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EFFECT OF THYMOL AND CINNAMALDEHYDE ACTIVE COMPONENTS OF ESSENTIAL OILS ON RUMEN MICROBIAL FERMENTATION USING *In vitro* TECHNIQUE

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ABSTRACT

This study aimed to evaluate the effect of different supplementations of pure thymol (THY) and cinnamaldehyde (CIN) or their mixture (MIX) by equal proportion using batch culture system with diluted ruminal fluid containing 50:50; concentrate: roughage diet. Ruminal fermentation profile including total gas production, methane emission, total and individual volatile fatty acids (TVFAs) and pH value were determined. Treatments were: control (no additive), 30, 50, 100 and 200 mg/L buffered rumen fluid. Total gas production was recorded after 6, 12, 36 and 48 hr of incubation, while methane emission was recorded after 12 hr and the rumen pH values were determined at 48 hr. The obtained results showed that the different doses of THY, CIN and MIX reduced ($P < 0.01$) cumulative gas production and methane emission. CIN addition at 100 and 200 mg/L resulted in superior reduction in total gas production and methane emission. Concentration-dependent increase of pH values were recorded with all treatments. Different doses of both THY and CIN or their MIX had no effect on TVFA concentration, while rumen molar proportion of acetate and acetate: propionate ratio was decreased with high doses of all treatments. The lowest value of acetate propionate ratio was recorded with CIN and MIX supplementation. Conclusively, THY, CIN and their MIX usage in low to moderated doses have a potential to reduce rumen methanogenesis in ruminant diets without affecting rumen fermentation adversely.

Key words: Cinnamaldehyde, thymol, methanogenesis, rumen fermentation.

INTRODUCTION

In recent years, ruminant nutritionists have dedicated their research to modify ruminal fermentation in the way that enhancing feed utilization efficiency and productivity, while diminishing the methane (CH_4) emissions (martin *et al.*, 2010).

Methane is a potent green house gas with high global warming potential (Abd El-Rahman, 2012). Enteric fermentation from domestic ruminants shares with approximately 15% of global CH_4 emissions (IPCC, 2007). In Egypt, agricultural CH_4 emissions increased from 10.48 (in 1990) to 13.29 Tera grams in 2010 which represent 26.1% of the total CH_4 emissions (WDI and GDF, 2013).

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In this regard, the well documented antimicrobial activity of essential oils (EO) and their active components beside their safety for human consumption has prompted a number of scientists to examine the potential of these secondary metabolites to manipulate rumen microbial fermentation to improve production efficiency in ruminants and mitigate CH_4 emissions to environment (FDA, 2004 and Benchaar *et al.*, 2008).

Among EO, cinnamaldehyde (CIN), the main active compound of cinnamon (*Cinnamomum verum*) essential oil (Màthé, 2009) and thymol (THY), one of the major compounds of thyme (*Thymus vulgaris*) (Benchaar and Greathead, 2011), have prompted great interest because of their marked antimicrobial activity against rumen microbes (Chaves *et al.*, 2011).

Despite the neumerous *in vitro* (Castillejos *et al.,* 2006; Martinez *et al.,* 2006; Fraser *et al.,* 2007) and *in vivo* studies (Cardozo *et al.,* 2006; Benchaar *et al.,* 2006 a;b and Yang *et al.,* 2010 a,b) concerning with THY and CIN effects on ruminal fermentations and methanogenesis process, three main issues are still exiting. Firstly, most results of these studies were contradictory. Secondly, little knowledge is available on the effect of their MIX on ruminal microbial ecosystem. Thirdly, most doses used are too high (*i.e.,* >300 mg/L of culture fluid) to be achieved *in vivo* and are impractical at feeding because of its probable detrimental effects on rumen fermentation and palatability.

Therefore, the objective of this trial was to test the effects of two purified secondary metabolites THY, CIN and their MIX at four different concentrations on the ruminal microbial fermentation profile in 50:50 C:R diet using *in vitro* batch culture fermentation system.

