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Comparative effects of addition of monensin, tannic acid and cinnamon essential oil on *in vitro* gas production parameters of sesame meal

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ABSTRACT: The aim of this experiment was to compare the effects of adding monensin, tannic acid and cinnamon essential oil on *in vitro* gas production parameters of sesame meal. Experimental treatments included sesame meal (control), sesame meal + 12 mg monensin/kg DM, sesame meal + 24 mg monensin/kg DM, sesame meal + 50 mg tannic acid/kg DM, sesame meal + 100 mg tannic acid/kg DM, sesame meal + 150 mg cinnamon essential oil/kg DM, sesame meal + 250 mg cinnamon essential oil/kg DM. The amount of gas produced by treatments fermentation was measured at 4, 6, 8, 12, 16, 24, 36, 48, 72, 96 and 120 hours after incubation. The results showed that gas production decreased significantly in 120 hours after incubation in the treatment containing monensin (at 12 and 24 mg/kg DM) and cinnamon essential oil (at 150 and 250 mg/kg DM) compared to the control treatment ($P<0.05$). The addition of monensin and cinnamon essential oil had a significant effect on increasing partitioning factor and fermentation efficiency compared to control treatment ($P<0.05$). Acid tannic at 100 mg/kg DM increased NEL, SCFA, OMDe and microbial protein compared to control ($P<0.05$). Ammonia nitrogen and total volatile fatty acids concentration at 120 h of incubation showed a significant increase in monensin and tannic acid supplementation compared to control ($P<0.05$), but cinnamon essential oil significantly decreased ammonia nitrogen concentration. In conclusion, cinnamon essential oil and monensin can be used in an environmentally conducive and acceptable way to diminish biogas emissions from ruminants; therewith ameliorate environmental conditions. However, the cinnamon essential oil can be easily used in livestock diets to improve fermentation and reduce biogas production.

***Keywords*:** Cinnamon essential oil, *in vitro* gas production, monensin, sesame meal, tannic acid

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INTRODUCTION

Sesame (*Sesame Indicum L*) belongs to the *Pedaliacea* family. Sesame has a straight stem of 150 to 180 cm in length and has single-branched and multi-branched varieties. The amount of oil from ranges 48 to 62 percent, in other sources, from 45 to 50 percent fat and some other sources mentioned a 50 to 55 percent range. Iranian sesame oil seeds have the highest amount of oil. Protein amounts of sesame arerange from 19 to 25 percent and carbohydrate content is from 10 to 15 percent (Jafari et al., 2019). This plant is cultivated to extract the oil (unsaturated containing several double bonds) present in its seeds.

By-products of agriculture industries such as sesame meal (SM) can have an important impact on diminishing production and ration costs. SM is a by-product of the sesame seed oil extraction. SM contains about 46% crude protein (CP) on dry matter basis, so it can be replacing soybean meal as a protein supplement in animal nutrition. Therefore, adding SM to livestock feeds in particular helps producers reduce the effect of a universal increase in feed costs. Numerable studies have appraised the efficacy of using SM on animal nutrition (Omer et al., 2019).SM is rich in methionine, arginine and leucine amino acids but has very little usable lysine (Rezaeipour et al., 2016).

In ruminants, feed efficiency is low due to rumen fermentation and gas production. Methane (CH_4) production has a direct relationship with feed consumption and about 7-9% of gross energy is consumed as CH_4. Ionophores such as monensin may be recognized to be effective in the rumen bio-hydrogenation process due to reducedCH_4 production by preventing the growth of H^+ bacteria. The utilization of ionophores as an oral antibiotic in livestock has been prohibited in some areas (such as the European Union). Therefore, secondary plant metabolites such as plant essential oils, saponins and tannins have been proposed as potential tools for manipulating the bacterial populations involved in gas production in rumen (Ishlak et al., 2015; Yao et al., 2020). Natural extracts from plants and tannic acid have been used to reduce methane production, reduce rumen degradation of protein and reduce ammonia production, increase rumen escaping protein for greater digestion in the gut and improve rumen fermentation efficiency (Al-Jumaili et al., 2017; Njidda et al., 2017).

Essential oils are used in animal nutrition for replacing growth promoting antibiotics (Besharati et al., 2020). In a study, researchers reported that among the chemical constituents of essential oils, oxygen monoterpens (especially alcohols) and monoterpene aldehydes strongly affect the growth and metabolism of rumen microbes, while hydrocarbons have lower monoterpene and inhibitory effects and sometimes arouse microbial activity (Taktak and Badawy, 2019). The chemical constituents of the essential oils have a great effect on the activity of rumen microorganisms and can improve energy and nitrogen consumption in the rumen. The researchers have found that adding cinnamon, thyme and clove essential oils (one µl per ml of culture medium) to the basal diet containing 80% forage and 20% concentrate reduced the rate of disappearance of dry matter (DM), crude protein (CP), and ammonia nitrogen concentrations. They as well as explained that the usage of cinnamon and thyme essential oils significantly reduced the rate of gas production (Jahani-Azizabadi et al., 2011).

