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Waste and Biomass Valorization

ISSN 1877-2641

Volume 12

Number 5

Waste Biomass Valor (2021)

12:2333-2342

DOI 10.1007/s12649-020-01167-2

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Influence of Cinnamon Essential Oil and Monensin on Ruminal Biogas Kinetics of Waste Pomegranate Seeds as a Biofriendly Agriculture Environment

Maghsoud Besharati¹ · Valiollah Palangi² · Mojtaba Moaddab¹ · Zabihollah Nemati¹ · Alberto Barababosa Pliego³ · Abdelfattah Z. M. Salem³

Received: 11 May 2020 / Accepted: 12 July 2020 / Published online: 25 July 2020
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Abstract

The objective of present experiment was to compare the effect of cinnamon essential oil with monensin on the environmental biogas production kinetics of pomegranate seeds. Experimental treatments were: pomegranate seeds (control), pomegranate seeds with 12 mg monensin/kg dry matter, pomegranate seeds with 24 mg monensin/kg dry matter, pomegranate seeds with 150 mg cinnamon essential oil/kg dry matter and pomegranate seeds with 250 mg cinnamon essential oil/kg dry matter. The biogas produced by treatments incubation was recorded at 4, 6, 8, 12, 16, 24, 36, 48, 72, 96 and 120 h after incubation. The results showed that biogas production decreased numerically at 120 h after incubation in the treatment containing monensin (at 24 mg/kg dry matter) and cinnamon essential oil (at 150 and 250 mg/kg dry matter) compared to the control treatment. The addition of monensin and cinnamon essential oil had a significant effect ($P < 0.05$) on increasing partitioning factor and fermentation efficiency compared to control treatment. Concentration of ammonia nitrogen at 120 h of incubation was reduced ($P < 0.05$) by adding different levels of cinnamon essential oil and monensin at the level of 24 mg, and total volatile fatty acids were significantly reduced in treatments containing cinnamon essential oil. Concentrations of ammonia nitrogen and total volatile fatty acids showed an incremental effect ($P < 0.05$) with the addition of 12 mg of monensin. 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.

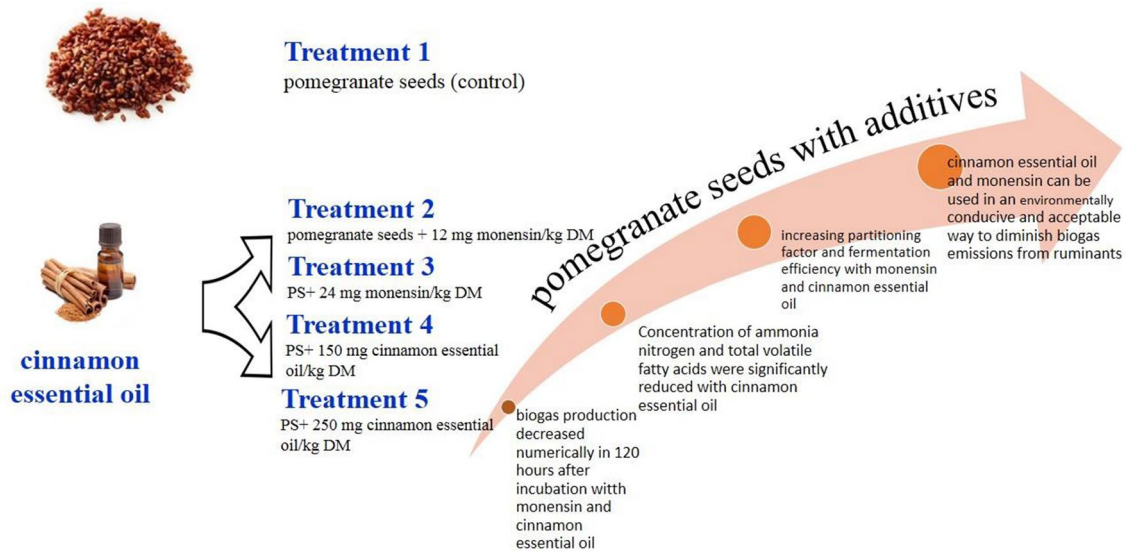
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Graphic Abstract



