EFFECT OF DIETARY ω-3, ω -6 AND ω -9 FATTY ACIDS ON PRODUCTIVE AND PHYSIOLOGICAL TRAITS OF LAYING HENS

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SUMMARY

This study investigated the effect of various sources and levels of vegetable oil as a source of omega- 3, omega- 6 and omega- 9 fatty acids on productive performance, egg quality, fatty acids profile and some physiological traits of laying hens. Two hundred and seventy Bovens laying hens at 45 weeks of age were randomly divided equally into nine treatments resulting from factorial design (3×3); three sources of vegetable oil (soybean, sunflower and linseed oil) with three levels (1, 1.5 and 2%). Obtained results showed that, final body weight, hen-day egg production percentage, feed consumption, egg quality characteristics, plasma total protein, globulin, aspartate transaminase, alanine aminotransferase, total lipids and very low density lipoprotein concentrations were not significantly affected (P>0.05) by various sources or levels of vegetable oils. The use of linseed oil in laying hens' diets led to a significant increase (p≤0.05) in the egg mass and weight of eggs produced from those hens. Feed conversion ratio of laying hens fed either linseed or sunflower oil diets were significantly (P≤0.05) improved compared to those received dietary soybean oil. There was a significant increase in the level of MUFAsin the egg yolk occurred as a result of using sunflower or linseed oil, compared to soybean oil. The inclusion of sunflower oil in the layer diets was able to improve significantly (P≤0.01) the concentration of γ-linolenic (C18:3n6) and arachidonic (C20:4n6) fatty acids in the egg yolk. The use of different levels of sunflower or linseed oil up to 2% led to a significant (P≤0.01) improvement in the level of high density lipoprotein. Moreover, layer fed diet with different levels of linseed oil significantly (P≤0.01) had the lowest concentration of low density lipoprotein. Significant (P≤0.01) improvement in the level of antioxidants was observed for the groups fed on diet containing linseed oil, followed by those fed dietary sunflower oil, compared to the control group that fed on soybean oil. In conclusion, the use of both sunflower oil or linseed oil up to 2% in the diet of laying hens are able to improve egg production, egg quality, the level of essential fatty acids in egg yolk, in addition improving the level of antioxidants and blood biochemical parameters.

Keywords: *laying hens, vegetable oil, omega-3, omega-6, omega-9, egg quality and physiological status*

INTRODUCTION

Poultry farming has seen a significant increase in the agriculture industry over the past several years. The egg-laying branch has distinguished itself in this market, as seen by the rising egg output at levels never previously seen in Egypt (FAO, 2020). Corn and soybean meal, which are the primary sources of energy and protein, respectively, have been the most important elements of poultry diets for decades. Modern laying chickens require more nourishment and consume less feed during the first stages of production since they are early-maturing animals. Therefore, dietary inclusion of lipid sources is a crucial step to guarantee their proper intake of energy (Silva *et al.*, 2014). Numerous studies have attempted to boost poultry productivity using concentrated energetic diets that are focused on increasing the carbohydrate content without regard to the maximum amount of starch or the minimum amount of fiber, but this has led to an increase in digestive issues (Gidenne *et al*., 2005). For commercial broiler and laying diets to meet required energy needs and ensure optimal productive performance, fats and oils must now be supplemented. The most frequent source of energy in feed diets for laying hens is oil, which has an impact on energy output, nutritional absorption, resistance to heat stress, dust reduction, and immune system enhancement. Additionally, they enhance feed intake (FI) and egg production performance (Chwen *et al*., 2013, and Stevanovi *et al*., 2018). The amount of oil added to hen feed is minimal in comparison to that consumed by broilers because laying hens have a different physiological structure and are more susceptible than broilers to problems of lipid metabolism. Hence, for laying hens' production

efficiency, lipid metabolism, and egg quality, the right amount and kind of oil addition are crucial. Additionally, diets with larger proportions of MUFAs(MUFAs) or poly unsaturated fatty acids (PUFAs) and lower proportions of saturated fatty acids were linked to improvements in body weight, FI, feed conversion ratio FCR (g feed/ g egg), egg production, egg quality, and mortality rate. The alteration of dietary fatty acids (FAs) has also been successful in inducing the accumulation of PUFAs in chicken meat and eggs (Küçükersan *et al*., 2010; Moura *et al*., 2019; and Gao *et al*., 2022).

However, eggs are an excellent source of proteins and are packed with compounds that serve biological purposes above and beyond mere nourishment. The biggest nutritional issue with eggs is that they are high in cholesterol (the average level ranges from 195 to 230 mg/egg), which can have a detrimental effect on the onset of atherosclerosis. As a result, many individuals choose to limit or restrict their intake of eggs (Laca *et al*., 2010 and Liu *et al*., 2010). Nutritionists are currently working to create functional food by incorporating some essential elements into human diets (meat, and eggs). Essential fatty acids (EFAs), notably omega-3, omega-6, and omega-9, may be one of these nutrients. It is feasible to enhance the quantity of these FAs in the eggs by raising the concentration of PUFAs in the laying hens' diet (Gonzalez-Esquerra and Leeson, 2000). It has been documented that feeding chickens various lipids can alter the yolk fatty acids (FAs). The capacity of chickens to deposit dietary lipids into egg yolks is also supported by research, making eggs a possible source of (PUFAs) (Filardi *et al*., 2005). So, Linseed and sunflower oils have been utilized to alter the FA profile of the lipids in egg yolks from laying chickens. (Milinsk *et al*., 2003).

As a result, this research was conducted to investigate the potential effects of different vegetable oil as a source of ω-3, ω-6, and ω-9 fatty acids on the FAs content of egg yolks and several metrics, such as egg quality, laying efficiency, and some blood biochemical characteristics in laying hens.

MATERIALS AND METHODS

Ethical Approval:

The institutional ethical rules of Agriculture College - Tanta University (No. AY 2019-2020/Session 6/ 2020.01.13) in dealing with animals for scientific purposes were followed during the experiment period.

Birds and management:

Under the direction of the Animal Production Department - Tanta University, this study was conducted in a private poultry farm at ElSanta El-bald town – near Tanta - Gharbia Governorate, Egypt (latitude of 30.75°N, longitude angle of 31.22°E, and mean altitude above sea level of 7 m), during the period from 1 September to 30 November 2020. Two hundred and seventy brown Bovens laying hens at 45 weeks of age were randomly divided equally into nine treatments resulting from factorial design (3×3); three sources of vegetable oil (soybean, sunflower and linseed oil) with three levels (1, 1.5 and 2%). Birds were individually kept in laying cages in an open-sided building under a 16h light 8h dark lighting schedule.

The experimental diets:

Birds were fed nine experimental layer diets in a mash form (18% crude protein; 2750 kcal ME, 4% Ca per kg diet) including three different oil sources (soybean oil, sunflower oil, and linseed oil) 3 inclusion levels (1.0, 1.5 and 2.0%). Diets were formulated to meet the recommendations of brown Bovens laying hens manual as showed in Table (1).

Measurements:

Performance traits:

Body weight, feed consumption (FC) and FCR (g of feed consumed/g of egg produced) were recorded and determined weekly. Eggs were collected daily; their weight and mass were recorded. Egg production was expressed as hen-day production and calculated using the following equation:

$$\text{Egg production} = \frac{\text{Number of eggs produced}}{\text{Number of live hens housed}} \times 100$$

Egg quality and chemical analysis:

At 57 weeks of age, 30 eggs per treatment were collected to assessed egg quality traits includes (egg weight, shape index, shell thickness, albumen height, albumen width, yolk height, yolk width, yolk color, and Haugh unit) within 12h postoviposition. Furthermore, another 30 random samples of eggs from each treatment were sent to the Laboratory of National Company for Fishery & Aquaculture (NCFA) – Ghalioun – Egypt, for FAs analysis. Egg yolk samples were prepared according to the instructions of (Ayerza and Coates, 2000) for the FAs analysis. Fatty acids profile of egg-yolk was determined by gas liquid chromatography (GLC) according to the method of (Radwan, 1978).

