




Chemical composition, and in vitro fermentation of ripe mango silage with molasses

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Abstract The objective was to evaluate the in vitro fermentation in silage of ripe mango with pangola grass hay and levels of sugar cane molasses as additive. The treatments were: 0 (T0%), 3 (T3%), 6 (T6%) and 9% (T9%) molasses. The ensilage was fermented for 21 days. Dry matter (DM), pH values, lactic acid, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ashes (As), partial and the accumulated biogas and methane production, DM degradation (DMD) and NDF degradation (NDFD) were determined in the ensilages. Variables were analyzed in a completely random design. T0% showed lower DM and As content, as well as a higher lactic acid concentration. T3% and T6% had different CP content. The higher NDF and

ADF content was found in T0% and T3%. With regard to partial biogas production, T0% had the higher partial biogas production at 6 h, while T3% and T6% had the higher production at 9 and 48 h; meanwhile, T0% and T3% had the higher production at 24 h, and T9% at 72 h. The higher partial methane production happened in T0% at 48 h and in T3% and T9% at 72 h. T0% and T3% had a higher DMD and NDFD. The lower accumulated biogas production took place in T3% and T6% ($p < 0.05$). Therefore, ripe mango ensilages (using pangola grass) do not require an additive (such as molasses) to improve the bromatological and fermented quality of the ensilage.

Keywords Molasses · Ensilage · Mango · In vitro · Pangola

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Introduction

Each year, the agri-food industry produces large amounts of waste all over the world, causing a serious pollution problem. These agricultural residues decompose in the fields—as part of their natural process—or they are burned. An agricultural residue that causes pollution problems due to its overproduction is the fruit of the mango tree (*Mangifera indica* L.). This fruit has: 74–94% moisture, 13.5–21% carbohydrates, 0.4–0.8% proteins, and 0.4% lipids; it is a source of calcium, phosphorus, iron (Yahía et al. 2006),

potassium, magnesium (Guha et al. 1996), and vitamins C, A, B₁, and B₃. The agro-industry uses 80% of the fresh mango to produce pulp, juice, and nectar. However, during the selection process, 28–43% of the fruit is wasted (Magaña et al. 2006), and this waste could potentially be used as fodder for ruminants, because the pulp and the skin are a source of carbohydrates (Cavallini et al. 2015).

The seasonal variation of mango production requires preservation methods, such as ensilage. This is a preservation method for fodder and agro-industrial by-products that are potentially suitable for feeding livestock (Guzmán et al. 2012), because soluble carbohydrates ferment in lactic acid, in order to retain the nutritional value of the ensilaged material (Bezabih and Tamir 2014). Additionally, including additives to the ensilage process improves fermentation and increase the nutritional value (Valencia et al. 2011). Dry fodder and agricultural urea are two of the additives used in mango ensilage (Razzaghzadeh et al. 2007; Halik et al. 2014); dry fodder controls the excess of moisture in the mango, while agricultural urea is a source of non-protein nitrogen (Guzmán et al. 2012). Additionally, molasses is a final crystallization residue of the physical process of sugar extraction. It has 80% DM, 35% sucrose, 15% glucose, and 4.5% nitrogenated compounds (Anaya-Reza and López-Arenas, 2018). Molasses is used as additive in ensilages, due to its soluble carbohydrates content, and it is also frequently used in 4–5% concentrations in tropical leguminous plants and pulses ensilages (Henderson 1993; Bolson et al. 1996).

Mango ensilages evaluations have been carried out in Brazil (Lima et al. 2007) and Venezuela (Cavallini et al. 2015) where ensilages were prepared using mango, corn stubble, green grass, hays, and ground corn, among other agro-industrial by-products. The use of molasses is in order to increase the amount of non-structural carbohydrates to improve the quality characteristics of the mango ensilage in the fermentation process, because mango does not have enough non-structural carbohydrates, so the hypothesis was that the increasing addition of molasses in ripe mango ensilage with pangola grass hay improves the content of non-structural carbohydrates. Accordingly, the objective of this study was to evaluate the quality, the bromatological composition, and the in vitro fermentation of ripe mango ensilages, with increasing

levels of sugar cane molasses as an additive in the ensilage process.

