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# Bovine Digesta as Organic Fertilizer in Gliricidia Fodder Banks: Agronomic Responses and Nutrient Composition

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## ABSTRACT

**Bovine digesta is an innovative by-product from slaughterhouses to fertilize forage crops, but applying excessive amounts can be inefficient in terms of dry matter yield (DMY) and nutritional characteristics. A two-year trial, which encompassed two rainy and two dry seasons, was conducted to assess the agronomic responses and nutrient composition of gliricidia fertilized with increasing levels of slaughterhouse bovine digesta (0, 1.25, 3.12, 6.25, 9.37, and 12.50 t/ha). Gliricidia DMY enhanced linearly from 8.0 to 15.9 t/ha/yr of DM as the bovine digesta dosage increased from 0 to 12.50 t/ha (p=0.0003). The DMY stability variance increased from 0 ($\sigma_i^2$ = 0.10) to 12.50 t/ha ($\sigma_i^2$ =14.09), so the bovine digesta reduced the DMY stability. Plant height also responded linearly to the fertilizer levels (p<0.0001). Consistent gains in leaf crude protein concentration (21.8, 22.5, 23.0, 23.7, 24.4, and 24.9 % DM for 0.00, 1.25, 3.12, 6.25, 9.37 and 12.50 t/ha, respectively) were observed because of the fertilizer levels (p<0.0001). As the gliricidia responds linearly to the bovine digesta fertilization regarding important agronomic and nutrient-composition traits, we recommend applying the top required dose (12.50 t/ha) to combine maximum forage yield and great roughage nutrient composition. It is not worth saving the organic fertilizer by using lower dosages.**

***Keywords:** arboreal legume; forage crops; Gliricidia sepium; nutritional value; slaughterhouse byproduct*

## INTRODUCTION

Gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) is an arboreal legume native to Mexico and Central America used in ruminant feeding in tropical and semiarid regions worldwide (Alamu *et al*., 2023). The species is naturalized in various regions (e.g., South America, Occidental Africa, and Southern Asia) with adaptations to water paucity conditions (from 365 to 800 mm of yearly rainfall) (Rusdy *et al*., 2021). The plant is extensively grown in fodder banks in countries, such as Mali, Burkina Faso, India, Brazil, and Indonesia (Rusdy *et* al., 2021; Amole *et al*., 2021).

Gliricidia is an excellent forage resource for ruminant feeding because of its elevated dry matter yield (DMY) and crude protein (CP) concentration (Silva *et al*., 2024). The species stands out by its biomass accumulation, desirable morphological traits for a good nutritional value (Bayala *et al*., 2023), and stability for producing forage in rainfed regimes (Dhillon *et al*., 2023). Gliricidia shows great nutrient composition with high leaf CP content (22.50% of DM, on average) and digestibility (66.30% of digestible DM, on average) (Silva *et al*., 2024).

Moreover, gliricidia responds very well to soil fertilization (Fungo *et al*., 2020), but chemical fertilizers might increase the cost of fodder bank maintenance. The indiscriminate use of chemical fertilizers can reduce soil fertility and the enzymatic activities of soil microbiota (Ansari & Mahmood, 2017). Conversely, organic fertilizers in fodder banks can reduce costs and be efficient in sustainable aspects, mainly because they are often potential polluter byproducts from the agribusiness production chain (Urra *et al*., 2019). Moreover, organic fertilizers from animal sources can improve nutrient cycling, soil organic matter concentration, and adequate substrates for the soil microbiota. Animal waste fertilizers generally have a low C/N ratio and are rich in readily plant-available N (Bergstrand, 2022). For these reasons, using organic manures in agriculture is an age-old practice in tropical areas like Brazil and Indonesia (Lestari *et al*., 2024). However, using organic fertilizers might not be efficient if their compositions are poor in minerals such as N, P, and K (Bhunia *et al*., 2021a).

In this scenario, bovine digesta is an important byproduct of ruminant slaughtering largely used as organic fertilizer in agriculture and horticulture crops, with great agronomic responses considering its rich mineral composition, mainly the higher N content and solubility than other organic manures (Roy *et al.*, 2016; Sankar *et al.*, 2022; Bhunia *et al.*, 2021b). Bhunia *et al*. (2021a) found more fruits per plant in bell pepper plants (*Capsicum annuum* L. var. Arka Basant) fertilized with bovine digesta (6.0) than with vermicomposting (1.6) and chemical salts (3.3). Bovine digesta is safe even without heat treatment or composting. Unlike other organic fertilizers, only dehydration is enough because the raw material is previously digested in the rumen. Also, it does not produce unpleasant odors during or after dehydration because of the rumen fluid evaporation (Edvan & Carneiro, 2011). Bovine digesta is not largely sought by farmers to fertilize forage crops, so its use can be innovative in the meat production chain (Bhunia *et al.*, 2021b).

Collection and transportation from the slaughterhouse are probably the main challenges of using bovine digesta as fertilizer, but it has many productive and socioeconomic advantages (Edvan & Carneiro, 2011). The use of bovine digesta as fertilizer can create proper disposal to avoid water contamination and eutrophication, and reduce risks of selling contaminated carcasses (Bhunia *et al.*, 2021b). Furthermore, the waste-to-fertilizer conversion has the potential to boost the economy in rural communities (Bhunia *et al.*, 2021b).

