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Effect of Thyme Essential Oils (*Thymus hyemalis* and *Thymus zygis*) and Monensin on in Vitro Ruminal Degradation and Volatile Fatty Acid Production

S. MARTÍNEZ,[†] J. MADRID,*,[†] F. HERNÁNDEZ,[†] M. D. MEGÍAS,[†]
J. A. SOTOMAYOR,[§] AND M. J. JORDÁN[§]

Department of Animal Production, University of Murcia, Campus de Espinardo, 30071 Murcia, Spain, and Murcian Institute of Investigation and Agricultural Development, C./Mayor s/n, 30150 La Alberca, Murcia, Spain

The effect of the essential oils of thyme on the in vitro ruminal degradability of a barley seed/alfalfa hay substrate was studied. Two essential oils were used, one from *Thymus hyemalis* (TH), rich in carvacrol, and the other from *Thymus zygis* (TZ), rich in thymol. Four experimental treatments of in vitro degradability, using the Daisy II$^{200/220}$ incubator, were conducted including a negative control (CO), a positive control at 7.5 μg/mL of monensin (MO), and two treatments with essential oils (TH or TZ) at 1.35 μL/mL. The material was incubated at 39.5 °C for various lengths of time. At each time, the disappearance of dry matter, crude protein, and neutral detergent fiber was measured. Volatile fatty acids (VFAs) were determined after 48 h of incubation. CO and MO provided ($p < 0.01$) higher values of potential degradability ($a + b$) of DM than the TH and TZ treatments (72.6 and 70.8 vs 53.2 and 48.2%, respectively). Also, crude protein degradability was lowest in the essential oil treatments. The CO treatment showed the highest potential degradability of NDF. The values of VFA production obtained ($p < 0.001$) with CO and MO treatments were higher than those obtained with TH and TZ treatments (21.0 and 19.1 vs 11.2 and 10.1 mM). The essential oils decreased the molar proportion of propionate, increasing the acetate/propionate ratio. In conclusion, the effects of essential oils at assayed doses would not be nutritionally beneficial to the ruminal energetic metabolism.

KEYWORDS: **In vitro degradability; monensin; *Thymus hyemalis*; *Thymus zygis*; essential oils**

INTRODUCTION

Many attempts have been made to modify ruminant digestion to improve animal production. For example, ruminal fermentation has been modulated with additives, such as monensin (antibiotic ionophore). This compound is widely used, but has been forbidden in the European Union because it can originate problems related with resistance to antibiotics in humans. The inclusion of monensin in the diets of ruminant prevents digestive disturbances and enhances feed efficiency due to its selective antimicrobial activity (*1*). Thus, monensin supplementation inhibits most lactate-producing ruminal bacteria, increasing the ruminal pH in animals consuming high-grain diets and decreasing the acetate/propionate ratio. Also, these effects are accompanied by a decrease in methane production (*2*).

As a result of monensin prohibition, a goal for nutritionists in this area would be to find new additives to modulate microbial activity in the rumen. Among the proposed alternatives, plant extracts are being studied because they are known to contain active principles with antimicrobial activity (*3*). Among plant extracts, the differences that have been observed in the antimicrobial activities of essential oils suggest that the susceptibility of microorganisms to various chemical components of the oils might also differ. The phenolic derivatives, carvacrol and thymol, present in thyme essential oil (as well as other Lamiaceae species, such as *Origanum vulgare* and *Rosmarinus officinalis*) exhibit considerable antimicrobial activity (*4*). Evans and Martin (*5*) studied the effects of thymol on ruminal microorganisms and found that thymol was a potent inhibitor of L-lactate production by *Streptococcus bovis* and *Selenomonas ruminantium*. In addition, Cardozo et al. (*6*) indicated that the effects of plant extracts on ruminal fermentation may differ according to ruminal pH.

The chemical composition of the essential oil depends not only on plant species but also on chemotypes, climatic conditions, and place of origin. Therefore, their antimicrobial activities might also vary. Thus, Rasooli and Mirmostafa (*7*) found that *Thymus pubescens* essential oil demonstrated a greater bactericidal effect against *Pseudomona aeroginosa* than *Thymus serpillum* essential oil, a finding they related to the differences in the composition of the essential oils.

