

Effect of Adding Linseed and Selenium to Diets of Layer Hen's on Performance, Egg Fatty Acid Composition and Selenium Content

Emel GÜRBÜZ *  Tahir BALEVİ * Behiç COŞKUN * Özcan Barış ÇİTİL *

* Department of Animal Nutrition & Nutritional Diseases, Faculty of Veterinary Medicine, Selcuk University, TR-42075 Konya - TURKEY

Makale Kodu (Article Code): KVFD-2011-5816

Summary

In a 4×3 factorial experiment, six hundred 31-week-old laying hens were fed diets containing 0, 25, 50 and 100 g/kg linseed with control, 0.3 mg/kg organic selenium (OrSe) and 0.3 mg/kg inorganic selenium (InSe) to determine the effects of diet on performance, eggshell parameters, egg fatty acid composition and selenium (Se) content of eggs. Laying hens were randomly assigned to 12 experimental groups with 5 replications. Linseed supplementation decreased egg production (d 1-30), feed consumption (d 1-30 and 1-90) and egg weight (d 60-90 and 1-90). Shell weight and thickness decreased when dietary linseed was increased. There were interactions between Se and linseed in feed consumption, feed efficiency, damaged egg ratio and shell thickness. The inclusion of InSe and OrSe increased the Se content of the eggs. Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), α -linolenic acid (ALA) and n-3 fatty acid concentrations in egg yolks increased with increasing dietary linseed. There were also interactions between linseed and Se in ALA and n-3 fatty acid concentrations. In conclusion, the addition of linseed to the diets increased DHA, EPA, ALA and n-3 fatty acid concentrations in egg yolks, and the addition of Se increased the Se content of the eggs. Egg fatty acids (ALA, n-3 fatty acid) were increased by supplementing dietary linseed with OrSe.

Keywords: *Egg, Linseed, n-3 fatty acid composition, Performance, Selenium*

Yumurtacı Tavuk Rasyonlarına Keten Tohumu ve Selenyum İlavesinin Performans, Yumurta Yağ Asidi Kompozisyonu ve Selenyum İçeriğine Etkisi

Özet

Dizaynı 4×3 faktöriyel olarak yapılan bu çalışma, 31 haftalık yaşta 600 adet yumurtacı tavuk rasyonlarına 0, 25, 50 ve 100 g/kg keten tohumu ile birlikte kontrol, 0.3 mg/kg organik selenyum (OrSe) ve 0.3 mg/kg inorganik selenyum (InSe) ilave edilmesinin performans, yumurta kabuğu parametreleri, yumurta yağ asidi kompozisyonları ve yumurta selenyum (Se) içeriğine etkilerinin belirlenmesi amacıyla yapıldı. Yumurtacı tavuklar rastgele herbiri 5 alt grup içeren 12 gruba ayrıldı. Keten tohumu ilavesi yumurta verimini (1-30 gün), yem tüketimini (1-30 ve 1-90 gün) ve yumurta ağırlığını (60-90 ve 1-90 gün) düşürmüştür. Yumurta kabuk ağırlığı ve kalınlığı rasyona keten tohumu ilavesiyle birlikte azalmıştır. Yem tüketimi, yemden yararlanma, hasarlı yumurta oranı ve yumurta kabuk kalınlığında Se ve keten tohumu arasında intereaksiyon bulunmuştur. InSe ve OrSe ilavesi yumurta selenyum içeriklerini arttırmıştır. Yumurta sarısında dokosaheksaenoik asit (DHA) ve eikosapentaenoik asit (EPA), α -linolenik asit (ALA) ve n-3 yağ asidi konsantrasyonları rasyona keten tohumu ilavesiyle artmıştır. ALA ve n-3 yağ asidi konsantrasyonlarında keten tohumu ve Se arasında intereaksiyon bulunmuştur. Sonuç olarak rasyonlara keten tohumu ilavesi yumurta sarısında DHA, EPA, ALA ve n-3 yağ asidi konsantrasyonlarını, Se ilavesi ise yumurta Se içeriğini arttırmıştır. Yumurta yağ asitleri (ALA, n-3 yağ asitleri) rasyona keten tohumu ile birlikte OrSe ilave edilmesiyle birlikte artmıştır.

Anahtar sözcükler: *Yumurta, Keten tohumu, n-3 yağ asidi kompozisyonu, Performans, Selenyum*



İletişim (Correspondence)



+90 332 2232705



vet_em@yahoo.com

INTRODUCTION

The poultry industry has been developing rapidly, and the production of eggs, which are a very important source of animal protein in the human diet, has increased significantly. However, because eggs contain high levels of cholesterol, their consumption has not been increasing proportionally. To alleviate this problem, the composition of diet given to poultry should be altered by the addition of different feedstuff. The amount of n-3 fatty acid in eggs can be increased by adding feedstuff that is rich in n-3 fatty acid¹⁻⁴.

When 50, 100, and 150 g/kg linseed were added to the diets of laying hens, statistically high levels of 2.31, 4.1 and 6.83% DHA were found in their eggs, respectively³. In another trial, although there was an increase in C18:3 n-3 and its metabolites EPA and DHA, there was a decrease in arachidonic acid, a metabolite of linoleic acid (C18:2n-6), and in n-6/n-3 fatty acids in egg yolks of laying hens fed with diets containing high amounts of linseed⁵. Feeding laying hens diets with enriched n-3 fatty acid led to increased n-3 fatty acid levels and decreased n-6/n-3 fatty acid levels in eggs^{6,7}.

