RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

Effects of supplementation of poopy seed and poopy seed oil at various quantities on oxidant-antioxidant balance in laying hens

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SUMMARY

This study was conducted to determine the effect of rations containing different amounts of Poppy seed (PS) and Poppy seed oil (PSO) on oxidant-antioxidant balance in laying hens. There was a control (unsupplemented) and six experimental groups (supplemented with 0.5,1.0 and 1.5 % PS and 0.5,1.0 and 1.5, % PSO). Totally 360 and 24 weeks old laying hens were utilized. The experiment was lasted for 8 weeks. The blood malondialdehyde (MDA), nitric oxide (NOx), vitamin C, β-carotene and vitamin A and levels, between PS groups did not differ. The reduced glutathione (GSH) levels in 1.0% and 1.5 % PS groups increased significantly compared to controls group. The MDA level was found to be significantly lower in the 0.5 % PSO group than in the controls and other PSO groups. The GSH levels in 0,5 % and 1.0% PS groups increased significantly compared to controls group. The NOx levels in 0,5% PSO group significantly decreased compared to other experimental groups. The Vitamin A levels in 0,5%, 1,0% and 1,5 PSO groups increased significantly compared to control group. The vitamin C and β-carotene levels between PSO groups did not differ. According to the findings of this study, the 0.5% supplementation of PSO to the diets of laying hens decrease the blood MDA levels and increase the GSH and vitamin A concentrations. It is thought that 0.5% PSO supplementation can be used effectively as an antioxidant supplement in laying hens.

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Yumurtacı Tavukların Diyetlerine Eklenen Değişik Düzeylerdeki Haşhaş Tohumu ve Haşhaş Tohumu Yağının Oksidan-Antioksidan Denge Üzerine Etkileri

ÖZET

Bu araştırma, yumurtacı tavuklarda yeme farklı oranlarda katılan haşhaş tohumu (HT) ve haşhaş yağının (HY) oksidan-antioksidan dengeye etkilerini belirlemek amacıyla yapılmıştır. Araştırmada HT ve HY içermeyen bir kontrol grubu (% 0 HT ve HY) ile % 0.5, 1.0, 1.5 düzeylerinde HT ve % 0.5, 1.0, 1.5, 2.0 ve 2.5 oranlarında HY içeren 9 farklı grup oluşturulmuştur. Araştırmada 35 haftalık yaşta, toplam 360 adet yumurta tavuğu (Hy-Line/ brown) kullanılmıştır. Deneme 8 hafta sürdürülmüştür. Kan malondialdehid (MDA), nitrik oksit (NOx), vitamin C, β-karoten ve vitamin A düzeylerinde HT grupları arasında anlamlı bir farklılık izlenmemiştir. Redükte glutasyon (GSH) düzeyleri % 1.0 ve % 1.5 HT gruplarında kontrol ve %0,5 HT grubuna oranla anlamlı düzeyde artmıştır. Kan MDA düzeyi %0,5 HY grubunda kontrol ve diğer HY gruplarına oranla anlamlı olarak azalmıştır. % 0,5 ve 1,0 HY grubunda GSH düzeyleri kontrol ve % 1,5 HY grubuna ve kontrole oranla anlamlı düzeyde artmıştır. %0,5 HY grubunda NOx düzeyi diğer gruplara oranla anlamlı olarak azalırken, vitamin A düzeyleri ise 0,5, 1,0 ve % 1,5 HY gruplarında kontrole oranla anlamlı düzeyde artmıştır. HY gruplarında vitamin C ve β-karoten yönünden anlamlı bir değişim izlenmemiştir. Sonuç olarak çalışmada elde ettiğimiz bulgular yumurtacı tavukların rasyonlarına katılan % 0.5 HY'nın kan MDA düzeyi üzerine azaltıcı, GSH ve vitamin A düzeyleri üzerine ise artırıcı etkilere sahip olması yumurtacı tavuklar için etkin bir antioksidan olarak kullanılabileceğini göstermektedir.

INTRODUCTION

Papaver somniferum (poppy) is cultivated as an annual crop in Asia Minor but is now grown in locations with similar climates throughout the world, including Anatolia; Turkey.[1] Poppy has been grown since ancient times for its oil-rich seeds and the opium, which is exuded from its incised seed capsules. While alkaloids from poppy capsules and straw are widely used in the pharmaceutical industry, its seeds are used extensively in various food products.[2] Poppy seed is generally rich in polyunsaturated fatty acids[1] and has the highest percentage of (85.9%) unsaturated fatty acid.[3,4] Nergiz and Ötles reported that poppy seed oil is also a good source of essential fatty acids.[3] Azcan et al. demonstrated that poppy seed oils contain much less stearic (2.5–3.2%) and linolenic (0.4–0.6%) acids than palmitic (10.0–13.0%), oleic (16.1–24.7%), and linoleic (56.4–69.2%) acids.[4]