Blood biochemical and antioxidants indices:

Through the wing vein, a blood sample from 9 hens chosen randomly from per treatment was collected at the end of the experimental period. The blood samples were divided into two portions; the first portion was collected on heparin to separate plasma by centrifugation at 3000 rpm for 5 minutes; the second portion was taken in a plain centrifuge tube, allowed to clot, and then centrifuged at 3000 rpm for 5 minutes to separate the serum; it was then stored in sterile screw-capped vials at -200C until it was subjected to some biochemical analysis. Using commercial kits (Diamond Diagnostics, Egypt) and following the manufacturer's instructions, concentrations of total cholesterol, triglycerides, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, aspartate transaminase (AST), alanine amino transferees (ALT), total protein, albumin, globulin, total antioxidants capacity (TAC), super oxide dismutase (SOD) and malondialdehyde (MDA) were determined calorimetrically.

Table (1): Composition and proximate analyses of the experimental diets.

Feed ingredients	Soybean oil			Sunflower oil			Linseed oil		
	1%	1.5%	2%	1%	1.5%	2%	1%	1.5%	2%
Yellow corn	57.30	57.30	55.20	57.30	57.30	55.20	58.30	57.30	55.70
Corn gluten meal, 62%	6.80	3.75	4.00	6.80	3.75	4.00	6.80	4.00	4.00
Soybean meal, 46%	18.30	23.00	22.00	18.30	23.00	22.00	18.30	23.00	22.50
Wheat bran	4.00	1.70	5.00	4.00	1.70	5.00	3.00	1.45	4.00
Limestone	9.50	9.8	9.25	9.50	9.8	9.25	9.50	9.8	9.25
Di-calcium Phosphate	1.80	1.65	1.75	1.80	1.65	1.75	1.80	1.65	1.75
NaCl	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL- methionine, 99%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
L-lysine, 75%	0.20	0.20	--	0.20	0.20	--	0.20	0.20	--
Vegetable oil	1.00	1.50	2.00	1.00	1.50	2.00	1.00	1.50	2.00
Sodium bicarbonate	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Potassium carbonate	0.30	0.30	--	0.30	0.30	--	0.30	0.30	--
Layer Premix *	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100	100	100	100	100
Proximate Analysis									
ME (Kcal/kg diet)	2740	2750	2750	2745	2755	2760	2750	2750	2750
Crude protein, %	18.12	18.04	18.07	18.12	18.04	18.07	18.05	18.13	18.11
EE%	4.1	3.91	4.23	4.1	3.91	4.23	4.1	3.91	4.23
Fiber, %	2.8	3.17	3.25	2.8	3.17	3.25	2.8	3.17	3.25
Calcium, %	4.08	4.17	3.99	4.08	4.17	3.99	4.08	4.17	3.99
Available Phosphorus, %	0.43	0.43	0.46	0.43	0.43	0.46	0.43	0.43	0.46
Methionine, %	0.46	0.41	0.46	0.46	0.41	0.46	0.46	0.41	0.46
Lysine, %	0.95	0.88	0.94	0.95	0.88	0.94	0.95	0.88	0.94

***** *Premix content; Vitamin mineral premix (units per kilogram of feed): vitamin A, 10,000 IU; vitamin D3, 3,500 IU; vitamin E, 35 IU; menadione, 1.5 mg; riboflavin, 5 mg; pantothenic acid, 8 mg; vitamin B12, 0.012 mg; pyridoxine, 1.5 mg; thiamine, 1.5 mg; folic acid, 0.5 mg; niacin, 30 mg; biotin, 0.06 mg; iodine, 0.8 mg; copper, 10 mg; iron, 80 mg; selenium, 0.3 mg; manganese, 80 mg; zinc, 80 mg.*

Statistical analysis:

Statistical Software for the Statistical Analysis System, SAS (2004), was used to conduct a two-way ANOVA statistical analysis of the differences between the treatments in a fully randomized design. The novel multiple-range test developed by Duncan was used to compare the significant changes in treatment mean values. The statistical model utilized for the variance analysis is:

$Y_{ijk} = \mu + T_i + R_{ij} + TR_{ij} + e_{ijk}$
Y_{ijk}= an observation
μ = overall mean
T_i= effect of using different sources of vegetable oil
R_{ij}= effect of using different levels of vegetable oil
TR_{ij} = effect of interaction between different sources and different levels of vegetable oil
e_{ijk} = residual error.

RESULTS AND DISCUSSION

Productive performance of laying hens:

Productive performances of Bovens hens as influenced by various sources and levels of vegetable oil are shown in Table 2. Regarding the effect of various oil sources on the productive performance of laying hens, it was noted that the use of linseed oil in laying hens' diets led to a significant increase ($P \leq 0.05$) in the egg weight and egg mass. The birds fed on soybean oil rations achieved the lowest average egg weight and egg mass. Feed conversion ratio of laying hens fed either linseed or sunflower oil diets were significantly ($P \leq 0.05$) improved compared to those received dietary soybean oil. Considering the effect of different levels of vegetable oils, significant differences are noted for hen-day egg production, egg weight, egg mass, FI and FCR. A significant improvement ($p \leq 0.05$) in hen-day egg production%, egg weight, egg mass, and FCR was observed in the group fed diet containing 2% vegetable oil, followed by those fed 1.5%, and then those received 1% vegetable oil. In addition, FI was significantly ($P \leq 0.05$) decreased by increasing the level of vegetable oil from 1 to 2%. Regarding the effect of interaction between oil sources and oil levels on productive performance of laying hens, percentage of hen-day egg production was significantly ($p \leq 0.05$) improved for groups fed diet with various oil sources at the level of 2% followed by those fed 1.5% oil and then received 1% vegetable oil. Additionally, laying hens treated with sunflower or linseed oil at the level of 2 and 1.5 % had significantly ($P \leq 0.05$) the higher egg weight. Whereas, the groups fed at 2% of either linseed or sunflower oil had significantly ($P \leq 0.01$) the highest average egg mass. Moreover, the lower ($P \leq 0.01$) FI was observed in the groups that were fed 2% of different vegetable oils (sunflower or linseed oil). While, the best ($P \leq 0.01$) FCR was observed for group fed 2% sunflower or linseed oil.

Identifying the potential influences of different sources and levels of vegetable oil on laying performance and egg quality features was the main goal of the current study. In the present study, there were no significant variations in laying hen body weights between treatment groups, as shown in Table 2. The absence of nutritional effects from using various oils sources and levels on laying hen body weights in this investigation supported the findings of prior studies that indicated no significant differences between dietary oil sources on laying hen body weight (Shafey *et al*., 1992). According to previous research of (Moura *et al*., 2019), none of the lipid sources had an effect on body weight and egg production; this was most likely due to the fact that the diets provided equivalent levels of energy and nutrients, guaranteeing appropriate nutrient delivery across all treatments.

Moreover, it was discovered that hen-day egg production, egg weight and FCR were significantly improved by using sunflower and linseed oil at the level of 2% compared to soybean oil (Table 2). This was most likely due to the fact that adding lipids to laying hen diets increases the density of the diet, enhances palatability, increases metabolic energy efficiency, boosts egg production, and enhances FCR (Brugalli, 1998), which is comparable to the findings in this study. Also, linseed oil is strong in omega-3 FAs and has a low omega-6 to omega-3 FAs ratio. According to Dalton (2000), lowering the omega-6 to omega-3 FAs ratio in the Japanese quail diet resulted in a considerable increase in egg size and production. According to Balevi and Coskun (2000), the type of vegetable oil used in layer feeding can have a significant impact on egg weight. However, the findings of my own research show that soybean oil in diet mixes have a detrimental influence.

These results are in agreement with (Al-Daraji *et al*., 2010) who observed that, supplementation of different dietary oil sources (3% sunflower, linseed, corn, and fish oil) did not significantly ($P > 0.05$) modify feed consumption. On the other hand, dietary fish oil at the inclusion level of 30 g/kg enhanced FCR, egg weight, hen-day egg production, egg mass, and cumulative egg production in quail hens. The improvement in performance may be due to the ability of vegetable oil to increase palatability (Silva *et al*., 2014), hence increasing consumption and productive performance . In addition, Küçükersan *et al*., (2010) reported that supplementation of different dietary oil (3% sunflower, fish, soybean and hazelnut oil) significantly ($P \leq 0.05$) affect egg production%. Groups feed diet supplemented with 3% fish or

soybean oil had significantly (P≤0.05) the highest rate of egg production compared to those received sunflower or hazelnut oil. At the same time, egg weight and egg mass were significantly (P≤0.01) increased for group fed on diet supplemented with 3% soybean oil followed by those treated with sunflower oil and then those received fish or hazelnut oil (Küçükersan *et al*., 2010). On the other hand, our results are not compatible with those observed by Wei *et al*., (2021) who reported that egg weight and egg mass were not significantly influenced by 3% soybean oil. Also, Dos-Santos *et al*. (2009) employed soybean, linseed, and cottonseed oils in experimental diets for white-egg layers at inclusion levels of 2 and 4% and discovered that adding vegetable oils to the diets, regardless of source, had no impact on the performance of the layers.