Selenium is a natural antioxidant, and it is an essential component of several major metabolic pathways, including thyroid hormone metabolism, antioxidant defence systems, and immune function. The adult human's requirement for Se ranges from 30 to 40 µg depending on daily food intake. Most of this Se requirements can be met by a Se-enriched foods such as Se-enriched eggs. Also Se-enriched eggs contain selenium ranges from 9.6 to 15 µg⁸⁻¹⁰. Historically, the Se source that has been used is the inorganic sodium selenite for production Se-enriched eggs. However, in these days, an organic source of Se was approved for use as a feed supplement in poultry diets for production Se-enriched eggs^{9,11}. This organic source is a Se-enriched yeast that is produced by growing the yeast *Saccharomyces cerevisiae* in a high-Se medium^{9,12}. Researches comparing InSe with OrSe in laying hens have been published, and the results from these experiments are consistent. Whole-egg Se concentration is increased by InSe and OrSe supplementation as dietary level increases, but OrSe supplements have been reported to increase Se levels in eggs more than InSe^{8,13-15}. Additionally, Se supplementation with linseed might be increase the concentrations of EPA and DHA in the egg because of Se and PUFA may interact, possibly through the action of the Se-dependent Glutathione peroxidase (GSH-Px)¹⁶.

The goal of this research was to determine the effects of adding different levels of linseed to layer hens diets, either alone or in combination with OrSe and InSe on egg production, daily feed intake, feed conversion, egg weight, damaged egg ratio, specific gravity, shell weight, shell thickness, oil ratio, egg fatty acid composition and Se content of eggs.

MATERIAL and METHODS

Experimental Design and Animals

A total of 600 *Lohmann LSL* hybrid laying hens aged 31 wk were placed in a completely enclosed, fan-ventilated light- (15L:9D) and temperature- (20°C) controlled room. The hens were housed in metal cages (55x45x40 cm) with 5 hens in each cage (2 cages per replication). Food and water were available *ad libitum*. Hens were reassigned to cages according to a randomised complete block design consisting of 12 treatments in 5 blocks (10 hens per replication, giving 50 hens per experimental group). The experimental period was 100 d.

Diets and Feeding

The composition of all the diets is shown in [Table 1](#). Trials were carried out according to a 4x3 factorial arrangement. The four groups received diets supplemented with 0, 25, 50, and 100 g/kg linseed, respectively. Because linseed has very little grains, it may not be milled properly, and the oil extracted may stay in the feed breaker. To avoid this, linseed was milled with corn so that the oil extracted was absorbed by the corn. Diets were formulated to be equal in protein, metabolisable energy, lysine, threonine, methionine, calcium and phosphorus. Afterwards, 0.3 mg/kg of OrSe (Sel-Plex 50, Alltech Inc., USA) and InSe (sodium selenite) were added to the feed of each control group. Limestone was first added to the Se supplement, then was increased gradually and mixed with the feed. Commercial vitamin and mineral premixes without Se were also mixed into the feeds.

Hen-d Egg Production

Hen-d egg production was recorded for 10 d, and similar pre-production values for treatments were ensured. Hen-d egg production was then recorded daily at the same time and calculated as the total number of eggs collected divided by the total number of live hens per day in each group. The collected eggs were classified as normal or damaged; the latter included the following: broken eggs (an egg with a broken shell and destroyed membrane), cracked eggs (an egg with a broken shell but intact membrane) and eggs without shells (an egg without a shell but with an intact membrane). Feed consumption (FC) and feed efficiency were determined at 15-d intervals.

Egg Weight and Eggshell Parameters

Egg weight and specific gravity were determined monthly using the methods described by Hamilton¹⁷ and Hempe et al.¹⁸. Ten eggs were taken from each replication at 0, 30, 60 and 90 d for two consecutive d. The shell thickness and shell weight of these eggs were measured¹⁹.

Table 1. Components and chemical composition of the diet**Tablo 1.** Rasyonun bileşimi ve kimyasal kompozisyonu

Components (g/kg Mixture)	Dietary Treatments			
	0 g/kg Linseed	25 g/kg Linseed	50 g/kg Linseed	100 g/kg Linseed
Maize	393.0	375.0	358.0	260.2
Wheat	277.9	284.2	287.3	357.3
Soybean meal	202.9	194.6	188.5	172.3
Linseed	0.00	25.0	50.0	100.0
Sunflower oil	16.0	11.0	6.0	0.00
Shell	50.0	50.0	50.0	50.0
Limestone	36.6	36.6	36.6	36.6
Dicalcium phosphate	15.4	15.4	15.4	15.4
Sodium chloride	3.0	3.0	3.0	3.0
Vit.-Min. Premix ¹	2.5	2.5	2.5	2.5
Antioxidant (etoxiquin)	1.0	1.0	1.0	1.0
Methionine	1.7	1.7	1.7	1.7
Nutrient Content				
ME(MJ/kg) ²	11.4	11.4	11.4	11.4
Dry matter (g/kg)	901.8	911.1	907.3	908.4
Crude protein (g/kg)	165.3	164.8	166.0	165.2
Crude fat (g/kg)	35.0	35.2	34.5	33.9
Crude fiber (g/kg)	65.6	63.4	61.2	63.4
Ash (g/kg)	79.5	75.3	71.9	76.5
Calcium (g/kg)	37.4	37.4	37.2	37.5
Phosphorus (g/kg)	6.1	6.1	6.1	6.1
Lysine(g/kg) ²	8.1	8.0	8.0	8.0
Methionine(g/kg) ²	4.3	4.4	4.4	4.5
Threonine(g/kg) ²	6.0	6.1	6.3	6.5

¹ Per 2.5 kg of vitamin-mineral premix contains 3.6mg retinly acetate, 0.05 mg cholecalciferol, 30 mg α-tocopheryl acetate, 3 mg menadione dimethylpyrimidiol bisulphite, 3 mg thiamin, 6 mg riboflavin, 5 mg piridoksal, 0.015 mg cyanocobalamin, 25 mg niacin, 0.04 mg biotin, 8mg carotenoid (carophyl redşcarophyl yellow), 1 mg folasin, 300 mg choline chloride, 50 mg ascorbic acid, 80 mg manganese, 35 mg iron, 50 mg zinc, 5 mg copper, 2 mg iodine, 0.4 mg cobalt

² Calculated value

Feed Analyses

All feed samples were analysed for dry matter, ash, crude protein, ether extract, crude fiber, calcium and total phosphorus²⁰.

