

# Plant density and bacterial inoculants on *Canavalia brasiliensis* yield and silage quality<sup>1</sup>

Luis Fernando Gómez-Ramírez<sup>2</sup>, Esteban Burbano-Erazo<sup>2</sup>,  
José Edwin Mojica-Rodríguez<sup>2</sup>, Sandra Carolina Perdomo Ayola<sup>2</sup>, Juan Ricardo Zambrano Ortiz<sup>3</sup>

## ABSTRACT

The use of legumes as a protein source in mixed silages presents a promising alternative for high-quality feed production. This study evaluated the potential of *Canavalia brasiliensis* as a forage legume through two experiments: the first tested four planting densities (166,500; 100,000; 71,500; and 55,500 plants ha<sup>-1</sup>) under dry tropical conditions, whereas the second assessed the effects of 5 % of molasses, synthetic and native microbial inoculants, as well as their combinations, on silage fermentation. The planting density did not affect the dry matter yield, although the density of 100,000 plants ha<sup>-1</sup> showed the greatest plant height and that of 55,500 plants ha<sup>-1</sup> the longest time to reach full ground cover. The additives and fermentation time significantly influenced the pH, soluble carbohydrates (°Brix), microbial counts and nutritional traits. The pH remained below 5, the soluble carbohydrates above 8.9 °Brix, and the lactic acid bacteria declined over time. The addition of molasses improved the dry matter content and digestibility. The crude protein decreased during fermentation, whereas the soluble protein increased and the acid detergent fiber decreased more than the neutral detergent fiber, especially in the inoculated treatments. The density of 55,500 plants ha<sup>-1</sup> and the use of 5 % of molasses are recommended to optimize the forage production and silage quality under tropical conditions.

**KEYWORDS:** Ensiling process, forage legumes, planting density, silage additives.

## RESUMO

Densidade de plantio e inoculantes bacterianos na produtividade e qualidade de silagem de *Canavalia brasiliensis*

O uso de leguminosas como fonte proteica em silagens mistas representa uma alternativa promissora para a produção de alimentos de alta qualidade. Avaliou-se o potencial forrageiro de *Canavalia brasiliensis* por meio de dois experimentos: o primeiro testou quatro densidades de plantio (166.500, 100.000, 71.500 e 55.500 plantas ha<sup>-1</sup>) sob condições tropicais secas e o segundo os efeitos da adição de 5 % de melaço, inoculantes microbianos sintéticos e nativos, e suas combinações, sobre a fermentação da silagem. A densidade de plantio não influenciou na produção de matéria seca, embora a densidade de 100.000 plantas ha<sup>-1</sup> tenha apresentado maior altura de plantas e a de 55.500 plantas ha<sup>-1</sup> maior tempo para atingir a cobertura total do solo. Os aditivos e o tempo de fermentação influenciaram significativamente no pH, carboidratos solúveis (°Brix), contagens microbianas e características nutricionais. O pH permaneceu abaixo de 5, os carboidratos solúveis acima de 8,9 °Brix e os lactobacilos diminuíram com o tempo. A adição de melaço melhorou o teor de massa seca e a digestibilidade. A proteína bruta reduziu-se durante a fermentação, enquanto a proteína solúvel aumentou, e a fibra em detergente ácido diminuiu mais do que a fibra em detergente neutro, especialmente nos tratamentos com inoculantes. Recomenda-se a densidade de 55.500 plantas ha<sup>-1</sup> e o uso de 5 % de melaço para otimizar a produção forrageira e a qualidade da silagem em condições tropicais.

**PALAVRAS-CHAVE:** Processo de ensilagem, leguminosas forrageiras, densidade de plantio, aditivos para silagem.

## INTRODUCTION

In dual-purpose cattle systems of the Colombian dry Caribbean, low nutrient availability in forage - particularly during the dry season - limits both yield and reproductive performance (Castro et al. 2017).

Native and cultivated grasses typically yield under 2,000 kg ha<sup>-1</sup> of dry matter per rotation, with low protein content and digestibility (Castro-Rincón et al. 2018). Consequently, milk yield remains low (4.5 L animal<sup>-1</sup> day<sup>-1</sup>), with extended calving intervals (434 days) and delayed first calving (38.3 months)

<sup>1</sup> Received: May 27, 2025. Accepted: July 16, 2025. Published: Sep. 02, 2025. DOI: 10.1590/1983-40632025v5582830.

<sup>2</sup> Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Centro de Investigación Motilonia, Agustín Codazzi, Colombia. E-mail/ORCID: lfgomez@agrosavia.co/0000-0001-9847-0606; eburbano@agrosavia.co/0000-0001-5056-9893; jmojica@agrosavia.co/0000-0001-7751-8631; sperdomo@agrosavia.co/0000-0002-6583-8088.

<sup>3</sup> Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Centro de Investigación La Suiza, Rionegro - Santander, Colombia. E-mail/ORCID: jzambrano@agrosavia.co/0000-0003-3748-6211.

(Burbano-Erazo et al. 2020). Additionally, prolonged dry periods reduce forage availability, threatening system sustainability (Bernabucci 2019). Addressing these constraints requires improving both the quality and accessibility of forage resources.

High-protein forage legumes enhance cattle diets and contribute to nitrogen fixation, thereby reducing fertilizer use and improving sustainability (Barbieri et al. 2023). They also help to lower greenhouse gas emissions (Gaviria et al. 2022), supporting integrated livestock systems (Santos et al. 2023). In Colombia's dry Caribbean, their use is limited to a few species, such as *Leucaena leucocephala*, *Clitoria ternatea* and *Bothriochloa pertusa* (Roncallo et al. 2012), making it necessary to explore other legumes adapted to arid conditions.

*Canavalia brasiliensis* has emerged as a promising forage legume due to its adaptability to tropical conditions, tolerance to environmental stress and high biomass production (Burbano-Erazo et al. 2019). Its integration into livestock systems offers a strategy to mitigate seasonal forage shortages, especially during dry periods, and to enhance resilience under climatic variability (Castro et al. 2017, Burbano-Erazo et al. 2020). However, effective conservation methods, such as ensiling, are necessary to guarantee year-round availability and preserve the nutritional quality and palatability of the product.