Materials and methods

Study site

The study was done at the Animal Nutrition Laboratory of the Faculty of Veterinary Medicine and Zootechny No. 2, Autonomous University of Guerrero (Cuajinicuilapa, Guerrero, Mexico).

Ensilages

The ingredients used to prepare the ensilages were ripe Ataulfo mango (*Mangifera indica*), pangola grass hay (*Digitaria decumbens*)—harvested and packed at 120 days of regrowth—, sugar cane molasses, and urea. The ripe mango and the pangola grass were crushed in a M.A.GRO[®] TR-3500 (Mexico) mixed-use mill and their bromatological composition can be found in Table 1. The following treatments were used (2 kg per silo): T0% = 89% mango, 9% pangola straw, and 2% urea; T3% = 86% mango, 9% pangola straw, 3% molasses, and 2% urea; T6% = 83% mango, 9% pangola straw, 6% molasses, and 2% urea; T9% = 80% mango, 9% pangola straw, 9% molasses, and 2% urea. Propylene bags (40 × 40 cm) were used to prepare the ensilage; the air was extracted using a Koblenz[®] (Spain) vacuum cleaner, in order to achieve the anaerobiosis conditions. The bags were sealed with raffia to preserve the anaerobiosis

Table 1 Bromatological composition of mango and pangola grass hay

Variable	Mango	Pangola grass hay
Dry matter (%)	24.35	92.36
Ashes (%)	2.79	6.13
Organic matter (%)	97.21	93.87
Crude protein (%)	5.59	6.57
Water soluble carbohydrates (%)	41.98	7.44
Ethereal extract (%)	2.25	–
Neutral detergent fiber (%)	28.95	70.75
Acid detergent fiber (%)	15.65	39.68

conditions and the fermentation process lasted for 21 days in a shed, at an average 33 °C room temperature.

Ensilage quality indicators

The pH was determined pouring 20 g of an ensilage in a 100 mL Kimax® beaker, adding 50 mL of distilled water (pH 7); the beaker was stirred for 3 h, at 15 min intervals. The beaker content was filtered with a double gauze and was placed in a 10 mL beaker in order to measure its pH using a Hanna® HI2211 potentiometer (Italy; pH calibration 7 and 4). Dry matter (DM, 930.15 Method) was estimated according to the AOAC Methodology (2005).

One g of ensilage and 10 mL of distilled water were poured into a 100 mL beaker. The beaker was refrigerated for 20 min and, subsequently, incubated at room temperature for 20 min, and stirred at 10 min intervals. The beaker content was filtered using a double gauze and 1 mL of the filtered material was poured into an Eppendorf tube (Neptune, Mexico) with 0.25 mL of Meyer® metaphosphoric acid at 25% (w/v). Lactic acid concentration was estimated according to Kinberley and Taylor Methodology (1996).

Bromatological analysis

The ensilage samples were dehydrated in a Riossa® HCF-41 stove (Mexico), at 60 °C during 72 h. The ensilages were crushed using a 1 mm sieve in a Thomas-Wiley Mill (Thomas Scientific®, Swedesboro, NJ, USA). The crude protein (CP; 920.105 Method) and ashes (As, 942.05 Method) content of the samples were determined using the methods described by the AOAC (2005). The method proposed by Van Soest et al. (1991) was used to determine the neutral detergent fiber (NDF) and the acid detergent fiber (ADF).

Biodigesters

In glass serological vials (120 mL), 0.5 g of DM of one type of treatment were added, along with 45 mL of culture medium. All the pipettes were kept in anaerobic conditions with CO₂ and each vial was considered as a biodigester and an experimental unit (five independent samples per treatment). The biodigesters were sterilized for 15 min in an All American®

1941X gas autoclave (USA), at 121 °C and 15 psi (Sánchez-Santillán et al. 2016). The biodigesters were inoculated with 5 mL of fresh rumen fluid (1157 g were centrifuged during 3 min) and incubated in a bath Marie at 39 °C for 72 h.

