-3 PUFA in their yolk lipids (Neijat et al., 2016; Ojekudo, et al., 2016).

Marine-derived oils, which are rich in Eicosapentaenoic acid (EPA) and DHA content (Zhang et al., 2024), may be used efficiently for egg fortification, rather than ALA and they can be used to enrich DHA concentration of eggs. Previous research revealed that DHA and EPA concentration in eggs may increase with 120 mg EPA and DHA supplementation from fish oil for 21 days when compared to 120 mg α-ALA supplementation from flaxseed per 100 g feed (Lemahieu *et al.*, 2015). Other researchers found that plasma triglyceride (TG) are decreased by enhanced n-3 PUFAs (Oscarsson and Hurt-Camejo, 2017).

Zhao *et al.* (2021) noted that the inclusion of 15 g/kg fish oil (FO) and 15 and 30 mg/kg krill oil inclusion to the diet caused an increase in egg's approximately 300 mg of EPA and DHA. Compared to krill oil addition, FO supplementation increased the retention of EPA and DHA in eggs. Another experiment showed that a 4 g/kg fish oil addition could increase 200 mg/kg of DHA in egg yolk (Cachaldora et al., 2006). Also, fish oil addition increased omega-3 fatty acid DHA and EPA contents of egg yolk (Antony et al., 2024).

This study was conducted to determine the effects of adding rich sources of n-3 FAs such as flaxseed oil, hempseed oil, fish oil, and DHA-rich sources as microalgae to laying hen feeds on the performance, egg quality, egg yolk fatty acid composition, and blood values of laying hens.

CHAPTER 1

GENERAL INFORMATION

Omega-3 and ω-6 FAs (FAs) are essential for the membranes of the cells, and they are necessary for human health and the proper functioning of the body. Essential FAs can't be generated endogenously. If poultry feed is supplemented with oil sources high in n-3 PUFAs, so, they can be a good source of PUFAs for humans who consume them (Alagawany et al., 2019). According to several studies, feed ingredients high in n-3 FAs can be used to generate poultry eggs and meat rich in PUFAs. Generally, research has been done to evaluate the impact of different n-3 FA sources in poultry diets on the cholesterol and fatty acid levels of eggs and meat (Van Elswyk, 1997; Gonzalez-Esquerra and Leeson, 2000). The research revealed that egg yolk FA concentrations may be modified by changing fat sources in the feeds with the addition of linseed oil (Oliveira et al., 2010).

Antony et al. (2024) found that, after 180 days of feeding hens a diet supplemented with 0, 5 g, 10 g, 15 g, and 20 g/ton fish oil omega-3, the EPA, DHA, and omega-3 content in eggs varied from 11.4 to 28.71 mg/100 g, 116.41 to 206.62 mg/100 g, and 172.03 to 327.78 mg/100 g, respectively, based on the supplement level.

Long-chain PUFA has been studied for at least three decades for its positive actions in biological processes in poultry. Dietary intervention with n-3 FAs may affect hen immunity and result in the creation of products with consumer health advantages. Using PUFA in chicken diets substantially decreases total lipid and cholesterol levels in the yolk and blood. Several research has been carried out to reduce the negative effects of total cholesterol and triglycerides in chicken products (Ahmad et al., 2012; Konieczka et al., 2017).

According to Sharifi et al. (2024) supplementing the diet with n-3 fatty acids increased egg production. The DHA and total n-3 fatty acids in the yolk increased linearly as the dietary Salomega level increased. Serum cholesterol and triglycerides dropped but HDL levels improved as the Salomega level in the diet increased. The MDA level in the yolks of bird eggs given 3 or 4% Salomega was higher than that of the control group. The oxidative stability of yolk lipids is reduced by salomega at 3 and 4 %.

1.1. Sunflower oil (SFO) properties and use in poultry diets

Sunflower oil (SFO) mainly contains oleic and linoleic fatty acids, while they have a low content of stearic and palmitic acids. Although sunflower varies depending on the variety, it is very rich in stearic (18:0) and oleic (18:1) FAs. The high-oleic-SFO kernels contain approximately 90% oleic acid. In newly developed varieties, the values of the same fatty acid in the other variety are highly variable and these values may change several times (Garcés et al., 2009). A previous study indicated that SFO contains the highest amount of long-chain mono-unsaturated fatty acid (MUFA) and poly-unsaturated fatty acid (PUFA)s (91.49 ± 1.91%) compared to coconut oil (7.12 ± 0.51%), palm (53.30 ± 0.36%), soybean (81.14 ± 1.49%), and mustard oils (86.80± 3.07%) (Chowdhury et al., 2007).

Ceylan et al. (2011) noted that the addition of 1.5 or 3% SFO, FO, and rapeseed oil had no impact on egg production, egg weight, feed intake, feed conversion ratio, body weight, and egg cholesterol levels of laying hens. However, yolk color was decreased in chickens fed SFO than other groups. Dietary treatments changed the FA content of the yolk. The LA composition increased with feeding 3% linseed and rapeseed oil and, the DHA deposition increased in the fish oil feeding group.

Alvarez et al. (2005) noted that laying hens fed 10, 20, and 30 g/kg SFO and 0, 1, and 2 conjugated linoleic acid (CLA) g/kg, did not affect feed intake, egg yield, egg weight, albumen height, shell thickness, and yolk color. CLA inclusion into the diet increased the concentrations of CLA and saturated fatty acids (SFA), but reduced MUFA, arachidonic acid, and DHA concentrations in the egg yolk. The MUFA concentrations in yolk were boosted by high-oleic acid SFO supplementation to diet, while those of CLA and SFAs were lowered. Egg quality characteristics and yolk CLA concentrations decreased as the amount of high-oleic acid SFO increased in the diet.

Kale (2021) found that the 3% sunflower and organic and inorganic selenium (Se, 0.3 mg/kg) additions to the diet of laying hens, did not affect egg yield, feed intake, FCR, Haugh unit, specific gravity, yolk, and white ratio. However, egg weight, shell ratio, and thickness, yolk color, egg shape index, and yolk and white index were significantly affected by the treatments. Egg yolk thiobarbituric acid reagents (TBARS) were increased in the SFO-fed hens than in the other groups. The addition of SFO and different selenium sources to the diet of hens did not generally affect egg quality and performance parameters, but SFO addition increased yolk TBARS value.

1.2. Flaxseed oil (FLO) properties and use in poultry diets

Flaxseed is one of the important functional foods and it is the source of a-linolenic acid. It contains high-quality protein and soluble fiber (Oomah, 2001). FLO has a favorable FA profile with a high amount of ALA (over 60%) compared to other plant oils (25%). The proportion of FAs, particularly unsaturated FAs, reported in various research for linseed oil varies greatly. This diversity is primarily due to differences in the investigated varieties and industrial processing procedures (Nykter et al., 2006). FLO contains a large amount of oleic 18.51%, linolenic 53.21%, linoleic 17.25% acids, and stearic 4.43% (Popa et al., 2012). Due to the high ALA content of FLO, EPA, and DHA can be produced from ALA and then deposited in the egg during metabolism. Humans had no efficient process to synthesize EPA and DHA from ALA like laying hens. ALA-rich FLO is a successful way to produce EPA, DHA, and n-3-enriched eggs (Keten, 2017).

Oliveira et al. (2010) reported that a fed diet added with FLO, SFO, or soybean oil, showed that the ratio of n-3 FAs was high in hen egg yolks from the FLO treatment group, but n-6 FAs was high in the soybean-added group. As the hens aged, the amount of PUFAs in egg yolks dropped. In the FLO-added group's omega-6 to n-3 ratio was 2.17 in older chickens and 2.1 in younger ones.

Soliman and El-Afifi (2020) noted that the hens fed 3% palm oil (PO), 2% PO+1% FLO, 1% PO+ 2% FLO, or 3% FLO supplementations, boosted the egg mass and egg production by 3% FLO addition, but FLO inclusion did not effect on yolk color index, yolk, shell, and albumin ratios. However, FLO supplementation substantially decreased plasma and yolk cholesterol, plasma triglyceride, SFAs, and MUFAs levels in the yolks, while the amounts of PUFAs increased in eggs. Dietary treatments resulted in a small rise in linoleic FA ratios, while 3% FLO increased the n-3 α-linolenic fatty acid ratio.

Celebi and Macit (2009) found that the addition of 2% FLO+2% tallow, 4% SFO, and 4% FLO, caused a decrease in feed consumption and feed efficiency ratio, while the FLO group had the lowest feed intake. The oil supplementations to diets increased egg production, but egg weight was not affected by treatments. The tallow addition enhanced the concentration of SFAs in the yolk. Amounts of linoleic and arachidonic acids in yolks were lower in the FLO group compared to the 4% SFO group. The oleic acid was the highest in layers fed with 2% tallow+2% FLO group. The inclusion of FLO enhanced a-linolenic and DHA ratio in yolks while decreasing other unsaturated FAs.

Bean and Leeson (2003) noted that feeding laying hens 0 or 10% flaxseed added diets feed consumption was lower in birds given flaxseed than in those fed a basal diet. Egg weight, egg yield, shell weight, eggshell thickness, and albumen height were not substantially different between 10% flaxseed and control birds. Moreover, in chicken-fed flaxseed, yolk weight was reduced. Flaxseed-fed chickens produced considerably more n-3 FAs in their eggs.

The laying hens fed supplementation of whole and ground flaxseed with different amounts (5%, 10%, or 15%) and 1.5% FO, showed that flaxseed addition lowered feed intake, egg weight, and weight gain than the control group. Furthermore, the addition of FO and flaxseed increased egg yield and egg white but decreased egg yolk percentage according to the control. Linolenic acid absorption improved linearly when dietary flaxseed intake increased. Dietary flaxseed and FO supplementations had no impact on egg cholesterol levels. Compared to golden flaxseed, brown flaxseed dramatically enhanced egg weight and egg yield (Scheideler and Froning, 1996).

Neijat et al. (2016) reported that hens fed diets containing FLO or algal DHA (0.20%, 0.40%, and 0.60% total n-3 FAs), there were no significant variations found in hen feed consumption, weight gain, production rate%, yolk and egg weight, eggshell index, eggshell thickness, specific gravity and Haugh unit, or yolk and plasma cholesterol level. While total n-3 polyunsaturated FA levels increased proportionally in the yolk and plasma. Algal DHA-fed chickens incorporated three times more docosahexaenoic acid than in the FLO group. The enrichment of total n-3 polyunsaturated FAs was highest in week two for algal DHA and FLO-fed hens.

Ehr et al. (2017) found that supplementation of FLO (0.5, 1.0, 2.0, 3.0, or 5.0%) and milled flaxseed (0.5, 1.0, 2.0, 3.0, 5.0%), showed that egg yolk EPA, DHA and ALA fatty acid content in FLO was twice greater than those milled flaxseed under similar dietary additions. The contents of EPA and DHA in yolks were unaffected by both flaxseed sources. Increasing the consumption ratio of FLO and milled flaxseed boosted yolk EPA and DHA. Birds consumed milled flaxseed and FLO had less body weight change as the dietary concentrations rose. Feed efficiency improved as the amount of FLO was boosted; however, feeding milled flaxseed lowered feed efficiency when compared to milled flaxseed, and the dietary FLO enhanced feed efficiency and ALA deposition in the yolks. These results suggest that FLO can be a useful choice for producing alpha-linolenic acid-enriched eggs.

Studies on brown vs white laying hens given diets added with various oils (flax seed oil, olive, soy, or tallow) at different concentrations (0, 5, and 10%), indicate that white laying hens generated yolks with high levels of saturated FAs and low amounts of polyunsaturated and monounsaturated FAs. Dietary oil inclusions enhanced feed conversion ratio and egg mass yield, but did not affect egg yield%. Soy and flax seed oil groups generated larger egg masses than those in tallow or olive oil groups. As the amount of additional oil increased, the level of saturated FA in the yolk reduced. The amounts of arachidonic (AA), DPA, and DHA acids in the yolks increased as the ALA content of the diets improved from 0 to 0.8%. Dietary improvements of alpha-linolenic acid intake above 2.3% had no additional impact on the yolk DPA or DHA concentrations and, indeed, reduced the amount of AA. Eggs with detectable levels of EPA were only produced in the flax seed oil treatment group (Grobas et al., 2001).

1.3. Hempseed oil (HSO) properties and use in poultry

Hempseed (HS) is produced industrial type of hemp plant. It is high in n-3 FAs, accounting for approximately 17 % of total FAs /100 g. As a result, hemp seed and its oil is useful sources to enhance poultry product's n-3 FAs concentration. In a study (Goldberg et al., 2012), laying hens fed a diet added with 0% hemp, 10, 20% hemp seed, and 4, 8, and 12% HSO for 12 weeks, indicating that the 12% HSO group had the greatest n-3 PUFAs content in the yolks (15.3 mg/g) compared to the control group (2.4 mg/g). According to the basal diet, hemp addition resulted in significant increases in egg yolk yellowness value and yolk redness value, but decreases in egg yolk brightness value, with the biggest alterations shown in the 20% hemp seed treatment. Hemp inclusion increased the n-3 PUFA level and color intensity of egg yolk, but it had no negative impacts on the sensory characteristics of cooked eggs.

Neijat et al. (2014) reported that hempseed supplementation (10%, 20%, or 30%) and HO oil (4.5% or 9%) or control (corn oil) to laying hen diets, had no appreciable impact on the laying performance, egg quality, and plasma glucose, protein, cholesterol, electrolytes, and uric acid concentrations. Comparing the 4.5% HSO the 10% and 20% hemp seed concentrations group's total plasma enzyme contents, especially gamma-glutamyl transferase were considerably reduced. Also, diets containing hemp seeds and HSO, particularly at concentrations of 10%, 20%, and 4.5%, may prevent liver damage or injury. Indicators of plasma clinical chemistry show that both HS and HO are well tolerated by laying hens; this demonstrates the safety and effectiveness of including hemp products in chicken diets.

Gakhar et al. (2012) supplemented 4%, 8%, and 12% hempseed oil (HSO), and 10%, and 20% hempseed did not affect average egg yield. However, egg weight was greater in chickens fed a 20% hemp seed diet than in the control group, while feed intake was reduced in birds fed the 4% HSO compared to controls. Treatments did not influence body weight (BW), except in chickens fed the 12% HSO which decreased final body weight than the control group. Total n-3 FA concentration in yolks raised when dietary α -α-linolenic acid supplementation hemp products upgraded. A quadratic effect was shown for docosahexaenoic acid contents in the yolk caused by upgrading α-linolenic acid supplementation. The results reveal that using HSO in laying hen meals with maximum concentrations of 20 and 12 percent, has no negative impacts on laying performance and increased n-3 FA concentrations of the yolk.

Jing et al. (2017) discovered that chicken layers given a diet added with 0% hemp, 4%, and 8% hemp oil, and Hemp omega, layer, and broiler performance were unaffected by dietary interventions. In comparison to the control group, birds fed either hemp oil or Hemp Omega diets showed higher amounts of total n-3 polyunsaturated fatty acids, DHA, EPA, and a-linolenic acids in the yolk. Moreover, hemp oil or Hemp Omega treatments did not influence the concentrations of total n-6 PUFAs, linoleic acid, and AA in eggs and meat. The addition of 8% hemp oil in layer diets provided by hemp oil or Hemp Omega has no adverse impact on total hen performance and increased n-3 fatty acid in eggs.

Kanbur et al. (2023) reported that laying hens given the same amount of different n-3 rich seed oils (hemp seed, soybean, corn, sunflower, and canola oils) the integrated seed oils had no effects on weight gain, feed consumption, FCR, egg mass, egg yield, egg and shell weight, eggshell thickness and strength. However, HSO increased the brightness value while reducing the egg yolk redness value but there were no significant variations between yellowness values in all groups. Hemp oil, like SFO, corn, and soybean oils, raised the polyunsaturated FA ratio in the yolk, and it also enhanced the n-3 fatty acid concentration. This indicates that HSO, like other seed oils, can be utilized safely in laying hens' feed without causing an adverse impact on egg production or egg quality. Moreover, HSO can create n-3-FA enriched eggs by increasing the desired FA concentration in the yolk.

Two investigations of 7-day-old broiler quail and 8-week-old laying quail were given a feed supplemented with varying levels of hempseed (0%, 5%, 10%, and 20%). Dietary HS addition resulted in increased redness of hip meat. Hempseed inclusion into the feed reduced oleic and palmitoleic FAs in breast meat while increasing linolenic and linoleic acid ratio. In investigation 2, egg yolk linolenic and linoleic fatty acid concentrations increased linearly as the HS addition increased in the diets. Quail with HS included had lower levels of oleic and palmitoleic acids in their egg yolk (Yalcin et al., 2018).

Konca et al. (2014) found that different amounts of hemp seed (0, 5, 10, and 20%) supplementations in the diets of Japanese quail, the results indicate that the body weight of treatment groups was not affected at 7 and 21 days, but it reduced at 42 days in the 20% hempseed group. There was no substantial variance in feed conversion ratio or feed intake between the experiment groups. The levels of glutathione peroxidase (GSHPx), malondialdehyde (MDA), catalase (CAT), nitric oxide (NO), total protein, and superoxide dismutase (SOD) were determined in the blood serum at the end of the experiment. The serum levels of NO and MDA dropped by hempseed addition, while it raised the levels of serum CAT, GSH-Px, and SOD. Finally, adding hemp seeds to quail diets may raise blood antioxidant activity without necessarily improving quail performance attributes.

Park et al. (2014) noted that laying hens fed with hempseed, corn, and evening primrose oils, revealed that egg production, HU, and yolk color of oil-supplemented groups were higher than those of controls. However, dietary interventions had no effect on feed intake, eggshell thickness, and shell-breaking strength. Gamma-linolenic acid was raised in eggs produced by the 1.09% HSO supplemented group and the birds given 4.87% evening primrose oil inclusion but, it was not detected in eggs from control and corn oil hens.

Laying hens fed dietary supplementations of HSO (2%, 4%, 6%), or hempseed (5, 10%, 15%, 20%), the findings demonstrated that hens fed 6% HSO had the best egg mass and egg production, and the lowest FCR and feed intake. HSO concentration increasing from 4 to 6% improved the HU in period 3. Although hempseed or HSO inclusions reduced the egg yolk color index, hempseed had a higher effect. The total protein content in plasma was unaffected by the dietary supplementations. The maximum addition of 6% HSO substantially lowered triglycerides, aspartate aminotransferase (AST), and blood plasma cholesterol when compared to the greatest amount of 20% hempseed. Chickens fed the 6% HSO diet had greater blood HDL-C amounts than those of the 20% hemp group. The 5% and 10% hempseed addition had appropriate impacts on the post-peak performance of laying hens. Adding HSO to the diet of laying hens improved egg qualities more than adding hempseed (Bazdidi et al., 2016).

Konca et al. (2019) hens fed a diet supplemented with raw hempseed or heat-treated hemp seed (0 or 15%), the feed intake of the raw hempseed group was decreased compared to other groups. Roche color values for the raw hempseed birds were higher than those for the HHS group. The HHS group had considerably higher egg weight, eggshell weight, egg mass, eggshell thickness, and shell surface area, than the raw hempseed group. Palmitoleic, oleic, and Palmitic acids in eggs were reduced in both treatment groups, compared to the controls. The linoleic acid, ALA, and DHA levels increased in both HS groups than the control group. Supplementing raw hempseed and heat-treated hemp seed to hen diets did not alter the levels of MDA, SOD, and blood lipid profile.

1.4. Fish oil properties and used in poultry

Fish oil is a source of n-3 fatty acids, DHA, and EPA, however, their amounts might vary among fish species. The EPA and DHA amounts of commercial fish oil varied between 2.743 and 15.448% and 3.758 and 10.717%, respectively. The EPA content in horse mackerel oil was 6.264% and 8.842% in anchovy oils. The DHA ratio in anchovy oils was 15.243% and 11.513% in horse mackerel oils. The n-3 content including fish oil, is about 25%, which many have good health impacts such as anti-carcinogenic, anti-diabetic, anti-atherogenic, and immunological stimulatory (Kartal et al., 2003; Balta et al., 2021). According to a study arranged by Mariod et al. (2015), feeding birds with a diet supplemented with 3% fish oil considerably boosts egg weight and egg production more than the birds' given 3% oleic FA-rich oil. The UFAs ratio in yolk lipids of birds given fish oil was dramatically raised, and monounsaturated and PUFAs as fish oil were added.

Lawlor et al. (2010) added 0, 20, 40, and 60 microencapsulated fish oil to diets, the egg yolk weight was significantly decreased. However, findings also show that adding microencapsulated FO to the diet may effectively improve the amount of long-chain n-3 FAs in egg yolk. However, there were no significant variations in feed intake, egg yield, or egg weight across all treatment groups (Lawlor et al., 2010).

Cachaldora et al. (2008) discovered that laying hens fed 0, 15, 30 g/kg of fish oil or 4 basal diets (0, 50g/kg of linseed, lard, and soybean oils), had no effect on feed consumption, egg weight, egg yield, HU, yolk weight and eggshell thickness. Yolk color was raised in SO, lard oil, and LO inclusions, while FO inclusion dropped linearly. The dietary treatments did not affect yolk fat composition but affected the fatty acid ratio. The FO addition enhanced the amount of EPA and DHA in egg yolks linearly. The inclusion of SO and LO raised the levels of ω-6 to n-3 FAs in egg yolk.

Study of FO (0, 14, 20g/kg) or CLA (1, 3 and 5g/kg) addition in laying hens' diet enhanced yolk fat content of long-chain n-3 FAs while reducing those of CLA, saturated, and LC ω-6 FAs. While the CLA addition increased linearly the yolk moisture, total egg weight, and yolk pH and fat contents of conjugated linoleic acid, saturated, and total long-chain n-3 FAs, but decreased to those of MUFA, total LC n-6 FAs and albumen pH (Alvarez et al., 2004).

Saleh (2013) claimed that FO and vegetable oil (0+5% VO, 1.25% FO+3.75% VO, 2.5% FO+2.5% VO, 3.75% FO+ 1.25%VO and 5% FO+ 0% VO), the 3.5% FO supplementations improved body weight gains by 9.2% compared to the control diet. Feed intake was raised by 26.4% in hens given 5% FO as opposed to the control group, and the egg weight was also reduced when fish oil addition increased. The yolk cholesterol level was decreased by 3.5% fish oil addition in a 14.5% reduction. In addition, it was shown that 3.5% FO supplementation had raised LA contents in eggs by 30.5%. FO addition increased eggshell ratio, shell thickness, and yolk color. However, the albumen%, yolk%, and their index and HU were not affected by the treatments.

In a study on the supplementation of FO, flaxseed, or chia seeds, no substantial variation was found in yolk fat composition across the groups, but dietary interventions have a considerable impact on the FA content of egg yolk. The highest n-3 fatty acid composition in eggs was found in the 300 g/kg chia-added group. DHA and EPA were exclusively found in fish oil-fed chickens. The types and amounts of n-3 FAs in feed affect the amount of n-3 FAs in the yolk (Coorey et al., 2015).