Ensiling is a method widely employed for preserving forage, particularly in regions characterized by marked seasonality (Silva et al. 2017). Ensiling tropical legumes, on the other hand, poses distinctive obstacles as a result of their low soluble carbohydrate concentration, high lignin levels, and the presence of undesirable epiphytic microorganisms (Jaurena & Pichard 2001, Kung et al. 2018). Additionally, the high protein content of tropical legumes can buffer the silage process and hinder the pH reduction necessary for efficient fermentation (López-Herrera & Briceño-Arguedas 2018).

Lactic acid bacteria inoculants can improve fermentation, silage stability and nutritional quality by converting soluble carbohydrates into organic acids (Guo et al. 2023). This study aimed to determine the optimal planting density for *C. brasiliensis* and evaluate the effects of bacterial inoculants on silage quality. It was hypothesized that higher densities would boost biomass yield, whereas

inoculants would enhance fermentation and nutrient preservation, offering sustainable feed alternatives.

## MATERIAL AND METHODS

The studies were conducted at the AGROSAVIA's Motilonia Research Station (Agustín Codazzi, Cesar, Colombia; 10°00'03.0''N and 73°14'53.3''W), under tropical dry conditions (rainfall of 1,585 mm; 28.7 °C; 70 % of relative humidity). A soil analysis was conducted in the experimental plot, which was characterized by a clay loam texture and an almost neutral pH (6.63). For soil preparation, one pass of a disk harrow was performed at a depth of 10 cm. The first study with the *C. brasiliensis* accession 17009 (CIAT) was conducted from July to October 2019 and evaluated under four planting densities (166,500; 100,000; 71,500; and 55,500 plants ha<sup>-1</sup> - with spacings between rows x between plants of 30 × 20, 50 × 20, 70 × 20 and 90 × 20 cm, respectively). A randomized complete block design, with three replicates, was used in 126-m<sup>2</sup> plots.

Seeds were manually sown at a depth of 2-3 cm and fertilized at 30 days (60 kg of N; 20 kg of K<sub>2</sub>O; 15 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>), with fertilization carried out manually by applying the fertilizer along the length of each planting furrow. Manual weeding and drip irrigation were applied as needed. Measured variables included days to 60 % of germination (time required for 60 % of the plants to germinate), true leaf emergence (time required for the appearance of true leaves in the germinated plants), ground cover (%; using Canopeo app.), days to full cover, plant height to total soil cover, dry matter content (60 °C; 48 h) and forage dry matter yield at 90 days (Mg ha<sup>-1</sup> from 1 m<sup>2</sup>). For each treatment, data were collected from three sampling points per 1 m<sup>2</sup> in each plot.

In the second study, conducted immediately after the first (from September to December 2019), green forage from a 3,000-m<sup>2</sup> plot of *C. brasiliensis* (accession 17009, density of 55,500 plants ha<sup>-1</sup>) was harvested at pre-flowering (60-70 days post-emergence) and chopped (~2 cm) using a gasoline-powered chipper (model CF 52) for six silage treatments: control; 5 % of molasses (w/w); synthetic consortium (C1); native consortium (C2); C1 + molasses; and C2 + molasses. The used molasses was derived from sugarcane, composed of 40-60 % of sugars, on a dry matter basis. C1 comprised five *Lactobacillus* and *Pediococcus*

strains ( $1 \times 10^{10}$  CFU mL<sup>-1</sup>) from the AGROSAVIA's collection; C2 was isolated from *Megathyrus maximus* via dilution-to-stimulation in modified MRS with 1 % of forage (Díaz-García et al. 2021). Inoculants and molasses were applied directly to the fresh forage after chopping it. Biological inoculum additives were sprayed at a concentration of  $1 \times 10^6$  CFU g<sup>-1</sup> of fresh matter, whereas molasses was incorporated at 5 % (w/w), based on the fresh weight of the forage in the respective treatments. The treated forage was then ensiled in airtight PVC mini-silos, each measuring 50 cm in length and 4 inches ( $\approx 10.16$  cm) in diameter, and fermented at room temperature for 20 days.

The pH, soluble carbohydrates (°Brix) and microbial populations (lactic acid bacteria and pathogens) were measured before ensiling and at 1, 3, 5, 10 and 20 days of fermentation. For each treatment, three replicates were used, and three samples were collected per replicate to ensure accuracy and statistical robustness. The pH was determined by mixing 1 g of silage with distilled water and measuring the supernatant with a Hanna HI99163 pH meter. Soluble carbohydrates were assessed with a digital refractometer (ATAGO® PAL-1) from aqueous extracts of 100 g of forage. Lactic acid bacteria and pathogens were quantified by plating serial dilutions on selective media: MRS agar (lactic acid bacteria), VRBA (coliforms) and YGC (molds and yeasts), followed by incubation under appropriate conditions and colony counting.

At the end of fermentation, silage quality was assessed by scoring odor, color and texture on a 0-4 scale (Boschini-Figueroa et al. 2014). For nutritive analysis, 300-g silage samples were dried, ground and analyzed by near-infrared spectroscopy for crude protein, soluble protein, fiber fractions (neutral detergent fiber - NDF and acid detergent fiber - ADF) and dry matter digestibility.

A randomized complete block design, with three replicates and four planting densities, was used for the *C. brasiliensis* trial. The Anova and Duncan's test ( $\alpha = 0.05$ ) assessed the treatment effects, and linear regression analyzed the ground cover over time. For the silage study, a  $6 \times 6$  factorial design (six treatments  $\times$  six fermentation times) was used, with 108 mini-silos. Anova evaluated the treatment, time and their interaction, with means compared by the Duncan's test ( $p < 0.05$ ). The analyses were performed using the SAS 9.4 software.

## RESULTS AND DISCUSSION

Seed germination (6 days) and early plant height (12-14 cm at 14 days) were similar across the treatments ( $p > 0.05$ ) (data not shown). While canopy closure was faster at higher densities (40.6 days for 166,500 plants ha<sup>-1</sup>; 51 days for 100,000 plants ha<sup>-1</sup>; 47.8 days for 71,500 plants ha<sup>-1</sup>) than at the lowest density (67 days for 55,500 plants ha<sup>-1</sup>), the dry biomass was not significantly affected by planting density ( $p > 0.05$ ) (Table 1). Seed requirements, however, varied significantly, from 32.2 to 96.6 kg ha<sup>-1</sup>, respectively for 55,500 and 166,500 plants ha<sup>-1</sup>, implying higher costs at denser sowings. Overall, the species maintained strong biomass production under semi-arid conditions across all densities.