Cinnamon is a dried and crushed cinnamomum tree bark. The original cinnamon species is scientifically named *Cinnamomum zeylanicum*. Cinnamon is a plant that extract of its stem, young shoots and leaves have therapeutic application. Cinnamaldehyde is one of the major active ingredients in cinnamon essential oil. Cinnamaldehyde is a compound of phenylpropanoid with strong antimicrobial activity that comprises about 75% of cinnamon essential oil (Sharma et al., 2016). In one study, researchers reported that, among other secondary metabolites (including thymol and carvacrol), cinnamaldehyde had no effect on cell membrane stability. The researchers (Nayanathara et al., 2018) suggested that the antimicrobial effects of cinnamaldehyde are exerted by the reaction between its carbonyl group with the proteins in the pre-plasm and inactivation of microbial enzymes.

The aim of this study was to compare the effects of adding monensin, tannic acid and cinnamon essential oil on gas production parameters of SM.

MATERIAL AND METHODS

Chemical composition of SM, comprisingDM, ether extract (EE), crude ash (CA), acid detergent insoluble fiber (ADF) and neutral detergent fiber (NDF) were determined according to the proposed AOAC (2002) methods (Table 1). Crude protein was measured using a Kjeldahl analyzer (Foss Tecator AB analyzer, Hoganas, Swede Kjeltec 2300 Auto analyzer) according to AOAC standard method (AOAC, 2002).

Experimental treatments included SM (200 mg

DM, control), SM+12 mg monensin/kg DM, SM+24 mg monensin/kg DM, SM+50 mg tannic acid/kg DM, SM+100 mg tannic acid/kg DM, SM+150 mg cinnamon essential oil/kg DM, SM+250 mg cinnamon essential oil/kg DM.

Table 1. Chemical composition of sesame meal (% of DM)

Chemical composition	Sesame meal
DM	93.21
CA	9.93
OM	83.28
CF	14.30
CP	40.95
NDF	41.50
ADF	18.40

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; CA: crude ash; OM: organic matter; CF: crude fat

Extracting Cinnamon essential oil

Cinnamon essential oil extraction was performed according to standard method using water distillation using Clevenger apparatus (Jahani-Azizabadiet al. 2011). For this purpose, the researcher first crushed cinnamon and then 70 g of the crushed sample was placed into the balloon of the Clevenger apparatus and 750 ml of distilled water was added to each balloon. The samples were then boiled for 3.5 hours after boiling. After this period, the essential oil was collected at the appropriate location in sterile glass (Jahani-Azizabadiet al. 2011). The cinnamon essential oils were determined by GC-mas (Agilent Technologies 7890B). Composition of cinnamon essential oil is shown in Table 1. Cinnamaldehyde content of cinnamon was obtained 72.32%.

***In vitro* gas production (IVGP) test**

Fedorak and Hrudy (1983) method was used to measure gas production. A 200 mg milled SM was weighed and transferred into 50 ml sterile serum bottles.

Rumen fluid was collected in a slaughter house using a four-layer cheese cloth and transferred rapidly to the laboratory in a 39° C water flask. Prior to transferring the rumen fluid into the serum bottles, they were mixed with 1:2 buffer prepared by McDougall (1948) method (one portion of rumen fluid and two parts of buffer). In each glass containing the experimental treatment, 20 ml of rumen and buffer mixture was added and after the anaerobic injection into the glass, the glass lids were tightly closed by rubber cap and metal press.

All bottles were transferred to the incubator shakerset at 39° C to measure gas production, and record the amount of gas produced by food fermentation using the Fedorak and Hrudy (1983) method was recorded at the time of 4, 6, 8, 12, 16, 24, 36, 48, 72, 96 and 120 hours after incubation.

Gas production parameters were calculated using the following mathematical model in the SAS package program according to the model reported by Palangi et al. (2020).

$$P = a + b\ (1\text{-}e^{-ct})$$

where 'P' is the disappearance at time 't', 'a' quickly degradable fraction (or washing loss), 'b' denotes slowly degradable fraction and 'c' is constant rate of degradation of 'b' (Palangi and Macit, 2019). Metabolizable Energy (ME) amounts of gas production (GP) and organic matter digestibility (OMD) were calculated using the equality reported by Menke et al. (1979) as:

$$ME(MJ/kg\ DM) = 2.20+0.136GP+0.057CP+0.0029CP^2$$

$$OMD(\%)=14.88+0.889GP+0.45CP+0.0651XA$$

where, XA ash in g 100 g-1 DM and GP is the net gas production (mL) at 24h short chain fatty acid was calculated using blow equation as:

$$SCFA(mmoL)=-0.00425+0.0222GP$$

where,

Gas is 24 h net gas production (mL/g DM).

The Makkar method was used to determine the partitioning factor (representing the amount of microbial protein synthesis). The partitioning factor (PF) is the mg of degraded organic matter per ml of gas produced in according with the following equation (Makkar, 2010).

$$PF= \frac{c-(a-b)}{IVGP}$$

where,

c= OM (mg), a= undegraded OM (mg), b= ash of fraction a and IVGP= amount of produced gas at 24 h.

