



Fermentation Profile and Nutrient Composition of Rehydrated Dry Mesquite Pod (*Prosopis* spp.) Silage with Increasing Moisture Levels

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ABSTRACT

Mesquite pods are traditionally used to feed livestock in arid regions. However, they are highly susceptible to beetle and other insect spoilage, so they must be preserved as silage for use during critical periods of feed scarcity. This study aims to assess the effects of increasing moisture levels from dry ground mesquite pods on fermentation parameters and nutrient composition of the silage as a method of preservation. The treatments consisted of adding 0%, 10%, 20%, 30%, 40%, and 50% water (w/w). Each treatment received four PVC mini silos under a completely randomized design. On average, each mini silo was filled with 1,325 g of ground mesquite pods for ensiling. All mini silos were stored at room temperature (17-22.5 °C) and opened after 40 days of fermentation. The treatments were compared by polynomial orthogonal contrasts. Fermentation results indicated that increasing the amount of added water reduced the pH ($p = 0.003$) and linearly increased lactic acid ($p = 0.0002$) in silage. Ammonia-N and the concentration of acetic and propionic acids had a quadratic effect ($p = 0.004$). Silage with 20-30% water had an intermediate pH (4.65-4.53) and lower ammonia-N levels (5.59-6.51). Losses of gas, effluent, and dry matter exhibited linear and quadratic effects ($p < 0.0001$) as water content increased, but after the 40% water treatment, these losses increased considerably. Results of nutritive value showed that crude protein was not significantly affected by the treatments ($p > 0.05$). However, neutral and acid detergent fiber increased both linearly ($p < 0.05$) and quadratically ($p < 0.0001$), while non-fiber carbohydrates decreased linearly ($p = 0.007$) and quadratically ($p < 0.0001$) as the water content increased. These changes resulted in a quadratic decline in metabolizable energy ($p = 0.05$) and net energy for gain ($p = 0.008$), along with a negative linear response to dry matter digestibility. Overall, adding 20%-30% water to dry mesquite pods during silage production appears optimal for preserving mesquite pods as a feed resource for ruminants. This range allows favorable nutrient concentrations and appropriate fermentation variables, while maintaining intermediate levels of gas, effluent, and dry matter losses.

Keywords: arid regions; fermentation; preservation; *Prosopis* spp.; ruminants

INTRODUCTION

Mesquite (*Prosopis* spp.) is a tree distributed in arid and semiarid regions of the world as a native or introduced species (Shackleton *et al.*, 2014). Indeed, mesquites may become one of the most abundant plants in the future of these hot climate regions because of global climate change (Mengistu *et al.*, 2023). For this reason, it is necessary to explore the different uses of mesquite to benefit the population and its potential use in feeding livestock (Ruíz-Nieto *et al.*, 2020).

One of the primary uses of mesquite is the use of its pods to feed livestock. The pods have been successfully included in feeding cattle, sheep, camels, buffaloes, rabbits, and poultry (Sawal *et al.*, 2004). Grazing animals consume fresh mesquite pods still on the tree, and when they have lost moisture and are found on the ground (Angeles-Hernández *et al.*, 2024). Moreover, it is common for farmers to collect and store mesquite pods to offer as feed to their animals in a pen (Ruíz-Nieto *et al.*, 2020). However, because the pods are susceptible to beetles and other insect pests when they are in the

field or stored (Parra-Gil *et al.*, 2020), their availability is seasonal, and consequently, the use of pods during the dry season is limited.

One alternative for preventing the deterioration of the pods and using them in periods of feed scarcity is their preservation as silage. Nevertheless, for proper preparation of the silage, grinding the pods is advisable. Grinding permits better results during the silage process, and it is beneficial for the animals that consume it since the protein content in the seed embryo is readily available for digestion (Ruíz-Nieto *et al.*, 2020). Moreover, grinding the mesquite pod prevents problems such as bloating (Cook *et al.*, 2008) caused by an excess of seeds that obstruct the passage of feed through the reticulum-omasal orifice and neural because of excessive chewing (Tabosa *et al.*, 2000). However, because mesquite pods lose moisture during maturation from 60% to 10% (Montañez-Valdez *et al.*, 2021), it is necessary to add water to promote fermentation in the silo. To achieve an adequate fermentation profile during ensiling, the plant or the material to be ensiled must have an appropriate moisture and carbohydrate content (Viana *et al.*, 2023). Under these conditions, sufficient lactic acid is produced to reduce the pH enough and preserve the material in the silo. This rehydration technique has been used successfully with dry corn grain; functioning appropriately, it improves nutritive value and extends preservation time (Ferrareto *et al.*, 2015; Andre Arcari *et al.*, 2016; da Silva *et al.*, 2018; Mombach *et al.*, 2018; Carvalho-Estrada *et al.*, 2020). Despite its positive results, the use of this technique is not widespread in other plant species, including mesquite. Therefore, our objective was to assess the effects of increasing moisture levels from dry ground mesquite pods on fermentation parameters and nutrient composition of the silage as a method of preservation. Based on the premise that moisture is a crucial factor in achieving good fermentation in silage, without significantly affecting its fermentation and nutritive value.