Fatty Acid Analyses

On d 0, 30, 60 and 90 of the trial, five eggs were randomly taken from each replication. Total lipid was extracted from the egg yolk samples by the method suggested by Folch et al.²¹. Egg yolk samples (4 g) were homogenised with 80 ml of a 2:1 (v/v) mixture of chloroform and methanol, after which 4 ml 0.88% sodium chloride was added. The liquid was mixed and left to stand for two h to allow phase separation²². The chloroform-methanol extract was evaporated to dryness in a water bath at 50°C under N₂ flow. Lipid extracts were then converted to fatty acid methyl esters by using a boron-trifluoride-methylation solution (Supelco, catalogue no. 3-3021).

Gas Chromatographic Analysis Procedure

The fatty acid methyl esters were separated and analysed by a Shimadzu 15-A gas chromatograph equipped with a dual flame ionisation detector and a 1.8 m×3 mm internal diameter packed glass column containing a 100/120 Chromosorb WAW coated with 10% SP 2330. The injector and detector temperatures were 225 and 245°C, respectively. The column temperature program began at 190°C for 35 min and was then increased by 30°C min⁻¹ to a final temperature of 220°C, which was maintained for 5 min. Nitrogen supplied at a flow rate of 20 ml/min was used as the carrier gas. Conditions were chosen to separate fatty acids of carbon chain lengths 14 to 22. The fatty acids were identified by comparison of retention times with known external standard mixtures and were quantified by a Shimadzu Class-VP Software System. Results were expressed as percentage distribution of fatty acid methyl esters. All chemicals used for gas chromatography analysis

procedures were obtained from Supelco Inc. (Bellefonte, PA, USA).

Determination of Selenium Content in Eggs

Five eggs from each replication were collected at d 30, 60 and 90. To determine their Se content, whole eggs were digested in a mixture of HNO₃ and H₂O₂. Mineralisates were diluted with deionised water, and Se content was determined by inductively coupled plasma mass spectrometry using an ICP-MS Varian instrument (Varian Australia, Cleyton South, MDC)²³.

Statistical Analysis

Statistical analyses for FC, feed efficiency, damaged egg ratio, egg weights, eggshell parameters, and the Se and fatty acid concentrations of eggs were performed using the GLM procedure. Data were analysed by ANOVA for a randomised complete block design with 12 dietary treatments in a 4x3 factorial arrangement. The ANOVA model included the effects of linseed, Se, and the interaction of linseed and Se. Any significant ($P < 0.05$) differences were further analysed by Duncan's multiple range test²⁴.

Experimental procedures were approved by the Selcuk University Veterinary Faculty Ethics Commission.

RESULTS

The value of the performance and eggshell parameters of the groups are shown in [Table 2](#). The fatty acid compositions of egg yolks at d 30 and 60 are shown in [Tables 3](#) and [4](#), respectively. The final fatty acid composition of the egg yolks at the end of the trial are shown in [Table 5](#), and the selenium content of the eggs is shown in [Table 6](#).

Composition of Linseed

The linoleic acid, ALA, n-3 fatty acid, n-6 fatty acid, SFA and UFA levels of linseed were determined to be 15.57, 50.64, 53.34, 15.57, 14.31 and 85.69 g/100 g, respectively.

The dry matter, ash, crude protein, dry fiber and ether extract parameters were determined to be 962.3, 29.9, 207.7, 261.9 and 376.8 g/kg, respectively and metabolizable energy value were calculated as 15.65 MJ/kg.

Performance

Linseed supplementation to the diet at 100 g/kg significantly decreased egg production during d 1-30 ($P < 0.05$). Feed consumption was significantly lower in the linseed groups than the control group during the d 1-30 ($P < 0.001$) and also was significantly lower 25 g/kg group than the control group at d 1-90 ($P < 0.001$). There was no difference in feed efficiency between the linseed fed and Se-added groups. There was some interaction ($P < 0.05$)

between the Se and linseed groups on d 60-90 and 1-90 in egg production, feed consumption (except for d 1-90) and feed efficiency ([Table 2](#)).

Egg Weight and Eggshell Parameters

There were differences in egg weight throughout the whole trial period except during d 30-60 in the linseed groups ($P < 0.05$). Linseed supplementation decreased shell weight in the all linseed groups and thickness in the 100 g/kg linseed group. ($P < 0.05$). The addition of Se did not change the eggshell parameters, but there was an interaction ($P < 0.05$) between Se and linseed in shell thickness ([Table 2](#)).

Fatty Acid Composition of Egg Yolk

Fatty acid composition analysis of egg yolks for linoleic acid, ALA, n-3 fatty acid, n-6 fatty acid, saturated fatty acids (SFA) and unsaturated fatty acids (UFA) was completed on d 1 of the trial. The measured values were 0.37, 21.03, 1.35, 22.00, 32.77 and 67.23 g/100 g fatty acids, respectively.