Each planting density exhibited distinct soil cover patterns. The highest density (166,500 plants ha<sup>-1</sup>) achieved over 50 % of soil cover by 20 days after emergence, following a logarithmic growth trend, whereas the other densities showed more linear trends (Figure 1).

*Canavalia brasiliensis* achieved full ground cover in 40-67 days, outperforming previous reports (Solis et al. 2019). While coverage may vary with soil and topography (Douxchamps et al. 2012), its rapid growth offers key agronomic benefits such as improved moisture retention, erosion control and reduced heat stress (Calero Hurtado et al. 2018). Its drought tolerance and biomass production in well-drained, clay-rich soils with high organic carbon make it well-suited for dry tropical areas like the Colombian Caribbean (Renté Martí et al. 2020).

The vigorous climbing habit of *C. brasiliensis* enables a rapid soil coverage (Douxchamps et al. 2012). As with other tropical legumes, higher planting densities promote increased plant height due to competition for light (Calero Hurtado et al. 2018).

Table 1. Growth and productive response of *Canavalia brasiliensis* under plant densities.

Plant density (plants ha <sup>-1</sup> )	Total soil cover (Days)	Plant height (cm)	Dry matter (Mg ha <sup>-1</sup> )
166,500	40.60 b*	62.20 b	11.30
100,000	51.00 b	73.10 a	11.53
71,500	47.80 b	55.50 b	9.53
55,500	67.00 a	55.00 b	9.84
P-value	0.02	0.005	0.13

\* Different letters in the same column indicate significant statistical differences ( $p < 0.05$ ). Duncan's test ( $\alpha = 0.05$ ).

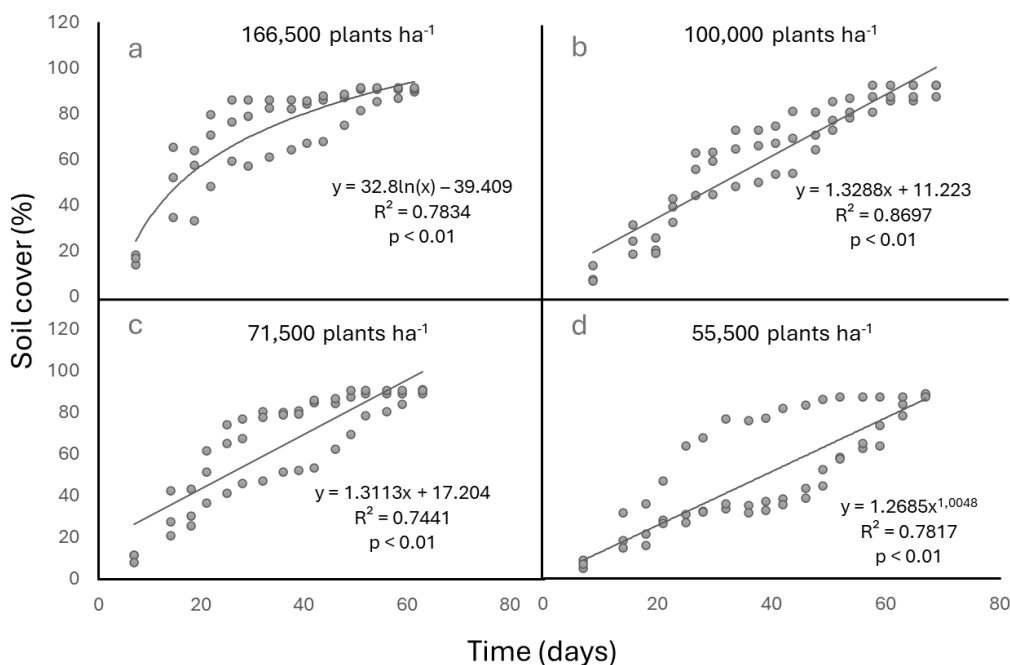


Figure 1. Soil cover trends under plant densities. The soil cover was measured in *Canavalia brasiliensis* at time points using a soil cover model.

The data in this study suggest that a greater density enhances vertical growth in *C. brasiliensis*. The observed plant heights surpassed those of other cover legumes such as *C. ensiformis* (Zapata et al. 2017) and align with previous findings of vigorous growth near 70 cm (Burbano-Erazo et al. 2019), where plants were grown at a spacing of 0.5 m between rows and 0.5 m between plants.

*C. brasiliensis* achieved a maximum dry matter yield of 11.53 Mg ha<sup>-1</sup>, surpassing values reported in Valle del Cesar (Castro-Rincón et al. 2018), likely due to differences in cutting age and rainfall. The lowest yield (9.53 Mg ha<sup>-1</sup>) was consistent with reports from Cuba (Martín et al. 2007), supporting its potential as a high-yielding forage in the Colombia's dry Caribbean region. Moreover, *C. brasiliensis* outperformed other tropical legumes such as *Mucuna*, *Lablab*, *Centrocema* and *Psophocarpus*, which required longer periods to reach a comparable ground cover (Abayomi et al. 2001).

An interaction between additive application and fermentation time was observed ( $p < 0.05$ ), affecting both the pH and soluble carbohydrates in the preserved forage. Molasses, alone or combined with microbial inoculants, reduced the pH below 5.0 in *C. brasiliensis* silage throughout the 20-day fermentation, when compared to the control

(6.13) and inoculants without molasses ( $pH > 5.0$ ) (Figure 2A). Additionally, molasses treatments significantly increased soluble carbohydrates ( $p < 0.05$ ), reaching or exceeding 8.9 °Brix at days 1, 3, 5, 10 and 20, whereas treatments without molasses remained below 8.0 °Brix (Figure 2B).