Each 100 mL of medium had: 30 mL clarified rumen fluid (12,857 g fresh rumen fluid were centrifuged during 10 min and sterilized for 15 min at 121 °C and 15 psi), 5 mL mineral solution I [6 g K₂HPO₄ (Sigma-Aldrich®) in 1000 mL distilled water], 5 mL mineral solution II [6 g KH₂PO₄ (Sigma-Aldrich®) + 6 g (NH₄)₂SO₄ (Merck®) + 12 g NaCl (Sigma-Aldrich®) + 2.45 g MgSO₄ (Sigma-Aldrich®) + 1.6 g CaCl₂·2H₂O (Sigma-Aldrich®) in 1000 mL distilled water], 0.1 mL resazurin at 0.1% (Sigma-Aldrich®), 0.2 g soya peptone (Merck®), 0.1 g yeast extract (Sigma-Aldrich®), 4 mL cysteine-sulfide solution [2.5 g L-cysteine (Sigma-Aldrich®) in 15 mL 2 N NaOH (Meyer®) + 2.5 g Na₂S·9H₂O (Merck®) gauged in 100 mL distilled water], 5 mL solution at 8% of Na₂CO₃ (Merck®), and 52.6 mL distilled water (Herrera-Pérez et al. 2018). The fresh rumen fluid was obtained from a bovine with a rumen cannula—previously fed in pangola grass prairies—and it was filtered using a cheesecloth in order to remove organic matter macroparticles. The bovine was handled according to the internal bioethics and welfare guidelines of the UAGro, which are based on two Mexican official standards (NOM-051-ZOO-1995).

Biogas and methane production

The *in vitro* production of biogas was measured using the movement of the plunger of a glass syringe (50 mL; BD Yale®, Brazil) at 3, 6, 9, 12, 24, 36, 48, and 72 h.

The CH₄ was captured using NaOH (2 N) [80 g NaOH (Merk®) in 1000 mL distilled H₂O] solution traps. The 60 mL serological vial were completely filled with the NaOH (2 N) solution and hermetically sealed. The traps were changed every 24 h, during the 72 h of the fermentation. The biodigesters were connected to the capture traps by a 3/32" wide and 45 cm long Taygon® hose. The hose was adapted with 20 G × 32 mm hypodermic needles at both ends. The trap had a needle with the above-mentioned characteristics, which acted as a release valve for the solution; additionally, the trap was placed upside-

down in a plastic test tube, with a V-cut, which enable the collection of the solution displaced by the CH_4 produced during the incubation (Torres-Salado et al. 2018).

Fermentation characteristics

The bacteria count, the dry matter degradation (DMD), the neutral detergent fiber degradation (NDFD), and the ammoniacal nitrogen concentration ($\text{NH}_3\text{-N}$) were determined 72 h after the biodigesters were incubated. The bacteria count was evaluated by direct count in a Petroff-Houser[®] chamber (Hernández-Morales et al. 2018). The $\text{NH}_3\text{-N}$ concentration was estimated according to McCullough (1967). The biodigester content was filtered in ANKOM[®] bags, at constant weight, in order to recover the non-degraded DM, and the difference in weights was used to calculate the DMD and NDFD was estimated according to Hernández-Morales et al. (2018).

Experimental design

The experimental design was completely random, with five replicates per variable. The results were analyzed using the GLM of SAS (SAS Inc. 2011) and the means were compared using the Tukey test ($p < 0.05$). The response to the increasing molasses content was calculated through orthogonal linear and quadratic contrasts.

Results

The DM in the mango ensilages showed a linear increase (Table 2; $p = 0.0001$) as an increasing amount of molasses was added, in comparison with the control. The content of lactic acid, NDF and ADF showed a linear decrease as an increasing amount of molasses was added to mango ensilages ($p = 0.0001$). In contrast, the amount of As showed a linear increase (Table 2) in mango ensilages as an increasing amount of molasses was added, in comparison with the control treatment ($p = 0.0001$). pH did not have any orthogonal effect or showed any difference between treatments ($p > 0.05$) as a result of the increasing level of molasses in ensilages; therefore, the ensilages had an average pH of 4.20 (Table 2). The content of crude protein (CP) did not show orthogonal effect of the

treatments with respect to the control ($p > 0.05$); but, T3% presented 12.40% higher CP content than T6% ($p < 0.05$) and both treatments did not show differences with the rest of the treatments ($p > 0.05$, Table 2).

