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Research Article

The Effect of Oregano Oil *(Origanum vulgare)* on the Fattening Performance and Blood Oxidant-Antioxidant Balance in Post-weaned Tuj Lambs

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Abstract

The purpose of this study is to investigate the effect of oregano oil (Origanum vulgare) at on fattening performance and blood oxidant-antioxidant balance such as glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) catalase (CAT) enzyme activities, malondialdehyde, ceruloplasmin, nitric oxide, albumin, total protein and globulin in post-weaned Tuj breed lambs. The lambs were average 155 days old, and were divided into 3 groups regardless of gender, and in total 18 lambs were used. In the study the control group (C) was fed with basal ration; while the experimental groups were orally fed with oregano oil supplementation as a commercial product in daily doses of 1 mL/lamb/day (T1) and 2 mL/lamb/day (T2), respectively, in addition to the basal ration, via sterile injections. The experiment was maintained for a total of 52 days of which 10 days were adaptation period. In the study, it has been seen in post-weaned Tuj breed lambs that using different doses of oregano oil had no effect on live weight gain, daily live weight gain, daily feed consumption, and feed conversion ratio ($P>0.05$). In the study, it was determined that the addition of oregano oil to the ration made no significant difference on fattening performance and MDA, GSH, CAT, ceruloplasmin, nitric oxide, albumin, total protein and globulin values of blood oxidant-antioxidant parameters. SOD and GPx values were significantly affected by the addition of oregano oil to the lamb rations at the point of examining the effect on blood oxidant-antioxidant balance ($P<0.05$). As a result, it has been determined that oregano oil did not affect the performance parameters, it significantly affected the oxidant-antioxidant balance in lambs.

Keywords: *Oxidant-antioxidant balance, Tuj, Lamb, Performance, Oregano oil*

Sütten Kesilmiş Tuj Irkı Kuzularda Kekik Yağı *(Origanum vulgare)* Kullanımın Besi Performansı ve Kan Oksidan-Antioksidan Denge Üzerine Etkisi

Öz

Bu çalışmanın amacı sütten kesilmiş Tuj ırkı kuzularda kekik yağının performans ve glutatyon (GSH), süperoksit dismutaz (SOD) oranları, glutatyon peroksidaz (GPx) katalaz (CAT) enzim aktiviteleri, seruloplazmin, nitrik oksit, albumin, total protein ve globulin kan oksidan-antioksidan denge üzerine etkisinin araştırılmasıdır. Kuzular ortalama 155 günlük yaşta olup cinsiyet gözetilmeksizin 3 gruba ayrılmış ve toplamda 18 kuzu kullanılmıştır. Çalışmada kontrol grubu (K) bazal rasyonla beslenirken deneme gruplarına bazal rasyona ek olarak sırasıyla 1 mL/kuzu/gün (T1) ve 2 mL/kuzu/gün (T2) dozlarında ticari ürün olan kekik yağı ilavesi steril şırıngalar ile hayvanlara günlük olarak oral şekilde verilmiştir. Deneme, 10 gün adaptasyon dönemi olmak üzere toplam 52 gün sürdürülmüştür. Çalışmada kesilmiş Tuj ırkı kuzularda kekik yağının farklı dozlarda kullanımının canlı ağırlık artışı, günlük canlı ağırlık artışı, günlük yem tüketimi ve yemden yararlanma oranını etkilemediği görülmüştür ($P>0.05$). Çalışmada rasyona kekik yağı katılmasının, besi performansı ve kan oksidan antioksidan parametrelerinden MDA, NO, GSH, CAT, seruloplazmin, albümin, total protein ve globulin değerlerinin üzerinde önemli bir farklılık oluşturmadığı saptanmıştır. Kan oksidan antioksidan denge üzerine etkinin irdelenmesi noktasında SOD ve GPx değerleri kuzu rasyonlarında kekik yağı ilavesinden anlamlı düzeyde etkilenmiştir ($P<0.05$). Sonuç olarak kekik yağının kuzularda performans parametrelerini etkilemediği, kan oksidan-antioksidan denge üzerine olumlu etkilerinin olduğu tespit edilmiştir.