Kucukersan et al. (2010) revealed that supplementations with 3% oils of fish, sunflower, soybean, and hazelnut oils) in chicken feed, feed efficiency, feed consumption, egg HU, egg yolk index, egg breaking strength, eggshell thickness and egg albumen index was unaffected by the dietary oil supplementations. Likewise, no noticeable variations have been found in egg cholesterol levels. Egg production and weight values were considerably increased by 3% soybean oil inclusion to hen's diet.

Kralik et al. (2008) found that FLO and FO (1.50% LO + 3.5% FO, 2.5% LO +2.5% FO, or 3.5% LO + 1.5% FO) additions increasing FLO addition to the laying hen feed raised yolk's n-3 PUFA and ALA, which was most beneficial in 3.5% LO + 1.5% FO fed group. Egg yolks from 1.50% LO and 3.5% FO fed group contained more EPA than those from 2.5% linseed +2.5% fish oil and 1.5% fish oil + 3.5% LO groups, while DHA concentration did not differ dramatically across treatment groups. The group with 3.5% FO + 1.5% LO had the greatest ω-6 to n-3 polyunsaturated FAs ratio.

Poultry diets were supplemented with different amounts of menhaden FO (0, 1, 2, and 3%), the FCR, feed intake, and weight gain were not affected by the dietary interventions, however, the DHA and EPA in egg yolk were significantly higher in FO group (Huang et al., 1990).

Kehui et al. (2004) noted that with the supplementations of 5% of different oils, (basal + fish, basal + palm, and basal diet + soybean) in the poultry diet, the final BW, FCR, egg white weight was unaffected by the supplementations. The n-3 FA contents in egg yolk had increased in the FO and soybean oil groups. While feed intake and yolk cholesterol levels were decreased in the FO group compared to other groups. Egg mass increased in palm oil compared to soybean oil, but decreased yolk weight than the FO group. Average egg weight raised in the FO group than control. Hen weight gain was higher in FO than in SO addition. EPA was not found in the palm oil group, while the highest DHA and EPA ratios on egg yolks were found in the FO group.

A study of supplementation of flaxseed and FO (0% FO and FS, 1.5% FO+4.32% FS, 4.32% FS, 1.5% FO, and 8.64% flaxseed) for 8 weeks, showed that the total amount of n-3 FAs in egg yolks dramatically raised in flaxseed and FO fed chickens, and the yolk cholesterol levels were lowered in 1.5% FO or 8.64% flax seed fed birds compared to the basal diet. FO and flax seed addition to the diet raised the total n-3 FA in eggs at the 56 and 28 days of the study. In period 1, egg total ω-6 FAs were unaffected by FO or flax seed diets. But dietary supplementation of 4.32% FS, 1.5% FO and 4.32% FS+1.5%FO had lower total n-6 FAs than the control in period two. Eggs with the greatest LA content were obtained from flax seed-fed birds, while eggs with the highest DHA content from FO addition. FO and flax seed dietary additions had no impact on serum high-density lipoprotein and triglyceride contents. Serum cholesterol levels were lower in 1.5% FO + 4.32% FLO and 8.64% flaxseed birds compared in the controls. FO and flax seed additions had no negative influence on egg, albumen, yolk and albumen weights, yolk, albumen and shell ratios, shell thickness, and eggshell strength. Egg yield was considerably higher in 4.32% FS chickens than in the control. All supplementations had no impact on feed conversion ratio and feed intake (Basmacioglu et al., 2003).

Cherian (2008) revealed that broiler hens given 3.5% FO-containing feed had a substantial drop in egg weight, eggshell weight, yolk weight, and color when compared to 1.75% FO + 1.75% yellow grease-fed laying hens. Dietary interventions had no impact on the total yolk contact. When compared to other weeks, egg yolk fat was highest at 42 weeks for 3.5% FO and FO + yellow grease eggs. Total n-3 FAs and the docosahexaenoic FAs and DHA to AA ratios were greater in 3.5% FO eggs than in 1.75% FO + 1.75% yellow grease.

Meluzzi et al. (2018) discovered that hens fed 3% of (FO or lard) and vitamin E (0, 50, 100, and 200 ppm), and the dietary interventions did not affect laying rate, feed intake, FCR, and egg weight. But yolk total n-3 FAs increased by FO supplementation, especially EPA FAs, and DHA FAs. The dietary vitamin E, slightly altered yolk fatty acid composition. Yolk α–tocopherols raised linearly as dietary vitamin E upgraded to the laying hen's diet.

A study of laying hens fed diet (control, 0 FO), (0.3% FO + 4.7% soybean), (0.6% FO + 4.4% soybean), (0.9% FO + 4.1% soybean), (1.2% FO + 3.8% soybean), and (1.5% FO+ 3.5% soybean). FO addition increased the EPA FAs, DHA FAs, and n-3 PUFAs levels in eggs. The ω-6 PUFA to n-3 PUFA ratio dropped from 8.69 in the control to 4.54 in the 1.5% FO + 3.5% soybean group (Kralik et al., 2021).

Zhao et al. (2021) discussed that 15 and 30 g/kg krill oil and 15 g/kg FO inclusion to the laying hen diet, could produce more than 300 mg of docosahexaenoic FAs and EPA FAs per one egg. At equal dietary levels, FO supplementation showed a higher retention efficiency of docosahexaenoic FAs and EPA FAs in eggs than KO. As a result, KO may increase the amount of egg docosahexaenoic FAs containing phospholipids, while FO has a high docosahexaenoic FAs content and conversion rate. In comparison to the control, the egg weight was reduced by 5.92% in the 1.5% krill oil group, while the 3%KO and 1.5% FO showed no effect. Feeding 3%KO during the intervention period resulted in a 5.25% drop in feed consumption compared to the control group, while 1.5% krill oil and 1.5% FO addition showed no negative effect on feed consumption.

An investigation of laying hens given a diet with 8% FO addition performed poorly when compared to soybean or coconut oil. The dietary FO was primarily responsible for the substantial decrease in egg weight. Hens fed FO showed consistently and significantly better shell traits than soybean or coconut oil groups. However, according to soybean oil group, yolk color average was higher in coconut oil, while average albumen height and HU were increased in FO than in SO or coconut oil. For the majority of the first two months of the experiment, FO raised the levels of serum UA, AST, and MDA (Dong *et al.*, 2018).

Koreleski et al. (2003) discussed that in laying hens given 0.3% of FO with or without vitamin E, the dietary FO had raised n-3 polyunsaturated fatty acid level and lowered n-6 to n-3 polyunsaturated fatty acid ratio (from 7.75 to 4.97) with no impact on egg cholesterol levels. The fatty acid contents in eggs were unaffected by the treatments; moreover, vitamin E supplementation raised α-tocopherol in egg yolk and upgraded fat stability during 15 and 42 days of storage. However, there was no significant difference between treatment groups in egg-weight and FCR. Feed intake and laying rate % were increased in the control group.

Omidi *et al.* (2015) demonstrated that supplementation of 0% or 3% fish, olive, canola, grape, and soybean oils did not affect feed intake, egg production, egg mass, egg weight, and feed efficiency. Dietary FO supplementation drastically decreased ω-6 FAs but increased docosahexaenoic and EPA in yolks. Finally, FO enhanced n-3 long-chain FAs in egg yolks and reduced the ω-6 to n-3 ratio, which may have good impacts on human wellness.

1.5. Microalgae properties and use in poultry diets

Microalgae have recently received much attention as an important source of animal feed and generator of n-3, DHA, and EPA (Lum et al., 2013). These n-3 LC PUFAs, DHA, and EPA can be used to prevent Alzheimer's disease and cognitive aging. Moreover, n-3 polyunsaturated FAs control the immune system and reduce inflammation, which has a good impact on both human and animal growth and productivity (Mori and Beilin, 2004; Dacks et al., 2013).

Ao et al. (2015) found that laying hens fed 1, 2, or 3% All-G-RichTM microalgae, the increasing ratio of algae addition in the feed enhanced the DHA content of egg yolks. Throughout the experiment, layer productive performance hen weight, feed consumption, FCR, egg production, egg weight, shell quality, yolk%, shell%, HU and yellowness value were not influenced by the dietary interventions. However, 2% and 3% ALL-G-RICHTM supplements decreased the lightness and raised the redness value of yolk.

Herber and Van Elswyk (1996) demonstrated that in two investigations of 24-wks-old or 56-wks-old laying hens in the same species fed marine algae (4.8%, 2.4%) and 1.5% menhaden oil, the yolk and egg weight were unchanged in 56-wks-old birds, the egg production was considerably decreased in 4.8% marine algae group. While 24-week-old laying hens did not influence egg production. However dietary treatments had a considerable influence on PUFA compositions of yolks in both groups. Dietary n-3 FA addition raised total n-3 fatty acids in the egg yolk while omega-6 FAs in the yolk. Although birds that consumed menhaden oil were primarily given EPA, the DHA was the main FA deposited in egg yolk. The greatest ratio of DHAs was shown in eggs from chickens fed a 4.8% Marine algae diet, which also enhanced effective yolk DHA deposition.

Lemahieu et al. (2013) noted that supplementation of 4 various n-3 rich microalgae (*Isochrysis*, *Chlorella fusca*, *Nannochloropsis oculata* and *Phaeodactylum tricornutum*), with 2 ratios: 250 mg and 125 mg + n-3 PUFA/100g diet of laying hens, there was no significant variations between treatment groups in egg-weight, albumen-weight, eggshell-strength yolk-weight, shell-weight and shell-thickness. The addition of these microalgae promoted the amounts of various ω -3 FAs in egg yolks, primarily the enrichment of DHA. Only the addition of chlorella resulted primarily in ALA enrichment. Phaeodactylum and Isochrysis inclusions produced the highest rate of n-3 PUFA enrichment. Moreover, Isochrysis, Phaeodactylum and nannochloropsis addition led the yolk's color to change from yellow to redness.

Szczurek et al. (2020) noted that hens fed diets contained 2% of various oil sources (algal, soybean, rapeseed, linseed, fish, camelina, or coconut oils) showing the fat sources did not affect the laying performance. However, yolk FA composition was strongly altered by dietary fat sources. The algal oil supplementation resulted in modifications to the yolk's lipid profile that raised the contents of EPA and DHA. Finally, the inclusion of marine algae oil in the layer chickens' diet is a successful approach to improve the deposition of n-3 polyunsaturated FAs in eggs, with no adverse effect on egg performance.

Sefer et al. (2011) reported that there was significant variation in yolk fatty acid content and ratio among groups when laying hens fed 0.5, 0.7, and 1.0% of microalgae

(Schizochytrium spp) supplementations. The egg yolks from the 1.0% group had the highest content of n-3 FUFA and the control group had the lowest n-3 content. Microalgal feed addition improved the egg yolk polyunsaturated FAs, ω-6 to n-3 polyunsaturated FAs ratio in the 1.0% group compared to the control group. Feeding marine algae (Schizochytrium spp.) to laying hens in levels greater than 0.5% had a substantial effect on egg fatty acid content and ω-6 polyunsaturated fatty/ n-3 polyunsaturated FAs concentrations.

Kaewsutas et al. (2016) fed hens 1% or 2% microalgae (Schizochytrium spp) or 4% FO-containing diet, there were no appreciable variations in hen day egg production, EW, feed-intake albumin height, egg mass, or HU between the control and treatment groups. Although the 1% and 2% algae groups had a greater DHA content in their eggs than layers consumed in control and 4% FO added groups. The n-3 to n-6 fatty acid ratios were dropped by adding 2% Schizochytrium spp, while the DHA level was raised. Dietary management of layers can increase the amount of DHA in eggs by feeding algae-supplemented diets.

Cheng *et al.* (2004) found that layers fed a diet included 0+20g butter/kg, 5g DHA + 15g butter/kg, or 20 g/kg of algae-derived DHA, showing that dietary DHA oil did not affect day egg number, egg weight, yolk weight and the amounts of triacylglycerol and cholesterol in the plasma. The DHA content in egg yolks and plasma rose considerably after DHA addition compared to control hens. Laying hens can give algal DHA oil to boost egg yolk DHA content and produce DHA-enriched eggs. According to these findings, dietary DHA oil can be absorbed into plasma and egg yolks.

Supplementation of 0.5%, 1.0%, or 0% microalgae powder to laying hens, there was no variation in egg production between 40 and 43 weeks. However, the microalgae powder-supplemented group’s serum triglyceride and total cholesterol were decreased according to the control group. In comparison to the control birds in weak 46, the egg yolk color was improved by 0.5% microalgae powder added group. Eggshell thickness rose in microalgae powder groups in the 46th week. Furthermore, the n-6/n-3 fatty acid concentration and unsaturated FA/ saturated fatty acid were decreased and yolk DHA levels were raised with microalgae powder (Park and Upadhaya, 2015).

Nia et al. (2021) noted that the percentage of broken eggs, egg weight, eggshell breaking strength, FCR, shell relative weight, daily feed intake, shell thickness, albumen height, HU, heterophil/ lymphocyte ratio, red, and white blood cells, platelets, and hemoglobin composition were not affected by 0.3%, 0.6%, and 0.9% *Spirulina platensis* inclusions. However, egg mass in the 0.6% algae-supplemented group was higher than control group. Furthermore, 0.6% and 0.9% algae/kg fed hens produced more eggs than those fed 0.3% algae. However, eggshell weight and egg-yolk color were improved due to increasing the dietary amounts of Spirulina. In conclusion, adding 0.6% Spirulina platensis algae to laying hens boosted egg mass and yolk color.

Abbas et al. (2022) found that the birds fed a diet supplemented with soybean or various amounts of microalgae (spirulina platensisis) (0%, 3%, 6%, 9%, and 12), the dietary inclusion of Spirulina platensisis enhanced hematological parameters, blood metabolites, egg quality and productive performance of the laying hens. In addition, findings demonstrated that 1% of *Spirulina platensisis* could replace 4.7% soybean meal component in the layer feed. Finally, adding *Spirulina platensisis* to layer diets in place of some soybean meal appears to be a viable nutritional strategy for raising laying hens with the best possible performance.

Wen et al. (2019) found that brown laying hens 37-wks-age were fed (0%, 1%, 2%, 4%, and 8%) of Microalgae (Nannochloropsis sp.) containing diets for 4 weeks. The nannochloropsis addition had no impact on egg quality and performance parameters except egg yolk color. The egg yolk color score raised with Nannochloropsis intake and peaked around day seven. With rising levels of Nannochloropsis in the diet, total ω-6 polyunsaturated FAs, and n-6/n-3 ratio dropped, while total n-3 polyunsaturated FAs level in egg yolk increased. The highest concentration of total n-3 polyunsaturated FAs and DHA were found in the 8% Nannochloropsis group. On day 13 of the Nannochloropsis treatments, the maximum DHA, total n-3 polyunsaturated FAs, very long-chain n-3 and polyunsaturated FAs were determined. Finally, dietary supplementation with Nannochloropsis enriched yolks with n-3 polyunsaturated FAs particularly DHA and improved yolk color without adverse influence on laying hen performance or egg qualities.

Zahroojian et al. (2013) revealed that Hy-line W36 laying hens given 4 different microalgae (Spirulina) supplementations in their diets (0%, 1.5%, 2.0%, and 2.5%), the addition of spirulina did not affect daily feed consumed, FCR, egg production, egg weight, HU, specific gravity, yolk-index, shell-thickness, shell-weight, or yolk cholesterol. However, egg yolk color in the spirulina added groups was increased. Using 2.0 and 2.5% of spirulina in laying hens' diet may generate visually attractive yolk color.

Keegan et al. (2019) revealed that laying hens given various amounts of DHA-rich Aurantiochytrium limacinum (0.25, 0.5, and 1%), to produce n-3 enriched eggs, the findings showed that there were no appreciable variations in feed intakes, feed conversion, EP, EW, EM, and total egg numbers of the laying hens between the treatment groups. During four weeks, the DHA levels raised and the n-6 to n-3 concentrations dropped; thus enhancing the nutrient content of the eggs for human consumption.

Chen et al. (2014) demonstrated that brown laying hens given feed added with varying concentrations of Schizochytrium powder (0%, 1, 2, or 3%), the mean egg weight, feed conversion ratio, laying rate, egg-shape index, shell-strength, shell thickness, HU, albumen height and yolk EPA were not affected by dietary Schizochytrium powder supplementations. However, the yolk color of the 1% Schizochytrium powder treatment group was noticeably higher than all other groups. The 1% and 2% Schizochytrium powder groups' serum levels of cholesterol, triglycerides, and low-density lipoproteins were considerably reduced than those of the control group. As dietary Schizochytrium powder supplementation increased, the amount of n-3PUFAs and DHA in egg yolk enhanced linearly, and the n-6 / n-3 ratio decreased. On the other hand, when the supplemented amount of Schizochytrium powder exceeded 2%, the yolk DHA content did not grow further, and the DHA enrichment in eggs might reach saturation after 15 days. The addition of 2% Schizochytrium powder to hens' diets can raise the amount of DHA in their yolks without affecting their health or egg quality.

As can be seen from the above studies, fat sources and microalgae's may show some variability in performance and egg yield of laying hens and fatty acid content in eggs depending on the fat sources. In this study, the effects of adding different oil sources rich in n-3 to laying hen diets on performance, egg quality, and fatty acid composition were investigated.

CHAPTER 2

METHOD AND MATERIAL

2.1. Animals, experimental design and diets

The protocol for animal use was reviewed and approved Erciyes University Institutional Animal Care and Use Committee (protocol 03.05.2023, no 23/101). This investigation was performed at the Erciyes University Agricultural Research and Application Center (ERÜTAM).

2.2. Animal Grouping

In the experiment, a total of 60 Super Nick white 54-week-old egg-laying hens were used. Animals were allocated into 5 different treatment groups as shown Table 2.1.

Table 2.1. Animal grouping

Groups	Feed supplemented	Cages /animal	Total number of animals
Group 1	Control feed + SFO	12 cages/1 laying hens	12
Group 2	Control feed +FLO	12 cages/1 laying hens	12
Group 3	Control feed + HSO	12cages/1 laying hens	12
Group 4	Control feed + fish oil	12 cages/1 laying hens	12
Group 5	Control feed + MALG	12 cages/1 laying hens	12
Total			60

2.3. Animal care and feeding

The hens were housed in individual laying hen cages (L45xW32xH35 cm) with 4 floors and 3 cages in every cage. Laying hens were divided into five separate cage blocks (1*12) (sunflower seed, flaxseed, hempseed, fish oil, and microalgae). The partitions were placed between the feeders to prevent hens eating next cages feeds. The egg production of hens was recorded for 14 days before starting the experiment, and they hens were distributed according to their egg production and body weight to minimize differences between the groups. All birds were raised in a semi-controlled environmental poultry house. Before data collection diets were given for 10 days for adaptation. There were 7 data collection periods, and each was 14 days. The experiment lasted for 98 days (7 periods*14 days). Cages bases and divided by the number of animals and calculated individually. Cages furnished with one nipple drinker in each cage.

Figure 2.1. Animal experimental house

2.4. Ingredients and nutritional composition of basal diet

The experimental diets were produced isocaloric and isonitrogenic nutrient composition for laying hens and used in the experiment. The five ration were prepared with different oil types (sunflower, flaxseed, hempseed fish oil) and microalgae+sunflower oil. Feeds were ground with 6 mm mill and all feed components mixed homogenously in a machine (Kocamaz 500, İzmir, Turkiye) the experimental feed unit. The feed material and chemical components are provided in Table 2.2. Feeds were sealed with plastic caps and stored in a shaded area with the lids closed.

Table 2.2. Composition of experimental diets

	Groups/quantity kg/ton				
	Sunflower oil	Flaxseed oil	Hemp oil	Fish oil	Microalgae
Yellow corn	500.00	500.00	500.00	500.00	500.00
Wheat	33.97	33.97	33.97	33.97	30.00
Sunflower meal	120.00	120.00	120.00	120.00	120.00
Soybean meal 46	151.69	151.69	151.69	151.69	150.48
Vegetable oils	30.00	30.00	30.00	30.00	26.00
Salt, NaCl	3.19	3.19	3.19	3.19	3.20
Vitamin and mineral premix*	2.00	2.00	2.00	2.00	2.00
Di-calcium phosphate	4.80	4.80	4.80	4.80	4.30
Limestone	94.15	94.15	94.15	94.15	94.47
Methionine	0.55	0.55	0.55	0.55	0.55
Multi-enzyme	0.50	0.50	0.50	0.50	0.50
Wheat bran	59.15	59.15	59.15	59.15	58.5
Microalgae	0	0	0	0	15
Total	1000	1000	1000		1000
Analyzed Chemical composition					
Dry matter, %	92.02	92.26	92.41	92.16	92.61
Crude protein, %	17.56	17.45	17.18	17.86	17.88
Crude fat, %	5.37	5.38	5.38	5.38	5.38
ADF	1.59	1.59	1.59	1.59	1.89
NDF	4.14	4.14	4.14	4.14	4.42
Ash, %	14.35	14.41	14.65	14.05	14.38
Calculated Chemical composition**					
Methionine, %**	0.36	0.36	0.36	0.36	0.36
Lysine, %**	0.77	0.77	0.77	0.77	0.77
Calcium, %**	3.70	3.70	3.70	3.70	3.70
Available phosphorus, %**	0.36	0.36	0.36	0.36	0.36
Metabolic Energy Kcal ME/kg***	2730	2730	2730	2730	2730

§: Oil type was changed according to treatment as shown top of column. *Vitamin-mineral premix per kilogram of the diet, Vitamin D3 2000 IU; Vitamin A, 15.000 IU; Vitamin E, 40.0 mg; Vitamin B1 (thiamine), 3.0 mg; Vitamin K, 5.0 mg; Vitamin B6, 5.0 mg; Vitamin B12, 0.03 mg; Vitamin B2 (riboflavin), 6.0 mg; Biotin, 0.1 mg; Niacin, 30.0 mg; Folic acid, 1.0 mg, Calcium D-pantothenate, 12 mg; Choline chloride, 400 mg, Iron, 35.0 mg; Manganese, 80.0 mg; Zinc, 50.0 mg; Iodine 2.0 mg; Cobalt, 0.4 mg; Copper, 5.0 mg; Selenium, 0.15 mg assures. **Compositions are calculated according to NRC feedstuff tables (1994). ***ME values calculated according to TSE (1994).

2.5. Determination of live weight, feed consumption, and feed conversion ratio

The body weight of animals was determined at the beginning and end of the study individually with the help of a precise balance (±1 g sensitivity). Feed consumption of laying hens was determined for each cage at two-week intervals and calculated by the difference between given and removed feeds. The average daily feed consumption per animal was obtained by dividing the amount of feed consumed between two weighing by the number of days (14 days) of feeding and the number of animals belonging to that group. Every two weeks, FCR was calculated by dividing feed consumption, g: egg mass, g, and egg mass were calculated by this formula; egg mass: (egg yield (%) × mean egg weight) /100.

2.6. Evaluation of egg yield, egg weight, and internal quality traits

Egg production was recorded daily, and egg yields were calculated as both number and percentage (%) every 14 days. The egg yield percentage was calculated as follows: total number of eggs to be produced every 14 days of each bird / 14 days x 100. Egg mass per period was calculated as egg weight (g) × egg yield %.

