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Influence of Dietary Thyme Extract (*Thymus vulgaris*) on Performance, Purine Derivatives, Cellulase Activity and Ruminal Fermentation Parameters in Fattening Lambs and Goat Kids

Research Article

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ABSTRACT

This study was investigated to compare the effects of thyme extract (*Thymus vulgaris*) on performance, ruminal fermentation, cellulase enzyme activity and microbial protein synthesis in fattening lambs and goat kids. For this purpose, 15 kids (average initial BW of 17.3±1.2 kg, 3 month-old) and 15 lambs (average initial BW of 21.4±1.5 kg, 3 month-old) were randomly assigned to 3 dietary treatment: 1) control (without thyme extract), 2) supplemented with 250 mg thyme extract, 3) supplemented with 500 mg thyme extract. Animals were housed in individual pens for 84 days. Experimental results showed that different levels of thyme extract had a significant effect on daily weight gain and feed intake in both species, so that in animals fed 250 mg of thyme extract was higher than the other treatments (P<0.01). Rumen pH was not affected by experimental treatments. The lowest NH_3-N was observed in the treatment containing 250 mg of thyme extract (P<0.01). The total concentrations of volatile fatty acids (VFA) as well as butyrate and acetate in ruminal fluid decreased significantly with increasing thyme extract; but propionate was increased in kids and lambs fed with 250 mg of thyme extract (P<0.01). Microbial protein produced in the rumen were significantly affected by experimental treatments (P<0.01). The activity of cellulolytic enzymes was not affected by the treatments. Our findings indicate that 250 mg thyme extract can be used in diet without adverse effect on lambs and kids. The lambs were also more affected by the thyme extract than the kids.

KEY WORDS enzyme activities, microbial protein, ruminal fermentation, thyme extract.

INTRODUCTION

Low feed efficiency is one of the problems in the rumen of ruminants, which results in reduced growth and production of livestock and, most importantly, environmental pollution due to the release of methane and nitrogenous compounds (Budak and Yilmaz, 2013). Changes in ruminal fermentation can be made to improve fermentation efficiency and substrate utilization and optimize feed nutritional value (McIntosh *et al.* 2003; Wanapat *et al.* 2008).

Antibiotics have been successful in reducing energy loss (as methane) and protein loss (as ammonia nitrogen) in the rumen (Calsamiglia *et al.* 2007). However, today, due to general concerns about the remains of these compounds in milk and meat and thus increasing the bacterial resistance to them has banning the use of materials in feed as additive in some countries (Tekeli *et al.* 2007). Therefore, the tendency to find alternatives with natural origin for antibiotics has increased over recent years (Benchaar *et al.* 2008). Plant secondary metabolites are among the natural alternatives that have been studied in recent years that have antimicrobial properties (Acamovic and Brooker, 2005). Essential oils are a form of secondary metabolites that are volatile in nature and contain a complex mixture of organic compounds, including terpenoids and phenylpropanoids (Wallace, 2004). One of the plant essential oils that have a great potential for use in ruminant diets is the essential oil of thyme (*Thymus vulgaris*). Thyme extract contains 0.6-8.8% (usually 1%) of essential oil, most of which are phenols (20-80%) and monoterpene hydrocarbons (such as P cymen and γ terpinen) and alcohols (such as Linalool and terpinen α). They show that sometimes these compounds make up 80% of the extract compounds. Naturally, thymol is also a major phenolic component in thyme and carvacrol is a sub–element (Leung and Foster, 1996). Although goats and sheep have often been fed and managed in a similar manner (goats being browsers and sheep being grazers), they are different, especially in their digestive capability, because of differences in the morphology of digestive system, including the mouth structure and gut, which may influence their ability to ingest and digest feed materials (Shipley, 1999). Available information on efficiency of feed utilization between goat and sheep has been inconsistent with several studies reported sheep had higher growth performance than goats (Abdullah *et al.* 2013). In a recent *in vitro* study, Candyrine *et al.* (2016) reported that goats had better rumen fermentation characteristics, including higher volatile fatty acids (VFA) production and higher population of cellulolytic bacteria compared to sheep, suggesting that goats could be more superior in digesting feed materials.

The results of studies performed on lambs (Biricik *et al.* 2016; Baytok *et al.* 2017) and goats (Ganjkhanlou *et al.* 2014) that have examined the effects of thyme extract or its major compounds (thymol and carvacrol) are very contradictory. Thus more research is needed to determine their effects on rumen fermentation and metabolism in lambs and goats. There is also little research on direct comparisons of the use of plant extracts *in vivo* between lambs and goats. Therefore, the aim of the present study was to compare these two species and the effects of thyme extract on performance and some ruminal parameters in fattening lambs and goat kids.