The °Brix value reflects the concentration of sucrose and other soluble compounds in forage juice. Forages contain soluble carbohydrates like glucose, fructose, sucrose and minor sugars (Rooke & Hatfield 2003). Efficient silage fermentation requires 60-80 g soluble carbohydrates per kg of dry matter (Silva et al. 2017). In this study, °Brix values in fresh and inoculated *C. brasiliensis* silage remained below 8 °Brix (equivalent to 8 g 100 g<sup>-1</sup> of solution) and stable over 20 days, whereas the pH stayed above 5 - suggesting poor fermentation. This is likely due to the legume's high buffering capacity from high crude protein and low sugar content (Costa Araujo et al. 2023).

However, the addition of molasses or its combination with microbial additives increased the concentration of soluble sugars and reduced the pH, indicating a successful fermentation process. This outcome is likely due to the use of soluble carbohydrates by lactic acid bacteria, leading to higher organic acid production (Sabertanha et al.



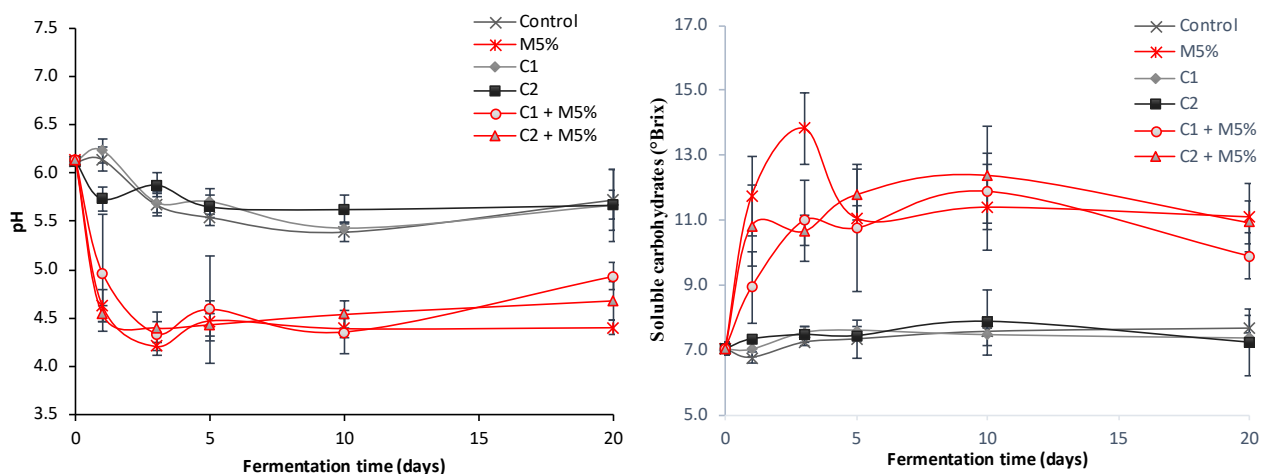


Figure 2. Variation in pH (A) and soluble carbohydrates (B) during the fermentation process in *Canavalia brasiliensis* silage with the application of additives. M5%: 5 % of molasses; C1: synthetic consortium; C2: native consortium.

2021). The progressive reduction in °Brix values over time supports this process. Similar results were reported by López-Herrera & Briceño-Arguedas (2018), who observed that molasses application in legume silage lowers the pH to 4.5-4.8, comparable to the values obtained here for *C. brasiliensis*.

Although lactic acid bacteria-based additives can improve legume silage (Ertekin 2023), their effect on pH in this study was similar to the control. This may be due to a stable native lactic acid bacteria population suppressing exogenous strains (Seppälä et al. 2019) or a lack of inoculant specificity for *C. brasiliensis*. Additionally, low soluble sugar levels likely limited the lactic acid production, reducing fermentation efficiency, especially in treatments without molasses.

A significant interaction between additives and fermentation time ( $p < 0.05$ ) was observed for lactic acid bacteria populations, with peak counts (7.03-7.40 Log CFU g<sup>-1</sup> of fresh matter) in inoculated treatments within the first three days (Table 2). Molasses-only treatments had the lowest lactic acid bacteria levels at days 3 and 20. By day 20, inoculated treatments retained higher lactic acid bacteria counts, though differences with the control were not significant. Enterobacteria and fungi increased initially but declined after day 3, stabilizing around 2.2 Log CFU g<sup>-1</sup> of fresh matter by the day 20 (Table 2).

Lactic acid bacteria populations in fresh *C. brasiliensis* forage were comparable to those reported for soybean and red clover (3.8-4.9 Log CFU g<sup>-1</sup> of fresh matter), but lower than in alfalfa

(6.75-7.56 Log CFU g<sup>-1</sup> of fresh matter) (Nkosi et al. 2016, Dong et al. 2020, Wang et al. 2022). Lactic acid bacteria counts increased within the first 24 hours and again by day 20 in most treatments, aligning with trends observed by Cheng et al. (2022). Notably, the M5% treatment showed a decline in lactic acid bacteria at day 20, despite the greater pH reduction, a pattern also observed by Eliasson et al. (2023).

Although fungi and enterobacteria are commonly found on forage surfaces, they are undesirable in silage, due to their negative impact on fermentation quality (Soundharrajan et al. 2021). In this study, their initial counts were lower than those reported for legumes like alfalfa (Silva et al. 2016). Both groups increased during the first 24 hours, likely due to early aerobic activity, but declined sharply after day 3. This suggests effective lactic acid bacteria proliferation and acidification, which inhibited spoilage microorganisms (Soundharrajan et al. 2021).

Organoleptic evaluation showed higher quality scores in treatments with molasses. The C2 + M5% treatment stood out, achieving scores of 4.0 (odor), 2.7 (color) and 2.0 (texture), indicating excellent to fair quality. C1 + M5% also performed well, with scores ranging from good to fair (3.3, 3.0 and 2.3, respectively). Similarly, M5% alone showed good results (3.7, 2.7 and 2.0). In contrast, the control and C2 treatments had the lowest scores, reflecting a lower silage quality (Table 3).

The organoleptic evaluation complemented the chemical and microbiological analyses by revealing

Table 2. Effect of additives on the count of lactic acid bacteria, molds and yeasts, and enterobacteria in *Canavalia brasiliensis* silage during the fermentation process.

