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The effects of carvacrol and/or thymol on the performance, blood and rumen parameters, and carcass traits of Merino sheep

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Abstract: The objective of this study was to investigate the effects of increasing doses of carvacrol (C) and/or thymol (T) on the performance, rumen fermentation, and blood and carcass parameters of Merino sheep. Eighty-four 12-week-old male Merino lambs were randomly assigned to 7 treatment groups. The sheep were fed with the same concentrate mixtures including a control diet, carvacrol 100 mg/kg (C_{100}), carvacrol 300 mg/kg (C_{300}), thymol 100 mg/kg (T_{100}), thymol 300 mg/kg (T_{300}), carvacrol+thymol 100 mg/kg (C_{50}+T_{50}), and carvacrol+thymol 300 mg/kg (C_{150}+T_{150}). The C and/or T supplementation did not affect the feed conversion. The lambs fed with C and/or T diets had higher rumen pH, NH_3-N, and total volatile fatty acid (VFA) compared to those in the control group. However, essential oil supplementation did not change the molar concentration of VFA. The serum urea and glucose in C and/or T groups were not found significant on days 0, 35, and 70 compared to the control group. Slaughter weights and other carcass parameters were similar between the groups. The effects of C and/or T supplementation on the rumen and production parameters showed limited effects when lambs were fed with the high concentrate diets.

Key words: Carcass, carvacrol, Merino sheep, rumen parameters, thymol

1. Introduction

Essential oils (EOs) have been reported to have both antimicrobial and fungicidal activities (1). Burt (1) suggested a number of possible modes of action of EOs against bacterial cells. EO can also cause cytoplasmic coagulation and eventually lead to cell lysis of bacteria (1). It has been suggested that gram-negative bacteria tend to have a higher resistance to EOs than gram-positive bacteria (2).

EOs have been reported to affect rumen fermentation (3) and some EOs are currently being investigated as rumen modifiers in ruminants. Studies on EOs have been conducted using in vitro methods such as batch culture and continuous culture (4). The effects of EOs include increased volatile fatty acid (VFA) concentrations and protein synthesis in vitro, and inhibition of protein degradation by decreasing deaminative activities in the rumen (5). Furthermore, increases in the ruminal true organic matter and nitrogen digestibility (6), total tract acid detergent fiber (ADF) and starch digestibility (7), dry matter intake, and milk and fat yield (8) in dairy cows have also been reported for EOs. However, Benchaar et al. (7) and Castillejos et al. (4) reported that a blend of EOs had no effect on ruminal ammonia. The data from these studies are often inconclusive and lead to conflicting results due to variation of the dosages, the chemical structure of the EO compounds, diets, blends, and EO providers (5). Although in vitro results are useful in screening bioactive compounds for antimicrobial activity, the true value of these plant extracts for altering the rumen microbial fermentation must be assessed in live animal trials.

Despite these conflicting results, several EO products are currently being marketed for ruminants as feed additives due to the lack of legal availability of ionophores in the European Union. Despite the increased interest in naturally occurring compounds that are relative to 'organic' production of ruminant products, it is crucial that the efficacy of EOs based on naturally occurring EOs should be assessed with in vivo studies.

Thymol (T) and carvacrol (C) are primary active ingredients isolated from thyme (9). T is a monoterpene with strong antimicrobial activity against a wide range of gram-positive and gram-negative bacteria, and it is one of the most researched active components of EOs (1). C is a

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phenolic compound that has strong antimicrobial activity (10). The objective of this study was to investigate the effects of increasing doses of C and T based on a natural blend of EOs on performance, rumen fermentation, and blood and carcass parameters in Merino sheep.

2. Materials and methods

This study was carried out at the Sheep Breeding Research Station, Bandırma, Balıkesir, Turkey. The EO was supplied from a commercial EO company (NBT Company, Alanya, Antalya, Turkey). The blends of the EOs used were thyme oil and *Origanum vulgare* oil. The constituents included T, C, cymene, and terpinene (Table 1). These blends contain high amounts of active C and T. The blend of the EO was obtained through steam distillation of two different thyme plants.

