EVALUATION OF THE EFFECT OF FOUR ESSENTIAL OILS AS POTENTIAL ALTERNATIVES FOR MONENSIN ON RUMEN FERMENTATION CHARACTERISTICS AND NUTRIENT DEGRADABILITY

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(Received 2/4/2019, accepted 27/6/2019)

SUMMARY

The objective of this study was to evaluate the effect of four essential oils (EOs) from guava (*Psidium guajava*), citronella (*Cymbopogon nardus*), lemongrass (*Cymbopogon citratus*), and geranium (*Pelargonium graveolens*) on gas production and rumen fermentation *in vitro* as a natural substitute for the ionophore antibiotic monensin. These EOs are chemically characterised by Gas Chromatography Mass and evaluated *in vitro* at four different concentrations (0, 15, 30, and 45 µl per 45 ml buffered rumen fluid) regarding their effects on gas production and rumen fermentation characteristics and were compared to those of monensin. Compared to the negative control, monensin significantly depressed gas production and truly degraded dry matter (TDDM) but enhanced propionate production. All EOs except *P. graveolens* significantly decreased gas production with increasing concentrations. TDDM was significantly reduced with *C. citratus* (at 45 µl) and *P. graveolens* (at 30 and 45 µl). No significant change was detected in the ammonia nitrogen concentration with all assayed EOs except *C. nardus* and *C. Citrus*. Compared to monensin and the negative control, *C. nardus* and *C. Citrus* reduced the ammonia concentration at high levels. High levels of all tested EOs significantly reduced protozoa counts. The EOs of *C. citratus* (at 45 µl) and *P. graveolens* (at 30 and 45 µl) also significantly increased the acetate proportion. Moreover, the acetate to propionate ratio was significantly increased by 30 µl *P. graveolens*. The results of the current study concluded that the tested EOs, except *P. graveolens*, efficiently diminished gas production with a similar potency to monensin. Furthermore, they exceed the monensin in their ability to reduce the ammonia nitrogen concentration and protozoa count without adversely affecting volatile fatty acid levels. But, they were less effective than monensin in modifying ruminal volatile fatty acid profile especially propionate and acetate to propionate ratio. Hence, *P. guajava*, *C. nardus*, and *C. citratus* EOs could be a safe and promising rumen manipulator.

Keywords: *Essential oils, monensin, nutrient degradability and rumen fermentation.*

INTRODUCTION

For decades, ionophores such as monensin have been effectively adopted as feed supplements to modulate ruminal fermentation and boost feed efficiency in livestock production (Khorrami *et al*., 2015; Melchior *et al*., 2018). Monensin may modify ruminal fermentation mainly by enhancing energy status via an antiporter action (Newbold *et al*., 2013) and inhibiting ruminal fungi, protozoa, and Gram-positive bacteria rather than Gram-negative bacteria (Duffield *et al*., 2008). The selective inhibition of Gram-negative bacteria resulted in an elevated propionate percentage and a concurrent reduction in acetate, butyrate, and methane production (Johnson *et al*., 2009).However, the use of ionophores such as monensin in ruminant nutrition is confronted by reduced social approval because of antimicrobial resistance in animals and humans together with the presence of residues in meat and milk, which pose a serious hazard to public health (Chesson, 2006; Yang *et al*., 2010). Accordingly, more regulations to prohibit their use are appearing and ruminant nutritionists are keenly trying to find suitable safe substitutes for these feed supplements (Anassori *et al*., 2011; Khorrami *et al*., 2015).

Currently, the use of plant bioactive ingredients as safe feed supplements to enhance nutrient utilisation and ruminal fermentation has gained substantial attention among ruminant nutritionists (Ye *et al*., 2018). Essential oils (EOs) are volatile aromatic complexes generated from plants by steam and/or water distillation (Calsamiglia *et al*., 2007). EOs reportedly manipulate the rumen fermentation pattern (Schären *et al*., 2017) as a response of their antimicrobial actions against both Gram-negative and Gram-positive bacteria due to the presence of phenolic and terpenoid compounds (Dorman and Deans, 2000). Essential oils show important biological activities, which account for the development of aromatherapy used in complementary and alternative medicine. Nevertheless, to our knowledge, information about the impact of the tested EOs compared with monensin to manipulate ruminal fermentation is still scarce. The goal of the current study was to assess the inclusion effects of four EOs in different doses on the rumen fermentation profile, nutrient degradability, and gas production (GP) *in vitro*.