MATERIALS AND METHODS

Animals and experimental treatments

This study was conducted in Kimia Dasht agro-industry farm (20 km from Gonbad Kavous city, Golestan province, Iran) for 84 days. Fifteen male goat kids (17.3±1.2 kg) and 15 male lambs (21.4±1.5 kg) of three months old were used in this study.

Animals were randomly allocated to a 2 (species) × 3 (thyme extract) factorial design experiment of six treatment groups: 1) lamb fed control diet, 2) lamb fed 250 mg thyme extract, 3) lamb fed 500 mg thyme extract, 4) goat kids fed control diet, 5) goat kids fed 250 mg thyme extract, and 6) goat kids fed 500 mg thyme extract. Each treatment group consisted of five animals (replicates). The animals were housed in individual pens with free access to clean drinking water and mineral blocks. Diet was formulated (Table 1) to meet requirements for growing lambs and kids (NRC, 2007).

Table 1 Ingredients and chemical composition of the basal experimental diet (percentage of dry matter of the diet)

Ingredients	%
Corn silage	20.0
Barley grain	45.0
Bagasse	2.0
Wheat bran	25.0
Soybean meal	4.5
baking soda	0.5
Salt	0.5
Vitamin and mineral premix[1]	1.0
Oyster powder	1.5
Chemical compounds	
Metabolisable energy (Mcal/kg)	2.70
Crude protein (%)	14.12
Neutral detergent fiber (NDF) (%)	33.00
Acid detergent fiber (ADF) (%)	17.00
Ca (%)	0.78
P (%)	0.53

[1] Supplied per kg of diet: vitamin A: 250000 IU; vitamin D: 50000 IU; vitamin E: 500 IU; Mn: 2500 mg; Fe: 3000 mg; Zn: 4000 mg; Co: 60 mg; I: 200 mg; Mg: 50000 mg and Se: 25 mg.

Animals were assigned into six groups to evaluate three isocaloric and isonitrogenous total mixed rations. Which were offered twice daily at 7 a.m. and 5 p.m. The solvent of thyme extract was 70% ethanol, which was purchased from Gorgan Plant Essential Oil Company, which was prepared based on its main active compound (66% thymol) (Table 2).

Table 2 Compounds of thyme extract (percentage)

Ingredients	%
Thymol	66.32
Carvacrol	3.67
γ-Terpinene	8.80
P-Cymene	12.58
α- Terpinene	2.21
α-Pinene	3.19
Myrcene α-phellandrene	2.43
β-Caryophyllen	0.8

Also, the specified amount of extract was divided into two parts and sprayed on the concentrate in the morning and evening. To ensure complete consumption of feed containing the extract by animals, first a small portion of feed that should be consumed by animals is poured into the bucket and then using an injection syringe, the extract is sprayed on the concentrate. To calculate the daily weight gain, the animals were weighed every 4 weeks, before the morning feeding. Feed conversion efficiency was calculated using the data related to daily dry matter consumption and daily weight gain.

Determination of chemical composition of feed

Samples of diet were analyzed for dry matter (DM), crude protein (CP), calcium and phosphorus and acid detergent fiber (ADF) according to AOAC (2000) procedures. Also, the determination of neutral detergent fiber (NDF) was measured by Van Soest *et al.* (1991) method.

Rumen samples collection

On day 70 of the experiment, samples of whole ruminal contents were collected from 5 animals of each group, 4 hours after morning feeding by stomach tube. The samples were filtered through four layers of muslin cloth and were immediately transported to the laboratory by insulating flask.

Ruminal pH, NH_3-N and VFA Analysis

The ruminal pH was measured immediately after sampling with a portable pH meter. Ruminal ammonia nitrogen (NH_3-N) content was determined using a phenol-hypochlorite method described by Broderick and Kang (1980). The measurement of volatile fatty acids (VFA) (acetic acid, propionic acid, butyric acid, isovaleric acid and valeric acid) were analyzed using the method proposed by Makkar (2010). Samples for VFA analysis were prepared as described by Erwin *et al.* (1961) and analyzed by GLC (Sigma-Aldrich, USA) using a polyethylene glycol nitro-terephthalic acid-treated capillary column (1.65 M×4.6 Mm) at 200 ˚C in the injector and 1.2 mL/min gas flow rate (24 mL/sec gas velocity).