MATERIALS AND METHODS

Location of the Experiment

The experiment was conducted in 2022 in the La Laguna Experimental Station of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), located in Matamoros, Coahuila, Mexico (25° 32' N, 103° 14' W and 1,150 m altitude). Mesquite pod collection took place during early July 2022 in goat grazing areas in the municipality of Matamoros, Coahuila, Mexico. Pods were from *Prosopis glandulosa* and *Prosopis laevigata*. Prior to grinding, they were cleaned to remove twigs, leaves, and any unnecessary material not belonging to the target plant tissues.

Ensiling Process and Evaluation

The moisture treatments included 0, 10, 20, 30, 40, and 50% (w/w) water based on the dry weight of the ground pods. The variables that describe the fermentation profile of silage were considered in terms

of pH, ammonia-N, lactic acid, acetic acid, propionic acid, butyric acid, and losses of gas, effluent, and dry matter (DM) of the silage. Likewise, variables that define the nutritional value criteria of the feed used in ruminants were evaluated: dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, lignified NDF (LNDF), non-fibrous carbohydrates (NFC), total digestible nutrients (TDN), digestible energy (DE), metabolizable energy (ME), net energy for maintenance (NE_m), and net energy for gain (NE_g). Additionally, the *in situ* digestibility of the dry matter (DMD, 48) and the NDF (NDFD, 48) were measured after 48 h of incubation.

Sample and Silage Preparation

The collected mesquite pods were ground using a mill (Model JF5; Terramark, San Nicolás de los Garza, Nuevo León, Mexico) to obtain a theoretical particle size of 4–5 mm. Before initiating the mini silo experiments, a dry matter (DM) curve was established to ensure uniform density across all treatments. For this purpose, 18 samples of 200 g of ground pods were placed in aluminum trays. The designated amount of water for each treatment (three replicates per treatment) was applied using a manual atomizer. The samples were subsequently dried in a forced-air oven (SHEL-LAB FX28) at 60 °C for 72 h. The average pre-experimental DM contents were 95.92 ± 0.02%, 86.42 ± 0.45%, 77.07 ± 0.22%, 67.44 ± 0.36%, 58.01 ± 0.06%, and 48.41 ± 0.17% for treatments containing 0%, 10%, 20%, 30%, 40%, and 50% added water, respectively.

Mini silos were constructed from PVC pipes (10.5 cm in diameter × 18 cm in length), sealed at both ends with lids made of the same material. An orifice (2.78 mm diameter) was drilled in the center of each lower lid to allow drainage during compaction of the rehydrated mesquite pods. A nylon bag (50-micron nylon mesh, Hebei Reking Wire Mesh Co., Ltd., Hebei China) containing 280 g of sand—previously dried at 60 °C for two hours—was placed at the bottom of each mini silo. Four mini silos were prepared per treatment. The ground mesquite pods were manually mixed in plastic containers with the corresponding treatment water using a wooden spoon. Deionized water was applied with a manual atomizer in a closed room without air currents to prevent losses of water or feed particles, and mixing continued until complete homogenization was achieved. Each mini silo was filled with an average of 1,325 g of mesquite pods for ensiling.

The rehydrated ground pods were then packed into the mini silos in layers of 10 cm, using a density of 720 kg DM/m³. The target density for each treatment was calculated using the pre-determined DM content and the volume of the mini silos, with volume estimated according to the formula: $V = \pi \times r^2 \times h$, where r is the radius and h is the height of the mini silo, considering the space occupied by the sand-filled nylon bag. Compaction was performed with a manual press consisting of a fixed metal arm inserted into the upper opening of the mini silo and a four-ton hydraulic jack that applied upward pressure. After filling, the mini

silos were hermetically sealed with adhesive tape and allowed to ferment at room temperature (17–22.5 °C) for 40 days. The experiment was arranged under a completely randomized design with four replications per treatment.