It is important to evaluate the effects of essential oils of well-known chemical composition on ruminal parameters, in order

* Author to whom correspondence should be addressed (telephone +34 968 36 47 50; fax +34 968 36 41 47; e-mail alimen@um.es).
[†] University of Murcia.
[§] Murcian Institute of Investigation and Agricultural Development.

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to confirm their usefulness as an alternative to growth promoter antibiotics in ruminants. The aim of this study was to assess the influence of two essential oils of thyme (*Thymus hyemalis* L. and *Thymus zygis* subsp. *gracilis*) on the in vitro ruminal degradability of dry matter, crude protein, and neutral detergent fiber and on the production of fermentation end products such as volatile fatty acids in comparison with the effects produced by monensin.

MATERIALS AND METHODS

Additives Used and Chemical Analyses of Essential Oils. The additives used were monensin sodium salt ($C_{36}H_{61}NaO_{11}$, 69864, Fluka BioChemika, Steinheim, Germany) and two essential oils of thyme, one obtained from *T. hyemalis* L. and the other from *T. zygis* subsp. *gracilis*. The chemical characterization of the essential oils was made at the Murcian Institute of Investigation and Agricultural Development (Murcia, Spain). For the quantitative analysis, samples of 0.1 μL were subjected to analysis by capillary gas chromatography, using a Hewlett-Packard 5890 gas chromatograph (GC) (Palo Alto, CA), equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm HP-5 (cross-linked Phenyl-Methyl Siloxane) column with 0.25 μm film thickness (Hewlett-Packard) according to the methods developed by Jordán et al. (*8*). Percentage compositions of samples were calculated according to the area of FID chromatographic peaks.

Gas chromatography−mass spectrometry (GC-MS) was used to identify the volatile components of the thyme essential oils (*6*). For that, a Hewlett-Packard 5890 series II Plus gas chromatograph (GC), equipped with a 30 m × 0.25 mm HP-5 column with 0.25 μm film thickness, was used. The GC was linked to a Hewlett-Packard model 5972 mass spectrometry detector. The chromatographic conditions were identical to those used for the gas chromatography analysis.

The individual peaks were identified from the retention times and retention index (relative to C_6−C_{17} *n*-alkanes), compared with those of known compounds, and by comparison of mass spectra using the NBS75K library (U.S. National Bureau of Standards, 1986) and spectra obtained from the standards, except for α-thujene, γ-cadinene, and (*Z*)-sabinene hydrate, which were tentatively identified considering the NBS75K library spectra and their corresponding retention indices. The percentage composition of samples was calculated according to the area of the chromatographic peaks.

Authentic standards were obtained from Sigma-Aldrich (Madrid, Spain) and Acros Organics (Morris Plains, NJ).

In Vitro Experimental Design. The in vitro experiment was conducted at the Animal Nutrition Experimental Unit of the University of Murcia (Murcia, Spain). In vitro incubation in a commercial incubator (Daisy II$^{200/220}$ incubator, ANKOM Technology Corp., Fairport, NY) was conducted to determine degradability. The substrate (DM basis) consisted of alfalfa hay (300 g/kg) and barley seed (700 g/kg) and was ground though a 1 mm screen.

The Daisy II$^{200/220}$ incubator consists of four independent digestion jars and allows the fermentation medium to be kept in continuous agitation and at a specific temperature (39.5 ± 0.5 °C). Thus, four treatments on total digestion media were carried out: no additive, control (CO); the monensin antibiotic, 7.5 μg/mL (MO); *T. hyemalis* L. essential oil, 1.35 μL/mL (TH); and *T. zygis* subsp. *gracilis* essential oil, 1.35 μL/mL (TZ). The doses of the evaluated additives were set to meet or exceed the minimum inhibitory concentrations (MICs) indicated by some authors for antimicrobial activity, such as Domescik and Scott (*9*) for monensin and Burt (*4*) for thyme oil.