Extraction of enzymes

The method described by Agarwal (2000) was used to measure the effect of the extract on the activity of carboxy methyl cellulase (CMC) and micro-crystalline cellulase (MCC) enzymes. Extraction of enzymes from three different parts of ruminal fluid including cellular (C) enzymes (bacteria, fungi and protozoa), extra cellular (EC) (floating and free microbes in ruminal fluid) and enzymes secreted from attached microbes particles (particulate material) (PM) were extracted and their activity was measured according to Hristov *et al.* (1999) method. The particulate material (2 g) was suspended in 10 mL phosphate buffer (0.1 M, pH 6.8), 2 mL of 0.4% lysozyme solution and 2 mL carbon tetrachloride were added to it. Glucose released by enzyme activity was estimated as described by Miller (1959) using the dinitrosalicylic acid (DNS) method. Adding 3 mL of DNS reagent prevents the reaction of rumen fluid enzymes with the substrate. Under experimental conditions, enzyme activity was expressed as µmole of sugars released per minute per ml.

Estimation of microbial protein

The measurement of microbial protein produced in the rumen was performed using the method of estimating excreted purine derivatives based on colorimetric method (Chen and Gomez, 1992). The calculation of total purine derivatives (TPD) excreted in the urine in mmol per day was obtained from the sum of allantoin, uric acid, xanthine and hypoxanthine. Daily urine volume was collected in a plastic bucket containing 100 ml of 10% sulfuric acid solution (10% solution or one molar to prevent nitrogen loss at pH of less than 3). Every morning, all the urine produced by the animal collected and to prevent sedimentation (especially uric acid) of the urine sample during storage, a 10 mL sample of the daily amount is diluted with 40 mL of distilled water and then to estimate PD is stored at 20 ˚C until the test.

Statistical analysis

This experiment was analyzed in a completely randomized design with 2 × 3 factorial arrangement including three levels of thyme extract (0, 250 and 500 mg/DM) and two species (lamb and goat kid). Data were analyzed using the MIXED procedure of SAS (2003), using model 1. The mean of treatments was compared by Tukey test at a significant level of 5%. For performance data, the initial weight of the animals was considered as covariate (model 2). The animal effect was assumed to be random in both models. The statistical models were as follows:

$$Y_{ijk} = \mu + T_i + S_j + TS_{ij} + E_{ijk} \quad (1)$$

$$Y_{ijk} = \mu + T_i + S_j + TS_{ij} + b(w_k - w) + E_{ijk} \quad (2)$$

In model 1

Where:

Y_{ijk}: observation for dependent variable.

µ: overall mean.

T_i: effect of thyme extract.

S_j: effect of animal species.

TS_{ij}: effect of interaction between T_i and S_j.

E_{ijk}: residual error.

In model 2

Where:

Y_{ijk}: observation for dependent variable.

µ: overall mean.

T_i: effect of thyme extract.
S_j: effect of animal species.
TS_{ij}: effect of interaction between T_i and S_j.
B: initial weight regression coefficient and performance traits.
w_k: initial weight k of animal.
w: average initial weight of animal.
E_{ijk}: residual error.

RESULTS AND DISCUSSION

The results of the effect of different levels of thyme extract on feed intake and performance are given in Table 3. During the experimental period, diets containing 250 mg of thyme extract had more daily weight gain (DWG) than other treatments (P<0.01). The results also showed that lambs gained better weight than kids, which is probably due to more feed intake by lambs. According to Table 3, the final weights of both species (lamb and kid) were affected by experimental treatments (P<0.01). The lowest final weight was observed in the treatment containing 500 mg of thyme extract and the highest final weight was observed in the treatment containing 250 mg of thyme extract. The results also showed that dry matter intake (DMI) in both species was affected by experimental treatments (P<0.01). Diets containing 250 mg of thyme extract had higher feed than other treatments (P<0.01) and the lowest feed intake related to the treatment contained 500 mg of thyme extract. Lambs also ate more feed intake than goat kids. But feed conversion ratio (FCR) was not affected by treatments.