The undegraded organic matter was also calculated based on the following equation (Makkar, 2010):OMDe (mg) = *c - (a -b)*

After measuring the volume of gas produced

during 24 hours of incubation, the contents of the bottle were transferred to a container and washed for 1 hour with neutral detergent. The contents of the detergent solution were then filtered with ash-free filter paper and the residue was dried by oven at 100 ° C for 10 hours and calculated fraction b. With subtracting b from a, undegraded OM was calculated in mg (Makkar, 2010; Vercoe et al., 2010).

The values of produced microbial mass and the efficiency of microbial mass synthesis were also calculated using the following equation (Makkar, 2010; Vercoe et al., 2010).

$MM\ (mg) = [c- (a- b)]-[NG_{ml} \times 2.2]$

where;

MM, mg produced microbial mass, NG, ml of produced pure gas and 2.2, stoichiometric coefficient.

Measurement of ammonia

20 ml of the boric acid reagent was poured into a 100 to 150 ml Erlenmeyer flask. 0.8 g of magnesium oxide was placed in a 150 ml Erlenmeyer flask and it was fixed below the sample inlet. 20 ml of rumen fluid was poured from the sample inlet. Distillation continued until about 50 ml of distilled material was collected in the collection tank. Then the solution was titrated with 0.1N sulfuric acid (Markham, 1942).

Determination of volatile fatty acids

One ml of 25% metaphosphoric acid (v/w) was added to 5 ml of filtered extract to determine total volatile fatty acids. The prepared samples were stored at -20 ° C. Prior to analysis, the samples were incubated at room temperature overnight to melt the frozen samples (Markham, 1942). Then tVFA determined colorimetric method.

Statistical analysis

Data were analyzed withina completely randomized design with General Linear Model (GLM) using-SAS (2018), with Duncan's multiple range test used for the comparison of means.

RESULTS

Composition of cinnamon essential oilwas presented in Table 2. The effect of addition of monensin, tannic acid and cinnamon essence on gas production (GP) of SM was shown in Table 3. The GP characteristics, GP potential and rate of GP were presented in Table 4. The effects of adding monensin, tannic acid and cinnamon essential oil on the fermentation parameters estimated in GP, PF, and microbial biomass production efficiency of SM were shown in Table 5. Effect of treatments on VFA, N-NH_3 and pH after 120 h incubation were shown in Table 6. The graph of GC-mas of cinnamon essential oil was presented in Figure 1.

Table2. Composition of cinnamon essential oil in 100 ml.

Composition	Percentage
C_8H_8	0.55
C_7H_6O	1.31
C_9H_8O	72.32
$C_9H_{10}O$	0.1
$C_{10}H_8O$	0.13
$C_{10}H_{10}O$	3.2
$C_{10}H_{12}O$	0.69
$C_{10}H_{16}O$	3.17
$C_{10}H_{18}O$	0.16
$C_{13}H_{18}$	2.24
$C_{15}H_{20}$	0.75
$C_{15}H_{22}$	1.21
$C_{15}H_{24}$	14.17

