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Inclusion of molasses to garlic foliage silage and its effect on *in vitro* ruminal fermentation and gas production

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ABSTRACT. The aim of this study was to evaluate the inclusion of molasses in garlic (*Allium sativum*) foliage silages and its effect on *in vitro* ruminal fermentation parameters and gases production including methane (CH_4) and carbon dioxide (CO_2). To this end, fermentation was carried out in 16 microsilos with the addition of molasses (T1 – garlic foliage 90%, ground maize 10%, molasses 0%; T2 – garlic foliage 85%, ground maize 10%, molasses 5%; T3 – garlic foliage 80%, ground maize 10%, molasses 10%; T4 – garlic foliage 75%, ground maize 10%, molasses 15%; n = 4) for 50 days. Subsequently, fermentation was carried out in microsilos using rumen fluid for nutritional evaluation. The inclusion of molasses affected protein, neutral detergent fibre (NDF), non-structural carbohydrates (NSC) and lactic acid contents, and pH ($P < 0.05$); protein and NDF decreased 22 and 12%, respectively, with the inclusion of 15% of molasses, and pH was generally reduced after the addition of molasses to the experimental treatments. However, molasses increased the NSC content and *in vitro* dry matter digestibility. Regarding ruminal fermentation, no changes were recorded in the proportions of volatile fatty acids ($P > 0.05$), while the concentration of total volatile fatty acids increased with the addition of molasses ($P < 0.05$). Ammonia-N levels decreased with the inclusion of molasses ($P < 0.05$), while maximum gas production increased up to 48%. Similarly, methane production increased by 46% at the maximum dose of molasses addition ($P < 0.05$), but no changes were recorded in the CH_4:CO_2 ratio ($P > 0.05$). These results suggested that the addition of molasses to garlic foliage silages did not result in significant changes in ruminal fermentation parameters such as volatile fat acid (VFA) levels. Therefore, garlic foliage silage can be applied without additional supplementation to reduce agricultural waste and as a non-conventional alternative in ruminant nutrition.

Introduction

Population growth has generated increased demand for food and natural resources to meet human needs. Therefore, researchers worldwide are struggling to develop new strategies to reduce waste in human and animal nutrition.

Garlic (*Allium sativum*) is mainly consumed by humans and diverse benefits have been attributed to this plant (Shang et al., 2019). With a worldwide production exceeding 20 million tonnes per year, major producers include China, India and Korea (Lee et al., 2017); Mexico alone produced 75000 tonnes in 2018. However, farmers market mainly the bulbs, while there is no commercial interest in the foliage (stems and leaves). It is well known that garlic cloves (the inner part of the bulb) contain certain metabolites with potent biological activity (Shang et al., 2019); nevertheless, some of these compounds are also present in the foliage but at lower concentrations.

Cattle farming is an anthropogenic activity that contributes to higher greenhouse gas (GHG) emissions such as methane (CH_4) and carbon dioxide (CO_2). Furthermore, livestock is responsible for almost 39% of the global methane production, with the highest amounts of this gas produced by intestinal synthesis in the rumen (Carro et al., 2018). Consequently, researchers are attempting to utilise non-conventional forage sources as feedstuffs, which are not intended to be in direct competition with human nutrition. In fact, Panthee et al. (2017) reported a reduction in the amount of enteric methane production when they supplied garlic leaves to ewes. Torres-Fraga et al. (2020) proposed diets with partial and complete substitution of alfalfa hay for garlic foliage, which resulted in reduced *in vitro* methane production by about 50%. Unfortunately, garlic is an annual plant, which makes it challenging to use as fresh forage throughout the year. The latter problem can be solved by preserving fresh foliage in the form of silage, which may prove to be a unique and cheap alternative. Silage ensures good quality of preserved forage sources through the fermentation of lactic-acid bacteria (LAB). However, LAB need rapidly fermentable carbohydrates (RFC) to achieve the desirable fermentation level and pH in a short time. Unfortunately, the aroma emitted by garlic leaves is very strong and may be rejected by animals. Rapisarda et al. (2012) observed that the presence of sulphur compounds responsible for garlic's unpleasant aromas in different ingredients decreased feed palatability in lambs and ewes. Additionally, the content of starch in garlic, a rapidly fermentable carbohydrate, is limited when compared to other crops such as maize (Putridinanti et al., 2019). Thanks to the high starch content in other crops like maize, there is no need for extra ingredients when preserving forage as silage. In addition, molasses appears to be a reliable ingredient as it has a high RFC content and can contribute to the pleasant aroma of the final product, while reducing repugnant odours by diluting sulphur compounds (Tuyen et al., 2014). Therefore, the objective of this study was to evaluate the inclusion of molasses in garlic (*A. sativum*) foliage silage and its effect on *in vitro* ruminal fermentation parameters, and gas production production.