Variable	Additive	Pre-silage	Fermentation time (Days)				
			1	3	5	10	20
Lactic acid bacteria population (Log CFU g <sup>-1</sup> of fresh matter)	Control	4.00 bcde*	6.60 ab	6.87 ab	5.92 abcd	5.74 abcd	5.00 abcde
	M5%	4.00 bcde	5.25 abcd	2.00 e	5.35 abcd	3.03 de	2.00 e
	C1	4.00 bcde	7.25 a	7.04 ab	6.37 abc	6.33 abc	6.00 abcd
	C2	4.00 bcde	7.03 ab	7.40 a	6.31 abc	6.15 abcd	6.07 abcd
	C1 + M5%	4.00 bcde	6.88 ab	5.88 abcd	5.09 abcde	3.94 bcde	5.92 abcd
	C2 + M5%	4.00 bcde	6.39 ab	3.26 cde	5.62 abcd	4.05 bcde	5.02 abcde
	Mean	4.00	6.57	5.41	5.78	4.87	5.00
Mold and yeast Population (Log CFU g <sup>-1</sup> of fresh matter)	Control	4.88	8.29	2.00	2.00	2.58	2.46
	M5%	4.88	7.96	2.00	2.00	2.00	2.00
	C1	4.88	8.06	2.00	2.00	2.00	2.00
	C2	4.88	7.51	2.00	2.00	2.46	3.85
	C1 + M5%	4.88	7.52	3.90	2.00	2.00	2.00
	C2 + M5%	4.88	8.01	2.00	2.00	2.00	2.00
	Mean	4.88 B	7.88 A	2.31 C	2.00 C	2.17 C	2.38 C
Enterobacteria Population (Log CFU g <sup>-1</sup> of fresh matter)	Control	3.17	4.38	2.00	2.00	2.81	2.00
	M5%	3.17	4.98	2.00	2.00	2.00	2.00
	C1	3.17	5.79	2.43	2.20	2.00	2.00
	C2	3.17	5.64	2.00	2.57	2.00	2.79
	C1 + M5%	3.17	5.64	2.00	2.00	2.00	2.30
	C2 + M5%	3.17	4.31	2.00	2.00	2.00	2.00
	Mean	3.17 B	5.12 A	2.07 C	2.12 C	2.13 C	2.18 C

Values are means of three replicates per treatment and sampling time (n = 3). \* Different lowercase letters indicate significant additive × time interactions; uppercase letters denote differences across fermentation times (Duncan's test;  $\alpha = 0.05$ ). Log: log<sub>10</sub>; CFU: colony-forming units; M5%: 5 % of molasses; C1: synthetic consortium; C2: native consortium.

clear differences in silage quality (Kung et al. 2018). Treatments with molasses, alone or combined with inoculants, exhibited pleasant, lactic acid-related odors and light vinegar-like notes, suggesting a good fermentation. In contrast, the control, C1 and C2 silages had strong, unpleasant odors and viscous textures, indicative of clostridial activity and associated with butyric acid and ammonia production - markers of poor fermentation (Kung et al. 2018, Li et al. 2020).

Table 3. Sensory parameters of *Canavalia brasiliensis* silage at 20 days of fermentation with the use of additives.

Additive	Parameter		
	Odor	Color	Texture
Control	2.0 c*	1.7 bc	1.7
M5%	3.7 a	2.7 ab	2.0
C1	2.7 bc	2.7 ab	1.7
C2	2.0 c	1.3 c	1.7
C1 + M5%	3.3 ab	3.0 a	2.3
C2 + M5%	4.0 a	2.7 ab	2.0

Values represent the average of three experimental replicates per treatment. \* Different letters indicate significant differences among additives for each parameter (Duncan's test;  $\alpha = 0.05$ ). M5%: 5 % of molasses; C1: synthetic consortium; C2: native consortium.

The interaction between additive application and fermentation time significantly affected nutritive parameters ( $p < 0.05$ ) (Table 4). The dry matter content increased during fermentation in the treatments with microbial inoculants and molasses, reaching 24.26 % (C1 + M5%) and 23.77 % (C2 + M5%), if compared to 20.66 % in pre-silage forage. In contrast, the control and C2 treatments had the lowest final dry matter values (20.44 and 20.29 %, respectively), showing slight, non-significant reductions (Table 4). This suggests limited degradation, despite of the high forage moisture, whereas the increase with molasses likely reflects a concentration effect from its higher dry matter content (Alonso-Galeana et al. 2023).

Crude protein decreased significantly ( $p < 0.05$ ) in all treatments. However, losses were minimized with M5% (15.41 %) and C2 + M5 % (14.59 %), when compared to the pre-silage level of 17.63 %. The control showed the greatest crude protein loss (12.18 %). Soluble protein increased in all treatments ( $p < 0.05$ ), with the highest values on day 20, in M5% (53.16 %), C1 + M5% (49.41 %) and C2 + M5% (51.50 %), versus 36.04 % for pre-silage. These

Table 4. Nutritional quality of *Canavalia brasiliensis* silage with additives at various days of fermentation.