Variable Measurement and Laboratory Analyses

Losses of gas, effluent, and dry matter (DM) were quantified following the methodology described by Gandra *et al.* (2017). On day 40 of ensiling, each mini silo was weighed to determine gas losses. Subsequently, each silo was opened, and the silage material, the silo structure, and the sand-filled nylon bag were weighed. Loss of gas (LG) was calculated using the following equation:

$$LG = (WME - WMSO) / (EDM \times 100)$$

where LG represents gas loss (% DM), WME is the weight of the mini silo at ensiling (kg), WMSO is the weight of the mini silo at opening (kg), and EDM denotes the amount of ensiled dry matter (quantity of ensiled mesquite pods \times % DM).

Effluent production was calculated as follows:

$$PE = (WMSAO - WMSBE) / (EDM \times 100)$$

where PE represents effluent production (kg effluent per metric ton of rehydrated mesquite pods before ensiling), WMSAO is the weight of the mini silo components after opening (kg), WMSBE is the weight of the mini silo components before ensiling (kg), and EDM is the ensiled DM (quantity of ensiled mesquite pods \times % DM).

Recovery of dry matter was calculated as:

$$RDM = (DMAOMS / DMBOMS) \times 100$$

where RDM is the recovery of dry matter (% DM), DMAOMS is the dry matter after opening the mini silo (kg), and DMBOMS is the dry matter before opening the mini silo (kg).

The loss of DM was calculated as follows:

$$LDM = \{[(TWMNE - WMSBE) \times EDM] - [(TWMSO - WMSAO) \times DMAE]\} / [(TWMNE - WMSBE) \times EDM] \times 100$$

where LDM is DM loss (% DM), TWMNE is the total mini silo weight at ensiling (kg), WMSBE is the weight of the mini silo components before ensiling (kg), EDM is the ensiled DM, TWMSO is the total mini silo weight at opening (kg), WMSAO is the weight of the mini silo components after opening (kg), and DMAE is the DM content after ensiling (kg).

After opening the mini silos, the top 5 cm of ensiled mesquite pods were removed, and 200 mL of deionized water was added to a 20-g sample, which was then mixed for 30 s in a blender (Waring Commercial Blender 7011 model 31BL92, Spacefood Tech., Monterrey, NL., Mexico) at high speed. The mixture was filtered through three layers of cheesecloth, and the liquid phase was used to determine the pH using a portable potentiometer (OHAUS Model ST2100, Parsippany, NJ, USA; Contreras-Govea *et al.*, 2016). Then, a second 20-g sample of fresh silage was mixed

with 80 mL of distilled, deionized water for 4 hours in an oscillating shaker (Eberbach Corporation, Ann Arbor, Michigan). After this time, the mixture was filtered with Whatman® #1 filter paper. The filtered liquid was centrifuged at 1048 \times g for 15 min, and the separated supernatant was subjected to ammonia-N analysis by the Kjeldahl method (AOAC, 1984). Ten mL of a third 20-g sample of filtered silage per mini silo was centrifuged at 2504 \times g for 15 min, and 5 mL of the supernatant was conserved at -20 °C in amber vials (Supelco®) plus 1 mL (250 μ L mL⁻¹) of metaphosphoric acid to determine the concentrations of lactic and organic acids (acetic, propionic, and butyric). The obtained samples were defrosted at ambient temperature the night before and filtered with cellulose acetate filters with 0.45-micron pores (non-sterile AC filter 25 mm \times 0.45 μ m, PROLAB, Tlajomulco, Jal. Mexico) to identify and quantify volatile fatty acids (acetic, propionic, and butyric), and lactic acid using high performance liquid chromatography (HPLC) in an Agilent 1200 chromatograph equipped with a diode detector and quaternary pump. Separation was achieved in an Aminex HPX-87H (300 mm \times 7.8 mm) Ion Exclusion column at a wavelength of 210 nm. The mobile phase consisted of 5.0 mM sulfuric acid at a flow rate of 0.6 mL min⁻¹. The acids were identified and quantified by comparison with pure standards (lactic acid, acetic acid, propionic acid, and butyric acid) and calibration curves ($R^2 \geq 0.99$) (Canale *et al.*, 1984).

The remaining mesquite pod silage, approximately 500 g from each mini silo, was dried at 60 °C to a constant weight in a forced-air oven for bromatological and digestibility analyses. The dry samples were ground in a Wiley mill (Arthur T. Thomas, Swedesboro, NJ) with a 1 mm screen. In each ground sample, the total N content was determined using the Dumas method by dry combustion (Leco FP-528, St. Joseph, MO), and the percentage of CP was calculated as total N \times 6.25.