Poultry diets are usually formulated around a cereal as the main source of energy, with an oilseed meal as the major supplement to the protein in the cereal. Soybean meal is the most commonly used protein source, but other oilseed meals may also be used.[5] The oil remained from the oilseeds contributes to the energy of the diet. Fats, including oils, contained more energy than starch are valuable in balancing the nutrients when formulating diets. In addition to energy, oilseed contains linoleic acid which has been shown to be important for obtaining maximum egg size, and linolenic acid which has beneficial effects on human health. [6,7]

A wide variety of reactive oxygen species (ROS) are produced in the course of the normal metabolism in biological systems and they have several important physiological functions, but their accumulation beyond the needs of the cell can potentially damage lipids, proteins, and nucleic acids. The utilization of excessive energy in the hens metabolisms due to egg production may led to an increased oxidative stress in the body. Oxidative stress plays an important role in the parthenogenesis of many chronic diseases and leads to a decreased production in laying hens.[8] In addition, the same free radicals that are generated during oxidative stress are produced during normal metabolism and thus are involved in both health and disease. Overwhelming evidences indicate that oxidative stress causes to cell and tissue injury. However, the cells possess an intricate network of defence mechanisms to neutralize excessive ROS accumulation, including antioxidant compounds and antioxidant enzymes. Therefore, under physiological conditions, cells are able to cope with the flux of ROS. Oxidative stress describes a condition in which cellular antioxidant defenses are insufficient to keep the levels of ROS below a toxic threshold.[9,10,11] Therefore, determination of oxidant-antioxidant balance in the blood of laying hens fed with diet contained oilseeds or their oils may provide the important data on their using in poultry and feed industry.

There have been no published articles investigating the effects of poppy seed and poppy seed oil on oxidant-antioxidant balance in Laying Hens by using biomarkers of oxidative stress. The purpose of the present study was to evaluate the effect of supplementation of poopy seed and poopy seed oil to diets at various quantities on oxidant-antioxidant balance in laying hens.

MATERIALS and METHODS

Chemicals: Vanadium(III) chloride (VCl_3), NaCl, EDTA Na_2, NaOH, thiobarbituric acid (TBA), trichloroacetic acid (TCA), HCl, sulfanilamide (SULF), and N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) were obtained from Merck (Darmstadt, Germany). Other chemicals used in the study were purchased from Sigma-Aldrich Chemical.

Animals and Protocol Design: Three hundred sixty, 35 week-old Hy-Line / Brown hens, were obtained from a commercial Laying facility. Hens were housed in cages (109 x 68 x 39 cm) in controlled environment laying house with 16 h of light and 8 h of darkness and temperature was between 26 and 32°C. The hens were divided into seven groups of 40 hens each. Each group of hens was further divided into four subgroups (10 hens in each subgroup). There was a control (C) (unsupplemented) and six experimental groups (supplemented with 0.5, 1.0 and 1.5 % poppy seed (PS) and 0.5, 1.0 and 1.5 % poppy seed oil (PSO)). The formulation of diets is shown in Table 1. The diets was a typical layer diet containing 17,5% crude protein (CP) and 2825 kcal metabolizable energy (ME/kg) and was calculated to cover or slightly exceed the nutrients requirements recommended by the National Research council.[5] Hens received the experimental diets and water ad libitum for 8 weeks.

At the end of the experimental period, blood samples were collected from vena breachialis from 10 birds randomly chosen from each groups. Blood samples were taken into heparinized tubes. Two milliliters of blood were immediately pipetted into another tube to measure malondialdehyde (MDA) and reduced glutathione (GSH). Remaining blood was centrifuged at 3000 rpm for 10 min at 4°C for plasma separation. Plasma samples were stored at -

30 °C for the analysis of Nitric oxide, vitamin C, vitamin A and β-carotene .

Biochemical analysis: MDA levels, an index of lipid peroxidation, were measured by the double heating method of Draper and Hadley.[12] The blood GSH concentration was measured as described by Beutler *et al.*.[13] Nitric oxide decomposes rapidly in aerated solutions to form stable nitrite/nitrate products (NOx). Plasma nitrite/ nitrate concentration was measured by a modified method of Griess assay, described by Miranda *et al.*.[14] The principle of this assay is reduction of nitrate by vanadium combined with detection by the acidic Griess reaction. Vitamin A and β-carotene concentrations were estimated by the method of Suzuki and Katoh[15] , and vitamin C was measured by Kyaw [30].