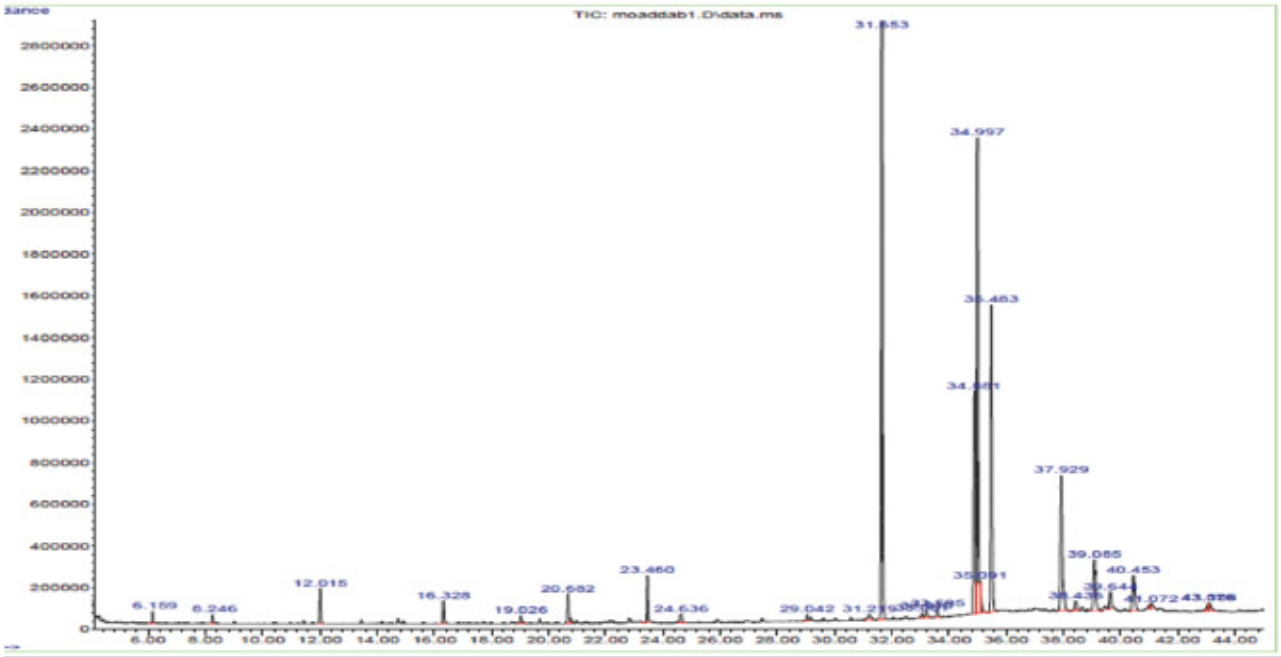

Figure 1. The graph of GC-mas of cinnamon essential oil

The use of agricultural oil seed meal in addition to the antioxidant approach from a nutritional point of view is also important. In the early hours of incubation (4 h and 6 h) the control had higher gas production than the other treatments (monensin, tannic acid and cinnamon essential oil) (P<0.05). Also, in the early hours of incubation (4 h and 6 h) SM treatments containing cinnamon essential oil (at 150 and 250 mg/kg DM) compared with monensin treatment (at levels 12 and 24 mg) and tannic acid (at 50 and 100 mg/kg DM) reduced gas production (P<0.05). After 24 hours of incubation, tannic acid treatment (at 100 mg/kg DM) showed the highest amount of GP, in the event that treating with monensin (at 12 and 24 mg/kg DM) and cinnamon essential oil (at level 150 and 250 mg/kg DM) reduced the amount of GP compared to the control group (P<0.05). After 120 hours of incubation, it was observed that treatment with cinnamon essential oil (at 150 and 250 mg/kg DM) showed the lowest GP and tannic acid treatment (at 100 mg/kg DM) had the highest amount of GP (P<0.05), whereasthere was significantly variation in GP of monensin (at 12 and 24 mg/kg DM) compared to the control group at all incubation times, that treating with monensin reduced the GP.

Table 3. Effects of adding monensin, tannic acid and cinnamon essential oil on gas productionof sesame meal (ml/ 0.2 g DM)

Treatments[1]	Incubation times (h)										
	4	6	8	12	16	24	36	48	72	96	120
SM	1.96^{a}	4.46^{a}	6.12^{a}b	8.66^{b}	11.32^{b}	15.79^{b}	18.17^{b}	20.72^{b}	21.98^{b}	23.25^{bc}	24.40^{bc}
SM+M12	1.68^{b}	4.17^{ab}	5.73^{b}	7.61^{d}	9.44^{c}	12.92^{c}	14.60^{c}	16.91^{c}	19.23^{c}	21.06^{cd}	22.24c^{d}
SM+M24	1.70^{b}	4.08^{ab}	5.76^{b}	7.86^{cd}	9.84^{c}	13.34^{c}	15.14^{c}	17.03^{c}	19.05^{c}	20.73^{d}	21.79^{d}
SM+TA50	1.56b	3.92^{b}	5.70^{b}	8.42^{bc}	11.03^{b}	15.55^{b}	18.21^{b}	20.60^{b}	22.30^{b}	23.87^{b}	24.86^{b}
SM+TA100	1.60^{b}	4.28^{ab}	6.50^{a}	9.88^{a}	13.41^{a}	18.61^{a}	21.19^{a}	23.68^{a}	25.56^{a}	27.03^{a}	28.12^{a}
SM+CEO150	1.50^{b}	3.25^{c}	4.90^{c}	7.10^{d}	9.60^{c}	11.88^{c}	14.88^{c}	16.93^{c}	18.68^{c}	19.68^{d}	20.18^{d}
SM+CEO250	1.50^{b}	3.52^{c}	5.17^{c}	7.37^{d}	9.87^{c}	12.09^{c}	15.09^{c}	17.08^{c}	18.83^{c}	19.83^{d}	20.33^{d}
SEM	0.071	0.139	0.169	0.252	0.336	0.514	0.653	0.771	0.762	0.787	0.784
P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Means within same column with different superscripts differ (P <0.05).

[1]SM: SM (control), SM+M12: SM+12 mg monensin/kg DM, SM+M24: SM+24 mg monensin/kg DM, SM+TA50: SM+50 mg acid tannic/kg DM, SM+TA100: SM+100 mg acid tannic/kg DM, SM+CEO150: SM+150 mg cinnamon essential oil/kg DM, SM+CEO250: SM+250 mg cinnamon essential oil/kg DM.

The GP characteristics, GP potential and rate of GP are presented in Table 4. Comparison of the GP process at different times and the GP parameters shows that the amount of gas obtained from the soluble fraction (a), (b) fraction, the gas production rate (c) and delay phase (L) of the treatments were significantly different (P<0.05). The protein content of the oilseeds was highly degradable, which is cause to the shortening of the delayed phase of SM containing monensin additive (at 12 and 24 mg/kg DM), cinnamon essential oil (at levels 150 and 250 mg/kg DM) and tannic acid (50 mg/kg DM) were significantly different from control and tannic acid treatment (at 100 mg/kg DM) (P<0.05). It is also observed that tannic acid (at 100 mg/kg DM) increased the lag time of SM.