Docosahexaenoic acid, EPA, ALA and n-3 fatty acids in egg yolks increased with increasing dietary linseed ($P < 0.05$). There were also interactions between the linseed and Se groups during d 60-90 involving ALA and n-3 fatty acids ([Table 4, 5](#)).

On d 30, there were differences in the levels of C14:0, C16:0, C18:3n-3, C20:4n-6, C20:5n-3, C22:5n-3, C22:6n-3, n-3 and n-6 fatty acids, PUFA, SFA and UFA in the linseed fed groups and C14:0, C20:4n-6 fatty acids in the Se-added groups ($P < 0.05$) ([Table 3](#)).

On d 60, there were differences in the levels of C14:0, C16:0, C18:0, C18:1n-9, C18:3n-3, C20:4n-6, C20:5n-3, C22:5n-3, C22:6n-3, n-3 and n-6 fatty acids, PUFA, mono-unsaturated fatty acids (MUFA), SFA and UFA in the linseed fed groups and C18:3n-3 and n-3 fatty acids in the Se-added groups ($P < 0.05$) ([Table 4](#)).

On d 90, there were differences in the levels of C14:0, C16:0, C18:3n-3, C20:4n-6, C20:5n-3, C22:5n-3, C22:6n-3 and n-3 fatty acids, PUFA, SFA and UFA in the linseed fed groups and C18:3n-3 and n-3 fatty acids in the Se-added groups ($P < 0.05$) ([Table 5](#)).

Selenium Content of Eggs

The inclusion of InSe and OrSe increased the Se content of eggs ($P < 0.05$). Egg selenium content was higher in the OrSe added group than in the control and InSe added groups on d 60 and 90 ([Table 6](#)).

DISCUSSION

Differences in egg production were found among groups fed with diets containing different amounts

Table 2. Yield performance, ratio of yolk fat and eggshell parameters of groups**Tablo 2.** Grupların verim performansı, yumurta sarısı yağ oranları ve yumurta kabuk parametreleri

Item	Linseed Groups					Selenium Groups ¹				Int ²	SEM
	0 g/kg	25 g/kg	50 g/kg	100 g/kg	P-value	0 mg/kg Se	0.3 mg/kg OrSe	0.3 mg/kg InSe	P-value		
Egg production ³, %/hen d											
1-30 d	86.38 ^a	84.60 ^{ab}	84.71 ^{ab}	83.45 ^b	0.019	84.26	85.25	84.75	0.533	0.636	0.330
30-60 d	81.79	81.48	81.07	80.81	0.824	81.34	81.61	80.91	0.767	0.176	0.390
60-90 d	81.24	82.24	82.24	81.90	0.822	81.26	82.63	81.84	0.421	0.043	0.440
1-90 d	83.14	82.77	82.68	82.06	0.424	82.32	83.16	82.50	0.295	0.010	0.240
Feed consumption ³, g/hen/d											
1-30 d	100.76 ^a	95.26 ^{bc}	96.05 ^b	92.62 ^c	0.000	96.49	96.87	95.16	0.359	0.291	0.570
30-60 d	90.05	88.77	90.16	89.85	0.653	89.47	89.82	89.83	0.924	0.097	0.430
60-90 d	101.75	97.56	98.91	101.71	0.627	98.52	101.5	99.86	0.661	0.050	1.440
1-90 d	97.52 ^a	93.87 ^b	95.04 ^{ab}	94.73 ^{ab}	0.001	94.82	96.09	94.95	0.224	0.603	0.001
Feed efficiency ³, kg feed/kg egg											
1-30 d	1.94	1.89	1.90	1.89	0.412	1.91	1.91	1.90	0.745	0.149	0.010
30-60 d	1.79	1.81	1.83	1.83	0.580	1.81	1.82	1.82	0.927	0.067	0.010
60-90 d	2.00	1.92	1.95	2.05	0.323	1.96	1.99	1.99	0.902	0.010	0.030
1-90 d	1.91	1.87	1.89	1.92	0.435	1.89	1.91	1.90	0.895	0.000	0.010
Egg weight ⁴, g											
1-30 d	60.50 ^a	59.57 ^{ab}	59.56 ^{ab}	58.95 ^b	0.121	59.73	59.56	59.64	0.957	0.074	0.230
30-60 d	61.72	60.52	60.97	60.75	0.302	61.00	60.68	61.29	0.573	0.236	0.240
60-90 d	62.77 ^a	61.86 ^{ab}	61.72 ^{ab}	60.90 ^b	0.030	62.04	61.89	61.51	0.605	0.161	0.220
1-90 d	61.66 ^a	60.65 ^b	60.75 ^b	60.20 ^b	0.002	60.92	60.71	60.81	0.820	0.166	0.140
Specific gravity ⁴											
1-30 d	1.080	1.079	1.079	1.078	0.366	1.078	1.080	1.079	0.305	0.738	0.000
30-60 d	1.079	1.079	1.080	1.080	0.578	1.079	1.080	1.080	0.935	0.797	0.000
60-90 d	1.080	1.080	1.079	1.079	0.776	1.079	1.080	1.080	0.858	0.378	0.000
1-90 d	1.080	1.080	1.080	1.080	0.977	1.080	1.080	1.080	0.633	0.866	0.001
Damaged egg ratio ³, %											
1-30 d	2.70	2.32	1.77	2.57	0.162	2.04	2.54	2.44	0.400	0.413	0.160
30-60 d	2.31	2.03	2.28	1.71	0.524	2.34	1.83	2.07	0.434	0.421	0.160
60-90 d	2.21	1.96	2.48	1.72	0.287	1.89	2.02	2.36	0.406	0.068	0.150
1-90 d	2.40	2.10	2.17	2.00	0.422	2.09	2.13	2.29	0.632	0.034	0.150
Ratio of yolk fat ⁵, %											
30 th d	32.10	32.00	32.51	32.60	0.658	32.06	32.63	32.22	0.607	0.711	0.390
60 th d	32.62	32.50	32.08	32.13	0.869	32.74	32.16	32.13	0.587	0.938	0.390
90 th .d	32.23	31.93	32.04	31.90	0.923	31.64	31.92	32.51	0.163	0.640	0.390
Eggshell parameters ⁴, 90 d											
Shell weight, g	5.66 ^a	5.41 ^b	5.44 ^b	5.43 ^b	0.008	5.46	5.50	5.49	0.777	0.148	0.003
Shell weight, %	9.03	8.88	8.82	9.00	0.331	8.93	8.93	8.94	0.994	0.684	0.004
Shell thickness, mm	0.368 ^a	0.368 ^a	0.359 ^{ab}	0.357 ^b	0.057	0.366	0.360	0.363	0.339	0.050	0.002