In two runs, four total digestion media for the incubator instrument were prepared with 1584 mL of a buffer solution, 400 mL of the ruminal inoculum, and 16 mL of an ethanol solution with the additive under study for each treatment. The buffer solution was made up from two buffer solutions (A and B) in a ratio of 1:5 to obtain a final pH of 6.8 at 39 °C. Buffer solution A contained KH_2PO_4 (10 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), NaCl (0.5 g/L), $CaCl_2 \cdot 2H_2O$ (0.1 g/L), and urea (0.5 g/L); and buffer solution B contained Na_2CO_3 (15.0 g/L) and $Na_2S \cdot 9H_2O$ (1.0 g/L). Ruminal fluid was collected ≈4 h after feeding from four goats of the Murciano-Granadina breed consuming alfalfa hay ad libitum. Ruminal fluid was transported to the laboratory in a prewarmed and sealed flask and was immediately squeezed through four layers of cheesecloth. The fluid resulting from the mixture of ruminal contents was used as ruminal inoculum.

Table 1. Chemical Composition of Ingredients and Experimental Substrate (Grams per Kilogram of Dry Matter)[a]

	DM	OM	CP	NDF	ADF	ADL	ash
barley seed	911.6	981.1	100.4	216.9	40.8	1.5	18.9
alfalfa hay	924.6	891.0	201.2	494.8	332.1	67.3	109.0
substrate (70:30)	916.5	952.2	130.1	261.7	112.9	21.1	47.7

[a] DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

Substrate samples of each treatment group were directly weighed (0.5 g) into filter bags (F57 filter nags with 25 μm of porosity, ANKOM Technology), prepared in duplicate for each selected incubation times (0, 4, 8, 12, 24, and 48 h) and placed in each jar of treatment. At each time, two filter bags per treatment group were randomly removed, after which the jars were flushed with CO_2 and sealed. The removed bags were washed under tap water until the rinsewater was clear. The washed bags were then dried and weighed to determine dry matter residues. In addition, VFA production was determined after 48 h of incubation. Thus, a 50 mL duplicate of each fermentation medium was removed from each jar, placed into tubes, and then centrifuged at 3000*g* for 20 min. Supernatants were transferred into plastic containers, 1 mL of 50% sulfuric acid was added, and they were frozen at −20 °C until analysis.

Substrate and residues were analyzed for dry matter (DM) by drying at 60 °C for 48 h, for crude protein (CP) by Kjeldahl × 6.25 as indicated by AOAC (*10*), and for neutral detergent fiber (NDF) according to the method (neutral detergent digestion with heat-stable α-amylase) described by Van Soest et al. (*11*). In vitro DM, CP, and NDF disappearance data were then calculated from the concentration of each nutrient in the residues and the original samples. In addition, the ash content of the substrate was analyzed by ashing at 550 °C for 3.5 h as indicated by the AOAC (*8*), and acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were analyzed as described by Van Soest et al. (*11*). The composition of the experimental substrate is shown in **Table 1**. VFA concentration was measured in the supernatants of fermentation media by capillary gas chromatography, as described by Madrid et al. (*12*), using 4-methyl-*n*-valeric acid as internal standard.

Degradation Kinetics and Statistical Analyses. In vitro nutrient disappearance data were used to estimate DM, CP, and NDF degradation parameters using nonlinear equations. The model used for DM and CP degradability was $p = a + b(1 - e^{-ct})$, described by Ørskov and McDonald (*13*), where p is the in vitro disappearance (%) at time t, a is the soluble fraction (%), b is the slowly degradable fraction (%), c is the rate at which the b fraction is degraded (h^{-1}), and $a + b$ is the potential degradability. Effective degradabilities (ED) of DM and CP were estimated using the equation $ED = a + bc/(c + k)$ described by Ørskov and McDonald (*13*), where k is the rumen flow rate (0.06 h^{-1}). Ruminal degradation of NDF was determined by model with a discrete lag phase: $p = a + b(1 - e^{-c(t-L)})$, described by McDonald (*14*), where L is the lag time before the beginning of degradation. Effective degradability (ED) of NDF was estimated using the equation $ED = a + [bc/(c + k)]\, e^{[-(c+k)L]}$, where k is the rumen flow rate (0.06 h^{-1}).