Table (2): Effects of different sources and levels of vegetable oil on productive performance of laying hens.

Treatment	Initial body weight, g	Final body weight, g	Hen-day egg production, %	Egg weight, g	Feed intake, g/d	Egg mass, g of egg/hen/d	Feed conversion ratio, g feed/g egg
Effect of oil sources on productive performance of laying hens							
Soybean oil	1899.45	1954.07	71.72	65.41^{b}	115.47	46.94^{b}	2.47^{a}
Sunflower oil	1949.44	2082.04	72.34	67.85^{ab}	114.63	49.094^{a}	2.34^{b}
Linseed oil	1961.11	2055.18	72.44	68.18^{a}	114.06	49.404^{a}	2.31^{b}
MSE	±25.69	±52.33	±0.61	±0.87	±0.95	±0.44	±0.41
Sig.	NS	NS	SN	*	NS	*	*
Effect of oil levels on productive performance of laying hens							
1 %	1940.56	2032.226	70.58^{b}	66.36^{b}	117.41^{a}	46.84^{b}	2.51^{a}
1.5 %	1920.556	2022.04	72.09^{ab}	65.75^{b}	115.20^{b}	47.41^{b}	2.43^{b}
2 %	1948.89	2037.03	73.83^{a}	69.33^{a}	111.54^{b}	51.18^{a}	2.18^{c}
MSE	±32.41	±56.33	±0.68	±0.91	±0.77	±0.76	±0.48
Sig.	NS	NS	*	*	*	*	*
Effect of interaction between oil sources and oil levels on productive performance of laying hens							
Soybean oil *1	1846.67	1838.89	70.22^{b}	64.27^{c}	118.1^{a}	45.13^{c}	2.62^{e}
Soybean oil *1.5	1900	1950	71.39^{ab}	64.42^{c}	115.23^{a}	45.99^{c}	2.51^{d}
Soybean oil *2	1951.67	2073.33	73.56^{a}	67.55^{abc}	113.07^{ab}	49.69^{b}	2.27^{b}
sunflower oil *1	1993.33	2096.67	70.71^{b}	68.47^{abc}	117.22^{a}	48.41^{b}	2.42^{c}
sunflower oil *1.5	1898.33	2105	72.21^{ab}	64.78^{bc}	115.41^{a}	46.78^{bc}	2.46^{c}
sunflower oil *2	1956.67	2044.44	74.11^{a}	70.3^{a}	111.26^{b}	52.09^{a}	2.13^{a}
Linseed oil *1	1981.67	2161.11	70.82^{b}	66.35^{abc}	116.91^{a}	46.99^{bc}	2.48^{cd}
Linseed oil *1.5	1963.33	2011.11	72.67^{ab}	68.05^{abc}	114.97^{a}	49.45^{b}	2.32^{b}
Linseed oil *2	1938.33	1993.33	73.83^{a}	70.13^{ab}	110.3^{b}	51.77^{a}	2.13^{a}
MSE	±44.63	±69.70	±0.88	±1.56	±1.87	±0.78	±0.74
Sig.	NS	NS	*	*	**	**	**

^{a,b,c,d,e} *Mean values followed by different letters in the same column are significantly different*
* *Significant differences at the level of 0.05* ** *Significant differences at the level of 0.01*
^{NS} *Not significant* ^{MSE} *mean standard error*

Egg quality of laying hens:

Data presented in Table 3 showed the effect of different sources and levels of vegetable oil on the egg quality of Bovens hens. It was observed that all egg quality characteristics include (shape index, shell thickness, albumen height, albumen width, yolk height, yolk width, yolk color, and Haugh unit) were not significantly affected (P>0.05) by different sources or levels of vegetable oils. Regarding the effect of interaction between oil sources and oil levels on egg quality of laying hens, no significant differences (P>0.05) were observed for shape index, yolk height, yolk width, and yolk color. On the other hand, shell thickness, albumen height, albumen width and Haugh unit were significantly affected (P≤0.05). Group fed diet containing 1.5% soybean oil had significantly (P≤0.05) the higher shell thickness, while those fed diet containing 2% sunflower oil had significantly (P≤0.05) the lower shell thickness. However, there are no significant differences between these two groups and the other experimental groups. As for albumen height, birds fed dietary 1% linseed oil achieved significantly (P≤0.05) the highest average for albumen height followed by those received 1.5% soybean oil and then those treated with 1% sunflower

oil. While, the lowest (P≤0.05) value was observed for the group fed diet containing 2% soybean oil. As for albumen width, the group fed diet containing 2% soybean oil significantly (P≤0.05) had the higher value. While, the lowest was observed for those received 1% linseed oil, with no significant differences between the other groups.

In recent years, Haugh unit scores have become widely regarded as a measure of albumen quality in egg quality investigations. In this study, the results indicate a significant improvement in Haugh unit as a result of using 1% linseed oil, with insignificant differences between them and those fed a diet containing either 1 or 1.5% soybean oil, or those fed 1 or 2% of sunflower oil.

Table (3): Effects of different sources and levels of vegetable oil on egg quality of laying hens

Treatment	Shape index %	Shell thickness µm	Egg weight g	Albumen height, mm	Albumen width, cm	Yolk height, mm	Yolk width, cm	Yolk color	Haugh unit
Effect of oil sources on egg quality of laying hens									
Soybean oil	75.72	350.55	65.41^{b}	7.26	9.28	18.14	4.09	7.72	97.58
Sunflower oil	77.04	339.44	67.85^{ab}	7.43	9.88	18.51	4.15	8.17	98.45
Linseed oil	76.01	343.33	68.18^{a}	7.66	9.27	18.78	4.17	8.05	99.33
MSE	±0.62	±2.18	±1.88	±0.18	±0.71	±0.33	±0.12	±0.09	±0.84
Sig.	NS	NS	*	NS	NS	NS	NS	NS	NS
Effect of oil levels on productive performance of laying hens									
1 %	74. 7	346.66	66.36^{b}	7.91	8.78	18.39	4.12	8.11	100.73
1.5 %	76.78	346.11	65.75^{b}	7.46	9.38	18.07	4.09	7.72	98.75
2 %	77.22	340.55	69.33^{a}	6.98	10.26	18.96	4.2	8.11	95.88
MSE	±0.88	±2.41	±1.43	±0.28	±0.47	±0.64	±0.21	±0.22	±0.79
Sig.	NS	NS	*	NS	NS	NS	NS	NS	NS
Effect of interaction between oil sources and oil levels on egg quality of laying hens									
Soybean oil *1	73.44	348.33^{ab}	64.27^{c}	7.65^{a}	8.43^{b}	17.72	3.98	7.83	99.88^{a}
Soybean oil *1.5	76.41	355.00^{a}	64.42^{c}	7.98^{a}	8.54^{b}	17.86	4.13	7.50	101.34^{a}
Soybean oil *2	77.32	348.33^{ab}	67.55^{abc}	6.16^{b}	10.86^{a}	18.83	4.16	7.83	91.51^{b}
sunflower oil *1	77.12	343.33^{ab}	68.47^{abc}	7.83^{a}	9.51^{ab}	18.32	4.14	8.17	100.08^{a}
sunflower oil *1.5	76.85	345.00^{ab}	64.78^{bc}	6.91^{ab}	10.05^{ab}	18.00	4.10	8.17	96.39^{ab}
sunflower oil *2	77.14	330.00^{b}	70.30^{a}	7.54^{ab}	10.07^{ab}	19.21	4.21	8.17	98.87^{a}
Linseed oil *1	73.74	348.33^{ab}	66.35^{abc}	8.24^{a}	8.40^{b}	19.14	4.23	8.33	102.22^{a}
Linseed oil *1.5	77.09	338.33^{ab}	68.05^{abc}	7.50^{ab}	9.56^{ab}	18.34	4.04	7.50	98.52^{a}
Linseed oil *2	77.19	343.33^{ab}	70.13^{ab}	7.25a^{b}	9.84^{ab}	18.85	4.23	8.33	97.26^{ab}
MSE	±1.53	±4.77	±2.47	±0.35	±0.85	±0.46	±0.23	±0.27	±1.63
Sig.	NS	*	*	*	*	NS	NS	NS	*