Considering this background, we hypothesized that increasing the dosage of bovine digesta used as an organic fertilizer would improve gliricidia forage yield and protein concentration. In addition, we hypothesized that rainy seasons contribute to high forage yield and high leaf CP concentration. Thus, the objective of this study was to evaluate agronomic responses and nutrient composition of gliricidia in response to increasing levels of bovine digesta applied as fertilizer and obtained from a slaughterhouse (0.00, 1.25, 3.12, 6.25, 9.37, and 12.50 t/ha) in a rainfed condition. This study was conducted to find the most suitable dose to fertilize gliricidia fodder banks, aiming for high forage yield and great nutrient composition.

## MATERIALS AND METHODS

### Site Description and Weather Details

The trial was conducted at the Professor Ignacio Salcedo Research Station of the Instituto Nacional do Semiárido (INSA), located in the municipality of Campina Grande, state of Paraíba, Brazil (07º14’00” S, 35º57’00” W; 491 m above sea level). The climate is characterized as As’ or dry tropical. The average rainfall is 503 mm annually, and the soil is classified as Solonetz (IUSS Working Group 2015; Santos *et al*., 2018). The experiment was conducted from 18 April 2022 to 9 December 2023. Figure 1 shows soil water balance (Camargo & Camargo, 2000) and weather data during the trial period.

### Field Experiment, Treatments, and Experimental Design

Gliricidia fodder bank used in the trial was grown in 2017 with no fertilization. The fodder bank area is comprised of 0.15 ha, with 500 trees planted after producing seedlings in the greenhouse of Semiarid National Institute of Brazil (INSA). Seedlings were transplanted to the site at 50 cm height, spaced 1.5 m apart between rows and plants. Trees were assessed in the fodder bank regarding morphological traits (plant height and canopy diameter) from 14 February 2020 to 24 August 2021 and then harvested at 50 cm stubble height.

The experimental design was a randomized complete block with three replications (experimental plots measuring 20 m² formed by 16 plants each) and six treatments comprised 0%, 10%, 25%, 50%, 75%, and 100% of the recommended dose of bovine digesta based on soil chemical properties, bovine digesta chemical properties, and technical guidelines. For that, ten soil samples were taken from the trial site in a zigzag way at a 40-cm layer and homogenized to form a composite sample. After that, the composite sample was analyzed for chemical properties.

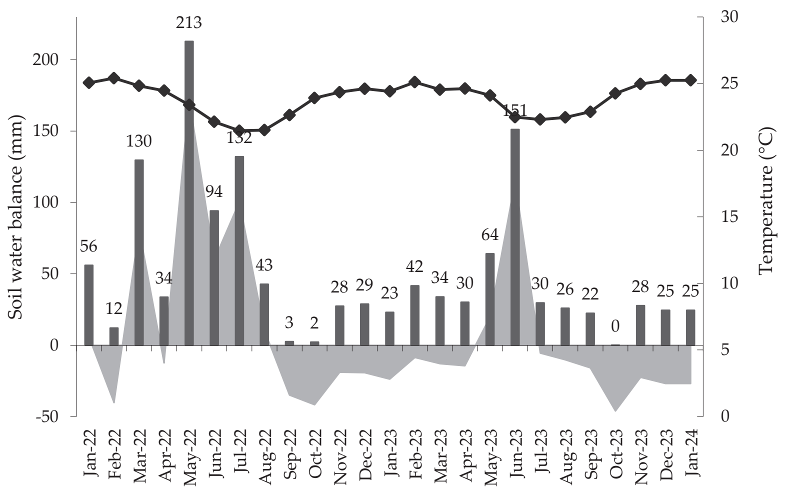


Figure 1. Soil water balance, rainfall, and monthly average temperature at the study site (Experimental Station of the Instituto Nacional do Semiárido, Campina Grande, PB) from January 2022 to January 2024. Soil water balance (mm), Rainfall (mm), Average temperature (ºC).

Soil chemical properties on 10 January 2022 were 1.00 and 58.65 mg/dm$^3$ for P and K, respectively, besides 0.04, 0.93, 2.17, 3.15, and 0.26 cmol$_c$/dm$^3$ for Na, $Ca^{2+}$, $Mg^{2+}$, $H^+$, and $Al^{3+}$. Organic carbon concentration was 0.59%, pH was 5.22, the organic matter concentration was 1.02%, the effective cation exchange capacity was 3.55%, and the soil base saturation was 48.95%. On 7 January 2023, soil chemical properties were 11.4 and 132.9 mg/dm$^3$ for P and K, respectively, besides 0.09, 2.30, 1.40, 1.60, and 0.10 cmol$_c$/dm$^3$ for Na, $Ca^{2+}$, $Mg^{2+}$, $H^+$, and $Al^{3+}$. Organic carbon concentration was 3.10%, pH was 5.94, the organic matter concentration was 2.30%, the effective cation exchange capacity was 4.23%, and the soil base saturation was 70.84%.