¹ OrSe = Organic selenium; InSe = Inorganic selenium² Int = Interaccion³ Data are means of 10 hens for each replications⁴ Data are means of 10 eggs for each replications⁵ Data are means of 5 eggs for each replications^{a-c} Means within the same row bearing different superscripts are significantly different (P<0.05)

Table 3. Fatty acids composition of eggs in groups at 30 d (g/100 g fatty acids)¹**Tablo 3.** Grupların 30. gün yumurta yağ asidi kompozisyonları (g/100 g yağ asidi)

Fatty Acids	Linseed Groups					Selenium Groups ²				Int ³	SEM
	0 g/kg	25 g/kg	50 g/kg	100 g/kg	P-value	0 mg/kg Se	0.3 mg/kg OrSe	0.3 mg/kg InSe	P-value		
C14:0	0.25 ^a	0.24 ^{ab}	0.24 ^{ab}	0.21 ^b	0.015	0.25 ^a	0.23 ^{ab}	0.22 ^b	0.012	0.945	0.010
C16:0	25.20 ^a	24.32 ^a	23.24 ^b	21.97 ^c	0.000	23.76	23.57	23.72	0.885	0.288	0.220
C18:0	7.52	7.92	7.11	7.52	0.236	7.71	7.08	7.94	0.053	0.833	0.150
C18:1n-9	38.85	38.49	39.17	39.53	0.321	38.66	38.48	38.38	0.936	0.143	0.330
C18:2n-6	20.76	19.94	20.14	19.55	0.423	19.97	20.75	19.58	0.176	0.378	0.260
C18:3n-3	0.74 ^d	2.25 ^c	3.92 ^b	6.87 ^a	0.000	3.21	3.32	3.80	0.179	0.336	0.390
C20:4n-6	1.76 ^a	1.57 ^a	1.22 ^b	1.16 ^b	0.001	1.39 ^{ab}	1.27 ^b	1.62 ^a	0.042	0.675	0.070
C20:5n-3	0.01 ^b	0.01 ^b	0.03 ^b	0.06 ^a	0.000	0.04	0.03	0.04	0.135	0.187	0.003
C22:5n-3	0.07 ^b	0.10 ^b	0.14 ^a	0.17 ^a	0.000	0.14	0.10	0.13	0.103	0.532	0.070
C22:6n-3	0.71 ^b	1.15 ^a	1.19 ^a	1.40 ^a	0.001	1.09	0.99	1.25	0.184	0.153	0.060
n-3 ⁴	1.65 ^d	3.72 ^c	5.43 ^b	8.61 ^a	0.000	4.58	4.90	5.08	0.441	0.505	0.370
n-6 ⁵	23.22 ^a	22.07 ^{ab}	21.65 ^{ab}	20.93 ^b	0.041	21.74	22.47	21.69	0.438	0.340	0.290
PUFA ⁶	25.03 ^b	25.95 ^b	27.22 ^b	29.68 ^a	0.000	26.46	26.75	27.69	0.383	0.514	0.430
MUFA ⁷	41.74	41.43	42.10	40.20	0.295	41.62	41.29	41.20	0.882	0.266	0.370
SFA ⁸	33.16 ^a	32.61 ^a	30.69 ^b	30.12 ^b	0.000	31.86	31.02	32.05	0.186	0.625	0.290
UFA ⁹	66.77 ^b	67.39 ^b	69.31 ^a	69.88 ^a	0.000	68.08	68.98	67.95	0.177	0.665	0.290

¹ Data are means of 5 eggs for each replications² OrSe = Organic selenium; InSe = Inorganic selenium³ Int = Interaction⁴ n-3 = C18:3n-3+C20:3n-3+C20:5n-3+C22:5n-3+C22:6n-3⁵ n-6 = C18:2n-6+C20:2n-6+C20:4n-6+C22:4n-6⁶ PUFA (Polyunsaturated fatty acids) = C20:2n-6+C20:3n-3+C20:4n-6+C20:5n-3+C22:3n-3+C22:4n-6+C22:5n-3+C22:6n-3⁷ MUFA (Mono-unsaturated fatty acids) = C14:1n-5+C16:1n-7+C18:1n-9⁸ SFA (Saturated fatty acids) = C14:0+C16:0+C17:0+C18:0+C20:0⁹ UFA (Unsaturated fatty acids) = C14:1n-5+C16:1n-7+C18:1n-9+C20:2n-6+C20:3n-3+C20:4n-6+C20:5n-3+C22:3n-3+C22:4n-6+C22:5n-3+C22:6n-3^{a-d} Means within the same row bearing different superscripts are significantly different (P<0.05)