To determine eggshell and internal quality a total 24 of eggs were collected in the last two days of each period (14^{th}, 28^{th}, 42^{nd}, 56^{th}, 70^{th}, $84^{th,}$ and 98^{th} days) and a total of 120 eggs were evaluated. Egg weights were determined with a precise scale (±0.1 g sensitivity). Internal quality traits (albumin height, HU, Roche color scale) were evaluated by the Egg Analyzer® (Orka Food Technology, USA). Color parameters of egg yolk (L*: brightness, b*: yellowness, and a*: redness) were evaluated with a Minolta CR-400 (Minolta Co, Japan) color meter every 14 days (Figure 2.2).

Figure 2.2. Determination of egg quality traits

2.7. Determination of eggshell properties

The shells of the broken eggs (g) were washed with tap water and dried at 75 ºC for 24 h in an oven. The dried eggshells were weighed on a precise scale (±0.1 g).

Eggshell (%) was assessed by dividing the shell weights: egg weight ×100.

Eggshell thickness (µm) was measured with a digital micrometer (±0.01 mm) from the sharp, blunt, and middle parts of the broken eggshells, and shell thickness value was determined by taking the arithmetic mean of these three measurements.

To calculate egg-shell surface area (SSA) the formula reported by Carter (1975) was used. According to formula Shell Surface Area = 3.9782 × egg weight^{7056} and shell weight of per unit area (USW, mg/cm2) was calculated by dividing the absolute shell weight: egg surface area.

2.8. Chemical analysis of feed samples

Feed's crude protein (CP), dry matter (DM), crude ash (CA), and crude oil (CO) analyses were performed according to AOAC (2013). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by Ankom 2000 automated fiber analyzer (Ankom Technology, USA).

2.8.1. Protein analysis of feeds

The amount of nitrogen (N) was determined by the Velp protein analyzer (Nitrogen analyzer NDA 702, Italy). This amount was multiplied by the coefficient of 6.25 and the crude protein (HP) ratio was calculated. Where samples were produced with an average weight of 0.400 mg and placed in a specific aluminum sheet before being placed in the device's assigned location.

2.8.2. Crude ash analysis of feeds

A dried and ground 1 g sample of feed was weighed and burned for 4 hours at 550 °C. After cooling, the samples were reweighted again, and the percentage of weight difference between the two weights was calculated to measure the amount of raw ash present in the ash furnace (Protherm, RLF, 120/6, Turkiye).

2.8.3. Dry matter analysis of feeds

The dry matter ratio was calculated by differencing the percentage change in the starting weight by the wet weight of the feed samples. They were reweighed after being dried at 70°C for 72 hours in an oven (Nüve, KD 400, Ankara, Turkiye) to stabilize their weight.

2.8.4. Crude oil analysis of feeds

To determine the crude oil of feed samples, 1g sample were used and put through a 1mm filter to extract the oil using petroleum ether. The amount of crude oil in the feed was calculated by calculating the percentage of the difference after the feeds were dried at 105 °C and weighed again with the help of a Velp fat extractor (SER-148, Italy) (Figure 2.3).

Figure 2.3. Determination of feed crude oil

2.9. Determination of the fatty acid composition of feed and egg yolk

The FA profiles of treatment diets and eggs were determined according to the Shimadzu application catalog. To determine FA composition periods 1, 2, 3, 5, and 7 were collected eggs from all treatment groups. In every period, 40 eggs from all groups were used (8 eggs for each group). Every 2 egg yolks from some groups were put in the bag, then cooked with boiling water, after cooling it was stored at −20°C until analysis. 1g of egg yolk was poured into the 15ml tube added 5ml hexane and shaken with a vortex for 3 min, then the transparent part was poured into the 5ml tube and added with 0.5ml µl KOH. The mixture was vigorously shaken with a vortex for 5 min and then 0.5ml of mixture was poured into the vial. The FA composition was analyzed by gas chromatograph (Shimadzu GC-2010 Plus, Japan) as described before Konca et al. (2019). The results were expressed as g FA/100 g total FAs. The rate of transfer of fatty acids from feed to the egg was calculated by proportioning the values of fatty acids in the egg to the values of fatty acids in the feed (according to the overall period).

Figure 2.4. Fatty acid Gas chromatography device

2.10. Determination of blood components

At the end of the experiment, blood samples (10 ml) were collected from the wing vein of randomly selected 8 laying hens from each group. Blood samples were centrifuged (5 min, 2500 rpm) and serum was separated and stored in Eppendorf tubes at -80 ° C until analysis day. The serums in day of analysis, thawed and serum glucose, total protein, triglyceride, cholesterol, alanine aspartate aminotransferase (AST), alanine aminotransferase (ALT) was analyzed by auto-analyzer by using commercial kits.

2.11. Statistical analyses

The study was designed and carried out in a completely randomized design and s data were analyzed according to the plan by using One-way Anova procedure in the SPSS statistics program Version 25. Duncan tests were used to identify differences between averages. The significance level was accepted $P < 0.05$. The data was presented as Means ± Standard errors in Tables.

CHAPTER 3

RESULTS

In this study, the effects of different n-3-rich oil sources additions on laying hen diets on performance, egg quality, and fatty acid composition were investigated and presented below.

3.1. Fatty acid composition of feeds

Effects of n-3 fatty acid oil sources on FAs composition in the feed are shown in Table 3.1. There was a strong difference between groups in FAs composition of different oil-added feed groups in terms of palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1n9c), linoleic acid (C18:2n6c), alpha-linolenic acid (C18:3n3), arachidonic acid (C20:4n6), EPA (C20:5n3) and DHA (C22:6n3) ($P<0.01$). The palmitic acid ratio on the feed was higher in the FO and MALG groups than in other groups. However, the palmitic acid composition on feed between the control, FLO, and HSO groups was not significantly different. The palmitoleic acid amount on the feed was highest in the FO group than the other groups, while the control group had the lowest palmitoleic acid; however, there were no significant variations between the FLO, HSO, and MALG group's palmitoleic acid ratio on feed. The oleic acid composition on feed in the control group was higher than all other groups and the lowest oleic acid ratio was found in the HSO group. The linoleic acid (LA) concentration on feed was high in control, HSO, and MALG groups compared to FLO and FO groups, and the lowest LA ratio was observed in the FLO group. The a-linolenic acid (ALA) amount on feed in FLO was increased, while the control diet had the lowest ALA ratio. However, there were no significant variations between the FO and MALG group's a-linolenic acid composition on diet. According to all other groups, the arachidonic acid content of the diet was high in the FO-added feed, and the lowest arachidonic acid was found in the FLO-added feed. Fish oil (FO) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) composition were higher than the other feeds. However, the control, FLO, HSO, and MALG group's EPA and DHA ratios on feed were not significantly different ($P<0.01$).

Table 3.1. Effects of omega-3 fatty acid oil sources on fatty acid (FAs) composition in feed of laying hens

	Oil sources					
Fatty Acids	SFO	FLO	HSO	FO	MALG	P
(C14:0) Myristic acid	0.08±0.00	0.46±0.33	0.52±0.44	1.18±0.01	0.69±0.32	0.236
(C16:0) Palmitic acid	8.82±0.12[b]	9.31±0.87[b]	10.33±0.79[b]	14.02±0.10[a]	13.52±0.63[a]	0.005**
(C16:1) Palmitoleic acid	0.11±0.01[c]	0.30±0.06[bc]	0.30±0.08[bc]	1.92±0.00[a]	0.51±0.08[b]	0.001**
(C18:0) Stearic acid	3.09±0.02	4.69±0.68	4.29±0.95	3.23±0.02	4.40±0.41	0.276
(C18:1n9c) Oleic acid	34.79±0.02[a]	23.39±0.25[d]	22.34±0.33[e]	31.44±0.09[b]	28.19±0.28[c]	0.001**
(C18:2n6c) Linoleic acid	50.80±0.10[a]	26.64±1.28[c]	50.09±1.55[a]	40.41±0.34[b]	48.90±1.00[a]	0.001**
(C18:3n3) a-Linolenic acid	1.81±0.03[d]	34.98±0.38[a]	11.85±0.36[b]	3.48±0.18[c]	3.42±0.02[c]	0.001**
(C20:4n6) Arachidonic acid	0.43±0.02[b]	0.10±0.01[e]	0.18±0.02[d]	0.70±0.01[a]	0.25±0.02[c]	0.001**
(C20:5n3) Eicosapentaenoic acid	0.02±0.02[b]	0.02±0.02[b]	0.03±0.01[b]	1.17±0.03[a]	0.04±0.02[b]	0.001**
(C22:6n3) Docosahexaenoic acid	0.08±0.01[b]	0.15±0.02 [b]	0.09±0.02[b]	2.48±0.16 [a]	0.11±0.02[b]	0.001**

[a,b,c]: Values with different superscripts in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

3.2. Effects of omega-3 fatty acid sources on body weight (BW), feed intake (FI), and feed conversion ratio (FCR)

Effects of n-3 fatty acid-rich oil sources in feed on body (BW) of laying hens are shown in Table 3.2. Different n-3-rich FA sources did not affect final body weight, body weight gain, and change percentage of hens (P>0.05). Figure 3.1. Shows body weight change% of groups in overall period.

Table 3.2. Effect of omega-3 fatty acid rich oil sources in feed on body weight (BW) of laying hens

Oil sources	Initial weight	Final weight	BWG	Change, %
SFO	1619.00±34.86	1660.75±36.65	41.75+9.91	2.59±0.63
FLO	1548.83±31.50	1578.50±31.13	29.67+7.12	1.94±0.46
HSO	1639.50±27.67	1684.75±23.68	45.25+10.90	2.84±0.69
FO	1605.00±28.54	1665.00±29.14	60.00+22.06	3.85±1.46
MALG	1620.67±33.49	1656.92±33.70	36.25+10.01	2.26±0.63
P	0.315	0.157	0.557	0.573

BWG: bodyweight gain, SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

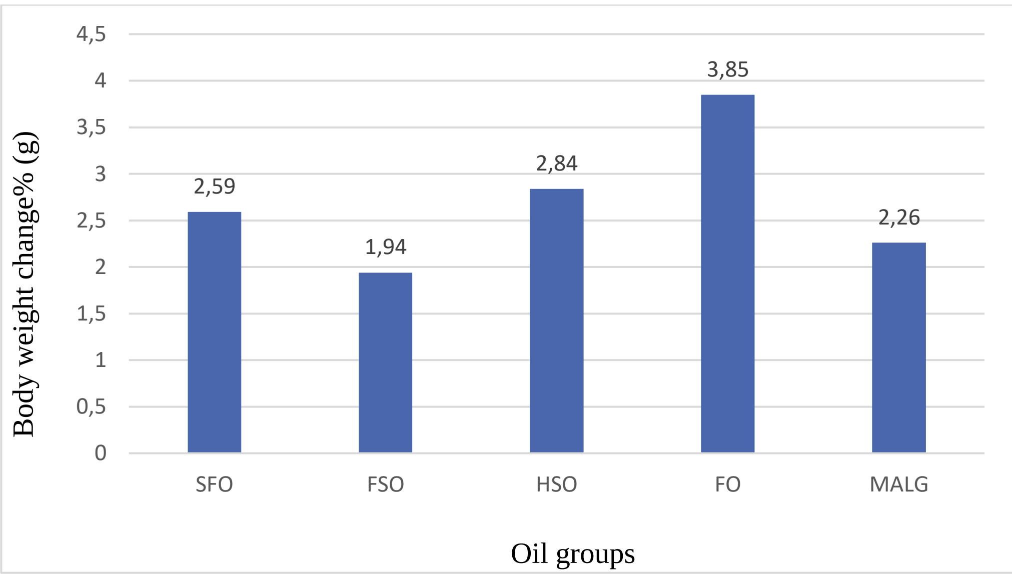

Figure 3.1. Effect of omega-3 fatty acid oil sources in feed on body weight change% of laying hens

Effects of different oil sources addition on feed intake (FI) of laying hens are given in Table 3.3. Different n-3-rich FAs addition did not affect FI of hens in most of periods and overall periods except period 5. The SFO and MALG group's feed intake in the 5th period was higher than those of all groups, and the FO addition caused a decrease in FI of hens compared to other groups (P<0.01). Figure 3.2. Shows feed intake in the overall period.

Table 3.3. Effects of n-3 rich oil sources on average daily feed intake (FI) of hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	116.99±2.19	115.77±1.62	114.09±2.77	115.66±2.34	118.96±1.42	0.578
2	114.13±2.23	113.98±2.44	108.74±2.19	108.52±1.80	110.38±2.63	0.227
3	113.10±2.45	116.16±1.65	116.23±2.13	114.14±2.03	114.47±2.05	0.790
4	115.56±1.86	117.89±1.07	119.56±0.56	118.60±0.95	116.52±1.79	0.240
5	118.53±0.39^{a}	112.26±1.47^{b}	112.01±1.78^{b}	107.79±2.21^{c}	118.10±0.46^{a}	0.001**
6	114.26±1.88	112.00±1.50	116.27±1.34	116.71±0.86	117.27±1.03	0.053
7	114.17±1.90	117.21±0.58	111.51±2.89	118.43±1.27	114.46±1.50	0.071
Overall	115.19±1.10	115.07±0.67	113.93±0.98	114.08±0.64	115.60±1.12	0.647

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

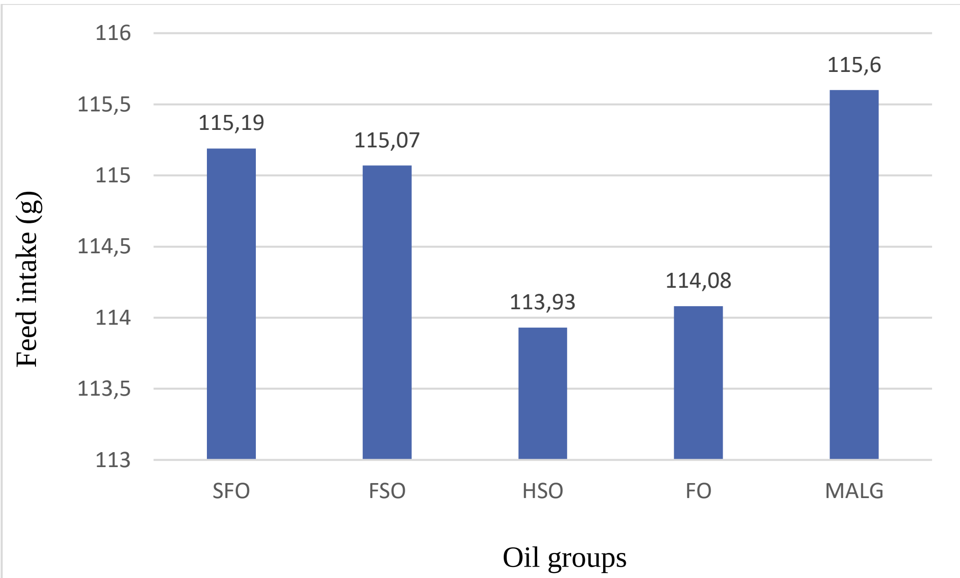

Figure 3.2. Effects of omega-3 fatty acid oil sources on feed intake in the overall period

Effects of omega-3-rich oil sources on feed conversion ratio (FCR) are given in Table 3.4. There were no significant differences among the groups in terms of FCR in all periods, and overall period, except period 5. In the control group, FCR values were higher than those of all groups; the MALG group's FCR value was higher than the FO-added group, however, it was similar to FLO and HSO groups in the 5th period ($P<0.01$). In the 5th period, the fish oil-added group's FCR value was lower than all the groups. Figure 3.3. Shows conversion ratio in the overall period.

Table 3.4. Effects of omega-3 rich oil sources on feed conversion ratio (FCR) of hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	1.81±0.04	1.81±0.05	1.83±0.06	1.77±0.06	1.85±0.05	0.842
2	1.74±0.04	1.78±0.05	1.71±0.08	1.66±0.03	1.72±0.04	0.585
3	1.72±0.04	1.80±0.05	1.75±0.03	1.72±0.04	1.80±0.05	0.516
4	1.88±0.07	1.88±0.04	1.96±0.09	1.85±0.04	1.85±0.06	0.758
5	1.87 ± 0.05^{a}	1.72 ± 0.03^{bc}	1.71 ± 0.04^{bc}	1.61 ± 0.04^{c}	1.76 ± 0.03^{b}	0.001**
6	1.81±0.04	1.75±0.02	1.82±0.08	1.84±0.06	1.85±0.03	0.647
7	1.87±0.03	1.87±0.03	1.75±0.05	1.84±0.06	1.76±0.05	0.218
Overall	1.81±0.03	1.80±0.02	1.79±0.04	1.75±0.03	1.80±0.03	0.713

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:$p<0.01$. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

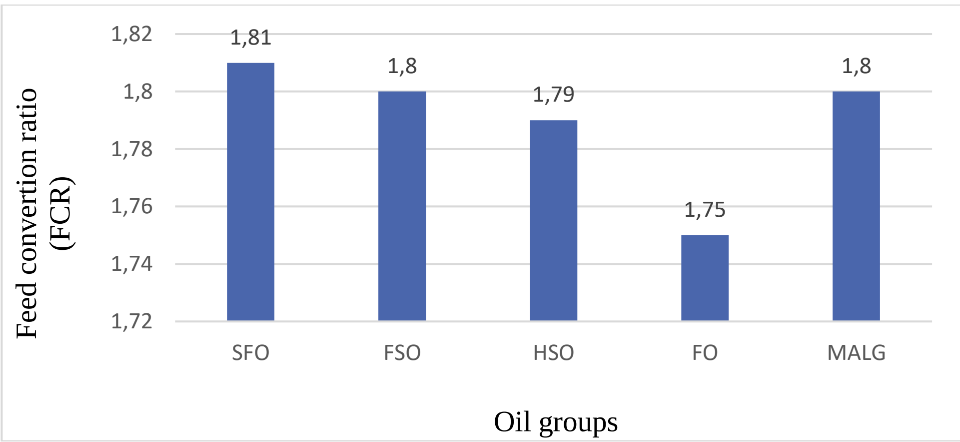

Figure 3.3. Effects of omega-3 fatty acids oil sources on Feed conversion ratio (FCR) in overall period

3.3. Effects of omega-3 fatty acid rich oil sources on egg production and egg yield (%)

Effects of n-3 fatty acid-rich oil sources on egg production number (EP) are given in Table 3.5 and egg yield in Table 3.6. There was no significant difference between the groups in terms of EP and EY% of groups except period 7. In the MALG and FO groups, EP and EY% values were higher than those of all other groups; however, there were no significant differences between the control, FLO, and HSO groups in the 7th period ($P<0.01$). Figure 3.4, and 3.5. Show EP and EY% values, respectively.

Table 3.5. Effects of n-3 fatty acid oil sources on egg production number (EP) of hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	13.67±0.19	13.92±0.08	13.58±0.23	13.75±0.13	13.92±0.08	0.450
2	13.83±0.11	13.67±0.22	13.42±0.26	13.58±0.15	13.67±0.14	0.621
3	13.92±0.08	13.58±0.19	13.92±0.08	13.92±0.08	13.67±0.19	0.242
4	13.33±0.33	13.58±0.19	13.50±0.34	13.83±0.11	13.50±0.26	0.746
5	13.75±0.13	13.83±0.11	13.75±0.13	13.92±0.08	13.83±0.17	0.878
6	13.42±0.29	13.75±0.18	13.42±0.34	13.42±0.26	13.83±0.11	0.596
7	$13.08±0.23^{b}$	$13.58±0.19^{ab}$	$13.50±0.19^{ab}$	$13.67±0.19^{a}$	$13.92±0.08^{a}$	0.039*
Overall	13.61±0.11	13.71±0.10	13.58±0.12	13.73±0.08	13.77±0.05	0.582

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

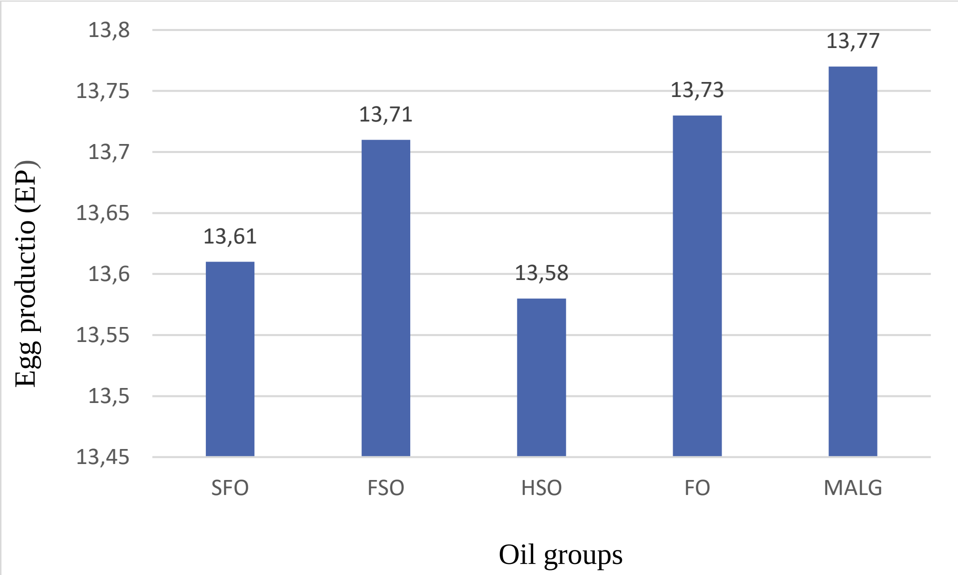

Figure 3.4. Effects of omega-3 fatty acid oil sources on egg production (EP) in overall period

Table 3.6. Effect of omega-3 fatty acid oil sources on egg yield ratio (EY %) of hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	97.62±1.34	99.41±0.60	97.02±1.64	98.22±0.93	99.41±0.60	0.450
2	98.81±0.80	97.62±1.61	95.83±1.86	97.03±1.06	97.62±1.01	0.621
3	99.41±0.60	97.02±1.38	99.41±0.60	99.41±0.60	97.62±1.34	0.242
4	95.24±2.38	97.02±1.38	96.43±2.41	98.81±0.80	96.43±1.87	0.746
5	98.22±0.93	98.81±0.80	98.22±0.93	99.41±0.60	98.81±1.19	0.878
6	95.83±2.05	98.21±1.28	95.83±2.40	95.83±1.86	98.81±0.80	0.596
7	93.45±1.64[b]	97.02±1.38[ab]	96.43±1.39[ab]	97.62±1.34[a]	99.41±0.60[a]	0.039*
Overall	97.15±0.76	97.89±0.73	97.07±0.83	98.07±0.57	98.29±0.36	0.615

[a,b,c]: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

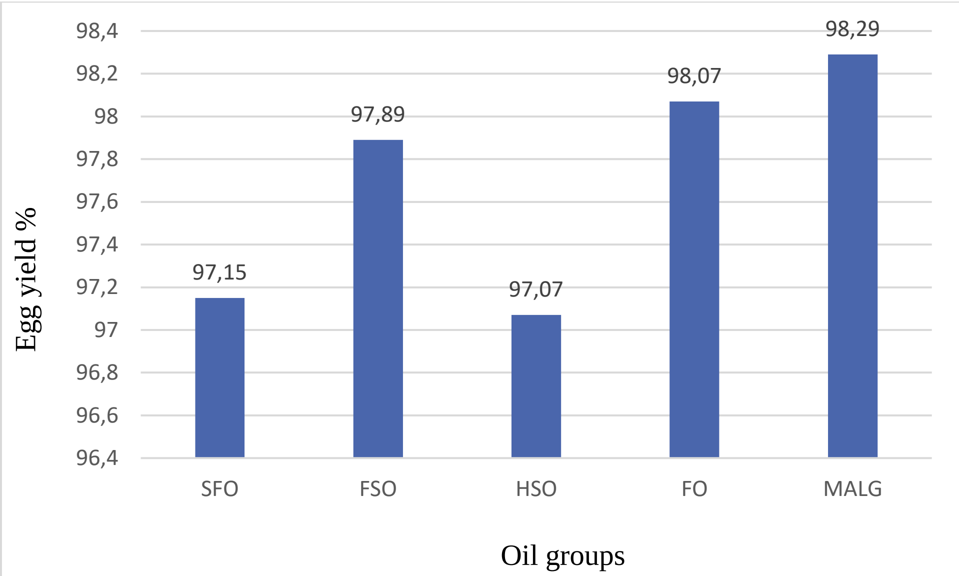

Figure 3.5. Effects of omega-3 fatty acid oil sources on egg yield percentage (EY %) in overall period

3.4. Effects of omega-3 fatty acid rich oil sources on egg external quality traits

The effect of n-3 FA-rich oil sources on egg weight (g) of laying hens is given in Table 3.7 and egg mass data is presented in Table 8. There were no statistically significant differences between the treatment groups in terms of egg weight and egg mass in all periods (P>0.05). Figures 3.6, and 3.7. Show egg weight and egg mass values of groups in the overall period, respectively.