Anahtar sözcükler: *Oksidan-antioksidan denge, Tuj, Kuzu, Performans, Kekik yağı*

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INTRODUCTION

Because the antibiotics and the antimicrobial agents, used in animal feeding for many years as growth stimulants, have given rise to the development of resistance in pathogenic bacteria in humans, and due to the residues they can generate in animal products, the demands for such products is decreasing day by day [1,2]. In recent years, with the prominence of consumer demand in terms of product characteristics, the desire to obtain more natural and more suitable products for human health has made the field of animal feeding with new searches a current issue, and many of the researchers have focused their attention on natural and aromatic plants, and essential oils obtained from them [3,4]. Oregano is a common term for the plant family *Lamiaceae*, which has more than 60 species, and known by their general aroma and taste [5]. Among these species *Thymus, Origanum, Satureja, Thymbra* and *Coridothymus* are of great importance both prevalently and economically [6]. Turkey is the country with the world's most important exporter thyme. A large part of the thyme exported from Turkey is *Origanum* species [7]. Both oregano itself and its extracts (oregano essential oil) contain substances that induce such as antiseptic, antioxidant, antimicrobial and aroma-regulating effects. It has been reported that oregano contains *phenols, thymo, carvacrol, monoterpene hydrocarbons, p-cymene and γ-terpinene* [8]. Oregano is rich in *carvacrol*, and at a lesser extent in *phenolic monoterpenoids* (particularly thymol) [9].

The short-lived reactive atoms and molecules with un-conjugated electrons in their outer orbitals are known as free radicals and the enzymatic and non-enzymatic structures trying to prevent radicals and their reactions are defined as antioxidants [10]. In normal functioning cells, there is a balance between the free radicals produced as a by-product of cell function and the antioxidants which inactivate them, and is described as an oxidant-antioxidant balance. Oxidative stress occurs when there is a free radical production in the cells that overcomes the antioxidant defense system; leads to protein and DNA oxidation, and lipid peroxidation in cells; and consequently causes cell destruction and tissue damage [10]. Therefore, studies on the potential antioxidant effects of plant extracts have accelerated especially in recent years. Farag et al.[11] examined the relationship between the chemical composition and antioxidant properties of essential oils, and stated that due to the presence of phenolic OH groups acting as a hydrogen donor to the peroxide radicals released during the first step of the lipid oxidation, thymol owns high antioxidant activity by reducing the formation of hydroxy peroxide.

In recent years, several studies focused on the aromatic plants and their extracts. Oregano oil has antioxidant properties. Therefore, oregano oil has been used in many studies because of its antioxidant properties. Turkey is very important in the world in the export of oregano. Oregano oil used in our study were obtained from the Mediterranean region of Turkey. There are many studies in the literature on the use of oregano in lambs. However, no study was seen in the literature about the use of oregano oil in Tuj breed lambs. Therefore, the aim of this study is to investigate the effect of oregano oil on fattening performance and blood oxidant-antioxidant balance such as glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) catalase (CAT) enzyme activities, malondialdehyde (MDA), ceruloplasmin, nitric oxide (NO), albumin, total protein and globulin in post-weaned Tuj breed lambs.

MATERIAL and METHODS

Animals, Experimental Design and Feed

This study was carried out with the permission of the Kafkas University Animal Experiments Local Ethics Committee (Decision No: KAU-HAYDEK/2018-086) report. In the study, Tuj breed lambs, being raised in Kars, Ardahan and Igdir provinces, which is a local sheep breed in Turkey were used as an animal material. The average age of the lambs was 155 days. Lambs were divided into 3 groups, regardless of gender, with a mean live weight of 34.75±0.22 kg, and a total of 18 lambs were used. The Experiment was conducted in Kafkas University Faculty of Veterinary Medicine, Prof. Dr. Ali Riza AKSOY Training, Research and Implementation Farm. The experiment was maintained for a total of 52 days of which 10 days were adaptation period. During the study, the animals were housed in individual boxes (180 cm × 150 cm × 120 cm; height, length, width, respectively) equipped with feeders dispensing pasture grass and concentrate feed separately. During the study, the animals were fed with pasture grass and lamb grower feed.

