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Araştırma Makalesi ***Research Article***

Effect of Different Levels of Oregano Essential Oil on Some Rumen Parameters in Lambs#

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Abstract

This study was conducted to determine the effects of different levels of oregano (*Origanum vulgare*) essential oil (OEO) on ruminal fermentation in lambs. Thirty-six weaned male Kivircik lambs were used as trial materials. Lambs were divided into one control and two treatment groups. For the two treatment groups, OEO was added into grower feed at a level of 250 and 500 ppm, respectively. Treatment period lasted for 9 weeks, including adaptation period. Feed and water was supplied *ad libitum* during the trial. Rumen fluid samples were collected from 9 lambs of each group, before the morning feeding and 3 and 6 hours after the feeding. From the ruminal fluid, pH, total volatile fatty acids (VFA), acetic acid, propionic acid, butyric acid and ammonia-nitrogen concentrations were determined. There were no effects of OEO on ruminal pH and concentrations of ammonia. In some of the rumen parameters (pH and total VFA), the results were statistically different periodically between groups and it was established that the time of measurement had an important role on the results of the rumen parameters. Furthermore, the quantitative increase of the total volatile fatty acid concentrations in the treatment groups according to the control was directly proportional to the increase of the oregano levels, in the periods except for the beginning. This situation makes us think that the oregano essential oil could have positive effects on digestion in lambs.

Özet

Yeme Farklı Düzeylerde Katılan Kekik Yağının Kuzularda Bazı Rumen Parametreleri Üzerine Etkisi

Bu çalışma, kuzularda, rasyona farklı düzeylerde katılan kekik (*Origanum vulgare*) uçucu yağının rumen fermantasyon parametreleri üzerine etkilerini saptamak amacı ile yürütülmüştür. Bu amaçla 36 baş sütten kesilmiş, erkek Kıvırcık ırkı kuzu deneme materyali olarak kullanılmıştır. Kuzular, biri kontrol, ikisi deneme grubu olmak üzere üç gruba ayrılmıştır. Deneme gruplarından birinin kuzu büyütme yemine 250 ppm, diğerine ise 500 ppm kekik uçucu yağı (OEO) eklenmiştir. Deneme, ilk haftası adaptasyon dönemi olmak üzere, toplam 9 hafta sürdürülmüş; deneme süresince büyütme yemi, kuru ot ve su *ad libitum* olarak verilmiştir. Yemlemeden önce ve yemlemeden sonraki 3. ve 6. saatlerde, her gruptan 9 adet kuzudan rumen sıvısı örnekleri alınmıştır. Rumen sıvılarında; pH, toplam uçucu yağ asitleri (UYA), asetik asit, propiyonik asit, bütirik asit ve amonyak azotu düzeyleri belirlenmiştir. Rumen parametrelerinden bazılarında (pH ve toplam UYA) ve dönemsel olarak gruplar arasında istatistiksel önemde farklılıkların olduğu ve ölçüm zamanının da bu parametrelerin üzerinde önemli bir etkiye sahip olduğu belirlenmiştir. Bunun yanı sıra, başlangıç dönemi dışındaki diğer dönemlerde, kekik esansiyel yağının dozunun artması ile orantılı olarak deneme gruplarının toplam uçucu yağ asidi düzeylerinin kontrole göre her zaman için sayısal bir artış içinde olması, kekik esansiyel yağının sindirim üzerinde olumlu bir etki meydana getirdiğini düşündürmektedir.

Introduction

For the past few decades, phytogenic feed additives such as plant extracts have received increased attention as potential alternatives to growth promoters for animal production (Benchaar et al., 2008). The use of essential oils (EO) in animal nutrition is growing in Turkey, as well as all over the world, because their consumption by humans and animals is recognized as safe (OJEU of 10/18/2003), and they have many beneficial effects (Ünal and Kocabağlı, 2014a).

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There are numerous studies showing beneficial effects of herbs and plant extracts on feed intake, immune functions and health, rumen fermentation and productivity of calves, dairy cows, heifers and also beef cattle (Cardozo et al., 2006; Devant et al., 2007; Fandiño et al., 2008; Greathead, 2003; Vakili et al., 2013; Yang et al,. 2007). Moreover, EO have been evaluated for their antimicrobial activity and they are being investigated as rumen modifiers in ruminants (Wallace, 2004). The chemical composition and dosage rate of EO may also affect the manner in which they alter ruminal N metabolism (Castillejos et al., 2006). However, most experiments conducted on EO have been laboratory based (*in vitro*) and of short-term nature (Busquet et al., 2006; Hristov et al., 2008). There are very limited *in vivo* studies that have evaluated the effectiveness of EO on rumen parameters of ruminants, especially in lambs.