2.1. Animals, treatments, and experimental procedures

Eighty-four 12-week-old male Merino lambs (22 ± 0.5 kg of BW at the beginning of the study) were assigned to 1 of 7 treatments in a completely randomized design. During a 10-week trial period, the lambs were fed with their assigned treatment diet (Sheep Breeding Research Station, Bandırma, Balıkesir, Turkey). The lambs were then housed in individual pens (0.80 × 1.40 m). Lambs were dewormed and vaccinated before the trial. During the study, the lambs were fed with a 0:100 forage:concentrate ratio (Table 2). Seven concentrate mixtures used in this trial were (on a dry matter (DM) basis): 1) control, 2) control feed plus 100 mg carvacrol/kg (C_{100}), 3) control feed plus 300 mg carvacrol/kg (C_{300}), 4) control feed plus 100 mg thymol/kg (T_{100}), 5) control feed plus 300 mg thymol/kg (T_{300}), 6) control feed plus 100 mg/kg carvacrol+thymol (50+50) (C_{50}+T_{50})), and 7) control feed plus 300 mg carvacrol+thymol (150+150)/kg (C_{150}+T_{150}). The lambs had ad libitum access to fresh water. The use and handling of the animals for this study was approved by the ethics council of Uludağ University (2010-06/11).

Twelve lambs were randomly assigned to each experimental treatment. The lambs were weighed at 15-day intervals throughout the study. The average daily gain was determined by dividing the weight gain by the number of the days. The feed conversion was calculated as the ratio between the dry matter intake and the average daily gain.

The lambs were offered the concentrate ad libitum (15% in excess of the previous day's intake) twice a day at 0900 and 1800 hours. The concentrate refusals were taken daily (0830 hours), pooled weekly for each animal, and sampled for DM, organic matter (OM), crude protein (CP), natural detergent fiber (NDF), and ADF determination. Samples of the offered concentrate were taken weekly for chemical composition analysis.

On day 70, the ruminal pH was measured in rumen fluid from each lamb at 0, 3, and 6 h after feed was offered. A speculum was inserted into the mouth, and a lubricated rubber tube was inserted through the speculum into the rumen via the esophagus. The ruminal contents (25–50 mL) were removed using an electric pump. The samples were monitored to ensure that they were not contaminated with saliva. The pH was measured immediately using a pH meter. The entire contents were squeezed through four layers of cheesecloth and were divided into subsamples for the determination of VFA and NH_3-N and for the counting of protozoa. The samples for VFA were acidified with 2 mL of 25% metaphosphoric acid and centrifuged at 5000 rpm for 10 min. The supernatants were frozen (–20 °C) for subsequent analyses.

For the counts of protozoa, 1 mL of filtrate was transferred to a vial containing 5 mL of methyl green-formalin-saline solution (11). The number of the protozoa × 10^5 per milliliter was counted on a microscope at a magnification of 100× in a 0.2-mL counting chamber after serial dilution. Duplicate measurements were made from each sample. The average of these measurements was used to determine the number of protozoa present in the initial sample.

On days 0, 35, and 70, blood samples were collected from each lamb via jugular venipuncture in nonheparinized tubes immediately before the morning feeding. The

Table 1. Essential oil compositions.

Composition, %	Thyme oil	*Origanum vulgare* oil
Thymol, %	55.33	0.4
Carvacrol, %	3.57	71.2
p-Cymene, %	13.47	9.8
γ-Terpinene, %	8.86	8.2
α-Pinene, %	3.08	1.7
Myrcene α-phellandrene, %	2.38	1.8
α-Terpinene, %	2.37	1.2
β-Caryophyllene, %	1.17	1.0

Table 2. Chemical compositions of the concentrate mixture (dry matter basis).

Nutrient composition (%)	Concentrate mixture[1]
Dry matter (DM)	89.2
Ash	10.3
Crude protein (CP)	18.6
Ether extract (EE)	4.8
Neutral detergent fiber (NDF)	33.8
Acid detergent fiber (ADF)	13.9
Acid detergent lignin (ADL)	8.9

[1]Concentrate mixture included: wheat bran: 40.0%, barley: 24.4%, sunflower meal (28% CP): 12.0%, full fat soy: 10.0%, corn: 9.6%, limestone: 2.9%, salt: 0.6%, and vitamin-mineral premix: 0.5% (DM basis).

samples were centrifuged at 1000 × *g* for 15 min at 4 °C. The collected serum was immediately placed on ice for transportation to the laboratory. In these samples, glucose and urea-N were determined by using an Auto Technicon DAX 72 autoanalyzer.