Rumen pH was not affected by experimental treatments but a decreasing trend in ruminal pH was observed by adding thyme extract to the diet. The concentration of ammonia nitrogen (NH_3-N) in the ruminal fluid of lambs and goats fed diets containing thyme extract showed a significant decrease compared to other treatments (P<0.01; Table 4). The lowest amount of ammonia nitrogen was observed in the treatment containing 250 mg of thyme extract. Species effect and interaction (extract×species) were not significant. The total concentrations of VFA and butyrate, acetate proportions in ruminal fluid decreased significantly with increasing thyme extract (P<0.01; Table 4). But propionate proportions was increased in goat kids and lambs fed with 250 mg of thyme extract and the lowest concentration of propionate was present in the treatment of 500 mg of the extract. The ratio of acetate to propionate (A:P) in the VFA of rumen fluid in the 250 mg treatment was lower compared to other treatments (P<0.01). Species effect and interaction (extract×species) were not affected by experimental treatments.

The results of measuring the excretion of purine derivatives (PD) and the amount of microbial protein (MP) pro duced are shown in Table 5. According to this table, the amount of excretion of each purine derivative (allantoin, uric acid, xanthine+hypoxanthine) and the total excretion and absorption of purine derivatives from urine and the amount of microbial protein produced in the rumen were significantly affected by experimental treatments (P<0.0001). In the treatment containing 250 mg of thyme extract, the highest amount of microbial protein and purine derivatives was observed. No significant differences were observed between lambs and goat kids as well as interactions.

The activity of cellulolytic enzymes (carboxy methyl cellulase (CMC), micro-crystalline cellulose (MCC)) of different rumen sections of lambs and goats tested is shown in Table 6. As the results showed, the experimental treatments could not have a significant effect on the activity of cellulolytic enzymes. But numerically, the activity of both CMC and MCC enzymes was higher in all three parts, Particulate material (PM), extracellular (EC) and cellular (C) and the total (total of all three parts) rumen of animals fed with 250 mg of thyme extract.

The findings of the present study showed that the inclusion of thyme extract in the level of 250 mg increases DWG, final weight, DMI of animals compared to the control. In agreement with the results of this study, Candyrine *et al.* (2019) reported that the highest daily weight gain, more feed intake and better feed conversion ratio were observed in sheep consuming flaxseed extract compared to goats. The researchers said the higher BWG and better FCR in sheep compared to goats could be attributed to the higher intake of sheep, which been grazers, are less selective in what were offered to them compared to goats (browsers). According to the results of this experiment Chaves *et al.* (2008) reported that cinnamaldehyde and Juniper essential oil caused a significant increase in daily weight gain of lambs (254 and 250 g) compared to the control treatment (216 g). In a study Khamisabadi *et al.* (2016) also observed that the use of 3% thyme extract and 3% mint extract caused a significant increase in mean daily gain, daily feed intake and dry matter intake and also no significant increase in feed conversion ratio (FCR) in male lambs. Also Okoruwa and Edoror (2019) observed that garlic extract at levels of 25 and 30 g had a significant effect on the daily weight gain in goats. However, in a study conducted by Baytok *et al.* (2017) on the effect of thyme essential oil on lamb performance, they found that daily weight gain, feed intake and feed conversion ratio were not affected by the treatments. Biricik *et al.* (2016) also found that supplementation of fattening lambs with carvacrol (100 or 300 mg/kg DM) and thymol (100 or 300 mg/kg DM) or their mixture had no effect on daily weight gain and coefficient Feed conversion.

Table 3 Mean daily weight gain and body weight in lambs and kids fed different levels of thyme extract

Parameter	Species		SEM	Treatment			SEM	P-value		
	Lamb	Kid		Control	250 mg	500 mg		SP	T	SP × T
DWG (g/day)	219.89[a]	111.75[b]	3.327	163.94[b]	180.65[a]	152.87[c]	0.808	0.0001	0.0001	0.0001
DMI (g/day)	1269.0[a]	801.60[b]	0.769	1023.70[b]	1067.50[a]	1014.70[c]	1.055	0.0001	0.0001	0.0006
FBW (kg)	37.86[a]	28.77[b]	0.055	33.16[b]	34.56[a]	32.23[c]	0.067	0.0001	0.0001	0.0001
FCR	6.133	6.48	0.078	6.26	5.99	6.66	0.111	0.1734	0.2467	0.3681

DWG: daily weight gain; DMI: dry matter intake; FBW: final body weight and FCR: feed conversion ratio.
SP: species; T: treatment; SP × T: species × treatment.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Ruminal fermentation parameters in lambs and kids fed different levels of thyme extract