^{a,b,c,d,e} *Mean values followed by different letters in the same column are significantly different*
* *Significant differences at the level of 0.05* ^{NS} *Not significant* ^{MSE} *mean standard error*

Our results of egg quality are compatible with those observed by Dos-Santos *et al.,* (2009) who reported that the inclusion of different vegetable oils in the diets of laying hens did not change the egg quality. Also, Küçükersan *et al.,* (2010) cited that, egg quality traits included (egg shell thickness, egg shell breaking strength, egg yolk index, egg albumen index, Haugh unit, and cholesterol content) did not significantly (P>0.05) modify by different dietary oil (3% sunflower, fish, soybean and hazelnut oil) supplementation. At the same time, Moura *et al*. (2019) discovered that lipid sources had no significant (P>0.05) influence on any of the egg-quality indicators studied (percentages of albumen, yolk, shell, eggshell thickness and specific gravity). In addition, dietary soybean oil supplementation had no effect on egg quality (P>0.05), according to Wei *et al*. (2021). Furthermore, as a consequence of employing different oil types and fat concentrations, (Gao *et al*., 2022) discovered no significant variations in eggshell index, eggshell strength, or eggshell thickness across different experimental groups. However, the Haugh unit was influenced differently (P≤0.05). On the other direction, supplemental linseed oil in the diet enhanced the quality of eggs and enriched them with ω-3, like EPA and DHA. Moreover, linseed oil is superior to other plant oils in terms of effectiveness (Farag *et al*., 2017; Mousa *et al*., 2017 and Alagawany *et al.,* 2018). Regarding to the color of the yolk, the results obtained by (Cachaldora *et al*., 2005) reported contrary results, claiming that adding soybean, linseed, or fish oils increased yolk color intensity.

Yolk fatty acid content:

Tables 4, 5, and 6 shows the FAs profile in egg yolks produced from all experimental groups of laying hens. The effect of oil sources on saturated FAs content in egg yolk of laying hens are presented

in Table 4. Seven saturated FAs were discovered (C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, and C21:0) in the analyzed eggs. The following FAs (Pentadecanoic, palmitic, Heptadecanoic, Arachnidan, and Heneicosanoic) were not affected (P>0.05) by the use of different sources of vegetable oils (soybean, sunflower and linseed oil) in laying hens' diets. While, a significant decrease in the level of (Myristic and Stearic) occurred as a result of using sunflower or linseed oil by 19.4, 51.2, 28.6, and 60.8% respectively, compared to soybean oil.

Regarding to the effect of oil levels on saturated FAs content in egg yolk of laying hens, there were no significant differences in the level of saturated FAs in egg yolk between the different levels of vegetable oils, except for stearic FA, which had a significant (P≤0.05) increase by increasing the level of vegetable oil in laying hens' diets from 1 to 2%. As for the effect of interaction between oil sources and levels on saturated FAs content in egg yolk of laying hens, significant differences were observed between treatments for all saturated FAs found in egg yolks except for the heptadecanoic and arachidic acids. Laying hens fed different levels of soybean oil produced eggs containing significantly (P≤0.05) the highest level of myristic acid. While the lowest (P≤0.05) content of myristic acid was found in egg yolks produced from chickens fed at a level of 1 or 2% linseed oil .

Egg yolks of birds fed diet containing 1% sunflower oil or 1.5% linseed oil achieved significantly (P≤0.05) the highest content of pentadecanoic acid. On the other hand, there were no traces of this acid in the egg yolk produced from layers fed on a diet containing 2% soybean oil. Egg yolks of birds fed diet containing 2% soybean oil achieved significantly (P≤0.05) the highest content of palmitic acid. While, the lowest was observed in the yolk of layers fed diet containing 2% sunflower oil. It is noted that, stearic acid was highly affected (P≤0.01) by the interaction between different sources and levels of vegetable oils, so that its level was as high as possible with egg yolk produced from chickens fed at the level of 1.5 or 2% soybean oil, while its lowest levels were observed with different levels of linseed oil or 1% sunflower oil. The use of linseed oil at different levels in the diets of laying hens led to the disappearance of heneicosanoic acid from the egg yolk, the same direction was observed with the level of 2% sunflower oil. On the other direction, egg yolks of birds fed diet containing 1.5% soybean oil achieved significantly (P≤0.05) the highest content of heneicosanoic acid.

The effects of different oil sources on MUFAs content in egg yolk of laying hens are presented in Table 5. Eight MUFAs were discovered (C14:1, C15:1-Cis 10, C16:1, C17:1-Cis 10, C18:1n9t, C18:1n9c, C20:1- Cis 11, and C24:1- Cis 15) in the analyzed eggs. The (Heptadecanoic) FA was not affected (P>0.05) by the use of different sources of vegetable oils (soybean, sunflower and linseed oil) in laying hens' diets. On the other direction, a significant increase in the level of (Myristoleic, Pentadecanoic, Palmitoleic, Oleic, Eicosenoic, and Tetracosenoic) occurred as a result of using sunflower or linseed oil, compared to soybean oil. While, Elaidic acid was significantly (P≤0.05) decreased as a result of using sunflower or linseed oil, compared to soybean oil. Regarding to the effect of different oil levels on mono unsaturated fatty acid content in egg yolk of laying hens, there were no significant differences in the level of MUFAs in egg yolk between the different levels of vegetable oils, except for palmitoleic fatty acid, which had a significant (P≤0.05) decrease by increasing the level of vegetable oil in laying hens' diets from 1 to 2%. At the same context, there was no significant difference (P>0.05) between the level of 1 and 1.5% of vegetable oil. As for the effect of interaction between oil sources and levels on mono unsaturated fatty acid content in egg yolk of laying hens, significant differences were observed between treatments for all MUFAs found in egg yolks except for the heptadecanoic acids. Hens fed different levels of soybean oil failed to produce eggs containing either of myristoleic and pentadecanoic acids. Whereas, hens fed at the level of 1% of sunflower oil achieved the highest level of myristoleic acid, followed by those fed 1 or 1.5% of linseed oil with a non-significant difference. Additionally, laying hens fed 1.5% of linseed oil produced eggs containing significantly (P≤0.01) the highest level of pentadecanoic and palmitoleic acid. Laying hens fed different levels of soybean oil produced eggs containing significantly (P≤0.05) the highest level of elaidic and eicosenoic acid. While the lowest (P≤0.05) was found in egg yolks produced from chickens fed at a level of 1.5% sunflower or linseed oil. On the other hand, laying hens fed different levels of linseed oil produced eggs containing significantly (P≤0.01) the highest level of tetracosenoic acid. While the lowest (P≤0.01) was found in egg yolks produced from chickens fed at a level of 1% soybean oil.

It is noted that, oleic acid was highly affected (P≤0.01) by the interaction between different sources and levels of vegetable oils, so that its level was as high as possible with egg yolk produced from chickens fed at the level of 1% linseed oil, while its lowest levels were observed with different levels of linseed oil or 1% sunflower oil, followed by those received different levels of sunflower oil. On the other direction, egg yolks of birds fed diet containing different levels of soybean oil achieved significantly (p≤0.01) the lowest content of oleic acid.

Table (4): Effects of different sources and levels of vegetable oil on saturated fatty acid profile in egg of laying hens.