The effect of treatment type on the kinetic parameters of DM, CP, and NDF degradability (each run was modeled as one experimental unit) and the results obtained for VFAs after 48 h of incubation were subjected to one-way analysis of variance. The comparison between means was analyzed by LSD test. The SPSS (*15*) software was used.

RESULTS

Chemical Composition of Essential Oil. The composition of the volatile fractions of the essential oils is shown in **Table 2**. The essential oil of *T. hyemalis* L. had 13 components present in concentrations exceeding 1%, the four most prevalent being

Table 2. Major Components (Relative Concentration, Percent) Identified in Thyme Essential Oils

component	RI[a]	*T. zygis* subsp. *gracilis*	*T. hyemalis* Lange
α-thujene[b]	940	1	1.81
α-pinene	949	0.53	0.87
camphene	969	0.19	0.57
sabinene	1001	tr[c]	0.41
β-pinene	1007	0.14	0.24
1-octen-3-ol	1009	0.25	0.13
3-octanone	1019	0.14	tr
myrcene	1026	1.02	1.14
α-phellandrene	1042	0.13	0.19
α-terpinene	1058	0.88	2.23
p-cymene	1068	17.03	20.93
limonene	1073	0.44	0.92
cineole	1076	tr	0.76
γ-terpinene	1109	3.13	18
(*E*)-sabinene hydrate	1116	0.54	2.52
terpinolene	1141	0.21	0.39
(*Z*)-sabinene hydrate[b]	1151	tr	3.71
linalool	1152	2.96	0.86
(*E*)-pinocarveol	1186	tr	0.18
(*Z*)-verbenol	1188	tr	0.33
camphor	1192	tr	0.63
borneol	1213	0.36	1.83
terpinen-4-ol	1220	0.62	2.29
p-cymen-8-ol	1227	0.12	0.24
α-terpineol	1231	0.15	0.38
dihydrocarvone	1235	tr	0.14
decanal	1243	nd	0.19
verbenone	1245	tr	2.35
carvacrol methyl ether	1272	0.84	1.42
geranial	1291	0.1	0.35
bornyl acetate	1302	0.1	0.1
thymol	1308	62.1	4.79
carvacrol	1314	3.13	24.3
(*E*)-caryophyllene	1419	0.73	0.71
α-humulene	1457	0.1	tr
valencene	1510	tr	0.36
γ-cadinene[b]	1537	tr	0.13
spathulenol	1640	tr	0.14
caryophyllene oxide	1650	0.38	0.13
total (%)		97	97

[a] Kovats index (HP-5). [b] Tentative identification. [c] Traces.

carvacrol (24.3%), *p*-cymene (20.93%), γ-terpinene (18.0%), and thymol (4.79%). The essential oil of *T. zygis* subsp. *gracilis* had 7 components above 1%, the four principal components being thymol (62.1%), *p*-cymene (17.03%), γ-terpinene (3.13%), and carvacrol (3.13%).

Degradation Characteristics. The kinetic parameters of in vitro DM, CP, and NDF degradability in the different treatments are given in **Table 3**. The TH and TZ treatments diminished ($p < 0.001$) the slowly degradable fractions (b) of DM, compared with the CO and MO treatments (37.4 and 33.1 vs 57.3 and 62.4%, respectively). Nevertheless, the degradation rates (c) of the b fractions with TH and TZ were higher ($p < 0.05$) than in CO and MO. However, CO and MO provided ($p < 0.01$) higher values of potential degradability ($a + b$) of DM than the TH and TZ treatments (72.6 and 70.8 vs 53.2 and 48.2%, respectively). Thus, the effective degradabilities of DM, as estimated by the parameters obtained from the mathematical model used, showed a tendency ($p = 0.081$) to diminish in the treatments that contained essential oils (34.6% for TH treatment and 34.5% for TZ treatment).