On day 70, a total of 35 male lambs from the seven fattening groups were slaughtered at a commercial slaughter facility. The lambs were weighed after fasting for 12 h with free access to water. After complete evisceration and dressing, the carcasses were weighed (the warm carcass weight). The head, skin, feet, and other offal were then removed and separately weighed. The internal fat deposited on top of the kidneys and around the gastrointestinal tract was separated and weighed. The carcasses were chilled at 4 °C for 24 h and then weighed (the cold carcass). The cold carcass was split into two symmetrical parts along the backbone. The left half of the carcass was cut into six parts, as described by Colomer-Rocher et al. (12), and weighed. The dressing percentage was calculated as the ratio of 12-h fasting weight prior to slaughter to chilled carcass weight.

2.2. Chemical analyses

The chemical composition analyses were conducted on the samples of the feeds and the refusals. The dry matter, ether extract, ash, and N were determined according to AOAC (13) procedures (AOAC official methods 943.01, 920.39 942.05, and 976.06, respectively). The NDF and ADF analyses were carried out as described by Van Soest et al. (14) and Goering and Van Soest (15), respectively.

Before the preparation of the concentrate mixtures, samples of the EO mixes were sent to the Plant, Drug, and Scientific Research Center of Anadolu University (Eskişehir, Turkey) for analysis of the EO composition profile.

The VFAs were determined by using a gas chromatograph (Hewlett Packard Agilent Technologies 6890N Network GC System, Serial CN10447002, Beijing, China). A column (6 × 2 mm ID glass) was packed with 10% SP-1200/1% H3P O4 80/100 Chromosorb WAW (Supelco, Bellefonte, PA, USA). The carrier gas (N_2) flow was 30 mL/min, the inlet temperature was 175 °C, the column temperature was 110 °C, and the detector temperature was 170 °C. The detection was performed by flame ionization. The ammonia-N concentration was determined by the phenol hypochlorite method (16).

2.3. Statistical analyses

Data were analyzed using the PROC MIXED procedure of SAS (17). The model fattening performance and the carcass traits included the groups (control, thymol, carvacrol, and thymol+carvacrol) and the treatments (C_{100}, C_{300}, T_{100}, T_{300}, $T+C_{50+50}$, and $T+C_{150+150}$) as fixed effects and the lamb as a random effect within the group. For statistical analysis of the ruminal fermentation characteristics (pH, VFA, NH_3-N) and protozoa enumeration, the sampling time and all interactions were added to the model and analyzed as repeated measures using the PROC MIXED procedure of SAS. A probability of $P \leq 0.05$ was considered significant and the trends are discussed at $P \leq 0.10$ unless otherwise stated.

3. Results

3.1. Analysis of feed and essential oils

The composition of the EO is presented in Table 1. This table reports the yields of the major constituents, carvacrol or/and thymol, p-cymene, γ-terpinene, α-pinene, myrcene α-phellandrene, α-terpinene, and β-caryophyllene. The composition values are similar to those reported by Bampidis et al. (9). The chemical composition of the concentrate is presented in Table 2.

3.2. Dry matter intake and growth performance

No differences in the concentrate intake were found (Table 3). The average daily gain (ADG) was not affected during the entire study by C and/or T supplementation. The C and/or T supplementation did not affect the final weight of the lambs or the feed conversion (Table 3). The feed conversion was calculated as the ratio between the dry matter intake (DMI) and the ADG and was averaged as 4.99 kg DMI per kg ADG.

3.3. Ruminal fermentation characteristics

There were significant effects of C and/or T on the rumen NH_3-N, pH, and total VFA concentrations between the groups (Table 4). There was no difference between the C and/or T in the group and the doses for ruminal NH_3-N at hour 6. There was a significant difference between the C and/or T in the groups ($P < 0.02$), but not in the doses at hour 0. However, the ruminal NH3-N was significantly

Table 3. Dry matter intake (DMI) and growth performance of lambs.