Keyword Waste pomegranate seeds · Cinnamon essential oil · Monensin · Biogas production

Abbreviations

ADF	Acid detergent insoluble fiber
BGP	Biogas production
CEO	Cinnamon essential oil
CP	Crude protein
DM	Dry matter
EE	Ether extract
GP	Biogas production
IVGP	Amount of produced biogas at 24 h
ME	Metabolizable energy
MM	Microbial mass
NDF	Neutral detergent fiber
NE _L	Net energy of lactation
OM	Organic matter
OMD	Organic matter digestibility
OMDe	Undegraded organic matter
PF	Partitioning factor
PS	Pomegranate seeds
SCFA	Short chain fatty acids
VFA	Volatile fatty acids

Statement of Novelty

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. Ionophores 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.

Introduction

The use of agricultural waste and agro-industrial sub-products in animal nutrition, in addition to reducing costs, reduces the use of human food in animal consumption [1, 2]. Furthermore, the consumption of such wastes as an animal feed, also reduces the environmental hazards caused by their disposal [3, 4]. Therefore, the proper use of agro-industrial by-products in livestock nutrition and identification of new and inexpensive feed resources can be considered as one of the important priorities of the livestock industry. In the industrial processing of pomegranate (*Punica granatum* L.), significant amounts of wastes are constituted the seeds, peels and pulp, remains as unused parts that able to use as ruminants feed [5]. In ruminants, efficiency of feed is low due to ruminal fermentation and

biogas production. Methane production is directly related to feed consumption, and about 2–12% of gross energy intake is lost as methane [6].

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 [7, 8]. Ionospheres such as monensin, may have been shown to be effective in the biodegradation process of rumen by inhibiting the growth of biomethane emissions by inhibiting the growth of gram-positive bacteria that produce hydrogen [9, 10]. The use of ionophores as an oral antibiotic in livestock has been banned in some areas (such as the European Union). Therefore, secondary plant metabolites such as plant essential oils [11, 12] saponins [13, 14] and tannins [15–17] have been suggested as potential tools for manipulating bacterial populations [18] involved in biogas production. Natural essential oils extracted from plants and tannic acid have been used to reduce biomethane production, ruminal protein decomposition and ammonia production; increase ruminal protein passage for further digestion in the gut, and improve ruminal fermentation efficiency. Pomegranate (*Punica granatum*) is a momentous fruitage crop in tropical and subtropical regions [19]. About 3% of the pomegranate is composed of its seeds that the content of compounds such as Hydroxybenzoic acids, Ellagic acid, Conjugated and Non-Conjugated fatty acids, Sterols and Triterpenes [20], that leads the pomegranate seeds can be used as animal feed [21].

Biogas production from ruminant livestock are one of the sources responsible for greenhouse gas emission due to fermentation of feedstuff in the rumen resulting in a loss of digested energy [22–24].

Recently, the use of essential oil and monensin additives in ruminant diets has attracted considerable interest [11, 25]. Many research studies have shown that supplementing essential oil in livestock diets improved silage quality, increase digestibility, rumen fermentation [26–28], ruminant production [29] and reduce biogas production [30–34]. The finding of Zhou et al. [11] suggested that oregano essential oil supplementation can modify ruminal fermentation to alter volatile fatty acids (VFA) concentrations and reduce biomethane emissions by extensively altering the ruminal bacterial community, suggesting an optimal feeding rate for future animal studies of approximately 52 mg/l for mature ruminants.

The hypothesis of this study was the addition of cinnamon essential oil and monensin improves ruminal fermentation and decrease biogas production. However, the aim of this study was to determine the comparative efficacy of cinnamon essential oil and monensin additives on biogas production of pomegranate seeds and their effects on cleaner environment.

Materials and Methods

Chemical Composition

Chemical composition of pomegranate seeds (PS), comprising dry matter (DM), ether extract (EE), ash, acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined according to the proposed AOAC [35] and Van Soast et al. [36] methods. Crude protein (CP) was measured using a Kjeldahl analyzer (Foss Tecator AB analyzer, Hoganas, Swede Kjelttec 2300 Auto analyzer) according to AOAC standard method [35].