Treatment	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C21:0
Effect of oil sources on saturated fatty acid content in egg yolk of laying hens							
Soybean oil	0.42^{a}	0.03	25.79	0.23	19.55^{a}	0.03	0.05
Sunflower oil	0.34^{b}	0.03	24.27	0.18	9.55^{b}	0.06	0.01
Linseed oil	0.30^{b}	0.04	22.73	0.17	7.66^{c}	ND	ND
MSE	±0.01	±0.01	±0.38	±0.02	±0.47	±0.06	±0.02
Sig.	*	NS	NS	NS	**	NS	NS
Effect of oil levels on saturated fatty acid content in egg yolk of laying hens							
1 %	0.36	0.04	24.88	0.15	10.69^{b}	0.06	0.02
1.5 %	0.35	0.04	24.36	0.2	12.74^{ab}	0.02	0.03
2 %	0.34	0.02	23.55	0.23	13.35^{a}	0.01	0.01
MSE	±0.01	±0.01	±0.22	±0.02	±0.37	±0.02	±0.02
Sig.	NS	NS	NS	NS	*	NS	NS
Effect of interaction between oil sources and oil levels on saturated fatty acid content in egg yolk							
Soybean oil *1	0.43^{a}	0.04^{ab}	25.54^{abc}	0.14	16.85^{b}	0.02	0.05^{ab}
Soybean oil *1.5	0.41^{a}	0.04^{ab}	25.25^{abcd}	0.28	20.66^{a}	0.05	0.07^{a}
Soybean oil *2	0.41^{a}	ND^{c}	26.58^{a}	0.28	21.15^{a}	0.03	0.04^{ab}
sunflower oil *1	0.38^{ab}	0.05^{a}	25.83^{ab}	0.16	7.67^{d}	0.17	0.02^{ab}
sunflower oil *1.5	0.33^{ab}	0.03^{ab}	25.11^{abcd}	0.15	10.08^{c}	0.01	0.01^{ab}
sunflower oil *2	0.31^{ab}	0.02^{bc}	21.86^{d}	0.22	10.91^{c}	ND	ND^{b}
Linseed oil *1	0.28^{b}	0.04^{ab}	23.26^{abcd}	0.16	7.54^{d}	ND	ND^{b}
Linseed oil *1.5	0.32^{ab}	0.05^{a}	22.73^{bcd}	0.17	7.47^{d}	ND	ND^{b}
Linseed oil *2	0.29^{b}	0.04^{ab}	22.20^{cd}	0.18	7.98^{d}	ND	ND^{b}
MSE	±0.02	±0.01	±0.49	±0.03	±0.52	±0.16	±0.05
Sig.	*	*	*	NS	**	NS	*

^{a,b,c,d,e} *Mean values followed by different letters in the same column are significantly different*
* *Significant differences at the level of 0.05* ** *Significant differences at the level of 0.01*
^{NS} *Not significant* ^{MSE} *mean standard error*

The effects of various oil sources on PUFAs content in egg yolk of laying hens are presented in Table 6. Nine PUFAs were discovered (C18:2n6t, C18:2n6c, C18:3n6, C18:3n3, C20:2, C20:3n6, C20:4n6, C20:3n3, and C22:6ns) in the analyzed eggs. The linolic fatty acid (C18:2n6c) was not affected (P>0.05) by the use of different sources of vegetable oils (soybean, sunflower and linseed oil) in laying hens' diets. On the other direction, linolelaidic FA (C18:2n6t) content was significantly (P≤0.05) decreased by using sunflower or linseed oil in laying hens diet by 51.7 and 91.4% as compared with soybean oil. The same direction was observed for the content of eicosadienoic (C20:2) fatty acid, which was significantly (P≤0.01) decrease by using sunflower or linseed oil in laying hens diet by 43.5 and 56.5% as compared with soybean oil. In addition, layer fed diet containing either sunflower or linseed oil produced eggs not containing significantly (P≤0.01) any concentration of DHA (C22:6ns) as compared to those fed soybean oil. The inclusion of sunflower oil in the layer diets was able to improve significantly (P≤0.01) the concentration of γ-Linolenic (C18:3n6) FA in the egg yolk. At the same time, there were no significant differences (P>0.05) between those fed on soy oil and those fed on linseed oil. Moreover, there was a non-significant increase in the level of eicosatrienoic acid with the use of sunflower oil. Layer birds fed linseed oil had the lowest content of eicosatrienoic acid in there egg yolk. The inclusion of sunflower oil in the layer diets was able to improve significantly (P≤0.01) the concentration of arachidonic (C20:4n6) FA in the egg yolk by 22.9% as compared to those fed on soybean oil. While, the inclusion of linseed oil in the layer diets was reduced significantly (P≤0.01) the concentration of arachidonic (C20:4n6) FA in the egg yolk by 27.1% as compared to those fed on soybean oil. Additionally, the inclusion of linseed oil in the layer diets was able to improve significantly (P≤0.01) the concentration of α-linolenic (C18:3n3) and eicosatrienoic (C20:3n3) FA in the egg yolk as compared to those fed on soybean or sunflower oil. Regarding to the effect of different oil levels on PUFAs content in egg yolk of laying hens, there were no significant differences in the level of PUFs in egg yolk between the different levels of vegetable oils, except for linolelaidic (C18:2n6t) and α-linolenic (C18:3n3) FAs, which were significant increased by increasing the level of vegetable oil in laying hens' diets from 1 to 2% .

As for the effect of interaction between oil sources and levels on PUFAs content in egg yolk of laying hens, significant differences were observed between treatments for all PUFAs found in egg yolk. Egg yolk of laying hens fed soybean oil at the levels of 1.5 or 2% had significantly (P≤0.05) the highest concentration of linolelaidic FA (C18:2n6t) followed by those received 2% sunflower oil as compared

with other treatments. Hens fed at the level of 2% of sunflower oil achieved the highest level of linoleic acid (C18:2n6c), followed by those fed 1.5% soybean oil. While, laying hens fed 1% of linseed oil produced eggs containing significantly (P≤0.05) the lowest level of linoleic FA. As for the content of γ-Linolenic FA in egg yolk, group fed diet containing 1 or 2% sunflower oil had significantly (P≤0.01) the highest content of γ-Linolenic. While, the lowest was found for those fed on diet with 2% linseed oil. On the other hand, laying hens fed diet with 1.5 or 2% linseed oil significantly (P≤0.01) produced the highest content of α-Linolenic FA in their yolk. While, the lowest was found for those fed different levels of soybean or sunflower oil. Additionally, it is noticed through this study that there was a significant increase in the yolk content of oleic acid as a result of feeding on high levels of linseed oil (2%). In the same context, this FA completely disappeared from the egg yolk of chickens fed with different levels of soy or sunflower oil or with a level of 1% of linseed oil. Hens fed different levels of sunflower oil significantly (P≤0.01) succeeded in depositing the largest amount of arachidonic acid in the egg yolk produced from it. Whereas, the egg yolk produced from hens fed different levels of linseed oil, especially at the level of 2%, achieved significantly (P≤0.01) the lowest content of the same acid. As for the content of DHA (C22:6ns) in egg yolk, group fed diet with different levels of soybean oil had significantly (P≤0.01) the highest content of DHA. In addition, no traces of this acid were found in the groups fed on different levels of either sunflower or linseed oil.

Table (5): Effects of different sources and levels of vegetable oil on mono unsaturated fatty acid profile in egg of laying hens

Treatment	C14:1	C15:1 Cis -10-	C16:1	C17:1 Cis -10-	C18:1n9t	C18:1n9c	C20:1 Cis -11-	C24:1 Cis -15-
Effect of oil sources on mono unsaturated fatty acid content in egg yolk of laying hens								
Soybean oil	ND^{b}	ND^{b}	0.99^{c}	0.06	0.18^{a}	34.67^{b}	0.27^{a}	0.09^{c}
Sunflower oil	0.05^{a}	0.03^{a}	2.47^{b}	0.06	0.09^{b}	45.83^{a}	0.24^{b}	0.24^{b}
Linseed oil	0.06^{a}	0.03^{a}	3.13^{a}	0.07	0.12^{ab}	47.08^{a}	0.19^{c}	1.02^{a}
MSE	±0.01	±0.01	±0.12	±0.01	±0.02	±0.41	±0.02	±0.18
Sig.	**	**	**	NS	*	**	*	*
Effect of oil levels on mono unsaturated fatty acid content in egg yolk of laying hens								
1 %	0.05^{a}	0.02	2.57^{a}	0.05	0.12	44.07	0.25	0.45
1.5 %	0.04^{ab}	0.03	2.26^{a}	0.06	0.11	42.05	0.22	0.47
2 %	0.02^{b}	0.01	1.77^{b}	0.08	0.15	41.46	0.23	0.42
MSE	±0.01	±0.01	±0.16	±0.02	±0.03	±0.12	±0.06	±0.04
Sig.	NS	NS	**	NS	NS	NS	NS	NS
Effect of interaction between oil sources and oil levels on mono unsaturated fatty acid content in egg yolk								
Soybean oil *1	ND^{c}	ND^{c}	1.24d^{e}	0.06	0.15^{ab}	38.15^{c}	0.28^{a}	ND^{c}
Soybean oil *1.5	ND^{c}	ND^{c}	0.88^{e}	0.06	0.18^{ab}	33.47^{d}	0.26^{ab}	0.12^{bc}
Soybean oil *2	ND^{c}	ND^{c}	0.86^{e}	0.07	0.22^{a}	32.39^{d}	0.28^{a}	0.14^{bc}
sunflower oil *1	0.09^{a}	0.02^{bc}	3.46^{ab}	0.05	0.11^{bc}	44.79^{b}	0.25^{abc}	0.26^{b}
sunflower oil *1.5	0.05^{b}	0.04^{ab}	2.38^{c}	0.06	0.05^{c}	47.50^{ab}	0.23^{abc}	0.24^{bc}
sunflower oil *2	0.02^{c}	0.04^{ab}	1.57^{d}	0.08	0.10^{bc}	45.21^{ab}	0.23^{abc}	0.21^{bc}
Linseed oil *1	0.06^{ab}	0.03^{ab}	3.02^{ab}	0.05	0.11^{bc}	49.28^{a}	0.21^{abc}	1.08^{a}
Linseed oil *1.5	0.07^{ab}	0.05^{a}	3.51^{a}	0.05	0.11^{bc}	45.17^{b}	0.18^{c}	1.05^{a}
Linseed oil *2	0.05^{b}	ND^{c}	2.87^{c}	0.10	0.14^{b}	46.78^{ab}	0.191^{bc}	0.92^{a}
MSE	±0.01	±0.01	±0.19	±0.02	±0.02	±0.45	±0.08	±0.06
Sig.	**	**	**	NS	*	**	*	**