	Control	Carvacrol		Thymol		Carvacrol + thymol		SEM	P
		C_{100}	C_{300}	T_{100}	T_{300}	$C+T_{100}$	$C+T_{300}$		
DMI (g/day)	1260	1232	1200	1191.3	1212	1225	1237	37.85	NS
	1260	1216		1202		1231		26.76	NS
Initial live weight (kg)	22.1	22.0	22.0	22.5	22.2	22.4	22.3	0.568	NS
	22.1	22.0		22.4		22.4		0.402	NS
Final live weight (kg)	40.1	38.9	38.7	39.4	39.6	39.1	40.3	0.889	NS
	40.1	38.8		39.4		39.6		0.628	NS
Average daily gain (g)	257.6	244.6	238.0	241.3	247.7	239.2	256.3	0.008	NS
	257.6	240.2		244.5		247.7		0.006	NS
Feed conversion*	4.9	5.1	5.1	4.9	4.8	5.17	4.8	0.13	NS
	4.9	5.1		4.9		5.0		0.09	NS

Treatments were carvacrol and thymol concentrations of 0 (control), 100 mg/kg carvacrol (C_{100}), 300 mg/kg carvacrol (C_{300}), 100 mg/kg thymol (T_{100}), 300 mg/kg thymol (T_{300}), 100 mg/kg carvacrol + thymol ($C+T_{100}$; 50+50 mg/kg), and 300 mg/kg carvacrol + thymol ($C+T_{300}$; 150+150 mg/kg). SEM: Standard error of the mean, NS: nonsignificant. *: Calculated as DMI/average daily gain.

different among control and T- and C-supplemented groups ($P < 0.05$) and also between the groups dosed at different levels after 3 h of feeding ($P < 0.05$). The highest value of the ruminal NH_3-N was determined in the C and T ($C_{50}+T_{50}$, $C_{150}+T_{150}$) groups.

The rumen pH was unaffected by EO type and by the doses of the T and/or C at hour 0. The rumen pH levels of C_{100} and $C+T_{50+50}$ were found higher than that of the control at hours 3 and 6 ($P < 0.05$). The total VFA concentrations were higher in the ruminal fluid of the sheep fed with diets supplemented with carvacrol, C_{100} and C_{300}, as compared with the other groups at hours 0 and 3. In addition, the total VFA concentration was higher in the control group compared with the others at hour 6, except T_{300} ($P < 0.05$, Table 4). There were no significant effects of carvacrol and/or thymol on the molar proportions of acetate, propionate, or butyrate and the acetate/propionate ratio in trial groups. Addition of C and/or T did not modify *Isotricha prostoma*, *Entodinium* spp., *Diplodinium*, and total protozoa numbers (Table 5).

3.4. Blood parameters

The serum urea and glucose in the sheep fed with carvacrol and/or thymol diets did not differ significantly from the control values on days 0, 35, and 70 (Table 6).

3.5. Carcass characteristics

The lambs fed with the control diet had no more significant cold carcass weights than those fed with C and/or T (Table 7). The slaughter weights were similar for the groups with C and/or T supplementation. EO type and different dose combinations of those two EOs had no effect on the warm carcass; dressing percentage; offal items; weight of head, four feet, heart, lungs, liver, kidney, spleen, and kidney; and pelvic and internal fat weights.

4. Discussion

4.1. Dry matter intake and growth performance

Addition (or supplementation) of lamb diets with C and/or T did not affect the DMI, ADG, or feed conversion (Table 3). Benchaar et al. (7) reported no changes in the DMI when dairy cows were supplemented with EO. Similarly, Beauchemin and McGinn (18) determined that EO did not affect feed intake in beef cattle. Bampidis et al. (9) observed no change in the DM intake, the ADG, or the feed conversion when growing lambs were fed with diets supplemented with oregano leaves. Our study shows that addition of C and/or T to lamb diets does not appear to alter the DMI, ADG, or feed efficiency.

Our results differed from the findings of Chaves et al. (19), who found higher ADG in lambs when cinnamaldehyde or juniper berry was added to a barley-based diet at a level of 200 mg/kg of dietary DM, but same researchers reported no effects on DMI when essential oils (cinnamaldehyde, garlic, juniper berry) were included in lambs diets. The reason for increased ADG after cinnamaldehyde or juniper berry supplementation may be an increased proportion of propionate, which has a role in energy metabolism. In our study, carvacrol and/or thymol had no effect on the proportion of the propionate when fed in different doses. The same researchers (20) investigated the effects of EO compounds on feed intake and growth performance using either a barley- or corn grain-based

Table 4. Effect of C and/or T on rumen fermentation characteristics of lambs.

