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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Effects of Oregano Essential Oil (*Origanum onites L.*) Supplementation on Growth and Biochemical Metabolites in Merino Lambs

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Abstract: After the prohibition of antibiotic usage to improve performance, the breeders have guided to natural resources for this purpose. Essential oils have many beneficial effects and they are ideal to use as a growth promoter feed additive in livestock. In our study for this reason, we used oregano essential oil (*Origanum onites L.*) in the treatment groups. Thirty Merino lambs (aged of 2.5 month and weighted average 30±0.1 kg) were housed in 45-day to determine the effects of oregano oil on the growth and biochemical metabolites. The main ration includes consisting of concentrate and alfalfa hay. In the control group oregano essential oil was not added. The second group (ORG 250) was supplemented with 250 mg/d oregano essential oil, third group (ORG 500) also were supplemented with 500 mg/d. Bloods were taken from 8 animals for each group in the initial and end of the study. Samples were collected into anticoagulant tubes and plasma were allocated into microtubes and stored until the analysis day. There were statistically differences in growth hormone (GH), total protein, triglyceride, AST concentrations in ORG 500 group at 45th day ($p<0.05$). However IGF-1, BHBA, albumin, cholesterol ALT, Ca, P levels had no statistically differences. It was defined that the increases in growth hormone, IGF-1 and some biochemical metabolites had positive effects on performance of oregano essential oil at a dose of 500 mg/day in small ruminants. It was concluded that the data that determined in this study will be beneficial for further researches.

Keywords: Biochemical metabolites, growth hormone, lamb, oregano, supplementation.

Merinos Kuzularında Kekik Esansiyel Yağı (*Origanum onites L.)* Takviyesinin Büyüme ve Biyokimyasal Metabolitler Üzerine Etkileri

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Öz: Performansı artırmak için antibiyotik kullanımının yasaklanmasından sonra yetiştiriciler, bu amaçla doğal kaynaklara yönelmişlerdir. Esansiyel yağların pek çok yararlı etkiye sahiptir ve çiftlik hayvanlarında büyümeyi hızlandırıcı yem katkı maddesi olarak kullanımı idealdir. Bu nedenle, çalışmamızda deneme gruplarında kekik esansiyel yağı (*Origanum onites L.)* kullandık. Otuz Merinos kuzusu (2.5 aylık ve ortalama ağırlıkları 30±0.1kg), kekik yağının büyüme ve biyokimyasal metabolitler üzerindeki etkilerini belirlemek için 45 günlük süreyle bakıldı. Ana rasyon konsantre ve yonca samanından içermektedir. Kontrol grubuna kekik esansiyel yağı ilave edilmedi. İkinci gruba (ORG 250) 250 mg/gün, üçüncü gruba (ORG 500) 500 mg/gün kekik esansiyel yağı ilave edildi. Çalışmanın başında ve sonunda her grup için 8 hayvandan kan alındı. Numuneler antikoagülan tüplere alındı, plazma mikrotüplere ayrıldı ve analiz gününe kadar saklandı. ORG 500 grubunda 45. günde büyüme hormonu (GH), total protein, trigliserid, AST konsantrasyonlarında istatistiksel olarak farklılık vardı ($p<0.05$). Ancak IGF-1, BHBA, albümin, kolesterol ALT, Ca, P düzeylerinde istatistiksel olarak fark bulunamadı. Küçükbaş hayvanlarda 500 mg/gün dozunda kekik esansiyel yağının büyüme hormonu, IGF-1 ve bazı biyokimyasal metabolitlerdeki artışları performans üzerinde olumlu etkilere sahip olduğu belirlendi. Bu çalışmada belirlenen verilerin daha sonraki araştırmalar için faydalı olacağı sonucuna varılmıştır.

Anahtar kelimeler: Biyokimyasal metabolitler, büyüme hormonu, ekleme, kekik, kuzu.

INTRODUCTION

In livestock, antibiotics had been used for prevention diseases and improvement growth performance since extended years. After the prohibition of antibiotic feed additives in Europe by the Union (EU) in 2006, increased the interest in research on alternative substances and began the search for different natural additives in ration (Placha et al., 2014). The purpose of these alternative additives is to decrease mortality rate, keep healthy and provide better animal yield while protecting the environment and consumer health. A lot of research has been done to look for natural products with beneficial effects similar to those of growth promoters (Cristo et al., 2022). Using phytogenic and natural products in ruminant rations can change rumen microbial fermentation and influence animal performance (Farghaly et al., 2021). One of the phytogenic and recently most studied products are essential oils. Essential oils are obtained from various aromatic plants. They are usually localized in temperate to warm countries and they are an important part of the traditional plants in the Mediterranean and tropical countries (Bakkali et al., 2008). *Origanum onites L.* that we studied has lots of beneficial effects. *Origanum onites L.* presents a wide distribution area in the south and west of Turkey and south of Greece. Buttons that make up the leaves and flowers of the *Origanum onites L.* plant are consumed as spice (Ietswaart, 1980).