MATERIALS AND METHODS

The current work was carried out at the Advanced Laboratory of Animal Nutrition, Department of Animal and Fish Production, Faculty of Agriculture (El-Shatby), Alexandria University.

Plant material and extraction of essential oils:

Fresh leaves from citronella (*Cymbopogon nardus*), guava (*Psidium guajava*), geranium (*Pelargonium graveolens*) and lemongrass (*Cymbopogon citratus*) were gathered during the flower stage from different locations in the Alexandria governorate and El-Kanater El-Khayria city, Qalyubia Governorate, Egypt. Plant species were verified and authenticated by the book Flora of Egypt (Tackholm 1974). The plants leaves were washed with distilled water and dehydrated for five days at room temperature. A Clevenger-type apparatus was used for EO isolation by hydro distillation for 4 h. Collected EOs were stored in amber glass bottles at 4°C for biological and gas chromatography-mass spectrometry (GC-MS) analyses.

Analysis of essential oils using GC-MS:

The chemical natures of the individual constituents of citronella, guava, geranium and lemongrass following dilution with hexane were identified using GC-MS (Thermo ISQ, Waltham, MA, USA). A GC-MS system equipped with a TG-5MS capillary column (Thermo, P/N 26098. 1430; 30 m × 0.32 mm × 0.25 µm) was used. The injection volume was 1 µL using an autosampler, and the injector temperatures were set at 240°C. The carrier gas was helium at a flow rate of 1 mL/min with a split ratio of 1:10. The initial oven temperature was 50°C and was maintained for 8 min and then raised to 130°C at a rate of 8°C/min. Then, the temperature was increased to 200°C at a rate of 5°C and ultimately raised to 280°C at 15°C/min, which was maintained for 4 min. The ion source temperature was 230°C, and the electron impact ionisation (ET) was 70 eV. Mass spectra were analysed in the SCAN mode over the range of 33 at 400 amu, with an emission current of 34.6 VA and electron multiplier voltage of 1392 V.

***Substrate description and* treatments:**

The diet used as a substrate was a total mixed ration (50% roughage: 50% concentrate). The formulation of the basal diet was 50% berseem hay, yellow corn 20%, what bran 12.5%, soybean meal 5%, crushed horse bean 10.75%, limestone 1%, salt 0.5% and a mixture of minerals and vitamins 0.25%. The basal diet was milled using a Wiley mill to permit a 1 mm screen and was adopted as a substrate. The chemical analysis of the experimental diet was conducted for organic matter (OM), dry matter (DM), ether extract (EE), and crude protein (CP) according to the procedure of the AOAC (2006). Acid detergent fibre (ADF), neutral detergent fibre (NDF), and acid detergent lignin (ADL) were estimated following the method of Van Soest *et al*. (1991) with an ANKOM 220 fibre analyser (ANKOM Technology Corporation, Macedon, NY, USA). NDF analysis was performed with heat stable a-amylase. The proximate analysis of the substrate was 89.61% OM, 17.00% CP, 3.97% EE, 50.63% NDF, 20.61% ADF, 4.34% ADL, 16.27% cellulose, 30.02% hemicellulose, and 10.39% Ash. EOs from guava, citronella, lemongrass, and geranium were supplemented at four levels (0, 15, 30, and 45 µl per 45 ml buffered rumen fluid). In the present study, monensin (Rumensin®, Elanco, Itapira, Brazil) was used as a positive control at a concentration of 93.6 µg per 300 mg basal diet.

Inoculum donors and in vitro gas production assay:

The inoculum donors were four adult rumen-fistulated Barki sheep with an average body weight of 46.0±1.6 kg. Sheep were fed 0.75 kg as-fed concentrate feed mixture and berseem hay *ad lib*.head/daily. The proximate analysis of the concentrate feed mixture was 14.5, 2.7, 89.5, 22.6, and 38.2% for OM, CP, EE, NDF, and ADF, respectively, on a DM basis. Before the morning feeding, liquid and solid rumen contents were obtained individually via the cannula by a stainless steel probe connected to a large volume syringe. Collected rumen contents were kept in pre-heated (39°C) shielded flasks and transferred to the laboratory under anaerobic conditions. The collected rumen contents were compressed via four layers of cheese cloth and placed in a water bath at 39°C with CO_2 saturation until inoculation.