	Time (h)	Control	Carvacrol		Thymol		Carvacrol + thymol		SEM	P
			C_{100}	C_{300}	T_{100}	T_{300}	$C+T_{100}$	$C+T_{300}$		
NH_3-N (mg/dL)	0	29.75	27.53	29.05	35.33	33.75	32.97	30.05	2.06	NS
		29.75^{ab}	28.29^{b}		34.54^{a}		31.5^{ab}		1.46	0.02
	3	34.6^{bc}	36.7^{b}	30.9^{c}	36.1^{bc}	38.5^{b}	33.7^{bc}	44.1^{a}	1.96	0.05
		34.6^{ab}	33.8^{b}		37.3^{ab}		38.9^{a}		1.88	0.05
	6	24.9	24.35	24.38	20.95	31.12	24.37	28.1	2.19	NS
		24.9	24.36		26.03		26.23		1.55	NS
pH	0	7.36	7.36	7.31	7.37	7.29	7.43	7.42	0.05	NS
		7.36	7.34		7.33		7.42		0.03	NS
	3	5.57^{bc}	5.94^{a}	5.82^{ab}	5.55^{c}	5.41^{c}	5.63^{bc}	5.64^{bc}	0.09	0.05
		5.74^{b}	5.88^{a}		5.48^{b}		5.64^{b}		0.06	0.05
	6	5.71^{bc}	6.00^{ab}	5.96^{abc}	5.98^{abc}	5.67^{c}	6.13^{a}	5.83^{abc}	0.11	0.05
		5.71	5.98		5.83		5.98		0.07	NS
Total VFA (mM)	0	31.6^{bc}	29.9^{bc}	42.6^{a}	28.9^{bc}	35.8^{ab}	24.9^{c}	35.4^{ab}	2.88	0.05
		31.6^{ab}	36.2^{a}		32.4^{ab}		30.2^{b}		2.04	0.05
	3	138.5^{ab}	159.3^{a}	114.7^{bc}	134.6^{ab}	135.3^{ab}	79.1^{d}	96.3^{cd}	12.4	0.05
		138.5^{a}	137.0^{a}		134.9^{a}		87.7^{b}		9.00	0.05
	6	186.3^{a}	147.9^{bc}	154.9^{bc}	155.3^{bc}	191.1^{a}	142.8^{c}	171.2^{ab}	9.26	0.05
		186.3^{a}	151.4^{c}		173.2^{ab}		157.0^{bc}		6.54	0.05
Acetate (mol/100 mol)	0	34.9	36..3	34.0	37.8	33.8	38.5	35.9	1.5	NS
		34.9	35.2		35.8		37.2		1.0	NS
	3	32.68	30.23	32.81	31.38	32.56	32.78	31.01	1.3	NS
		32.68	31.52		31.97		31.89		0.9	NS
	6	30.69	32.46	33.43	32.96	32.51	33.37	31.02	0.69	NS
		30.69	32.95		32.73		32.19		0.49	NS
Propionate (mol/100 mol)	0	19.9	19.3	21.1	18.8	19.3	19.2	18.7	1.12	NS
		19.9	20.2		19.0		18.9		0.7	NS
	3	35.81	41.77	32.51	32.41	32.40	35.18	33.83	2.43	NS
		35.81	37.14		32.40		34.51		1.76	NS
	6	33.46	30.65	33.12	33.36	34.97	32.56	32.20	1.54	NS
		33.46	31.89		34.16		32.38		1.09	NS
Butyrate (mol/100 mol)	0	14.2	14.3	13.7	14.1	15.4	14.1	16.2	1.7	NS
		14.2	13.9		14.7		15.1		1.7	NS
	3	14.3	12.9	13.7	16.5	14.3	13.1	14.3	2.88	NS
		14.3	13.3		15.4		13.8		2.08	NS
	6	13.58	13.08	14.09	14.25	13.24	12.8	15.6	0.83	NS
		13.58	13.58		13.75		14.21		0.58	NS
Acetate/propionate	0	1.76	1.94	1.54	2.03	1.84	2.08	1.98	0.11	NS
		1.76	1.74		1.93		2.03		0.08	NS
	3	0.92	0.85	1.04	0.97	1.04	0.95	0.92	0.06	NS
		0.92	0.95		1.01		0.93		0.04	NS
	6	0.94	1.19	1.04	0.99	0.94	1.04	0.97	0.07	NS
		0.94	1.11		0.97		1.00		0.05	NS