Parameter	Species		SEM	Treatment			SEM	P-value		
	Lamb	Kid		Control	250 mg	500 mg		SP	T	SP × T
pH	6.52	6.50	0.003	6.67	6.65	6.62	0.004	0.8923	0.7341	0.4985
NH_3-N (mg/dL)	18.81	18.06	0.084	20.68[a]	15.23[c]	19.13[b]	0.104	0.8152	0.0001	0.7503
VFA (Mmol/L)										
Acetic	63.93[a]	63.78[b]	0.035	66.52[a]	64.41[b]	60.65[c]	0.043	0.0073	0.0001	0.2669
Butyric	11.81[a]	11.60[b]	0.053	13.56[a]	11.43[b]	10.11[c]	0.065	0.0087	0.0001	0.2803
Propionic	22.22	22.13	0.067	21.53[b]	24.84[a]	20.15[c]	0.082	0.3251	0.0001	0.3761
Acetic:propionic	2.89	2.90	0.009	3.08[a]	2.59[c]	3.00[b]	0.011	0.6304	0.0001	0.1465
Total VFA	95.00[a]	94.76[b]	0.019	98.53[a]	94.59[b]	91.52[c]	0.023	0.0001	0.0001	0.2981

VFA: volatile fatty acids.
SP: species; T: treatment; SP × T: species × treatment.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of different levels of thyme extract supplementation on urinary purine derivatives (PD) and microbial protein supply (MPS) in lambs and kids fed experimental diets

Parameter	Species		SEM	Treatment			SEM	P-value		
	Lamb	kid		Control	250 mg	500 mg		SP	T	SP × T
Urinary purine derivatives, (mmol/d)										
Allantoin	4.68	4.31	0.024	3.53[b]	6.43[a]	3.53[c]	0.029	0.0768	0.0001	0.0680
Uric acid	0.973	0.800	0.010	0.498[c]	1.400[a]	0.763[b]	0.012	0.0731	0.0001	0.0679
Xan. + Hypox.	0.733	0.697	0.009	0.518[c]	0.999[a]	0.629[b]	0.011	0.0551	0.0001	0.8750
Total PD absorbed	8.61	8.10	0.037	6.30[c]	11.51[a]	7.25[b]	0.045	0.6293	0.0001	0.5550
Total PD excreted	5.38	5.81	0.025	4.54[c]	8.83[a]	4.92[b]	0.031	0.0643	0.0001	0.5763
Microbial N (g/d)	6.26	6.19	0.027	4.58[c]	8.37[a]	5.27[b]	0.033	0.0751	0.0001	0.5551
MPS (g/d)	53.80	52.29	0.084	50.05[b]	58.99[a]	50.10[b]	0.103	0.0734	0.0001	0.0792

Xan. + Hypox: xanthine + hypoxanthine and MPS: microbial protein supply.
SP: species; T: treatment; SP × T: species × treatment.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effect of levels of thyme extract on changes in activities of enzymes in various fractions of rumen contents (μmol glucose released/min/mL)

Parameter	Species		SEM	Treatment			SEM	P-value		
	Lamb	kid		Control	250 mg	500 mg		SP	T	SP × T
Carboxymethyl cellulase										
C	170.41	169.41	0.035	176.50	182.28	150.96	2.806	0.5478	0.0792	0.4318
EC	79.57	77.01	2.672	80.65	86.20	73.02	3.27	0.2963	0.0963	0.8170
PM	255.35	250.95	4.909	244.13	251.12	242.00	6.012	0.4441	0.0654	0.6334
Total	505.34	496.39	5.705	501.28	520.30	496.01	6.987	0.3897	0.1327	0.4621
Microcrystalline cellulase										
C	151.02	154.74	5.091	157.91	165.90	144.83	6.235	0.6090	0.4981	0.2834
EC	88.69	85.91	0.623	94.86	88.78	78.77	0.763	0.5131	0.2800	0.1605
PM	285.79	276.84	0.736	288.10	273.73	277.12	0.901	0.4491	0.2981	0.6721
Total	506.56	523.45	5.628	520.87	513.41	490.73	6.893	0.4438	0.7610	0.5491