The *in vitro* GP technique was conducted following the technique of Theodorou *et al*. (1994) with the modification of the semi-automatic system of Mauricio *et al*. (1999), utilising a pressure transducer in 120 ml serum tubes incubated for 24 h at 39°C. The tested diets (300 mg as-fed) were incubated with 30 ml MB9 medium and 15 ml assorted rumen fluid. MB9 was composed of 2.8 g NaCl, 0.1 g $CaCl_2$, 0.1 g $MgSO_4.7H_2O$, 2.0 g KH_2PO_4 and 6.0 g Na_2HPO_4 per litre. Then, the CO_2 was rushed for 30 min, and the pH was set to 6.8 (Onodera and Handerson, 1980). Bottles were sealed by rubber stoppers, agitated, and incubated at 39°C. After 6-, 12- and 24-h incubations, the gas headspace pressure was recorded by a pressure transducer, and the bottles were shaken by hand. Based on the regression obtained in our system and conditions, the GP volume at every incubation time was estimated. For each GP assay, three GP runs were used. GP measurements were performed in triplicate for each run. Each run had six blank tubes comprising buffered rumen fluid lacking substrate, six negative control tubes with substrate only, six positive control tubes with substrate fortified monensin, and six tubes with substrate for each EO dose. GP volumes were expressed as ml per g incubated DM. After the end of the incubation, three bottle contents were adopted for the estimation of TDOM and TDDM based on the technique of Blümmel and Becker (1997). The NH3-N concentration was determined in another three bottles (Preston, 1995). For determining protozoal numbers, rumen fluid was mixed with methyl green–formalin saline solution and kept for later counting based on the method of Galyean (1989). Following the method of Palmquist and Conrad (1971), VFAs were estimated using a gas chromatograph (GC Thermo TRACE 1300). The VFA of known concentrations was used as a standard for calibration, and no internal standard was used.

Statistical analysis:

Because positive and negative controls were used for all treatment combinations, the design of the current study did not have a factorial arrangement. Hence, to compare EOs at different concentrations with monensin and the negative control, an analysis we performed using the four EOs with three concentrations each combined with monensin and the negative control to form one treatment variable with 14 levels (4 × 3 + monensin + negative control). The proc mixed procedure of SAS (2002) was used to analyse the obtained results. The data were analysed within each sample as time (run) that considered a random effect. Significance was declared at $P < 0.05$, and Dunnett's multiple comparison test (Dunnett, 1955) was used to compare the negative control, monensin and tested EOs. The contrast statement was used to determine the linear and quadratic variable response to increasing doses of EOs.

RESULTS AND DISCUSSION

Composition of the tested essential oils:

EOs extracted from diverse plant species can differ in stereochemistry, chemical structures and bioactive activities (Burt, 2004). Numerous components were recognised from the chromatograms of the tested EOs of *P. guajava*, *C. nardus*, *C. citratus*, and *P. graveolens*. As shown in Table (1), based on the relative percentages of the chromatogram areas, the chief compounds of *P. guajava* EO were cyclopentene,3-(2-propenyl) (24.26%), jasminlactone (11.10%), lactic acid pentamethylbenzyl ester (10.8%), and 4-acetoxyquinolin-2-one (10.7%). Citronellol (33.6%), geraniol (27.2%), oleic acid (2.9%), and epoxy-linalooloxide (2.4%) were the major components of *C. nardus* EO, as shown in Table (2). As depicted in Table (3), β-citral (31.8%) and α-citral (25.8%) represented the major components of *C. citratus* EO. In contrast, á-myrcene, cyclopentene, 1-butyl-, 5-hepten-2-one, 6-methyl-, β-linalool, cis-geranyl acetate, trans-13-octadecenoic acid, and linoleic acid were minor components. α Citronellol, β citronellol, linalool, geranyl

formate, and caryophyllene constituted 18.3, 8.41, 6.98, 5.16, and 4.84%, respectively, of *P. graveolens* EO chromatogram areas (Table 4).

Table (1): Main bioactive components of guava (*Psidium guajava L.*) essential oil detected by gas chromatography/mass spectrometry.