of linseed in the first 30 d of the trial, as seen in [Table 2](#). Although the group fed a diet containing 100 g/kg linseed produced the lowest number of eggs, the control group produced the highest. In different periods of the trial, as well as throughout the whole trial time, there were no differences in egg production between the groups. An interaction was observed between the groups fed with linseed and Se. It is reported that adding linseed in different ratios to the diet did not affect egg production²⁵. Similar results were also reported by other trials with the addition of 150 g/kg linseed⁵. On the other hand, egg production was increased in those fed with different amounts of linseed^{26,27}. The damaged egg ratio did not differ between the control, linseed fed and Se-added groups. However, there was an interaction between the groups fed with linseed and the groups fed with Se.

The lowest feed intake was in the 25 g/kg linseed group during d 1-90. There were interactions on d 60 and 90 for the groups fed with linseed and Se. The addition of 50, 100 and 150 g/kg linseed to the diet decreased feed intake compared to the control groups, as reported by Scheideler et al.²⁶. Similarly, decreased feed intakes have

been reported by others^{28,29}.

There were no differences in feed efficiency between the linseed-fed and Se-added groups. However, there was an interaction between the Se and linseed groups during d 60-90 and 1-90 in egg production, feed consumption and feed efficiency. Augustyn et al.²⁹ reported significantly decreased feed efficiency in the 50 g/kg linseed fed group.

The addition of linseed to the diets decreased egg weight during d 1-90. Although similar results were reported by some authors^{3,27}, no alteration was reported in another study²⁸. Novak and Scheideler²⁵ also reported increased egg weights when laying hens were fed with 100 g/kg linseed. In the present study, increasing the amount of linseed in the diets led to decreased egg weight. The smaller egg size might be due to decreased n-6 fatty acid and increased n-3 fatty acid levels in the diets. It might also be due to a decrease in linoleic acid, commonly known as n-6 fatty acid, in the diets. Linoleic acid is an essential fatty acid that influences egg size³

Shell weight decreased in the linseed groups and thickness decreased in the 100 g/kg linseed group. Similar

Table 4. Fatty acids composition of eggs in groups at 60 d(g/100 g fatty acids) ¹**Tablo 4.** Grupların 60. gün yumurta yağ asidi kompozisyonları (g/100 g yağ asidi)

Fatty Acids	Linseed Groups					Selenium Groups ²				Int ³	SEM
	0 g/kg	25 g/kg	50 g/kg	100 g/kg	P-value	0 mg/kg Se	0.3 mg/kg OrSe	0.3 mg/kg InSe	P-value		
C14:0	0.26 ^a	0.26 ^a	0.24 ^{ab}	0.22 ^b	0.031	0.24	0.24	0.24	0.972	0.843	0.010
C16:0	24.28 ^a	23.97 ^{ab}	23.16 ^b	21.52 ^c	0.000	23.24	23.11	23.35	0.855	0.530	0.220
C18:0	7.59 ^a	7.34 ^{ab}	7.00 ^b	7.43 ^a	0.039	7.23	7.31	7.48	0.348	0.355	0.150
C18:1n-9	39.72 ^a	40.21 ^a	39.35 ^a	37.05 ^b	0.000	38.93	39.05	39.25	0.836	0.140	0.270
C18:2n-6	21.57	20.33	20.35	20.00	0.184	20.98	20.34	20.37	0.545	0.805	0.260
C18:3n-3	0.67 ^d	2.13 ^c	4.06 ^b	8.33 ^a	0.000	3.57 ^b	4.29 ^a	3.54 ^b	0.001	0.000	0.400
C20:4n-6	1.57 ^a	1.35 ^b	1.15 ^c	1.03 ^d	0.000	1.27	1.27	1.27	0.944	0.795	0.030
C20:5n-3	0.03 ^b	0.02 ^b	0.03 ^b	0.07 ^a	0.000	0.04	0.04	0.03	0.393	0.137	0.004
C22:5n-3	0.07 ^c	0.13 ^b	0.13 ^b	0.17 ^a	0.000	0.13	0.12	0.13	0.740	0.767	0.006
C22:6n-3	0.63 ^c	0.90 ^b	1.09 ^a	1.17 ^a	0.000	0.98	0.92	0.94	0.480	0.322	0.030
n-3 ⁴	1.47 ^d	3.26 ^c	5.52 ^b	9.96 ^a	0.000	4.85 ^b	5.51 ^a	4.80 ^b	0.031	0.005	0.440
n-6 ⁵	23.71 ^a	22.11 ^b	21.78 ^b	21.28 ^b	0.022	22.59	22.01	22.06	0.644	0.820	0.290
PUFA ⁶	25.36 ^b	25.51 ^b	27.43 ^b	31.39 ^a	0.000	27.59	27.67	27.01	0.730	0.405	0.480
MUFA ⁷	42.15 ^a	42.79 ^a	42.07 ^a	39.31 ^b	0.000	41.48	41.51	41.76	0.872	0.170	0.290
SFA ⁸	32.48 ^a	31.69 ^a	30.49 ^b	29.40 ^c	0.000	30.93	30.82	31.23	0.698	0.860	0.250
UFA ⁹	67.51 ^c	68.30 ^c	69.51 ^b	70.70 ^a	0.000	69.07	69.18	69.78	0.699	0.860	0.250

