

EFFECT OF ADDING TWO LEVELS OF OIL CINNAMON (*CINNAMOMUM ZEYLANICUM*) AND GARLIC (*ALLIUM SATIVUM*) IN TOTAL GAS, METHANE, DRY MATTER AND ORGANIC MATTER *IN VITRO* DIGESTIBILITY

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Abstract

Experiment was performed to determine the effect addition two herbal plants oil. The treatments were: 1- control: (80:20) concentrate: roughage without additives, 2- control diet + cinnamon oil either 300 or 600 mg/kg DM, 3- control diet + garlic oil either 300 or 600 mg/kg DM. The results was showed decrease ($p<0.05$) total gas for cinnamon compared to garlic oil by 30.66, 58.50 and respectively and decreases methane production was lower ($p<0.01$) for cinnamon oil compared to garlic oil with 300 and 600 mg/kg DM have reached 6.00 and 14.66, 2.00 and 8.33 ml/200 mg DM after 24 h incubation respectively compared with control treatment. While, organic matter digestibility increased significantly ($P<0.05$) reached to (69.16 and 72.71%) by cinnamon and garlic oil respectively, include the level 300mg. These results showed that essential oils such as garlic and cinnamon reduced the total methane gas emissions, net energy at the level of 300 and 600mg/kg DM and had no negative impact on the rumen environment.

Keywords: Oil Garlic ,Oil cinnamon, methane gas production, *in vitro* digestibility

1. Introduction

Currently, it has been directed nutrition researchers to some additives in ruminant diets such as aqueous extracts, oils of some medicinal plants or enzymes (Molho-Ortiza et al. 2022; Mustafa, et al. 2022) to reduce the production greenhouse gases such as CO₂ and CH₃, to which ruminants contribute with 39% of the total gases (Gerber, et al 2013) The methane gas heat was produced raising the degree of Global warming estimated to be about 21-28 times more than carbon dioxide (IPCC,2014). Ruminants lose about 12% of the feed intake energy as methane emission (Kobayashi 2010). Also observed through studies essential oils which was found in medicinal plants an effective role in improving the rumen environment and animal performance (Cobellis et al., 2016). Ribeiro-Santos et al., (2017) indicated the most affective substances for cinnamon are cinnamaldehyde and polyphenols, while, Garlic is rich in sulfur compounds 84.3–98.9% (Dziri, et.al., 2014). For that, we did a study to evaluate the effect of cinnamon oil (*Cinnamomum zilanicum*) and garlic oil (*Allium sativum*) on methane (CH₃) production and *in vitro* digestibility of dry matter and organic matter.

Materials and Methods 2.

Work done in the nutrition lab , department of animal production /college of agricultural engineering science/ university of Baghdad , to show the results of adding the (cinnamon and

garlic) oil with two levels (300 and 600)mg/kg DM into the ration (80 : 20). Table 1. The ingredients of concentrate % (Barley grain, wheat bran, yellow corn, soybean meal, salts and lime) and roughage (alfalfa hay). Table 2. The chemical analysis of concentrate and roughage (on DM basis %)

Table 1. Formulation of the concentrate diet(%)

Ingredients	Barley grain	Wheat bran	Yellow corn	Soybean meal	Salts and lime
%	46	32	10	10	2

Table 2. Chemical composition of concentrate diet and roughages (on DM basis %)

Factors	DM %	OM	CP	EE	CF	NFE	Ash	ME*(MJ/Kg DM)
Concentrate: Roughage(80:20)								
	93.06	95.78	14.12	2.57	12.19	66.90	4.22	12.46

ME = 0.012 [C.P] + 0.005 [C.F] + 0.031 [E.E] + 0.014 [NFE] (MAFF,1975)

2.1. Methane emission

Estimation of total gas and methane according to by Menke and Steingass, (1988), 0.2 g of diet were taken and placed inside a 100 ml glass syringe with the addition of 10 ml of rumen fluid and 20 ml of artificial saliva with the addition of CO₂ gas, it was incubated in a bath water at 39 ° C for 24 hours, 4 samples were taken for each stage of incubation, then injections were withdrawn after end of period gas production incubation. 4 ml of NaOH concentration of 4% is added to 2 glass syringe to calculate the production of methane according to (Fievez et al 2005). After estimating the total gas within 24 hours, some parameters were estimated using the equations mentioned below

$$ME(MJ/kg \text{ DM}) = 1.06 + 0.157GV + 0.084CP + 0.22CF - 0.081 A(\text{Ash})$$

$$IVOMD (\%) = 14.88 + 0.889 GV + 0.45CP + 0.651 x A(\text{ASH})$$

$$SCFA (\text{m mol}/100\text{ml}) = 0.0239 GV - 0.061 (\text{Menke and Steingass}1988)$$

$$NEL (MJ/Kg \text{ DM}) = 0.096 x GV + 0.0038 x CP + 0.000173 x EE^2 + 0.54(\text{Getachew ,et.al., 1999})$$