In the study; dry matter, energy and other nutrient requirements of animals were calculated according to NRC [12] standards. The daily amount of concentrate feed provided to the lambs was 600 g per animal. Pasture grass and water was supplied *ad libitum* during the trial. Animals were fed mainly with roughage. The content of the concentrated feed is given in *Table 1*. In adaptation period that lasted 10 days lambs got accustomed to the experimental diet. All lambs were treated against internal and external parasites. The study was conducted in Kars. Kars is a place where important pastures are at for Turkey. Tuj lambs used in the study are a region-specific race. Tuj lambs are fed with pasture-based feed in the region. For this reason, in our study, while feeding tuj lambs with the pasture *ad libitum,* 600 g of concentrated feed were given daily. In this wise, the effect of using oregano oil based on pasture feeding method on performance and blood oxidant and antioxidant balance was investigated.

In the study, control group (C) was fed with basal ration. In addition to basal ration, the experimental groups were fed with a supplementation of oregano oil *(Origanum vulgare,* BOTALIFE®), a commercial product at doses of 1 mL/lamb/

Table 1. *Ingredient composition of concentrate feed*

Ingredients	Amount, %
Wheat	3.75
Barley	17.5
Corn	12.5
Soya bean meal	2.5
DDGS	2.5
Sunflower seed meal (28-30 CP)	2.5
Sunflower seed meal (36 CP)	2.5
Safflower meal	6.25
Cotton seed meal (28-30 CP)	4.6
Wheat bran	20
Corn bran	6.3
Leaf cotton (25 CP)	6.25
Molasses	9
Marble powder	3.25
Salt	0.5
Vitamin mineral premix	0.1

[1] CP: Crude Protei
[2] DDGS: Dried Distillers Grains with Solubles
[3] The vitamin & mineral premix provided the following (per kg): 4.000.000 IU Vit. A, 800.000 IU Vit. D_3, 5.000 IU Vit. E, 400 mg Vit. B_2, 2 mg Vit. B_{12}, 5.000 mg Vit. PP, 1.000 mg D-pantothenic acid, 20.000 mg choline, 50 mg Co, 5.400 mg Fe, 185 mg I, 6.900 mg Mn, 800 mg Cu, 6.400 mg Zn, 14 mg Se

day (T1) and 2 mL/lamb/day (T2), respectively. Oregano oil was given orally by sterile syringes to the experimental groups daily. Oregano oil used in the study, *Origanum vulgare* belong to the species of plants are grown in Turkey. Oregano oil used in the study contains 4.15% thymol and 75.3% carvacrol. The essential oil acid profile of the commercial oregano oil is presented in *Table 2*.

Feed Analysis

Nutrient analysis of the feed was determined according to the method reported in AOAC [13]; while NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) analyses for pasture grass were determined according to the method reported by Goering and Van Soest [14].

Determination of Performance Parameters

Animals were weighed before morning feeding in the beginning and on the 0th, 10th, 31st and 52nd days of the trial. At the end of the trial, daily feed consumption and feed conversion ratios of each group were calculated. Feed convertion ratio was calculated as the proportion of daily feed consumption to daily weight gain (kg/kg).