Fiber was analyzed sequentially, beginning with the determination of NDF in 0.5 g of sample in filter bags with 25 μ pores (F57, Ankom Tech., Macedonia, NY), using thermo-stable α -amylase and sodium sulfite in the fiber analyzer (A200, Ankom Tech., Macedonia, NY). ADF was then determined with CTAB and H₂SO₄ in the same fiber analyzer. Finally, using the same bags, the lignin content was determined using 72% H₂SO₄. Ash content was determined by incinerating two grams of the dry samples in crucibles placed in a muffle at 550 °C for six hours. The NFC content was obtained as follows: NFC (%) = 100 - (% CP + % NDF + % Ash + % EE), where EE (ether extract) was assumed to be 2.0% for all the samples (Del Valle *et al.*, 1983). Calculations for TDN, DE, ME, NE_m, and NE_g were based on Armijo-Nájera *et al.* (2019).

In Situ Ruminant Digestibility Assay

All procedures and animal handling were approved in strict adherence to the Mexican Official Standard NOM-062-ZOO-1999. For digestibility analysis, 4.5 g of a dry sample was introduced into a 10 \times 20 cm nylon bag with 50 μ m porosity (R1020, Ankom

Tech., Macedonia, NY) for incubation in duplicate for 48 h in the ventral sac of two bovines with ruminal fistulas (ENLS, Zapotlanejo, Jal.). All the bags were removed simultaneously and submerged for 10 min in a bucket of ice water. All the samples were then rinsed manually until the water was clear. The bags were then left to drain and subsequently dried in a forced-air oven at 55 °C for 48 h. The difference between the initial weight and the final weight accounts for digestible DM (DMD, 48h). Finally, approximately 0.5 g of the remaining sample was extracted and introduced into a F57 bag (Ankom Tech., Macedonia, NY) to determine the residual NDF and calculate the NDFD, 48h (Santana *et al.*, 2025).

Statistical Analysis

All statistical analyses were conducted using the statistical program R (R Studio Inc., Boston, MA) and the agricolae package, employing the aov instruction for analysis of variance (Mendiburu, 2023). The treatments were compared by polynomial orthogonal contrasts searching for linear and quadratic effects in each of the moisture treatments. Means were separated with the Tukey test ($\alpha = 0.05$).

RESULTS

The percentages of DM and moisture in the mesquite pods with different treatments of water are presented in Table 1. Significant differences are observed among all the treatments evaluated. Moisture increased and DM decreased linearly ($p < 0.0001$) in the mesquite

pods at the beginning of and after ensiling as the water added to the treatments increased.

The results for pH and ammonia-N in the mesquite pod silage differed among the treatments of applied water (Table 2). Silage pH decreased linearly ($p = 0.003$) as the quantity of added water increased. Higher pH levels (5.03 and 4.94) were observed in silage with 0% and 10% added water, intermediate pH levels (4.65 and 4.53) were observed when 20% and 30% were added, and lower pH levels (4.29 and 4.07) were observed with 40% and 50% added water. Unlike pH, the addition of water to the mesquite pods resulted in a quadratic increase in ammonia-N ($p = 0.004$) in the silage. The highest concentration of ammonia-N (8.66% and 8.16%) in the mesquite pod silage occurred with 40% and 50% added water, respectively, and was lower (5.59%) with 20% added water. Adding 0% or 10% water to the mesquite pods can provide the same ammonia-N concentration in silage as adding 20% or 30% water.

Regarding the concentration of lactic acid ($p = 0.0002$) and total VFAs ($p = 0.03$) in the silage (Table 2), they increased linearly as the amount of water added increased. Additionally, acetic acid ($p = 0.0007$) and propionic acid ($p = 0.03$) exhibited a decreasing quadratic effect in their concentration as the added water increased. The content of butyric acid in the silage did not differ among treatments ($p > 0.05$).

The orthogonal analysis indicated differences in losses of gas and effluent and loss and recovery of DM in the mesquite pod silage (Table 3). The increase in added water had linear and quadratic effects ($p < 0.0001$ - 0.0002) on losses of gas, effluent, and DM in the silage. Losses in the form of gas and DM in the silage

Table 1. Dry matter content in rehydrated mesquite pod silage with increasing levels of moisture

Variables	Added water (%)						SEM	P-value ¹	
	0	10	20	30	40	50		L	Q
At ensiling									
Moisture (%)	4.09 ^f	13.58 ^e	22.14 ^d	30.92 ^c	42.38 ^b	51.06 ^a	0.50	<0.0001	0.43
DM (%)	95.91 ^a	86.42 ^b	77.86 ^c	69.08 ^d	57.62 ^e	48.94 ^f	0.50	<0.0001	0.43
After ensiling									
Moisture (%)	5.03 ^f	13.97 ^e	28.26 ^d	34.69 ^c	42.12 ^b	50.69 ^a	0.88	<0.0001	0.33
DM (%)	94.97 ^a	86.03 ^b	71.74 ^c	65.31 ^d	57.88 ^e	49.31 ^f	0.88	<0.0001	0.33

Note: ^{abcdet} Values within each row with different superscripts are significantly different ($p \leq 0.05$). SEM = standard error of the mean; ¹L = linear; Q = quadratic; DM = dry matter.

