RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

Effects of Supplementation of Aniseed *(Pimpinella anisum L.)* at Various Amounts to Diets on Lipid Peroxidation, Antioxidant Activity and Some Biochemical Parameters in Laying Quails (*Coturnix coturnix japonica*)

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SUMMARY

Aniseed (*Pimpinella anisum L.*), an aromatic plant, is an annual herb cultivated in many countries but indigenous to Iran, India and Turkey. Aniseed has been used effects of carminative, antiseptic, antispasmodic, expectorant, stimulant and stomachic. This study was carried out to determine the effects of Aniseed (*Pimpinella anisum L.*) on blood malondialdehyte (MDA), antioxidant activity (AOA), glutathion (GSH), total cholesterol (TC), glucose, total protein (TP) and triglyceride (TG) in laying quails. A total of 180 laying quails were divided into six groups. One basal diet was used in the experiment. There were 5 experimental groups which were supplemented with aniseed as follows: Group I; 10 g/Kg, Group II; 20 g/Kg, Group III; 30 g/Kg, Group IV; 40 g/Kg, Group V; 50 g/Kg, respectively. The Control Group received no aniseed throughout the experiment lasted for 13 weeks. Aniseed supplementation reduced blood MDA significantly ($p<0.05$) in Groups I, II and III. Whereas plasma AOA level was higher in Groups III and V compared to the CG. Blood GSH increased significantly ($p<0.05$) in Groups III, IV and V. TC, glucose and total protein concentrations were not affected by supplemental aniseed. Plasma TG level was lower in only Group I than in the CG. In conclusion, the results of this study demonstrate that aniseed could be used at 30 g/Kg level in quail diets for an increased antioxidant activity with GSH and a decreased MDA levels.

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Yumurtacı Bıldırcınların (*Coturnix coturnix japonica*) Diyetine Farklı Miktarlarda İlave Edilen Anasonun *(Pimpinella anisum L.)* Lipid Peroksidasyonu, Antioksidan Aktivite ve Bazı Biyokimyasal Parametrelere Etkisi

ÖZET

Bir aromatik bitki olan anason (*Pimpinella anisum L.),* başta İran, Hindistan ve Türkiye olmak üzere birçok ülkede yetiştirilen yıllık bir bitkidir. Anason tohumu karminatif, antiseptik, antispazmodik, ekspektoran, stimulant ve midevi etkileri nedeni ile kullanılmaktadır. Bu çalışma yumurtacı bıldırcınlarda (*Coturnix coturnix japonica*) anason tohumunun kan malondialdehit (MDA), antioksidan aktivite (AOA), glutatyon (GSH), total kolesterol (TK), glukoz, total protein (TP) ve trigliserid (TG)'leri üzerine etkilerini belirlemek amacı ile yapılmıştır. Çalışmada 180 adet yumurtacı bıldırcın kullanılmış, biri anason tohumu ilave edilmemiş bazal rasyonu tüketen Kontrol grubu (KG) olmak üzere sırası ile Grup I; 10 g/Kg, Grup II; 20 g/Kg, Grup III; 30 g/Kg, Grup IV; 40 g/Kg, Grup V; 50 g/Kg düzeyinde anason tohumu ilave edilmiş rasyonları tüketen deneme grupları olacak şekilde 6 gruba ayrılmıştır. Çalışmada bir temel rasyon kullanılmış ve çalışma 13 hafta sürdürülmüştür. Anason ilavelerinin kan MDA düzeyini anlamlı olarak ($p<0.05$) Grup I, II ve III te düşürdüğü, plasma AOA düzeyinin, KG ile karşılaştırıldığında Grup III and V te daha yüksek olduğu ($p<0.05$), kan GSH düzeyinin ise Grup III, IV and V te anlamlı olarak ($p<0.05$) yüksek olduğu, bununla birlikte kan glukoz ve total protein düzeylerinin ise anason tohumu ilavesinden etkilenmediği görülmüştür. Plazma TG düzeyinin ise sadece Grup I de control grubundan düşük olduğu belirlenmiştir ($p<0.05$). Bu sonuçlar, temel rasyona 30 g/Kg düzeyinde anason tohumu ilavesinin GSH ile birlikte AOA düzeyini artırdığını ve MDA seviyesini azaltabileceğini göstermektedir.