C: cellular fraction; EC: extracellular fraction and PM: particulate material.
SP: species; T: treatment; SP × T: species × treatment.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Yesilbag *et al.* (2016) found no significant difference in daily weight gain and feed intake in growing Sanan goats fed different levels of Juniper essential oil. In another study Abdullah *et al.* (2013) reported that dry matter intake in sheep was higher than in goats, and the reason for the tendency of goats to eat sweet foods that had less dry matter than other parts. However, in the study of these researchers, feed conversion ratio and daily weight gain were similar in both species and were not significant. Factors affecting growth rate in lambs and goats include age, sex, breed, genetics, dry matter intake, feed composition, weaning age, ambient temperature, activity, energy and protein concentration of diet and feed additives (NRC, 2007). Decreased daily weight gain in lambs and goats fed a diet containing 500 mL of thyme extract may be associated with increased concentrations of plant secondary compounds, reduced growth of microorganisms, and decreased rumen fermentation. Also, another reason for daily weight loss in the experimental treatment of 500 mg of thyme extract compared to the other two treatments can be considered as high consumption of this extract and the decrease in the ability of digestion and the reduction of feed intake. However, the improvement in the average daily weight gain in lambs and kids goat receiving a diet containing 250 mg of thyme extract could indicate an appropriate level of use of this extract and a possible improvement in their intestinal fermentation processes.

Gram-positive bacteria are more sensitive to the antimicrobial effects of plant secondary compounds (such as essential oils) than gram-negative bacteria due to the lack of a polysaccharide layer. Because gram-positive bacteria convert dietary nutrients into low-yield compounds such as acetate and methane, plant secondary compounds improve rumen fermentation and animal function by reducing the production of these compounds. Essential oils in moderate doses are selectively able to improve the production pattern of volatile fatty acids to increase propionate production, but at higher doses they have an inhibitory effect on a large population of microorganisms (Calsamiglia *et al.* 2007). Also, due to the significant interaction effects, it can be said that thyme extract caused the most changes in lambs compared to goat kids, and lambs had better performance in terms of final weight and daily weight gain compared to goats. The reason for this difference may be due to differences in the morphology of the digestive system, including the structure of the mouth and intestines of lambs and goats, which may affect their ability to digest food (Shipley, 1999).

Dry matter intake is the most important factor in the growth of growing lambs and goat kids. Factors affecting dry matter intake include palatability and digestibility, rumen excretion rate, dietary protein and fiber content, feed water content, dietary mineral level, ruminal pH, weight and age of livestock, space and access to noted feed and ambient temperature (NRC, 2007). The plant active compounds are expected to slow down the digestion of nutrients (especially fiber) by inhibiting rumen microorganisms, and thus reducing the flow rate of nutrients through the rumen and food, but the results of the present experiment showed that this depends on the dose of the extract. Higher doses (500 mg) reduced feed intake in lambs and kids goat, but increased feed intake at lower doses (250 mg). Perfume from essential oils can stimulate the olfactory and taste nerves, which in turn stimulates the appetite. These compounds can also stimulate feed intake digestion by stimulating digestive enzymes (Perdok *et al.* 2003; Pulina *et al.* 2013). Another factor that stimulates feed intake is the absorption of soluble compounds in food. Essential oil compounds have a positive effect on the absorption of nutrients from the intestine (Ozdogan *et al.* 2011). Based on the above, it can be said that in this study, thymol may have been able to increase feed intake by affecting the absorption of nutrients in the intestine. Considering the factors that can be evaluated such as palatability, weight and age of animals in this study, it can be inferred that with increasing age and weight of lambs and goats, their need for nutrients to meet their needs increases and consequently dry matter consumption increases. The amount of essential oil consumption is also important because in some reports, low doses stimulate feed intake and high doses have the opposite effect on feed intake (Patra, 2011).