Biochemical Analyses

On the 10th and 52nd days during the experimental period, after separating a fair amount of the blood samples, taken from the *V. jugularis* of the animals with anticoagulant (EDTA) tubes, as whole blood, plasma of the remaining

Tablo 2. *The essential oil acid profie of the commercial oregano oil (%)*

Ingredient	%	Ingredient	%
α-*pinene*	0.29	spathulenol	0.37
α-*thujene*	0.30	b-caryophyllene oxide	1.01
α-terpinene	0.66	carvone	0.25
b-myrcene	0.79	α -*terpineol*	0.32
α-*fellanderen*	0.30	borneol	0.99
g-terpinen	2.39	b-bisabolen	2.08
p-simen	2.89	timol	4.15
linalyl acetate	0.35	carvacrol	75.30
linalool	6.36		

blood was obtained. Samples taken were centrifuged at 3000 rpm for 15 min, and stored at -20ºC until the analyses were carried out.

Superoxide dismutase, GPx and CAT antioxidant enzyme activities in plasma were determined by ELISA device (Epoch, Biotek, USA) using commercial kits (Cayman Chemical Co., USA). Whole blood reduced glutathione (GSH) analysis was determined colorimetrically (Epoch, Biotek, USA) according to the method issued by Beutler et al.[15]; while MDA in plasma by Yoshoiko et al.[16], NO by Miranda et al.[17], ceruloplasmin by Colombo and Ricterich [18], and albumin and total protein levels by commercial test kit (Biolabo, France). The globulin was determined by subtraction of the albumin from the total protein according to Doumas et al.[19].

Statistical Analysis

For the significance of the differences between the statistical calculations belonging to the groups and the mean values of the groups, one-way analysis of variance (ANOVA) method was used, and for the significance control of the difference between the groups, the appropriate next stage test (Tukey) was applied. For this purpose, SPSS packaged software was used [20].

RESULTS

The amounts of nutrients and metabolizable energy values of concentrated feed and pasture grass are shown in *Table 3*. The performance parameters of the study are given in *Table 4*. In the study, statistically significant difference between the groups regarding average live weights, live weight gains, daily live weight gains, daily feed consumption and feed conversion ratio was not found (P>0.05). It has been observed that for the oregano oil use in post-weaned Tuj lambs, the difference between the groups, in terms of the performance parameters, was not statistically significant (P>0.05). It has been observed that the addition of oregano oil to lamb rations does not affect the performance parameters, but improves it.

Blood parameters of the study are given in *Table 5*. The differences between the groups on MDA, NO, GSH, CAT,

Table 3. *Nutrient and energy* levels of the feeds (%)*

Feeds	DM	CP	EE	CF	CA	Calcium	Phosphorus	ADF	NDF	ME
Concentrate	87.36	16.25	2.36	11.03	7.99	0.64	0.42	-	-	2531
Pasture grass	92.1	9.13	-	-	7.6	0.65	0.16	38.70	62.60	1767

DM: *Dry matter,* ***CP:*** *Crude protein,* ***EE:*** *Ether extract,* ***CF:*** *Crude fiber,* ***CA:*** *Crude ash;* ***ADF:*** *Acid detergent fiber,* ***NDF:*** *Neutral detergent fiber,* ***ME*:*** *Metabolic Energy (kcal/kg)*

Table 4. *The effect of oregano oil on the live weight, live weight gain, daily live weight gain, daily feed consumptions, feed consumptions and feed conversion ratio of lambs*

Fattening Performance Parameters	Control		T1 1 mL/day/lamb		T2 1 mL/day/lamb		Significance
	X	Sx	X	Sx	X	Sx	P
Live Weight, kg Days							
0	34.58	0.23	34.83	0.42	34.83	0.52	0.886
10	35.81	0.25	36.13	0.34	36.06	0.47	0.814
31	38.18	0.2	38.7	0.23	38.93	0.34	0.160
52	41.05	0.07	41.4	0.33	41.65	0.44	0.441
Live Weight Gain, kg, Days							
0-10	1.23	0.12	1.3	0.11	1.23	0.12	0.889
10-31	2.36	0.08	2.56	0.19	2.86	0.24	0.195
31-52	2.86	0.23	2.7	0.18	2.71	0.18	0.818
0-52	6.46	0.25	6.56	0.37	6.81	0.48	0.805
Daily Live Weight Gain, g Days							
0-10	123.33	12.01	130	11.25	123.33	12.01	0.889
10-31	112.7	3.82	122.22	9.25	136.51	11.64	0.195
31-52	136.5	11.24	128.57	8.60	129.36	8.98	0.818
0-52	124.39	4.98	126.28	7.24	131.09	9.29	0.805
Daily Feed Consumption (dry matter), g Days							
0-52	1511.77	43.56	1448.30	101.34	1441.01	97.95	0.814
Feed Convertion Ratio Days							
0-52	12.32	0.84	11.91	1.56	11.46	1.37	0.898