Peak	Compounds	MW†	Formula	RT†	Area%
1-	Cyclopentene,3-(2-propenyl)-	108	C_8H_{12}	4.80	24.26
2-	Jasminlactone	168	$C_{10}H_{16}O_2$	13.88	11.10
3-	Lactic acid, pentamethylbenzyl ester	250	$C_{15}H_{22}O_3$	11.23	10.77
4-	4-Acetoxyquinolin-2 one	203	$C_{11}H_9NO_3$	14.23	10.71
5-	1H-Cycloprop[e]azule ne, decahydro-1,1,7-trimethyl-4-methylene-,[1aR-(1aà,4aà,7à,7aá, 7bà)]	204	$C_{15}H_{24}$	11.52	3.68
6-	à-Caryophyllene	204	$C_{15}H_{24}$	11.76	2.42
7-	Naphthalene, decahydro-4a-methyl-1 -methylene-7-(1-methy lethenyl)-, [4aR-(4aà,7à,8aá)]-	204	$C_{15}H_{24}$	12.35	2.21
8-	9-Octadecenoic acid, methyl ester, (E)-	296	$C_{19}H_{36}O_2$	23.83	2.00
9-	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	20.66	1.95
10-	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1à,3aá,4à,7á)]-	204	$C_{15}H_{24}$	12.50	1.93
12-	à-Cadinol	222	$C_{15}H_{26}O$	15.50	1.80
13-	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methyle thyl)-, (1S-cis)-	202	$C_{15}H_{22}$	13.00	1.60
14-	Cyclopentane, 2-(1,1-dicyanomethyl)-1-isopropenyl-3-methyl	188	$C_{12}H_{16}N_2$	14.54	1.42
15-	Ylangene	204	$C_{15}H_{24}$	10.41	1.37
16-	Caryophyllene	204	$C_{15}H_{24}$	11.88	1.37
17-	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-1R*,3E,7E,11R*)]	220	$C_{15}H_{24}O$	14.65	1.23
18-	Ledol	222	$C_{15}H_{26}O$	14.35	1.05

†MW = molecular weight, RT = retention time, minutes.

Table (2): Main bioactive components of citronella (*Cymbopogon nardus*) essential oil detected by gas chromatography/mass spectrometry.

Peak	Compounds	MW†	Formula	RT†	Area%
1-	Citronellol	156	$C_{10}H_{20}O$	8.40	33.55
2-	Geraniol	153	$C_{10}H_{19}N$	8.97	27.21
3-	Oleic Acid	282	$C_{18}H_{34}O_2$	24.65	2.85
4-	Epoxy-linalooloxide	186	$C_{10}H_{18}O_3$	10.44	2.40
5-	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	20.71	2.19
6-	11-Octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$	23.87	2.17
7-	Linalool	154	$C_{10}H_{18}O$	5.84	2.11
8-	5-Hepten-2-one, 6-methyl-	126	$C_8H_{14}O$	4.05	1.87
9-	3-(4,8,12-Trimethyltridecyl) furan	292	$C_{20}H_{36}O$	9.85	1.62
10-	Erucic acid	338	$C_{22}H_{42}O_2$	28.14	1.41
12-	cis-Verbenol	152	$C_{10}H_{16}O$	7.21	0.91
13-	Octadecanoic acid	284	$C_{18}H_{36}O_2$	24.90	0.75
14-	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	21.42	0.71
15-	8-Hydroxycarvotanace tone	168	$C_{10}H_{16}O_2$	10.25	0.66
16-	Geranyl acetate	196	$C_{12}H_{20}O_2$	10.55	0.57
17-	2-Tridecanone	198	$C_{13}H_{26}O$	12.50	0.51
18-	cis-Verbenol	152	$C_{10}H_{16}O$	6.91	0.50
19-	Citronellal	154	$C_{10}H_{18}O$	6.71	0.39
20-	Octadecanoic acid, methyl ester	298	$C_{19}H_{38}O_2$	24.31	0.31

†MW = molecular weight, RT = retention time, minutes.

Table (3): Main bioactive components of lemongrass (*Cymbopogon citratus*) essential oil detected by gas chromatography/mass spectrometry.

Peak	Compounds	MW†	Formula	RT†	Area%
1-	β-Citral	152	$C_{10}H_{16}O$	8.91	31.86
2-	α-Citral	152	$C_{10}H_{16}O$	8.36	25.82
3-	á-Myrcene	136	$C_{10}H_{16}$	4.11	4.11
4-	Cyclopentene, 1-butyl-	124	C_9H_{16}	8.60	3.90
5-	5-Hepten-2-one, 6-methyl-	126	$C8H_{14}O$	4.04	2.12
6-	β-Linalool	154	$C_{10}H_{18}O$	5.83	1.86
7-	cis-Geranyl acetate	196	$C_{12}H_{20}O_2$	10.55	1.81
8-	trans-13-Octadecenoic acid	282	$C_{18}H_{34}O_2$	24.55	1.57
9-	Linoleic acid	280	$C18H32O_2$	24.45	1.48
10-	1-(Cyclopropyl-nitro-methyl)-cyclopentanol	185	$C_9H_{15}NO_3$	10.42	1.23
12-	(Z)-Geranic acid	196	$C_{12}H_{20}O_2$	20.19	1.15
13-	Methyl 10,11-tetradecadienoate	238	$C_{15}H_{26}O_2$	9.83	1.03

†*MW = molecular weight, RT = retention time, minutes.*

Table (4): Main bioactive components of geranium (*Pelargonium graveolens*) essential oil detected by gas chromatography/mass spectrometry.