The objective of the present study was to investigate the effects of oregano essential oil (OEO), which is produced in Turkey and added in different levels into the feed, on ruminal fermentation characteristics of growing lambs.

Materials and Methods

Animals, feeding, and feed analyses

This experiment, conducted with the principles of ethical committee, was approved by Istanbul University Local Ethical Committee on the Care and Use of Experimental Animals (No: 05). In this study, 36 weaned male Kivircik lambs with an initial live weight of 22.71 ± 0.71 kg obtained from the research farm of Istanbul University were used. The study lasted for 9 weeks including one-week adaptation period. At the end of adaptation period, live weights of the lambs were recorded and they were randomized in three groups, two treatment groups and one control group each of which consisted of 12 lambs.

During the study, hay was used as forage and lamb grower feed was used as concentrate pellets. Concentrate feed was specially produced (oregano essential oil sprayed on to pellets) on a monthly basis in a factory, in order to prevent the spoiling of oregano essential oil (OEO) while it was stored. The oregano oil was added into lamb grower feed of two treatment groups, for the first treatment group 250 ppm, and 500 ppm for the second treatment group. The oregano oil used in the study, was provided from a commercial company (Türer Tarım ve Orman Ürünleri A.Ş., İzmir) in Turkey and analyzed in BIBAM Laboratory of Anadolu University and it was reported that analyzed material contained 65.0% carvacrol and 0.3% thymol.

Feed ingredients were formulated by regarding maintenance and performance rate for weaned lambs and in accordance with nutrient and energy requirements specified by NRC (1985). In the study, group feeding method was used and a diet consisting of 60% concentrate pellets + 40% hay (metabolizable energy (ME) = 2.65 Mcal/kg) and *ad libitum* water were offered. Dry matter, crude ash, crude protein, crude fat (ether extracts) and crude cellulose contents of lamb grower feed and hay were analyzed according to AOAC (1995) procedures, colorimetric calcium level analysis and spectrophotometric phosphorus level analysis were performed in the laboratory of Animal Nutrition and Nutritional Diseases department (Veterinary Faculty, Istanbul University). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were carried out as described by Van Soest et al. (1991) and Goering and Van Soest (1970), respectively (Table 1).

Rumen samples collection and analyses

On day 1, 28, and 56 of the experiment, samples of whole ruminal contents were collected from 9 lambs of each group, before the morning feeding and 3 and 6 hours after feeding. The ruminal pH was measured immediately with a portable pH meter (Hanna Instruments pH 211 microprocessor pH meter, Romania).

Ruminal fluid was strained through 4 layers of cheesecloth, and 2 sub-samples of the filtrate were acidified to pH 2 with 50% H_2SO_4 and frozen at -20°C for later determination volatile fatty acids, and ammonia nitrogen (NH_3-N) analyses. Ammonia nitrogen and total VFA concentrations of ruminal fluid were measured according to steam distillation method described by Markham (1942). Samples for VFA analysis were prepared as described by Erwin et al. (1961) and analyzed by GLC (Sigma-Aldrich, USA) using a polyethylene glycol nitroterephthalic acid-treated capillary column (Column 80/120 Carbopack B-DA / 4% Carbowax 20M, USA) at 200°C in the injector and 1.2 mL/min gas flow rate (24 mL /sec gas velocity). Ammonia N concentration was analyzed by spectrophotometry (Chebios UV-VIS, Italy).

Statistical Analysis

For the statistical analysis of ruminal fermentation characteristics (pH, VFA, and NH_3-N), sampling time and sampling time × treatment were added to the model and analyzed using repeated measures variance analysis method. In the statistical model; group was indicated as “between subject factor”, and measurement time was indicated as “within subject factor”. For statistical calculations, SPSS 10.0 software was used (SPSS, 1999).

Table 1. Ingredient and nutrient composition of the basal diet fed to lambs (Dry Matter, %).