Material and methods

Study area

This research was carried out at the Faculty of Veterinary Medicine and Husbandry of the Durango State Juarez University. Garlic foliage (*A. sativum*) was harvested randomly from irrigated crops located in Zacatecas, Mexico (22°58'44.6"N 102°43'20.4"W) in late May 2019 and separated from the bulbs. Other ingredients such as maize and molasses were purchased from a local store in the area. This study was approved by the Livestock Protection and Promotion of the State of Durango (OF 2019-011-3).

Silages as experimental treatments

Silages were prepared based on garlic foliage as the main forage source and ground maize. The chemical composition of garlic foliage is given in Table 1. Four levels of molasses inclusion (T1 – 0, T2 – 5, T3 – 10 and T4 – 15%) as a direct substitution of garlic foliage were evaluated in the experimental silages (Table 2). Thus, 16 experimental microsilos (in quadruplicate per experimental treatment) were placed in plastic containers and hermetically sealed (30 cm diameter × 50 cm height) for 50 days. Following this period, the microsilos were opened and the content subjected to various analyses.

Table 1. Chemical composition of garlic foliage

Nutrients	%, DM
Organic matter	90.1
Crude protein	11.5
Ether extract	2.1
NDF	35.1
ADF	26.7
THC	66.3
NSC	31.2
Lignin	6.8

THC – total carbohydrates, NSC – non-structural carbohydrates, NDF – neutral detergent fibre, ADF – acid detergent fibre

Table 2. Ingredients of experimental treatments

Ingredients, % DM	T1	T2	T3	T4
Garlic foliage	90	85	80	75
Ground maize	10	10	10	10
Molasses	0	5	10	15

T1 – (garlic foliage 90%, ground maize 10%, molasses 0%), T2 – (garlic foliage 85%, ground maize 10%, molasses 5%), T3 – (garlic foliage 80%, ground maize 10%, molasses 10%), T4 – (garlic foliage 75%, ground maize 10%, molasses 15%)

Lactic fermentation analysis of silages

The pH (model HI 83142, Hanna Instruments, Woonsocket, RI) and lactic acid level (Borshchevskaya et al., 2016) were determined shortly after opening the microsilos. In addition, the levels of volatile fatty acids (VFA) were determined using the procedures outlined by Galyean (2010).

Chemical analysis of silages

Samples from each experimental treatment were dried in a forced-air oven at 55 °C for 72 h and ground to a particle size of 1 mm using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) for further analyses. Dry matter (DM), crude protein (CP), ether extract and ash content, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin fraction were evaluated according to the AOAC International (2005). In addition, *in vitro* dry matter digestibility (IVDMD) was estimated following the manufacturer's manual (Daisy II, ANKOM Technology, Macedon, NY, USA).