The partial production of biogas at 3 and 6 h of fermentation in mango ensilages showed a linear decrease (Table 3), as the content of molasses increased in comparison with the control treatment ($p = 0.022$ and 0.0001). Additionally, biogas production at 6 h had a quadratic effect, and the production of T0% and T6% was higher. At 9 h, biogas production had a quadratic effect and T3% had the highest production. Nevertheless, adding molasses did not modify the partial production of biogas at 12 h of fermentation. However, the partial production of biogas at 24 h showed a linear decrease (Table 3), as the content of molasses increased in comparison with the control treatment ($p = 0.0001$); meanwhile, at 48 and 72 h of incubation, the production showed a linear increase in mango ensilages, as an increasing amount of molasses was added, in comparison with the control witness ($p = 0.019$ and 0.0001). Therefore, the accumulated biogas production at 72 h of fermentation showed a linear decrease when molasses was added ($p = 0.0002$), and most of the biogas was produced by T0% and T3%.

Adding molasses did not affect the partial production of CH_4 in mango ensilages at 24 h of incubation, reaching an average of 21.63 mL g^{-1} . CH_4 production showed a linear decrease at 48 h, as the content of molasses increased, in comparison with the control ($p = 0.0001$). In contrast, the partial production of methane at 72 h of incubation showed a linear increase in mango ensilages, as an increasing amount of molasses was added, in comparison with the control treatment ($p = 0.0014$). Therefore, adding molasses did not modify the accumulated methane production at 72 h of incubation and its average was $36.78 \text{ mL g MS}^{-1}$ (Table 3). Consequently, whether molasses is added or not does not affect CH_4 production in ripe mango and pangola grass hay ensilages.

The total bacteria count ($p = 0.0001$), DMD ($p = 0.0007$), NDFG ($p = 0.0376$), and ammoniacal nitrogen concentration showed a linear decrease (Table 3), as the content of molasses increased, in comparison with control. This is a sign that ripe mango-pangola grass hay ensilages do not require

Table 2 Quality and bromatological composition of ripe mango silages, using molasses as additive

	T0%	T3%	T6%	T9%	SEM	Tukey test	Linear	Quadratic
DM	22.52 ^b	25.15 ^a	24.62 ^a	25.17 ^a	0.303	< 0.0001	0.0001	0.0013
pH	4.58	4.01	3.99	4.21	0.111	0.2129	0.2442	0.0826
Lactic	11.96 ^a	9.67 ^b	9.76 ^b	8.06 ^b	0.408	0.0004	0.0001	0.5039
CP	32.37 ^{ab}	34.53 ^a	30.72 ^b	31.92 ^{ab}	0.515	0.031	0.1444	0.5172
NDF	53.47 ^a	52.53 ^a	47.16 ^b	45.30 ^b	0.919	< 0.0001	0.0001	0.3542
ADF	31.19 ^a	30.52 ^a	27.89 ^b	25.80 ^b	0.602	< 0.0001	0.0001	0.1946
As	5.40 ^c	6.31 ^b	7.25 ^a	7.01 ^a	0.188	< 0.0001	0.0001	0.0001

Means in the same row with different superscript letters are different ($p < 0.05$)

T0% = 89% mango, 9% pangola straw, and 2% urea; T3% = 86% mango, 9% pangola straw, 3% molasses, and 2% urea; T6% = 83% mango, 9% pangola straw, 6% molasses, and 2% urea; T9% = 80% mango, 9% pangola straw, 9% molasses, and 2% urea; DM = dry matter (%); Lactic = percentage of lactic acid with regard to dry matter (%); CP = crude protein (%); NDF = neutral detergent fiber (%); ADF = acid detergent fiber (%); As = ashes (%); SEM = standard error of the mean