MATERIALS AND METHODS

In vitro Batch Fermenters and Experimental Design

Rumen content was obtained from two fistulated sheep (34.85 kg ± 1.35) before feeding. The animals were fed tall fescue hay and concentrates mixture (1:1) and chemical composition of the concentrate and hay is presented in Table 1. The rumen fluid was transported in a prewarmed thermos and strained through four layers of cheese cloth to obtain ruminal fluid inoculums free from feed particles. The filtered ruminal fluid was centrifuged at 650×g for 5 minutes then the supernatant was mixed with 1:4 with phosphate bicarbonate buffer (McDougall, 1948), maintained at 39°C and flushed with CO_2 saturated gas for 15 minutes. Forty millimeters of buffered ruminal fluid were placed in glass tubes, containing 400 mg of the same diet fed to sheep but previously ground to pass *via* 1mm screen, quickly closed with gas release rubber stopper fitted as described by Tilley and Terry (1963) using tree way valve connected with calibrated plastic syringe to collect produced gas. Treatments were, control without addition, CIN (C_9H_8O, purity ≥ 98%, Flaka chemical, Switzerland), THY ($C_{10}H_{14}O$, purity of 99%, Oxford laboratory Company Mumbai, India) and MIX (50:50). Four different doses were tested for each group: 30, 50, 100 and 200 mg/L of the total culture fluid. Compounds were tested in triplicate at each dose. All compounds were dissolved in ethanol and a total of 0.2 ml was added to the culture fluid. Tubes containing treatments and control were incubated into horizontal shaking water bath 48 hr at 39°C.

Chemical Analysis

By the end of the incubation period (48 hr), the final pH of the batch was measured with a pH meter (model F-51, Horiba, Kyoto, Japan) and samples were collected and kept at - 20°C for VFA determination. Total gas production was recorded from visual assessment of calibrated scale on the plastic syringes at 6, 12, 36, 48 hr of incubation and calculated by deducting gas produced from blank tube (without substrate). Methane was determined after 12 hr of incubation by closing the tree way valve and get the syringe for analysis. Gas chromatography model GC-2014 (Shimadzu, Kyoto, Japan) equipped with a Porapak-QS column (50-80, 2 m) and thermal conductivity detector (TCD) was used for gas determination. Temperature of column and detector were 70 and 100 °C, respectively. Carrying gas (Helium) flow was adjusted to 32 ml/min. Based on the CH_4 percentage estimated in the gas produced, CH_4 production in mL was calculated in each sample [CH_4 volume (mL/ g DM) = CH_4 % × total gas produced (mL) /sample weight on dry matter basis] according to Natel *et al.* (2012) .

Total and individual VFA were determined by GC (model GC-14B, Shimadzu, Kyoto, Japan) with crotonic acid as internal standard (Ottenstein and Bartley, 1971). Feed samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE) and ash according to (AOAC, 2000). Neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash (aNDFom) was analyzed according to Van Soest *et al.* (1991).

Statistical Analysis

Obtained data were analyzed using the MIXED procedure of SAS (1998). The mathematical model was $Y_{ijk} = \mu + T_i + C_j + TC_{ij} + e_{ijk}$, where μ= the overall mean, T_i = the fixed effect of treatment, C_j=the fixed effect of concentration, TC_{ij}= the fixed effect of interaction between treatment and concentration and e_{ijk} = residual error.

Table 1. Chemical composition (% on DM basis) of the forage and the concentrate

	Concentrate *	Tall fescue hay
Crude protein	14.03	8.30
Ether extract	4.18	0.88
aNDFom**	22.81	73.53
ADFom	7.28	43.77
Ash	7.19	7.68

* Composition (g/kg): wheat bran 140, rice bran 90, soybean meal 50, crushed barley 380, crushed maize 320, calcium 10, salt 10 and vitamins mixture 2.

** Neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash

RESULTS AND DISCUSSION

Total Gas Production

It is well known that gas production is the sum of direct gas produced as a result of fermentation (CO_2 and methane) and the indirect gas produced from buffering of VFA (CO_2) released from bicarbonate buffer (Blummel and Ørskov, 1993). In the current experiment, the different values of the total gas production during different periods of incubations (6, 12, 24, 36 and 48 hr) were decreased ($P<0.01$) with THY, CIN or MIX addition (Table 2). Lower values were recorded with CIN at all times in comparison with other groups ($P< 0.01$). Also, gas production level was decreased ($P < 0.01$) as a result of increasing the concentration from 50 to 200 mg/L at all duration incubation time (Table 2). Also, the effect of the interaction between EO source and its concentration showed significant ($P<0.05$) lower cumulative gas production than the control group, especially at the highest levels (100 and 200 mg/L) (Table 3). A similar effect was recorded in other studies but with different percentages. For instance, Kamalak *et al.* (2011) found that addition of 200 mg/L THY decreased total gas production by 22.77%. In the contrast, Benchaar *et al.* (2007) reported only 4% reduction with the same concentration. Both Doane *et al.* (1997) and Kamalak *et al.* (2011) attributed the reduction of total gas production to the depressed levels of total VFA in their study. But in the current study, no significant reduction of total VFA was observed so we hypothesized that the reduction of fermentation gas observed in the present trial could be related to the concentration of fermentation gases rather than to VFA. In the same line, Macheboeuf *et al.* (2008) showed in *in vitro* study that, supplying 264 mg of CIN /L was associated with a decrease of CH_4 production by 13% without altering VFA and acetate or propionate production, suggesting that, at this dose, it inhibited rumen methanogenesis by acting directly against rumen methanogens.

Methane Emission

Reducing enteric CH_4 emissions from ruminants is beneficial from the nutritional side of view (*i.e.,* improve feed efficiency and animal productivity) beside environmentally reduce contribution of agricultural sector to total GHG emissions perspective. A few reports have specifically demonstrated that THY and CIN impacts on rumen methanogenesis (Benchaar and Greathead, 2011).

In the present trial, the concentrations of CH_4 ($P < 0.01$) were significantly lowered with THY, CIN and MIX supplementation compared with the control group as presented in Table 4 expressed as percentage and mL/g DM. The maximum reduction of CH_4 production was observed with CIN group (70.69%) followed by MIX group (62.50%) and THY group (41.38%). In the same trend, a significant ($P < 0.01$) decrease was observed with increasing the concentration from 30 to 200 mg/L (Table 4). The highest reduction in methane production was clearly detected with high levels (83.19 and 100% with the concentrations of 100 and 200 mg/L respectively). The interaction effect between EO sources and their concentrations showed that CIN and MIX had a strong effect on reducing CH_4 emissions, especially at the high levels (100 and 200 mg/L) compared with control (Table 5).

Table 2. Effect of cinnamaldehyde (CIN), thymol (THY) or their mixture (MIX) and its concentrations on *in vitro* gas production ($\overline{X}$ ± SE)

Item	Gas production (mL/g DM)				
	6 hr	12 hr	24 hr	36 hr	48 hr
Effect of treatments					
Control	22.87±1.23 ^{a}	50.39±1.40 ^{a}	86.79±2.14 ^{a}	104.51±2.33 ^{a}	117.11±3.06 ^{a}
CIN	15.98±0.97 ^{c}	36.16±2.52 ^{c}	62.06±4.48 ^{c}	72.56±6.61 ^{c}	79.90±8.44 ^{c}
THY	18.90±0.97 ^{b}	43.10±2.64 ^{b}	70.87±4.40 ^{b}	80.78±6.11 ^{b}	88.48±7.49 ^{b}
MIX	17.73±0.74 ^{bc}	42.34±2.63 ^{b}	68.47±4.73 ^{b}	81.77±6.80 ^{b}	90.52±8.14 ^{b}
Significance	**	**	**	**	**
Effect of concentrations (mg/L)					
0	22.87±1.23 ^{a}	50.39±1.40 ^{a}	86.79±2.14 ^{a}	104.51±2.33 ^{a}	117.11±3.06 ^{a}
30	20.69±0.73 ^{ab}	50.08±1.38 ^{a}	83.68±1.79 ^{ab}	100.94±1.99 ^{ab}	113.69±1.74 ^{ab}
50	18.51±0.65 ^{bc}	46.19±1.36 ^{a}	77.92±1.62 ^{b}	95.96±1.34 ^{b}	108.25±1.34 ^{b}
100	16.18±1.21 ^{cd}	35.85±2.36 ^{b}	58.40±3.49 ^{c}	65.56±4.28 ^{c}	71.62±5.37 ^{c}
200	14.78±0.41 ^{d}	30.02±0.78 ^{c}	48.52±0.81 ^{d}	51.01±1.02 ^{d}	51.63±0.95 ^{d}
Significance	**	**	**	**	**

Means within columns with unlike superscript differ significantly (P<0.01).