Table 3.7. Effect of omega-3 FA oil sources on egg weight (EW) of laying hens, g

		Oil sources				
Periods	SFO	FLO	HSO	FO	MALG	P
1	66.40±1.03	64.65±0.83	64.70±0.91	67.18±1.16	64.94±1.02	0.263
2	66.50±0.87	66.05±0.86	67.35±1.08	67.73±0.77	65.81±0.92	0.507
3	66.12±0.88	66.99±0.68	66.96±0.95	66.82±0.64	65.62±0.93	0.703
4	65.18±0.89	65.03±0.66	64.43±0.99	65.15±0.80	65.78±0.88	0.863
5	64.93±1.15	66.08±0.85	66.95±0.69	67.48±0.73	68.16±0.77	0.078
6	66.11±0.76	65.38±0.72	67.55±0.93	66.62±0.68	64.33±0.89	0.060
7	65.71±0.83	64.97±0.80	66.22±0.78	66.43±0.99	65.90±1.04	0.811
Overall	65.91±0.76	65.62±0.62	66.31±0.76	66.83±0.68	65.76±0.82	0.777

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

Table 3.8. Effect of omega-3 fatty acid oil sources on egg mass (EM) of laying hens, g

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	64.78±1.53	64.26±1.19	62.77±1.57	66.02±1.87	64.57±1.52	0.690
2	65.68±1.22	64.44±1.39	64.57±1.90	65.68±1.04	64.27±1.47	0.918
3	65.68±1.04	65.04±1.46	66.60±1.48	66.42±0.90	64.03±1.45	0.619
4	62.12±2.06	63.08±1.25	62.26±2.32	64.41±1.35	63.43±1.68	0.891
5	63.80±1.63	65.22±0.83	65.73±1.02	67.07±1.05	67.31±1.11	0.202
6	63.32±1.60	64.17±0.90	64.90±2.37	63.88±1.59	63.56±1.31	0.966
7	61.33±1.18	62.93±0.82	63.94±1.70	64.92±1.82	65.54±1.63	0.290
Overall	64.01±1.14	64.22±0.75	64.43±1.35	65.55±1.11	64.63±1.20	0.889

[a,b,c]: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

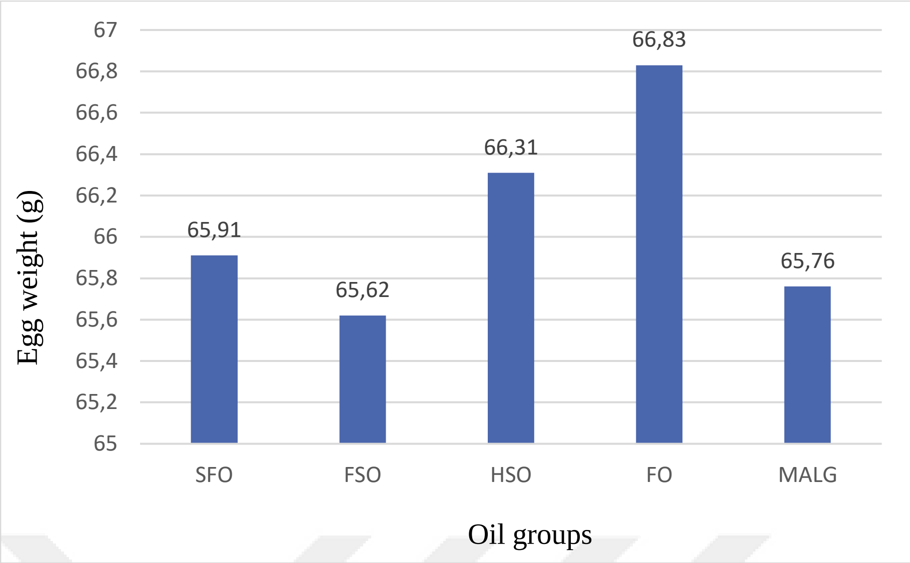

Figure 3.6. Effects of omega-3 fatty acid oil sources on egg weight in overall period

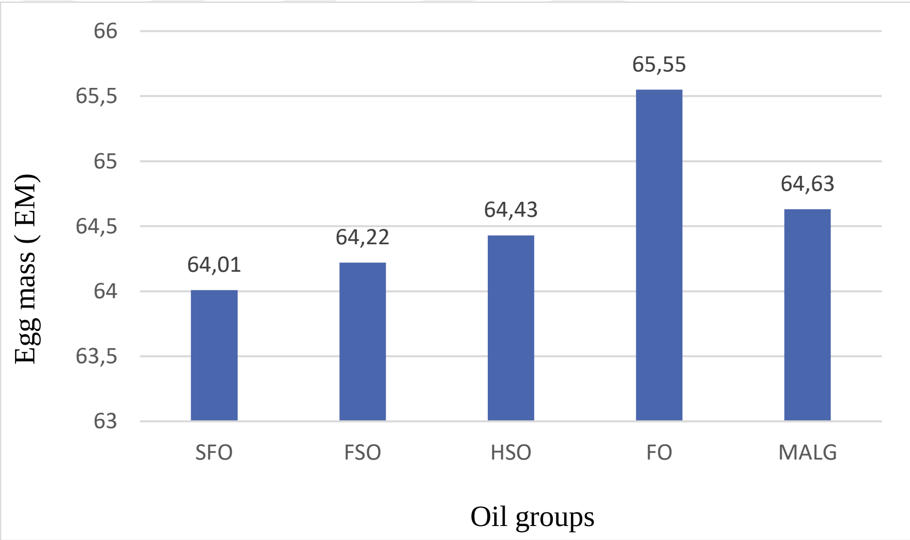

Figure 3.7. Effects of omega-3 fatty acid oil sources on egg mass (EM) in overall period

Effects of omega-3 FA-rich oil sources on Haugh unit (HU) are given in Table 3.9. Except period 3 and 4, the treatments had no significant effect on the HU values of laying hens. In period 3, the HU values of the MALG group were higher than those of other groups, and the FO group's HU values were higher than the HSO-supplemented group. However, there was no significant differences existed between the control, FLO, and HSO-added groups. During period 4, the HSO and MALG groups had higher HU values than the FO and FLO groups ($P < 0.01$). Figure 3.8. Show Haugh unit (HU) in the overall period.

Table 3.9. Effects of omega-3 fatty acid rich oil sources on egg Haugh unit (HU) of laying hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	59.90±3.16	65.13±2.83	64.78±3.22	70.14±1.38	64.26±3.40	0.184
2	55.06±4.22	59.45±3.76	58.68±4.02	59.88±2.65	62.98±2.28	0.616
3	59.11±3.47^{bc}	58.91±3.64^{bc}	55.53±3.82^{c}	66.11±2.44^{ab}	68.70±1.09^{a}	0.015*
4	61.09±3.66^{ab}	56.79±3.84^{b}	67.26±1.01^{a}	58.36±3.36^{b}	67.80±1.36^{a}	0.020*
5	66.62±1.43	58.81±3.01	58.69±1.67	62.31±3.25	61.17±2.79	0.170
6	67.42±1.61	63.40±2.84	61.13±2.73	63.13±2.77	65.44±1.56	0.400
7	66.05±2.05	63.78±1.88	61.75±1.93	62.08±1.87	64.02±2.09	0.545
Overall	61.66±1.51	60.94±1.64	60.94±1.30	63.43±0.87	64.96±1.17	0.140

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01.SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

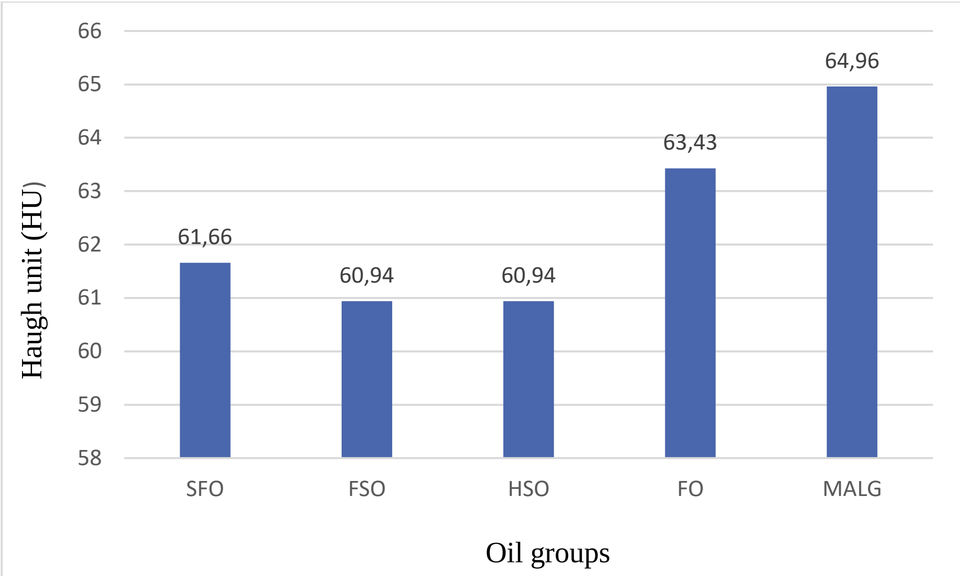

Figure 3. 8. Effects of omega-3 fatty acid oil sources on Haugh unit (HU) in the overall period

The effect of omega-3 fatty acid oil sources on eggshell weight (ESW) is given in Table 3.10. There was no significant difference in ESW between the groups except periods 1 and 4. In the first period, the MALG and FO group's ESW values were greater than the HSO group. However, no significant differences were observed between the control, FLO, MALG, and FO-supplemented groups. In period 4, the control, FLO, FO, and MALG groups had greater ESW values than the HSO group ($P<0.01$), however, no significant differences existed between the control, FLO, MALG, and FO groups. Figure 3.9. Show eggshell weight in overall period.

Table 3.10. Effect of omega-3 fatty acid oil sources on eggshell weight (ESW) of hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	6.15±0.13^{ab}	6.14±0.08^{ab}	5.81±0.17^{b}	6.36±0.10^{a}	6.18±0.09^{a}	0.025*
2	6.31±0.10	6.23±0.07	6.11±0.18	6.34±0.09	6.25±0.07	0.651
3	6.26±0.13	6.34±0.07	6.16±0.16	6.40±0.05	6.38±0.11	0.547
4	6.01±0.10^{a}	6.07±0.09^{a}	5.63±0.16^{b}	6.06±0.10^{a}	6.18±0.08^{a}	0.010*
5	6.00±0.09	6.16±0.07	6.09±0.16	6.36±0.10	6.35±0.08	0.078
6	6.16±0.08	6.24±0.08	6.20±0.18	6.31±0.09	6.23±0.09	0.908
7	5.95±0.09	5.78±0.09	5.64±0.19	6.07±0.11	6.00±0.11	0.094
Overall	6.14±0.08	6.14±0.06	5.97±0.16	6.28±0.07	6.23±0.07	0.201

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

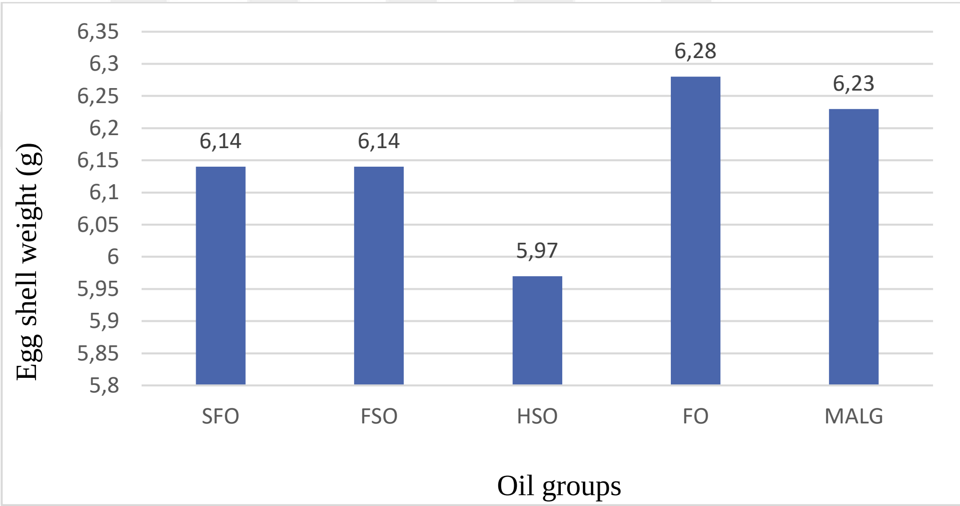

Figure 3.9. Effects of omega-3 fatty acid oil sources on eggshell weight (ESW) in overall period.

Effect of omega-3 fatty acid oil sources on the eggshell ratio (ESR %) is given in Table 3.11. There was no significant difference between the groups except periods 3, 4, 7, and in overall period in terms of ESR%. In the period 1, 4, 7 and overall period MALG, FO, and FLO groups had greater ESR% values than the HSO group. However, there were no significant differences between the control, FLO, FO, and MALG-supplemented groups in ESR% (P<0.01) values in these periods. Figure 3.10. Show eggshell ratio in overall period.

Table 3.11. Effect of omega-3 fatty acid oil sources on eggshell ratio (ESR %) of hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	9.26±0.13[ab]	9.52±0.08[a]	8.98±0.21[b]	9.50±0.09[a]	9.53±0.07[a]	0.010 *
2	9.50±0.12	9.45±0.11	9.05±0.20	9.38±0.12	9.50±0.09	0.097
3	9.48±0.15	9.47±0.09	9.19±0.20	9.60±0.09	9.72±0.09	0.072
4	9.24±0.15[a]	9.34±0.14[a]	8.77±0.23[b]	9.31±0.16[a]	9.41±0.07[a]	0.042*
5	9.32±0.25	9.34±0.08	9.08±0.21	9.43±0.11	9.35±0.12	0.667
6	9.33±0.12	9.56±0.12	9.17±0.23	9.48±0.14	9.68±0.09	0.133
7	9.06±0.11[a]	8.90±0.11[ab]	8.52±0.26[b]	9.15±0.15[a]	9.10±0.08[a]	0.028*
Overall	9.32±0.11[a]	9.37±0.07[a]	8.98±0.21[b]	9.42±0.09[a]	9.48±0.05[a]	0.029*

[a,b,c]: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

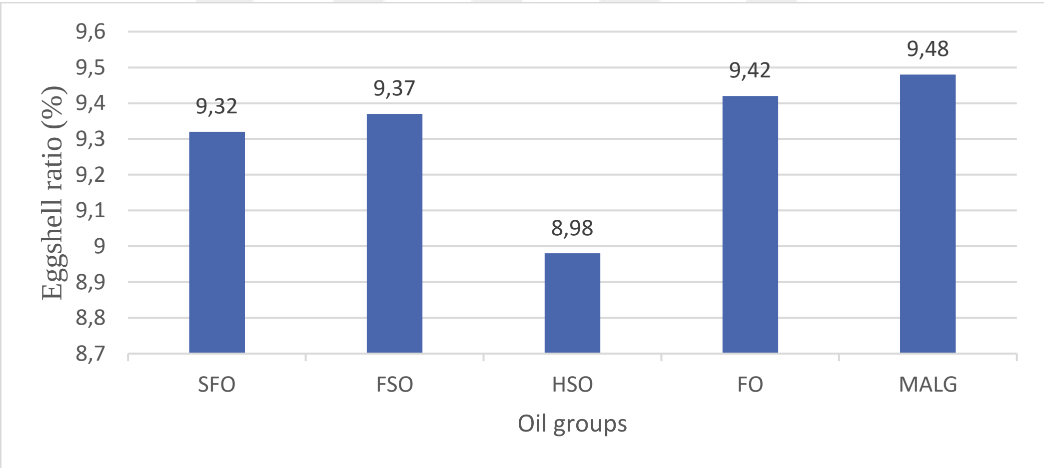

Figure 3.10. Effect of omega-3 fatty acid oil sources on the eggshell ratio (ESR %) in overall period

The effect of omega-3 fatty acid oil sources on eggshell thickness (EST) is given in Table 3.12. There was a significant difference between treatment groups in periods 1, 2, 4, 5, 6, and 7. In periods 1 and 7 the MALG, FO, FLO, and control groups had greater EST values than the HSO-supplemented group. In period 2, the EST values of the control group were greater than those of the HSO and MALG groups, and the FO group was higher than the HSO-supplemented group. However, no significant differences exist between the control, FLO, and FO-added groups. In period 4, the FO group had higher EST values than the control, HSO, and FLO groups. However, no significant differences exist among the control, FLO, and HSO groups. In period 5, the MALG and FO groups had higher EST values than the HSO and FLO groups. In period 6, the FO group had higher EST values than the control, HSO, and MALG groups. Figure 3.11. Show eggshell thickness in the overall period.

Table 3.12. Effect of omega-3 fatty acid oil sources on eggshell thickness (EST) of laying hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	0.34±0.00^{a}	0.33±0.00^{a}	0.32±0.01^{b}	0.34±0.00^{a}	0.34±0.00^{a}	0.001**
2	0.36±0.00^{a}	0.35±0.01^{abc}	0.34±0.01^{c}	0.36±0.00^{ab}	0.35±0.00^{bc}	0.014*
3	0.37±0.05	0.34±0.00	0.32±0.01	0.33±0.00	0.33±0.00	0.428
4	0.33±0.00^{bc}	0.33±0.00^{bc}	0.32±0.01^{c}	0.35±0.00^{a}	0.34±0.00^{ab}	0.001**
5	0.34±0.00^{ab}	0.33±0.00^{b}	0.33±0.01^{b}	0.34±0.00^{a}	0.34±0.00^{a}	0.002**
6	0.33±0.00^{b}	0.34±0.00^{ab}	0.34±0.01^{b}	0.35±0.00^{a}	0.34±0.00^{b}	0.035*
7	0.35±0.00^{a}	0.34±0.00^{a}	0.33±0.01^{b}	0.35±0.00^{a}	0.35±0.00^{a}	0.004**
Overall	0.35±0.01	0.34±0.00	0.33±0.01	0.35±0.00	0.34±0.00	0.068

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

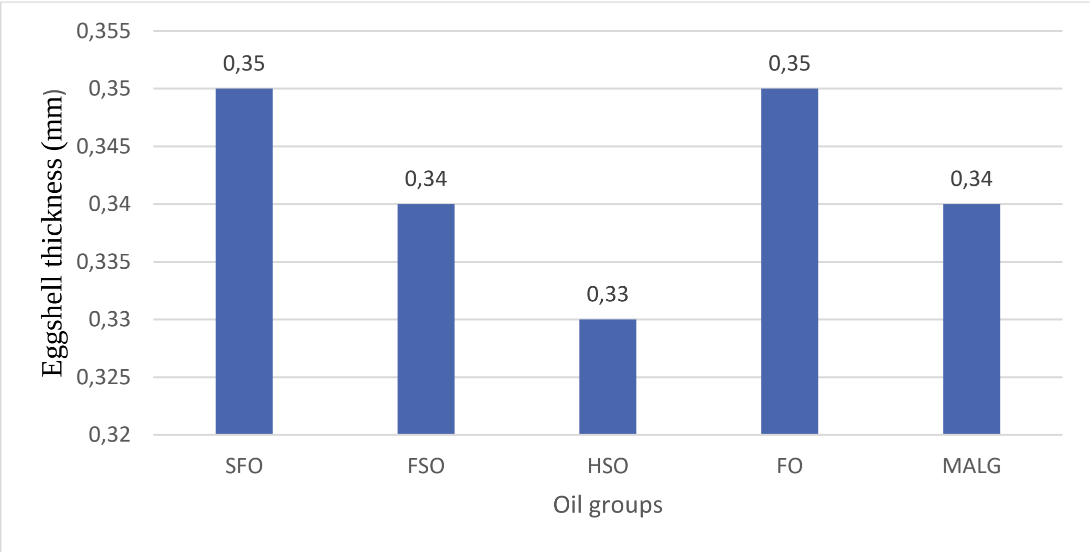

Figure 3.11. Effect of omega-3 fatty acid oil sources on eggshell thickness (EST) in overall period

Effect of omega-3 fatty acid oil sources on eggshell surface area (ESA) is given in Table 3.13. There was no significant difference period in terms of ESA between treatment groups in all periods and the overall (P>0.05). Figure 3.12. Show eggshell surface area in overall period.