Table 4. Effects of adding monensin, tannic acid and cinnamon essential oil on b, c and L parameters of sesame meal

Treatments[1]	Parameters[2]		
	b (ml/0.2 g DM)	c (ml/h)	L (h)
SM	23.70^{b}	0.045	1.67^{b}
SM+M12	21.90^{bc}	0.033	0.90^{cd}
SM+M24	21.21^{c}	0.038	0.75^{cd}
SM+TA50	23.87^{b}	0.091	1.51^{bc}
SM+TA100	27.24^{a}	0.048	2.50^{a}
SM+CEO150	20.91^{c}	0.037	1.22^{bc}
SM+CEO250	21.00^{c}	0.038	1.08^{bc}
SEM	0.775	0.018	0.266
P-value	<.0001	0.350	<.0001

Means within same column with different superscripts differ (P<0.05).
[1]SM: SM (control), SM+M12: SM+12 mg monensin/kg DM, SM+M24: SM+24 mg monensin/kg DM, SM+TA50: SM+50 mg acid tannic/kg DM, SM+TA100: SM+100 mg acid tannic/kg DM, SM+CEO150: SM+150 mg cinnamon essential oil/kg DM, SM+CEO250: SM+250 mg cinnamon essential oil/kg DM.
[2]b: Potential of gas production, c: rate of gas production, L: lag time.

The effects of adding monensin, tannic acid and cinnamon essential oil on the fermentation parameters estimated in GP, PF, and microbial biomass production efficiency of SM are shown in Table 5. The results show that by increasing the gas production (24h of incubation periods) of SM treated with tannic acid (100 mg/kg DM), increased ME and microbial protein (MP) (P<0.05). Treatments containing monensin and cinnamon essential oil had a significant difference with the control treatment by reducing the ME and the estimated MP content. The levels of short chain fatty acids (SCFAs), organic matter digestibility (DOM) and net energy of lactation (NE_L) were presented in Table 5. The highest amounts of SCFAs, DOM and NE_L were in the tannic acid treatment (100 mg/kg DM) and the lowest in the treatment containing cinnamon essential oil (150 mg/kg DM). There was no significant difference between treatment with monensin (12 and 24 mg/kg DM), cinnamon essential oil (150 and 250 mg/kg DM) and control. Significant differences were found in the content of tannic acid (50 and 100 mg/kg DM) (P<0.05), indicating that the levels of short-chain fatty acids, OMD, and NE_L were high. The decrease has been shown to be due to a decrease in GP within 24 hours after incubation. Maximum feedstuff fermentation efficiency (FFE) in monensin-containing treatment at levels 12 and 24 mg/kg DM was 10.82 and 10.94 mg/ml, respectively, and the lowest in control and treatment containing 100 mg tannic acid/kg DM were 7.95 and 7.77 mg/ml, respectively.

Table 5. Effects of adding monensin, tannic acid and cinnamon essential oil on estimated gas production parameters of sesame meal

Treatments[1]	Parameters										
	GP	SCFA	DOM	NE_L	FFE	ME	MP	OMDe	PF	MM	EMBS
SM	15.79^{b}	0.34^{b}	28.85^{b}	1.74^{b}	7.9^{5c}	4.35^{b}	34.80^{b}	79.61^{d}	5.07^{c}	44.87^{c}	32.05^{c}
SM + M12	12.97^{c}	0.28^{c}	26.01^{c}	1.42^{c}	10.82^{a}	3.89^{c}	31.37^{c}	90.69^{b}	7.02^{a}	62.27^{a}	44.48^{a}
SM + M24	13.34^{c}	0.29^{c}	26.43^{c}	1.47^{c}	10.94^{a}	3.96^{c}	31.87^{c}	92.31^{a}	7.05^{a}	62.96^{a}	44.97^{a}
SM + TA50	15.55^{b}	0.34^{b}	28.62^{b}	1.72^{b}	8.85b^{c}	4.31^{b}	34.51^{b}	87.47^{c}	5.67^{bc}	53.26^{b}	38.04^{b}
SM + TA100	18.61^{a}	0.40^{a}	31.64^{a}	2.07^{a}	7.77^{c}	4.79^{a}	38.17^{a}	93.51^{a}	5.03^{c}	52.57^{b}	37.55^{b}
SM + CEO150	12.09^{c}	0.26^{c}	24.98^{c}	1.30^{c}	9.91^{ab}	3.76^{c}	30.13^{c}	70.23^{e}	5.95^{b}	44.10^{c}	31.50^{c}
SM + CEO250	11.88^{c}	0.27^{c}	25.19^{c}	1.32^{c}	9.38^{b}	3.73^{c}	30.38^{c}	62.21^{f}	5.58b^{c}	40.60^{d}	30.01^{c}
SEM	0.560	0.012	0.555	0.064	0.398	0.088	0.669	0.494	0.249	1.161	0.829
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means within same column with different superscripts differ (P<0.05).
[1]SM: SM (control), SM+M12: SM+12 mg monensin/kg DM, SM+M24: SM+24 mg monensin/kg DM, SM+TA50: SM+50 mg acid tannic/kg DM, SM+TA100: SM+100 mg acid tannic/kg DM, SM+CEO150: SM+150 mg cinnamon essential oil/kg DM, SM+CEO250: SM+250 mg cinnamon essential oil/kg DM.
GP: gas production (ml/0.2 g DM), SCFA: short chain fatty acid, DOM: digestible organic matter (%), NE_L: net energy for lactation (MJ/kg DM), FFE: feed fermentation efficiency, ME: metabolizable energy (MJ/kg DM), MP: microbial protein,OMDe: truly digestible OM (mg/0.2 DM), organic matter; PF: partitioning factor (mg DOM/ml GP), MM: microbial biomass production (mg). EMBS: microbial biomass production efficiency.