Table 3. Effect of the interaction between cinnamaldehyde (CIN), thymol (THY) or their mixture (MIX) and its concentrations on *in vitro* gas production ($\overline{X}$ ± SE)

Item	Concentration	Gas production (mL/g DM)				
	(mg/L)	6 hr	12 hr	24 hr	36 hr	48 hr
CIN	**30**	18.67±1.68 ^{bcde}	45.72±2.03 ^{cde}	79.3±3.99 ^{bc}	96.58±3.52 ^{cd}	111.05±3.27 ^{bcd}
	50	17.73±1.23 ^{cdef}	41.99±2.14 ^{e}	72.79±2.14 ^{cd}	91.46±0.93 ^{d}	104.05±1.68 ^{d}
	100	12.60±2.14 ^{h}	28.00±2.91 ^{g}	47.12±4.15 ^{f}	50.39±4.28 ^{g}	51.79±3.70 ^{f}
	200	14.93±0.47 ^{fgh}	28.93±0.47 ^{g}	48.99±0.00 ^{f}	51.79±0.00 ^{g}	52.72±0.47 ^{f}
THY	**30**	21.93±0.47 ^{ab}	50.86±1.23 ^{ab}	83.99±1.40 ^{ab}	98.91±1.23 ^{bc}	111.05±1.68 ^{bcd}
	50	20.07±1.23 ^{abc}	49.46±0.47 ^{abc}	82.12±0.47 ^{ab}	97.51±1.23 ^{cd}	108.71±0.47 ^{cd}
	100	19.60±0.81 ^{abcd}	43.16±0.23 ^{de}	69.76±0.84 ^{d}	77.69±0.40 ^{e}	84.69±1.21 ^{e}
	200	14.00±0.81 ^{gh}	28.93±0.93 ^{g}	47.59±1.62 ^{f}	48.99±1.62 ^{g}	49.46±1.23 ^{f}
MIX	**30**	21.47±0.47 ^{ab}	53.66±0.93 ^{a}	87.72±1.68 ^{a}	107.31±1.23 ^{a}	118.98±1.40 ^{a}
	50	17.73±0.47 ^{cdef}	47.12±1.68 ^{bcd}	78.86±2.03 ^{bc}	98.91±1.87 ^{bc}	111.98±1.62 ^{abc}
	100	16.33±0.47 ^{defg}	36.39±0.80 ^{f}	58.32±0.93 ^{e}	68.59±2.91 ^{f}	78.39±5.05 ^{e}
	200	15.40±0.81 ^{efgh}	32.19±1.61 ^{fg}	48.99±2.14 ^{f}	52.26±2.60 ^{g}	52.72±2.33 ^{f}
Control	**0**	22.87±1.23 ^{a}	50.39±1.40 ^{abc}	86.79±2.14 ^{a}	104.51±2.33 ^{ab}	117.1±3.06 ^{ab}
Significance		*	**	**	**	**

Means within columns with unlike superscript differ significantly.

Significant level: * p<0.05; ** p<0.01.

Table 4. Effect of cinnamaldehyde (CIN), thymol (THY) or their mixture (MIX) and its concentrations on methane emissions ($\overline{X}$ ± SE)

Item	CH_4		CH_4 reduction potential (%)
	(mL/g DM)	(%)	
Effect of treatments			
Control	2.32±0.32 [a]	4.57±0.50 [a]	-
CIN	0.68±0.2 [c]	1.53±0.51 [c]	70.69
THY	1.36±0.27 [b]	2.80±0.54 [b]	41.38
MIX	0.87±0.26 [c]	1.72±0.51 [c]	62.50
Significance	**	**	-
Effect of concentrations			
0 mg/L	2.32±0.32 [a]	4.57±0.50 [a]	-
30 mg/L	1.99 ±0.12 [a]	3.97±0.20 [a]	14.22
50 mg/L	1.50±0.17 [b]	3.19±0.32 [b]	35.34
100 mg/L	0.39±0.19 [c]	0.90±0.43 [c]	83.19
200 mg/L	ND [c]	ND [d]	100
Significance	**	**	-

Means within columns with unlike superscript differ significantly.