2.2 In vitro apparent digestibility

In vitro digestibility determination according to Terry and Tilley (1963). The first solution NaHCO₃(49 gm) + Na₂HPO₄(18.6 gm) dissolved in 800 ml distilled water ,second Solution KCl (28.5) gm + NaCl (23.5) gm+ MgCl₂.7H₂O(6) gm+ CaCl₂(2) gm dissolved in 1 liter of distilled water then take 100 ml of solution (II) and add to the solution (I) then complete the volume to one liter. putting 0.5 gm of ration and then adding 40 ml of saliva and 10 ml of rumen fluid. Secondly, carbon dioxide (CO₂) was injected into each tube to get rid of oxygen gas, incubate the tube in a water bath at 39 ° C for 24 hr. In addition of a blank tube without sample to estimate the DM and the OM. Deposit was taken and dried at 105 ° C for overnight to measure the digestibility of DM and then they burned at 550 ° C for 3 hr. to calculate the digestibility of OM .

3. Chemical analysis : All chemical analyses were conducted according to (AOAC, 2010).

4. Statistical analysis

Statistical Analysis Using Complete Random Design (SAS,2012). The means were compared using Duncan's multiple range test (Duncan, 1955).

$$Y_{ijkl} = \mu + R_i + E_j + e_{ijkl}$$

Where: Y_{ijk} = the response; μ = the overall mean

R_i = Source of oil ($i=1$ and 2); E_j = levels of oil ($j=1$ and 2); e_{ijkl} = the experimental error ijk

5. Results

.Total gas and methane production1.

Table3. Refers that total gas was decrease ($p<0.05$) for cinnamon compared to garlic oil and control by 30.66 and 58.50 and 55.33 ml /200 mg DM at 24 hr. incubation respectively. While it was observed that methane decreased with cinnamon oil for levels 300 and 600 ml /200 mg DM compared with garlic oil .It was 6.00, 14.66, 2.00 and 8.53 ml/200mg DM 24 h incubation respectively .

5.2 Metabolizable energy MJ/kg DM, and Net energy /MJ at 24 h incubation

Results (table 4) were pointed no significant differences for cinnamon and garlic oil to both dose 300and 600mg/kg DM for metabolizable energy MJ/kg DM, and Net energy /MJ at 24 h incubation.

5. 3 Short chain fatty acid mmol/100ml, and estimation in vitro organic matter digestibility

Table 5 illustrated that no significant differences in Short chain fatty acid (mmol/100ml) between treatments compared with control . While increase ($P<0.05$) for organic matter digestibility. It reached to (69.16 and 72.71%) for cinnamon and garlic oil respectively, when addition for the level 300mg/ kg DM.

5.4 .Dry and organic matter digestibility .

Table.6 refers that no significant differences between dry and organic matter digestibility, when cinnamon and garlic oil were added at 300 and 600 mg/kg DM.

6. Discussion

6. 1.Total gas and methane production ml /200 mg DM at 24 hr. incubation

The results appeared that in this study, total gas and methane was decreased with Cinnamon oil (CEO) may be caused by the increase in the number of proteolytic bacteria consuming hydrogen, nitrogen and carbon dioxide, which are used by microorganisms to methane-producing this result agree with (Kim et al 2015). On the other hand , Some studies indicated that garlic oil at 500 mg/kg DM perhaps seemed good natural supplement to improve rumen microorganisms activity and to manipulate rumen fermentation (Kongmun and others 2010, Anassoriet .et.al. 2011) .While, Jahani Azizabadi and colleagues (2011) cleared that garlic oil decreased total gas and methane production with high level of roughage :concentrate (80:20). Patra and Yu (2015) pointed that garlic oil in two diet: high and low F/C total gas and methane production were decreased by GO in both diets . Doreau and colleagues (2017) found that no significant differences

in total gas an in vitro study using 8 mg of garlic essential oil with high level of concentrate(30:70) F:C. Rofiq and others (2012) evaluated cinnamon and clove oil and their mixture at a rate of 300 ppm to the diet (60:40) concentrate: alfalfa hay led to a decrease in methane production. when addition two levels of cinnamon powder to maize stover 5 to 30 mg/g DM reduced Methane production In vitro (Aderinboye,et.al.2020).In another study Besharat , and colleagues (2021) revealed cinnamon essential oil improve rumen characteristic and reduce methane production in ruminants diets. Molho-Ortiz et al (2022) pointed that decreases in DM digestibility , total VFA concentration are associated with reductions pH and total gas and methane production, when supplementing (500 mg) garlic, and cinnamon, with basal diet (50% concentrate,20% alfalfa,30% corn silage

6.2. In vitro DM and OM digestibility

Present study appeared no differences between treatments in IVDMD and IVOMD this results may be due to the using same dose of cinnamon and garlic and appositive affect of essential oil for the two plants in rumen fermentation and microbes compared with control. Anassori et al (2011) pointed that slightly depressed by garlic oil in organic matter digestibility . Cobellis et al (2016) found that garlic content essential oil and phenols a decrease IVDMD. Another study found that decrease in the dry matter digestibility coefficient of garlic oil is attributed to its effect on the bacterial cell wall(Roy et al ,2014) .