The essential oils, which contain significant amounts of carvacrol and thymol, possess antibacterial, antispasmodic, antiseptic, antimicrobial, cytotoxic, antioxidant and antifungal activities. Also, the essential oil of the plant is used in pharmacy and perfumery apart from food (Lagouri et al.,1993; Sivropoulou et al., 1996). Froehlich et al., (2017) declared that lower than recommended feeding rates of an EO combination resulted in improved dairy calf growth performance, which suggests that previous EO studies may have fed too much EO that resulted in similar or reduced animal performance. It was reported that there is an improvement in performance fed with some essential oil mixture also including oregano in livestock (Wu et al., 2021). Besides, it is seen that the use of essential oils as small ruminants' growth and performance enhancers have limited data.

A study conducted in cattle, it was observed when an energy-enhanced ration is used and feed intake was reduced, feed efficiency, serum GH and IGF-1 levels could be developed. GH and IGF-1 markers may work together or independently on the control of animal metabolism (Zhang et al., 2015). GH is secreted from the pituitary gland and regulates gluconeogenesis, lipogenesis, lipolysis, and insulin secretion. It effects on liver and adipose tissue in animals (Sørensen et al., 1992; Baumann, 1994). IGF-1 is a hormone that synthesis from liver and one of the important duty to provide protein synthesis Also this somatomedin conduce to increase the rate of feed conversion (Gao et al., 2006; Yang et al., 2019). The axis of between GH and IGF-1 play an important role in the regulation of metabolism and GHR combines with GH to stimulate a lot of metabolic activities by producing IGF-1 in the liver tissues (Akis et al., 2010; Ayuk & Sheperd, 2006).

The aim of the current study to investigate the changes of plasma growth and biochemical parameters in lambs supplemented with different doses of oregano essential oil.

MATERIAL AND METHOD

Management and Diets: The study carried out at the Dak Agricultural Products Trading and Limited Company in Kırklareli, Turkey. The total of 30 (aged of 2.5 month and weighted average 30±0.1 kg) lambs were housed in individual blocks with approximately 2.0 m^2. Beddings were made from wheat straw. All animals were in the same condition and access to ad libitum mix lamb ration and water. The compositions of grower concentrate feed and alfalfa hay are given in Table 1. The oregano essential oil was given to the lambs orally using syringe. Thirty merino lambs were divided three group. Each group was included ten animals. The oregano essential oil was not added to the control group. The second group (ORG 250) was supplemented with 250 mg/d oregano essential oil, third group (ORG 500) also were supplemented with 500 mg/d. The doses of the oregano essential oil were determined by previous study (Yesilbag et al. 2017). This study was approved by Animal Care Use and Committee from Tekirdag Namik Kemal University and it was enumerated T2021-765. The animals were fed for 45 days and then the study was completed.

Table 1. Nutrient composition of grower concentrate and alfalfa hay on a dry matter basis*.

Item	Grower concentrate†,‡	Alfalfa hay
Dry matter, %	89.6	91.8
CP, %	16.74	17.20
Ether extract, %	3.80	1.43
NDF, %	27.89	40.83
ADF, %	11.32	31.87
ADL, %	4.28	9.56
NFC§, %	42.47	29.87
Ash, %	9.1	10.67
Ca, %	1.69	1.45
P, %	0.63	0.30

*Nutrient analyses of the feeds were performed according to AOAC (2016).

†C.P. Feed Industry, Tekirdag, Turkey.

‡Contained the main ingredients: wheat brain, ground corn grain, corn gluten meal, sunflower meal,ground wheat grain, ground barley grain, DDGS, molasses, calcium carbonate, wheat flour, soya bean meal, vinasse, vegetable oil, ammonium chloride, vitamin and mineral mix, salt.

§NFC (Non-Fibre Carbonhydrate): 100 – (% NDF + % CP + % Ether extract + % Ash)

Content of Oregano Essential Oil: Oregano essential oil was obtained from commercial company (Nativital Natural Life and Health Products Company). The composition of the *Origanum onites L.* essential oil is shown in Table 2. In the content of the oregano essential oil carvacrol ratio was 73.4% amount.

Sample Collection: Blood samples were taken from the jugular vein, 8 animals for each group in the initial and end of the study. Samples were collected into Ethylenediaminetetraacetic acid (K_3 EDTA) anticoagulant tubes and they were centrifuged in the same day at 3000 rpm for 10 minutes to separate the plasma. The plasma samples were allocated into microtubes and were stored at -20°C until the analysis day.