Tablo 1. Kuzulara verilen temel rasyonun içeriği ve besin maddeleri kompozisyonu (%, KM'de).

Ingredient (%)	**Basal diet**	
Barley		
Corn	19.40	
Corn Gluten	11.30	
Wheat Bran	8.50	
Soybean Meal	31.80	
Sunflower Meal	6.50	
Limestone	13.90	
Molasses	2.85	
Salt	5.10	
Mineral Premiks[1]	0.54	
Vitamin Premiks[2]	0.10	
	0.01	
Total	100.00	
Nutrient composition (%)	**Basal diet**	**Hay**
Dry Matter	87.30	93.40
Crude Protein	17.86	8.89
Crude Fat	3.55	0.95
Crude Cellulose	5.13	40.32
Crude Ash	6.15	7.79
NDF	19.56	60.95
ADF	7.83	43.04
Calcium	1.05	1.31
Phosphorus	0.54	0.20

[1]1 kg premix contained 10.000 mg Cu, 50.000 mg Fe, 50.000 mg Mn, 50.000 mg Zn, 50 mg Co, 800 mg I, 150 mg Se, 340.000 mg Ca.
[2]1 kg premix contained 66.700.000 IU Vitamin A, 16.700.000 IU Vitamin D_3, 167.000 IU α-tocopherol acetate, 42000 mg Vitamin B_1, 25.000 mg Vitamin B_2, 125 mg Vitamin B_{12}, 12,500 mg Niacin

Differences in P values less than 0.05 were considered to be significant.

Results

Chemical compositions of the diets and formulation of grain diet have shown in Table 1. The effect of different levels of OEO on performance and some blood parameters in lambs were previously published elsewhere (Ünal and Kocabağlı, 2014b). Results of different dosages OEO on fermentation parameters at the 28th and at the 56th days of the experimental period in the rumen of sheep are presented in Table 2 and in Table 3, respectively. No difference was observed in rumen pH, acetate, propionate and ammonia nitrogen concentration among treatments during the study, except ruminal pH (sampling time: before feeding) at the 56th days of the experimental period. Total VFA concentration increased in 500 ppm OEO ($P<0.05$) group at 28th days of the experiment (sampling time: 3h after feeding) and also 56th days of the experiment (sampling time: 6h after feeding). Butyrate proportions were influenced by addition of OEO (Table 2, Table 3). It was established that the time of measurement had an important role on the results of the rumen parameters. Furthermore, the quantitative increase of the total volatile fatty acid concentrations in the treatment groups according to the control, were directly proportional with the increase of the oregano doses at the 56th days of the experiment, except beginning.

Discussion

Various studies have been conducted to determine the effects of EO and their components on rumen microbial fermentation in ruminants (Newbold et al., 2004; Biricik et al., 2016; Busquet et al., 2006; Cardozo et al., 2006; Castillejos et al., 2006). These studies used a wide range of EO and EO compounds, dosages, diets and, not surprisingly, results have been inconsistent. The varied response among EO products evidently reflects differences in chemical structure, which influences their effects on microbial activity. In this study, different levels of OEO, which has been produced in Turkey, were examined.

Table 2. Effect of Oregano essential oil (OEO) on fermentation parameters at 28th days of the experimental period in the rumen of lambs.

Tablo 2. Kekik uçucu yağının denemenin 28. gününde kuzuların rumen parametreleri üzerine etkisi.