Significant level: ** $p<0.01$. ND= Not detectable.

Table 5. Effect of the interaction between treatments and its concentrations on methane emissions

Treatment	Concentration	CH_4		CH_4 reduction potential (%)
	(mg/L)	(mL/g DM)	(%)	
CIN	30	1.75±0.23 [bc]	3.80±0.40 [ab]	24.57
	50	0.99±0.27 [f]	2.30±0.56 [d]	57.33
	100	ND [g]	ND [e]	100
	200	ND [g]	ND [e]	100
THY	30	2.32±0.08 [a]	4.57±0.15 [a]	0
	50	1.99±0.15 [ab]	4.03±0.33 [ab]	14.22
	100	1.12±0.09 [df]	2.60±0.21 [cd]	51.72
	200	ND [g]	ND [e]	100
MIX	30	1.90±0.10 [abc]	3.53±0.12 [b]	18.10
	50	1.52±0.07 [cd]	3.23±0.15 [bc]	34.48
	100	0.04±0.04 [g]	0.10±0.10 [e]	98.28
	200	ND [g]	ND [e]	100
Control	0	2.32±0.32 [a]	4.57±0.50 [a]	-
Significance		**	**	-

Means within columns with unlike superscript differ significantly.

Significant level: ** $p<0.01$. ND= Not detectable.

These findings were in accordance with previous *in vitro* observations of Macheboeuf *et al.* (2008) and Benchaar and Greathead (2011) in a 24 hr batch culture who confirmed high doses of THY (470 and 300 mg/kg, respectively) were required to inhibit CH_4 production. In contrast, no change in methane concentration was reported with THY (50, 100, 200 mg/L of culture fluid) or CIN (132 mg/L) in 24 hr batch cultures (Evans and Martin, 2000 and Macheboeuf *et al.*, 2008).

Noteworthy, Calsamiglia *et al.* (2007) previously demonstrated that antagonistic, additive or synergistic effects could be resulted at combination of the active components of essential oils. Interestingly in the current experiment, a synergistic anti-methanogenic activity observed at combination of THY and cinnamaldehyde. The same trend was previously reported but with other combination. Burt (2004) has reported more pronounced anti-methanogenic activity at combination between CIN and eugenol. We suggested that this activity might mediate through the inhibition of methane-producing microorganisms.

Ruminal Volatile Fatty Acids and pH Values

It is well known that the VFA production in the rumen is the main source of energy for ruminant animals and so the decrease in VFA production in rumen may yield adverse nutritional consequences (Benchaar *et al.*, 2007). In the current experiment, neither total ruminal VFA concentration nor the proportion of propionate were affected by THY, CIN and MIX addition. While, the molar proportion of acetate was significantly ($P<0.01$) decreased (Table 6). A significant ($P<0.05$) decrease was observed in the acetate propionate ratio (C_2:C_3) as affected by CIN and MIX addition. The obtained values of the TVFAs, acetate and propionate showed a significant ($P<0.01$) decrease related to increasing the concentration of tested compounds, whereas C_2:C_3 ratio was not significantly affected (Table 6). However, the interaction effect between THY, CIN or MIX and concentration showed no significant effect (Table 7).

Although the lack effect on TVFAs in the present trial agrees with Newbold *et al.* (2004) who reported no change in total VFA concentrations *in vitro* in rumen fluid removed from sheep fed on specific blend of essential oil compounds containing THY as major component. Similarly, Cardozo *et al.* (2004) suggested that the effects of cinnamon oil (0.22 mg/L of rumen fluid) in a continuous culture experiment on VFA concentration were negligible. However, Busquet *et al.* (2006) recorded notable reduction of total VFA in an *in vitro* batch culture supplied with CIN, but this may be attributed to the high doses used in this study. Notably, the lack effect of plant extracts on total VFA indicates that the doses used were not toxic to ruminal microbes (Cardozo *et al.*, 2004). Unlike total VFA and propionate, the proportion of acetate was decreased with CIN and MIX addition at all concentrations. Similar pattern of individual volatile fatty acid were reported in studies of Evans and Martin (2000); Cardozo *et al.* (2004) and Castillejos *et al.* (2008).