The bovine digesta was obtained from the Vera Cruz slaughterhouse in Campina Grange municipality, Paraíba state, Brazil. The bovine digesta fertilizer was obtained from the rumen emptying at the evisceration made by the employers, followed by sun drying. The top average temperatures in Campina Grande in 2022 (year 1) and 2023 (year 2) were 24.2 and 24.5 °C, respectively. The bovine digesta was analyzed in terms of concentrations of N (Horwitz, 2005), P, K, Ca, and Mg (Bezerra Neto & Barreto, 2011). P and K values were transformed into $P_2O_5$ and $K_2O$ (Table 1) to calculate the necessary dose to fertilize gliricidia fodder banks. P, K, Ca, and Mg contents were analyzed in the Multipurpose Laboratory of INSA (LABINSA). In contrast, the N content was obtained in the Laboratory of Animal Feed and Nutrition of INSA (LANA-INSA).

According to the Cavalcanti *et al*. (2008) handbook, the levels of N, P, and K required to fertilize the gliricidia fodder banks were 1, 30, and 20 kg/ha/yr of N, $P_2O_5$, and $K_2O$, respectively (1-30-20 of NPK). Thus, considering the soil's chemical properties and the bovine digesta potential as fertilizer (Table 1), we calculated the entire bovine digesta level needed to address 1, 30, and 20 kg/ha/yr of N, $P_2O_5$, and $K_2O$ using the rule of three, and found 12.50 t/ha/yr for that. This value (12.50 t/ha/yr) was converted into 25 kg per plot per year, considering the 20-m$^2$ plot size. The increasing doses were calculated from that: 0, 10, 25, 50, and 75% of the total recommended dose were applied at 0.00, 1.25, 3.125, 6.25, and 9.375 t/ha/yr, converted into 0.00, 2.50, 6.25, 12.50, and 18.75 kg per plot per year also considering the 20-m$^2$ plot size. The organic fertilizer was weighed on a digital scale with 1-g precision. Gliricidia plots were fertilized on 18 April 2022 and 13 February 2023, respectively. A top-dressing fertilization was performed, and the dehydrated bovine digesta was directly applied to the soil. We did not record stink issues during the trial, probably because of the rumen fluid evaporation (Edvan & Carneiro, 2011).

Table 1. Chemical properties of slaughterhouse bovine digesta applied as organic fertilizer to fodder banks of gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) on 21 February 2022 and 19 January 2023

| Bovine digesta collected on | P | N | K | Ca | Mg | OM |
|---|---|---|---|---|---|---|
| | mg/dm$^3$ | g/kg | | | | |
| Monday, February 21, 2022 | 184 | 11.0 | 7.1 | 13.6 | 3.7 | 336 |
| Thursday, January 19, 2023 | 186 | 8.7 | 8.9 | 12.1 | 5.4 | 456 |
| | $P_2O_5$ | N | $K_2O$ | Ca | Mg | MO |
| | % | | | | | |
| Monday, February 21, 2022 | 4.21 | 1.10 | 0.85 | 1.36 | 0.37 | 33.6 |
| Thursday, January 19, 2023 | 4.26 | 0.87 | 1.07 | 1.21 | 0.44 | 45.6 |

Note: P= phosphorous, N= nitrogen, K= potassium, Ca= calcium, Mg= magnesium, OM= organic matter.

### DMY and Agronomic Responses

Dry matter yield (DMY) evaluations and morphological traits were collected from 18 April 2022 to 9 December 2023, totaling four harvests. Two were performed in rainy seasons, and the others were made in dry seasons. The harvest frequency was 120 days during the rainy and 180 days during the dry seasons. The cutback to attain uniform height prior to the evaluations was made on 18 April 2022, while the first harvest occurred on 16 August 2022, the second one on 12 February 2023, the third was performed on 12 June 2023, and the last was made on 9 December 2023. The regrowth intervals from the first and third harvests were classified as "rainy season", while the regrowth periods of the second and fourth cuts were the "dry season". This classification followed the soil water balance observed in the trial (Figure 1). For all evaluated plants, the cut intensity was 50 cm of stubble height. All biomass was reaped from the fodder bank at each harvest time. Plots were 20 m$^2$, and the net plot area comprised four plants centrally positioned on each plot (2.25 m$^2$).

The total DMY, including the DMY in leaves and stems, was estimated based on the dry matter (DM) concentration and the four plants' weight within the net plot area. A 1-kg aliquot was separated from the cut fodder to be fractionated into leaves and stems, which were dried in a forced-air oven at 55 °C for 72 h. The DM content (934.01) was determined according to the AOAC methods (Horwitz, 2005). In addition, parameters of Shukla's stability variance for DMY (Shukla, 1972) have been evaluated owing to environmental variations over seasons and years. From the DMY of leaves and stems, we could calculate the leaf/stem ratio by dividing the leaf biomass by the stem biomass. Plant height and canopy diameter were assessed using a 2-m long stick graduate in centimeters.

### Forage Nutrient Composition

Leaf samples were collected, identified, and dried in a forced-air oven at 55 °C for 72 hours to analyze the chemical composition. The analyses were performed in the Laboratory of Animal Feed and Nutrition of INSA (LANA-INSA). Concentrations of DM, ashes, crude protein (CP), and ether extract (EE) were analyzed using AOAC methods (AOAC, 2005). Concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest *et al*. (1991). The NDF was analyzed without thermostable amylase, including residual ashes and proteins. All chemical variables were expressed as percentages.