¹ Data are means of 5 eggs for each replications² OrSe = Organic selenium; InSe = Inorganic selenium³ Int = Interaction⁴ n-3 = C18:3n-3+C20:3n-3+C20:5n-3+C22:5n-3+C22:6n-3⁵ n-6 = C18:2n-6+C20:2n-6+C20:4n-6+C22:4n-6⁶ PUFA (Polyunsaturated fatty acids) = C20:2n-6+C20:3n-3+20:4n-6+C20:5n-3+C22:3n-3+C22:4n-6+C22:5n-3+C22:6n-3⁷ MUFA (Mono-unsaturated fatty acids) = C14:1n-5+C16:1n-7+C18:1n-9⁸ SFA (Saturated fatty acids) = C14:0+C16:0+C17:0+C18:0+C20:0⁹ UFA (Unsaturated fatty acids) = C14:1n-5+C16:1n-7+C18:1n-9+C20:2n-6+C20:3n-3+C20:4n-6+C20:5n-3+C22:3n-3+C22:4n-6+C22:5n-3+C22:6n-3^{a-d} Means within the same row bearing different superscripts are significantly different (P<0.05)

results were also reported in another study ³. It has been hypothesised that the anti-nutritional compounds present in linseed impair the digestion and absorption of nutrients ³⁰. Thus may result in decreased calcium absorption and eggshell quality.

Adding increased linseed to the diet increased the C18:3n-3 levels in the egg yolk. Similar results were reported by some authors ^{3,31,32}. Scheideler and Froning ³ reported that 50, 100 and 150 g/kg linseed supplementation to diets increased the amount of DHA to 2.31, 4.18 and 6.83%, respectively. In the present study, the DHA amount in the linseed groups was significantly higher than that of the control group. Supplementation with 0, 100 and 200 g/kg linseed increased the amount of DHA to 51, 81 and 87 mg/egg in another study ³².

When the feedstuff was enriched with n-3 fatty acid, the n-3 fatty acid content of the eggs increased. Aymond and Elswyk ³¹ reported that when 50 and 150 g/kg linseed were added to the diet, the n-3 fatty acid levels in the eggs increased depending on the linseed amount in the diet. The result of this study is similar to our findings.

When the amount of linseed in the diet increased, n-6 fatty acid amounts in the egg yolk decreased proportionally. Although the highest SFA amount was obtained from the control group, the lowest one was obtained from the group fed with a 100 g/kg linseed diet. Our observation that there were no differences in the SFA ratios in the eggs from the control group and the group fed with 25 g/kg linseed diet are similar to the findings of others ³³. Additionally, these results also mean that increased dietary linseed decreases the SFA ratio, as found in our study and reported by others ³³.

The enrichment of feedstuff with UFA increased the egg UFA ratios significantly. The highest ratio was obtained from the group that consumed 100 g/kg linseed diet. Similar results were found at d 30, 60 and 90. An interaction was found between the linseed and Se groups in terms of C18:3n-3 and n-3 fatty acid amounts at d 60. The addition of OrSe to the diet increased the levels of C18:3n-3; as a consequence, n-3 fatty acid levels increased. The same results were obtained at d 90. When the linseed amount in the diet increased, the n-3 fatty acid in egg yolks increased and n-6 fatty acid in egg yolks decreased. Some researchers ³⁴⁻³⁶ have reported that a positive correlation

Table 5. Fatty acids composition of eggs in groups at 90 d (g/100 g fatty acids) ¹**Tablo 5.** Grupların 90. gün yumurta yağ asidi kompozisyonları (g/100 g yağ asidi)

Fatty Acids	Linseed Groups					Selenium Groups ²				Int ³	SEM
	0 g/kg	25 g/kg	50 g/kg	100 g/kg	P-value	0 mg/kg Se	0.3 mg/kg OrSe	0.3 mg/kg InSe	P-value		
C14:0	0.28 ^a	0.24 ^b	0.25 ^b	0.24 ^b	0.045	0.25	0.25	0.26	0.721	0.095	0.010
C16:0	25.75 ^a	24.70 ^b	24.03 ^b	22.84 ^c	0.000	24.09	24.67	24.24	0.260	0.034	0.210
C18:0	7.44	6.93	7.24	7.08	0.307	7.34	7.01	7.16	0.393	0.377	0.150
C18:1n-9	41.79	40.92	40.74	40.35	0.404	41.32	40.61	41.52	0.099	0.072	0.330
C18:2n-6	17.92	19.13	18.07	17.09	0.129	18.12	18.29	17.75	0.751	0.100	0.310
C18:3n-3	0.58 ^d	1.92 ^c	3.27 ^b	6.11 ^a	0.000	2.79 ^b	3.40 ^a	2.72 ^b	0.011	0.000	0.290
C20:4n-6	1.33 ^a	1.26 ^a	1.22 ^a	1.00 ^b	0.001	1.24	1.19	1.19	0.672	0.977	0.003
C20:5n-3	0.01 ^b	0.02 ^b	0.03 ^a	0.06 ^a	0.000	0.02	0.03	0.03	0.604	0.590	0.004
C22:5n-3	0.07 ^c	0.10 ^c	0.15 ^b	0.19 ^a	0.000	0.12	0.13	0.13	0.603	0.850	0.007
C22:6n-3	0.52 ^c	0.85 ^b	1.11 ^a	1.26 ^a	0.000	0.94	0.91	0.95	0.859	0.715	0.050
n-3 ⁴	1.31 ^d	2.97 ^c	4.69 ^b	7.68 ^a	0.000	3.96 ^b	4.59 ^a	4.07 ^b	0.017	0.000	0.330
n-6 ⁵	19.89	20.79	19.57	18.33	0.062	19.75	19.86	19.32	0.761	0.107	0.340
PUFA ⁶	21.33 ^b	23.90 ^a	24.39 ^a	26.13 ^a	0.001	23.85	24.59	23.37	0.439	0.051	0.460
MUFA ⁷	44.76	44.10	43.99	43.61	0.698	44.34	43.31	44.70	0.250	0.134	0.360
SFA ⁸	33.90 ^a	32.00 ^b	31.62 ^b	39.26 ^c	0.000	31.81	31.93	32.09	0.812	0.020	0.250
UFA ⁹	66.10 ^c	68.00 ^b	68.38 ^b	69.74 ^a	0.000	68.19	67.91	68.07	0.811	0.020	0.250