Peak	Compounds	MW†	Formula	RT†	Area%
1-	α Citronellol	156	$C_{10}H_{20}O$	8.20	18.28
2-	β Citronellol	156	$C_{10}H_{20}O$	8.63	8.41
3-	Linalool	182	$C_{11}H_{18}O_2$	8.85	6.98
4-	Geranyl formate	182	$C_{11}H_{18}O_2$	6.96	5.16
5-	Caryophyllene	204	$C_{15}H_{24}$	14.94	4.84
8-	Geranyl tiglate	236	$C_{15}H_{24}O_2$	16.43	2.21
9-	δ-Cadinene	204	$C_{15}H_{24}$	13.05	2.14
10-	trans-13-Octadecenoic acid	282	$C_{18}H_{34}O_2$	24.58	2.07
12-	ë-Neoclovene	204	$C_{15}H_{24}$	11.20	1.94
13-	β-Bourbonene	204	$C_{15}H_{24}$	10.61	1.67
14-	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	20.17	1.38
	8-Octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$	23.87	1.35
15-	D-Limonene	136	$C_{10}H_{16}$	4.67	1.09
16-	Geranyl propionate	210	$C_{13}H_{22}O_2$	12.12	1.14
17-	Germacrene D	204	$C_{15}H_{24}$	12.28	1.11
18-	Geraniol butyrate	224	$C_{14}H_{24}O_2$	13.71	1.10

†*MW = molecular weight, RT = retention time, minutes.*

Effects on gas production and feed degradability:

Herein, monensin was chosen as a positive control treatment due to its favourable roles in modulating ruminal fermentation. Table (5) demonstrates the effect of the tested EOs at different concentrations compared to that of monensin on both GP and feed degradability. A significant reduction in truly degraded organic matter (TDOM) and GP, expressed as mL/g dry matter (DM) (P=0.001) and mL/g TDOM (P=0.039), was obvious following the addition of monensin compared to that following the addition of the negative control. These results are in agreement with those of earlier *in vitro* trials (Callaway *et al.*, 1997; Shen *et al.*, 2017). The reduction in GP following monensin treatment is highly linked to its lipophilic nature, which causes a disturbance in the intracellular K^+ and Na^+ balance, which is injurious to Gram-positive bacteria that produce higher amounts of hydrogen and lactate (Russell and Strobel, 1988). The suppressive action of monensin on feed degradability is mostly due to its cellulolytic bacteria inhibition, which is the main contributor to fibre degradability in the rumen (Narvaez *et al.*, 2013; Jeyanathan *et al.*, 2014).

Additionally, a tendency comparable to the effect of monensin on GP (mL/g DM and mL/g TDOM) was observed for *P. guajava*, *C. nardus*, and *C. citratus* EOs (Table 5). Compared with the negative control, EOs achieved the maximum reduction in GP at 30 and 45 µl; however, there were no significant differences (mL/g DM) between the EO and monensin groups. Moreover, compared to the negative control, *P. graveolens* EO did not elicit a significant change in GP. However, this decline is not detected for minor doses of these EOs and not for all added doses of EOs from both *P. guajava* and *C. nardus*.

Table (5): Effect of different levels of essential oils (EOs) on gas production and feed degradability after a 24-h *in vitro* incubation.