Table 2. Fermentation profile in rehydrated mesquite pod silage with increasing levels of moisture

Variables	Added water (%)						SEM	P-value ¹	
	0	10	20	30	40	50		L	Q
pH	5.03 ^a	4.94 ^a	4.65 ^b	4.53 ^b	4.29 ^c	4.07 ^c	0.04	0.003	0.43
Ammonia-N (% of total N)	6.59 ^{ab}	6.72 ^{ab}	5.59 ^b	6.51 ^{ab}	8.66 ^a	8.16 ^{ab}	0.52	0.14	0.004
Fermentation products (% DM)									
Lactic acid	1.80 ^c	2.16 ^c	3.21 ^{bc}	3.02 ^{bc}	4.44 ^{ab}	5.12 ^a	0.36	0.0002	0.10
Acetic acid	0.11 ^a	0.05 ^{ab}	0.00 ^b	0.00 ^b	0.02 ^b	0.00 ^b	0.02	0.05	0.0007
Propionic acid	1.65 ^a	0.81 ^{ab}	0.96 ^{ab}	0.79 ^{ab}	0.79 ^{ab}	0.62 ^b	0.19	0.08	0.03
Butyric acid	0.24	0.20	0.03	0.02	0.06	0.05	0.08	0.77	0.29
Total VFA	3.80 ^{ab}	3.23 ^b	4.17 ^{ab}	3.84 ^{ab}	5.32 ^{ab}	5.79 ^a	0.45	0.03	0.02

Note: ^{abc} Values within each row with different superscripts are significantly different ($p \leq 0.05$). VFA = volatile fatty acids; SEM = standard error of the mean; ¹L = linear; Q = quadratic; N = nitrogen; DM = dry matter.

were intermediate, with 20% to 30% water. Likewise, the highest losses were observed with the addition of up to 50% water to the mesquite pods, with a significant increase noted with 40% added water. Moreover, the recovery of DM in the silage decreased linearly at a rate of 2.9 units as the added water in the treatments increased ($p < 0.01$).

The effects of adding water to mesquite pods on the nutritive value of the silage are shown in Table 4. The content of CP was not affected by the treatments ($p > 0.05$). However, the contents of NDF and ADF of the silage increased linearly ($p < 0.05$) and quadratically ($p < 0.0001$). A quadratic effect was observed when the water content increased from 0% to 50%, although a plateau phase was evident between 30% and 40% moisture. From the addition of 20% to 40% water, the fibrous fractions of the silage differ from those of the other treatments. Lignin and LNDF of the silage showed quadratic ($p = 0.002$) and linear ($p = 0.02$) increases, respectively. The content of NFC in the silage, such as NDF and ADF, decreased quadratically with the addition of water, exhibiting a plateau between 20% and 40% added water. The concentrations of DE and NE_m were not affected by the treatments ($p > 0.05$). However, the increase in added water to the mesquite pods caused a quadratic reduction of ME ($p = 0.05$) and NE_g ($p = 0.008$) in the silage.

In situ digestibility of DM and NDF of the mesquite pod silage is shown in Figure 1. Increasing the amount

of added water did not modify NDF digestibility after 48 h of incubation. Nevertheless, *in situ* DM digestibility decreased linearly after 48 h of incubation as the added water increased. Similarly, DM digestibility decreased by 65% with treatments of 0% and 10% added water, and up to 50% with the highest amount of added water (50%).

DISCUSSION

This study aimed to preserve dry ground mesquite pods through silage, testing different moisture levels achieved by adding water. The results revealed that the optimum amount of added water for preserving mesquite pods through silage is between 20% and 30% moisture. The addition of these levels of water promoted adequate fermentation of the mesquite pods, resulting in lower total losses and no impact on the nutritive composition of the silage. This coincides with the literature that indicates that quality silage, appropriately preserved, should have acid fermentation (Kung *et al.*, 2018), with low losses in the form of gas and effluents (Tuovinen *et al.*, 2025), good recovery of dry matter (Borreani *et al.*, 2018), and minimal changes in the nutrient composition, relative to the original material (Kung *et al.*, 2018).