Table 3.13. Effect of omega-3 fatty acid oil sources on eggshell surface area (ESA) of laying hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	76.77±0.84	75.33±0.68	75.38±0.75	77.38±0.94	75.57±0.83	0.269
2	76.85±0.71	76.49±0.70	77.53±0.88	77.87±0.62	76.29±0.75	0.501
3	76.55±0.72	77.27±0.55	77.23±0.77	77.14±0.52	76.13±0.76	0.688
4	75.77±0.72	75.67±0.54	75.13±0.81	75.75±0.66	76.27±0.72	0.853
5	75.55±0.98	76.51±0.69	77.23±0.56	77.67±0.59	78.22±0.63	0.076
6	76.55±0.62	75.96±0.59	77.69±0.75	76.98±0.55	75.07±0.73	0.060
7	76.23±0.68	75.62±0.65	76.63±0.64	76.80±0.80	76.35±0.85	0.816
Overall	76.37±0.62	76.14±0.51	76.70±0.62	77.13±0.55	76.24±0.67	0.772

[a,b,c]: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

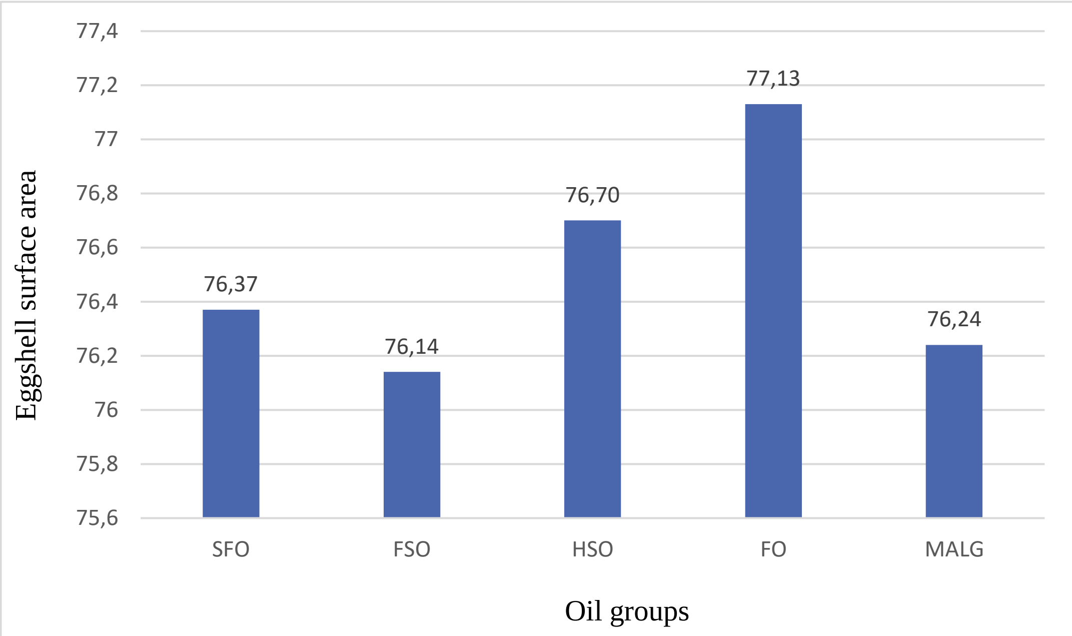

Figure 3.12. Effect of omega-3 fatty acid oil sources on eggshell surface area (ESA) in overall period

The effect of omega-3 fatty acid oil sources on eggshell per unit (ES/U) is given in Table 3.14. There was no significant difference in ES/U between the treatment groups except periods 1, 4, 7, and overall. In the 1st, 4th, 7th, and overall periods, the HSO group's ES/U values were higher than the control, FLO, FO, and MALG groups. However, no significant differences exist between the control, FLO, FO, and MALG-added groups (P<0.01). Figure 3.13. Show eggshell per unit in overall period.

Table 3.14. Effect of omega-3 fatty acid rich oil sources on eggshell per unit (ES/U) of laying hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	12.55±0.18^{b}	12.29±0.10^{b}	13.21±0.38^{a}	12.18±0.10^{b}	12.24±0.09^{b}	0.002**
2	12.23±0.1	12.31±0.13	12.93±0.38	12.33±0.15	12.23±0.09	0.095
3	12.32±0.22	12.22±0.11	12.73±0.35	12.07±0.09	11.98±0.13	0.091
4	12.68±0.20^{b}	12.51±0.16^{b}	13.61±0.45^{a}	12.58±0.23^{b}	12.35±0.10^{b}	0.006**
5	12.65±0.24	12.44±0.10	12.91±0.38	12.27±0.15	12.35±0.15	0.269
6	12.46±0.16	12.21±0.14	12.78±0.39	12.25±0.17	12.09±0.12	0.188
7	12.85±0.15^{b}	13.13±0.16^{b}	14.02±0.60^{a}	12.71±0.20^{b}	12.78±0.12^{b}	0.018*
Overall	12.51±0.14^{b}	12.43±0.09^{b}	13.14±0.38^{a}	12.33±0.11^{b}	12.26±0.07^{b}	0.015*

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

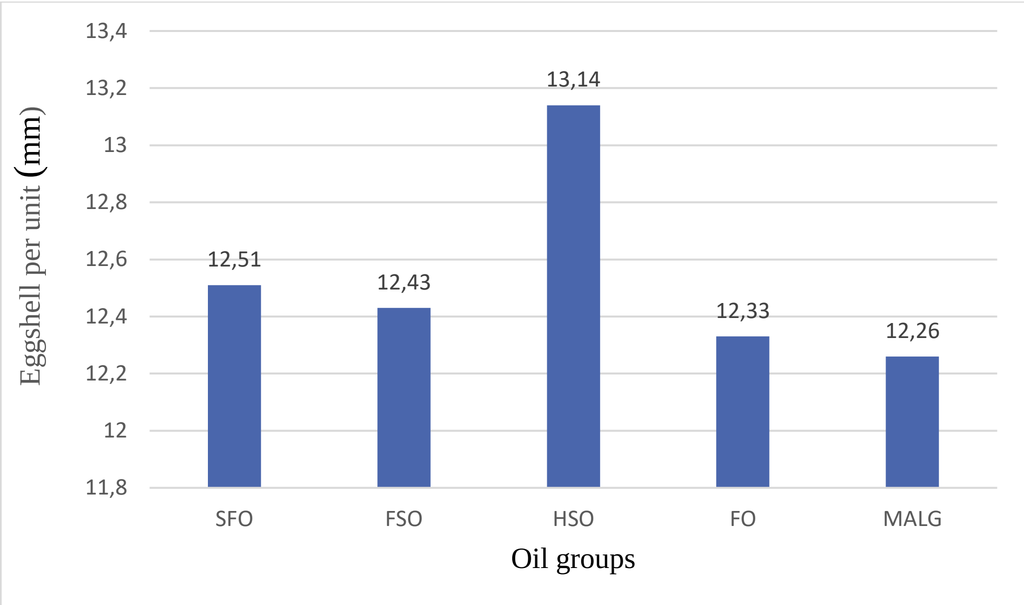

Figure 3.13. Effect of omega-3 fatty acid oil sources on eggshell per unit (ES/U) in overall period

3.5. Effects of omega-3 fatty acid rich oil sources on egg internal traits

Effect of omega-3 fatty acid oil sources on egg albumen height (Hmm) is given in Table 3.15. There was no significant difference in Hmm between the treatment groups all periods and the overall period except periods 3 and 4. In the third period, the MALG and FO groups had higher HMM values than the HSO group, but no significant differences existed between the control, FLO, and FO-added groups. In period 4, the HSO and MALG groups had higher HMM values than the FO and FLO groups. However, there were no significant differences between the control, MALG, and HSO groups ($P<0.01$). Figure 3.14. show albumen height in overall period.

Table 3.15. Effect of omega-3 fatty acid oil sources on egg albumen height (HMM) of laying hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	4.61±0.26	5.02±0.25	5.03±0.26	5.55±0.14	4.98±0.24	0.090
2	4.32±0.31	4.68±0.27	4.69±0.33	4.60±0.18	4.79±0.20	0.769
3	4.55±0.26^{bc}	4.68±0.26^{abc}	4.30±0.27^{c}	5.17±0.21^{ab}	5.28±0.12^{a}	0.016*
4	4.73±0.23^{ab}	4.35±0.29^{b}	5.12±0.12^{a}	4.48±0.25^{b}	5.20±0.14^{a}	0.020*
5	4.96±0.16	4.41±0.23	4.38±0.17	4.88±0.23	4.75±0.23	0.159
6	5.22±0.17	4.87±0.24	4.72±0.19	4.88±0.19	4.92±0.15	0.458
7	5.09±0.19	4.81±0.19	4.64±0.19	4.68±0.15	4.92±0.17	0.392
Overall	4.75±0.15	4.71±0.14	4.69±0.11	4.91±0.07	4.98±0.11	0.332

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01.SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

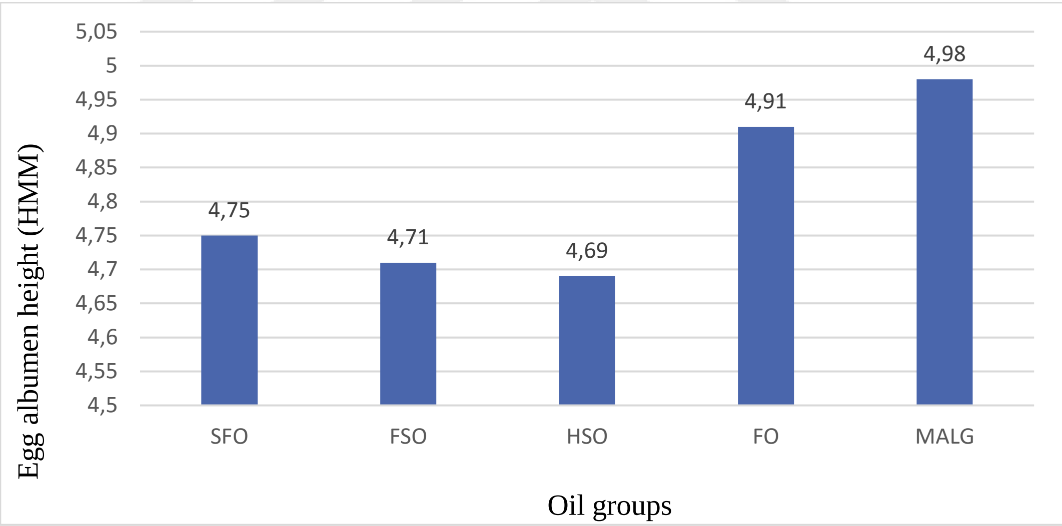

Figure 3.14. Effect of omega-3 fatty acid oil sources on egg albumen height (HMM) in overall period

Effect of omega-3 fatty acid oil sources on Roche color scale values (RRS) is given in table 3.16. There was strong significant in RRS different between groups in period 5, 6, 7 and Overall. In period 5, HSO, FLO, and FO groups had greater RRS values than the control and MALG groups. In the sixth and overall periods, the RRS values of the control, FLO, HSO, and FO groups were greater than those of the MALG group. However, there is no significant difference between the control, FLO, FO, and HSO groups. In the seventh period, the RRS values of the control and FO groups were higher than those of the HSO and MALG groups. However, no significant differences existed between the control, FLO, and FO groups (P<0.01). Figure 3.15. Show Roche color scale values in overall period.

Table 3.16. Effect of omega-3 fatty acid oil sources on Roche color scale (RRS) values of laying hens

	Oil sources					
Periods	SFO	FLO	HSO	FO	MALG	P
1	9.08±0.12	8.67±0.25	8.42±0.32	8.83±0.08	8.88±0.11	0.186
2	8.96±0.07	8.83±0.26	9.42±0.13	9.38±0.12	9.08±0.22	0.078
3	8.92±0.20	9.17±0.13	9.17±0.14	9.25±0.16	9.08±0.20	0.693
4	9.54±0.13	9.17±0.12	9.46±0.12	9.29±0.11	9.25±0.09	0.136
5	9.00±0.21^{b}	10.17±0.16^{a}	10.42±0.12^{a}	10.25±0.14^{a}	8.75±0.28^{b}	0.001**
6	10.58±0.13^{a}	10.38±0.10^{a}	10.50±0.10^{a}	10.46±0.13^{a}	8.58±0.25^{b}	0.001**
7	10.63±0.15^{a}	10.42±0.10^{ab}	10.13±0.26^{b}	10.83±0.12^{a}	8.79±0.12^{c}	0.001**
Overall	9.46±0.06^{a}	9.47±0.07^{a}	9.57±0.10^{a}	9.68±0.05^{a}	8.93±0.07^{b}	0.001**

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

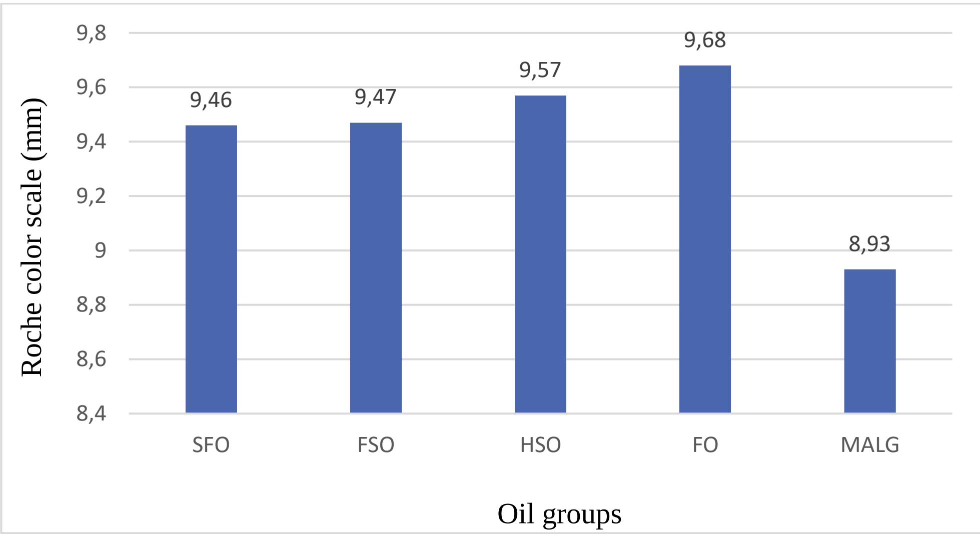

Figure 3.15. Effect of omega-3 fatty acid oil sources on Roche color scale (RRS) in overall period

The effect of omega-3 fatty acid oil sources on egg yolk brightness (L*) is given in Table 3.17. There was a strong significant difference in L* between groups in period 2, 3, 5, 6, 7, and Overall . In the 2nd period, the control group's L* values were higher than those of all other groups. In the 3rd, 5th, and overall periods control group's L* values were higher than the FO, HSO, and FLO groups and there were no significant differences between the control and MALG-supplemented groups. In period 6 the MALG group's L* values were higher than those of FO, HSO, And FLO groups. However, there are no significant differences between the control and MALG groups. In the 7th period, the control group's L* values were higher than those of the HSO and FO groups. However, there are no significant differences between the control, FLO, and MALG groups (P<0.01). Figure 3.16. show egg yolk brightness values in overall period.

Table 3.17. Effect of omega-3 fatty acid oil sources on egg yolk brightness (L*) of laying hens

Periods	Oil sources					P
	SFO	FLO	HSO	FO	MALG	
1	54.70±0.39	53.38±0.35	53.26±0.44	53.59±0.38	53.68±0.43	0.096
2	54.86±0.32^{a}	53.59±0.29^{b}	53.38±0.62^{b}	52.94±0.33^{b}	53.56±0.40^{b}	0.019*
3	55.88±0.37^{a}	54.40±0.35^{b}	54.44±0.41^{b}	54.30±0.46^{b}	55.13±0.48^{ab}	0.040*
4	55.62±0.32	55.38±0.32	55.52±0.42	55.86±0.48	55.70±0.30	0.920
5	56.50±0.40^{a}	55.19±0.27^{b}	53.88±0.38^{c}	54.80±0.31^{bc}	55.91±0.49^{ab}	0.001**
6	55.94±0.39^{ab}	55.15±0.45^{bc}	54.63±0.38^{c}	54.63±0.36^{b}	56.42±0.42^{a}	0.005**
7	56.18±0.43^{a}	55.15±0.44^{ab}	54.42±0.53^{b}	54.82±0.42^{b}	56.39±0.38^{a}	0.006**
Overall	55.60±0.24^{a}	54.50±0.22^{bc}	54.15±0.33^{c}	54.32±0.23^{c}	55.11±0.26^{ab}	0.001**

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

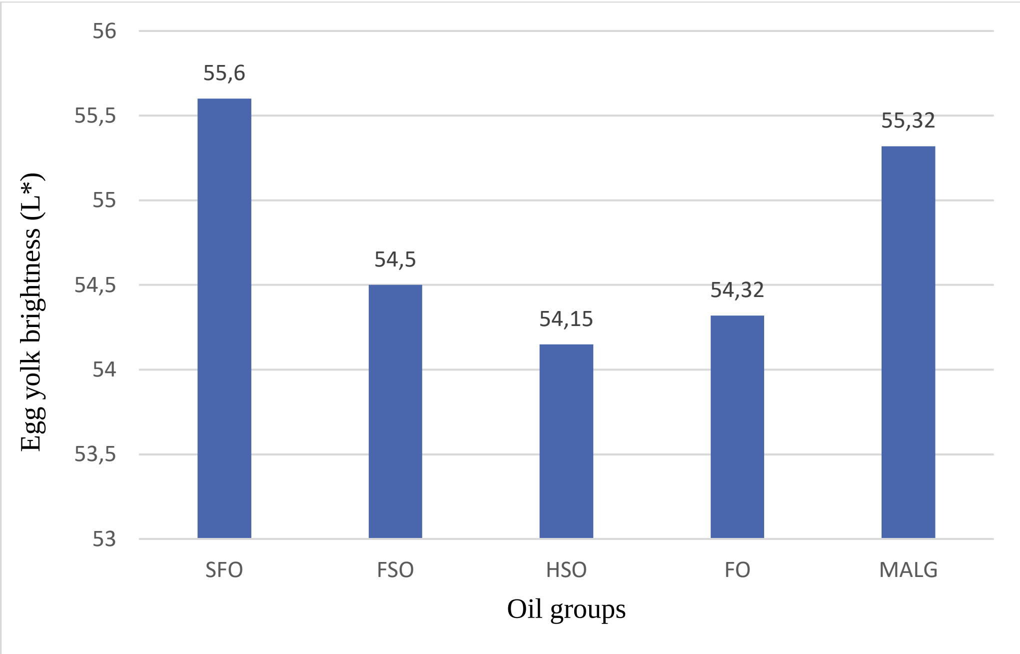

Table 3.16. Effect of omega-3 fatty acid oil sources on egg yolk brightness (L*) in overall period

Effect of omega-3 fatty acid oil sources on egg yolk redness (a*) is given in Table 3.18. There was a significant difference in a* between groups in periods 1, 4, 5, 6, 7, and Overall. During the first period, the control group had greater a* values than the HSO, FO, and MALG groups. However, there are no significant differences between control and FLO. In the fourth period, the HSO group had higher a* values than the MALG and FLO groups, but there were no significant differences between the control, HSO, and FO groups. In period 5, the HSO and FO groups had greater a* values than the control, MALG, and FLO groups. During the sixth period, the control group had higher a* values than all other groups, and there were no significant differences between the FLO, HSO, and FO groups. In the seventh period control, the FO group had greater a* values than the HSO, MALG, and FLO groups. In the Overall, the control, FLO, HSO, and FO group's a* values were higher than the MALG group. However, there are no significant differences between the control, FLO, HSO, and FO groups (P<0.01). Figure 3.17. Show egg yolk redness values in the overall period.

Table 3.18. Effect of omega-3 fatty acid oil sources on egg yolk redness (a*) of laying hens

Periods	Oil sources					P
	SFO	FLO	HSO	FO	MALG	
1	1.68±0.21^{a}	1.52±0.12^{ab}	1.02±0.19^{c}	0.91±0.10^{c}	1.17±0.08^{cb}	0.001**
2	1.38±0.07	1.36±0.07	1.57±0.13	1.64±0.10	1.45±0.09	0.173
3	1.17±0.12	0.95±0.14	1.00±0.11	1.04±0.10	1.28±0.10	0.250
4	1.23±0.14^{abc}	1.04±0.14^{bc}	1.62±0.12^{a}	1.47±0.18^{ab}	0.82±0.15^{c}	0.002**
5	1.05±0.15^{c}	2.89±0.16^{b}	3.94±0.17^{a}	3.79±0.18^{a}	1.04±0.14^{c}	0.001**
6	5.21±0.24^{a}	4.41±0.19^{b}	3.96±0.11^{b}	4.36±0.22^{b}	0.86±0.12^{c}	0.001**
7	4.67±0.23^{a}	4.13±0.14^{b}	4.13±0.18^{b}	4.87±0.14^{a}	0.96±0.13^{c}	0.001**
Overall	2.22±0.10^{a}	2.19±0.08^{a}	2.30±0.10^{a}	2.41±0.08^{a}	1.11±0.06^{b}	0.001**

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

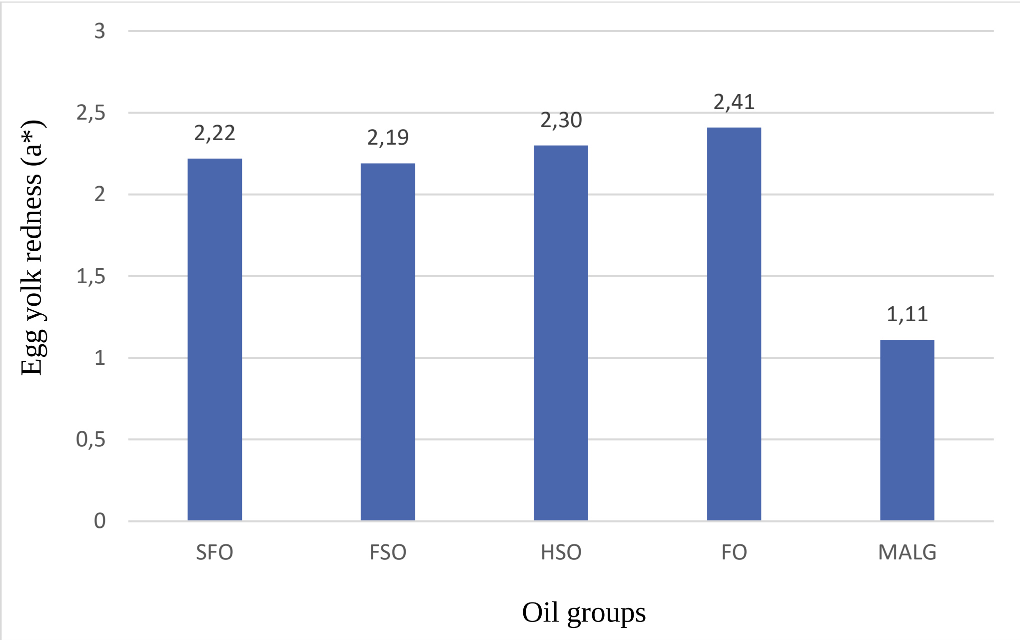

Figure 3.17. Effect of omega-3 fatty acid oil sources on egg yolk redness (a*) in overall period

The effect of omega-3 fatty acid oil sources on egg yolk yellowness (b*) is given in Table 3.19. There was a significant difference in b* between groups in 1,2,3,4, 5, and 7 periods and the overall period. In the 1st period, the control group's b* values were higher than those of the FLO and FO groups, but there are no significant differences between control, MALG, and HSO. In period 2, the control and HSO group's b* values were higher than the MALG and FLO groups, but there were no significant differences between the control, HSO, and FO groups. In period 3 the HSO group's b* values were higher than those of the control group, but there were no significant differences between the control, HSO, FLO, MALG, and FO groups. In the 4th period, the HSO and FO group's b* values were higher than those of all other groups. During the 5th period, the control, FO, and HSO group's b* values were higher than the MALG group. However, there were no significant differences between the control, FLO, HSO, and FO groups. In the 7th period, the control group's b* values were higher than those of the FO and MALG groups and there were no significant differences between the control, FLO, and HSO groups. In the overall period, the control and HSO groups' b* values were higher than the FLO and MALG groups. However, there were no significant differences between the control, HSO, and FO groups in b* value (P<0.01). Figure 3.18. Show egg yolk yellowness values in the overall period.