The non-fiber carbohydrates (NFC) were determined according to Sniffen *et al*. (1992) using the formula:

Furthermore, the total digestible nutrient content (TDN) was calculated using the ADF concentration and the following formula proposed by Patterson (2000):

$$NFC = 100 - (NDF + CP + ashes + EE)$$

Furthermore, the total digestible nutrient content (TDN) was calculated using the ADF concentration and the following formula proposed by Patterson (2000):

$$TDN = 88.90 - (7.79 \times ADF)$$

### Statistical Analysis

Data were subjected to the normality test of residuals (covtest residual panel) and analysis of variance (ANOVA) using the PROC MIXED of SAS On Demand for Academics (SAS Institute Inc., 2014). The effects of season, bovine digesta level, and the season-bovine digesta interaction were fixed, while the block and evaluation year effects and their interactions were random. Bovine digesta levels within each season of the year were subjected to regression analyses (p<0.05) using the PROC GLM of SAS OnDemand for Academics. The seasons were analyzed as repeated measures in time. The mathematical model was:

$$Y_{ijkl} = \mu + X\alpha_i + X\beta_j + Z\tau_k + Z\delta_l + X(\alpha\beta)_{ij} + Z(\alpha\tau)_{ik} + Z(\beta\tau)_{jk} + Z(\alpha\beta\tau)_{ijk} + Z(\alpha\delta)_{il} + Z(\beta\delta)_{jl} + Z(\alpha\beta\delta)_{ijl} + Z(\tau\delta)_{kl} + e_{ijkl}$$

Where the $Y_{ijk}$ is the dependent variable; $\mu$ is the overall mean; $X\alpha_i$ is the fixed effect of bovine digesta level (1 to 6); $X\beta_j$ is the fixed effect of season of the year (1 to 2); $Z_{\tau k}$ is the block random effect (1 to 3); $X(\alpha\beta)_{ij}$ is the season-bovine digesta interaction effect; $Z(\alpha\tau)_{ik}$, $Z(\beta\tau)_{jk}$, and $Z(\alpha\beta\tau)_{ijk}$ are the interaction effects of block with bovine digesta level and season of the year; $Z(\alpha\delta)_{il}$, $Z(\beta\delta)_{jl}$, and $Z(\alpha\beta\delta)_{ijl}$ are the interaction effects of evaluation year with bovine digesta level and season of the year; $Z(\tau\delta)_{kl}$ is the interaction between random effects; and $e_{ijk}$ is the residual error.

When the F-test was significant (p<0.05), means of year's seasons were compared using the probability of difference ("pdiff") adjusted by the Tukey test. Means of the bovine digesta levels were compared using linear and quadratic effects of polynomial orthogonal contrasts, in addition to the regression analysis to formulate the equations. All tests were considered significant at 5% of error probability (p<0.05).

Parameters of stability variance ($\sigma_i^2$) for DMY were calculated considering the bovine digesta levels and the four harvests over the two evaluation years. The model of Shukla's stability variance (Shukla, 1972) was applied using the PROC MIXED procedure of SAS OnDemand for Academics, according to the statements proposed by Piepho (1999). In this analysis, lower variance indicates better stability (Reckling *et al*., 2021).

## RESULTS

### DMY and Agronomic Responses

There was an interaction effect of season of the year and bovine digesta level on DMY per harvest and year (p<0.05). Considering the seasons within each bovine digesta level, higher DMY values were recorded in the rainy period. The DMY increased linearly regardless of the season of the year (Table 2). No quadratic effects of bovine digesta levels were observed on the gliricidia biomass production (p>0.05).

Table 2. Dry matter yield (DMY) and parameters of Shukla's stability variance ($\sigma i^2$) in gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) fodder banks fertilized with slaughterhouse bovine digesta levels in the rainy and dry seasons of years 1 and 2

| Bovine digesta level (t/ha) | Season of the year | | Mean | Annual DMY (t/ha/yr) | Shukla's stability variance ($\sigma i^2$) |
|---|---|---|---|---|---|
| | Rainy | Dry | | | |
| | DMY (t/ha) | | | | |
| 0.00 | 5.29 ᵃ | 2.74 ᵃ | 4.02 | 8.03 | 0.10 |
| 1.25 | 6.39 ᵃ | 2.50 ᵃ | 4.45 | 8.89 | 4.26 |
| 3.12 | 6.92 ᵃ | 2.45 ᵃ | 4.69 | 9.37 | 6.65 |
| 6.25 | 8.12 ᵃ | 3.58 ᵃ | 5.85 | 11.70 | 6.95 |
| 9.37 | 11.67 ᵃ | 6.08 ᵃ | 8.87 | 17.75 | 12.22 |
| 12.50 | 10.89 ᵃ | 4.98 ᵃ | 7.93 | 15.87 | 14.09 |
| Mean | 8.22 | 3.72 | | 11.94 | - |
| SEM | 0.738 | | | 0.841 | - |
| *P-value* | | | | | |
| S | 0.0139 | | | - | |
| BD | 0.0062 | | | 0.0003 | |
| BD × S | 0.0209 | | | - | |
| L | 0.0187 | <0.0001 | | <0.0001 | |
| Q | 0.4339 | 0.8587 | | 0.7410 | |
| EQ | Y = 5.23 + 0.704x | Y = 2.24 + 0.296x | | Y = 7.48 + 0.999x | |
| $R^2$ | 0.77 | 0.68 | | 0.77 | |

Note: Means followed by the lowercase letter within rows do not differ by the Tukey test (p<0.05) for year's season effect. Linear (L) and quadratic (Q) effects on bovine digesta levels were significant at a 5% probability of error (p<0.05), according to the polynomial orthogonal contrast test. SEM= standard error of the mean, BD= effect of bovine digesta level, S= year's season effect, BD × S= interaction effect (bovine digesta level × season of the year), EQ= equations for linear and quadratic models, $R^2$= determination coefficient.