Cinnamon essential oil (CEO) extraction was performed according to standard method using water distillation using Clevenger apparatus [37]. For this purpose, 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 h after boiling. After this period, the essential oil was collected at the appropriate location in sterile glass [37].

Biogas Production Evaluation

Experimental treatments included PS (control), PS with 12 mg monensin/kg DM, PS with 24 mg monensin/kg DM, PS with 150 mg cinnamon essential oil/kg dry matter and PS with 250 mg cinnamon essential oil/kg dry matter. Fedorah and Hrudý [38] method was used to measure biogas production. 200 mg of milled experimental treatments 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 [39] 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. In order to correct the biogas produced by rumen fluid, 5 serum bottles were considered without adding samples and containing only rumen and buffer fluid. Bottles were transferred to the incubator shaker at 39 °C for measuring biogas production, and record the amount of biogas produced by food fermentation using the Fedorah and Hrudý [38] method were performed at times of 4, 6, 8, 12, 16, 24, 36, 48, 72, 96 and 120 h after incubation.

Biogas production parameters were calculated using the following mathematical model in the SAS package

program according to the model reported by Ørskov and McDonald [40].

$$P = a + b(1 - e^{-c(t+L)})$$

where 'P' is the disappearance at time 't', 'a' quickly degradable fraction (or washing loss), 'b' denotes slowly degradable fraction, 'c' is constant rate of degradation of 'b' and 'L' is lag phase [41]. Metabolizable Energy (ME) amounts of biogas production (GP) and organic matter digestibility (OMD) were calculated using the equality reported by Menke et al. [42] as:

$$\begin{aligned} \text{ME(MJ/kg DM)} &= 2.20 + 0.136\text{GP} + 0.057\text{CP} + 0.0029\text{CP}^2 \\ \text{OMD (\%)} &= 14.88 + 0.889\text{GP} + 0.45\text{CP} + 0.0651\text{XA} \end{aligned}$$

where, XA is crude ash in g 100/g dry matter (DM) and GP is the net biogas production (ml) at 24 h. The short chain fatty acids (SCFA) were calculated using following equation as:

$$\text{SCFA (mmol)} = -0.00425 + 0.0222\text{GP}$$

where, GP is 24 h net biogas 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 biogas produced in according with the following equation [43].

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

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

The undegraded organic matter (OMDe) was also calculated based on the following equation [43]:

$$\text{OMDe (mg)} = c - (a - b)$$

After measuring the volume of biogas produced during 24 h of incubation, the contents of the bottle were transferred to a container and washed for 1 h 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 h and calculated fraction b. With subtracting b from a, undegraded OM was calculated in mg [43, 44].

The values of produced microbial mass (MM) and the efficiency of microbial mass synthesis were also calculated using the following equation [43, 44].

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

where: MM, mg produced microbial mass, NG, ml of produced pure biogas and 2.2, stoichiometric coefficient.

Twenty ml of the boric acid reagent was poured into a 100 to 150 cc Erlenmeyer flask. Place 0.8 g of magnesium

oxide in 150 ml Erlenmeyer flask and fix it 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 0.1 N sulfuric acid [45].

One ml of 25% metaphosphoric acid (volume/weight) was added to 5 ml of filtered extract to determine volatile fatty acids. The prepared samples were stored at – 20° C. Prior to analysis, the samples were incubated at room temperature overnight to melt frozen samples [45].

Data were analyzed as a CRD with GLM using SAS [46], with Duncan's multiple range test used for the comparison of means. Following mathematical model was used for statistical analysis.

$$Y_{ijk} = \mu + T_i + e_{ij}$$

where; Y_{ij} represents dependent variable; μ is general mean; T_i is effect of treatment (Feed Samples); e_{ij} is error.