INTRODUCTION

In livestock production systems, antibiotics are commonly fed to animals to prevent disease and metabolic disorders, as well as improve feed efficiency. However in recent years, public concern over routine use of antibiotics in livestock nutrition has increased due to the emergence of antibiotic resistant bacteria that may represent a risk to human health. Consequently, considerable effort has been devoted towards developing alternatives to antibiotics. Plants and their extracts offer a unique opportunity in this regard since aromatic plants and their extracts have been used traditionally in the therapy of some diseases for a long time through the world.[1] Therefore, the utilization of plant or plant extracts in livestock production has expanded in livestock nutrition. The banning in 2006 of the use of antibiotics as animal growth promoters in the European Union has increased demand from producers for alternative feed additives that can be used to improve animal production.[2]

As an aromatic plant, anise (*Pimpinella anisum L.*) is an annual herb indigenous to Iran, India, Turkey and many other warm regions in the world. Anise is cultivated in Turkey for domestic consumption and export with a planting area of about 51.870 acres and annual seed production of about 11.000 tons).[3] Aniseed and aniseed volatile oil are used in alcoholic beverages industry, cosmetic, medicine and as aroma and additive in food industry. While it is used in drink industry in Turkey, the beverages that are produced by using aniseed are also common in France (Pastis, Pernod, Anisette ve Ricard), Greece (Ouzo), Russia (Allasch), South America (Aguadiente), Germany and Spain. Aniseed and aniseed oils are employed also in food industry as flavoring agents (e.g., Greek Ouzo, Lebanese arak, anisette) and a concoction of seeds in hot water is used as a carminative, antiseptic, diuretic, digestive, and a folk remedy to insomnia and constipation.[4] *P. anisum* may have therapeutic effects in diseases such as digestive, gynaecologic, neurologic, and respiratory disorders.[5] However, if it is ingested at high quantities, aniseed oil may induce nausea, vomiting, seizures, and pulmonary edema.[6] Chemical studies have demonstrated that the aniseed contains *anethole* (85%) as an active ingredient, in addition to *eugenol, methylchavicol, anisaldehyde* and *estragole*.[6,7] Previous studies have shown confusing results about using aniseed in livestock animals. Ciftci et al.[8] showed that supplementation of anise oil to hen diets at 400 mg/kg increased weight gain. On the other hand, Simsek et al.[9] reported that adding mix oil including anise oil to diet of broiler unchanged body weight, feed conversion and carcass characteristics, except for gizzard weight. Bayram et al.[10] found that the supplementation of aniseed to the diet of quails at 4% of total diet improved immune response against Newcastle virus. However, Erdogan et al.[11] suggested that anise has not a potent hepatoprotective effect against carbon tetrachloride (CCl_4) induced hepatic damage in rats.

At this time, very little is known about the effects of aniseed on oxidant-antioxidant balance and biochemical parameters. The objective of the present study was to determine the effects of supplementation of Aniseed (*Pimpinella anisum L.*) to diet at various quantities on blood lipid peroxidation, antioxidant activity, glutathione, total cholesterol, glucose, total protein and triglyceride in laying quails.

MATERIAL AND METHOD

Animal and protocol design

A total of 180 laying quails (Coturnix coturnix japonica) used in the experiment. They were 8 week age at the start of experiment and divided into six groups of 30 birds. Each treatment group was further subdivided into six subgroups of 5 birds (4 female ans 1 male) per replicates. There were 5 experimental groups which were supplemented with aniseed as follows: Group I; 10 g/Kg, Group II; 20 g/Kg, Group III; 30 g/Kg, Group IV; 40 g/Kg, Group V; 50 g/Kg, respectively. The Control Group received no aniseed. Feed and water provided as *ad libitum*. Artificial light was supplied 16 hours per day. The trial was lasted in 13 weeks.

Diet compatation

Nutrient requirements of the diet determined according to NRC.[12] The basal diet used in the experiment nutrient contents as follows 20% crude protein, 2900 kcal/kg metabolisable energy, 2.5% calcium, 0.35% available phosphorus, 3% crude cellulose, 4% crude fat, 9.44% crude ash, 1.00% lysine, 0.38% methionine, 2.1% linoleic acid, respectively.