Moreover, the fermentation parameters revealed a linear reduction of pH as moisture increased in the silo. The linear reduction in pH observed in our study

Table 3. Gas, effluent, and DM losses in rehydrated mesquite pod silage with increasing levels of moisture

Variables	Added water (%)						SEM	P-value ¹	
	0	10	20	30	40	50		L	Q
Loss of gas (% DM)	0.00 ^d	0.59 ^d	8.62 ^{bc}	6.38 ^c	10.65 ^b	36.16 ^a	0.61	<0.0001	<0.0001
Loss of gas (%)	0.0 ^c	0.50 ^c	6.67 ^b	5.37 ^b	8.60 ^b	25.17 ^a	0.84	<0.0001	<0.0001
Effluent (kg/t)	0.35 ^c	0.43 ^c	1.42 ^c	1.67 ^c	8.01 ^b	16.10 ^a	0.68	<0.0001	<0.0001
Effluent (%)	0.30 ^c	0.37 ^c	1.07 ^c	1.40 ^c	6.47 ^b	11.13 ^a	0.47	<0.0001	<0.0001
Total (gas + effluent)	0.30 ^d	0.95 ^d	9.69 ^c	7.78 ^c	17.11 ^b	47.29 ^a	0.69	<0.0001	<0.0001
Loss of DM (%)	1.55 ^d	1.74 ^d	19.66 ^{bc}	14.50 ^c	22.51 ^b	35.52 ^a	1.12	0.0002	<0.0001
Recovery of DM (%)	98.73 ^a	99.12 ^a	86.09 ^b	89.99 ^b	89.79 ^b	81.42 ^c	1.24	<0.0001	0.09

Note: ^{abcde} Values within each row with different superscripts are significantly different ($p \leq 0.05$). SEM = standard error of the mean; ¹L = linear; Q = quadratic; DM = dry matter.

Table 4. Nutritive value of rehydrated mesquite pod silage with increasing levels of moisture

Variables	Added water (%)						SEM	P-value ¹	
	0	10	20	30	40	50		L	Q
CP (% DM)	10.43	10.17	10.95	11.21	11.26	11.8	0.41	0.28	0.39
NDF (% DM)	38.52 ^c	37.97 ^c	43.49 ^{bc}	45.54 ^b	45.40 ^b	52.54 ^a	1.22	0.02	<0.0001
ADF (% DM)	24.26 ^c	24.17 ^c	29.58 ^b	30.76 ^b	30.74 ^b	36.46 ^a	0.98	0.03	<0.0001
Lignin (% DM)	6.46 ^b	7.06 ^b	7.98 ^b	8.61 ^b	8.50 ^b	8.61 ^a	0.54	0.10	0.002
LNDF	16.77 ^b	18.64 ^{ab}	18.29 ^{ab}	18.91 ^{ab}	18.71 ^{ab}	22.37 ^a	0.90	0.02	0.12
Ash (% DM)	4.33	4.19	4.65	4.57	4.45	4.11	0.22	0.59	0.30
NFC (% DM)	45.72 ^a	46.67 ^a	39.91 ^b	37.68 ^b	37.89 ^b	30.73 ^c	1.14	0.007	<0.0001
TDN (% DM)	87.96	88.33	87.23	86.86	86.79	86.03	0.58	0.27	0.09
DE (Mcal/kg DM)	3.87	3.89	3.84	3.82	3.82	3.79	0.02	0.30	0.08
ME (Mcal/kg DM)	3.17 ^{ab}	3.19 ^a	3.15 ^{ab}	3.13 ^{ab}	3.13 ^{ab}	3.10 ^b	0.02	0.21	0.05
NE_m (Mcal/kg DM)	1.82	1.82	1.81	1.8	1.79	1.78	0.01	0.22	0.09
NE_g (Mcal/kg DM)	1.14 ^{ab}	1.15 ^a	1.13 ^{ab}	1.13 ^{ab}	1.13 ^{ab}	1.12 ^b	0.006	0.16	0.008

Note: ^{abc} Values within each row with different superscripts are significantly different ($p \leq 0.05$). SEM = standard error of the mean; ¹L = linear; Q = quadratic; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; LNDF = lignified NDF; NFC = non-fiber carbohydrates; TDN = total digestible nutrients; DE = digestible energy; ME = metabolizable energy; NE_m = net energy for maintenance; NE_g = net energy for gain.