The effect of treatment type on in vitro CP degradability showed no differences in the kinetic parameters among the four treatment groups ($p > 0.05$). However, the slowly degradable fractions and the potential degradabilities were numerically smaller in the treatments containing essential oils, an effect that had statistically significant consequences when the effective degradability of CP was considered. Thus, TH and TZ treatments provided ($p < 0.01$) lower ED values than CO and MO treatments (24.6 and 29.5 vs 40.1 and 38.6%, respectively).

The values of in vitro NDF degradability were fitted by the model with lag time. No differences were noted between the NDF soluble fractions (a) or between lag times (L) for the beginning of NDF degradation in the different treatments. However, an effect of the additives on the slowly degradable fractions (b) of NDF was detected; the CO treatment showed a tendency ($p = 0.0708$) toward a highest b value (39.2%) compared with the MO, TH, and TZ treatments (25.9, 21.0, and 18.3%, respectively). This fact was reflected statistically ($p < 0.05$) in the NDF potential degradability (41.0 vs 29.3, 21.6, and 19.56%, respectively). In addition, it was observed that additives tended ($p = 0.0607$) to diminish NDF effective degradability, although the degradation rate (c) of NDF b fraction in treatment TZ was greater than in other treatments.

Volatile Fatty Acid Production. The average concentration of volatile fatty acids and the molar proportions of each individual acid in the in vitro rumen liquor at 48 h of incubation are presented in **Table 4**. As can be seen, the total VFA concentration at 48 h was influenced by treatment type ($p < 0.001$), the values obtained with CO and MO treatments being higher than with TH and TZ treatments (21.0 and 19.1 vs 11.2 and 10.1 mM). The molar proportions of acetate, propionate butyrate, isobutyrate, and isovalerate were also influenced ($p < 0.05$) by the treatment type. Thus, MO treatment provided the highest molar proportion of propionate (24.6%) at 48 h, thus lowering the acetate/propionate ratio. The essential oils, on the other hand, substantially decreased the molar proportion of propionate, increasing the acetate/propionate ratio. The molar proportions of butyrate, isobutyrate, and isovalerate of MO, TH, and TZ treatments were lower than in the CO treatment.

DISCUSSION

Chemical Composition of Essential Oils. The genus *Thymus* comprises ≈150 species. Furthermore, the composition of the essential oils that are obtained from them varies according to the species and, also, between chemotype, harvesting season, and geographical origin (*16*). *T. hyemalis* Lange, winter thyme, is an endemic shrub of the southeastern Iberian peninsula (*8*). The volatile fraction of the essential oils of *T. hyemalis* used in our experiment was rich in carvacrol (24.3%) and contained many components with a concentration of >1%. *T. zygis* subsp. *gracilis* (red thyme) used in our experiment was rich in thymol (62.1%) but with fewer components.

In Vitro Degradability. The effects of *T. hyemalis* and *T. zygis* essential oils on rumen fermentation were extensive and of similar intensity at the assayed doses, despite the differing compositions of the volatile fractions of essential oils. Thus, DM and CP degradabilities decreased in both essential oil treatments. This effect on DM degradability was also reported by Newbold et al. (*3*) when they determined the effects of specific essential oil preparation (with thymol, guajacol, and limonene) on the in situ degradability of soybean meal, although the effect was dependent on the material incubated in the nylon bag. In addition, a study of in situ ruminal degradability reported that a commercial blend of essential oils decreased CP degradation in three of the five protein sources used (*17*).