K: *Control,* ***T1:*** *1mL/day/lamb oregano oil,* ***T2:*** *2 mL/day/lamb oregano oil. Statistically not significant (P>0.05). All values are given as mean ± standard error of mean (SEM), (n=6)*

ceruloplasmin, albumin, total protein and globulin values of oregano oil use in weaned Tuj lambs were not statistically significant (P>0.05). According to the results, in terms of SOD and GPx, the use of oregano oil in post-weaned Tuj breed lambs was found to be statistically significant (P<0.05).

DISCUSSION

The use of aromatic plants and extracts in ruminant breeding is increasing each passing day. It has been reported that essential oils stimulate the digestive system of animals and increase the efficiency of digestive secretions [21]. Due to these features, essential oils have a positive effect on performance parameters. In the study conducted, it was observed that the use of oregano oil in different doses in post-weaned Tuj lambs did not affect live weight gain, daily live weight gain, daily feed consumption, and feed conversion rate. There are many current studies using thyme and other plant extracts that support the results of our study [22-26]. In another study, it has been reported that the use of *cinnamic aldehyde* or *carvacrol* (200 mg/kg diet) does not affect dry matter consumption, live weight gain, and feed conversion ratio parameters [27]. In contrast to the results obtained, it was reported in a different study that the use of oregano oil in lamb feeding did not affect the feed conversion rate, and that the daily feed consumption and daily live weight gain were higher [28]. There are also studies reporting that the use of plant extracts in lambs has positive effects on dry matter consumption, live weight gain and feed conversion parameters [29-31].

The observed differences between the results are considered to be affiliated with the factors such as: plant species from which the oregano oil is obtained, oregano oil dose, the *carvacrol* and *thymol* ratio in oregano oil, the composition of the ration, the roughage-weighted feeding method, race

Table 5. *The effect of oregano oil on MDA, NO, GSH, CAT, SOD, GPx, ceruloplasmin, albumin, total protein and globulin*

Oxidant-Antioxidant Parameters	Days	Control		T1		T2		Significance
		X	Sx	X	Sx	X	Sx	P
MDA (µmol/L)	10	2.70	0.04	2.75	0.03	2.77	0.04	0.479
	52	2.69	0.04	2.73	0.02	2.76	0.03	0.407
NO (µmol/L)	10	12.24	0.94	12.32	1.30	12.75	1.14	0.948
	52	12.48	1.10	12.93	1.01	12.97	0.97	0.934
GSH (mg/dL)	10	38.21	1.93	38.16	2.14	38.24	1.95	1.000
	52	37.84	1.67	37.51	1.89	39.80	2.89	0.736
SOD (U/mL)	10	1.06^{c}	0.07	1.72^{b}	0.07	2.40^{a}	0.08	**0.000***
	52	1.02^{c}	0.07	1.70^{b}	0.07	2.27^{a}	0.02	**0.000***
CAT (nmol/min/mL)	10	28.07	1.39	32.23	1.55	32.42	2.05	0.156
	52	27.80	0.89	30.36	1.18	31.81	1.35	0.077
GPx (nmol/min/mL)	10	297.31^{b}	8.47	311.08^{ab}	4.22	32.54^{a}	6.00	**0.046***
	52	294.81^{b}	6.04	320.97^{ab}	7.10	321.72^{a}	7.41	**0.022***
Ceruloplasmin (mg/dL)	10	21.75	1.46	22.12	1.54	19.48	1.40	0.413
	52	19.57	0.9	18	1.02	18.14	1.08	0.488
Albumin (g/dL)	10	2.9	0.06	2.91	0.11	2.94	0.08	0.944
	52	2.87	0.07	2.95	0.08	2.87	0.06	0.709
Total protein (g/dL)	10	7.15	0.05	7.06	0.08	7.19	0.05	0.404
	52	7.18	0.10	7.16	0.13	7.20	0.06	0.952
Globulin (g/dL)	10	4.25	0.1	4.15	0.16	4.24	0.07	0.819
	52	4.31	0.16	4.21	0.14	4.33	0.07	0.789