Table 3 Production of biogas, methane, and in vitro fermentative characteristics of ripe mango ensilages which various percentages of molasses were added

	T0%	T3%	T6%	T9%	SEM	Tukey test	Linear	Quadratic
PB ₃	36.37	30.19	27.71	21.54	2.253	0.1225	0.022	0.999
PB ₆	42.05 ^a	19.03 ^c	32.27 ^b	23.48 ^c	2.353	< 0.0001	0.0001	0.0002
PB ₉	24.92 ^b	33.37 ^a	26.73 ^{ab}	24.04 ^b	1.232	0.0116	0.2639	0.0084
PB ₁₂	12.46	9.00	10.08	10.51	0.555	0.1592	0.3116	0.0775
PB ₂₄	56.61 ^a	56.57 ^a	35.28 ^b	28.48 ^b	3.476	< 0.0001	0.0001	0.244
PB ₄₈	48.27 ^{ab}	56.56 ^a	53.95 ^a	38.05 ^b	2.209	0.0022	0.019	0.0009
PB ₇₂	11.43 ^d	16.92 ^c	21.67 ^b	36.98 ^a	2.500	< 0.0001	0.0001	0.0005
CH ₄₋₂₄	22.22	24.09	22.22	17.98	1.955	0.7671	0.4503	0.4784
CH ₄₋₄₈	11.34 ^a	7.38 ^b	5.05 ^b	5.01 ^b	0.716	< 0.0001	0.0001	0.0059
CH ₄₋₇₂	4.14 ^b	3.69 ^b	6.82 ^{ab}	8.52 ^a	0.644	0.0064	0.0014	0.2446
PAB ₇₂	218.53 ^a	219.10 ^a	200.36 ^{ab}	173.59 ^b	5.594	0.001	0.0002	0.0568
CH _{4-A72}	35.64	38.83	37.11	35.51	1.218	0.7881	0.8588	0.3779
DMD	74.41 ^a	70.60 ^{ab}	69.63 ^b	71.56 ^{ab}	0.616	0.0011	0.0007	0.8943
NDFD	59.41 ^a	52.36 ^a	44.76 ^b	41.64 ^b	1.957	0.0164	0.0376	0.0084
[B]	7.2 ^a	7.6 ^a	5.2 ^b	5.5 ^b	0.315	< 0.0001	0.0001	0.2967
NH ₃ -N	46.56 ^a	42.81 ^{ab}	41.42 ^b	43.67 ^{ab}	0.666	0.0236	0.047	0.0121

Means in the same row with different superscript letters are different ($p < 0.05$)

T0% = 89% mango, 9% pangola straw, and 2% de urea; T3% = 86% mango, 9% pangola straw, 3% molasses, and 2% urea; T6% = 83% mango, 9% pangola straw, 6% molasses, and 2% urea; T9% = 80% mango, 9% pangola straw, 9% molasses, and 2% urea; PB₃ = partial production of biogas at 3 h (mL g⁻¹ DM); PB₆ = partial production of biogas at 6 h (mL g⁻¹ DM); PB₉ = partial production of biogas at 9 h (mL g⁻¹ DM); PB₁₂ = partial production of biogas at 12 h (mL g⁻¹ DM); PB₂₄ = partial production of biogas at 24 h (mL g⁻¹ DM); PB₄₈ = partial production of biogas at 48 h (mL g⁻¹ DM); PB₇₂ = partial production of biogas at 72 h (mL g⁻¹ DM); CH₄₋₂₄ = partial production of methane at 24 h (mL g⁻¹ DM); CH₄₋₄₈ = partial production of methane at 48 h (mL g⁻¹ DM); CH₄₋₇₂ = partial production of methane at 72 h (mL g⁻¹ DM); PAB₇₂ = accumulated production of biogas at 72 h (mL g⁻¹ DM); CH_{4-A72} = accumulated production of methane at 72 h (mL g⁻¹ DM); [B] = rumen bacteria count (10⁸ cells mL⁻¹); DMD = in vitro dry matter degradation (%); NDFD = in vitro neutral detergent fiber degradation (%); NH₃-N = ammoniacal nitrogen (mg dL⁻¹)

SEM standard error of the mean

molasses to improve neither their *in vitro* fermentative characteristics, nor the accumulated biogas production.