^{a,b,c,d,e} *Mean values followed by different letters in the same column are significantly different*

* *Significant differences at the level of 0.05* ** *Significant differences at the level of 0.01*

^{NS} *Not significant* ^{MSE} *mean standard error*

In general, it is noted that the addition of sunflower oil or linseed oil at its different levels led to an improvement in the level of MUFAs. These results are compatible with those observed by (Ceylan *et al.*, 2011) who cited that, dietary fish oil significantly (P<0.01) increased the deposition of docosahexaenoic acid in the egg yolk. Additionally, Oliveira *et al*., (2010) found that laying hens fed a soybean oil diet produced eggs with high levels of PUFAs omega-6 (n-6) and omega-3 (n-3) in the yolks (23.55, 2.30%, respectively), whereas hens fed a beef tallow diet produced eggs with higher levels of MUFAs (47.53%) compared to hens fed a control diet (38.72%).

The levels of trans fats in the egg yolks across all treatments (0.91, 0.11, and 0.05%) were regarded as being extremely low. According to Omidi *et al*., (2015), fish oil dramatically boosted DHA and EPA while considerably decreasing omega-6 FAs in egg yolks. The egg yolk's linolenic acid content was also

boosted by canola. The same conclusion was reported by Kamely *et al*., (2016) who cited that, supplementation of fish oil increased percentage of EPA and DHA in egg yolk (P<0.05). The addition of fish oil to the diet of quails raised the amount of omega-3 FAs and decreased the amount of omega-6 in the yolk (P<0.01) as well as lowered the n-6/n-3 ratios from 30.63 to 5.25. In addition, Batkowska *et al*., (2021) reported that the addition of soybean or linseed oil to feed mixtures of laying hens significantly increased the content of PUFAs in egg yolks and the content of n3 FAs group. Additionally, Gao *et al*., (2022) found that incorporated of 1.5% and 3% soybean oil to feed mixtures of laying hens increased the content of MUFAs/PUFAs in egg yolk.

Table (6): Effects of different sources and levels of vegetable oil on poly unsaturated fatty acid profile in egg of laying hens

Treatment	C18:2n6t	C18:2n6c	C18:3n6	C18:3n3	C20:2	C20:3n6	C20:4n6	C20:3n3	C22:6ns
Effect of oil sources on poly unsaturated fatty acid content in egg yolk of laying hens									
Soybean oil	0.58^{a}	15.07	0.08^{b}	0.30^{b}	0.23^{a}	0.14^{ab}	0.96^{b}	ND^{b}	0.18^{a}
Sunflower oil	0.28^{b}	14.55	0.11^{a}	0.21^{b}	0.13^{b}	0.16^{a}	1.18^{a}	ND^{b}	ND^{b}
Linseed oil	0.05^{c}	12.62	0.07^{b}	3.63^{a}	0.10^{b}	0.11^{b}	0.70^{c}	0.05^{a}	ND^{b}
MSE	±0.02	±0.40	±0.01	±0.08	±0.01	±0.01	±0.04	±0.01	±0.03
Sig.	*	NS	**	**	**	**	**	**	**
Effect of oil levels on poly unsaturated fatty acid content in egg yolk of laying hens									
1 %	0.05^{c}	13.76	0.09	0.89^{b}	0.16	0.13	1.00	ND	0.09
1.5 %	0.32^{b}	13.74	0.08	1.53^{a}	0.13	0.14	0.96	0.02	0.04
2 %	0.54^{a}	14.75	0.08	1.71^{a}	0.17	0.14	0.88	0.03	0.05
MSE	±0.03	±0.04	±0.01	±0.03	±0.02	±0.01	±0.03	±0.01	±0.02
Sig.	*	NS	NS	**	NS	NS	NS	NS	NS
Effect of interaction between oil sources and oil levels on poly unsaturated fatty acid content in egg yolk of laying hens									
Soybean oil *1	0.02^{b}	14.83^{abc}	0.09^{bc}	0.27^{c}	0.22^{ab}	0.17^{b}	1.02^{ab}	ND^{c}	0.28^{a}
Soybean oil *1.5	0.87^{a}	15.50^{ab}	0.08^{cd}	0.32^{c}	0.24^{a}	0.14^{bc}	0.94^{bc}	ND^{c}	0.13^{ab}
Soybean oil *2	0.85^{a}	14.89^{abc}	0.07^{cd}	0.31^{c}	0.22^{ab}	0.11^{c}	0.93^{bc}	ND^{c}	0.14^{ab}
sunflower oil *1	0.07^{b}	14.91^{abc}	0.11^{a}	0.28^{c}	0.15^{c}	0.13^{bc}	1.15^{a}	ND^{c}	ND^{b}
sunflower oil *1.5	0.05^{b}	11.96^{cd}	0.10^{ab}	0.18^{c}	0.05^{e}	0.14^{bc}	1.21^{a}	ND^{c}	ND^{b}
sunflower oil *2	0.71^{ab}	16.79^{a}	0.11^{a}	0.17^{c}	0.19^{bc}	0.22^{a}	1.19^{a}	ND^{c}	ND^{b}
Linseed oil *1	0.06^{b}	11.53^{d}	0.07^{d}	2.13^{b}	0.10^{d}	0.10^{c}	0.83^{bc}	ND^{c}	ND^{b}
Linseed oil *1.5	0.05^{b}	13.75^{abcd}	0.07^{d}	4.09^{a}	0.10^{d}	0.13^{bc}	0.74^{c}	0.07^{b}	ND^{b}
Linseed oil *2	0.05^{b}	12.58^{bcd}	0.06^{e}	4.66^{a}	0.10^{d}	0.10^{c}	0.53^{d}	0.09^{a}	ND^{b}
MSE	±0.04	±0.60	±0.01	±0.09	±0.02	±0.01	±0.06	±0.01	±0.05
Sig.	*	*	**	**	**	**	**	**	**

a,b,c,d,e Mean values followed by different letters in the same column are significantly different
** Significant differences at the level of 0.05 ** Significant differences at the level of 0.01*
NS Not significant MSE mean standard error

Blood chemical parameters and oxidative statues:

Data presented in Table 7 showed the effect of different oil sources and levels on some blood biochemical constituents of laying hens. Total protein, globulin, AST, ALT, total lipids and VLDL concentrations were not significantly (P>0.05) affected by different oil sources. However, plasma albumin, triglycerides, total cholesterol, HDL and LDL-cholesterol concentrations were significantly (P≤0.01) affected by different oil sources. Whereas, Plasma concentrations of albumin, triglycerides, total cholesterol and LDL were decreased significantly by using linseed oil (P≤0.01). In the opposite direction, Plasma concentrations of HDL was increased significantly (P≤0.01) by using sunflower or linseed oil. In general, linseed oil has the ability to improve the biochemical characteristics of blood, especially the lipid and cholesterol profile, followed directly by sunflower oil compared to soybean oil.