K: *Control,* ***T1:*** *1 mL/day/lamb oregano oil,* ***T2:*** *2 mL/day/lamb oregano oil. Statistically not significant (P>0.05). All values are given as mean ± standard error of mean (SEM), (n=6);* ^{a,b,c} *The differences between the mean values with a different letter in the same row were statistically significant (P<0.05) * P<0.001*

of the animal, oregano oil's administration route to the animal.

Oxidation events occur constantly in the metabolism of a living being, and reactive oxygen substances, taken from outside, accelerate these oxidation events. Antioxidants are substances that prevent autoxidation/peroxidation progress by reacting with the radicals very quickly [32]. Endogenic antioxidants are non-enzymatic antioxidants composed of glutathione, albumin and ceruloplasmin, and enzymatic antioxidants that form the SOD, CAT, GPx and glutathione reductase enzymatic defense line [33,34]. The SOD enzyme is the first line of the antioxidant defense system. It plays a critical role in eliminating superoxide radicals [35]. Glutathione peroxidase is found in the cytoplasm of cells, and protects cells against oxidative damage caused by H_2O_2 [36]. In the present study, it was observed that the use of oregano oil in lambs did not affect MDA, NO, GSH, CAT, ceruloplasmin, albumin, total protein, and globulin values, but significantly affected SOD and GPx values. Compared to the control group, SOD and GPx values increased in direct proportion to the increasing doses of oregano oil. The research results are consistent with current studies using thyme and other plant extracts. In the study in which 11 different plant extracts, including thyme, were used for lamb feeding, it was reported that the blood antioxidant activity was not affected [37]. Gumus et al.[24] reported in their study that the use of oregano oil in the lambs not effected liver SOD values but GSH and CAT values, and also affected GSH, SOD, and CAT enzyme activities in *M. longissimus dorsi* tissue muscle significantly. Furthermore, in some studies using lamb extract from lambs, glutathione peroxidase activity (P<.05) has been positively affected and antioxidant parameters have been improved [26,31,38].

In conclusion, it was determined that the addition of oregano oil into the ration did not have a significant effect on fattening performance and MDA, NO, GSH, CAT, ceruloplasmin, albumin, total protein, and globulin values of blood oxidant-antioxidant parameters. SOD and GPx values were significantly affected by the addition of oregano oil in lamb rations at the point of examining the effect on blood oxidant-antioxidant balance. Oregano oil had no effect on performance parameters in lambs, but significantly affects SOD and GPx values in blood. Therefore, it is determined that blood oxidant-antioxidant balance parameter results; have the potential to protect the cells against oxidative damage caused by free radicals, are able to decrease the peroxidation by strengthening the antioxidant structure in blood, and can be effective in protecting the oxidative stress which decreases the efficiency and resistance of the animals.

In the light of the data obtained from this study, it was concluded that the use of oregano oil, which is among the natural herbal products as an alternative to antibiotics, in Tuj lambs will make an important contribution to the breeders and the researchers, who will work later in this field, since it clarifies the changes occurred in the performance and blood parameters.

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