Table 2. Composition of the *Origanum onites L.* essential oil.

Components	(%)
Myrcene	1.2
γ-Terpinene	3.5
p-Cymene	2.7
Linalool	7.5
β-Bisabolene	1.6
Borneol	1.0
Thymol	1.1
Carvacrol	73.4

Biochemical and ELISA Analysis: Sandwich ELISA (enzyme-linked immunosorbent assay) method was used to quantify the IGF-1, GH and BHBA concentrations. The absorbance values from each plasma samples were plotted against the standard curve and calculated of standard curve with standard density and sample OD values. GH (Growth Hormone) ELISA Kit (Cat. No:201-07-0801) IGF-1 (Insulin Like Growth Factor) ELISA Kit (Cat. No:201-07-3008) and BHBA (Beta-Hydroxybutyric acid) ELISA Kit (Cat. No:201-07-2851) were defined with commercial kits (Sunred Biological Technology Co., Ltd., China)

Statistical Analysis: In the study, data were analyzed in the SPSS 20.0 software package (SPSS Inc., Chicago, IL, USA) One-Way Analysis of Variance (ANOVA) was used with General Linear Models (GLM) procedure. Significant differences within mean values of groups were exposed to Duncan test was used as a post-hoc test. Differences were considered statistically significant when $p<0.05$ (Dawson & Trapp, 2001). Results were stated as mean ± standard error of the mean.

RESULTS

As shown in Table 3, initial and last GH, IGF-1 and BHBA values were given in the experimental group of lambs. The statistical differences of GH values were determined in ORG 250, ORG 500 and control groups. The highest GH level (2.79 ng/ml) was in the 45th day for ORG 500 group compared with other groups and this value was statistically significant ($p<0.05$). The IGF-1value was evaluated the highest in ORG 500 group compared with control and ORG 250 group in the initial day. But this level was not found to be statistically significant ($p<0.05$). There were no statistical differences in BHBA values for two terms. However, no differences the highest levels in ORG 500 group.

The biochemical parameters were presented in Table 4. Total protein, Triglyceride, AST levels had statistically differences ($p<0.05$). The highest level of total protein was in the 250 ORG group at 45th day (5.91g/dl). Also, at 45th day, Triglyceride and AST were superior concentrations in the 500 ORG group compared with control group ($p<0.05$).

Table 3. The effects of oregano essential oil supplementation on GH, IGF-1, BHBA concentrations of Merino lambs (n:8).

Parameters	Experiment period (days)	Control	ORG 250	ORG 500	SEM	*P*-value
GH (ng/ml)	1st	1.59	1.63	2.62	0.257	0.179
	45th	1.57^{b}	1.60^{b}	2.79^{a}	0.211	0.018
IGF-1 (ng/ml)	1st	104.97	142.94	150.98	11.481	0.224
	45th	92.55	117.43	146.14	12.676	0.232
BHBA (nmol/ml)	1st	377.41	456.86	561.38	41.057	0.190
	45th	353.05	396.53	469.67	25.273	0.165

ORG 250: added 250 mg/d oregano esential oil; ORG 500: added 500 mg/d oregano essential oil; GH: Growth Hormone; IGF-1: Insulin Like Factor-1 and BHBA: Beta-Hydroxybutyric acid.
Different superscripts in each row show the significant difference between the groups $p<0.05$.

Table 4. The effects of oregano essential oil supplementation on biochemical parameters of Merino lambs (n:8).

Parameters	Experiment period (days)	Control	ORG 250	ORG 500	SEM	*P*-value
Total Protein (g/dL)	1st	5.80	6.34	5.78	0.155	0.169
	45th	5.11^{b}	5.91^{ab}	5.66^{a}	0.151	0.050
Albumin (g/dL)	1st	3.16	3.15	3.53	0.080	0.069
	45th	3.56	3.65	3.49	0.093	0.785
Total Cholesterol (mg/dl)	1st	73.64	71.30	68.69	2.538	0.745
	45th	61.60	62.99	64.30	1.222	0.687
Triglyceride (mg/dl)	1st	66.52	61.32	64.36	1.461	0.361
	45th	68.27^{b}	72.65^{ab}	77.03^{a}	1.535	0.050
ALT (U/L)	1st	26.16	20.34	23.55	1.201	0.140
	45th	25.70	21.84	25.12	1.145	0.348
AST (U/L)	1st	50.87	45.42	48.72	1.963	0.541
	45th	42.62^{b}	42.25^{b}	56.63^{a}	2.451	0.016
Calcium (mg/dl)	1st	8.82	9,11	9.31	0.140	0.376
	45th	9.31	9.34	9.52	0.134	0.800
Phosphorus (mg/dl)	1st	6.77	6.60	6.36	0.120	0.384
	45th	6.53	6.71	6.87	0.167	0.729

ORG 250: added 250 mg/d oregano esential oil; ORG 500: added 500 mg/d oregano essential oil;
Different superscripts in each row show the significant difference between the groups $p<0.05$.