Variables of ruminal fluid	Sampling times (hours)	Treatments			Sx	Significance
		Control	250 ppm OEO	500 ppm OEO		
Ruminal pH	0[1]	6.89^{x}	6.77^{x}	6.77^{x}	0.09	NS
	3[2]	5.86^{y}	5.83^{y}	5.88^{y}	0.09	NS
	6[2]	6.09^{y}	5.90^{y}	5.91^{y}	0.10	NS
	Time effect	***	***	***		
Acetate, Mmol/l	0[1]	50.14^{y}	54.04^{z}	52.34^{y}	4.81	NS
	3[2]	60.11^{xy}	67.06^{y}	78.62^{x}	4.35	NS
	6[2]	68.11^{x}	83.46^{x}	66.95^{xy}	5.01	NS
	Time effect	*	***	**		
Propionate, Mmol/l	0[1]	15.99^{y}	16.71^{y}	15.81^{z}	1.52	NS
	3[2]	34.30^{x}	28.78^{x}	37.48^{x}	3.31	NS
	6[2]	30.01^{x}	29.29^{x}	27.91^{y}	3.41	NS
	Time effect	***	**	***		
Butirate, Mmol/l	0[1]	8.86^{y}	9.54^{y}	9.25^{z}	0.94	NS
	3[2]	15.99^{b,x}	18.63^{ab,x}	22.0^{a,x}	1.31	*
	6[2]	16.48^{x}	17.81^{x}	18.01^{y}	1.30	NS
	Time effect	***	***	***		
Total volatile fatty acids, Mmol/l	0[1]	77.10^{y}	82.18^{z}	79.40^{y}	6.61	NS
	3[2]	114.44^{b,x}	117.16^{b,y}	142.32^{a,x}	7.00	*
	6[2]	117.91^{x}	133.32^{x}	116.57^{x}	7.40	NS
	Time effect	**	***	***		
Ammonia N mg/dl	0[1]	14.43^{y}	14.59^{y}	14.29^{y}	0.25	NS
	3[2]	17.86^{x}	18.09^{x}	17.84^{x}	0.41	NS
	6[2]	12.19^{z}	12.10^{z}	12.05^{z}	0.26	NS
	Time effect	***	***	***		

[1] Before feeding; [2] After feeding

$^{a-b}$ Values with different superscripts in a row differ significantly (P<0.05).

$^{x-z}$ Values with different superscripts in a column for each variables differ significantly (P<0.05).

No difference was observed in rumen pH and ammonia nitrogen concentration among treatments during this study (Table 2 and 3). Newbold et al. (2004) reported that, the addition of EO (Crina Ruminants, UK) to the diet had no effect (P>0.05) on ruminal pH or ammonia concentrations. Thus, results presented here are consistent with Newbold et al. (2004). The findings of the present study for ruminal pH were also in line with the results of previous studies in lambs (Chaves et al., 2008), calves (Vakili et al., 2013), dairy cows (Yang et al., 2007), and beef cattle (Cardozo et al., 2006; Fandiño et al., 2008, Yang et al., 2010) which showed that EO has no effect on ruminal pH. In contrast to these findings, Castillejos et al. (2006) reported an increase in pH in *in vitro* systems and Benchaar et al. (2006) found that an increase in ruminal pH when dairy cows received EO. Biricik et al. (2016) observed that lambs had higher rumen pH, NH_3-N, and total volatile fatty acid (VFA) compared to those in the control group when they were fed high concentrate diet with calvacrol and/or thymol. Canbolat et al. (2010) reported that, the inclusion of oregano oil in different doses (0, 50, 100, 200, 400, 600 and 800 mg/L) decreased the total volatile fatty acids (VFA), acetic acid, propionic acid, butyric acid, acetic acid/propionic acid ratio and ammonia level whereas it increased the ruminal pH. They used rams and fed the animals 40:60 (DM) concentrate: forage mix. In our study, animals were fed a diet consisting of 60:40 (DM) concentrate: hay mixture. The results of Canbolat et al. (2010) study opposed to the results of our study, which can be partially explained by the type of diets used; because different diets cause different proteolytic activities in microbial communities with different predominant species of proteolytic bacteria (Wallace et al., 1987).

The total VFA concentration increased significantly in the 500 ppm OEO (P<0.05) group compared to the control group on the 28th (sampling time: 3h after

Table 3. Effect of Oregano essential oil (OEO) on fermentation parameters at 56th days of the experimental period in the rumen of lambs.

Tablo 3. Kekik uçucu yağının denemenin 56. gününde kuzuların rumen parametreleri üzerine etkisi.