Regarding to the effect of different oil levels on some blood biochemical constituents, there were no significant differences (P>0.05) in the concentrations of total protein, albumin, globulin, HDL, LDL and VLDL between the different levels of vegetable oils. On the other hand, the activity of AST and ALT enzymes was significantly increased by increasing the level of vegetable oil from 1 up to 2%. The same direction was observed for the concentrations of total lipids, triglyceride, and total cholesterol which were significantly increased by increasing the level of vegetable oil from one up to 2%. As for the effect of interaction between oil sources and levels on some blood biochemical constituents of laying hens, there were no significant differences (P>0.05) in the concentrations of total protein, globulin and VLDL.

On the other hand, group fed dietary soybean oil at the level of 1% had significantly (P≤0.05) the highest concentration of albumin. However, the lowest was observed for groups fed different levels of linseed oil.

Regarding to the activity of ALT enzyme, group fed dietary soybean oil at the level of 1% had significantly (P≤0.01) the higher activity of ALT enzyme. While, the lower was observed for groups fed linseed oil at the level of 1.5%. Groups fed diet with the highest level (2%) of different oil sources significantly (P≤0.01) had the highest activity of AST enzyme. While, the lowest activity was observed for group fed linseed at the level of 1%. The concentrations of total lipids and triglyceride were significantly (P≤0.01) increased by using the highest level (2%) of soybean oil. At the same time, linseed oil at the level of 1% has the ability to decrease the concentrations of total lipids and triglyceride followed by sunflower oil and then soybean oil at the level of 1%. In addition, the use of different levels of sunflower or linseed oil up to 2% led to a significant (P≤0.01) improvement in the level of cholesterol, especially the type of HDL-cholesterol. Moreover, layer fed diet with different levels of linseed oil significantly (P≤0.01) had the lowest concentration of LDL-cholesterol.

These findings are consistent with those of Aly *et al.* (2003), who found that broiler chickens given dietary black seed oil had lower plasma cholesterol concentrations (P≥0.05) than those fed the control diet. The active substance that works as an inhibitor to the active enzyme hepatic 3-hydroxyl-3 methyglutaryl coenzyme A (HMG-CoA), which synthesizes cholesterol, is thought to be the cause of the fall in plasma cholesterol levels (Crowell, 1999). Additionally, this decrease in serum cholesterol may in some cases be attributable to a decrease in certain hormones secreted by the cortex of the adrenal glands, which in turn results in a decrease in the release of FAs from adipose tissues or a decrease in fat oxidation, which in turn results in a decrease in the level of FAs, including blood cholesterol (Ganong, 2005). It is possible that the hypocholesterolemic and hypolipidemic effects of sunflower or linseed oil, which inhibit the hepatic activities of lipogenic and cholesterogenic enzymes like malic enzyme, FA synthase, and glucose-6-phosphatase dehydrogenase, are the cause of the changes in the mean values of plasma HDL-cholesterol in the birds fed dietary sunflower or linseed oil (Chi *et al*., 1982) and 3 hydroxyl 3 methyl-glutaryl-CoA (HMG-CoA) reductase (Qureshi *et al.*, 1987).

Our results are compatible with those observed by Dong *et al*., (2018) who showed that, serum AST and uric acid concentrations were increased (P<0.05) by fish oil during the majority of the first 2 months of the trial. In addition, Ekine *et al*., (2020) showed that albumin, total bilirubin, sodium, potassium and urea were not significantly influenced by dietary treatments (soybean oil, fish oil and coconut oil).

Data presented in Table 8 showed the effect of different sources and levels of vegetable oil on the on antioxidants status of Bovens hens. The results indicate a significant (P≤0.01) improvement in the level of antioxidants for the groups fed on diet containing linseed oil, followed by those fed dietary sunflower oil, compared to the control group that fed on soybean oil. Total antioxidants capacity was significantly (P≤0.05) increased for group treated with linseed oil by 13.8% compared to those received soybean oil. At the same time, there were no significant differences between the group fed on linseed oil and those fed on sunflower oil, and there were no significant differences between the group fed on sunflower oil and those fed on soybean oil. The same trend was observed for SOD, which was significantly (P≤0.01) increased for group received linseed oil by 17.55% compared to those received soybean oil. On the other direction, MDA was significantly (P≤0.01) decreased by using linseed oil in Bovens hens diet's by 27.8% compared to those received soybean oil. Regarding to the effect of different levels of vegetable oil on antioxidants status of Bovens hens, there were no significant differences in the level of TAC and MDA between the different levels of vegetable oils, while the concentration of SOD was significantly (P≤0.05) increased by increasing the level of vegetable oil in laying hens' diets from 1 to 2%. As for the effect of interaction between oil sources and levels on the antioxidants status of laying hens, there were significant differences observed in the concentrations of TAC, SOD and MDA. Whereas, group fed dietary linseed oil at the level of 2% had the highest (P≤0.01) concentration of TAC and SOD followed by those received 1.5% linseed oil compared to other groups. In the opposite trend, layer received linseed oil at the level of 2% significantly (P≤0.01) had the lowest concentration of MDA followed by those received 1.5% and then fed 1% linseed oil compared to the control. By lowering the concentration of MDA and raising TAC and SOD, linseed oil has demonstrated an antioxidative impact in the current study. High levels of free radical generation in the body can result in oxidative stress, which in turn causes oxidative damage to vital biomolecules and many chronic illnesses (Poulsen *et al.*, 1998). The defensive mechanism of the living system includes antioxidant enzymes, and animals may consume antioxidants through their diet in the form of vitamins and minerals. Earlier research shown that the seeds' flavonoids, notably apigenin and luteolin, gave UFAs oils a high level of antioxidant activity (Leung, 1980).

Table (7): Effects of different sources and levels of vegetable oil on some blood biochemical parameters of laying hens

Treatment	TP g/dL	Alb. g/dL	Glob. g/dL	ALT U/L	AST U/L	Total lipids mg/dL	Trigly. mg/dL	Choles. mg/dL	HDL mg/dL	LDL mg/dL	VLDL mg/dL
Effect of oil sources on blood biochemical of laying hens											
Soybean oil	5.39	2.51^{a}	2.88	43.89	113.64	396.56	132.56^{a}	177.44^{a}	31.30^{c}	136.67^{a}	9.48
Sunflower oil	5.32	2.40^{a}	2.92	43.87	111.70	413.00	123.22^{a}	157.67^{b}	37.93^{b}	112.11^{b}	7.62
Linseed seed oil	5.29	2.17^{b}	3.12	42.26	113.46	379.00	112.78^{b}	158.44^{b}	48.93^{a}	100.78^{c}	8.73
MSE	±0.11	±0.07	±0.12	±1.13	±4.79	±11.43	±3.56	±2.14	±0.89	±2.16	±0.87
Sig.	NS	**	NS	NS	NS	NS	**	**	**	**	NS
Effect of oil levels on blood biochemical of laying hens											
1 %	5.48	2.50	2.98	42.21^{b}	100.20^{c}	357.44^{c}	112.00^{b}	157.67^{b}	36.32	112.44	8.90
1.5 %	5.14	2.25	2.89	42.22^{b}	110.31^{b}	397.33^{b}	124.11^{a}	165.67^{ab}	39.00	117.67	9.00
2 %	5.38	2.32	3.06	45.58^{a}	128.29^{a}	433.78^{a}	132.44^{a}	170.22^{a}	42.84	119.44	7.93
MSE	±0.14	±0.13	±0.16	±1.01	±3.02	±5.25	±1.75	±4.56	±0.19	±2.61	±2.15
Sig.	NS	NS	NS	*	**	**	**	*	NS	NS	NS
Effect of interaction between oil sources and oil levels on blood biochemical of laying hens											
Soybean oil *1	5.47	2.70^{a}	2.77	47.13^{a}	100.10^{cd}	356.67^{e}	121.67^{cd}	162.00^{c}	30.33^{f}	122.67^{c}	9.00
Soybean oil *1.5	5.20	2.30^{bc}	2.90	42.10^{ab}	110.00^{bc}	392.67^{c}	130.67^{b}	178.67^{b}	30.67^{f}	137.33^{b}	10.37
Soybean oil *2	5.50	2.53^{abc}	2.97	42.43^{ab}	130.83^{a}	440.33^{a}	145.33^{a}	191.67^{a}	32.60^{ef}	150.00^{a}	9.07
sunflower oil *1	5.47	2.60^{ab}	2.87	44.57^{ab}	101.10^{bcd}	380.67^{cd}	113.67^{d}	155.00^{c}	34.73^{e}	112.67^{d}	7.60
sunflower oil *1.5	5.13	2.30^{bc}	2.83	44.30^{ab}	109.93^{bc}	425.00^{b}	125.00^{bc}	157.33^{c}	38.27^{d}	11.33^{d}	7.73
sunflower oil *2	5.37	2.30^{bc}	3.07	42.73^{ab}	124.07^{a}	433.33^{ab}	131.00^{b}	160.67^{c}	40.80^{d}	112.33^{d}	7.53
Linseed seed oil *1	5.50	2.20^{c}	3.30	45.03^{ab}	99.40^{d}	335.00^{f}	100.67^{e}	156.00^{c}	43.90^{c}	102.00^{e}	10.10
Linseed seed oil *1.5	5.10	2.17^{c}	2.93	40.23^{b}	111.00^{b}	374.33^{d}	116.67^{cd}	161.00^{c}	47.77^{b}	104.33^{de}	8.90
Linseed seed oil *2	5.27	2.13^{c}	3.13	41.50^{ab}	129.97^{a}	427.67^{ab}	121.00^{cd}	158.33^{c}	55.13^{a}	96.00^{e}	7.20
MSE	±0.27	±0.15	±0.16	±0.98	±4.18	±8.96	±0.98	±2.08	±0.23	±1.76	±1.70
Sig.	NS	*	NS	**	**	**	**	**	**	**	NS