The effect of adding monensin, tannic acid and cinnamon essential oil, on the amount of total volatile fatty acid, ammonia nitrogen and ruminal pH using SM in the gastric test at 120 h after in vitro incubation was presented in Table 6. The pH of the control did not differ significantly (P>0.05). The highest pH reduction was observed in treatments containing tannic acid (100 mg/kg DM).

Table 6. Effects of treatments on VFA, N-NH3 and pH after 120 h incubation

Treatments[1]	Items[2]		
	tVFA (mmoL/l)	$N\text{-}NH_3$ (mmoL/l)	pH
SM[1]	226.33^{b}	368.67^{c}	6.76^{ab}
SM+M12	385.00^{a}	518.00^{b}	6.72^{b}
SM+M24	413.00^{a}	742.00^{a}	6.70^{b}
SM+TA50	434.00^{a}	679.00^{a}	6.71^{b}
SM+TA100	238.00^{b}	709.33^{a}	6.66^{b}
SM+CEO150	205.33^{b}	196.00^{d}	6.68^{b}
SM+CEO250	212.33^{b}	156.33^{d}	6.81^{a}
SEM	39.143	27.153	0.029
P-value	0.0006	<.0001	0.030

Means within same column with different superscripts differ (P<0.05).
[1]SM: SM (control), SM+M12: SM+12 mg monensin/kg DM, SM+M24: SM+24 mg monensin/kg DM, SM+TA50: SM+50 mg acid tannic/kg DM, SM+TA100: SM+100 mg acid tannic/kg DM, SM+CEO150: SM+150 mg cinnamon essential oil/kg DM, SM+CEO250: SM+250 mg cinnamon essential oil/kg DM.
[2]tVFA: total volatile fatty acids, $N\text{-}NH_3$: ammonium.

DISCUSSION

The results showed that SM treatment with tannic acid (100 mg/kg DM) produced more gas than control and cinnamon essential oils treatment. Moheghi et al. (2013) found that addition of tannic acid in the diet containing a mixture of forage (50%) and concentrate (50%) increased the amount of cumulative gas production at all incubation times compared to the control group. A possible reason for this increase in GP may be due to its anti-protozoa characteristic that results in more fermentation and *in-vitro* GP. Hence, in another study- on the ability of tannic acid to inhibit methanogenesis and bio-hydrogenation of unsaturated fatty acid, they found that the addition of 25 mg tannic acid at 250 mg DM was not different from control group, but addition of 50 mg tannic acid to 250 mg DM reduced GP and had significant difference with control (Al-Jumaili et al., 2017).

The results of *in vitro* and *in vivo* trials mostly show contradictory results from rumen hydrolyzed tannins. This is maybe due to diversity in the nature of tannins and the amount of inclusion in the diet (Buccioni et al., 2015). Besharati et al. (2013) also found that monensin containing cottonseed meal (24 mg/kg DM) reduced the production of gas compared to the other treatments, which was in agreement to the findings of present study. These results agreed to the findings of Callaway and Martin (1996) which proved the in vitro culture with monensin supplementation produced lower gas production than monensin-free additive group. Though some studies also reported a decrease in methane production by the addition of monensin (Jafari et al., 2019). Monensin may have reduced GP by ionophores lipophilic compounds that was toxic to many bacteria, protozoas, and fungi (Haque, 2018; Broucek, 2018). The toxicity of these compounds was due to their penetration into the cell membrane and changes in ionic charge inside. Ionophores penetrate into the cell membrane by binding to positive ions and transporting them (sodium, potassium, magnesium and calcium) and causing them to die by changes in ionic charge inside the cells (Holmes et al., 2018; Novilla, 2018).