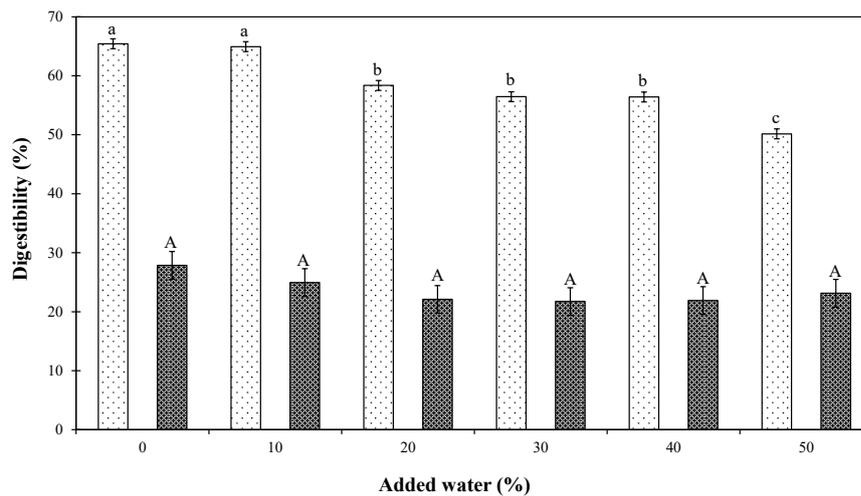


Figure 1. *In situ* digestibility of dry matter (DM) ($p = 0.01$, SEM = 0.84) and neutral detergent fiber (NDF) ($p > 0.05$, SEM = 2.34) in mesquite pod silage with incrementing moisture levels, after 48 h incubation. Note: \square = DMD, 48h; \blacksquare = NDFD, 48h.

is associated with a linear increase (1.80% to 5.12%) in silage lactic acid production as the water level increased. In an ideal fermentation, lactic acid bacteria use water-soluble carbohydrates, such as glucose, for growth and reproduction, resulting in a suitable decrease of pH in the silo (Rooke & Hatfield, 2003; Kung *et al.*, 2018). However, the quadratic effect on the concentrations of acetic acid, propionic acid, and ammonia-N suggests that the treatments of 0% to 10% and 40% to 50% water added to the silage do not provide adequate moisture content for efficient fermentation. Excessive moisture in silages exacerbates protein degradation into ammonia-N and reduces their quality (Tuovinen *et al.*, 2025; Rooke & Hatfield, 2003). Furthermore, adding too much moisture to the silage material allows soluble nutrients, such as protein and carbohydrates, to be excreted with the exudate, thereby reducing the silage's nutritional value (Yi *et al.*, 2023). In contrast, in silages with low moisture content, compaction is less efficient, resulting in a reduction in the activity of water in the silage medium. Under these conditions, the water available for the growth of lactic and other acid-producing bacteria becomes limiting, resulting in low acid accumulation and, subsequently, poor fermentation (Kung *et al.*, 2018; Yi *et al.*, 2023). Therefore, according to these parameters, rehydration with 20%-30% water promoted good fermentation for the mesquite pod silage. The pH found in our study with this level of moisture is higher (3.7-4.0) than that of silages of field corn with 30%-40% DM, but it is similar to the pH (4.3-4.5) of silages of legumes with 30%-35% DM (Kung *et al.*, 2018). Coutinho *et al.* (2013) reported a pH range of 3.7 to 3.8 in elephant grass silages to which they added 15% mesquite pods at 119 days of fermentation. Likewise, the addition of 20% water promoted a concentration of ammonia-N of 5.6, which is similar to ammonia-N (5%-7%) of corn silage with 30%-40% DM and to high moisture corn with 70-75% DM, but lower than legume silage (10%-15%) with 30%-40% DM (Kung *et al.*, 2018).

Although the treatments with 0 or 10% added water had lower losses of gas and DM, as well as of

effluent, in these treatments, the mesquite pods clearly did not undergo a fermentation process appropriate to silage. With a pH value of 5, it was not possible to preserve it adequately. In contrast, the treatments with 40% and 50% added water had the highest losses of gas (10.65%-36.16%), DM (22.51%-35.52%), and effluent (6.47%-11.13%) in the silo. These results are important since the effluent in the silages contains water-soluble carbohydrates, protein, minerals, and fermentation products (Muck *et al.*, 2018). For this reason, it is essential to prevent effluent runoff in the silos, as it is an indicator of nutrient loss and an inadequate fermentation process. Heterolactic fermentation in the silo, commonly promoted by excess moisture, causes greater loss of DM through elevated production of ethanol and gas (CO_2) (Tuovinen *et al.*, 2025), which is not desirable. In fact, high moisture content in the silage material promotes the production of effluent in the silo. Therefore, the moisture content for avoiding runoff should be around 70% (Kung *et al.*, 2018; Muck *et al.*, 2018). Mombach *et al.* (2018) rehydrated dry corn grain with a bacterial inoculant and maintained a moisture level of 30%, which resulted in higher losses of gas (15.40%) and effluent (26.5%). However, they reported DM losses (41.8%) like those in our study, with comparable moisture levels (30%). Mombach *et al.* (2019) concluded that rehydration of dry corn grain with 30% water resulted in lower losses of effluent and DM in the silo. Borreani *et al.* (2018) reported that ideal DM losses can vary from 5% to 13% during the ensiling process, including respiration, fermentation, and effluent, depending on management.