Furthermore, increasing values of parameters of Shukla's stability variance were found as the bovine digesta levels enhanced. Thus, gliricidia fertilized with lower bovine digesta levels were more stable for DM production than those treated with higher levels (Table 2).

An interaction effect (bovine digesta level × season of the year) was found in the leaf DMY ($p<0.05$). A higher leaf biomass was harvested in the rainy than in the dry period within all bovine digesta levels (Table 3). Moreover, the leaf DMY enhanced linearly as the bovine digesta level was increased in both seasons. An interaction effect was also observed in the stem DMY ($p<0.05$), with positive linear responses to the bovine digesta fertilizer in both seasons too. Stem biomass production was higher in the rainy season than in the dry season in all tested bovine digesta levels.

The interaction between the year's season and bovine digesta level affected gliricidia's leaf/stem ratio ($p<0.05$). Within each bovine digesta level, a

Table 3. Leaf and stem dry matter yields (DMY) of gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) fertilized with slaughterhouse bovine digesta levels in the rainy and dry seasons of years 1 and 2

| Bovine digesta level (t/ha) | Season of the year | | Mean | Season of the year | | Mean |
|---|---|---|---|---|---|---|
| | Rainy | Dry | | Rainy | Dry | |
| | Leaf DMY (t/ha) | | | Stem DMY (t/ha) | | |
| 0.00 | 3.03 [a] | 1.65 [b] | 2.34 | 2.26 [a] | 1.09 [b] | 1.68 |
| 1.25 | 3.97 [a] | 1.49 [b] | 2.73 | 2.42 [a] | 1.01 [b] | 1.72 |
| 3.12 | 3.58 [a] | 1.45 [b] | 2.52 | 3.34 [a] | 1.00 [b] | 2.17 |
| 6.25 | 4.64 [a] | 1.96 [b] | 3.30 | 3.48 [a] | 1.63 [b] | 2.55 |
| 9.37 | 6.51 [a] | 3.05 [b] | 4.78 | 5.16 [a] | 3.03 [b] | 4.09 |
| 12.50 | 5.75 [a] | 2.79 [b] | 4.27 | 5.14 [a] | 2.19 [b] | 3.66 |
| Mean | 4.58 | 2.06 | | 3.63 | 1.66 | |
| SEM | 0.349 | | | 0.360 | | |
| *P-value* | | | | | | |
| S | 0.0184 | | | <0.0001 | | |
| BD | <0.0001 | | | 0.0082 | | |
| BD × S | 0.0067 | | | 0.0016 | | |
| L | 0.0002 | <0.0001 | | 0.0044 | 0.0002 | |
| Q | 0.4631 | 0.6213 | | 0.4848 | 0.5191 | |
| EQ | $Y = 3.04 + 0.387x$ | $Y = 1.43 + 0.089x$ | | $Y = 2.18 + 0.316x$ | $Y = 0.81 + 0.206x$ | |
| $R^2$ | 0.61 | 0.72 | | 0.87 | 0.62 | |

Note: Means followed by the lowercase letter within rows do not differ by the Tukey test ($p<0.05$) for year's season effect. Linear (L) and quadratic (Q) effects on bovine digesta levels were significant at a 5% probability of error ($p<0.05$), according to the polynomial orthogonal contrast test. SEM= standard error of the mean, BD= effect of bovine digesta level, S= year's season effect, BD × S= interaction effect (bovine digesta level × season of the year), EQ= equations for linear and quadratic models, $R^2$= determination coefficient.

Table 4. Leaf/stem ratio of gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) fertilized with slaughterhouse bovine digesta levels in the rainy and dry seasons of years 1 and 2

| Bovine digesta level (t/ha) | Season of the year | | Mean |
|---|---|---|---|
| | Rainy | Dry | |
| 0.00 | 1.51 [a] | 1.46 [a] | 1.48 |
| 1.25 | 1.66 [a] | 1.40 [a] | 1.53 |
| 3.12 | 1.07 [b] | 1.57 [a] | 1.32 |
| 6.25 | 1.20 [a] | 1.15 [a] | 1.17 |
| 9.37 | 1.14 [a] | 0.92 [a] | 1.03 |
| 12.50 | 1.08 [a] | 1.08 [a] | 1.23 |
| Mean | 1.28 | 1.31 | |
| SEM | 0.188 | | |
| *P-value* | | | |
| S | 0.8107 | | |
| BD | 0.2208 | | |
| BD × S | 0.0022 | | |
| L | 0.0075 | 0.0112 | |
| Q | 0.0337 | 0.0370 | |
| EQ | $Y = 1.56 - 0.097x + 0.0049x^2$ | $Y = 1.56 - 0.105x + 0.0065x^2$ | |
| $R^2$ | 0.61 | 0.44 | |