Variables of ruminal fluid	Sampling times (hours)	Treatments			Sx	Significance
		Control	250 ppm OEO	500 ppm OEO		
	0[1]	6.79^{a,x}	6.54^{b,x}	6.94^{a,x}	0.07	**
Ruminal pH	3[2]	5.52^{z}	5.76^{y}	5.60^{z}	0.12	NS
	6[2]	6.44^{y}	6.46^{x}	6.18^{y}	0.15	NS
	Time effect	***	***	***		
Acetate,	0[1]	34.96^{y}	43.13^{y}	39.06^{y}	4.04	NS
Mmol/l	3[2]	55.68^{x}	58.68^{x}	65.63^{x}	4.45	NS
	6[2]	50.18^{x}	45.17^{y}	61.70^{x}	4.69	NS
	Time effect	***	**	**		
Propionate,	0[1]	14.04^{z}	16.40^{y}	13.69^{y}	1.09	NS
Mmol/l	3[2]	37.25^{x}	34.58^{x}	38.92^{x}	6.47	NS
	6[2]	21.83^{y}	27.16^{x}	31.07^{x}	3.38	NS
	Time effect	***	*	**		
Butirate,	0[1]	5.22^{b,z}	10.67^{a,z}	7.90^{ab,y}	1.12	**
Mmol/l	3[2]	14.03^{x}	19.13^{x}	21.06^{x}	2.14	NS
	6[2]	11.26^{y}	15.04^{y}	17.83^{x}	1.81	NS
	Time effect	***	***	***		
Total volatile	0[1]	55.89^{z}	72.77^{z}	63.54^{y}	5.44	NS
fatty acids,	3[2]	111.47^{x}	118.22^{x}	130.95^{x}	11.89	NS
Mmol/l	6[2]	85.90^{b,y}	91.78^{ab,y}	115.39^{a,x}	8.36	*
	Time effect	***	**	***		
Ammonia N	0[1]	15.29^{y}	15.79^{y}	15.40^{y}	0.21	NS
mg/dl	3[2]	18.97^{x}	18.96^{x}	18.68^{x}	0.21	NS
	6[2]	12.81^{z}	13.02^{z}	11.94^{z}	0.34	NS
	Time effect	***	***	***		

[1] Before feeding; [2] After feeding

[a-b] Values with different superscripts in a row differ significantly (P<0.05).

[x-z] Values with different superscripts in a column for each variables differ significantly (P<0.05).

feeding) and the 56th (sampling time: 6h after feeding) days of the experiment (Table 2-3). Wang et al. (2009) also reported a higher VFA concentration in sheep that were fed a hay/concentrate-based diet supplemented with oregano oil. In contrast to this, Castillejos et al. (2006) found that the antimicrobial activity of tyhmol used at a dose of 500 mg/L led to an increased pH, depression in total VFA concentration and a reduction in diet fermentability. However, in another study, Castillejos et al. (2008) reported a decrease in pH, associated with an increase in total VFA concentration at all doses (5, 50 and 500 mg/L) of thymol in the batch culture fermentation. According to these different results, it can be concluded that the optimum and toxic amounts in tyhmol are close and identifying the optimum dose is difficult. The different pH results obtained by using essential oils in *in vitro* and *in vivo* studies can be considered as the different responses of the rumen microorganisms to these additives (Benchaar and Greathead, 2011).

Besides their effects on VFA and pH concentrations, essential oils have also various influences on ruminal NH_3-N concentrations. Reduction of protein and starch degradation and inhibition of amino acid degradation are the main effects of EO in the rumen (Hart et al., 2008). Some studies show that the NH_3-N concentrations are dose dependent, while other studies state that the experimental method and length of the study also have an effect on the concentrations. When long-term *in vivo* studies and short-term *in vitro* studies are compared, the lack of effects of EO on nitrogen metabolism in long-term studies can be related to the longer exposure of rumen microorganisms to EO. Longer exposure time may cause an adaptation of rumen microorganisms to the EO, which may lead to a degradation of these compounds by ruminal bacteria (Busquet et al., 2005; Cardozo et al., 2004). An *in vitro* study, evaluating the effects of thymol and eugenol at different doses (0, 5, 50, 500 and 5.000 mg/L) on rumen fermentation, shows that only the highest dose of these

compounds decreased the ruminal concentrations of NH_3-N (Castillejos et al., 2006), whereas in another study by the same authors demonstrated that all doses of thymol decreased the NH_3-N concentrations (Castillejos et al., 2008). As seen in most *in vivo* studies (Benchaar et al., 2007; Devant et al., 2007; Yang et al., 2007), our study also showed that EO mostly does not affect ruminal NH_3-N concentrations.

The results of this study indicate that OEO, which is produced in Turkey, has a potential to improve rumen fermentation by increasing total VFA and that it might be useful as ruminal fermentation modifier in lambs. However, additional research is required to establish the optimal level of OEO considering the potential adaptation of microbial populations, and to demonstrate the effect of OEO on improvement in animal performance.

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