^{a,b,c,d,e,f} *Mean values followed by different letters in the same column are significantly different*
* *Significant differences at the level of 0.05* ** *Significant differences at the level of 0.01* ^{NS} *Not significant* ^{MSE} *mean standard error*
Tp = Total protein Alb.= Albumin Glob. = Globulin AST = Aspartate transaminase
ALT = Alanine amino transferees Trig.= Triglycerides Choles. = Cholesterol
HDL= High density lipoprotein LDL = Low density lipoprotein

Our findings are consistent with those made by Kamely *et al*. (2016), who reported that adding savory essential oil to the diet considerably (P<0.01) decreased MDA generation in egg yolks after 21 days of storage. In fresh eggs, eggs kept at room temperature, and eggs stored at 4°C, egg yolk lipid oxidation rates were greater (P<0.05) in the fish oil-containing groups. Additionally, According to Dong *et al.* (2018), the serum MDA concentrations in the fish oil group at weeks 2, 4, 6, and 12 were higher (P<0.05) than those in the soybean oil group. At weeks two and four, the fish oil group's serum MDA concentration was substantially (P<0.05) greater than that of the coconut oil group. The average MDA concentration varied significantly (P<0.05) across the three treatments, with the fish oil treatment having the greatest concentration and the soybean oil treatment having the lowest. Moreover, Gao *et al.* (2022) investigated how different oil types and fat concentrations affected egg quality, production efficiency, and laying hens' antioxidant capacity. They discovered that all groups' glutathione peroxidase and superoxide dismutase concentrations had increased, with the lard-treated group showing the greatest increase.

Table (8): Effects of different sources and levels of vegetable oil on antioxidants status of laying hens.

Treatment	TAC(m mol/l)	SOD (m mol/l)	MDA (m mol/l)
Effect of oil sources on antioxidants activity of laying hens			
Soybean oil	0.94b	4.67b	7.09a
Sunflower oil	0.99ab	4.73b	6.99a
Linseed seed oil	1.07a	5.49a	5.12b
MSE	±0.02	±0.20	±0.08
Sig.	*	**	**
Effect of oil levels on antioxidants activity of laying hens			
1 %	0.98	4.75b	6.35
1.5 %	0.99	4.84ab	6.43
2 %	1.03	5.30a	6.42
MSE	±0.02	±0.09	±0.08
Sig.	NS	*	NS
Effect of interaction between oil sources and oil levels on antioxidants activity of laying hens			
Soybean oil *1	0.90c	4.68c	6.92ab
Soybean oil *1.5	0.94bc	4.59c	7.05ab
Soybean oil *2	0.99bc	4.74c	7.31a
sunflower oil *1	1.06abc	4.68c	6.75b
sunflower oil *1.5	0.97bc	4.70c	6.97ab
sunflower oil *2	0.96bc	4.82c	7.26a
Linseed seed oil *1	0.98bc	4.88c	5.38c
Linseed seed oil *1.5	1.07ab	5.23b	5.28c
Linseed seed oil *2	1.15a	6.35a	4.70d
MSE	±0.02	±0.09	±0.11
Sig.	*	**	**

[a,b,c,d] Mean values followed by different letters in the same column are significantly different
[*] Significant differences at the level of 0.05 *[**] Significant differences at the level of 0.01*
[NS] Not significant *[MSE] mean standard error*

CONCLUSION

In conclusion, the use of both sunflower oil and linseed oil up to 2% in the laying hens are able to improve egg production, egg quality, the level of essential FAs in egg yolk, in addition improving the level of antioxidants and blood biochemical parameters.

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تأثير علائق غنية بالأحماض الدهنية من نوع الأوميجا 3 ، 6 ، 9 علي الأداء الانتاجي والخصائص الفسيولوجية للدجاج البياض

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تهدف هذه الدراسة الي تقييم تأثير مصادر ومستويات مختلفة من الزيوت النباتية كمصدر للأحماض الدهنية أوميجا 3 ، 6 و 9 على الأداء الإنتاجي وجودة البيض مستوي الأحماض الدهنية بصفار البيض وبعض الصفات الفسيولوجية للدجاج البياض. تم تقسيم مائتين وسبعين دجاجة لوهمان بياضة بعمر 45 أسبوعًا بشكل عشوائي إلى تسعة معاملات ناتجة عن تصميم عاملي (3 × 3) ثلاثة مصادر للزيت النباتي (زيت فول الصويا، عباد الشمس وزيت بذر الكتان) بثلاثة مستويات (1 ، 1.5 و 2٪). أظهرت النتائج المتحصل عليها أن وزن الجسم النهائي ، ونسبة إنتاج البيض اليومي ، واستهلاك العلف ، وخصائص جودة البيض ، والبروتين الكلي للبلازما ، والجلوبيولين ، وإنزيمات الكبد ، والدهون الكلية ، وتركيزات الكولسترول منخفض الكثافة لم تتأثر معنويا ($P>0.05$) بمصادر أو مستويات الزيوت النباتية. أدى استخدام زيت الكتان في علائق الدجاج البياض إلى زيادة معنوية ($P\leq0.05$) في كتلة البيض ووزن البيض المنتج من تلك الدجاجات. تحسينت نسبة التحويل الغذائي للدجاج البياض المغذاة على علائق بذر الكتان أو زيت عباد الشمس معنويا ($P\leq0.05$) مقارنة بتلك التي تناولت زيت فول الصويا في نظامها الغذائي. كانت هناك زيادة معنوية في مستوى الأحماض الدهنية الأحادية غير المشبعة في صفار البيض نتيجة استخدام زيت عباد الشمس أو الكتان مقارنة بزيت فول الصويا. أدى إدراج زيت عباد الشمس في العلف إلى تحسين معنوي ($P\leq0.01$) في تركيز الفا - لينولينك الأراكيدونك في صفار البيض. أدى استخدام مستويات مختلفة من زيت عباد الشمس أو زيت الكتان بنسبة تصل إلى 2٪ إلى تحسن كبير ($P\leq0.01$) في مستوى الكوليسترول عالي الكثافة. علاوة على ذلك ، فإن النظام الغذائي الذي تم تغذيته بمستويات مختلفة من زيت بذور الكتان قلل معنويا ($P\leq0.01$) من تركيز الكوليسترول منخفض الكثافة. لوحظ تحسن معنوي ($P\leq0.01$) في مستوى مضادات الأكسدة للمجموعات التي تتغذى على نظام غذائي يحتوي على زيت الكتان ، تليها تلك التي تغذت على زيت عباد الشمس ، مقارنة بالمجموعة الضابطة التي تغذت على زيت فول الصويا. في الختام ، فإن استخدام كل من زيت عباد الشمس أو زيت بذر الكتان بنسبة تصل إلى 2٪ في النظام الغذائي للدجاج البياض قادر على تحسين إنتاج البيض ، وجودة البيض ، ومستوى الأحماض الدهنية الأساسية في صفار البيض ، بالإضافة إلى تحسين مستوى مضادات الأكسدة و الخصائص الكيموحيوية للدم.

الكلمات المفتاحية: *الدجاج البياض ، الزيوت النباتية ، الأوميجا -3 ، الأوميجا -6 ، الأوميجا -9 ، جودة البيض والحالة الفسيولوجية*