Treatments were carvacrol and thymol concentrations of 0 (control), 100 mg/kg carvacrol (C_{100}), 300 mg/kg carvacrol (C_{300}), 100 mg/kg thymol (T_{100}), 300 mg/kg thymol (T_{300}), 100 mg/kg carvacrol + thymol ($C+T_{100}$; 50+50 mg/kg), and 300 mg/kg carvacrol + thymol ($C+T_{300}$; 150+150 mg/kg). SEM: Standard error of the mean. Means in a row with different superscripts differ at $P< 0.05$). NS: Nonsignificant.

Table 5. Effect of C and/or T on rumen protozoa.

	Time(h)	Control	Carvacrol		Thymol		Carvacrol + thymol		SEM	P
			C_{100}	C_{300}	T_{100}	T_{300}	$C+T_{100}$	$C+T_{300}$		
Isotricha prostoma	0	0.33	0.38	0.35	0.18	0.21	0.00	0.20	0.14	NS
		0.33	0.36		0.19		0.10		0.10	NS
	3	0.37	0.07	0.29	0.24	0.51	0.06	0.62	0.10	NS
		0.37	0.18		0.37		0.34		0.07	NS
	6	0.36	0.81	0.34	0.70	0.55	0.17	0.66	0.11	NS
		0.36	0.57		0.63		0.41		0.08	NS
Entodinium spp.	0	1.96	1.95	1.95	1.99	1.95	1.98	1.97	0.01	NS
		1.96	1.95		1.97		1.97		0.07	NS
	3	1.95	1.97	1.98	1.99	1.9	1.97	1.97	0.01	NS
		1.95	1.97		1.97		1.97		0.01	NS
	6	1.98	1.94	1.98	1.96	1.98	1.95	1.94	0.01	NS
		1.98	1.95		1.96		1.94		0.01	NS
Diplodinium	0	0.23	0.33	0.38	0.21	0.17	0.45	0.04	0.11	NS
		0.23	0.35		0.19		0.24		0.07	NS
	3	0.34	0.36	0.33	0.39	0.34	0.34	0.14	0.12	NS
		0.34	0.35		0.37		0.24		0.08	NS
	6	0.10	0.11	0.19	0.12	0.05	0.42	0.21	0.11	NS
		0.10	0.15		0.08		0.31		0.08	NS
Protozoa ($\times 10^3$) n/mL	0	5.42	5.44	5.48	5.45	5.45	5.41	5.45	0.09	NS
		5.42	5.46		5.45		5.43		0.06	NS
	3	5.46	5.39	5.50	5.51	5.53	5.53	5.45	0.08	NS
		5.46	5.45		5.52		5.49		0.05	NS
	6	5.60	5.54	5.47	5.45	5.47	5.40	5.50	0.09	NS
		5.60	5.50		5.46		5.45		0.06	NS

Treatments were carvacrol and thymol concentrations of 0 (control), 100 mg/kg carvacrol (C_{100}), 300 mg/kg carvacrol (C_{300}), 100 mg/kg thymol (T_{100}), 300 mg/kg thymol (T_{300}), 100 mg/kg carvacrol + thymol ($C+T_{100}$; 50+50 mg/kg), and 300 mg/kg carvacrol + thymol ($C+T_{300}$; 150+150 mg/kg). SEM: Standard error of the mean, NS: nonsignificant.

diet without supplementation or supplemented with 0.2 g/kg of carvacrol or cinnamaldehyde. They found that neither the source of the grain nor the EO supplementation affected the DM intake, live weight, or ADG.

4.2. Ruminal fermentation characteristics

There is evidence showing that EO affects the rumen bacterial population (21,22). McIntosh et al. (23) reported that EO inhibited the growth of some ammonia-hyperproducing bacteria. The effect of EO was due to the inhibition of ammonia formation from amino acids (21,24,25). The increase in ammonia-N concentration observed in this experiment is the opposite of the findings of most other studies (Table 4). Busquet et al. (10) reported that carvacrol (2.2 mg/L) decreased the large peptide concentrations and increased the ammonia-N concentrations in long-term continuous culture of rumen contents in 2 h after feeding. This is in consistence with recent reports suggesting that EOs increase the amount of ammonia-N (26).