Table 3.19. Effect of omega-3 fatty acid oil sources on egg yolk yellowness (b*) of laying hens

Periods	Oil sources					P
	SFO	FLO	HSO	FO	MALG	
1	38.03±0.71^{a}	35.06±0.48^{b}	36.69±0.77^{ab}	35.28±0.44^{b}	36.41±0.52^{ab}	0.004**
2	37.33±0.64^{a}	35.18±0.53^{b}	37.24±0.58^{a}	36.09±0.46^{ab}	35.52±0.64^{b}	0.024*
3	35.78±0.56^{b}	36.26±0.67^{ab}	37.94±0.46^{a}	36.14±0.60^{ab}	37.53±0.69^{ab}	0.048*
4	38.34±0.65^{b}	38.33±0.50^{b}	40.32±0.60^{a}	40.91±0.76^{a}	37.29±0.56^{b}	0.001**
5	38.55±0.79^{a}	37.69±0.54^{ab}	38.98±0.57^{a}	39.05±0.58^{a}	36.49±0.52^{b}	0.018*
6	39.93±0.85	40.45±0.62	38.12±0.76	38.77±0.72	39.51±0.71	0.184
7	40.52±0.62^{a}	39.26±0.62^{ab}	39.13±0.48^{ab}	38.33±0.69^{b}	37.57±0.53^{b}	0.010*
Overall	38.19+0.39^{a}	37.21+0.32^{b}	38.21+0.27^{a}	37.55+0.31^{ab}	37.10+0.33^{b}	0.039*

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

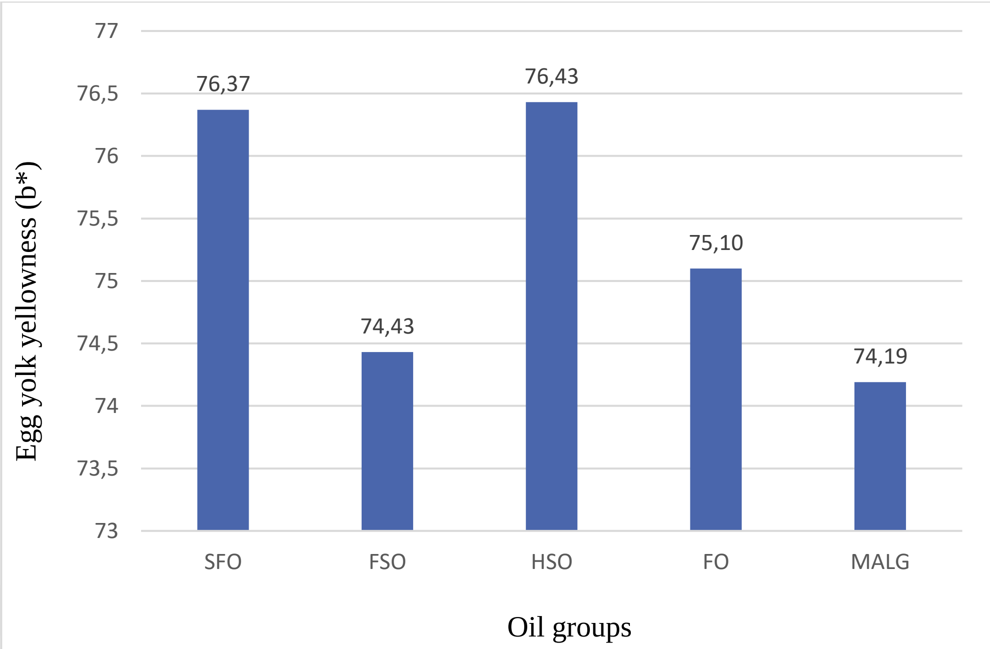

Figure 3.18. Effect of omega-3 fatty acid oil sources on egg yolk yellowness (b*) in overall period

3.6. Effects of omega-3 fatty acid-rich oil sources on egg yolk fatty acid compositions

The effect of omega-3 fatty acid oil sources on the fatty acids (FAs) composition of egg yolk on day 14 is shown in Table 3.20. There was no significant difference in yolk fatty acid concentration between the groups, except (C16:0) palmitic acid, (C18:3n3) a-Linolenic acid, and (C22:6n3) docosahexaenoic acid during period 1. The control groups' Palmitic acid composition was higher than the FLO, HSO, and MALG groups. However, there was no significant difference between FO, FLO, HSO, and MALG group's palmitic acid. FLO groups' a-linolenic acid ratio in egg yolk was increased than all other groups. However, there were no significant variations between the control, HSO, FO, and MALG group's ALA amounts. The DHA ratio in yolk was raised in the FLO, FO, and MALG groups than in the control group. However, there were no significant variations in terms of DHA in control and HSO groups ($P<0.01$).

Table 3.20. Effects of omega-3 fatty acid oil sources on fatty acids (FAs) composition of egg yolk on d 14

	Oil sources					
Fatty acids, %	SFO	FLO	HSO	FO	MALG	P
(C14:0) Myristic acid	3.30±0.95	1.30±0.58	0.30±0.21	1.30±0.32	0.30±0.26	0.056
(C16:0) Palmitic acid	29.30±1.10^{a}	25.30±0.92^{b}	26.30±0.25^{b}	27.30±0.33^{ab}	26.30±0.47^{b}	0.015*
(C16:1) Palmitoleic acid	2.30±0.11	2.30±0.06	2.30±0.24	2.30±0.22	2.30±0.13	0.075
(C18:0) Stearic acid	10.30±2.98	11.30±1.55	9.30±0.85	9.30±1.26	10.30±1.00	0.984
(C18:1n9c) Oleic acid	34.30±5.85	40.30±1.38	40.30±0.60	43.30±1.13	42.30±0.41	0.212
(C18:2n6c) Linoleic acid	17.30±6.08	11.30±1.36	17.30±0.35	13.30±0.86	15.30±1.27	0.656
(C18:3n3) a-Linolenic acid	1.30±1.22^{b}	5.30±0.53^{a}	1.30±0.05^{b}	0.30±0.03^{b}	0.30±0.26^{b}	0.001**
(C20:4n6) Arachidonic acid	0.30±0.11	0.30±0.06	0.30±0.04	0.30±0.06	0.30±0.07	0.055
(C20:5n3) Eicosapentaenoic acid	0.30±0.13	0.30±0.09	0.30±0.01	0.30±0.03	0.30±0.04	0.057
(C22:6n3) Docosahexaenoic acid	0.30±0.12^{b}	0.30±0.08^{a}	0.30±0.05^{ab}	0.30±0.06^{a}	0.30±0.12^{a}	0.026*

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

Effect of omega-3 fatty acid oil sources on the fatty acids (FAs) composition of egg yolk on day 28 are given in Table 3.21. There was a significant difference between groups in terms of (C16:0) palmitic acid, (c18:1n9c) oleic acid, (c18:2n6c) linoleic acid, c18:3n3) a-linolenic acid and (c20:5n3) eicosapentaenoic acid in period 2. The palmitic acid concentration of the FLO group was decreased than all other groups; however, there was no significant difference between the control, HSO, FO, and MALG groups. The oleic acid ratio in egg yolks from HSO groups was decreased than those of all other groups; however, there were no significant variations between the Control, FLO, FO, and MALG groups. The linoleic acid composition of yolks from the HSO and MALG groups was higher than those of control, FLO, and FO groups. However, there was no significant difference between the control, FLO, and FO group's linoleic acid ratio in yolk. The a-linolenic acid (ALA) amount in egg yolk was increased in the FLO group compared to all other groups. The lowest ALA ratio was found in the control, FO, and MALG groups; however, there were no variations between these groups in terms of ALA. The eicosapentaenoic acid (EPA) ratio of the control group was higher compared to all other groups, while the lowest EPA was found in the FLO, HSO, and FO groups. The docosahexaenoic acid composition of HSO, FO, and MALG groups was increased than those of control and FLO, while the control group had the lowest EPA ($P<0.01$).

Table 3.21. Effect of omega-3 fatty acid oil sources on fatty acids (FAs) composition of egg yolk on d 28

Fatty acids, %	Oil sources					P
	SFO	FLO	HSO	FO	MALG	
(C14:0) Myristic acid	1.65±0.46	0.58±0.13	1.65±0.50	1.37±0.47	0.67±0.11	0.155
(C16:0) Palmitic acid	26.94±0.36[a]	23.26±0.58[b]	26.26±0.43[a]	26.32±0.68[a]	25.56±0.29[a]	0.001**
(C16:1) Palmitoleic acid	2.47±0.16	2.55±0.09	2.21±0.09	2.19±0.32	1.84±0.10	0.086
(C18:0) Stearic acid	10.70±0.89	8.41±0.33	9.60±1.01	8.73±0.70	8.76±0.26	0.198
(C18:1n9c) Oleic acid	42.06±0.72[a]	43.45±1.24[a]	39.10±0.82[b]	44.70±1.41[a]	43.56±0.27[a]	0.010*
(C18:2n6c) Linoleic acid	14.27±1.14[c]	14.16±0.73[c]	17.92±1.21[a]	14.62±0.85[c]	17.26±0.30[bc]	0.022*
(C18:3n3) a-Linolenic acid	0.72±0.18[c]	6.22±0.49[a]	1.94±0.14[b]	0.58±0.03[c]	0.52±0.20[c]	0.001**
(C20:4n6) Arachidonic acid	0.79±0.16	0.53±0.02	0.75±0.08	0.68±0.03	0.82±0.01	0.150
(C20:5n3) Eicosapentaenoic acid	0.20±0.03[a]	0.04±0.01[c]	0.06±0.01[c]	0.05±0.01[c]	0.12±0.04[b]	0.001**
(C22:6n3) Docosahexaenoic acid	0.23±0.06[c]	0.81±0.04[a]	0.53±0.07[b]	0.78±0.06[a]	0.90±0.02[a]	0.001**

[a,b,c]: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

Effect of omega-3 fatty acid oil sources on the fatty acids (FAs) composition of egg yolk on day 56 is given in Table 3.22. There was a significant difference between groups in terms of (C14:0) myristic acid, (C18:0) stearic acid, (C20:4n6) arachidonic acid and (C22:6n3) docosahexaenoic acid during period 3. The myristic acid content of the MALG group was higher than all other groups, while the HSO myristic acid amount increased than the FLO group. However, there was no significant difference in myristic acid between the HSO, control, and MALG groups. During the third period, the FLO, HSO, and MALG group's stearic acid ratio were increased than the FO group; however, there was no significant difference between the control and FO groups in stearic acid composition in their egg yolks. The Arachidonic acid content of the control group was higher than all other treatment groups; however, there was no significant difference between the FLO, HSO, FO, and MALG group's arachidonic acid concentrations in their yolks. The Docosahexaenoic acid amount of the FO group was increased than the control, FLO, and MALG groups. However, there was no significant difference between the FO and HSO group's DHA ratio on egg yolk. Although the DHA amount was the lowest in the control and MALG groups, there was no significant difference between the control, MALG, and FLO groups ($P<0.01$). Effects of omega-3 fatty acid oil sources on fatty acids (FAs) composition on egg yolk on day 84 are given in Table 3.23. There was no significant difference between groups in terms of FAs composition in egg yolk except (C18:0) Stearic acid, (C18:2n6c) linoleic acid, (C18:3n3) a-Linolenic acid and (C22:6n3) Docosahexaenoic acid. During period 5, the Stearic acid content of FO and MALG groups was increased than those of the control and FLO groups; however, there was no significant difference between FO, MALG, and HSO group's stearic acid ratio on yolk. The control and FLO group's stearic acid amounts were the lowest. The linoleic acid concentration of the control, FO, and MALG groups was higher than those of the HSO group; however, there was no significant difference between the control, FLO, FO, and MALG groups, linoleic acid composition. The a-linolenic acid (ALA) content of HSO has increased more than that of all other groups, while the control, FLO, and MALG group's ALA concentrations were the lowest. The docosahexaenoic acid (DHA) ratio of the control group was decreased than all other treatment groups, however, there was no significant difference between the FLO, HSO, FO, and MALG group's DHA compositions on yolk ($P<0.01$).

Table 3.22. Effect of omega-3 fatty acid oil sources on fatty acids (FAs) composition of egg yolk on d 56

	Oil sources					
Fatty acids, %	SFO	FLO	HSO	FO	MALG	P
(C14:0) Myristic acid	0.45±0.05^{bc}	0.38±0.06^{c}	0.53±0.04^{b}	0.45±0.03^{bc}	0.68±0.04^{a}	0.004**
(C16:0) Palmitic acid	26.05±0.36	24.48±0.36	24.38±0.09	25.72±0.48	25.39±1.36	0.360
(C16:1) Palmitoleic acid	2.81±0.16	2.64±0.05	3.03±0.07	2.61±0.62	2.59±0.19	0.824
(C18:0) Stearic acid	8.27±0.22^{ab}	9.04±0.17^{a}	8.90±0.13^{a}	6.62±1.23^{b}	9.36±0.47^{a}	0.043*
(C18:1n9c) Oleic acid	44.45±0.57	43.69±0.63	42.95±0.23	35.81±9.38	47.67±1.49	0.417
(C18:2n6c) Linoleic acid	16.15±0.48	16.10±0.60	14.36±0.15	22.98±7.85	12.87±0.84	0.354
(C18:3n3) a-Linolenic acid	0.47±0.04	2.55±0.39	4.67±0.26	4.18±3.59	0.43±0.03	0.246
(C20:4n6) Arachidonic acid	0.94±0.03^{a}	0.70±0.06^{b}	0.58±0.01^{b}	0.67±0.02^{b}	0.66±0.05^{b}	0.001**
(C20:5n3) Eicosapentaenoic acid	0.17±0.03	0.05±0.05	0.02±0.01	0.23±0.16	0.07±0.02	0.291
(C22:6n3) Docosahexaenoic acid	0.26±0.04^{c}	0.39±0.03^{bc}	0.60±0.06^{ab}	0.74±0.05^{a}	0.32±0.12^{c}	0.001**

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

Table 3.23. Effect of omega-3 fatty acid oil sources on fatty acids (FAs) composition of egg yolk in period 84

	Oil sources					
Fatty acids, %	SFO	FLO	HSO	FO	MALG	P
(C14:0) Myristic acid	0.46±0.03	0.51±0.06	0.38±0.05	0.42±0.19	0.58±0.03	0.614
(C16:0) Palmitic acid	27.53±0.66	26.7±1.18	24.485±0.14	25.86±1.22	26.44±0.36	0.170
(C16:1) Palmitoleic acid	3.24±0.29	3.48±0.20	3.35±0.11	2.87±0.23	2.68±0.12	0.063
(C18:0) Stearic acid	7.51±0.10^{b}	8.10±0.21^{b}	8.42±0.17^{ab}	9.12±0.47^{a}	9.07±0.34^{a}	0.006**
(C18:1n9c) Oleic acid	42.64±0.69	41.90±0.78	41.52±0.72	40.91±0.87	43.08±0.51	0.273
(C18:2n6c) Linoleic acid	17.39±0.89^{a}	15.65±0.49^{ab}	14.11±0.31^{b}	16.66±0.93^{a}	16.32±0.27^{a}	0.027*
(C18:3n3) a-Linolenic acid	0.38±0.03^{c}	1.39±0.48b^{c}	6.66±0.46^{a}	3.10±1.33^{b}	0.60±0.10^{c}	0.001**
(C20:4n6) Arachidonic acid	0.73±0.04	0.63±0.03	0.45±0.08	0.62±0.14	0.71±0.02	0.115
(C20:5n3) Eicosapentaenoic acid	0.07±0.04	0.07±0.01	-0.03±0.04	0.02±0.03	0.05±0.02	0.153
(C22:6n3) Docosahexaenoic acid	0.07±0.05^{b}	0.49±0.08^{a}	0.66±0.05^{a}	0.43±0.10^{a}	0.49±0.08^{a}	0.001**

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

Effects of omega-3 fatty acid oil sources on fatty acids (FAs0 composition of egg yolk on day 14 are given in Table 3.24. There was a significant difference between groups in C16:0) palmitic acid, (C16:1) palmitoleic acid, (C18:1n9c) oleic acid, (C18:2n6c) linoleic acid, (C18:3n3) a-linolenic acid and (C22:6n3) docosahexaenoic acid compositions on egg yolk. The palmitic acid ratio of the FO group was increased than FLO and HSO groups; however, there was no significant between the control, FO, and MALG groups' palmitic acid amounts. The FLO groups' palmitic acid concentration decreased more than all other groups. Although the palmitoleic acid content of the control, HSO, FO, and MALG groups did not show significant differences, the palmitoleic acid ratio of FLO increased compared with that of the control, HSO, and MALG groups. The oleic acid ratio of HSO decreased compared to those of the Control, FLO, and MALG groups. However, there was no significant difference between the control, FLO, FO and MALG group's oleic acid composition on yolk. The linoleic acid (AL) amount of HSO group was increased than those of FLO and FO groups; however, the HSO, control, and MALG group's linoleic acid ration was no significantly different. While the lowest AL found in the FLO group. The a-linolenic acid (ALA) concentration of the FLO group was higher than those of all other groups, while the lowest ALA group was the control group; however, there was no significant difference between FO and MALG group's ALA contents in yolk. The docosahexaenoic acid (DHA) amounts of the FO group was higher compared to all other groups (except the MALG group), while the lowest DHA level was found in the control group. However, there was no significant difference between the FLO, HSO, and MALG group's DHA composition on egg yolk ($P<0.01$).

Table 3.24. Effect of omega-3 fatty acid oil sources on fatty acids (FAs) composition of egg yolk on d 98

	Oil sources					
Fatty acids, %	SFO	FLO	HSO	FO	MALG	P
(C14:0) Myristic acid	0.37±0.01	0.49±0.14	0.36±0.03	1.55±1.10	0.64±0.18	0.450
(C16:0) Palmitic acid	26.58±0.25^{ab}	23.57±0.19^{c}	25.31±0.24^{b}	27.60±0.95^{a}	26.07±0.41^{ab}	0.001**
(C16:1) Palmitoleic acid	2.63±0.09^{b}	3.29±0.15^{a}	2.66±0.05^{b}	2.97±0.31^{ab}	2.56±0.11^{b}	0.038*
(C18:0) Stearic acid	7.37±0.04	8.55±0.62	8.62±0.27	10.02±1.76	8.23±0.40	0.338
(C18:1n9c) Oleic acid	43.12±0.41^{a}	44.20±1.55^{a}	39.37±0.39^{b}	41.84±1.83^{ab}	44.00±0.52^{a}	0.048*
(C18:2n6c) Linoleic acid	18.65±0.37^{ab}	11.03±3.12^{c}	20.20±0.31^{a}	13.65±1.72^{bc}	16.65±0.41^{ab}	0.007**
(C18:3n3) a-Linolenic acid	0.26±0.03^{d}	7.04±0.26^{a}	2.04±0.09^{b}	0.75±0.02^{c}	0.40±0.05^{cd}	0.001**
(C20:4n6) Arachidonic acid	0.84±0.03	1.21±0.74	0.86±0.06	0.73±0.06	0.74±0.03	0.846
(C20:5n3) Eicosapentaenoic acid	0.12±0.04	0.08±0.03	0.06±0.04	0.08±0.01	0.07±0.01	0.601
(C22:6n3) Docosahexaenoic acid	0.08±0.00^{c}	0.56±0.09^{b}	0.53±0.08^{b}	0.83±0.08^{a}	0.67±0.02^{ab}	0.001**

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

Effects of oil sources on fatty acid (FAs) composition of egg yolk in the overall period are shown in Table 3.25. There was no significant difference between groups in terms of FAs composition of egg yolk (except, (C16:1) palmitoleic acid, (C18:3n3) a-linolenic acid, (C20:5n3) EPA and (C22:6n3) DHA). In the overall period, the palmitoleic acid content of FLO was greater than other groups, but the lowest palmitoleic acid content was found in the MALA group. However, there were no significant differences between MALG, HSO, and the FO group's palmitoleic acid ratio in yolks. The a-linolenic acid amount of the FLO group was higher than all other groups. The a-linolenic acid (ALA) ratio of FLO was higher than all other groups, also the HO group's ALA ratio was increased than control, FO, and MALG groups. However, the control and MALG groups had the lowest ALA contents on their egg yolks. The eicosapentaenoic acid composition of control groups was increased than all other groups; however, there were no notable variations between the FLO, HSO, FO, and MALG group's EPA amounts on yolk. The docosahexaenoic acid contents of the control group were significantly decreased than all other groups; however, there was no significant difference between the FLO, HSO, FO, and MALG group's DHA ratio on egg yolk ($P<0.01$). Figures 3.19, 3.20, and 3.21. Show a-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid content of egg yolk in overall period

Table 3.25. Effects of n-3 FA rich oil sources on fatty acid (FAs) content of egg yolk in overall period

	Oil sources					
Fatty acids, %	SFO	FLO	HSO	FO	MALG	P
(C14:0) Myristic acid	1.23±0.27	0.75±0.14	0.78±0.13	1.01±0.26	0.71±0.02	0.301
(C16:0) Palmitic acid	27.25±0.19	26.17±1.23	25.29±0.08	26.56±0.41	25.94±0.39	0.280
(C16:1) Palmitoleic acid	2.73±0.06^{b}	3.13±0.11^{a}	2.71±0.07^{bc}	2.64±0.20^{bc}	2.36±0.07^{c}	0.005**
(C18:0) Stearic acid	8.90±0.45	9.57±0.50	9.08±0.37	8.89±0.57	9.21±0.11	0.784
(C18:1n9c) Oleic acid	41.30±1.35	45.51±2.82	40.75±0.24	41.26±1.80	44.21±0.32	0.203
(C18:2n6c) Linoleic acid	16.74±1.21	14.68±1.42	16.73±0.29	16.35±1.57	15.65±0.02	0.645
(C18:3n3) a-Linolenic acid	0.72±0.25^{d}	4.76±0.34^{a}	3.41±0.13^{b}	1.84±0.66^{c}	0.51±0.05^{d}	0.001**
(C20:4n6) Arachidonic acid	0.78±0.02	0.73±0.14	0.66±0.04	0.67±0.03	0.72±0.01	0.724
(C20:5n3) Eicosapentaenoic acid	0.19±0.03^{a}	0.08±0.01^{b}	0.04±0.00^{b}	0.09±0.03^{b}	0.09±0.01^{b}	0.002**
(C22:6n3) Docosahexaenoic acid	0.17±0.04^{b}	0.60±0.05^{a}	0.56±0.04^{a}	0.68±0.04^{a}	0.61±0.02^{a}	0.001**