Biochemical analyses

At the 13th weeks of experiment, blood samples were collected from vena breachialis from 8 birds randomly chosen from each groups. Blood samples collected in heparinised tubes were centrifuged 1500g for 15 minutes at +4°C. Plasma was stored frozen at -20°C until analysis. Blood MDA was estimated according to method of Draper and Hardley[13], which is based on the coupling MDA with thiobarbituric acid. Plasma AOA was measured according to Koracevic et al.[14]. Briefly, a standardized solution of Fe–EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction, lead-

ing to the formation of hydroxyl radicals (•OH). These reactive oxygen species degrade benzoate, resulting in the release of TBARS. Antioxidants from the added sample cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of color development defined as the AOA. Blood GSH was assayed by colorimetric method Beutler *et al.*[15], respectively. Plasma TC, glucose, TP and TG concentrations were measured using the commercially available assay kit (Chema Diagnostica, Italy).

Statistical analysis of the data

Statistical analysis was made with SPSS statistical software (SPSS for Windows; Release 10.0 Standard Version). Comparisons between different groups were performed by one-way ANOVA by Duncan's multiple range tests. Differences between means of $P < 0.05$ were considered significant. The results are expressed as means ± standard errors.

RESULTS

All parameters and their levels are shown in tables 1. and 2. Blood MDA reduced significantly in Group I, II and III ($p<0.05$). AOA level was elevated in Group III and V compared to the CG ($p<0.05$). Addition of aniseed increased GSH in Group III, IV and V compared that of CG ($p<0.05$). TG level decreased in only Group I compared to the CG ($p<0.05$). All other parameters were not statistically significant difference in CG. Plasma TC, glucose and TP concentrations were not affected by supplementation of aniseed.

DISCUSSION

Oxygen radicals and other reactive oxygen species (ROS) are common products of cellular metabolism. However, during times of environmental stress ROS can increase dramatically and result in significant damage to cell structures. Polyunsaturated fatty acids (PUFAs), which are abundant in cellular membranes, allow for fluidity of cellular membranes. Since a free radical prefers to steal electrons from the lipid membrane of a cell, initiating a free radical attack on the cell known as lipid peroxidation, it is perhaps reasonable to advocate lipid peroxidation as a significant event in the development of membrane damage.[16] Lipid peroxidation is a complicated radical chain reaction leading to the formation of various products including lipid hydroperoxides, conjugated dienes and MDA.[17] Therefore, blood MDA concentration is often determined in some studies as an indicator of lipid peroxidation in the body.[18,19] Our results showed that the supplementation of aniseed to the diet at 10-30 g/Kg of total diet reduced blood MDA concentration, which indicates a decreased lipid peroxidation. Simon et al.[7] suggested that aniseed in the diet at high quantities may have a negative effect on digestive and pulmonary systems. In the present study, aniseed in the diet at 40-50 g/Kg of total diet unchanged blood MDA concentration. This effect of aniseed could be resulted from various bioactive elements especially the volatile oil acids such as eugenol and phenolic compounds with other phytochemical compounds that were found in aniseed. Gulcin et al.[6] reported that aqueus extract of aniseed had much more antioxidant capacity than the its ethanol extract. On the other hand, Al-Ismail and Aburjai[20] found that when compared with ethanol extract, the aqueus extract of aniseed was more effective in reducing the ability to create iron chelation.[21]

To prevent free radical formation or limit their damaging effects, the cell has a defense system of antioxidants. Cells are normally able to defend themselves against ROS damage through the use of enzymes such as superoxide dismutase and catalase. Small molecule antioxidants such as ascorbic acid (vitamin C), alpha tocopherol (vitamin E), uric acid, and glutathione also play important roles as cellular antioxidants.[17] Recently, interest has considerably increased to find a naturally occurring antioxidant for being used in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers.[22] Therefore, natural antioxidants can protect the organism from free radicals and retard the progress of many chronic diseases as well as lipid peroxidation.[6] Our results showed that AOA increased in the quails fed with aniseed. In the present study, both decreased or unchanged MDA and increased AOA indicate that the supplementation of aniseed to diet affects positively the antioxidant defense.