A notable dose dependent increase in the ruminal pH values were observed with THY, CIN and their MIX (Tables 6 and 7). In agreement with our finding, Evans and Martin (2000) and Castillejos *et al.* (2006) observed higher pH values at incubation of ruminal fluid in 24 hr *in vitro* batch cultures with 400 mg/L and 500 mg/L of THY respectively. Also, Kamalak *et al.* (2011) found that the addition of THY at 200 mg/L increased final pH value of *in vitro* batch culture after 96 hr incubation times. This may be related to the slight reduction of TVFA concentrations recorded in the current study. Allen (1997) had previously confirmed the negative relationship between TVFA concentrations and pH value in the rumen. In contrast, Castillejos *et al.* (2008) tested different doses of THY (5, 50 and 500 mg/L) in a batch culture fermentation experiment with 10:90 forage to concentrate diet, they recorded that there was a decrease of pH values at all used doses. In the later studies, decrease in VFA concentration was recorded which might explain the decrease in pH value.

In the current experiment, both THY and CIN were shown to have antimicrobial activities *in vitro*. This result is in agreement with the findings of Benchaar *et al.* (2007); Dorman and Deans (2000); Ultee *et al.* (2002) and Burt (2004), who found that phenolic compounds such as THY had a high microbial activity due

Table 6. Effect of cinnamaldehyde (CIN), thymol (THY) and their mixture (MIX) addition on ruminal volatile fatty acid profile and pH values ($\overline{X}$ ± SE)

	pH	Acetate (mM)	Propionate (mM)	TVFA (mM)	C_2:C_3 (mol : mol)
Effect of treatments					
Control	6.80±0.01^{c}	67.1±7.0^{a}	22.4±3.7	94.8±9.1	3.10±0.45^{a}
CIN	6.91±0.03^{b}	37.2±3.1^{b}	23.0±2.4	67.6±6.5	1.79±0.26^{b}
THY	6.92±0.03^{ab}	43.0±4.0^{b}	19.0±1.8	73.1±3.7	2.37±0.21^{ab}
MIX	6.95±0.02^{a}	35.3±2.6^{b}	24.0±1.6	67.9±3.1	1.51±0.12^{b}
Significance	**	*	NS	NS	*
Effect of concentrations					
0 mg/L	6.80±0.01^{d}	67.1±7.0^{a}	22.4±3.7^{abc}	94.8±9.1^{a}	3.10±0.45
30 mg/L	6.83±0.01^{cd}	51.7±3.0^{b}	28.5±1.8^{a}	87.9±4.1^{ab}	1.86±0.15
50 mg/L	6.84±0.02^{c}	41.0±2.5^{bc}	24.3±1.6^{ab}	76.2±2.6^{b}	1.77±0.18
100 mg/L	6.99±0.01^{b}	35.2±2.5^{cd}	19.3±1.5^{bc}	59.8±2.2^{c}	1.99±0.30
200 mg/L	7.04±0.01^{a}	26.2±1.1^{d}	16.0±1.9^{c}	54.3±2.5^{c}	1.94±0.37
Significance	**	**	**	**	NS

Means within columns with unlike superscript differ significantly.

* p<0.05; ** p<0.01. NS = Not signifiant.

Table 7. Effect of the interaction between cinnamaldehyde (CIN), thymol (THY) or their mixture (MIX) and its concentrations on volatile fatty acid profile and pH values ($\overline{X}$ ± SE)

Treatment	Conc. (mg/L)	pH	Acetate (mM)	Propionate (mM)	TVFA (mM)	C_2:C_3 (mol : mol)
CIN	**30**	6.82±0.02^{e}	48.3±4.3	32.2±3.2	92.4±9.1	1.50±0.04
	50	6.81±0.01^{e}	44.4±3.1	26.0±2.3	80.6±6.0	1.73±0.17
	100	6.96±0.02^{cd}	30.6±1.9	19.9±1.0	52.8±3.0	1.53±0.03
	200	7.05±0.00^{a}	25.6±2.2	14.1±3.8	44.7±1.2	2.40±1.09
THY	**30**	6.83±0.02^{e}	61.5±2.9	25.9±1.8	90.8±5.0	2.38±0.11
	50	6.80±0.01^{e}	42.3±3.3	18.9±0.8	75.1±1.6	2.26±0.26
	100	6.98±0.01^{bc}	41.9±5.2	18.0±4.5	65.9±2.7	2.70±0.77
	200	7.06±0.01^{a}	26.4±1.7	13.3±2.0	60.5±1.0	2.12±0.41
MIX	**30**	6.84±0.01^{e}	45.3±2.0	27.5±3.7	80.4±4.3	1.70±0.24
	50	6.92±0.02^{d}	36.3±6.2	27.9±1.7	72.9±5.0	1.32±0.27
	100	7.02±0.02^{ab}	33.3±03.1	19.9±2.2	60.6±0.3	1.73±0.26
	200	7.01±0.01^{ab}	26.5±2.5	20.8±2.9	57.8±0.1	1.30±0.12
Control	**0**	6.80±0.01^{e}	67.1±7.0	22.4±3.7	94.8±9.1	3.10±0.45
Significance		**	NS	NS	NS	NS