^{a,b,c}: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

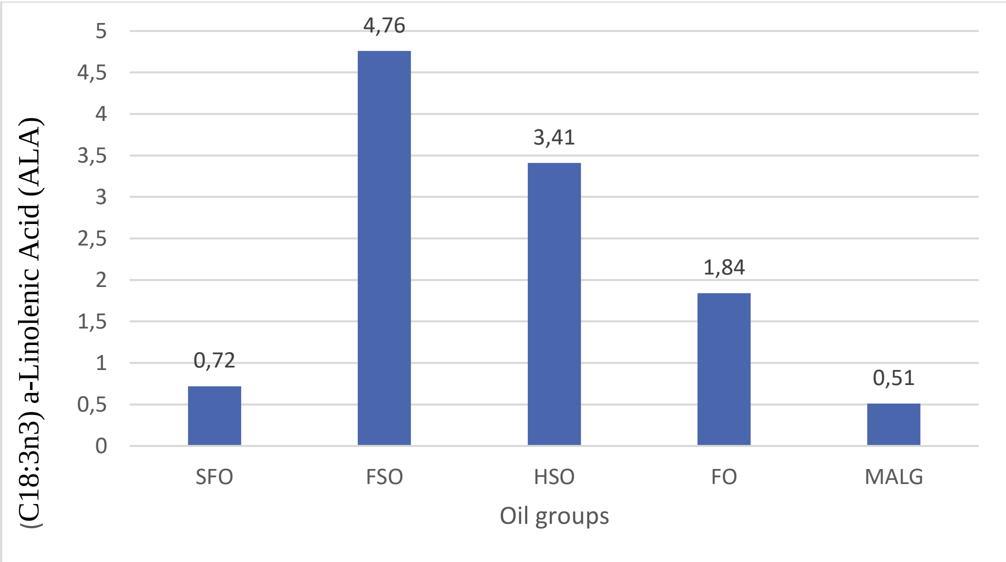

Figure 3.19. Effects of omega-3 fatty acid oil sources on a-linolenic acid content of egg yolk in overall period

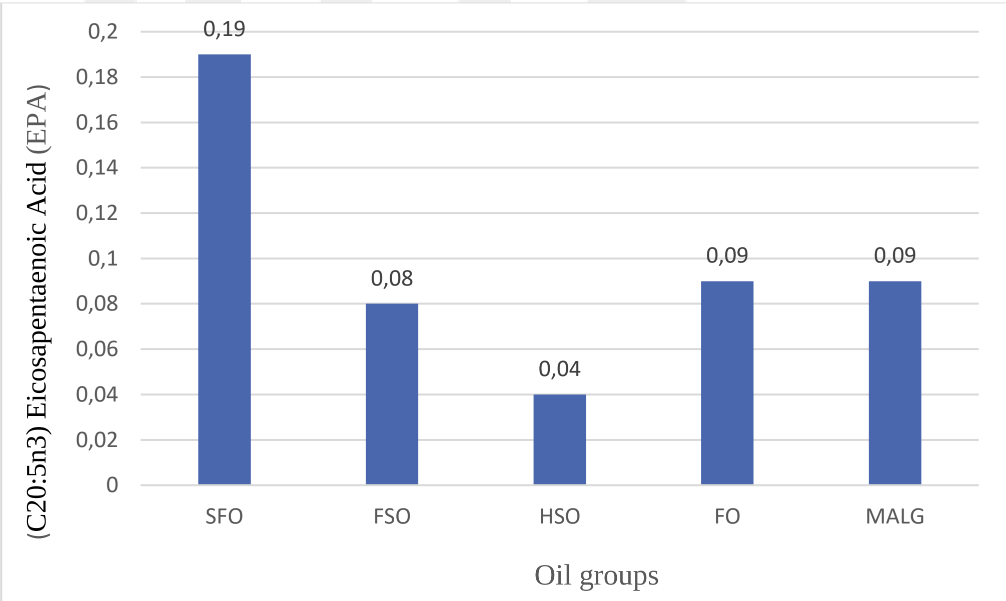

Figure 3.20. Effects of omega-3 fatty acid oil sources on eicosapentaenoic acid content of egg yolk in overall period

Effects of fatty acids transferring from feed to egg were calculated and given in Table 3.26. In this table, the values of fatty acids in the feed and their values in the egg (according to the overall period) were calculated by proportioning. Since no statistical analysis was performed, P (significance) values were not given.

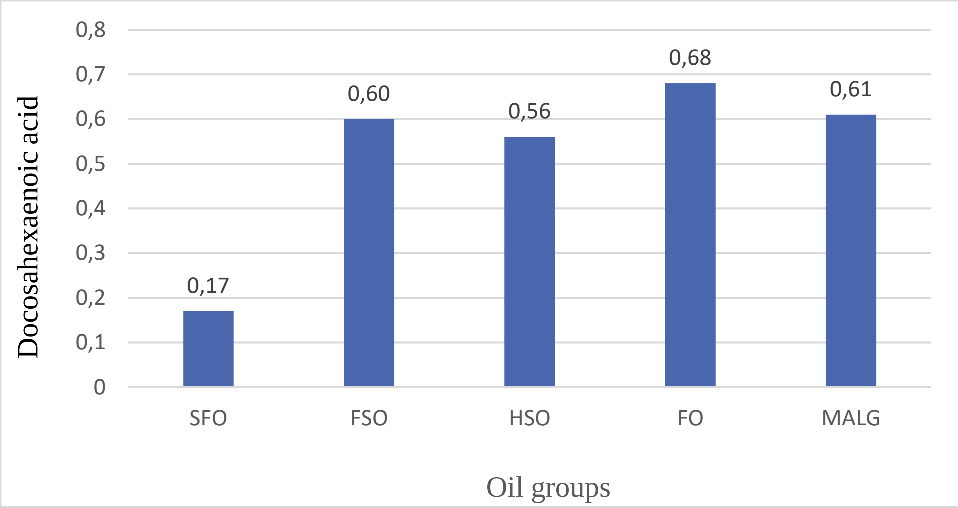

Figure 3.21. Effects of omega-3 fatty acid oil sources on docosahexaenoic acid content of egg yolk in overall period

Table 3.26. Effect of omega-3 fatty acid oil sources on the transfer rate of dietary omega-3 fatty acids into egg yolks

	Change ratio, egg FA/feed FA				
Fatty Acids	SFO	FLO	HSO	FO	MALG
(C14:0) Myristic Acid	15.38	1.63	1.50	0.86	1.03
(C16:0) Palmitic acid	3.09	2.81	2.45	1.89	1.92
(C16:1) Palmitoleic acid	24.82	10.43	9.03	1.38	4.63
(C18:0) Stearic acid	2.88	2.04	2.12	2.74	2.09
(C18:1n9c) Oleic acid	1.19	1.95	1.82	1.31	1.57
(C18:2n6c) Linoleic acid	0.33	0.55	0.33	0.40	0.32
(C18:3n3) a-Linolenic acid	0.40	0.14	0.29	0.53	0.15
(C20:4n6) Arachidonic acid	1.81	7.30	3.67	0.96	2.88
(C20:5n3) Eicosapentaenoic acid	9.50	4.00	1.33	0.08	2.25
(C22:6n3) Docosahexaenoic acid	2.13	4.00	6.22	0.27	5.55

P: probability, **:p<0.01. SFO: Sunflower oil, FLO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

3.7. Effects of omega-3 fatty acid rich oil sources on blood parameters

The effect of omega-3 fatty acid oil sources on blood parameters (BP) is shown in Table 3.26. There were no significant differences between the groups in terms of blood serum glucose, triglyceride, cholesterol, protein, AST, and ALT concentrations (P>0.05).

Table 3.27. Effect of omega-3 fatty acid oil sources on blood parameters (BP) of laying hens

	Oil sources					
Parameters	SFO	FSO	HSO	FO	MALG	P
Glucose, mg/dL	227.75±5.35	227.88±6.02	225.38±4.19	230.13±4.35	223.63±2.12	0.878
Triglyceride, mg/dL	951.18±164.15	1073.75±52.02	1256.75±50.01	1253.43±170.41	1120.00±101.25	0.183
Cholesterol, mg/dL	122.40±18.75	110.44±17.52	121.76±15.62	143.35±16.33	93.83±9.51	0.284
Protein, mg/dL	170.63±7.62	184.75±8.24	179.75±6.27	191.75±15.11	181.00±7.85	0.630
AST, IU/L	2.88±0.62	3.040±0.55	3.03±0.67	4.16±0.79	3.21±0.56	0.546
ALT, IU/L	6.12±0.21	5.59±0.20	5.83±0.18	5.99±0.12	5.63±0.10	0.131

[a,b,c]: Values with different superscript in a column differ significantly, P: probability, **:p<0.01. SFO: Sunflower oil, FSO: Flaxseed oil, HSO: Hempseed oil, FO: Fish oil, MALG: Microalgae

CHAPTER 4

DISCUSSION

4.1. Effect of omega-3 fatty acid-rich oil sources on the performance traits of laying hens

In this study, n-3 fatty acid-rich oil sources (sunflower oil, FLO, HSO, FO, and microalgae powder) were used to improve laying hen performance, egg quality, and fatty acid composition of egg yolk. The fat sources did not affect body weight, body weight gain, and body weight change percentage groups. Similar results were found in different experiments, Grobas et al. (2001) 0, 5, and 10 % tallow (TA), olive oil (OL), soy oil (SO) and linseed oil addition to diets had no influence on body weight of laying hens. The inclusion of FLO 2 and 3% in hen diets did not affect body weight change (Surai and Sparks, 2001). Similarly, Keegan et al. (2019) reported that MALG inclusion of 0.25, 0.5, and 1% to diets and. However, Kim et al. (2016) 3 and 5% FLO, Leeson et al. (2000) 10 and 20% FLO, and Scheideler and Froning (1996) 5, 10 and 15% FLO addition caused a decrease in body weight of layers. In another experiment, Gakhar et al. (2012) noted that the addition of HSO (12%) to laying hen diets decreased in BW of hens, but did not affect 4 and 8% HSO addition.

In the current study, the treatment groups did not affect the feed intake during the experiment (except period 5). The SFO and MALG added group's feed intake in the 5th period was higher than those of all other groups, and, the FO addition caused a decrease in FI compared to other groups. In this study, rations were prepared as isocaloric and isonitrogenic. The feed consumption of poultry may be affected by the energy concentration of the feed; while the consumption of feed may decrease with a high-energy diet, and feed may increase with a low-energy diet. Therefore, in this study, it can be thought that feed consumption was not affected since the energy levels of the diets were similar to nutrient composition. On the other hand, egg production, egg mass and live weight of the hens were similar and therefore feed consumption and live weight change values were not affected by the treatments. On the other hand different opil soruces did not affect feed intake of hens as shown by Ceylan et al. (2011) who noted that linseed oil, SFO, FO and rapeseed oil in 1.5% and 3% ratio, Ehr et al. (2017), FLO or milled flaxseed 0.5, 1.0, 2.0, 3.0, or 5.0%, and Szczurek et al. (2020) 2% of algal oil, FO, soybean oil, rapeseed oil, linseed oil, camelina oil, or coconut oil additions did not affect feed intake of laying hens. Similarly, Lawlor et al. (2010) microencapsulated FO 0, 20, 40, or 60 g/kg, Cachaldora and Garc (2008) FO, soybean oil, linseed oil, or lard 0, 15, 30 g/kg or 0, 50 g/kg, Basmacioğlu et al. (2003) FO and flaxseed 0, 1.5% FO, 4.32% FS, 1.5% FO+4.32% FS or 8.64% FS and Alvarez et al. (2004) did not affect feed intake of laying hens. Moreover, Kanbur et al. (2023) HSO, SFO, corn oil, canola oil, or soybean oil 3.70%, Konca et al. (2014) hemp seed 0, 5, 10, and 20%, Park et al. (2014) HSO, evening primrose oil, or corn oil 0%, CO, (HO 1.6 GLA)or (EPO 8.035 GLA), Alvarez et al. (2005) high-oleic acid SFO with conjugated linoleic acid 10, 20, 30 g/kg HS 0, 1, 2 g/kg and Kale and Bingöl (2021) SFO or selenium 3%, 0.3 mg/kg, did not affect feed intake of hens.

Abbas et al. (2022) noted that 12% microalgae (*Spirulina platensisis*) increased feed intake of laying hens. Other studies by Soliman and El-Afifi (2021) 3% Flax seed oil and Leeson et al. (2000) 20% flax seed supplementation increased feed intake. Although Grobas et al. (2001) found that 5, and 10% of linseed oil, olive, soy, or tallow, Celebi and Macit (2009) Flax seed oil, tallow or SFO 2% FO+2% TO,4% SO or 4%FO decreased feed intake of laying hens. similarly, Scheideler and Froning (1996) flaxseed and FO 5, 10, or 15%, Saleh (2013) 1.25, 2.5, and 3.5, 5% FO and Neijat et al. (2014) 4.5 and 9.0% HSO decreased feed intake. Furthermore, Kim et al. (2016) reported that the flax seed oil and/or microalgae 0, 3, and 5% FO and 7.5, 10, 3+7.5, 3+10, 5+7.5 or 5+10% full-fatted Staurosira sp., Herber and Van Elswyk (1996) 1.5% menhaden oil, or 2.4%, 4.8% marine algae addition in the diet, Kaewsutas et al. (2016) 1%, 2% MALG and 4% FO, Nia et al. (2021) 0.3%, 0.6%, and 0.9% of Spirulina platensis, and Ao et al. (2015) 1, 2, or 3% All-G-RichTM microalgae oil supplementations, had no effect on feed intake of laying hens.

In the current study, there were no significant differences among the groups in terms of FCR in all periods, and overall period, except period 5. In the control group, FCR values were higher than those of all groups; and the MALG group's FCR value was higher than FO FO-added group, however, it was similar to FSO and HSO groups in the 5th period (P<0.01). In the 5th period, the FO-added group's FCR value was lower than all of the groups. Similarly, Ceylan et al. (2011) noted that the 1.5% or 3% linseed oil, sunflower, fish and rapeseed oil 3% FO, SFO, soybean oil, or hazelnut oil, and Meluzzi et al. (2018) 3% FO and vitamin E did not affect FCR of laying hens. Also, Konca et al. (2014) found that 0, 5, 10, and 20% hemp seed in quail, and Kale and Bingöl (2021) 3%, 0.3 mg/kg SFO or selenium, and Jing et al. (2017) found that 4, 8%HO or 4, 8%HΩ hemp oil or hemp omega, addition had no effect on feed conversion ratio in hens.

Similarly, Ao et al. (2015) found that 1, 2, or 3% All-G-Rich™ microalgae oil, and Nia et al. (2021) 0.3%, 0.6%, and 0.9% of blue-green algae (Spirulina platensis), Zahroojian et al. (2013) 1.5%, 2.0%, and 2.5% Microalgae (Spirulina), and (Keegan et al., 2019) 0.25, 0.5, and 1% Microalgae (DHA-rich *Aurantiochytrium limacinum*) inclusion in laying hen's diet did not affect feed conversion ratio of laying hens. Zahroojian et al. (2013) reported that 0, 1.5%, 2.0%, and 2.5% Microalgae (Spirulina), Jing et al. (2017) found that 4, 8%HO or 4, 8%HΩ hemp oil or hemp Omega, and (Keegan et al., 2019) 0.25, 0.5, and 1% Microalgae (DHA-rich *Aurantiochytrium limacinum*) did not effect on FCR. However, Leeson et al. (2000) reported that the 20% flax seed, (Dong et al., 2018) 8% FO, Bazdidi et al. (2016) 5% HSO or 20% hempseed, and Abbas et al. (2022) 12% microalgae (Spirulina platensisis) additions increased FCR. Although, (Grobas et al., 2001) noted that 5, and 10% linseed oil, olive, soybean, or tallow and Celebi and Macit (2009) 0, 2%FO+2% TO, 4% SO or 4%FO, FLO, tallow or SFO, and Neijat et al. (2014) 4.5, 9.0% HSO decreased feed conversion ratio in hens.

In this study, the performance values of the animals were not significantly affected by the fat sources and microalgae addition. In order for the performance values of laying hens to be affected, it is related to the deficiency of some nutrients in the feed or the decrease in feed utilization due to the anti-nutritional elements of some substances in the feed. However, in this study, in addition to the similar protein and energy values of the feeds, there is no negative substance that will affect the feed consumption of the animals. Therefore, it shows that the addition of different fat sources to the feed can be used in the future without any problems in performance. The most important factor that will affect the performance values of the animal is the feed consumption. The decrease in feed consumption shows itself with the regression in the yield parameters.

4.2. Effect omega-3 fatty acid rich oil sources on egg production, egg weight and egg mass of laying hens

In this study, there was no significant difference between the treatment groups and the overall period in EP except period 7. In MALG and FO groups EP values were higher than those of all other groups; however, there is no significant differences between the control, FSO, and HSO groups in 7^{th} period ($P<0.01$). Ao et al. (2015) reported that 1, 2, or 3% All-G-Rich microalgae oil**,** Szczurek et al. (2020) 2% algal oil, FO, soybean oil, rapeseed oil, linseed oil, camelina oil, or coconut oil, Kaewsutas et al. (2016) 1%, 2% Microalgae (Schizochytrium spp) or 4% FO, and Park et al. (2015) 0, 0.5%, or 1.0% microalga had no impact on egg production.

Similarly, Cachaldora and Garc (2008) 0, 15, 30 g/kg FO or 0, 50 g/kg FO, linseed, lard or soybean oils, and Alvarez et al. (2004) 14, 20 g/kg FO, or 1,3 and 5g/kg CLA, and Meluzzi et al. (2018) 3% FO or vitamin E did not affect EP of laying hens. Furthermore, Kanbur et al. (2023) found that 3.70% HSO, SFO, corn oil, canola oil, or Soybean oil, Konca et al. (2019) 0 or 15% raw hempseed or heat-treated hemp seed, Alvarez et al. (2005) 10, 20, 30 g/kg HS 0, 1, 2 g/kg high-oleic acid SFO with conjugated linoleic acid, and Kale and Bingöl (2021) 3%, 0.3 mg/kg SFO or selenium supplementation had no impact on egg production.

In a study conducted by Celebi and Macit (2009) noted that flax seed oil, tallow, or SFO (0, 2%FO+2% TO,4% SO, or 4%FO) inclusion increased EP of laying hens. In another study, Scheideler and Froning (1996) discovered that 0, 5, 10, or 15% flaxseed and FO improved egg production of layers. Park et al. (2014) noted that HSO, evening primrose oil, or corn oil (0%, CO, HO1.6GLA or EPO 8.035 GLA), Bazdidi et al. (2016) 0, 2, 4, 6 HO or 5, 10, 15, 20 HS supplementations increased EP of laying hens. Also, Nia et al. (2021) showed that 0.6% and 0.9% Spirulina platensis addition improved hen EP. However Herber and Elswyk (1994) discovered that 4.8% marine algae, and Leeson et al. (2000) 10% or 20% flax seed inclusion decreased EP.

In the current study, there was no significant difference in EY% between the treatment groups except period 7. According to all other groups MALG and FO group's EY% values were highest; however, there is no variation among the control, FSO, and HSO groups in the 7^{th} period (P<0.01). Similarly, (Ceylan et al., 2011) found that 1.5% or 3% linseed, rapeseed, fish, and flaxseed oils addition was no impact on egg yield percentage. Another experiment by Scheideler and Froning (1996) found that 5, 10, or 15% flaxseed and FO supplementations did not influence on EY%. Also, Leeson et al. (2000) discovered that the addition of 10% or 20% flax seed, Grobas et al. (2001) 5, and 10% linseed oil, olive, soy, or tallow oil inclusions, and Neijat et al. (2014) (4.5, 9.0%HO or 10, 20, 30%HS HSO and hempseed addition did not affect EY%. Similarly, Keegan et al. (2019) noted that 0.25, 0.5, and 1% Microalgae (DHA-rich Aurantiochytrium limacinum) additions, and Zahroojian et al. (2013) 1.5%, 2.0%, and 2.5% Microalgae (Spirulina) supplementation had no impact on EY%. Although different results were found by Lawlor et al. (2010) 20, 40, or 60 g/kg microencapsulated FO supplementation increased EY%. (Kucukersan et al., 2010) discovered that 3% of FO and soybean oil additions raised EY% compared to 3% hazelnut oil while 3%SFO decreased EY%. Also Bazdidi et al. (2016) discovered that 2,4, 6 HSO and 5, 10, 15,20 hemp seed supplementations increased EY%. However, Dong et al. (2018) noted that 8% of FO, supplementation reduced EY% according to 8% soybean oil, and coconut oil.

In this experiment, there was no significant difference in EW and EM between treatment groups in all periods and the overall period (P>0.05). Similarly, Szczurek et al. (2020) reported that 2% algal oil, FO, soybean, linseed, rapeseed, camelina, or coconut oils addition did not affect EW and EM of laying hens. Also, Ehr et al. (2017) found that 0.5, 1.0, 2.0, 3.0, or 5.0% FLO, and milled flaxseed, Omidi1 et al. (2015) 3% FO, olive oil, or grape seed oil, canola oil, and soybean oil, and Kanbur et al. (2023) 3.7% HSO, SFO, corn oil, canola oil, or Soybean oil supplementations did not affect EW and EM. Kaewsutas et al. (2016) noted that the 1%, 2% microalgae (Schizochytrium spp), and 4% FO supplementations were no effect on EW and EM. Although, Abbas et al. (2022) reported that 3%, 6%, 9%, or 12% microalgae (*Spirulina platensisis)* supplementations did not effected EW except for the 9%, group which increased EW. Other research by Soliman and El-Afifi (2021) showed that 1, 2 or 3% FLO, palm oil, or mixture, only FLO inclusion increased EW. However, Celebi and Macit (2009) discovered that 2%FSO+2%

TO, 4% SO, or 4%FSO had increased EW of hens. Nia et al. (2021) found that 0.3%, 0.6%, and 0.9% blue-green algae (Spirulina platensis) additions increased egg mass in hens.

One of the most important aims of this study is to show how the fatty acid profile of eggs will change with oils containing different n-3 FA sources. The fact that egg yield and egg weight and mass do not change is an important advantage for this study. In addition to protein and amino deficiency in feeds, insufficient essential fatty acids are important factors affecting egg production and weight (Keshavarz and Nakajima, 1995; Novak et al., 2006). The protein and amino acid concentrations of the feeds used in this study were prepared according to the catalogue of the hens used in the study that would meet the nutrient needs of the relevant laying hen breed. The level of essential fatty acids is also a criterion taken into consideration and in this study, these essential oils (linoleic, linolenic and arachidonic fatty acids) were already met above the need. In addition, since feed consumption is also similar, it shows that these nutrients are consumed at similar levels by laying hens.

4.3. Effect omega-3 fatty acid rich oil sources on egg Haugh unit (HU) of laying hens

In this experiment, there were no notable differences in HU of eggs between the groups except for periods 3 and 4. In the third period, the HU values of the MALG group were higher than those of all other groups, and the FO group's HU values were higher than the HSO-supplemented group. However, there was no significant differences between the control, FSO, and HSO-added groups. During period 4, the HSO and MALG groups had higher HU values than the FO and FSO groups ($P < 0.01$). Szczurek et al. (2020) found that 2% algal oil, FO, soybean oil, rapeseed oil, linseed oil, camelina oil, or coconut oil inclusion did not affect egg HU. Experiments by Neijat et al. (2016) reported that 0.20%, 0.40%, and 0.60% FLO and algal DHA, Cachaldora and Garc (2008) supplementations 15, 30 g/kg or 50 g/kg FO, soybean oil, linseed oil, or lard, and Küçükersan et al. (2010) 35 FO, SFO, soybean oil, and hazelnut oil additions did not affect egg HU.

However Scheideler and Froning (1996) reported that 5, 10, or 15% flaxseed and FO supplementations increased HU of eggs. Dong et al. (2018) 8% FO, soybean oil, and coconut oil, only FO addition increased HU. Abbas et al. (2022) noted that 3%, 6%, 9%, or 12% microalgae (Spirulina platensisis) inclusion improved egg HU.