These effects could be related with the antibacterial properties of essential oils. Such oils have been used to inhibit bacterial growth, which explains why the addition *T. hyemalis* or *T. zygis*

Table 3. Kinetic Parameters of DM, CP, and NDF Degradabilities of Ration with Different Treatments

	treatment type[a]					
	control	monensin	*T. hyemalis*	*T. zygis*	SE[b]	*p* value
DM degradability[c]						
a (%)	15.2	15.4	13.2	15.2	0.63	0.5710
b (%)	57.3 b	62.4 b	37.4 a	33.1 a	0.97	0.0008
c (h^{-1})	0.0459 a	0.0378 a	0.0698 b	0.0801 b	0.00	0.0201
($a + b$)	72.6 b	70.8 b	53.2 a	48.2 a	5.73	0.0071
ED (%)	40.0 b	39.5 ab	34.6 a	34.5 a	0.72	0.0810
CP degradability						
a (%)	23.5	21.4	20.5	22.3	0.31	0.5664
b (%)	24.6	27.0	18.1	13.3	2.08	0.1917
c (h^{-1})	0.0914	0.1096	0.0760	0.1083	0.02	0.9456
($a + b$)	48.2	48.4	38.6	35.7	2.08	0.1704
ED (%)	40.1 b	38.6 b	24.6 a	29.5 a	0.81	0.0053
NDF degradability[d]						
a (%)	1.8	3.5	0.6	1.2	1.46	0.3509
b (%)	39.2 b	25.9 ab	21.0 a	18.3 a	2.16	0.0708
c (h^{-1})	0.0265 a	0.0268 a	0.0501 a	0.0849 b	0.00	0.0112
L (h)	1.81	2.48	2.48	0.98	0.27	0.2701
($a + b$)	41.0 b	29.3 ab	21.6 a	19.5 a	0.38	0.0252
ED (%)	12.0 b	10.2 ab	7.9 a	10.4 ab	0.37	0.0607

[a] Means in the same row with different letters are significantly different. [b] Standard error of the mean. [c] Kinetic parameters of nonlinear equation for DM or CP degradability, $p = a + b\,(1 - e^{-ct})$, and effective degradabilities (ED) of DM or CP, $ED = a + bc/(c + k)$, assuming a rate of passage (k) of 0.06 h^{-1}. [d] Kinetic parameters of nonlinear equation for NDF degradability, $p = a + b\,(1 - e^{-c(t-L)})$, and effective degradabilities (ED) of NDF, $ED = a + (bc/(c + k))\,e^{(-(c+k)L)}$, assuming a rate of passage (k) of 0.06 h^{-1}.

Table 4. Volatile Fatty Acid (VFA) Concentrations at 48 h of Incubation with Different Treatments[a]

	control	monensin	*T. hyemalis*	*T. zygis*	SE[b]	*p* value
total VFAs (mM)	21.0 b	19.1 b	11.2 a	10.1 a	0.33	0.0006
molar proportion (%)						
acetate	74.9 b	70.9 a	84.9 c	83.3 c	0.26	0.0001
propionate	16.6 c	24.6 d	10.3 a	11.7 b	0.18	0.0000
butyrate	7.3 b	3.7 a	3.8 a	4.0 a	0.06	0.0001
isobutyrate	0.62 c	0.37 a	0.50 b	0.52 bc	0.01	0.0186
isovalerate	0.44 c	0.26 a	0.29 ab	0.33 b	0.00	0.0046

[a] Means in the same row with different letters are significantly different. [b] Standard error of the mean.

essential oils decreased the activity of the ruminal microorganism. However, because of the large number of different groups of chemical compounds present in essential oil, the antibacterial activity cannot be easily attributed to one specific mechanism. In a bibliographical revision, Burt (*4*) described several ways different investigators had proposed to explain such antibacterial action, including degradation of the cell wall, damage to the cytoplasmatic membrane, leakage of cell contents, coagulation of cytoplasm, and depletion of the proton motive force.

Both oils used in our experiment contain powerful phenolic substances with antimicrobial effects (carvacrol and thymol). The antibacterial mechanism of action is probably related to the presence of phenolic substances with hydroxyl groups, which are able to increase membrane fluidity, altering and exhausting the microbial cell (*18*). However, other components of the essential oils may also be related to the microbial activity. For example, high levels of *p*-cymene (20.93 and 17.03%, respectively) were measured in *T. hyemalis* and *T. zygis* essential oils.