Statistical Analysis: All data were presented as mean ± SE for parametric variables. Parametric variables were compared using one-way analysis of variance with post-hoc analysis using the Duncan test. Data were analyzed using the SPSS® for Windows computing program (Version 11.0), and $p<0.05$ was considered statistically significant.[17]

Table 1. Chemical compositions of control and experimental diets.
Çizelge 1. *Kontrol ve deneysel diyetin kimyasal yapısı*

	Control	0.5 % PS	1.0 % PS	1.5 % PS	0.5 % PSO	1.0 % PSO	1.5 % PSO
Corn	555.86	556.63	557.39	558.14	560.99	566.15	570.74
Soybean meal	141.78	131.52	128.00	134.48	139.75	162.43	184.41
Full fat soya	115.06	121.53	121.27	111.01	109.10	76.42	44.61
Sunflower meal	75.04	73.37	71.70	70.04	73.76	72.48	72.53
Poppy seed	-	5.00	10.00	15.00	-	-	-
Poppy seed oil	-	-	-	-	5.00	10.00	15.00
Limestone	88.26	87.99	87.73	87.46	88.32	88.38	88.44
DCP	13.82	13.78	13.74	13.70	13.89	13.95	14.00
Salt	2.56	2.56	2.56	2.56	2.54	2.52	2.51
Vitamin*	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Mineral**	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine	1.12	1.12	1.12	1.12	1.15	1.17	1.19
$NaHCO_3$	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Toxin binding	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Calculated values							
Crude protein, %	17.50	17.50	17.50	17.50	17.50	17.50	17.50
ME, kcal/kg	2825	2825	2825	2825	2825	2825	2825
Calcium,%	3.75	3.75	3.75	3.75	3.75	3.75	3.75
Phosphorus,%	0.653	0.655	0.657	0.658	0.656	0.647	0.645

DCP: Dicalcium phosphate
*Provided by per kg of diet: Vitamin A, 10 000 IU; vitamin D3, 1 000 IU; vitamin E, 25 mg; vitamin K3, 3 mg; vitamin B1, 2 mg; vitamin B2, 6 mg; niacin 20 mg; vitamin B6, 4 mg; vitamin B12, 15 mg, folic acid, 0.8 mg; choline chloride,
**Provided by per kg of diet: 300 mg; Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg, I, 1 mg; Co, 0.2 mg; Se.

RESULTS

The results of biochemical parameters (MDA, GSH, NOx, vitamin C, Vitamin A and β-carotene levels) in experimental group were summarized in Table 2 and 3.

As shown in Table 2 MDA, NOx, vitamin C, β-carotene and Vitamin A and levels, between PS groups did not differ. The GSH levels in 1.0% and 1.5 % PS groups increased significantly compared to controls and 0,5% PS group ($p<0.05$).

The MDA level was found to be significantly lower in the 0.5 % PSO group than in the controls and other PSO groups ($p<0.05$). On the other hand the MDA levels in 1.0 % PSO group significantly increased compared to controls and other PSO groups ($p<0.05$). The GSH levels in 0,5 % and 1.0% PS groups increased significantly compared to controls and 1,5% PS group ($p<0.05$). The NOx levels in 0,5% PSO group significantly decreased compared to other experimental groups ($p<0.05$). The Vitamin A levels in 0,5%, 1,0% and 1,5 PSO groups increased significantly compared to control group. The vitamin C and beta carotene levels between PSO groups did not differ.

Table 2. Levels of biochemical parameters in control and PS groups. Results are expressed as means ± standard errors.
Çizelge 2. *Kontrol ve PS grubunda biyokimyasal parametrelerin seviyeleri. Sonuçlar means ± standard errors olaral verilmiştir.*

	C	0,5% PS	1% PS	1,5% PS
MDA (nmol/mL)	3,71±0,37	3,86±0,35	3,25±0,56	3,53±0,49
GSH (g/L)	42,85±1,47 ^{*b}	66,42±11,68 ^{*a,b}	74,12±10,65 ^{*a}	87,22±7,30 ^{*a}
NOx (µmol/L)	24,82±2,08	38,76±3,31	28,54±2,23	33,16±6,42
Vitamin C (mg/dL)	3±0,67	3,16±0,51	2,21±0,49	2,89±0,62
β-carotene (µg/dL)	47,95±6,89	47,94±1,57	46,62±4,66	58,63±3,4
Vitamin A (µg/dL)	7,32±1,26	7,98±0,47	7±0,77	8,86±0,89

Table 3. Levels of biochemical parameters in control and PSO groups. Results are expressed as means ± standard errors.
Çizelge 3. *Kontrol ve PSO grubunda biyokimyasal parametrelerin seviyeleri. Sonuçlar means ± standard errors olaral verilmiştir.*