Note: Means followed by the lowercase letter within rows do not differ by the Tukey test ($p<0.05$) for year's season effect. Linear (L) and quadratic (Q) effects on bovine digesta levels were significant at a 5% probability of error ($p<0.05$), according to the polynomial orthogonal contrast test. SEM= standard error of the mean, BD= effect of bovine digesta level, S= year's season effect, BD × S= interaction effect (bovine digesta level × season of the year), EQ= equations for linear and quadratic models, $R^2$= determination coefficient.

significant difference between rainy and dry periods was observed only in the 3.12 t/ha level (Table 4). There were reductions in the leaf/stem ratio as the bovine digesta was increased in both seasons under linear and quadratic effects. In the rainy season, the control treatment led to a leaf/stem ratio of 1.51, while the top fertilization level led to a 1.08 value. In the dry period, the mean decreased from 1.46 (control treatment) to 1.08 (12.50 t/ha level).

Gliricidia's canopy diameter was affected by the interaction between the year's season and bovine digesta level (p<0.05). Wider canopies were found in gliricidia trees harvested in the rainy than in the dry period in all tested organic fertilizer levels (Table 5). Canopies were more expansive as the bovine digesta levels increased in the rainy season under a linear effect. In the dry season, only a quadratic effect was found. This time, gliricidia canopies diminished from 0 (control) to 3.12 t/ha level. Then, progressive gains were recorded until the top-recommended dose (12.50 t/ha). Furthermore, an interaction effect was observed in the plant height. Within levels 0.00 (control), 1.25, and 9.37 t/ha of rumen digesta, there was no difference in plant size comparing the rainy to the dry period, contrary to what was observed at 3.12, 6.25, and 12.50 t/ha. Plants grew linearly as the bovine digesta level increased in both seasons (Table 5).

## Forage Nutrient Composition

Isolated effects of season and bovine digesta levels were observed in the DM content (p<0.05), but no interaction was found (p>0.05; Table 6). Higher DM content in the leaves was recorded in the rainy than in the dry season, regardless of the tested levels. Linear DM content reductions were found as the bovine digesta level increased (from 27.08 to 24.55% on average).

We could observe an interaction effect of season and bovine digesta levels on ash contents in gliricidia leaves (p<0.05). Results were variable when comparing the seasons within each level of organic fertilizer; that is, they were higher in rainy season for some levels but were higher in the dry season for others (Table 6). Higher ash content in leaves was recorded in the rainy than in the dry season for the control treatment. The opposite was recorded in the other bovine digesta treatments, except for the 6.25 t/ha level, for which we did not find a significant difference. Within the rainy season, no linear or quadratic effect was observed, and no differences between the means were reported. Within the dry season, we found a linear ash content increment from 6.38 to 7.54% of DM.

Only the season of the year changed the NDF concentration in gliricidia leaves (p<0.05), so the organic fertilizer did not affect this variable (p>0.05; Table 7). A higher NDF content was found in the dry than in the rainy season. Conversely, the year's season and bovine digesta levels had an interaction effect on the ADF content, but no linear or quadratic effects were found. Higher ADF content was found in rainy than in dry season within 0.00 (control), 3.12 and 6.25 t/ha bovine digesta levels, while no significant differences were observed in the other levels (1.25, 9.37, and 12.50 t/ha). Within the rainy season, the highest leaf ADF content was observed in the 6.25 t/ha level and the lowest in the top level (12.50 t/ha). Within the dry season, the highest

Table 5. Canopy diameter and plant height of gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) fertilized with slaughterhouse bovine digesta levels in the rainy and dry seasons of years 1 and 2

| Bovine digesta level (t/ha) | Season of the year | | Mean | Season of the year | | Mean |
|---|---|---|---|---|---|---|
| | Rainy | Dry | | Rainy | Dry | |
| | Canopy diameter (cm) | | | Plant height (cm) | | |
| 0.00 | 147 [a] | 128 [b] | 137 | 119 [a] | 103 [a] | 111 |
| 1.25 | 148 [a] | 106 [b] | 127 | 128 [a] | 94 [a] | 111 |
| 3.12 | 163 [a] | 108 [b] | 136 | 150 [a] | 88 [b] | 119 |
| 6.25 | 159 [a] | 114 [b] | 137 | 155 [a] | 110 [b] | 133 |
| 9.37 | 180 [a] | 122 [b] | 151 | 155 [a] | 120 [a] | 138 |
| 12.50 | 185 [a] | 124 [b] | 154 | 182 [a] | 120 [b] | 151 |
| Mean | 164 | 117 | | 149 | 106 | |
| EPM | 7.9 | | | 16.0 | | |
| *P-value* | | | | | | |
| S | 0.0496 | | | 0.170 | | |
| BD | 0.0939 | | | 0.0452 | | |
| BD × S | <0.0001 | | | 0.0004 | | |
| L | <0.0001 | 0.1176 | | <0.0001 | 0.0003 | |
| Q | 0.9841 | 0.0433 | | 0.4843 | 0.4388 | |
| EQ | $Y=146.71 + 3.140x$ | $Y = 118.85 – 2.883x + 0.2856x^2$ | | $Y=123.34 + 5.563x$ | $Y = 96 + 0.844x$ | |
| $R^2$ | 0.79 | 0.29 | | 0.85 | 0.59 | |

Note: Means followed by the lowercase letter within rows do not differ by the Tukey test (p<0.05) for year's season effect. Linear (L) and quadratic (Q) effects on bovine digesta levels were significant at a 5% probability of error (p<0.05), according to the polynomial orthogonal contrast test. SEM= standard error of the mean, BD= effect of bovine digesta level, S= year's season effect, BD × S= interaction effect (bovine digesta level × season of the year), EQ= equations for linear and quadratic models, $R^2$= determination coefficient.