Discussion

In this study, dry matter increased by 10.92% in the mango ensilages to which molasses was added (Table 2); this can be attributed to the additional DM (2.95 times) provided by each unit in which molasses was included (Baytok et al. 2005), in comparison with the 26.4% dry matter contained in mango (Guzmán et al. 2012). The DM in this study was 18.70 and 29.37% lower than the percentage published by Guzmán et al. (2010) and Guzmán et al. (2012), who included up to 85% mango plus fodder, with and without urea. Overproduction (Ajila et al. 2007; Jawad et al. 2013), fruit waste during its selection for commercialization purposes (Magaña et al. 2006) and its 8–4 °Brix soluble carbohydrates (Salamanca et al. 2007) enable the use of mango—along with other fodder sources—in ensilages that are used to feed ruminants in tropical regions.

Although molasses was used as an additive and a source of soluble sugars (Bolson et al. 1996), in order to increase the concentration of fermentable carbohydrates in the mango ensilage process, the lactic acid content diminished, without having an impact on the pH of the ensilage. These findings matched the results of Guzmán et al. (2010) who also recorded no modification of the pH of the ensilage when 3 and 2% molasses and urea were respectively added. The diminishing of lactic acid as the amount of molasses added to ensilage increased may be attributed to an inhibition caused by high sugar concentrations. Those levels inhibit the microbial growth of the homofermentative and heterofermentative lactic acid bacteria that can be found in ensilages, consequently diminishing the lactic acid concentration (Nancib et al. 2015; Anaya-Reza and López-Arenas, 2018). Martínez-Teruel et al. (2007) published lower values than those found out in this study for lactic acid in ensilages that include the husk, grains, and cobs of sweetcorn: they reported 5.3% concentrations with regard to DM, after 90 days of fermentation.

Likewise, the increasing amounts of molasses used to substitute mango diminished the NDF and ADF content in ensilages, because the full mango fruit

contains an average of 54.4 and 2.75 NDF and ADF, respectively (Sruamsiri and Silman 2009); meanwhile, molasses does not contribute any structural carbohydrates, because it lacks fibrous fraction. Likewise, the gradual increase of molasses in mango ensilages could have influenced the higher content of ashes, because molasses can include nine times more ashes per kg of DM, in comparison with the whole mango fruit (Guzmán et al. 2012).

Biogas production during the first 24 h after incubation indicates the amount of non-structural carbohydrates (Sánchez-Santillán et al. 2015; Torres-Salado et al. 2018) which contains feed that will be fermented during rumination; therefore, mango ensilages produced 62.24–78.90% of the total biogas (Table 3) produced during the first 24 h. Consequently, the content of structural carbohydrates resembles that of corn ensilages (Aragadvay-Yungán et al. 2015), given the biogas production in both types of ensilages. During the next 24 h of incubation, a 24.19% average of the total biogas (Table 3) was produced in the ensilages, because structural carbohydrates (such as hemicellulose) had started their fermentation process (Sánchez-Santillán et al. 2015). Seventy-two hours after incubation, biogas production increased as the content of molasses in the treatments increased: this is an indication that the fermentation of structural carbohydrates (such as cellulose) had improved (Sánchez-Santillán et al. 2015). In this study, the total biogas production in mango ensilages was higher than in sunflower fodder ensilages (Aragadvay-Yungán et al. 2015), and unripe, physiologically ripe, and ripe mango ensilages (Cavallini et al. 2015). However, they were lower than the corn ensilages evaluated by Aragadvay-Yungán et al. (2015) after 72 h of fermentation, and higher than the results reported by Antolín et al. (2009) for hybrid maize ensilages subject to 96 h of fermentation. However, the total biogas production shows that ripe mango ensilages that include pangola grass hay do not require the addition of molasses, since the molasses-free treatment had the highest biogas content and it showed a linear decrease as more molasses was added (Table 3).