The results of this experiment showed that the used cinnamon essential oil has the potential to affect ruminal fermentation of SM. The effects of natural plant essential oils and plant extracts on improving ruminal fermentation (e.g., reduced CH_4 production ruminal protein degradation, and-rumen ammonia nitrogen concentration) have been reported previously (Besharati et al., 2020). But in another study performed on a diet containing 80 % concentrate and 20 % forage, they found that adding cinnamon and thyme essential oil at all levels, reduced total GP and CH_4 production in comparison with control treatment ($P<0.05$). The results showed tannic acid at 50 and 100 mg/kg DM caused more gas production than those of treatments containing monensin and cinnamon essential oil. Reduction of GP in treatments containing cinnamon essential oils indicates that plant oils at low levels are useful for fermentation and but at high levels selectively restrict gram-positive and gram-negative bacteria (Oulkheir et al., 2017; Semeniuc et al., 2017).

The lag phase is required for the digestion of both parts that have the ability to degrade and the solve fractions. Moheghi et al. (2013) noted that the rate of gas production decreased with increasing levels of tannic acid in treatments. The highest potential of gas production (b) was obtained in the treatment with tannic acid (100 mg/kg DM) with 27.24 ml/0.2 g DM and the lowest in the treatment with cinnamon essential oil (at the level of 150 mg/kg DM) with 21.91 ml/0.2 g DM. The fraction b of SM significantly decreased with adding monensin (at 24 mg/kg DM) and essential oil (at 150 and 250 mg/kg DM) ($P<0.05$).

The rate of gas production (c) was not statistically affected by the additives ($P>0.05$). The study by El-Waziry et al. (2007) investigating the influence of different levels of tannic acid to soybean meal, statedthat there was no significant variation in the rate of GP. Tannins appear to inhibit the activity of microbial enzymes by forming protein complexes with enzymes in the bacterial cell wall, thereby reducing the digestibility of carbohydrates, especially structural carbohydrates, by cellulolytic bacteria, thus through this mechanism they exert their effect on the digestion of whole feedstuffs (Huang et al., 2018). The results of another study conducted by Besharati et al. (2013) on the effect of monensin supplementation (24 mg/kg DM) found that GP potential and GPrate were reduced, which was in agreement with the results of this study. Taghavi-Nezhad et al. (2011) reported that there is a negative relationship between peppermint essential oil at 250, 500, 750 and 1000 µg/ml and gas production parameters (gas production, gas production potential and rate, factor fractionation and microbial mass production), as the essential oil level increased, GP parameters decreased.

The results showed that monensin and cinnamon essential oil increased the fermentation efficiency and tannic acid (50 mg/kg DM) had not significant. Studies show that the addition of tannic acid to the experimental diet increases the digestibility of OM, the amount of short chain fatty acids and ME. There is a positive correlation between the amount of produced gas and the production of short-chain fatty acids (Menke, 1988) and GP can predict the amount of volatile fatty acids production that has a positive relationship with microbial mass production (Rabelo et al., 2017).

The partitioning factor (PF) index was the highest in monensin-containing treatments (12 and 24 mg/kg DM) with 7.02 and 7.05 mg of OM degraded per milliliter of gas, respectively, and the lowest value for control with 5.07 mg of degraded OM per ml gas ($P<0.05$), and there was a significant difference between treatments containing monensin with other

treatments (P<0.05). Treatments containing cinnamon essential oil (150 and 250 mg/kg DM) and tannic acid (50 mg/kg DM) also increased PF index, which showed a significant difference with control (P<0.05). Microbial mass index (MM) and microbial mass synthesis efficiency in treatment containing monensin (12 and 24 mg/kg DM) and tannic acid (50 and 100 mg/kg DM) were significantly different from others, but monensin-treated samples had a significant difference with tannic acid-treated ones. Control and cinnamon essential oil treatment (150 and 250 mg/kg DM) did not differ significantly (P>0.05). Makkar et al. (1995) reported that the effect of tannin on reducing feed degradation rate contributes to the simultaneous release of nutrients and this may be responsible for increased microbial efficiency. Research has shown that when consuming tannin-containing forages, the amount of non-ammonia nitrogen injected into the small intestine was higher than that consumed nitrogen, that part of which was attributed to increased MP production. Cinnamon essential oil and tannic acid reduced feed degradation rates, which can help coincide with the release of nutrients resulting in increased efficiency and in fact the amount of energy and ammonia available for MP synthesis has reached its equilibrium level, and the rumen microorganisms are able to grow better. In the Kiran and Kirishnamurti (2007) report, the range of PF content of feeds for protein sources ranged from 3.86 to 6.48 mg/ml and for energy sources between 3.28 and 4.53 mg/ml. In the present study, the PF parameter of SM was between 5.03 and 7.05 mg/ml, which was high and close to the reported by Kiran and Kirishnamurti (2007). The slight difference between treatments in this respect and the small variance of the results of the present experiment could be related to the low GP due to the protein degradation but not fermentation in the SM solution and increased the true digestible OM and consequently increased the PF parameter.