In vitro gas production

Approximately 1 g of each experimental treatment was placed into glass modules (ANKOM RF Gas Production System, Technology, Macedon, NY, USA) equipped with electronic transducers for pressure measurements. These modules were then incubated in triplicate with buffer solutions and rumen fluid in a 2:1 ratio, following the method described by Theodorou et al. (1994). Rumen fluid was obtained from two fistulated Angus steers and filtered through 4 layers of cheesecloth. This procedure was approved by the Livestock Protection and Promotion of the State of Durango. The incubations were carried out from 0 to 96 h during which time the pressure in each module was recorded. The *in vitro* gas production kinetics was estimated by fitting the volume of gas produced data to the Gompertz function according to the follow equation:

$$GP = Gmax*\exp[-A*\exp(-k*t)],$$

where: GP (ml) – the gas production at time t, Gmax (ml) – the maximum gas production, k (%/h) – the constant gas production rate, A (h) – the latency period before gas production begins.

In vitro ruminal fermentation parameters

Approximately 1 g of each experimental treatment was placed in triplicate in glass modules (ANKOM RF Gas Production System, Macedon, NY, USA) with a mixture of buffer solutions and rumen fluid in a 2:1 ratio (Theodorou et al., 1994). After 24 h of fermentation, the gases contained in the headspace fraction of each module were analysed for CH_4 and CO_2 composition using a gas analyser (Landtec, GEM5000 Gas Analyzer, QED Environmental Systems, Inc., Dexter, MI, USA) and procedures proposed by González-Arreola et al. (2019). After opening the modules, the pH was promptly measured using a portable pH/mV meter (model HI 83142, Hanna Instruments, Granjas, México). A 10-ml aliquot was taken from each module, filtered and centrifuged at 3000 *g* for 5 min. Subsequently, each aliquot was divided into two subsamples for the analysis of VFA and nitrogen ammonia (NH_3-N) (Galyean, 2010).

Statistical analysis

The data obtained for each variable were analysed using a completely randomized design with the GLM procedure of SAS. The means were compared using the Tukey test, and significant differences were declared at $P < 0.05$.

Results

Chemical composition

The chemical composition of different silages in experimental treatments is shown in Table 3. The addition of molasses to the experimental treatments had significant effects on DM, CP, ash, EE, NDF, pH, lactic acid, acetic acid, propionic acid and IVDMD ($P < 0.05$). The ash and protein contents decreased by 27% and 22%, with the addition of 15% molasses (T4), respectively, in comparison to T1; however, the same dose of molasses decreased ether extract and NDF contents by 10% and 12%, respectively. The addition of molasses also resulted in a decrease in pH in the experimental treatments relative to T1. In contrast, lactic and acetic acid levels increased significantly after molasses addition ($P < 0.05$). Notably, the highest level of molasses addition (T4) led to a 7% increase in IVDMD.

In vitro ruminal fermentation parameters

In vitro ruminal fermentation parameters are summarized in Table 4. The inclusion of molasses to the experimental treatments had a significant impact on VFA (individual and total) and N-NH_3 ($P < 0.05$) levels. Specifically, acetic and propionic acid levels increased by about 7% and 5%, respectively, compared to T1, whereas butyric acid concentration decreased by 17% in T4. The addition of 15% molasses to T4 led to a 44% increase in TVFA levels, and the Ac:P ratio increased by 4% after molasses supplementation ($P < 0.05$). N-NH_3 levels decreased approximately by 16% with the inclusion of molasses compared to T1.