The CP content in the mesquite pod silage was not modified by the applied treatments, with values ranging from 10% to 11.8%. In this respect, reports have been made of similar CP values in pods of different mesquite species that were not subjected to a fermentation process (Armijo-Nájera *et al.*, 2019; Ruíz-Nieto *et al.*, 2020). The technique of ensiling involves a process of proteolysis, or enzymatic degradation, of proteins into non-protein nitrogen forms, such as peptides, free

amino acids, and ammonia. This process is one of the most common during silage fermentation. However, it was not observed in our study, possibly because of the lower pH in the silage, which prevented the growth of proteolytic microorganisms (da Silva Ferreira *et al.*, 2021) or the presence of different inhibiting compounds in the mesquite, such as tannins (4.5 mg/g DM) and/or polyphenols (15.9 g/mg DM) (García-Azpeitia *et al.*, 2022). In any case, during ensiling, the protein content remained the same as in mesquite pods in their natural state.

The concentrations of NDF and ADF in the silage increased, while NFC decreased, as the moisture level increased. Nevertheless, a plateau phase was observed between 20% and 40% added water. Some reports state that during the ensiling process, carbohydrates are a key source of fermentable substrate (Kung *et al.*, 2018; Muck *et al.*, 2018). Non-structural carbohydrates are used to provide quantities of monosaccharides, which can increase fermentation and indirectly increase the concentrations of fiber components (Rooke & Hatfield, 2003). As a result, the pH in the mesquite pod silage of our study decreased linearly, thereby preventing deterioration, while also reducing the concentration of carbohydrates relative to fiber as moisture levels increased. However, it is essential to emphasize that the concentration of water-soluble carbohydrates, rather than NFC, in the silage would have provided a more straightforward explanation for the reduction in pH in the different treatments of our study.

Metabolizable energy and NE_g of the silage were higher in the treatment of 10% water and decreased quadratically with increasing additions of 40% and up to 50% water. This can also be explained by the reduction in carbohydrates in the silage as the levels of added water increased, since the NFC are important in estimating energy values in feed (Weiss & Tebbe, 2019). Proper fermentation begins in the liquid state of the silage when non-fibrous carbohydrates are the primary substrates in the silo. The combination of adequate moisture and carbohydrate concentration enables efficient utilization, facilitating an optimal transition to an anaerobic environment within the silo (Nunes *et al.*, 2024). Excessive moisture in the silo may still promote good carbohydrate utilization, but will result in excessive effluent and/or prolonged fermentation (Kung *et al.*, 2018). It is important to note that the differences in ME and NE_g among treatments in the present study may not be metabolically or physiologically relevant for the animal. The average ME content in the treatments with 10% to 40% water (1.15 Mcal kg^{-1} DM) is lower than that reported in corn silages (2.0 Mcal kg^{-1}) but similar to that of wheat straw (1.18 Mcal kg^{-1} DM; INRA, 2018).

Finally, in situ DM digestibility of the mesquite pod silage decreased linearly at the rate of 2.5 units in response to the increase in water. As the quantity of water increased, the concentration of NDF increased, while NFC decreased, affecting the total DM digestibility of the silage. This is because the fiber in a feed or forage is generally less degradable than the NFC fractions (Pinho *et al.*, 2019). Nevertheless, the addition of 20 to 40% water in our study obtained an

average DM digestibility of 57%. Camboim *et al.* (2025) found a higher degradability (90%-91%) of ground corn grain rehydrated with 20 to 30% whey than ground corn rehydrated with 30% water or 40% whey (84% degradability). Ground corn rehydrated with 30% water also had a higher NDF concentration (11%) than ground corn treated with 20% or 30% whey (6.6%-6.9% of NDF). Therefore, reducing the carbohydrates used as fermentation substrates can increase the fibrous fraction, thereby reducing silage digestibility.

CONCLUSION

This study successfully preserved mesquite pods as silage. Rehydration of ground, dry mesquite pods with 20%-30% water resulted in adequate lactic acid production, reducing the pH to 4.5-4.6, with low ammonia-N concentrations and acceptable effluent, gas, and dry matter losses. Furthermore, fermentation in the silo did not modify the protein content and maintained intermediate fiber and carbohydrate levels of the silage. However, further research is required to validate responses in farm-scale ensiling systems.

CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors declare that no artificial intelligence tools were used in any of the experimental stages, from the conception of the study to the final writing of the results presented in this manuscript.

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