Results and Discussion

Chemical Composition

The pomegranate seeds had high crude protein, ether extract, acid detergent fiber and neutral detergent fiber contents with reduction in its nutritional value due to high fiber contents (Table 1). In a study of Khoddami et al. [47], the crude protein, crude fiber and ash percentage of pomegranate seeds were 19.73, 12.54, and 1.47%, respectively. The percentage of ether extract, crude protein, acid detergent fiber, neutral detergent fiber and hemicellulose were reported 92.48, 9.39, 14.99, 47.80 and 61.47 and 13.67%, respectively [48], that were higher than the present study. Despite that pomegranate, seeds may be less affected by environmental factors, but the weather conditions and the amount of received water at the time of growth, may have caused these differences.

Table 1 Chemical composition of pomegranate seeds

Compounds	%
Dry matter	91.9
Organic matter	97.8
Ether extract	12.4
Crude protein	25.6
Neutral detergent fiber	50.3
Acid detergent fiber	35.8
Hemicellulose	14.6

Table 2 The effect of adding monensin and cinnamon essential oil on the cumulative biogas production of pomegranate seed (milliliters of produced biogas per 0.2 g dry matter)

Treatment	Incubation time (h)										
	4	6	8	12	16	24	36	48	72	96	120
1	0.32	1.46 ^a	2.28 ^a	3.42 ^{ab}	4.66 ^a	6.68 ^a	6.76 ^{ab}	7.51 ^{ab}	7.85 ^{ab}	8.06 ^{ab}	8.48 ^{ab}
2	0.48	1.52 ^a	2.50 ^a	3.58 ^a	5.02 ^a	7.42 ^a	7.94 ^a	9.03 ^a	9.83 ^a	10.49 ^a	11.15 ^a
3	0.30	1.16 ^b	1.96 ^{ab}	3.12 ^{ab}	3.62 ^b	4.71 ^b	5.71 ^b	6.46 ^b	6.76 ^b	7.01 ^b	7.31 ^b
4	0.30	1.00 ^b	1.45 ^b	2.75 ^b	3.50 ^b	4.65 ^b	5.65 ^b	6.40 ^b	6.70 ^b	6.95 ^b	7.25 ^b
5	0.30	1.00 ^b	1.45 ^b	2.75 ^b	3.50 ^b	4.79 ^b	5.79 ^b	6.54 ^b	6.84 ^b	7.09 ^b	7.39 ^b
SEM	0.059	0.079	0.174	0.223	0.304	0.515	0.650	0.772	0.831	0.884	0.954
P-value	0.18	< 0.0001	< 0.0001	0.04	0.003	0.001	0.04	0.01	0.006	0.04	0.04

Differences between the averages indicated by different letters in the same column are important. 1—Pomegranate seed (control), 2—pomegranate seed containing 12 mg of monensin per kg dry matter, 3—pomegranate seed containing 24 mg of monensin per kg dry matter, 4—pomegranate seed containing 150 mg of cinnamon essential oil per kg dry matter, 5—pomegranate seed containing 250 mg of cinnamon essential oil per kg dry matter

Biogas Production

The volume of biogas produced at 24 h of incubation reduced ($P < 0.05$) with the addition of monensin (24 mg/kg dry matter) and cinnamon essential oil (150 and 250 mg/kg dry matter). However, a significant reduction was not observed at low doses of mononuclear treatment (12 mg/kg dry matter) (Table 2). It is worth noting that cumulative biogas production at all hours of incubation for the treatments in which monensin (12 mg/kg dry matter) was used, were higher than the control.

The results of this experiment show that adding cinnamon essential oil to the pomegranate seeds was reduces the amount of biogas production compared to the control, which confirms the Jahani-Azizabadi et al. [49]'s findings.

In environments containing natural antioxidants, the biogas production curve increases significantly compared to other treatments. This increase could mean that the optimal culture environment for buffering is maintained for a longer period of time than other treatments, possibly due to the antioxidant role of pomegranate seeds compounds in maintaining environmental stability and eliminating radicals and harmful products of oxidation of unsaturated fatty acids [50]. Bakhshizadeh et al. [51] indicated the value of pomegranate pulps biogas production 48 h after incubation about 154.4 ml/g dry matter, this could be due to the difference between pomegranate pulp and pomegranate seeds. Besharti et al. [52] found that the cotton seeds meal containing monensin (24 mg/kg dry matter) reduced biogas production compared to other treatments, which was consistent with the results of the present experiment. These results were similar with Callaway and Martin [53]'s reports, who concluded that biogas production of treatments supplemented with monensin had lower biogas production than control. Agarwal et al. [54], in a study using mint essential oil, showed that a doses of 0.33 and 1 microL per milliliter of mint essential oil, the biogas production was increased,

whereas increasing the concentration of essential oil to 2 μ L per milliliters, biogas production was decreased. They have been attributed this reduction to inhibitory effect of essential oil on the fibro bacteria carcinogens and anaerobic fungi, as well as inhibition of the activity of carboxymethyl cellulose and xylanase enzymes.