Table 3. Chemical composition of experimental treatments

Parameter	Experimental treatments				
	T1	T2	T3	T4	SEM
DM, %	36.4 ± 0.29[ab]	37.8 ± 0.12[a]	35.9 ± 0.48[b]	35.5 ± 0.26[b]	0.251
Ash,% DM	17.3 ± 0.005[a]	14.1 ± 0.09[b]	12.9 ± 0.07[c]	12.5 ± 0.11[d]	0.081
CP, % DM	11.2 ± 0.287[a]	9.9 ± 0.10[ab]	8.7 ± 0.56[b]	8.0 ± 0.165[b]	0.330
EE, % DM	2.0 ± 0.009[a]	1.9 ± 0.03[ab]	1.7 ± 0.05[b]	1.8 ± 0.11a[b]	0.067
NDF, % DM	55.4 ± 1.781[a]	52.9 ± 0.69[ab]	49.2 ± 0.60[b]	48.4 ± 1.07[b]	1.141
ADF, % DM	45.2 ± 2.10	43.6 ± 0.15	43.2 ± 1.50	40.1 ± 0.30	1.304
Lignin, % DM	16.0 ± 1.03	15.9 ± 0.66	14.7 ± 0.93	12.5 ± 0.84	0.881
Silica, % DM	1.2 ± 0.058	1.2 ± 0.14	1.1 ± 0.03	0.9 ± 0.08	0.088
NSC, %, DM	14.1 ± 0.57[c]	21.7 ± 1.03[b]	28.3 ± 0.94[a]	28.3 ± 0.64[a]	0.823
pH	5.4 ± 0.02[a]	5.1 ± 0.011[b]	5.0 ± 0.005[c]	4.9 ± 0.005[d]	0.012
Lactic acid, % DM	2.2 ± 0.01[c]	2.7 ± 0.03[b]	2.9 ± 0.02[ab]	3.0 ± 0.09[a]	0.052
Acetic acid, % DM	0.6 ± 0.05[b]	0.6 ± 0.00[b]	0.7 ± 0.01[b]	0.9 ± 0.07[a]	0.044
Propionic acid, % DM	0.2 ± 0.00	0.2 ± 0.00	0.1 ± 0.01	0.1 ± 0.00	0.005
IVDMD, % DM	76.3 ± 1.58[b]	76.6 ± 0.25[b]	80.0 ± 1.01[ab]	82.4 ± 1.62[a]	1.249

T1 – (garlic foliage 90%, ground maize 10%, molasses 0%), T2 – (garlic foliage 85%, ground maize 10%, molasses 5%), T3 – (garlic foliage 80%, ground maize 10%, molasses 10%), T4 – (garlic foliage 75%, ground maize 10%, molasses 15%); DM – dry matter, CP – crude protein, EE – ether extract, NDF – neutral detergent fibre, ADF – acid detergent fibre, NSC – non-structural carbohydrates, IVDMD – *in vitro* dry matter digestibility, SEM – standard error of the mean; [a–d] – means within a row with different superscripts are significantly different at $P < 0.05$

Table 4. *In vitro* ruminal fermentation parameters in experimental treatments

Parameter	Experimental treatments				
	T1	T2	T3	T4	SEM
pH	6.9 ± 0.01	6.9 ± 0.01	6.9 ± 0.02	6.9 ± 0.02	0.019
AA, % TVFA	73.4 ± 0.47	74.5 ± 1.54	75.2 ± 4.56	76.1 ± 0.48	2.482
PA, % TVFA	12.0 ± 1.69	12.0 ± 0.04	12.5 ± 0.53	12.8 ± 0.24	0.898
BA, % TVFA	7.2 ± 0.11	7.1 ± 0.44	6.7 ± 1.34	6.4 ± 0.06	0.711
OSCFA, % TVFA	5.2 ± 0.37	5.7 ± 0.55	6.4 ± 0.10	6.0 ± 1.62	0.883
TVFA, mM	102.0 ± 7.75[b]	109.7 ± 4.39[b]	129.3 ± 10.13[ab]	147.1 ± 15.62[a]	10.87
Ac:P ratio	6.1 ± 0.10	6.2 ± 0.06	6.0 ± 1.29	5.9 ± 0.55	0.680
$N\text{-}NH_3$, mg/dl	6.0 ± 0.05[a]	5.5 ± 0.007[b]	5.3 ± 0.04[c]	5.0 ± 0.24[c]	0.361