Means within columns with unlike superscript differ significantly. * p<0.05; ** p<0.01.

Sig. = Signifiant level, NS = Not signifiant. Conc. = concentration.

to the presence of a hydroxyl group in the phenolic structure. Burt (2004) suggested that the antimicrobial activity of these phenol compounds is through the disturbance of cytoplasmic membrane, disrupting the proton motive force, electron flow active transport and coagulation of cell contents. However, unlike CIN does not have a hydroxyl or acid group to act as a proton carrier to disrupt the outer membrane or deplete the intracellular ATP pool (Helander *et al.,* 1998). Alternatively, the antimicrobial properties of CIN are thought to arise through its carbonyl group binding and inactivating microbial enzymes (Burt, 2004).

Overall, our results concluded that using low to moderated doses of THY, CIN and their mixture with medium concentrate diets (dairy diets) did not affect rumen fermentation adversely but have good anti-methanogenic activity which would encourage its potential use in ruminant diets. Further research is required to determine the effect of their addition on voluntary feed intake, digestibility, animal performance and the profitability of the supplementation *in vivo*.

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تأثير الثيمول والسينمالدهيد كمركبات فعالة فى الزيوت العطرية على تخمرات الكرش الميكروبية معملياً

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أجريت هذه الدراسة لإختبار تأثير إضافة إثنين من المركبات الفعالة فى الزيوت العطرية وهم الثيمول والسينمالدهيد ومخلوطهما (١:١) بإستخدام سائل كرش مخفف فى مخمر معملى (ذو الدفعات) ومخلوط علفى يحتوى على نسبة ٥٠:٥٠ (علف مركز :خشن) وذلك على تخمرات الكرش والتى تشمل الأس الهيدروجينى، الأحماض الدهنية الطيارة الكلية ومكونتها (الأسيتات والبروبيونات)، الإنتاج الكلى للغازات وإنتاج الميثان. تم تسجيل إنتاج الغاز بعد ٦، ١٢، ٢٤، ٣٦ و ٤٨ ساعة من التحضين بينما تم قياس الميثان بعد ١٢ ساعة والأس الهيدروجينى والأحماض الدهنية الطيارة بعد ٤٨ ساعة، أوضحت النتائج أن الجرعات المختلفة من الثيمول والسينمالدهيد ومخلوطهما أدت إلى إنخفاض معنوى فى إنتاج الغاز وإنبعاثات غاز الميثان، أدت إضافة السينمالدهيد بمستويات ١٠٠ و٢٠٠ ملجم/لتر من بيئة التحضين إلى إنخفاض حاد فى إنتاج الغاز وإنبعاثات الميثان، كما أدت زيادة تركيزات المواد المختبرة الى زيادة فى قيم الأس الهيدروجينى، لم تؤثر التركيزات المختلفة للثيمول والسينمالدهيد أو مخلوطهما على تركيز الأحماض الدهنية الطيارة بينما إنخفض معنوياً تركيز الأسيتات ونسبة الأسيتات للبروبيونات خاصة مع التركيزات المرتفعة للمركبات المختبرة، ويستخلص من النتائج المتحصل عليها أن إضافة جرعات منخفضة أو متوسطة من الثيمول أو السينمالدهيد أو مخلوطهما فى علائق المجترات له تأثير فعال فى خفض إنتاج الميثان بدون تأثيرات سلبية على تخمرات الكرش.

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