Component	Additive	Pre-silage	Fermentation time (days)	
			5	20
Dry matter; %	Control	20.66 bc*	19.57 c	20.44 bc
	M5%	20.66 bc	23.78 a	22.69 ab
	C1	20.66 bc	22.20 ab	20.98 bc
	C2	20.66 bc	19.61 c	20.29 bc
	C1 + M5%	20.66 bc	23.31 a	24.26 a
	C2 + M5%	20.66 bc	24.36 a	23.77 a
Crude protein; % DM	Control	17.63 a	13.72 def	12.18 f
	M5%	17.63 a	16.33 abc	15.41 bcd
	C1	17.63 a	13.77 def	13.70 def
	C2	17.63 a	16.44 ab	14.02 de
	C1 + M5%	17.63 a	16.02 abc	13.47 ef
	C2 + M5%	17.63 a	15.35 bcd	14.59 cde
Soluble protein; % crude protein	Control	36.04 g	41.93 de	46.00 d
	M5%	36.04 g	56.51 a	53.16 b
	C1	36.04 g	40.72 ef	43.00 e
	C2	36.04 g	38.50 fg	42.55 e
	C1 + M5%	36.04 g	49.14 c	49.41 c
	C2 + M5%	36.04 g	53.06 b	51.50 bc
NDF; % DM	Control	50.02 cde	54.07 a	51.59 bc
	M5%	50.02 cde	49.21 de	49.55 cde
	C1	50.02 cde	52.84 ab	49.66 cde
	C2	50.02 cde	48.3 e	49.14 de
	C1 + M5%	50.02 cde	50.30 cde	50.64 cd
	C2 + M5%	50.02 cde	49.76 cde	50.18 cde
ADF; % DM	Control	25.45 a	26.55 a	22.84 b
	M5%	25.45 a	20.00 de	17.40 f
	C1	25.45 a	25.47 a	22.17 bc
	C2	25.45 a	25.30 a	23.11 b
	C1 + M5%	25.45 a	21.62 bcd	19.11 ef
	C2 + M5%	25.45 a	20.49 cde	18.82 ef
Dry matter digestibility; % DM	Control	65.55 ab	62.09 d	62.04 d
	M5%	65.55 ab	66.24 a	66.32 a
	C1	65.55 ab	62.48 d	63.45 cd
	C2	65.55 ab	64.65 abc	63.42 cd
	C1 + M5%	65.55 ab	65.48 ab	64.24 bc
	C2 + M5%	65.55 ab	65.30 ab	65.22 ab

Values represent the average of three experimental replicates per treatment and sampling time (n = 3). \* Different letters within rows indicate significant differences for the additive × time interaction (Duncan's test;  $\alpha = 0.05$ ). DM: dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; M5%: 5 % of molasses; C1: synthetic consortium; C2: native consortium.

values were also significantly higher than for the control (46.00 %). In contrast, C1 and C2 had the lowest soluble protein levels (43.00 and 42.55 %), even below the control ( $p < 0.05$ ) (Table 4).

The crude protein content of fresh *C. brasiliensis* matched a previous report by Castro-Rincón et al. (2018). During fermentation, crude protein declined due to proteolysis, especially in treatments without molasses, where the pH > 4.3 promoted ammonia accumulation and increased soluble protein levels

(Man & Wiktorsson 2002). Molasses-treated silages retained more crude protein, despite the high soluble protein, likely due to reduced proteolysis and microbial use of non-protein nitrogen from molasses (~5 % of protein). Still, some proteolysis likely occurred, possibly driven by slightly elevated pH, even in these treatments.

The NDF concentrations remained mostly stable during fermentation, except for the control and C1 treatments, which showed significantly higher values (54.07 and 52.84 %, respectively) on day 5 ( $p < 0.05$ ). The lowest NDF (48.3 %) was recorded in C2, on day 5. In contrast, ADF varied significantly ( $p < 0.05$ ), with silages containing molasses (M5%; C1 + M5%; C2 + M5%) showing lower ADF levels on day 20 (17.40, 19.11 and 18.82 %, respectively), when compared to pre-silage (25.45 %) and silages without molasses (control: 22.84 %; C1: 22.17 %; C2: 23.11 %) (Table 4).

The dry matter digestibility significantly decreased ( $p < 0.05$ ) in the control, C1 and C2 treatments without molasses, dropping from 65.55 % in pre-silage to 62.04, 63.45 and 63.42 % by day 20, respectively. Although C1 + M5% and C2 + M5% also showed decreases, these were not statistically significant ( $p > 0.05$ ). The M5% treatment exhibited a slight, non-significant increase (66.32 %) in dry matter digestibility after fermentation. At day 20, dry matter digestibility values in M5%, C1 + M5% and C2 + M5% were significantly higher than in the control (Table 4).

No clear trend was observed for the NDF content during fermentation. Increases in some treatments (control and C1 at day 5) likely reflect a relative rise in fiber due to soluble compound loss. In contrast, NDF reductions (C2 at day 5 and inoculated treatments by day 20) suggest actual fiber degradation, particularly of hemicellulose and cellulose (Jaurena & Pichard 2001). Inoculated treatments without molasses showed smaller NDF-ADF gaps, indicating a greater hemicellulose breakdown. Digestibility dropped more markedly in the control, possibly due to spoilage microbes, despite the high lactic acid bacteria levels.

These findings support the use of *C. brasiliensis* at a density of 55,500 plants ha<sup>-1</sup> and the inclusion of molasses at 5 % for improved silage quality. Further studies are needed to assess the long-term effects of these practices on animal performance and economic feasibility.

## CONCLUSIONS

1. Under tropical dry conditions, *Canavalia brasiliensis* showed adequate adaptation as a forage legume;
2. While the planting density did not significantly influence the dry matter yield, the density of 55,500 plants ha<sup>-1</sup> resulted in the longest time to achieve full ground cover, suggesting a slower early development. However, the plant height was the highest for 100,000 plants ha<sup>-1</sup>, indicating a potential advantage for light interception and biomass formation;
3. In the ensiling experiment, the application of 5 % of molasses improved the dry matter content and digestibility of the silage, while maintaining the pH below 5 and soluble carbohydrate levels above 8.9 °Brix;
4. The use of microbial inoculants - either synthetic or native - did not consistently enhance fermentation quality, but contributed to reductions in fiber fractions in some treatments.

## ACKNOWLEDGMENTS

We extend our gratitude to AGROSAVIA C. I. Motilonia, for their administrative support. Special thanks to Dr. Diego Jiménez (Universidad de Los Andes), Hugo Jiménez and Fernando Rodríguez (AGROSAVIA), for supplying the microbial inoculants essential to this research. This study was funded by the 2018 joint research initiative between the Universidad de Los Andes and La Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), under the research project “Biological Additives as Technological Alternatives for the Conservation of Tropical Forages in Silage Processes, under Conditions of the Dry Colombian Caribbean”.

## REFERENCES

ABAYOMI, Y.; FADAYOMI, O.; BABATOLA, J.; TIAN, G. Evaluation of selected legume cover crops for biomass production, dry season survival and soil fertility improvement in a moist Savanna location in Nigeria. *African Crops Science Journal*, v. 9, n. 4, p. 615-627, 2001.