Chaves et al. (20) observed no changes in ammonia concentration but they found lower ruminal pH and higher total VFA concentrations in lambs supplemented with carvacrol or cinnamaldehyde in a barley- or corn-based diet compared to the lambs fed with a control diet. However, the same researchers found no effect of cinnamaldehyde, garlic, or juniper berry supplementation on ruminal pH, ammonia concentration, or total VFA content (19). Benchaar et al. (25) emphasized that these

Table 6. Effect of C and/or T on blood parameters of lambs.

	Day	Control	Carvacrol		Thymol		Carvacrol + thymol		SEM	P
			C_{100}	C_{300}	T_{100}	T_{300}	$C+T_{100}$	$C+T_{300}$		
Urea (mmol/L)	0	43.83	41.75	42.91	43.36	40.50	39.25	39.00	2.06	NS
		43.83	42.33		41.93		39.12		1.45	NS
	35	48.00	43.58	47.58	46.25	48.16	47.91	45.45	1.84	NS
		48.00	45.58		47.20		46.68		1.30	NS
	70	45.25	49.25	46.50	44.50	44.08	43.75	48.18	1.77	NS
		45.25	47.87		44.29		45.96		1.25	NS
Glucose (mg/dL)	0	92.33	95.66	95.16	87.81	93.75	92.16	93.00	2.55	NS
		92.33	95.41		90.78		92.58		1.80	NS
	35	91.54	87.66	86.25	85.75	89.16	90.50	87.36	2.42	NS
		91.54	86.95		87.45		88.93		1.71	NS
	70	90.00	84.91	88.33	90.58	87.50	87.83	86.54	2.45	NS
		90.00	86.62		89.04		87.18		1.73	NS

Treatments were carvacrol and thymol concentrations of 0 (control), 100 mg/kg carvacrol (C_{100}), 300 mg/kg carvacrol (C_{300}), 100 mg/kg thymol (T_{100}), 300 mg/kg thymol (T_{300}), 100 mg/kg carvacrol + thymol ($C+T_{100}$; 50+50 mg/kg), and 300 mg/kg carvacrol + thymol ($C+T_{300}$; 150+150 mg/kg). SEM: Standard error of the mean, NS: nonsignificant.

effects varied according to the concentration of the EO or the compound used, the pH of the incubation medium, and the in vitro technique used. Evans and Martin (27) reported that thymol at a dose of 400 mg/L increased the pH in in vitro fermentation cultures, whereas at doses of 50, 100, and 200 mg/L, no effects were observed.

The ruminal molar percentages of individual VFAs were not altered with the addition of C and/or T (Table 4). Newbold et al. (28) reported no change in ruminal total VFA concentration or molar percentages of individual VFAs in the sheep fed with 100 mg/day EO. Castillejos et al. (4) reported an increase in the ruminal total VFA concentration but no change in the molar proportions of individual VFAs when EO was added in continuous culture. The same research group (29) observed that 500 mg/L thymol decreased the ruminal total VFA concentration and the molar proportion of acetate, and increased the molar proportion of propionate, but no effects were reported at 5 and 50 mg/L. The findings of our study suggest that the inconsistencies among the other reports might be due to differences in the dietary inclusion level and the variety of the EOs, and interactions between the feed intake, the composition, and the EOs.

We lack sufficient data to comment on the effects of the EOs on ruminal protozoa populations. Cordoza et al. (30) observed that the addition of a mixture of cinnamaldehyde and eugenol (90 mg/day) to the diets of beef heifers increased the numbers of holotrichs and had no effect on the entodiniomorphs, and no effects were observed on numbers of these protozoal species after using higher levels of cinnamaldehyde (600 mg/day) and eugenol (300 mg/day) in a mixture. In agreement with our findings, Tekippe et al. (26), Benchaar et al. (7), Wallace et al. (24), and Newbold et al. (28) observed no change in protozoa counts as a result of adding a mixture of EOs to the diet (Table 5).

4.3. Blood parameters

Serum urea and glucose were unaffected by C and/or T (Table 6). Chaves et al. (19) reported no changes in the plasma glucose and urea and in serum glucose.