Species and extract had no effect on pH and it from 6.50 to 6.67. However, the amount of NH_3-N and the concentration of VFA were affected by the treatments. Ruminal deamination of the diet increases ammonia and urea excretion, which reduces the efficiency of dietary nitrogen consumption and is associated with groundwater pollution as well as the release of N_2O greenhouse gases (Tedeschi *et al.* 2003). Therefore, reduction of ammonia nitrogen, increase of microbial protein and consequently reduction of environmental pollution, is one of the main goals of studies on the use of medicinal plants in the diet of ruminants. Consistent with the results of this study, Khamisabadi *et al.* (2016) showed that lambs receiving mint and thyme extracts had 70% and 50% lower ammonia nitrogen concentrations at 0 and 2 hours after feeding, respectively, compared to the control treatment. But experimental treatments did not have a significant effect on ruminal pH. They stated that the decrease in ruminal ammonia concentration using peppermint and thyme extracts in the diets of lambs could possibly be due to high concentrations of carvacrol and thymol (the active ingredient in thyme), cinnamaldehyde (the active ingredient in peppermint); Which is effective in bacterial proteolysis and reducing feed protein proteolysis. Wang *et* *al*. (2009) also reported that the addition of 250 mg of oregano extract to sheep diets did not affect ruminal pH but reduced ruminal NH_3-N concentration. On the other hand Baytok *et al*. (2017) tested the extracts of two different species of thyme on fattening lambs. Their results showed that ruminal pH and ammonia nitrogen concentration were not affected by experimental treatments. The differences observed in these studies can be attributed to factors such as the essential oil supplementation method, addition time (just before feeding), active ingredient content, ambient temperature and the amount of other active ingredients in this study. Decreased ammonia concentration indicates the inhibitory effect of plant essential oils on the proteolytic activity of ruminal microorganisms. Essential oils have been reported to inhibit the deamination of amino acids by ruminal microbes (McIntosh *et al*. 2003). The use of different levels of juniper seed extract in growing goats in the study Yesilbag *et al*. (2016) showed that the amount of ruminal pH did not differ significantly between treatments, but the concentration of ammonia nitrogen in the first period (start of the experiment) was affected by experimental treatments and the goats that received the extract had less NH3-N than the control. The production of ammonia in the rumen is due to the predation of bacteria by protozoa and its nitrogen metabolism and the activity of some hyper-ammonia producing (HAP) bacteria. Decreasing ammonia concentration along with increasing plant essential oil concentration can be due to inhibition of amino acid deamination activity by bacteria or inhibition of ammonia-producing bacteria or decrease in urease activity (Benchaar *et al*. 2008).

Extensive studies have been performed on the effect of plant secondary metabolites on fatty acids and different results have been reported, which mainly reduce acetic and butyric acid and increase propionic acid while reducing methane. A study has shown that the main plant essential oils and their constituents in a behavior similar to monensin can reduced acetate and increased the proportion of propionate, which is considered as a favorable yield due to the addition of plant essential oils (McGuffey *et al*. 2001). Khorrami *et al*. (2015) investigated the effect of extracts of thyme and cinnamon (500 mg/kg dry matter) on the ruminal volatile fatty acids of cannulated cows and showed that the total concentration and ruminal molar ratio VFA were not affected by experimental treatments, while the molar proportion of propionate was increased with thyme. In both treatments, the ratio of acetate to propionate decreased compared to the control. Evaluation of the effect of juniper extract on the rumen parameters of growing Saanen goats by Yesilbag *et al*. (2016) showed that the total amount of VFA, molar ratio of acetic acid, propionic acid, butyric acid and the ratio of acetic acid to propionic acid in the rumen were not significantly different between control and other experimental treatments. Biricik *et al*. (2016) investigated the effect of adding carvacrol and thymol (active ingredient of thyme extract) on ruminal parameters of fattening lambs and observed that the concentration of VFA in ruminal fluid of lambs fed diets containing in two levels of 100 and 300 mg/kg at 0 and 3 hours after feeding were higher than other treatments, which were contrary to the findings of the present study. Candyrine *et al*. (2019) showed that sheep had the highest concentrations of VFA, acetic acid and acetic acid to propionic ratio compared to goats. With higher total VFA production and molar ratio of acetic acid, sheep are predicted to have higher available energy to support growth and possibly a greater tendency to biosynthesize fats. Candyrine *et al*. (2019) found that the higher bacterial population of *B. fibrisolvens* (fiber digesters) in sheep than in goats could be one of the reasons for the higher VFA and acetic acid in sheep. Contrary to the results of the present study, Candyrine *et al*. (2016) found that the total concentrations of VFA, acetic acid and butyric acid in goats were higher than in sheep. In total, the overall decrease in ruminal fermentation at high thyroid dose levels may be due to its active ingredients (thymol and carvacrol), which are more effective than other non-phenolic secondary plant metabolites due to the presence of antimicrobial agent, Because the presence of the hydroxyl group in the phenolic structure and the loss of bacterial cell membrane integrity, ultimately leads to a decrease in glucose uptake by bacteria. Changes in the concentration of VFA as a result of the antimicrobial effect of plant essential oils depend on their intake and dietary composition. At maximum concentrations of each plant essential oil, most treatments reduce total VFA (Hart *et al*. 2008). It is desirable not to change the concentration of volatile fatty acids or to increase them if it is accompanied by changes in the concentration of ammonia nitrogen and a decrease in methane production.