	C	0,5% PSO	1% PSO	1,5% PSO
MDA (nmol/mL)	3,71±0,37 ^{*b}	2,41±0,17 ^{*c}	4,87±0,36 ^{*a}	4,62±0,44 ^{*a,b}
GSH (g/L)	42,85±1,47 ^{*b}	88,84±8,38 ^{*a}	77,69±10,39 ^{*a}	66,07±7,86 ^{*b}
NOx (µmol/L)	24,82±2,08 ^{*a,b}	15,94±1,04 ^{*b}	29,36±1,68 ^{*a}	29,43±5,97 ^{*a}
Vitamin C (mg/dL)	3±0,67	2,32±0,33	2,39±0,43	1,78±0,29
β-carotene (µg/dL)	47,95±6,89	48,17±5,41	63,56±2,02	51,60±3,032
Vitamin A (µg/dL)	7,32±1,26 ^{*c}	9,1±1,07 ^{*b}	10,58±0,65 ^{*a}	9,48±0,51 ^{*a,b}

*$p<0.05$

DISCUSSION

ROS are produced as normal products of cellular metabolism. However, excessive generation of free radicals can occur due to differences in ingredients of diets.[18,19] When there is an imbalance between ROS production and various antioxidant systems, this leads to an injury in cells and tissues due to alterations of macromolecules (membrane lipids, proteins and DNA) [19,20]. Since membrane phospholipids are major targets of oxidative damage, lipid peroxidation (LP) is often the first parameter analyzed for proving the involvement of free radical damage. LP is a complicated radical chain reaction leading to the formation of various products including malondialdehyde.[21,22] Thus, the presence of MDA is considered as an indicator of free-radical damage through membrane lipid peroxidation.[22] The antioxidant defense system includes small molecular antioxidants, antioxidant enzymes, and metal chelating agents. The efforts of the endogenous antioxidant enzymes to remove the continuously generated free radicals that initially increased due to an induction but with later enzyme depletion resulted in oxidative cell damage.[23] GSH and its metabolizing enzymes provide the major defense against ROS-induced cellular damage.[24] GSH serves as a reductant in oxidation reactions resulting in the formation of GSSG and thus can protect cells against the damage of ROS arised during conditions of oxidative stress. A decrease of the GSH concentrations may reflect depletion of the antioxidant reserve.[9]

In the present study, the supplementation of poppy seed oil at only 0.5 % level to the diet significantly decreased the blood MDA concentration. However, more supplementation of PSO to the diet significantly increased the blood MDA concentration. The supplementation of poppy seed did not affect on the blood MDA concentration. Our results show that the supplementation of PS has no effect on lipid peroxidation in the laying hens. Although research is limited, diets high in fat have been shown in several studies to be associated with higher markers of oxidative stress in animals.[25,26] In our study the high levels of PSO increased the MDA but low levels of PSO decreased the blood MDA concentration in laying hens. These results in confirmed previous studies conducted in animals.[25,26] Also the supplementation of PO and POS to the diet significantly increased the blood GSH concentration. Furthermore the vitamin A levels in all PSO groups increased significantly compared to control group. Poppy oil has a desirable fatty acid composition, with 73% linoleic, 10% palmitic, and 13% oleic acid as the major fatty acids.[3,27] The reported health benefits of oils rich in linoleic acid, like corn or safflower oils, in lowering serum cholesterol levels is an indication of the nutritional importance of the use of poppy seed oil.[28,29] Ozoan and Atalay suggested that the poppy seed oils contained an appreciable amount of tocopherols[1]. Bollengier *et al.* reported that vitamin E increases the antioxidant enzyme activities too during stress and so removes the free radicals in the early phases of lipid peroxidation.[30] Sterols in poppy seed oil consist almost entirely of campesterol, stigmasterol, sitosterol and delta 5-avenasterol.[31] Yoshida and Niki reported that β-sitosterol, stigmasterol, and campesterol exerted antioxidant effects on the oxidation of methyl linoleate oil solution.[32] The results in present suggested that 0.5 % PSO supplementation exhibited direct antioxidant by reducing basal MDA formation and increased the GSH and vitamin A levels in blood. It could be explained by the phytosterols fraction and tocols contents. Its constituents showed the potential use of 0.5 % PSO supplementation as a source of antioxidant principle.

The role of nitric oxide (NO) appears controversial because a tissue dysfunction or injury could occur after inhibition of NO. However, high production level of NO has been suggested as a cause of tissue injury.[33] Stimulation of tissue NO production is also associated with adverse events such as hypotension, inhibition of intermediary metabolism, and the production of the potent oxidant peroxynitrite following radical–radical reaction with superoxide.[34] The bioavailability of NO is reduced due to the increased level of superoxide radical, which transforms NO to peroxynitrite.[35] The present study found the plasma NOx concentrations in 0,5% PSO group were significantly decreased compared to other expertimental groups but The NOx levels in PO groups did not differ.

According to the findings of this study, the 0.5% supplementation of PSO to the diets of laying hens decrease the blood MDA levels and increase the GSH and vitamin A concentrations. It is thought that 0.5% PSO supplementation can be used effectively as an antioxidant suplement in laying hens ■

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