4.4. Carcass characteristics

Information on the effects of EO supplementation on the carcass traits of ruminants is lacking. In this study, diets supplemented with C and/or T did not affect the dressing percentage, offal items, head weight, weight of four feet, heart weight, weight of lungs, liver weight, testes weight, kidney weight, spleen weight, or kidney, pelvic, and internal fat weights (Table 7). These results agree with the findings of Chaves et al. (20) and Bampidis et al. (9).

4.5. Conclusion

The addition of carvacrol and/or thymol did not affect the DMI, feed conversion, rumen protozoa counts and total protozoa, or blood and carcass parameters; however, it had effects on NH_3-N, rumen pH, and total VFA. The results of this study showed limited effects of C and/or

Table 7. Effect of C and/or T on carcass characteristics of lambs.

	Control	Carvacrol		Thymol		Carvacrol + thymol		SEM	P
		C_{100}	C_{300}	T_{100}	T_{300}	$C+T_{100}$	$C+T_{300}$		
Slaughter weight (kg)	41.16	39.18	37.54	40.94	39.88	39.82	39.94	0.53	NS
	41.16	38.36		40.41		39.88		0.38	NS
Warm carcass weight (kg)	20.38	19.20	18.34	20.00	19.82	19.54	19.46	0.40	NS
	20.38	18.77		19.91		19.50		0.28	NS
Cold carcass weight (kg)	19.88	18.62	17.84	19.40	19.16	18.90	18.86	0.39	NS
	19.88	18.23		19.28		18.88		0.28	NS
Dressing percentage (%)	49.60	49.10	48.97	49.05	49.80	49.15	48.80	0.84	NS
	49.60	49.04		49.43		48.98		0.59	NS
Offal items (kg)									
Head weight	2.41	2.48	2.23	2.41	2.38	2.61	2.46	0.08	NS
	2.41	2.36		2.39		2.53		0.06	NS
Skin weight	4.09	3.90	3.89	4.03	4.18	4.01	4.43	0.18	NS
	4.09	3.89		4.10		4.21		0.13	NS
Four feet weight	1.08	1.05	0.95	1.12	1.05	1.13	1.05	0.03	NS
	1.08	1.00		1.09		1.09		0.02	NS
Kidney weight	0.11	0.11	0.10	0.11	0.10	0.11	0.10	0.00	NS
	0.11	0.11		0.11		0.10		0.00	NS
Kidney and pelvic fat weight	0.19	0.17	0.16	0.15	0.12	0.16	0.18	0.02	NS
	0.19	0.16		0.14		0.17		0.01	NS
Long leg weight	3.19	3.11	2.32	3.22	3.19	3.23	3.12	0.23	NS
	3.19	2.71		3.20		3.17		0.16	NS
Internal fat weight	0.33	0.25	0.30	0.26	0.32	0.31	0.35	0.04	NS
	0.33	0.27		0.29		0.33		0.03	NS
Lungs weight	0.68	0.64	0.60	0.67	0.63	0.64	0.63	0.04	NS
	0.68	0.61		0.65		0.63		0.03	NS
Spleen weight	0.082	0.084	0.074	0.074	0.082	0.092	0.076	0.00	NS
	0.082	0.079		0.078		0.084		0.00	NS
Heart weight	0.20	0.21	0.21	0.20	0.25	0.20	0.21	0.01	NS
	0.20	0.21		0.23		0.20		0.01	NS
Liver weight	0.89	0.83	0.74	0.84	0.81	0.83	0.79	0.04	NS
	0.89	0.79		0.82		0.81		0.03	NS

Treatments were carvacrol and thymol concentrations of 0 (control), 100 mg/kg carvacrol (C_{100}), 300 mg/kg carvacrol (C_{300}), 100 mg/kg thymol (T_{100}), 300 mg/kg thymol (T_{300}), 100 mg/kg carvacrol + thymol ($C+T_{100}$; 50+50 mg/kg), and 300 mg/kg carvacrol + thymol ($C+T_{300}$; 150+150 mg/kg). SEM: Standard error of the mean, NS: nonsignificant.

T supplementation on the rumen and on the production parameters when lambs were fed with a high concentrate diet. However, since it was determined in the present study that adding C and/or T in the given dosages led to increases in the rumen pH and in the total VFA concentration, this is a positive result in terms of nutrition.

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