Microbial protein plays an important role in the supply of ruminant nitrogen requirements and provides most of the amino acids needed for growth, maintenance and production of the host animal (Vaithiyanathan *et al*. 2007).

Allantoin had the highest share in the estimation of the microbial protein in sheep, and accounting for about 80-60% of all urinary purine derivatives. Uric acid and xanthine + hypoxanthine also contain 10-30% and 5-10% of total purine derivatives, respectively (Vaithiyanathan *et al*. 2007). In this study, animals receiving thyme extract had more purine derivatives and microbial protein synthesis than controls. There was no significant difference in microbial protein synthesis between sheep and kids goat, although numerically the amount of microbial protein in lambs was higher than in goat kids. Decreasing the concentration of ammonia nitrogen in the treatments containing the extract along with increasing the amount of microbial protein produced indicates that nitrogen may have been used to produce microbial protein. Although the reduction of ammonia nitrogen is a favorable reaction for the fermentation process, but the reduction of more than the allowed, level of 15 L/mg is not appropriate and *in vivo* system make is difficult to meet the needs of animals. Therefore, in the treatment containing 500 mg of thyme extract, microbial protein was lower and as a result, they had weaker performance than other treatments. Results of tea saponin fed lambs (Zhou *et al.* 2011) and the use of tea saponin in goats (Hu *et al.* 2005) is in agreement with our findings. The effect of decreasing tea saponin on ammonia nitrogen and increasing microbial protein has also been reported by Mao *et al* (2010). In the rumen ecosystem in ruminants, protozoa play a negative role in nitrogen utilization because they ingest and digest large numbers of bacteria, thus reducing the net flow of microbial protein from the rumen to the intestine (Evans and Martin, 2000). Secondary metabolites and extracts have the ability to degrade cell membranes and also bind to protozoa cholesterol of the cell membrane and cause their degradation. Reducing the number of ruminal ciliated protozoa due to the use of plant extracts may increase the flow of microbial protein from the rumen, improve feed efficiency and reduce metanogenesis (Newbold *et al.* 2004). Shifting the path of fermentation reactions to increase microbial protein synthesis has significantly reduced the amount of total VFA.

The rumen is a source for the production of fiber-degrading enzymes. Cellulase and xylanase produced by ruminal fungi are known to be among the most active fiber-degrading enzymes. Fiber-degrading enzymes include total cellulase activity (filter paper degradation), CMC, and MCC. The activity of these enzymes is in three separate parts of the rumen contents, including small particles (microbes attached to the rumen particle), intracellular part (cells that are freely suspended in the liquid part of the ruminal fluid) and extracellular part. Among these three sections, the highest hydrolytic activity of enzymes was related to the part of microbes attached to fine particles, followed by intracellular enzymes and finally extracellular enzymes (Agarwal *et al.* 2000). Contrary to the findings of this study, Wina *et al.* (2005) observed that due to the use of *Sapindus rarak saponins* extract, the activity of carboxymethylcellulase and xylanase was higher in fistula goats than in controls, but in sheep the activity of these two enzymes decreased by 50% compared to the control. Wina *et al.* (2005) found that a higher proportion of entodiniomorphid protozoa were found in goat rumen compared to sheep. Because goats are selective, they encounter more secondary plant components than sheep. Therefore, goat rumen microorganisms may be more compatible with these substances than sheep rumen microorganisms. Wang and Wang (2016) found that the use of plant extracts in goat diets increased the activity of cellulosic digestive enzymes, especially microcrystalline and carboxymethylcellulase. Patra *et al.* (2009) observed that the activity of carboxymethylcellulase was reduced by clove essential oil. It is possible that the microbial population that breaks down fiber is inhibited by plant oil extracts and essential oils. However, in confirming our results, Wallace *et al.* (1994) did not observed any effect on carboxymethylcellulase activity in the rumen of cows in the presence of essential oils.

CONCLUSION

In general, the results of this study showed that the addition of thyme extract at the level of 250 mg to the diet of lambs and kids goat had a positive effect on growth performance and feed intake. Lambs and kids goat fed 250 mg of thyme extract had the best weight gain, the highest microbial protein and propionate production. Therefore, thyme extract up to 250 mg can be added to animal diets. As the extract in the diet increased, the concentration of VFA and NH_3-N decreased. FCR, ruminal pH and activity of MCC and CMC enzymes were not affected by experimental treatments. Between the two species of lambs and goat kids in terms of performance, lambs had better performance and the behavior of the extract was different in the two species, so that the extract caused the most changes in lambs compared to kids.

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