T1 – (garlic foliage 90%, ground maize 10%, molasses 0%), T2 – (garlic foliage 85%, ground maize 10%, molasses 5%), T3 – (garlic foliage 80%, ground maize 10%, molasses 10%), T4 – (garlic foliage 75%, ground maize 10%, molasses 15%); AA – acetic acid, PA – propionic acid, BA – butyric acid, OSCFA – other short chain fatty acids (sum of isobutyrate + valerate + isovalerate), TVFA – total identified volatile fatty acids, Ac:P – acetate:propionate ratio, $N\text{-}NH_3$ – ammonium, SEM – standard error of the mean; [abc] – means within a row with different superscripts are significantly different at $P < 0.05$

In vitro gas production

Gas production kinetics, rumen fermentation parameters, and methane and CO_2 production are summarised in Table 5. It could be observed that the inclusion of molasses affected all parameters of gas production kinetics ($P < 0.05$) relative to T1. The inclusion of 15% molasses in T4 increased the maximum gas production (Gmax) and gas production at 24 h (GP_{24}) by approx. 48% in both variables. In addition, the constant rate of gas production (k)

Table 5. *In vitro* gas production kinetics and CH_4 and CO_2 production in experimental treatments

Parameter	Experimental treatments				
	T1	T2	T3	T4	SEM
Gmax, ml/g DM	93.9 ± 1.89[b]	114.9 ± 8.45[ab]	117.4 ± 6.45[ab]	139.0 ± 7.20[a]	12.9712
A, h	2.9 ± 0.10[a]	2.8 ± 0.10[ab]	2.7 ± 0.001[ab]	2.5 ± 0.035[b]	0.1120
k, %/h	0.08 ± 0.071[b]	0.15 ± 0.001[a]	0.14 ± 0.000[a]	0.16 ± 0.032[a]	0.0773
GP_{24}, ml/g DM	81.5 ± 6.81[b]	91.9 ± 1.56[b]	104.4 ± 1.91[ab]	120.5 ± 10.35[a]	3.6469
CH_4, ml/g DM	8.2 ± 0.45[b]	9.4 ± 0.40[b]	9.9 ± 0.38[b]	12.0 ± 0.58[a]	1.5990
CO_2, ml/g DM	59.5 ± 3.26[b]	68.8 ± 2.94[b]	71.9 ± 2.75[b]	87.1 ± 4.21[a]	11.5911
CH_4:CO_2 ratio	0.137 ± 0.0001	0.137 ± 0.0002	0.137 ± 0.0001	0.137 ± 0.0004	0.0021

T1 – (garlic foliage 90%, ground maize 10%, molasses 0%), T2 – (garlic foliage 85%, ground maize 10%, molasses 5%), T3 – (garlic foliage 80%, ground maize 10%, molasses 10%), T4 – (garlic foliage 75%, ground maize 10%, molasses 15%); DM – dry matter, Gmax – maximum gas production, A – lag time before the gas production begins, k – constant rate of gas production, GP_{24} – gas production after 24 h of fermentation, SEM – standard error of the mean; [abc] – means within a row with different superscripts are significantly different at $P < 0.05$

increased up to 80% in T4, while the lag phase (A) decreased by 16% in T4 compared to T1. Moreover, CH_4 and CO_2 production increased up to 46% for both variables with the maximum inclusion of molasses in the experimental treatments ($P < 0.05$). However, no changes were observed in the CH_4:CO_2 ratio ($P > 0.05$).