Table 3. Effect of adding cinnamon and garlic oil (0 , 300 and 600) mg / kg DM to mixed ration (80 : 20) concentrate : roughage (alfalfa hay) on total gas and methane production ml /200 mg DM at 24 hr. incubation

Parameters Treatments	Concentrate: roughage (80:20)		
	Volume Gas ml /200 mg DM Total incubation 24hr		
	CON	300mg	600mg
Cinnamon oil	55.33 ± 0.913	30.66±1. 452b	42.00±2.00ab
Garlic oil	55.33±0.913	a 58.50 ±1.892	39.66±2.603ab
Significant	N.S	*	N.S
	Concentrate: roughage (80:20)		
	Methane gas ml/200mgDM 24 h incubation		
	CON	300mg	600mg
Cinnamon oil	±8.94523.83	6.00±0.00ab	0.00b±2.00
Garlic oil	23.83±8.945	14.66± 8.969ab	8.33±0.333ab
Significant	NS	**	**

(P< 0.05)*, (P<0.0.1)** ,N.S ,no significant

Table 4. Effect of adding cinnamon and of garlic oil (0, 300 and 600) mg/kg DM to mixed ration (80 : 20) concentrate : roughage (alfalfa hay) on Metabolizable energy MJ/kg DM, and Net energy /MJ at 24 h incubation

Parameters Treatments	Concentrate: roughage(80:20)		
	Determination Metabolizable energy (MJ/kg DM)		
	CON	300mg	600mg
Cinnamon oil	13.173± 0.2967	1.206 ± 11.916	12.0366±0.313
Garlic oil	0.2967 ±13.173	12.576±1.738	11.673±0.408
Significant	N.S	N.S	N.S
Concentrate: roughage (80:20)			
Determination Net energy (MJ)			
	CON	300mg	600mg
Cinnamon oil	a1.336 ± 046.	1.160 ±0.185a	ab 0.9400±0.050
Garlic oil	1.336± 0.046a	1.300± 0.280a	ab ±0.063 0.883
Significant	N.S	N.S	N.S

(P< 0.05)*, (P<0.0.1)** ,N.S ,no significant

Table 5 . Effect of adding cinnamon and garlic of oil (0,300 and 600) mg/kg DM mixed ration (80 : 20) concentrate : roughage (alfalfa hay) on Short chain fatty acid mmol/100ml, and in vitro organic matter digestibility determination at 24 hr incubation

Parameters Treatments	Concentrate: roughage(80:20)		
	Determination Short chain fatty acid (mmol/100ml)		
	Control	300mg	600mg
Cinnamon oil	3.136±0.060	4.973±0.738a	4.076± 0.193ab
Garlic oil	0.060 ±3.136	5.343±1.037a	3.846±0.248ab
Significant	N.S	N.S	N.S
Concentrate: roughage(80:20)			
Determination true on in vitro organic matter digestibility			

	Control	300mg	600mg
Cinnamon oil	1.290±52.396	69.163 ±6.836ab	62.4733±1.776ab
Garlic oil	52.396± 1.290	72.716±9.703a	60.473± 2.378ab
Significant	N.S	*	N.S

(P< 0.05)*, (P<0.0.1)** ,N.S ,no significant

Table 6. Effect of adding cinnamon and garlic oil (control , 300 and 600) mg / kg DM to mixed ration(80 : 20) concentrate : roughage (alfalfa hay) on in vitro dry matter and organic matter digestibility disappearance

Parameters Treatments	Concentrate: roughage (80:20)		
	In vitro dry matter disappearance		
	CON	300mg	600mg
Garlic oil	±4.0137 62.7666	65.216 ±2.778	64.513 ± 3.901
Cinnamon oil	4.013 ± 62.7666	72.196 ± 2.981	64.070 ±5.065
Significant	N.S	N.S	N.S
	Concentrate: roughage(80:20)		
	In vitro organic matter disappearance		
	CON	300mg	600mg
Garlic oil	65.226± 3.999	68.750 ± 1.181	64.963 ±3.837
Cinnamon oil	3.999 ±65.226	73.773 ± 4.354	66.603±2.684
Significant	N.S	N.S	N.S

N.S ,no significant

CONCLUSIONS 7.

In general, maybe can concluded that the addition of garlic and cinnamon oil at levels of 300 and 600 mg/kg DM .It does not have any negative effect on in vitro digestibility of DM and OM . The percentages used in this study are safe and harmless. Higher percentages can be used to learn about its effect

8.References

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