Table 6. Dry matter (DM) and ash content in leaves of gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) fertilized with slaughterhouse bovine digesta levels in the years 1 and 2

| Bovine digesta level (t/ha) | Season of the year | | Mean | Season of the year | | Mean |
|---|---|---|---|---|---|---|
| | Rainy | Dry | | Rainy | Dry | |
| | DM (% of fresh matter) | | | Ash (% of DM) | | |
| 0.00 | 25.62 | 28.54 | 27.08 | 6.77 [a] | 6.38 [b] | 6.58 |
| 1.25 | 25.17 | 26.47 | 25.82 | 6.28 [b] | 7.04 [a] | 6.66 |
| 3.12 | 24.85 | 27.15 | 26.00 | 6.45 [b] | 6.95 [a] | 6.70 |
| 6.25 | 24.09 | 27.04 | 25.56 | 6.70 [a] | 6.95 [a] | 6.83 |
| 9.37 | 23.10 | 24.73 | 23.91 | 6.71 [b] | 7.11 [a] | 6.91 |
| 12.50 | 23.07 | 26.04 | 24.55 | 6.92 [a] | 7.54 [a] | 7.23 |
| Mean | 24.22 [b] | 26.66 [a] | | 6.64 | 7.00 | |
| SEM | 2.50 | | | 0.18 | | |
| *P-value* | | | | | | |
| S | 0.0011 | | | 0.0037 | | |
| BD | 0.0002 | | | 0.0435 | | |
| BD × S | 0.0719 | | | 0.0415 | | |
| L | 0.0002 | 0.0081 | | 0.8540 | 0.0228 | |
| Q | 0.7675 | 0.3126 | | 0.0827 | 0.0918 | |
| EQ | Y = 25.36 – 0.244x | Y = 28.01 -0.421x | | Y = 6.75 | Y = 6.55 + 0.166x | |
| $R^2$ | 0.61 | 0.41 | | 0.20 | 0.45 | |

Note: Means followed by the lowercase letter within rows do not differ by the Tukey test ($p<0.05$) for year's season effect. Linear (L) and quadratic (Q) effects on bovine digesta levels were significant at a 5% probability of error ($p<0.05$), according to the polynomial orthogonal contrast test. SEM= standard error of the mean, BD= effect of bovine digesta level, S= year's season effect, BD × S= interaction effect (bovine digesta level × season of the year), EQ= equations for linear and quadratic models, $R^2$= determination coefficient.

Table 7. Concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in leaves of gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) fertilized with slaughterhouse bovine digesta levels in the years 1 and 2

| Bovine digesta level (t/ha) | Season of the year | | Mean | Season of the year | | Mean |
|---|---|---|---|---|---|---|
| | Rainy | Dry | | Rainy | Dry | |
| | NDF (% of DM) | | | ADF (% of DM) | | |
| 0.00 | 46.57 | 47.74 | 47.16 | 34.08 [a] | 27.06 [b] | 30.57 |
| 1.25 | 41.48 | 49.76 | 45.62 | 29.69 [a] | 29.61 [a] | 29.65 |
| 3.12 | 40.77 | 48.95 | 44.86 | 31.16 [a] | 25.43 [b] | 28.29 |
| 6.25 | 47.70 | 50.72 | 49.21 | 34.76 [a] | 29.50 [b] | 32.13 |
| 9.37 | 42.32 | 51.25 | 46.78 | 29.67 [a] | 28.64 [a] | 29.05 |
| 12.50 | 41.11 | 47.60 | 44.36 | 28.28 [a] | 26.87 [a] | 27.57 |
| Mean | 43.32 [b] | 49.34 [a] | | 31.24 | 27.87 | |
| SEM | 0.82 | | | 0.66 | | |
| *P-value* | | | | | | |
| S | 0.0055 | | | 0.0308 | | |
| BD | 0.0980 | | | 0.0044 | | |
| BD × S | 0.1520 | | | 0.0065 | | |
| L | 0.4875 | 0.7446 | | 0.6557 | 0.3848 | |
| Q | 0.3332 | 0.2942 | | 0.2188 | 0.3650 | |
| EQ | Y = 46.52 | Y = 47.69 | | Y = 34.05 | Y = 27.01 | |
| $R^2$ | 0.11 | 0.28 | | 0.41 | 0.05 | |

Note: Means followed by the lowercase letter within rows do not differ by the Tukey test ($p<0.05$) for year's season effect. Linear (L) and quadratic (Q) effects on bovine digesta levels were significant at a 5% probability of error ($p<0.05$), according to the polynomial orthogonal contrast test. SEM= standard error of the mean, BD= effect of bovine digesta level, S= year's season effect, BD × S= interaction effect (bovine digesta level × season of the year), EQ= equations for linear and quadratic models, $R^2$= determination coefficient.