Discussion

Chemical composition

Molasses is an ingredient with a high content of soluble carbohydrates, but is not a source of protein or structural carbohydrates. Therefore, the inclusion of molasses in the experimental treatments led to a reduction in the protein and structural carbohydrate fractions. The addition of molasses increased the amount of soluble carbohydrates in the silage process, which improved the fermentation process. Thus, a decrease in pH was a consequence of the improved fermentation process, which in turn increased the concentration of lactic acid. According to Morales-Querol et al. (2019), this increase is a result of the proliferation of lactic acid-producing bacteria, which are responsible for the decrease in pH values as a result of the fermentation process. Previous study by González-Arreola et al. (2019) obtained similar values of NDF and ADF in maize silage compared to those presented in this work (González-Arreola et al., 2019). In fact, even the treatments with garlic foliage and molasses had higher values of CP and IVDMD compared to the results reported by González-Arreola et al. (2019), presumably due to an increase in the soluble carbohydrate fraction. López-Herrera et al. (2014) also reported higher digestibility, which they attributed to a reduction in the structural carbohydrates fraction (NDF and ADF). Similarly, Araiza-Rosales et al. (2015) found that the inclusion of molasses in the diet increased digestibility. The present study showed lower values of lactic acid in T1, indicating a reduced presence of LAB when compared to maize silages. However, the addition of molasses to the experimental treatments increased lactic and acetic acids levels, but butyric acid was not detected in the fermentation products. Amer et al. (2012) reported similar findings, showing that lactic and acetic acid increased with higher concentrations of water-soluble carbohydrates and the absence of butyric acid. The lack of butyric acid is an indicator of the absence of *Saccharolytic clostridial* fermentation and can cause a putrid odour in lactic fermentations of dairy products (Kalac, 2017).

In vitro ruminal fermentation parameters

Regarding *in vitro* ruminal fermentation, no changes were observed in rumen pH and in the individual proportions of volatile fatty acids, such as acetic, propionic, butyric and other short-chain fatty acids (OSCFA – the sum of valeric, isovaleric and isobutyric acids). However, the final concentration of total volatile fatty acids (TVFA) increased with the inclusion of molasses, as shown in Table 4. The proportions of individual fatty acids (FA) remained similar among the treatments, but molasses increased FA synthesis, which has also been reported previously by several authors (Van Dung et al., 2014; Kumar et al., 2013). Araba et al. (2002) also observed an increase in TVFA when molasses was added to a barley-based diet for bulls. Furthermore, the latter authors also reported a 10% increase in the acetate:propionate ratio; however, in this study, this ratio remained similar between the treatments. Putridinanti et al. (2019) added cassava powder as an energy source to the diet of steers and observed no changes in individual FA fractions but an increase in the final concentration of TVFA, which was consistent with the results obtained in the present study. In addition, the same authors also reported a significant decrease in N-NH_3 levels due to reduced protein fraction in the ration. Therefore, an increase in the molasses fraction in the silage should result in a reduced protein fraction. Previous research demonstrated that higher NH_3 concentrations were needed to increase the microbial fermentation rate in the rumen. However, some studies pointed out that ammonia concentrations of 5 mg/dl were required for optimal digestion (Putridinanti et al., 2019). The results obtained in this study showed that the minimum concentration at maximum molasses inclusion was 5 mg/dl, while the higher concentration of ammonia (T1, 6 mg/dl) was reached with no molasses addition. Furthermore, Panthee et al. (2017) reported an enhancement of nitrogen utilisation with higher proportions of garlic foliage used as a supplement, which was consistent with the results of the current study.