Additive	Dosage [µl/45 ml rumen fluid]	Gas production		TDDM (g/100 g)	TDOM (g/100 g)
		[ml/g DM]	[ml/g TDOM]		
Negative control		124.47†	181.60†	69.61†	69.95
Monensin		93.64*	107.67*	66.56*	67.36
Psidium guajava	15	111.04†	156.21†	70.26†	71.17†
	30	104.73*	148.43*†	69.80†	70.52†
	45	92.39*	135.54*†	67.15	68.13
	Linear	0.140	0.015	0.109	0.138
	Quadratic	<0.001	<0.001	0.027	0.172
Cymbopogon nardus	15	103.40*	149.00*†	68.70	69.42
	30	94.95*	140.61*†	67.00	67.69
	45	89.09*	133.69*	67.40	67.96
	Linear	0.005	0.003	0.472	0.704
	Quadratic	<0.001	<0.001	0.055	0.103
Cymbopogon citratus	15	114.89†	161.78†	70.31†	71.08†
	30	103.68*	151.12*†	68.47	69.15
	45	95.75*	142.13*†	66.64*	67.47
	Linear	0.316	0.09	0.196	0.127
	Quadratic	<0.001	0.001	0.002	0.006
Pelargonium graveolens	15	118.60†	171.31†	68.40	69.17
	30	111.64	161.85†	66.89*	68.60
	45	108.44	151.34†	66.85*	68.19
	Linear	0.571	0.553	0.304	0.472
	Quadratic	0.049	0.013	0.011	0.061
Statistics					
SEM		3.43	4.21	0.66	0.59
P-value		<0.001	<0.001	0.001	0.039

**Means within a column that have a symbol are significantly different (P < 0.05) from the control.*
† Means within a column that have a symbol are significantly different (P < 0.05) from monensin.
DM = dry matter; SEM = standard error of the mean; TDDM= truly degraded dry matter; TDOM = truly degraded organic matter.

The decrease in GP with increasing concentrations of EOs detected in the current study is consistent with the findings of Patra and Yu (2012) and Sallam *et al.* (2011) who also reported a linear decrease in total GP with increasing EO doses. Notably, the reduction in GP associated with a reduction in the protozoa count observed here may be linked to the antimicrobial activity of EOs because of their active components (Calsamiglia *et al.*, 2007). For instance, citral, the main active ingredient detected in *C. citratus* herein, significantly reduces GP in the rumen with notable antimicrobial action (Lin *et al.*, 2013). Additionally, geraniol, a major component of *C. nardus*, exerts antimicrobial action against both Gram-negative and

Gram-positive bacteria (Dorman and Deans, 2000) and reduces total GP when tested at 500 mg l^{-1} (Pirondini *et al.*, 2015).

Compared to the negative control, EOs from *C. citratus* (45 µl) and *P. graveolens* (30 and 45 µl) decreased truly degraded dry matter (TDDM), but no significant difference was observed between these EOs and monensin (Table 5). Digestibility reduction is the outcome of the emulation between digestion and passage rates (Van Soest, 1994). In numerous reports, adding blended EOs reduced both the effective degradability and ruminal degradation rate of some protein supplements (Newbold *et al.*, 2004). In the current study, *C. citratus* markedly decreased TDDM together with GP. The antimicrobial activity of its major component citral could be responsible for the decline in TDDM detected in the current experiment. The hostile effect of *P. graveolens* on TDDM may be due to citronellol as it reportedly efficiently hinders rumen microbial activity and exhibits activity against both Gram-negative and Gram-positive bacteria (Wan *et al.*, 1998; Burt, 2004).

Effects on rumen fermentation products:

Generally, compared to the negative control, monensin did not elicit any significant alterations in the NH_3–N concentration, pH, and protozoa count (Table 6). All concentrations of the tested EOs did not alter (*P*=0.141) the ruminal pH.

Table (6): Effect of different levels of essential oils (EOs) on the ammonia concentration and protozoal count after a 24-h *in vitro* incubation.

Additive	Dosage [µl/45 ml rumen fluid]	NH_3–N (mg/100 mL)	pH	Protozoa (10^5/mL)
Negative control		27.95	5.76	6.80
Monensin		29.53	5.62	6.10
Psidium guajava	15	27.50	5.61	6.10
	30	25.29	5.55	5.10
	45	22.41†	5.55	4.40*
	Linear	0.834	0.526	0.583
	Quadratic	0.027	0.361	0.007
Cymbopogon nardus	15	27.22	5.59	5.70
	30	24.10	5.63	4.90
	45	20.88*†	5.66	3.30*†
	Linear	0.894	0.450	0.586
	Quadratic	0.007	0.839	0.005
Cymbopogon citratus	15	27.00	5.57	5.60
	30	23.66†	5.60	5.20
	45	22.45†	5.67	4.00*
	Linear	0.848	0.356	0.399
	Quadratic	0.030	0.845	0.018
Pelargonium graveolens	15	26.44	5.60	5.90
	30	25.68	5.62	5.10
	45	23.81†	5.63	4.60*
	Linear	0.643	0.470	0.363
	Quadratic	0.066	0.704	0.010
Statistics				
SEM		0.50	0.04	0.18
P-value		0.027	0.141	0.021

**Means within a column that have a symbol are significantly different (P < 0.05) from the control.*
† Means within a column that have a symbol are significantly different (P < 0.05) from monensin.
SEM = standard error of the mean.