GSH provides major protection in oxidative injury by participating in the cellular system of defense against oxidative damage.[23,24] GSH scavenges O_2 and protects protein thiol groups from oxidation. GSH also has a major role in restoring other free radical scavengers and antioxidants, such as vitamin E and ascorbic acid, to their reduced state. Because tissue GSH levels and the activities of glutathione reductase and glutathione peroxidase, which are critical constituents of the GSH-redox cycle, are significantly reduced due to oxidative stress, the authors propose that an impairment of antioxidant defense mechanisms could permit enhanced free radical induced tissue damage.[25,26] In this study, the supplementation of aniseed to diet increased blood GSH concentration but this in-

crease reached a significance ($p<0.05$) when supplemented aniseed to diet at 30 g/Kg and more.

Some plants or their extracts affect the plasma concentrations of glucose, TC and triglycerides absorbed from intestines.[18,27] In the present study, the plasma glucose, TC and TP concentrations were not affected by aniseed supplementation. However, aniseed supplementation at low levels (10-20 g/Kg of total diet) decreased the plasma triglyceride concentrations in this study.

In conclusion, aniseed could be used at 30 g/Kg level in quail diets with an increased antioxidant activity and a decreased lipid peroxidation and, it does not affect the biochemical parameters negatively as well. However, further studies should be carried out to determine the beneficial effects of aniseed supplementation to the diet ■

Table 1. Blood MDA, GSH and plasma AOA levels in the groups (mean±SE)
Çizelge 1. Gruplardaki kan MDA, GSH ve plazma AOA düzeyleri (mean±SE)

Parameters	Control $\overline{X}$ ±SE	Grup I $\overline{X}$ ±SE	Grup II $\overline{X}$ ±SE	Grup III $\overline{X}$ ±SE	Grup IV $\overline{X}$ ±SE	Grup V $\overline{X}$ ±SE
MDA (nmol/ml)	12.33 ± 0.51^{a}	10.46 ± 0.71^{bc}	9.09 ± 0.55^{c}	10.66 ± 0.31^{b}	11.58 ± 0.51^{ab}	11.70 ± 0.52^{ab}
AOA (mmol/L)	1.59 ± 0.04^{c}	1.63 ± 0.04^{c}	1.77 ± 0.08^{bc}	1.88 ± 0.10^{ab}	1.81 ± 0.02^{bc}	2.07 ± 0.12^{a}
GSH (mg/dl)	73.32 ± 2.62^{c}	82.04 ± 3.05^{abc}	76.86 ± 3.90^{bc}	86.81 ± 3.08^{ab}	90.60 ± 5.06^{a}	84.62 ± 2.95^{ab}

$^{a, b, c}$: Different superscripts in the same row indicate significant differences ($p<0.05$).

Table 2. Plasma TC, Glucose, TP and TC concentrations in the groups (mean±SE)
Çizelge 2. Gruplardaki plazma TC, glukoz, TP ve TC konsantrasyonları (mean±SE)

Parameters	Control $\overline{X}$ ±SE	Grup I $\overline{X}$ ±SE	Grup II $\overline{X}$ ±SE	Grup III $\overline{X}$ ±SE	Grup IV $\overline{X}$ ±SE	Grup V $\overline{X}$ ±SE
T-Cholesterol (mg/dl)	75.59±9.22	63.79 ± 10.54	69.99 ± 19.99	73.08 ± 12.34	82.77 ± 13.04	78.95 ± 11.08
Glucose (mg/dl)	160.16 ± 10.15	180.63 ± 5.38	194.49 ± 10.09	195.28 ±12.30	171.72 ± 4.19	210.01 ± 24.34
Total Protein (g/dl)	3.79 ± 0.51	3.45 ± 0.34	3.11 ± 0.34	3.10 ± 0.37	3.61 ± 0.49	4.12 ± 0.41
Triglycerides (mg/dl)	218.33 ± 5.07^{a}	156.67 ±12.56^{b}	173.73 ±21.20^{ab}	192.63 ±27.05^{ab}	220.79 ± 10.08^{a}	219.42 ±2.55^{a}

$^{a, b, c}$: Different superscripts in the same row indicate significant differences ($p<0.05$).

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