Therefore, one candidate is *p*-cymene (a precusor of carvacrol and thymol), because antibacterial synergism with carvacrol, whereby it seems to facilitate the transport of carvacrol through the cytoplasmic membrane of *Bacillus cereus*, has been observed (*19*). In addition, other components such as γ-terpinene and α-terpinene could have important effects, because it has been observed that α-terpinene can inhibit the growth of bacteria such as *Escherichia coli* (*20*), although the antimicrobial effects of γ-terpinene have not been verified. It is important to indicate that these components (γ- and α-terpinine) show a higher percentage in the *T. hyemalis* essential oil than in the *T. zygis* essential oil (18.0 and 2.23 vs 3.13 and 0.88%, respectively).

As a result of the different compositions of the oils, differences in antimicrobial effect might have been expected. Nevertheless, at the assayed dose (1.35 μL/mL) the effects on ruminal fermentation were similar because, at this level, synergism probably exists between the oil components or because the effects are so great that any difference between oils would be imperceptible.

Also, NDF degradability was decreased by the essential oils used in our experiment, whereas the addition of monensin treatment had a similar effect. Jalc et al. (*21*) showed also that monensin decreased the in vitro degradability of NDF. Ionophores, such as monensin, act by interrupting transmembrane movement and the intracellular equilibrium of ions, exhausting and causing the death of some microorganisms (*22*). In in vivo studies, monensin did not affect NDF degradation (*1*), because the expression of such as effect in an in vivo ruminal medium is possibly more complex, inhibiting bacteria such as *Streptococcus bovis* (lactic acid producers) and therefore preventing the pH from falling, favoring activity of the cellulolytic bacteria. Therefore, it could be hypothesized that the results of essential oils on degradability could be different between in vitro and in vivo tests.

Volatile Fatty Acid Production. The production of VFAs in the MO and CO treatments exceeded that observed in the TH and TZ treatments. This effect is related with the highest DM degradation of the CO and MO treatments. Khandaker et al. (*23*) indicated that a high VFA concentration suggests increased rumen microbial activity due to more quantities of matter being fermented in the rumen. Also, numerous studies have demonstrated that the main effect of monensin on energy metabolism in the rumen is to increase production of propionate and to reduce the production of acetate, thus diminishing the acetate/propionate ratio (*24*); such an effect was also observed in our experiment with MO. However, the essential oil treatments had an effect opposite that of monensin on the molar

proportion of propionate, perhaps due to a less specific antimicrobial action of the essential oils, which could have inhibited degradation of most of the nutrients and, consequently, of the VFAs.

The benefits of modifying degradability could be defined as an increase (or no change) in total VFA concentration and a decrease in the acetate/propionate ratio, because an alteration of ruminal microbial fermentation in favor of propionate is more energetically efficient (*6*). These effects are obtained in our experiment with monensin (acetate/propionate ratio of 2.9), but they were not found in the case of *T. hyemalis* (acetate/propionate ratio of 8.2) or *T. zygis* (with ratio of 7.1) essential oils.

It is necessary to emphasize that, although in our experiment monensin did not alter the degradation of the protein, the molar proportions of isobutyrate and isovalerate were smaller in the treatment with monensin than in CO. A reduction in these branch-chained VFAs is related with a reduction in amino acid catabolism (*25*). McGuffey et al. (*1*) indicated that numerous studies on monensin revealed that it had little effect on ruminal proteolysis, but ruminal degradation of peptides and amino acids was inhibited, causing an accumulation of them.

Our results demonstrate that *T. hyemalis* and *T. zygis* essential oils at 1.35 μL/mL had a similarly high effect on rumen fermentation, despite the differences in the composition of the volatile fraction of the essential oils. DM, CP, and NDF degradability decreased in both essential oil treatments. In addition, the action of monensin on ruminal degradability was as expected and as already demonstrated by other authors. However, the concentration of VFAs and their molar profile after the essential oil treatments differed from that observed with monensin. The effects of essential oils at the assayed dose on fermentation and on the end products would not be nutritionally beneficial to the rumen energetic metabolism. However, further investigations are necessary to consider the possible in vivo effect of these oils on in vivo ruminal fermentation.

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