In vitro gas production

The parameters of gas production kinetics are given in Table 5. It was notable that the maximum gas production (that the Gmax) increased with raising proportion of molasses in the silage. Specifically, Gmax increased by 48% with the addition of 15% molasses to the silage. This effect could be attributed to the increased digestibility resulting from the reduction of the structural carbohydrate fraction due to the addition of molasses in the treatments, which increased the availability of energy from the soluble carbohydrates in molasses. This observation was consistent with the findings of Sahoo and Walli (2008), who observed an increase in energy with incrementing molasses content in the diet of cattle utilised for milk and meat production. The addition of molasses to T4 in our study increased Gmax and made it comparable to that obtained from using solely maize silages (González-Arreola et al., 2019). Additionally, a shorter lag phase would be expected as a consequence of the reduction in structural carbohydrate levels, leading to increased digestibility. Tuyen et al. (2014) found that adding molasses to silage could increase microbial protein production, resulting in a reduction in the lag phase and an increase in gas production after 24 h of fermentation. This increase in gas production was due to higher proportions of molasses in the silage, which led to higher soluble carbohydrate levels and rapid gas production. As a consequence, the time for reaching the asymptotic phase in gas production (stationary phase) was shorter (Ferraro et al., 2009). Similarly, CH_4 and CO_2 production increased along with higher proportion of molasses in the silage, although higher content of garlic foliage in the silage could reduce generation of these gases. Likewise, Arndt et al. (2015) showed that increasing dry matter content and digestibility could enhance CH_4 and CO_2 production. In experiment T1, lower methane concentrations were obtained than in silages produced solely from maize; however, the maximum addition of molasses in experiment T4 resulted in a higher methane production compared to that obtained only from maize silage (González-Arreola et al., 2019). Previous studies have reported that higher concentrations of lactic acid can lead to a reduction in CH_4 synthesis. This is because higher concentrations of lactic acid stimulate propionate synthesis, which is a natural competitor of methanogenesis for hydrogens (Alli et al., 1984). In this study, propionic acid levels remained similar despite increasing molasses proportion in the experimental treatments. However, the concentration of TVFA increased with the addition of molasses, which lowered the level of propionic acid. The latter suggested that more hydrogens were being released and not contributing to the synthesis of propionic acid. Instead, more methane was being synthesised, which was consistent with findings of the current study, i.e. methane concentration increased with molasses addition. Previous research has described a symbiotic relationship between protozoa and methanogens, where methanogenic archaea take advantage of the hydrogen transfer due to protozoan activity and synthesise methane as a result of their metabolism (Francisco et al., 2019). The secondary metabolites and bioactive sulphur compounds contained in garlic foliage have been demonstrated to exhibit antimicrobial, antibacterial, antifungal and antiprotozoal activity. Thus, T1 which contained the highest concentration of garlic foliage (i.e. also sulphur compounds), was expected to show a higher antiprotozoal activity, and in consequence, lower methanogenesis due to reduced transfer of hydrogens, as detailed earlier (Sari et al., 2022). On the other hand, if garlic foliage and methanogenesis suppressors were reduced, a higher protozoal activity should be expected, and indeed it was observed in T4. Similarly, CO_2 production increased with molasses addition. This could be explained by the fact that molasses provided energy to the system, enhancing the fermentation process and increasing gas production. Indeed, this was observed in Gmax values, which exceeded 40 ml/g, representing nearly 50% of that obtained in T1. The increased production of CO_2 and methane due to hydrogen transfer would suggest minimal changes in the CH_4:CO_2 ratio, which was indeed the case. These results indicated that the presence of molasses increased the efficiency of energy utilisation. This theory was supported by some authors, who reported proportional increases in total gas, methane and CO_2 production levels (Castro et al., 2021; López-Aguirre et al., 2016). Although Limón-Hernández et al. (2019) observed no changes in gas production kinetics parameters, they reported an increase in methane production when 2% molasses was added to the experimental treatment in *in vitro* assays.

Conclusions

The addition of molasses to garlic foliage silage improved *in vitro* digestibility and gas production, as well as increased methane production to the same level as CO_2 due to improved fermentation. However, the addition of molasses did not change the proportion of VFA in rumen fermentation products, as this parameter increased in the same proportions during fermentation without molasses. Therefore, adding molasses may not be necessary in the production of garlic foliage silage as a non-conventional alternative for ruminant feed and agricultural waste reduction. It is recommended to conduct *in vivo* trials to assess the palatability of the silage to animals.

Conflicts of interest

The Authors declare that there is no conflict of interest.

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