DISCUSSION

In ruminant nutrition, the goal is to increase feed efficiency and productivity of animals. For this purpose, antibiotic additive to the ration as an additive have been used for a long time because they boost rumen fermentation and yield (Nagaraja et al., 1997). However, it was forbidden to participate in animal feeds in 2006 due to the fact that it also threatens human health because, it remains residue in animal products such as meat and milk and creates antibiotic resistance. After this process, the tendency towards natural compounds such as phytochemicals were rised, and it was improved the interest in essential oils for antioxidant and antimicrobial effects that arrange rumen fermentation (Macheboeuf et al., 2008). Essential oils are plant metabolites that have an important area of use as natural feed additives in animal nutrition due to their antibacterial, antifungal and antioxidant properties, and that give plants and spices their characteristic odor and color (Castillejos et al., 2006). Carvacrol and thymol the active ingredients of Origanum L. which we used in this study, are monoterpenoids with broad antimicrobial activity against gram-positive and gram-negative bacteria. These essential oils obtained from medicinal plants are scientifically and economically important for our country (Kırbağ & Bağcı, 2000). The carvacrol amount of oregano (73.1%) used in the study was determined the highest level compared with other content compounds. Chaves et al., (2008) found that the ruminal pH of the lambs was lower in the carvacrol and cinnamaldehyde essential oil were added to the experimental group than those fed with the control ration and the total VFA concentrations were higher than the control group. It has been reported that this condition occurs when carvacrol and cinnemaldehyde increase the digestibility of nutrients in the rumen.

Farghaly & Abdullah (2021) reported that the addition of oregano mint and rosemary to different sheep groups found the highest values of total protein, ALT, and globulin in the oregano group compared with the other medical plant groups. Khamisabadi et al., (2016) stated that adding of either oregano or peppermint to the diet of lambs reduced significantly triglyceride, glucose and urea when compared with control ration. In a small ruminant study, serum cholesterol and triglyceride levels decreased at additive essential oil group (Ran et al., 2018). However, in our study total protein and triglyceride concentrations were higher in ORG 500 group than control and other treatment group at 45th day. Guney et al., (2021) found that glucose and total protein concentrations increased significantly in the dietary supplementation with rosemary oil group.

Ran et al., (2018) claimed that plasma glucose, NEFA and BHBA concentrations can be used indicator of energy balance in ruminants. Also, they reported that essential oils did not affect the glucose but reduced the NEFA and BHBA concentrations in their study. This situation shows that essential oils improve the energy balance in small animals. Orzuna-Orzuna et al., (2022) acquired the similar results in a beef cattle study with supplemented with EOs.

Essential oil such as oregano oil is used as an alternative to antibiotics as growth promoters in ruminants. The study was concluded that, it was given the combination of oregano and monensin and oregano as a supplement in bulls, the growth performance could be increased without the use of antibiotics (Ran et al. 2018). In the same study similar to our results the GH concentrations were determined the highest values in essential oregano oil group. In a different study, Hassan & Hassan, (2009a) reported that Karadi lambs fed diet supplemented with rosemary significantly increased GH and daily gain compared with fed except rosemary additive.

Growth hormone (GH) secrets from anterior pituitary and the hypothalamus and its mediators regulate GH secretion. Main regulatory factors are GH releasing hormone (GHRH), somatostatin (SRIF), GH releasing peptide (ghrelin) and insulin-like growth factor (IGF-I). IGF-1 stimulates systemic body growth and it belongs growth-stimulating effects on almost every cell in the body, especially on skeletal muscle, cartilage, bone, lung, liver, kidney, nerve, skin cells and hematopoietic cells (Kato, 2002). Plasma IGF-1 concentration is an indicator of productivity and performance increment (Breier et al., 1988). So, IGF-1 is an important metabolite to evaluate the nutrient balance. In a study, serum IGF-1 level increased significantly with the supplementation of 250 mg/kg rosemary oil low dose group in lambs (Guney et al., 2021). Besides, in our study IGF-1 levels improved at ORG 500mg/kg group.

CONCLUSION

In this study we researched the effects of growth and biochemical metabolites in Merino lambs with oregano essential oil additive. Lamb meat is offered for human consumption, hence it would be appropriate to avoid residue products such as antimicrobial and turn to natural additive sources. Because of this reason, it is thought that essential oils will have beneficial effects by supplemented to the ruminant diets. It was concluded that the data revealed by this study will be useful for further researches.

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