¹ Data are means of 5 eggs for each replications² OrSe = Organic selenium; InSe = Inorganic selenium³ Int = Interaction⁴ n-3 = C18:3n-3+C20:3n-3+C20:5n-3+C22:5n-3+C22:6n-3⁵ n-6 = C18:2n-6+C20:2n-6+C20:4n-6+C22:4n-6⁶ PUFA (Polyunsaturated fatty acids) = C20:2n-6+C20:3n-3+20:4n-6+C20:5n-3+C22:3n-3+C22:4n-6+C22:5n-3+C22:6n-3⁷ MUFA (Mono-unsaturated fatty acids) = C14:1n-5+C16:1n-7+C18:1n-9⁸ SFA (Saturated fatty acids) = C14:0+C16:0+C17:0+C18:0+C20:0⁹ UFA (Unsaturated fatty acids) = C14:1n-5+C16:1n-7+C18:1n-9+C20:2n-6+C20:3n-3+C20:4n-6+C20:5n-3+C22:3n-3+C22:4n-6+C22:5n-3+C22:6n-3^{a-d} Means within the same row bearing different superscripts are significantly different (P<0.05)**Table 6.** Selenium content of egg in groups (mg/kg) ¹**Tablo 6.** Grupların yumurta selenyum içeriği (mg/kg)

Item	Linseed Groups					Selenium Groups ²				Int ³	SEM
	0 g/kg	25 g/kg	50 g/kg	100 g/kg	P-value	0 mg/kg Se	0.3 mg/kg OrSe	0.3 mg/kg InSe	P-value		
Se Content of Egg, mg/kg											
30 th d	0.146	0.141	0.141	0.125	0.920	0.093 ^b	0.198 ^a	0.151 ^a	0.024	0.961	0.012
60 th d	0.162	0.126	0.156	0.157	0.819	0.092 ^c	0.213 ^a	0.157 ^b	0.001	0.966	0.012
90 th d	0.167	0.176	0.154	0.152	0.859	0.095 ^c	0.236 ^a	0.159 ^b	0.000	0.960	0.014

¹ Data are means of 5 eggs for each replications² OrSe = Organic selenium; InSe = Inorganic selenium³ Int = Interaction^{a-c} Means within the same row bearing different superscripts are significantly different (P<0.05)

was observed between concentrations of dietary UFA and Se. Also, Pappas et al.³⁷ reported that diets enriched in PUFA with selenium had a lower concentration of n-6 fatty acid and a higher concentration of n-3 fatty acid in egg yolks. Vitamin E and Se are key components of the antioxidant system, serving to reduce lipid peroxidation ^{38,39}. Glutathione peroxidase (GSH-Px) is a selenoprotein and Se is an essential part of a variety of selenoproteins ⁴⁰.

Additionally, Wang et al.⁴¹ reported that Se yeast supplementation significantly increased the activity of GSH-Px in egg albumen. In the current study, the interaction between Se and linseed in C18:3n-3 and n-3 fatty acid levels in yolk suggests that Se and PUFA may interact, possibly through the action of the Se-dependent GSH-Px. ¹⁶.

The selenium content of eggs was different between

the control, OrSe and InSe groups on d 30, 60 and 90. The selenium content of eggs was higher in the OrSe group than in the control and InSe-added groups on d 60 and 90. The dietary supplementation of OrSe and InSe revealed 0.24 and 0.16 mg/kg Se, respectively, in eggs on d 90; these results are in agreement with other studies^{8-10,42}. Paton et al.⁴³ also reported that the supplementation of OrSe and InSe revealed levels of 0.25 and 0.16 mg/kg Se, respectively, in eggs. Se supplementation increased the Se concentration in eggs, and this increase was generally proportional to the levels of dietary and OrSe supplementation, resulting in a greater increase in egg Se content than InSe^{8,13,14,43,44}. Combs and Combs⁴⁵ reported that OrSe sources are actively absorbed and can be directly incorporated into protein, whereas InSe sources are passively absorbed by the body. Inorganic Se sources are required for selenocysteine synthesis, and this may explain why the Se content of eggs was higher in the OrSe-added group.

The addition of linseed to the poultry diet did not have any negative effects on egg production, feed conversion and damaged egg ratio, specific gravity and oil level in yolk. On the other hand, it decreased feed intake and eggshell weight and thickness. The fatty acid composition of the egg could change significantly depending on the diet. The addition of linseed to the diet resulted in the increase of C18:3n-3 and n-3 fatty acid levels in the egg yolks. In addition to these, OrSe and InSe addition to the diet increased Se content of eggs. Furthermore, our data indicate that OrSe results in a greater deposition of Se in the egg than does InSe, and that the addition of OrSe with linseed to the diet increased the C18:3n-3 and n-3 fatty acid levels in egg yolks.

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