ALONSO-GALEANA, J. A.; MARTÍNEZ, E. J. M.; SEGURA, I. G.; ALMAZÁN, M. T. V.; PLATA, I. J.; HUERTA, R. C.; TRUJILLO, V. G.; GOCHI, L. C.; PÉREZ, A. G.; HERNÁNDEZ, R. R. Comparación del pH y la materia seca en tres procesos de ensilaje con forraje de ajonjolí (*Sesamum indicum*) en el trópico seco. *Archivos*

*Latinoamericanos de Producción Animal*, v. 31 suppl. 1, p. 281-285, 2023.

BARBIERI, P.; STARCK, T.; VOISIN, A. S.; NESME, T. Biological nitrogen fixation of legumes crops under organic farming as driven by cropping management: a review. *Agricultural Systems*, v. 205, e103579, 2023.

BERNABUCCI, U. Climate change: impact on livestock and how can we adapt. *Animal Frontiers*, v. 9, n. 1, p. 3-5, 2019.

BOSCHINI-FIGUEROA, C.; PINEDA-CORDERO, L.; CHACÓN-HERNÁNDEZ, P. Evaluation of ratana grass (*Ischaemum indicum* Hoult.) silage with three different kinds additives. *Agronomía Mesoamericana*, v. 25, n. 2, p. 297-311, 2014.

BURBANO-ERAZO, E.; BROCHERO-ALDANA, G. A.; RODRÍGUEZ-JIMÉNEZ, D. M.; MOJICA, J. E.; LOMBO-ORTIZ, D. F. Evaluation of *Canavalia brasiliensis* Mart. ex Benth. genotypes for seed production in the Colombian Caribbean. *Nutrición Animal Tropical*, v. 14, n. 2, p. 75-84, 2020.

BURBANO-ERAZO, E.; MOJICA-RODRÍGUEZ, J. E.; BROCHERO-ALDANA, G. A.; CARDONA-IGLESIAS, J. L.; CASTRO-RINCÓN, E. Forage production in tropical legumes, in the dry Colombian Caribbean region. *Pastos y Forrajes*, v. 42, n. 2, p. 143-151, 2019.

CALERO HURTADO, A.; CASTILLO, Y.; QUINTERO, E.; PÉREZ, Y.; OLIVERA, D. Effect of four planting density in the agricultural yields on common beans (*Phaseolus vulgaris* L.). *Revista de La Facultad de Ciencias*, v. 7, n. 1, p. 88-100, 2018.

CASTRO, R. E.; SIERRA, A.; MOJICA, J. E.; CARULLA, J. E.; LASCANO, C. E. Effect of species and management of legumes used as green manures in the quality and yield of a forage crop used in livestock systems in the dry tropics. *Archivos de Zootecnia*, v. 66, n. 253, p. 99-106, 2017.

CASTRO-RINCÓN, E.; MOJICA-RODRÍGUEZ, J. E.; CARULLA-FORNAGUERA, J. E.; LASCANO-AGUILAR, C. E. Evaluation of legumes as green manure in forage crops for livestock in the dry Colombian Caribbean. *Agronomía Mesoamericana*, v. 29, n. 3, p. 597-617, 2018.

CHENG, Q.; LI, M.; FAN, X.; CHEN, Y.; SUN, H.; XIE, Y.; LI, P. Effects of epiphytic and exogenous lactic acid bacteria on fermentation quality and microbial community compositions of paper mulberry silage. *Frontiers in Microbiology*, v. 13, e973500, 2022.

COSTA ARAUJO, C. M.; JARA GALEANO, E. S.; ORRICO JUNIOR, M. A. P.; FERNANDES, T.; ALVES, J. P.; RETORE, M.; SILVA, S. J.; ORRICO, C. A.; GARCÍA, A.; MACHADO, L. A. Z. Fermentative