Biogas Production Kinetics

The highest ($P < 0.05$) potential for biogas production (b) was found with the monensin (12 mg/kg DM), and the lowest ($P < 0.05$) was found with the addition of cinnamon essential oil (150 mg/kg dry matter)-(Table 3). The constant biogas production rate (c) and the lag phase of pomegranate seeds showed difference ($P < 0.05$) in monensin and

Table 3 The effect of adding monensin and cinnamon essential oil on biogas production parameters of pomegranate seeds

Treatments	Estimated parameters		
	Biogas production potential (ml per 0.2 g of dry matter)	Constant biogas production rate (ml/h)	Lag phase (h)
1	8.08 ^{ab}	0.068 ^a	3.25 ^a
2	10.65 ^a	0.049 ^b	2.69 ^b
3	7.07 ^b	0.053 ^b	2.56 ^b
4	7.06 ^b	0.051 ^b	3.08 ^a
5	7.21 ^b	0.051 ^b	3.18 ^a
SEM	0.949	0.002	0.129
P-value	0.04	< 0.0001	0.002

Differences between the averages indicated by different letters in the same column are important. 1—Pomegranate seed (control), 2—pomegranate seed containing 12 mg of monensin per kg dry matter, 3—pomegranate seed containing 24 mg of monensin per kg dry matter, 4—pomegranate seed containing 150 mg of cinnamon essential oil per kg dry matter, 5—pomegranate seed containing 250 mg of cinnamon essential oil per kg dry matter

cinnamon essential oil treatments. Adding monensin (12 and 24 mg/kg dry matter), led to a reduction ($P < 0.05$) in the lag phase compared to control and cinnamon essential oil. Lag phase is the required time for digestion of both 'a' and 'b' parts has the ability, so that the feed is identified by microorganisms and begins to degradation.

Addition of monensin and cinnamon essential oil to the pomegranate seeds were reduced ($P < 0.05$) the constant biogas production rate (c).

Bakhshizadeh et al. [51] investigated the characteristics of pomegranate pulp using gas production method, in this study the determined gas production potential (a) and gas production rate (c) were 180.41 and 0.0795, respectively. These results were higher than the obtained data, and this difference can result due to the difference between pomegranate pulp and pomegranate seeds. Taghavi-Nezhad et al. [55] examined the effects of peppermint oil, at 250, 500, 750 and 1000 $\mu\text{m}/\text{ml}$ of cultivation environment, on gas production parameters and found that increasing the essential oil levels was decreased gas production produced.

There is a growing advantage to use of phytogetic products in livestock production as dietary supplements. This alternative interest between phytogetic products and livestock is not novel [56] but, its application in health and greenhouse gases mitigation productivity could be [57]. Pedraza-Hernandez et al. [33] show that inclusion *Moringa oleifera* extract in diet increased gas production and suggested it could be due to presence of fermentable and digestible saccharides in phytonstituents. Embay et al. [34] showed that methane production was reduced with the addition of *Origanum vulgare* L. essential oil. Rumen microorganisms can adapt to and/or degrade the active compounds of essential oil, particularly at low dosage rates [58, 59]. Mbiriri et al. [60] reported a transient mitigating effect on

the suppression of CH_4 with carvacrol and thymol supplementation at 300 mg/l. However, Fraser et al. [26] observed a persistent effect with 500 mg essential oil /l on CH_4 production in a continuous culture fermenter. Günel et al. [61] reported that the addition of anise oil and white thyme oil at the high doses concluded in biomethane mitigation and had negatively impacted on rumen microbial fermentation. Narvaez et al. [62] reported adding monensin reduced methanogens population. It has been reported that monensin can directly inhibit H_2 -producing bacteria, and indirectly decreasing biomethane production [63].