In the current study, compared to both negative and positive controls, EO from *C. nardus* (45 µl) significantly decreased the NH_3–N concentration. Compared to monensin, the four tested EOs at a concentration of 45 µl significantly reduced the NH_3–N concentration (Table 6). A similar trend was previously noted with other EOs, such as clove oil (Gunal *et al.*, 2013), eugenol (Busquet *et al.*, 2006; Castillejos *et al.*, 2006), *Eucalyptus citriodora,* and *Ocimum gratissimum* (Kouazounde *et al.*, 2015). However, according to Castillejos *et al.* (2008), 500 mg/l clove oil had no influence on NH_3–N concentrations. The small effects of *P. guajava*, *P. graveolens* and *C. citratus* EOs on the NH_3–N concentration may be due to the collaboration between their active components and proteins (Gunal *et al.*, 2013). Nonetheless, the strong suppressive effect of *C. nardus* EO on ammonia production could probably result from the reduction in hyper-ammonia producing bacteria such as *Peptostreptococcus anaerobius* and *Clostridium sticklandii* due to the antimicrobial activity of its major component geraniol (McEwan *et al.*, 2002). These changes lead to further inhibition of deamination of amino acids and subsequently decreased ammoniagenesis (Castillejos *et al.*, 2006).

Table (7): Effect of different levels of essential oils (EOs) on the volatile fatty acid concentration (mM) after a 24-h *in vitro* incubation.

Additive	Dosage [µl/45 ml rumen fluid]	Volatile fatty acids (mM)				C2/C3 ratio	Total
		Acetate (C2)	Propionate (C3)	Butyrate	Valerate		
Negative control		36.99	11.02†	9.08	1.03	3.38	58.13
Monensin		43.84	15.90*	8.81	0.92	2.77	69.47
Psidium guajava	15	41.79	10.54	8.71	0.45	3.98	61.47
	30	43.51	12.78	10.93	0.92	3.45	68.14
	45	44.74	11.24	8.59	1.03	4.04	65.60
	Linear	0.105	0.862	0.872	0.269	0.230	0.199
	Quadratic	0.094	0.185	0.700	0.394	0.839	0.059
Cymbopogon nardus	15	38.97	10.47	6.64	0.54	3.74	58.61
	30	45.29	13.30	9.18	1.06	3.43	68.83
	45	40.84	13.19	7.79	0.64	3.14	62.47
	Linear	0.173	0.947	0.934	0.398	0.128	0.375
	Quadratic	0.070	0.024	0.728	0.910	0.045	0.058
Cymbopogon citratus	15	41.48	10.57	9.68	0.41	4.03	62.14
	30	40.91	10.72	11.95	0.62	3.91	64.20
	45	48.89*	10.16	9.23	0.93	4.84†	69.20
	Linear	0.065	0.749	0.354	0.161	0.172	0.230
	Quadratic	0.001	0.644	0.657	0.625	0.025	0.028
Pelargonium graveolens	15	41.94	12.60	7.97	0.66	3.37	63.15
	30	49.81*	9.12	9.36	1.04	5.52*†	69.32
	45	48.71*	10.98	7.49	0.67	4.67†	67.84
	Linear	0.002	0.592	0.783	0.487	0.342	0.031
	Quadratic	<0.001	0.089	0.850	0.935	0.004	0.005
Statistics							
SEM		1.01	0.72	0.87	0.06	0.12	1.59
P-value		0.004	0.001	0.813	0.377	<0.001	0.067

**Means within a column that have a symbol are significantly different (P < 0.05) from the control.*
† Means within a column that have a symbol are significantly different (P < 0.05) from monensin. SEM = standard error of the mean.

Compared with the negative control, monensin significantly enhanced propionate (*P*=0.001) but not acetate production. These results are in agreement with those of earlier *in vitro* trials (Callaway et al., 1997; Shen et al., 2017). In the rumen, propionate is generated by the succinate or acrylate pathway, and the

succinate pathway is the predominant pathway (Jeyanathan et al., 2014). Monensin reportedly boosts propionate production via the succinate pathway by increasing propionate and succinate producer abundance and reducing non-producers (Schären et al., 2017).