Kralik et al. (2021) found that 0.3% FO + 4.7% SO (1) 0.6% FO + 4.4% SO (2) 0.9% FO + 4.1% SO (3), 1.2% FO + 3.8% SO and 1.5% FO+ 3.5% SO FO and soybean oil additions decreased egg HU. Bazdidi et al. (2016) noted that only 4% of hempseed oil decreased egg HU after feeding 2, 4, 6 HO or 5, 10, 15, 20 HS, supplementations of laying hens. Also, Kale and Bingöl (2021) reported that SFO caused a reduction of egg HU when supplemented 3%, 0.3 mg/kg SFO or selenium in laying hens feed.

4.4. Effect omega-3 fatty acid rich oil sources on eggshell quality traits of laying hens

In this study, there was no significant difference in ESW between groups in all periods and overall periods except periods 1 and 4. In the first period, the MALG and FO groups had greater ESW values than the HSO group. However, there are no significant differences between the control, FSO, MALG, and FO-supplemented groups. In the fourth period, the control, FSO, FO, and MALG groups had greater ESW values than the HSO group. However, there were no significant differences between the control, FSO, MALG, and FO groups ($P<0.01$). Soliman and El-Afifi (2021) noted that 1%, 2%, and 3% Flax seed oil, palm oil, or mixture inclusion did not affect the ESW of laying hens. Also, (Ceylan et al., 2011) reported that 1.5% or 3% linseed oil, SFO, FO, and rapeseed oil addition did not affect ESW. Other studies by Basmacioğlu et al. (2003) 1.5% FO, 4.32% Flax, 1.5% FO+4.32% Flax, and 8.64% Flax, and Lemahieu et al. (2013) 125 mg or 250 mg microalgae (*Isochrysis*, *Chlorella fusca*, *Nannochloropsis oculata*, and *Phaeodactylum tricornutum*) supplementation to the laying hen's diet did no affected ESW. Although, Saleh (2013) 1.25, 2.5 3.5, and 5% FO supplementation, Konca et al. (2019) 15% raw hempseed or heat-treated hemp seed addition, and Nia et al. (2021) 0.3%, 0.6%, and 0.9% blue-green algae (Spirulina platensis) did not affect eggshell weight. However, Scheideler and Froning (1996) noted that 5, 10, or 15% flaxseed and FO addition reduced ESW. Cherian (2008) 1.75% FO + 1.75% yellow grease**,** or 3.5% FO (high in omega-3 FA) supplementations decreased ESW of breeder broiler chicken.

In the current study, there was no significant difference between the treatment groups except periods 3, 4, 7, and the overall period in terms of ESR%. In the first, fourth, seventh, and overall periods, MALG, FO, and FSO groups had greater ESR% values than the HSO group. However, there were no significant differences between the control, FSO, FO, and MALG-supplemented groups in ESR% ($P<0.01$). Previous research by Bazdidi et al. (2016) discovered that 2, 4, 6 HSO and 5, 10, 15, 20 hemp seed supplementations did not affect eggshell ratio percentage. Also, Kralik et al. (2021) found that FO and soybean oil (0.3% FO + 4.7% SO,(1) 0.6% FO + 4.4% SO,(2) 0.9% FO + 4.1% SO (3), 1.2% FO + 3.8% SO and 1.5% FO+ 3.5% SO) did not affect ESR%. Other studies by Basmacioğlu et al. (2003) demonstrated that FO and flaxseed (1.5% FO, 4.32% FLS, 1.5% FO+4.32% FLS, and 8.64% FLS) inclusions had no impact on ESR%. Soliman and El-Afifi (2021) noted that 1% FLO+2% PO, 2% FLO+1%PO, and 3% FLO had not effected on ESR%. Also Szczurek et al. (2020) found that 2% of algal oil, FO, soybean oil, rapeseed oil, linseed oil, camelina oil, or coconut oil supplementations did not influence ESR%. Konca et al. (2019) reported that the 15% raw hempseed or heat-treated hemp seed did not influence ESR%. Nia et al. (2021) reported that 0.3%, 0.6%, and 0.9% blue-green algae (*Spirulina platensis*) inclusion did not affect ESR%.

The present experiment there was a significant difference between groups in periods of 1, 2, 4, 5, 6, and 7. MALG, FO, FSO, and control groups had greater EST values than the HSO-supplemented group in the first and seventh periods. In the second period, the EST values of the control group were greater than those of the HSO and MALG groups, and the FO group was higher than the HSO-supplemented group. However, there are no significant differences between the control, FSO, and FO-added groups. In period four, the FO group had higher EST values than the control, HSO, and FSO groups. However, there are no significant differences among the control, FSO, and HSO groups. In the fifth period, the MALG and FO groups had higher EST values than the HSO and FSO groups. In period six FO group had higher EST values than the control, HSO, and MALG groups.

Study by Saleh (2013) 1.25, 2.5 3.5, 5% FO supplementation increased EST. Kale and Bingöl (2021) 3%, 0.3 mg/kg SFO or selenium inclusion raised EST. Park et al. (2015) 0.5%, or 1.0% microalgae addition improved eggshell thickness. However, (Ceylan et al., 2011) 1.5% or 3% linseed oil, sunflower, fish, and rapeseed oils addition did not effect EST. Neijat et al. (2016) 0.20%, 0.40%, and 0.60% FLO or algal DHA supplementation did not affect EST. Cachaldora and Garc (2008) 15, 30 g/kg or 50 g/kg FO, soybean oil, linseed oil, or lard, and Küçükersan et al. (2010) 3% FO, SFO, soybean oil, or hazelnut oil inclusion did not affect EST.

In the current study, there was no significant difference between the treatment groups and the overall period in terms of eggshell surface area (ESA) (P>0.05). Similar results were found by Ceylan et al. (2011) addition of 1.5% or 3% linseed oil, sunflower, fish, and rapeseed oils to the diet of laying hens did not influence ESA. Furthermore, Neijat et al. (2016) 0.20%, 0.40%, and 0.60% FLO or algal DHA inclusion, and Ao et al. (2015) 1, 2, and 3% All-G-Rich™ microalgae oil supplementation did not affect ESA. Different results were found by, Grobas et al. (2001) found that the 5, and 10% linseed oil, olive, soy, or tallow, and Konca et al. (2019) 15% heat-treated hemp seed supplementations increased ESA compared to the 15% raw hempseed addition.

In this experiment, there was no significant difference in eggshell per unit (ES/U) between the treatment groups except periods 1, 4, 7, and the overall period. In the 1st, 4th, 7th, and overall periods, the HSO group's ES/U values were higher than the control, FSO, FO, and MALG groups. However, there is no significant differences between control, FSO, FO and MALG added groups (P<0.01). Konca et al. (2019) found 15% heat-treated hemp seed increased ES/U compared to that 15% raw hempseed. However, in another study by Neijat et al. (2016) 0.20%, 0.40%, and 0.60% FLO or algal DHA addition did not influence ES/U. Ao et al. (2015) reported that 1, 2, or 3% All-G-Rich™ microalgae oil inclusion into the diet did not influence ES/U.

In this study, egg shell quality parameters were generally not significantly affected by oil types and MALG addition. This situation shows that the added oil sources do not have negative effects on eggshell quality. Anti-nutrition factors such as oxalic acid found in some feeds affect the availability of calcium and phosphorus by binding them (Özen et al., 1999). Therefore, no factor affecting the bioavailability of calcium and phosphorus, which are the main components that form the shell, was found in these oil sources. At the same time, while the formation of soap or the binding of oils with calcium may have a negative effect, it is thought that the use of oils does not harm calcium and phosphorus metabolism in this study.

4.5. Effect omega-3 fatty acid rich oil sources on egg internal quality traits of laying hens

In the present study, there was no significant difference in Hmm between groups in all periods and overall period except periods 3 and 4. In the third period, MALG and FO groups had higher Hmm values than the HSO group, but there were no significant differences between the control, FSO, and FO added groups. In period 4, the HSO and MALG groups had higher Hmm values than the FO and FSO groups. However, there were no significant differences between the control, MALG, and HSO groups ($P<0.01$). Scheideler and Froning (1996) noted that 5, 10, or 15% flaxseed and FO inclusions did not influence Hmm. An experiment conducted by Cherian (2008) showed that 1.75% FO + 1.75% yellow grease or 3.5% FO addition did not affect Hmm. Also Alvarez et al. (2005) reported that 10, 20, 30 g/kg HS, 1, 2 g/kg high-oleic acid SFO with conjugated linoleic acid supplementation did not affect Hmm. Lemahieu et al. (2013) found that 125 mg and 250 mg microalgae (*Isochrysis*, *Chlorella fusca*, *Nannochloropsis oculata*, and *Phaeodactylum tricornutum*) additions did not influence Hmm.

However, Dong et al. (2018) noted that 8% FO supplementation increased egg Hmm compared 8% soybean oil, and coconut oil. Kale and Bingöl (2021) found that 3%, 0.3 mg/kg SFO or selenium inclusion did not affect egg Hmm. While other research by Kralik et al. (2021) showed that 0.3% FO + 4.7% SO, 0.6% FO + 4.4% SO, 0.9% FO + 4.1% SO (3), 1.2% FO + 3.8% SO and 1.5% FO+ 3.5% SO FO, Soybean oil addition decreased egg Hmm. Gakhar et al. (2012) reported that according to 4%, HO, 10 or 20%, HS supplementations 8% 12% HO addition decreased egg Hmm.

In the current experiment, there was strong significance in Roche scale color (RRS) differences between groups in the 5^{th}, 6^{th}, 7^{th}, and overall periods. In period 5, HSO, FSO, and FO groups had greater RRS values than the control and MALG groups. In the sixth and overall periods, the RRS values of the control, FSO, HSO, and FO groups were greater than those of the MALG group. However, there is no significant difference between the control, FSO, FO, and HSO groups. In the seventh period, the RRS values of the control and FO groups were higher than those of the HSO and MALG groups. However, there were no significant differences between the control, FSO, and FO groups ($P<0.01$). Saleh (2013) noted that different amounts of FO 1.25, 2.5 3.5, and 5% addition in laying hens' diet increased Roche color scale values. Another study by Dong et al. (2018) indicated that 8% FO and coconut oil inclusion increased egg RRS compared to 8% soybean oil. Park et al. (2014) CO (HO1.6GLA) or (EPO 8.035 GLA) HSO, evening primrose oil, or corn oil additions improved RRS. Also, Konca et al. (2019) reported that 15% of raw hempseed or heat-treated hemp seed supplementation raised egg RRS in hens.

Kale and Bingöl (2021) noted that 3%, 0.3 mg/kg SFO or selenium additions increased egg RRS. Although, Alvarez et al. (2004) found that 14, 20 g/kg FO, or 1,3 and 5g/kg CLA FO**,** conjugated linoleic acid supplementation did not influence egg RRS. Scheideler and Froning (1996) noted that 5, 10, or 15% flaxseed and FO inclusions did not affect RRS. Another experiment by Alvarez et al. (2005) shows that 10, 20, and 30 g/kg HS 1, and 2 g/kg high-oleic acid SFO with conjugated linoleic acid inclusions did not affect RRS. Szczurek et al. (2020) discovered that the 2% algal oil, FO, soybean oil, rapeseed oil, linseed oil, camelina oil, or coconut oil additions did not affect RRS.

There was a strong significant difference in L* between groups in 2, 3, 5, 6, 7, and overall periods. In the 2nd period, the control group's L* values were higher than those of all other groups. In the 3rd, $5^{th,}$ and overall periods, the control group's L* values were higher than the FO, HSO, and FSO groups; however, there are no significant differences between the control and MALG-supplemented groups. In period 6 the MALG group's L* values were higher than those of FO, HSO, and FSO groups. However, there are no significant differences between the control and MALG groups. In the 7^{th} period, the control group's L* values were higher than those of the HSO and FO groups. However, there are no significant differences between the control, FSO, and MALG groups. (P<0.01). Goldberg et al. (2012) found that 4%, 8%, 12% HO or 10%, and 20% HS, the hempseed supplementations decreased L* values. A study by Lemahieu et al. (2013) indicated that 125 mg and 250 mg microalgae (*Isochrysis*, *Nannochloropsis oculata*, and *Phaeodactylum tricornutum*) additions decreased L* value compared to *Chlorella fusca*. Ao et al. (2015) noted that 2, or 3% All-G-Rich^{TM} microalgae oil addition reduced L* than 1% All-G-Rich^{TM} microalgae oil. Although, Kanbur et al. (2023) noted that 3.70% HSO and canola oil additions increased L value compared to 3.70% SFO, corn oil, and or Soybean oil. However, Neijat et al. (2016) reported that 0.20%, 0.40%, and 0.60% FLO or algal DHA additions did not affect L value.

In this study, there was a strong significant difference in a* value between 1, 4, 5, 6, 7, and overall periods. During the first period, the control group had greater a* values than the HSO, FO, and MALG groups. However, there are no significant differences between control and FSO. In the fourth period, the HSO group had higher a* values than the MALG and FSO groups, but there were no significant differences between the control, HSO, and FO groups. In period 5, the HSO, and FO groups had greater a* values than the control, MALG, and FSO groups. During the sixth period, the control group had higher a* values than all other groups, moreover, there were no significant differences between the FSO, HSO, and FO groups. In the seventh period control, the FO group had greater a* values than the HSO, MALG, and FSO groups. In the overall period, the control, FSO, HSO, and FO group's a* values were higher than the MALG group. However, there were no significant differences between the control, FSO, HSO, and FO groups ($P<0.01$). Kanbur et al. (2023) found that 3.70% HSO inclusion reduced value compared to 3.70% SFO, corn oil, canola oil, and Soybean oil. Other study by Goldberg et al. (2012) noted that 10%, 20% Hempseed supplementation increased a value that 4%, 8%, 12% HSO. Also, Lemahieu et al. (2013) found that 125 mg and 250 mg microalgae *(*Isochrysis, Nannochloropsis oculata, *and* Phaeodactylum tricornutum*)* supplementations raised a value according to *Chlorella fusca.* Ao et al. (2015) reported that 2, or 3% All-G-Rich^{TM} microalgae oil inclusion improved a value than 1% All-G-Rich^{TM} microalgae oil. However, Neijat et al. (2016) discovered that 0.20%, 0.40%, and 0.60% FLO and algal DHA inclusions did not influence a value.

In the present study, there was a significant difference in b* between 1, 2, 3, 4, 5, and 7 periods and the overall period. In the 1st period, the control group's b* values were higher than those of the FSO and FO groups, but there are no significant differences between control, MALG, and HSO. In the 2nd period, the control and HSO group's b* values were higher than the MALG and FSO groups, but there were no significant differences between the control, HSO, and FO groups. In period 3 the HSO group's b* values were higher than those of the control group, but there were no significant differences between the control, HSO, FSO, MALG, and FO groups. In the 4th period, the HSO and FO group's b* values were higher than those of all other groups. In the 5th period, the control, FO and HSO group's b* values were higher than MALG group. However, there are no significant differences between the control, FSO HSO, and FO groups. In the 7th period, the control group's b* values were higher than those of the FO and MALG groups and there were no significant differences between the control, FSO, and HSO groups. In the Overall period, the control and HSO group's b* values were higher than FSO and MALG group. However, there were no significant differences between the control, HSO, and FO groups in b* ($P<0.01$). Previous research by Goldberg et al. (2012) noted that 10%, 20% hemp seed addition increased b value compared to 4%, 8%, and, 12% HSO. Lemahieu et al.

(2013) found that 125 mg and 250 mg microalgae (*Isochrysis, Chlorella fusca*, *Nannochloropsis oculata,* and *Phaeodactylum tricornutum*) inclusion increased value. Nevertheless, Neijat et al. (2016) reported that 0.20%, 0.40%, and 0.60% FLO or algal DHA additions did not effect a value. Kanbur et al. (2023) found that 3.70% HSO, SFO, corn oil, canola oil, and soybean oil supplementations did not influence b* value.

Egg yolk color can be provided by feeds, but color substances are not produced by the animal in the body. Egg yolk color is one of the most important visual factors affecting egg purchase. When the ratio of colorant feeds used in feeds increases, egg yolk color also increases. On the other hand, color substances (xanthophylls, zeaxanthin, olericin, etc.) are fat-soluble substances and are transferred to the egg with fat. In practice, the most important source that contains the most color substance in laying hen feeds and affects the color of egg yolk is yellow corn. Since yellow corn was used in similar ratios in this study, it is thought that the effect from here will be similar. However, in this study, microalgae addition to feeds caused a decrease in egg yolk color according to the Roche color scale and other groups were found to be similar to the control group. Similarly, MALG added group caused a statistical decrease in egg yolk a* and b* values. Although this is an unexpected situation, the decrease in the color of the egg yolk may cause a decrease in the preference of the eggs by the consumers. In this case, it is necessary to increase the amount of colorants in the feeds containing microalgae.

4.6. Effect omega-3 fatty acid rich oil sources on fatty acid composition of egg yolks

In the current study, the composition of yolk fatty acids was significantly affected by dietary omega-3-rich oil treatments. Yolk palmitic acid ratio was decreased in all treatment groups (except FO), but ALA increased in the FSO group, while DHA content increased in FSO, FO, MALG, and HSO groups during day 14. During period 2, the palmitic acid ratio on egg yolk decreased in the FSO group, and oleic acid decreased in the HSO group; however dietary HSO addition increased linoleic acid, while yolk ALA content raised by FSO and HSO-added diets, and the EPA ratio decreased in all treatment groups, however, DHA concentration increased in treatment groups. On day 42, egg myristic acid contents decreased in FSO and increased in the MALG group; however, the Stearic acid decreased in the FO group, and Arachidonic acid decreased in all treatment groups compared to the control, DHA was increased in HSO and FO groups than the control and MALG. In period 5, stearic acid in FO and MALG was higher than the control and FSO groups, and the linoleic acid decreased in the HSO group; however, the a-linolenic acid rose in FSO and FO groups, while DHA of all treatment groups increased. On d 84, FSO addition decreased the palmitic acid and linoleic acid, but it increased the ALA and Palmitoleic acid content. The lowest oleic acid was found in the HSO group, but it had the highest linoleic acid ratio in their eggs. The ALA and DHA were increased in all treatment groups compared to the control; however, the highest DHA was found in the FO and MALG groups. On overall period the Palmitoleic acid and ALA compositions were higher in FSO group, while the lowest palmitoleic acid found in MALG. ALA where increased in FSO, FO, and HSO groups than the control and MALG. EPA decreased in all treatment groups, but DHA increased compared to the control. Other studies by Szczurek et al. (2020) found that the addition of 2% of algal oil, FO, soybean oil, rapeseed oil, linseed oil, camelina oil, or coconut oil, Kim et al. (2016) FLO and microalgae, Huang et al. (1990) 1,2,3% menhaden fish oil, and Wen et al. (2019) 1%, 2%, 4%, and 8% microalgae, additions to the laying hens diet was increased the DHA and EPA content on egg yolk. Kim et al. (2016) also noted that the FLO inclusion increased ALA content of eggs than microalgae.

In this study, the ALA ratio in egg yolk oil was generally higher in the FLO group compared to the other groups, the EPA ratio was higher in the control group compared to the other groups, and the DHA ratio was significantly lower in the control group compared to the other groups. According to these results, when n-3 rich oils used in the nutrition of laying hens, FLO provided an effective increase in terms of increasing the ALA ratio, followed by HSO and FO, respectively, but in the case of MALG+SFO supplementation, an ALA ratio similar to the control group. Since ALA FA is essential in human nutrition and in cases where it is not always possible to reach and consume FLO, HSO and FO, it seems that the use of these oils can contribute to those who consume these eggs by increasing the ALA ratio in eggs. The increase in DHA in egg yolk was provided more with FO than with the MALG group, while MALG was the second most effective. In this study, MALG was used at only 1.5% in the laying hen diet, and it can be said that if this MALG rate is increased, it is possible to further increase the DHA rate in eggs.

4.7. Effect omega-3 fatty acid rich oil sources on blood components of laying hens

In the current experiment, there was no significant difference between the treatment groups in blood parameters (BP) ($P>0.05$). Similar results were found by Neijat et al. (2016) addition 0.20%, 0.40%, and 0.60% FLO and algal DHA did not affect serum triglyceride and cholesterol concentrations in hens. Also, Konca et al. (2019) noted that 15% raw hempseed or heat-treated hemp seed supplementations did not impact serum triglyceride, cholesterol, protein, AST, and ALT concentrations in laying hens. Konca et al. (2014) found that 5, 10, and 20% of hempseed supplementations did not impact serum protein in quail. Cheng et al. (2004) found algal DHA oil and butter (20 g butter/kg, 5 g+15 g butter/kg, or 20 g /kg DHA) additions did not affect blood parameters (triglyceride and cholesterol). However, Bazdidi et al. (2016) reported that 2,4, HO or 5, 10, 15,20 HS additions did not affect blood protein while 6% HO inclusion decreased blood cholesterol and AST in hens. Celebi and Macit (2009) noted that 2%FLO+2% TO, 4% SO, and 4%FLO additions increased blood ALT and Bean and Leeson (2003) noted that 10% flax seed increased blood ALT in laying hens.

There are studies showing that the addition of omega-3 oil improves heart health in humans (Zhang et al., 2022) and reduces cholesterol and triglyceride levels in the blood (Cabo et al., 2012) (Superko et al., 2014). Most of these studies have been evaluated in people with lipid-related diseases. Therefore, there may be factors related to lipid metabolism or lifestyle or previous inadequate, irregular intake of these n-3 fatty acids and age-related factors. In this study, there was no significant change in the cholesterol and triglyceride levels of the laying hens compared to the control group, and the amount of ALA in the feed of the control group animals was 1.81%, which was sufficient. In practice, the amount of linoleic acid was specified for laying hen diets (1.55% for Super Nick Chick and decreased with advancing age) and the amount of ALA and the amount of EPA and DHA were not determined. From this perspective, the amount of ALA in the feed for laying hens is not calculated in practical commercial diets.

4.8. Conclusion and Recommendations

As a consequence, the omega-3-rich oil sources additions to the laying hen diets had significantly influenced egg yolk fatty acid composition during the study, while the most effect was found in DHA which the dietary n-3 FA-rich oil addition increased in all periods of the experiment. The ALA composition of egg yolk increased in treatment groups than in the control, while the highest ALA was found in eggs from FSO-fed hens. EPA had no significant variation between treatment groups and control (except period 2 and overall period). Initial and final BW, BW gain and its percentage, feed intake and FCR (except p5), egg production and EY%, egg mass, egg weight, egg albumen height and HU, eggshell weight, eggshell surface area, and blood parameters were not affected by the treatments. However, dietary interventions affected the eggshell per unit, eggshell thickness, eggshell ratio, egg yolk Roche color scale values, egg yolk brightness (L*), egg yolk redness (a*), and egg yolk yellowness (b*).

Finally, we recommended the use of omega-3-rich oil sources additions to the laying hens' diets can increase docosahexaenoic acid (DHA) and a-linoleic acid (ALA) concentration in egg yolk without negative effects on laying performance, egg quality, and blood parameters.

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