Degradation Parameters

Increasing biogas production at 24 h, treatments containing monensin and cinnamon essential oil, was reduced the amount of metabolic energy and microbial protein content. The provided concentration of short-chain fatty acids (SCFA), organic matter digestibility (OMD) and net energy of lactation (NE_L) were show that the addition of monensin (24 mg/kg DM) and cinnamon essential oil (150 and 250 mg/kg DM) reduces the amount of SCFA, OMD and NE_L compared to the control. This reduction could be due to a reduction in biogas production 24 h of incubation (Table 4).

The highest rate of feed fermentation efficiency ($\text{FFE} = \text{mg IDMD}/\text{ml}$ accumulative biogas produced at 24 h post incubation) was observed in treatments containing monensin, and lowest in the control. The results showed that the addition of monensin and cinnamon essential oil were increased the fermentation efficiency of pomegranate seeds in comparison to control.

Decreased population of ruminal microorganisms such as *Butyrivibrio fibrosolvence* due to unsaturated oils in various

Table 4 Partitioning factor, production of microbial biomass, really digested organic matter and efficiency of microbial biomass production and metabolizable energy of pomegranate seed

Treatments	Parameters										
	GP	SCFA	OMD	NE_L	FFE	ME	MP	OMDe	PF	MM	EMBS
1	6.68 ^a	0.143 ^a	17.34 ^a	1.01 ^a	9.25 ^b	5.06 ^a	20.92 ^a	28.51 ^e	4.29 ^c	13.81 ^d	8.63 ^d
2	7.42 ^a	0.160 ^a	18.07 ^a	1.09 ^a	9.82 ^b	5.17 ^a	21.80 ^a	34.04 ^c	4.94 ^c	17.71 ^c	11.07 ^c
3	4.71 ^b	0.100 ^b	15.39 ^b	0.78 ^b	15.31 ^a	4.75 ^b	18.57 ^b	38.29 ^a	8.14 ^a	27.93 ^a	17.45 ^a
4	4.65 ^b	0.098 ^b	15.33 ^b	0.77 ^b	14.15 ^a	4.74 ^b	18.50 ^b	32.46 ^d	6.98 ^b	22.24 ^b	13.89 ^b
5	4.79 ^b	0.101 ^b	15.47 ^b	0.79 ^b	14.57 ^a	4.76 ^b	18.66 ^b	36.15 ^b	7.54 ^{ab}	25.61 ^a	16.00 ^a
SEM	0.516	0.011	0.511	0.059	0.621	0.081	0.616	0.446	0.306	1.017	0.636
P-value	0.001	0.001	0.001	0.001	< 0.0001	0.001	0.001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Differences between the averages indicated by different letters in the same column are important. 1—Pomegranate seed (control), 2—pomegranate seed containing 12 mg of monensin per kg DM, 3—pomegranate seed containing 24 mg of monensin per kg DM, 4—pomegranate seed containing 150 mg of cinnamon essential oil per kg DM, 5—pomegranate seed containing 250 mg of cinnamon essential oil per kg DM

GP biogas production (ml/0.2 g dry matter), SCFA short chain fatty acid, OMD organic matter digestibility (%), NE_L net energy for lactation, FFE feed fermentation efficiency, ME metabolizable energy, OMDe truly digestible OM (mg/0.2 dry matter), organic matter; PF partitioning factor (mg DOM/ml GP), MM microbial biomass production (mg), EMBS microbial biomass production efficiency