- parameters and chemical composition of mixed silages from corn-crotalaria intercropping. *Animal Feed Science and Technology*, v. 305, e115779, 2023.
- DÍAZ-GARCÍA, L.; CHAPARRO, D.; JIMÉNEZ, H.; GÓMEZ-RAMÍREZ, L. F.; BERNAL, A. J.; BURBANO-ERAZO, E.; JIMÉNEZ, D. J. Top-down enrichment strategy to co-cultivate lactic acid and lignocellulolytic bacteria from the *Megathyrus maximus* Phyllosphere. *Frontiers in Microbiology*, v. 12, e744075, 2021.
- DONG, Z.; SHAO, T.; LI, J.; YANG, L.; YUAN, X. Effect of alfalfa microbiota on fermentation quality and bacterial community succession in fresh or sterile Napier grass silages. *Journal of Dairy Science*, v. 103, n. 5, p. 4288-4301, 2020.
- DOUXCHAMPS, S.; FROSSARD, E.; UEHLINGER, N.; RAO, I.; VAN DER HOEK, R.; MENA, M.; OBERSON, A. Identifying factors limiting legume biomass production in a heterogeneous on-farm environment. *Journal of Agricultural Science*, v. 150, n. 6, p. 675-690, 2012.
- ELIASSON, T.; SUN, L.; LUNDH, A.; HÖJER, A.; SAEDÉN, K. H.; HETTA, M.; GONDA, H. Epiphytic microbiota in Swedish grass-clover herbage and the effect of silage additives on fermentation profiles and bacterial community compositions of the resulting silages. *Journal of Applied Microbiology*, v. 134, n. 9, elxad196, 2023.
- ERTEKIN, I. Effects of commercial bacterial inoculants on fermentation and nutritive quality of wheat and annual legume mixed silages. *Bangladesh Journal of Botany*, v. 52, n. 3, p. 775-782, 2023.
- GAVIRIA, X.; BOLÍVAR VERGARA, D. M.; CHIRINDA, N.; MOLINA-BOTERO, I. C.; MAZABEL, J.; ROSALES, R. B.; ARANGO, J. *In vitro* methane production and ruminal fermentation parameters of tropical grasses and grass-legume associations commonly used for cattle feeding in the tropics. *Livestock Research for Rural Development*, v. 34, n. 5, e17, 2022.
- GUO, X.; XU, D.; LI, F.; BAI, J.; SU, R. Current approaches on the roles of lactic acid bacteria in crop silage. *Microbial Biotechnology*, v. 16, n. 1, p. 67-87, 2023.
- JAURENA, G.; PICHARD, G. Contribution of storage and structural polysaccharides to the fermentation process and nutritive value of lucerne ensiled alone or mixed with cereal grains. *Animal Feed Science and Technology*, v. 92, n. 3-4, p. 159-173, 2001.
- KUNG, L.; SHAVER, R. D.; GRANT, R. J.; SCHMIDT, R. J. Silage review: interpretation of chemical, microbial, and organoleptic components of silages. *Journal of Dairy Science*, v. 101, n. 5, p. 4020-4033, 2018.
- LI, R.; JIANG, D.; ZHENG, M.; TIAN, P.; ZHENG, M.; XU, C. Microbial community dynamics during alfalfa silage with or without clostridial fermentation. *Scientific Reports*, v. 10, e17782, 2020.
- LÓPEZ-HERRERA, M.; BRICEÑO-ARGUEDAS, E. Effect of the legume species and the carbohydrate source on the protein fractionation of silage mixtures. *Nutrición Animal Tropical*, v. 12, n. 1, p. 19-39, 2018.
- MAN, N.; WIKTORSSON, H. Effect of molasses on nutritional quality of cassava and Gliricidia tops silage. *Asian-Australasian Journal of Animal Sciences*, v. 15, n. 9, p. 1294-1299, 2002.
- MARTÍN, G.; COSTA ROUWS, J.; URQUIAGA, S.; RIVERA, R. Crop rotation of *Canavalia ensiformis* green manure of maize and arbuscular mycorrhize in an Eutric Rodic Nitisol of Cuba. *Agronomía Tropical*, v. 57, n. 4, p. 313-321, 2007.
- NKOSI, B. D.; MEESKE, R.; LANGA, T.; MOTIANG, M. D.; MODIBA, S.; MKHIZE, N. R.; GROENEWALD, I. B. Effects of ensiling forage soybean (*Glycine max* (L.) Merr.) with or without bacterial inoculants on the fermentation characteristics, aerobic stability and nutrient digestion of the silage by Damara rams. *Small Ruminant Research*, v. 134, n. 1, p. 90-96, 2016.
- RENTÉ MARTÍ, O.; REYES, P. P.; CORRALES VILA, Y.; CUEVAS RODRÍGUEZ, M.; NÁPOLES GARCÍA, M. C. *Canavalia ensiformis* (L.): in the chemical properties of a Eutric differentiated soils. *Revista Científica Del Amazonas*, v. 3, n. 6, p. 65-75, 2020.
- RONCALLO, B.; MURILLO, J.; RODRÍGUEZ, G.; BONILLA, R. R.; GARRIDO, M. F. Forage production and animal response in soils in the Cesar valley under a recovery process. *Ciencia y Tecnología Agropecuarias*, v. 13, n. 1, p. 89-96, 2012.
- ROOKE, J. A.; HATFIELD, R. D. Biochemistry of ensiling. In: BUXTON, D. R.; MUCK, R. E.; HARRISON, J. H. (ed.). *Silage science and technology*. Madison: American Society of Agronomy, 2003. p. 95-139.
- SABERTANHA, E.; ROUZBEHAN, Y.; FAZAELI, H.; REZAEI, J. Nutritive value of sorghum silage for sheep. *Journal of Animal Physiology and Animal Nutrition*, v. 105, n. 6, p. 1034-1045, 2021.
- SANTOS, C. A.; MONTEIRO, R. C.; HOMEM, B. G. C.; SALGADO, L. S.; CASAGRANDE, D. R.; PEREIRA, J. M.; BODDEY, R. M. Productivity of beef cattle grazing *Brachiaria brizantha* cv. Marandu with and without nitrogen fertilizer application or mixed pastures with the legume *Desmodium ovalifolium*. *Grass and Forage Science*, v. 78, n. 1, p. 147-160, 2023.
- SEPPÄLÄ, A.; RINNE, M.; HUUSKONEN, A. Efficacy of different additives in ensiling faba bean and field pea

- based whole crop silages. *Agricultural and Food Science*, v. 28, n. 4, p. 165-175, 2019.
- SILVA, T. C.; SILVA, L. D. da; SANTOS, E. M.; OLIVEIRA, J. S. Importance of the fermentation to produce high-quality silage. In: JOZALA, A. (ed.). *Fermentation processes*. Rijeka: InTechOpen, 2017. p. 1-20.
- SILVA, V. P.; PEREIRA, O. G.; LEANDRO, E. S.; SILVA, T. C. da; RIBEIRO, K. G.; MANTOVANI, H. C.; SANTOS, S. A. Effects of lactic acid bacteria with bacteriocinogenic potential on the fermentation profile and chemical composition of alfalfa silage in tropical conditions. *Journal of Dairy Science*, v. 99, n. 3, p. 1895-1902, 2016.
- SOLIS, R.; PEZO, M.; ARÉVALO, L.; LAO, C.; ALEGRE, J.; PÉREZ, K. Evaluation of leguminous species as cover crops associated with sacha inchi. *Pesquisa Agropecuaria Tropical*, v. 49, e58011, 2019.
- SOUNDHARRAJAN, I.; PARK, H. S.; RENGASAMY, S.; SIVANESAN, R.; CHOI, K. C. Application and future prospective of lactic acid bacteria as natural additives for silage production: a review. *Applied Sciences*, v. 11, n. 17, e8127, 2021.
- WANG, S.; SHAO, T.; LI, J.; ZHAO, J.; DONG, Z. Fermentation profiles, bacterial community compositions, and their predicted functional characteristics of grass silage in response to epiphytic microbiota on legume forages. *Frontiers in Microbiology*, v. 13, e830888, 2022.
- ZAPATA, M. V.; ISAZA, J. G. L.; BETANCUR, L. F. R.; LOPERA, S. A.; SIERRA, M. M. Plant growth evaluation of *Cajanus cajan* (L.) Millsp., *Canavalia ensiformis* (L.) DC. and *Cratylia argentea* (Desvaux) O. Kuntze., in soils degraded by sand and gravel extraction. *Acta Agronomica*, v. 66, n. 4, p. 580-587, 2017.