Ammonia nitrogen concentration (mmol/l) increased at both monensin and tannic acid levels compared to control, but cinnamon essential oil at both levels showed a decrease that resulted from decreased ammonia nitrogen concentration and increased MP production. It indicates that there is a linear relationship between these two indices and that nitrogen is probably used in the production of microbial protein. The researchers found that monensin supplement (24 mg/kg DM) had less inhibitory effect on rumen fermentation compared to thyme essential oil (100 mg/l culture medium). High partitioning factor in the feed indicates more conversion of the degraded material to microbial mass, greater efficiency of microbial mass production, less methane production and greater feed consumption, so PF can be used to predict microbial mass and methane production in ruminants. The increase in PF value indicates that the degradedOM is more likely to move towards microbial mass production and to decrease volatile fatty acid production (Kiran and Kirishnamurti 2007).

The results obtained in this study regarding pH decrease were consistent with the addition of tannic acid in the study of Moheghi et al. (2013). The decrease in pH by tannic acid and tannin has also been observed in other studies (Jafari et al., 2019). Probably one of the reasons for the decrease in pH could be the change in the patterns of rumen bacteria, especially cellulolytic bacteria (Jafari et al., 2019). On the other hand, protozoa depletion drives the pattern of volatile fatty acids production to produce more propionate and less acetate and butyrate (Silanikove et al., 2006). Silanikove et al. (2006) stated that under in vitro conditions, decreased absorption of volatile fatty acids from the rumen wall and increased ruminal fatty acid production were the main causes of pH decrease.

Ammonia nitrogen production in treatments tannic acid (50 and 100 mg/kg DM) and monensin (12 and 24 mg / kg DM) was higher than those of cinnamon and control (P<0.05). Cinnamon essential oil significantly reduced the amount of ammonia nitrogen in all treatments (P<0.05). Moheghi et al. (2013) who examined the in vitro experiment with a 50:50% forage to concentrate ration, observed that the addition of tannic acid reduced the amount of ammonia nitrogen in 24 h of incubation. One of the goals of using essential oils was to reduce the concentration of ammonia nitrogen, which indicates a reduction in the amino acid deamination by ammonia-producing bacteria, which is useful in feeding ruminants because it increases the amount of protein passing through rumen and ultimately increases the efficiency of protein utilization in ruminants (Van Nevel and Demeyer, 1988). The effect of essential oils in different experiments on the ammonia concentration was different. The cause of these differences may be related to the type and amount of essential oil and substrate used. The decrease in ammonia concentration can be linked to a decrease in the number of rumen protozoa (Talebzadeh et al., 2012), the inhibition of high-ammonia-producing bacteria, a decrease in amino acid deamination, and an increase in protein flow into the small intestine (Tager, 2010).

Most of the essential oils reduce rumen ammonia concentration due to antimicrobial activity on a specific group of gram-positive bacteria. Ammonia-producing bacteria make up only one percent of the population of rumen bacteria but have high de-amination activity and inhibition of these bacteria by secondary plant compounds by increasing protein utilization efficiency and reducing rumen ammonia production (Patra, 2011). Monensin reduces deamination and ammonia depletion by controlling the protein-degrading bacteria in the rumen (McGuffey et al., 2001).

Volatile fatty acid production was significantly increased in treatments containing tannic acid (50 mg/kg DM) and monensin (12 and 24 mg/kg DM) ($P<0.05$). The amount of volatile fatty acid production of cinnamon essential oil was not significantly different from the control ($P>0.05$). Moheghi et al.(2013) stated that tannic acid at 24 h incubation reduced pH and ammonia nitrogen compared to control. In most studies, the use of essential oils or their compounds did not reduce or alter the total concentration of volatile fatty acids. Since volatile fatty acids are a major source of energy for ruminant tissues, reducing their production is not beneficial, but no change in the concentration of volatile fatty acids is desirable if accompanied by a decrease in ammonia concentration, a decrease in methane production or a change in the ratio of volatile fatty acids.

The effect of the essential oil on the concentration of volatile fatty acids is dependent on the type of diet, the dosage and the pH of the rumen (Kholif et al., 2018;Besharati et al., 2020). Benchaar et al. (2007) reported that adding cinnamon (400 mg/l), clove (200 mg/l) and thyme (200 mg/l) essential oils to the medium had a significant effect on total volatile fatty acid concentration. Busquet et al. (2006) pointed out that the decrease in total volatile fatty acid production by the addition of dill essential oil and its main active ingredient showed a decrease in feed degradability. In the present study, cinnamon essential oil reduced the disappearance of DM, OM, and CP in addition to the reduction of ammonia nitrogen concentration, which was in accordance with the Jahani-Azizabadi et al. (2011) study.

CONCLUSION

Although the use of antibiotics increases the efficiency of feed, but for some reason they are not used, nowadays, other additives such as herbal essential oils are used for this purpose. Ionospheres such as monensin, may have been shown to be effective in the biodegradation process of rumen by inhibiting the growth of methane emissions by inhibiting the growth of gram-positive bacteria that produce hydrogen. The use of ionophores as an oral antibiotic in livestock has been banned in some areas. Therefore, secondary plant metabolites such as plant essential oils have been suggested as potential tools for manipulating bacterial populations involved in biogas production. The use of cinnamon essential oil as a biogas reducer for the first time is a novelty of this study. It is for first time that the effect of monensin and cinnamon essential oil on biogas production has been compared.

CONFLICT OF INTEREST

None declared.

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