studies [64, 65] can be considered as one of the possible causes for increasing the delay time. The antioxidant role of biologically active compounds in pomegranate seeds in contrast the oxidizing compounds of oxidized fats were thought to be a factor in reducing the lag phase in the monensin and cinnamon essential oil treatments. Therefore, due to the same nutritional and buffering capacity of the used cultivation environment, the microbial population is expected to have the potential to multiply at the beginning of incubation and after the lag phase and neutralization of radical compounds during bio-hydrogenation process and other defense mechanisms [66]. Oxidative stress in eukaryotes and prokaryotes occurs when free radicals accumulate in the cell, and leads the proper functioning of the cell membrane, replicating DNA and reducing cell life [67]. The ruminal microorganisms, which are mostly anaerobic have a less developed antioxidant system than aerobic organisms [66]. In the case of fatty acid oxidation disorders is more likely to be affected by oxidative stress. Nutrition of oxidized fats increases the amount of peroxides in the ruminants, reduced microbial protein production and yield and negatively affect ruminal fermentation [68]. The use of antioxidants in the diet balances these negative effects by neutralizing peroxides and free radicals as well as reducing the peroxidation of fatty acids [68]. Jahani-Azizabadi et al. [49] to evaluate the effect of some natural essential oil on microbial-digestive activities and reactions, using of cinnamon essential oil was increased fermentation efficiency.

Ruminal Metabolites

The addition of monensin with pomegranate seeds increased the biogas production which indicates that the removal of the restriction created by tannin. Therefore, monensin was increase the volatile fatty acids production and fermentation in rumen, consequently, and leads to an improvment of animal performance and growth. This may be due to the decrease in ammonia nitrogen in 120 h of incubation, monensin (at 12 and 24 mg/kg dry matter) and cinnamon essential oil (at 150 and 250 mg/kg dry matter) were decreased the crude protein digestion due to fatty acid peroxidation derivatives (Table 5).

Increased ammonia nitrogen production over incubation time, is likely the optimal environmental conditions which will benefit the increase in protozoan population and further decomposition of microbial protein. Decreases in ammonia production due to the addition of cinnamon essential oil have also been reported in some previous studies [69, 70], in addition, our results were in agreement with the findings of Jahani-Azizabadi et al. [49]. The results reported by Vakili et al. [71] were inconsistent with present study findings. However, Busquet et al. [72] also reported that the addition of cinnamaldehyde (the main active ingredient in cinnamon

Table 5 Effects of monensin and cinnamon essential oil on volatile fatty acids, ammonia nitrogen and pH of pomegranate seed in 120 h of incubation

Treatments	tVFA (mmol/l)	N-NH ₃ (mmol/l)	pH
1	342.0 ^b	406.0 ^b	6.77 ^{cd}
2	403.7 ^a	536.7 ^a	6.81 ^{bc}
3	350.0 ^b	322.0 ^c	6.74 ^d
4	281.0 ^c	154.0 ^d	6.90 ^a
5	291.7 ^c	121.3 ^d	6.83 ^b
SEM	6.98	18.40	0.021
P-value	< 0.0001	< 0.0001	< 0.0003

Differences between the averages indicated by different letters in the same column are important. 1—Pomegranate seed (control), 2—pomegranate seed containing 12 mg of monensin per kg dry matter, 3—pomegranate seed containing 24 mg of monensin per kg dry matter, 4—pomegranate seed containing 150 mg of cinnamon essential oil per kg dry matter, 5—pomegranate seed containing 250 mg of cinnamon essential oil per kg dry matter

essential oil) to the culture medium in the two-way continuous culture system did not affect nitrogen metabolism. Benchaar et al. [73] reported that adding cinnamon essential oil (400 mg/l) to the cultivation environment did not affect the concentration of volatile fatty acids. Yang et al. [74] also reported that the addition of cinnamaldehyde leads to decrease in ammonia nitrogen.

Conclusion

Monensin and cinnamon essential oil at both levels had the the similar effect on biogas production and reduced its rate. Concentrations of ammonia nitrogen and total volatile fatty acids at 120 h of incubation were reduced by adding different levels of cinnamon essential oil. Cinnamon essential oil was more effective in reducing both of these parameters than monensin.

Cinnamon essential oil and monensin can be used in an environmentally friendly and sustainable way to reduce biogas emissions from ruminants; thereby improving environmental conditions. However, due to the ban of antibiotics and ionophores using in livestock feed within the European Union, the cinnamon essential oil can be easily used in livestock diets to improve fermentation and reduce biogas production.

Compliance with Ethical Standards

Conflict of interest The authors declare that there to be no competing interests.

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