There were no differences with regard to CH₄ production from one treatment to another, 24 h after incubation (Table 3); but, although there was a variation between treatments during the next 48 h (Table 3), what matters is how much biogas was

produced by CH₄. CH₄ accounted for 16.31% of the total biogas production of T0%, and it increased as more molasses was added to ensilages: for T9%, CH₄ amounted to 20.46% of the total biogas. This can be attributed to the partial production of biogas from 48 to 72 h. The biogas produced can be attributed to the fermentation of the cellulose. The main fermentation products of cellulose are acetate, CO₂, and H₂ (Zhang et al. 2015; Sánchez-Santillán et al. 2016; Sánchez-Santillán and Cobos-Peralta, 2016). Therefore—as a metabolic strategy to obtain energy—methanogenic *Archaea* use H₂ and CO₂ as a substrate to produce CH₄ (Araujo et al. 2011; Noguera et al. 2011; Ramírez et al. 2014). The production of CH₄ in this study was higher than the production reported by Navarro-Villa et al. (2012), who mentioned that a 22.9 mL production of CH₄ g⁻¹ DM was achieved in *Lolium perenne* grass ensilages. Therefore, including molasses in ripe mango ensilages with pangola grass hay does not affect the accumulated production of CH₄ at 72 h.

Various non-conventional feed products for ruminants were evaluated in vitro to develop strategies that diminish environmental pollutants and to improve the productive parameters of production units. Therefore, before carrying out in vitro evaluations, in vitro degradations are executed in order to determine if their use as fodder for ruminants is feasible. In this study, the average DMD in ensilages was 71.55% (Table 3); the values were greater for corn silages (Aragadway-Yungán et al. 2015) and lower for corn-cowpea ensilages (Castillo et al. 2009). This variability is attributed to the bromatological conditions of the products that will be used for the ensilage process (Posada and Noguera 2005). These conditions specifically include the composition of the cell wall (Ramírez et al. 2002) and the soluble sugars that are available (Araiza-Rosales et al. 2015); since, in this study, the results are higher than the DMD of the artichoke (Martínez-Teruel et al. 2007), pepper (De Haro et al. 2001), and passion fruit (Espinoza-Guerra et al. 2016) ensilages; however, they are lower than Chinese potato (Caicedo et al. 2015) and apple (Araiza-Rosales et al. 2015) ensilages.

The NDFD diminished as the levels of cane molasses used as an additive during the ensilage process increased; the value of T9% was lower in this study (Table 3), without being lower than 40%, because lower percentages indicate a negative impact on the energy content and the potential consumption of

DM (Hoffman et al. 2007b). NDFD values above 50% for T0% and T3% (Table 3) help to guarantee that the animal will receive a good level of nutrients, since—during the lignification process of the plant cell wall (NDF)—the adherence and hydrolysis of cellulolytic bacteria diminish (Hoffman et al. 2007a). The total bacteria count diminished by 31.6% when the amount of sugarcane molasses added to ensilages increased from 3 to 6%. An average population of 7.4×10^8 bacteria mL⁻¹ was quantified. These values were similar to the results of in vitro tests where fibrous substrates (Chanthakhoun et al. 2012; Sánchez-Santillán et al. 2016) and the pods and leaves of pulses (Hernández-Morales et al. 2018) were evaluated. The NH₃-N content in the cultivation medium varied between T0% and T9% (Table 2), indicating that, as a result of their molasses content, the nitrogenous fractions of the mango ensilages had various levels of degradation (Rodríguez et al. 2010; Khejornsart et al. 2011). The difference in the NH₃-N content can be attributed to the effect that adding molasses to the ensilage had on the microbial population of this study; a variation in the NH₃-N content when nitrogenous ingredients are included is expected (Christensen et al. 2016), but not as a consequence of adding energy ingredients.

Conclusion

Based on the ensilage quality, bromatological analysis, biogas production, methane, and in vitro fermentative characteristics variables, this study proves that it is not necessary to add molasses to improve the said variables in the production of ripe mango ensilages with pangola grass hay; on the contrary, adding more than 6% molasses has a negative effect on the above-mentioned characteristics.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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