As mirrored by VFA profiles, the current study showed that compared to no supplementation, supplementation with EOs from *C. citratus* (45 µl) and *P. graveolens* (30 and 45 µl) significantly increased acetate production (Table 7). Similarly, Kamalak et al. (2011) reported that 200–1200 mg L^{-1} *Citrus sinensis* EO altered the VFA profile by increasing acetate. Additionally, in the present study, the acetate to propionate ratio increased with increasing levels of *P. graveolens* EO, most likely due to molecular hydrogen accumulation. Here, the effects exhibited by *P. graveolens* on VFA are consistent with the results of Patra and Yu (2012) who found no alteration in TVFA level with *M. piperita*, whereas both acetate and the acetate to propionate ratio increased. Conversely, Canaes et al. (2017) reported that citral oil reduced the ruminal acetate proportion and acetate to propionate ratio. This discrepancy could be due to the variation in the concentration and activity of secondary metabolites within a given EO source.

In summary, the tested EOs manipulated the rumen fermentation characteristics but to varying extents. Differences in the properties and chemical structures of EOs might underlie these discrepancies. All tested EOs, except *P. graveolens*, efficiently diminished gas production with a similar potency to monensin. Furthermore, they exceed the monensin in their ability to reduce the ammonia nitrogen concentration and protozoa count without adversely affecting volatile fatty acid levels. But, they were less effective than monensin in modifying ruminal volatile fatty acid profile especially propionate and acetate to propionate ratio. Hence, *P. guajava*, *C. nardus*, and *C. citratus* EOs could be a safe and promising rumen manipulator.

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تقييم تأثير أربعة أنواع من الزيوت العطرية كبدائل محتملة للمونينسين على خصائص التخمر ومعدل تحلل العناصر الغذائية فى الكرش

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أجريت هذة الدراسة بهدف تقييم تأثير أربعة من الزيوت العطرية المستخلصة من أوراق نباتات الجوافة (*Psidium guajava*)، والسترونيلا (*Cymbopogon nardus*)، والليمون (*Cymbopogon citratus*)، والعطر (*Pelargonium graveolens*) كبديل طبيعي للمضاد الحيوى مونينسين. تم إختبار أربعة تركيزات مختلفة من الزيوت العطرية (0، 15، 30، و 45 ميكروليتر لكل 45 ملل من سائل الكرش) ومقارنة تأثيرها مع المونينسين على خصائص التخمر فى الكرش وإنتاج الغاز معمليا . أظهرت النتائج حدوث إنخفاض معنوى فى إنتاج الغاز ومعدل تحلل المادة الجافة الحقيقى وزيادة معنوية فى إنتاج حمض البروبيونيك مع إضافة المونينسين مقارنة بمجموعة الكنترول (بدون أى إضافات). حدث إنخفاض معنوى فى إنتاج الغاز بزيادة تركيز الزيوت العطرية المختبره ما عدا زيت العطر. إنخفض معدل تحلل المادة الجافة الحقيقى معنوياً مع إضافة زيت الليمون بتركيز 45 ميكروليتر وزيت العطر بتركيز 30 و 45 ميكروليتر. لم تحدث إضافة الزيوت العطرية أى إختلافات معنوية فى تركيز نيتروجين الأمونيا ما عدا زيت الليمون والسترونيلا . بشكل عام فقد أدت التركيزات المرتفعة من كل الزيوت العطرية إلى إنخفاض معنوى فى أعداد البروتوزوا. أدى إستخدام زيت الليمون بتركيز 45 ميكروليتر وزيت الجوافة بتركيز 30 و 45 ميكروليتر إلى زيادة معنوية فى تركيز حمض الخليك. أيضاً فقد زادت نسبة حمض الخليك إلى حمض البروبيونيك معنوياً مع تركيز 30 ميكروليتر من زيت العطر. وقد خلصت نتائج الدراسة الحالية إلى أن إستخدام الزيوت العطرية المختبرة بإستثناء زيت العطر قد أدى إلى إنخفاض إنتاج الغاز بكفاءة مماثلة للمونينسين كما أنها كانت أكثر فاعلية من المونينسين في قدرتها على تقليل تركيز نيتروجين الأمونيا وعدد البروتوزوا دون التأثير سلباً على إنتاج الأحماض الدهنية الطيارة. ولكن، كانت أقل فعالية من المونينسين فى التأثير على مكونات الأحماض الدهنية الطيارة فى الكرش وخاصة حمض البروبيونيك ونسبة حمض الخليك الى حمض البروبيونيك. ومن ثم، يمكن إستخدام الزيوت العطرية لأوراق نباتات الجوافة والليمون والسترونيلا كبدائل آمنة للمضادات الحيوية فى تغذية المجترات.