ADF content was found in 1.25 and 6.25 t/ha levels, while the lowest value was recorded in the 3.12 t/ha level.

Bovine digesta levels linearly increased the CP content in gliricidia leaves ($p<0.05$; Table 8). Conversely, no season effect was found. Consistent gains in the leaf CP concentration were observed due to the fertilizer-increasing levels in both rainy and dry seasons.

In addition, isolated effects of season and bovine digesta levels were observed on the NFC concentration in gliricidia leaves ($p<0.05$; Table 8). NFC content was higher in the rainy than in the dry season, regardless of the fertilizer level. Moreover, the leaf NFC concentration was higher in the 1.25 and 3.12 than in the 6.25 and 9.37 t/ha levels. The lowest NFC content was recorded in the 9.37 t/ha level, regardless of the season evaluated. No

Table 8. Concentrations of crude protein (CP), non-fiber carbohydrates (NFC) and total digestible nutrients (TDN) in leaves of gliricidia (*Gliricidia sepium* [Jacq.] Kunth ex Walp.) fertilized with slaughterhouse bovine digesta levels in the years 1 and 2

| Bovine digesta level (t/ha) | Season of the year | | Mean | Season of the year | | Mean | Season of the year | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | Rainy | Dry | | Rainy | Dry | | Rainy | Dry | |
| | CP (% of DM) | | | NFC (% of DM) | | | TDN (% of DM) | | |
| 0.00 | 21.16 | 22.44 | 21.80 | 19.91 | 18.67 | 19.29 | 59.63 $^{b}$ | 67.28 $^{a}$ | 63.45 |
| 1.25 | 22.18 | 22.77 | 22.47 | 24.67 | 16.00 | 20.34 | 65.00 $^{a}$ | 65.17 $^{a}$ | 65.09 |
| 3.12 | 22.87 | 23.16 | 23.01 | 24.90 | 16.32 | 20.61 | 62.37 $^{b}$ | 68.58 $^{a}$ | 65.47 |
| 6.25 | 23.41 | 24.06 | 23.74 | 17.18 | 13.44 | 15.31 | 60.28 $^{b}$ | 65.33 $^{a}$ | 62.80 |
| 9.37 | 24.36 | 24.44 | 24.40 | 21.24 | 11.93 | 16.58 | 65.27 $^{a}$ | 66.01 $^{a}$ | 65.64 |
| 12.50 | 25.29 | 24.57 | 24.93 | 21.18 | 15.92 | 18.53 | 66.17 $^{a}$ | 67.43 $^{a}$ | 66.80 |
| Mean | 23.21 | 23.57 | | 21.51 $^{a}$ | 15.38 $^{b}$ | | 63.12 | 66.63 | |
| SEM | 0.25 | | | 0.92 | | | 0.48 | | |
| *P-value* | | | | | | | | | |
| Y | 0.6175 | | | <0.0001 | | | 0.0005 | | |
| BD | <0.0001 | | | 0.0021 | | | <0.0001 | | |
| BD × Y | 0.1570 | | | 0.0910 | | | 0.0002 | | |
| L | 0.0026 | 0.0078 | | 0.7704 | 0.0008 | | 0.7780 | 0.3848 | |
| Q | 0.9997 | 0.1640 | | 0.9879 | 0.0032 | | 0.2832 | 0.3650 | |
| EQ | Y =22.04 + 0.255x | Y=22.34 + 0.334x | | Y = 19.88 | Y = 18.67 – 1.506x + 0.0990x² | | Y = 59.60 | Y = 67.20 | |
| $R^2$ | 0.92 | 0.73 | | 0.35 | 0.58 | | 0.40 | 0.05 | |

Note: Means followed by the lowercase letter within rows do not differ by the Tukey test ($p<0.05$) for year's season effect. Linear (L) and quadratic (Q) effects on bovine digesta levels were significant at a 5% probability of error ($p<0.05$), according to the polynomial orthogonal contrast test. SEM= standard error of the mean, BD= effect of bovine digesta level, S= year's season effect, BD × S= interaction effect (bovine digesta level × season of the year), EQ= equations for linear and quadratic models, $R^2$= determination coefficient.

linear or quadratic effects were observed on the NFC leaf content in the rainy season, but both effects were found in the dry one. In this case, the NFC decreased from 0.00 (control) to 9.37 t/ha but increased from 9.37 to 12.50 t/ha of bovine digesta.

The bovine digesta levels affected the TDN concentration within both evaluation seasons ($p<0.05$). An interaction effect was observed, but linear or quadratic effects were not, thus we did not find regression responses. During the rainy season, the 1.25, 9.37, and 12.50 t/ha levels led to higher TDN contents in leaves. An intermediate value was found at the 3.12 t/ha level, and the lowest concentrations were found at 0.00 and 6.25 t/ha levels. Within the dry season, the 3.12 and 12.50 t/ha levels led to the highest TDN, while the 1.25 t/ha treatment led to the lowest TDN content (Table 8).

## DISCUSSION

Gliricidia trees responded very well to the bovine digesta levels and seasons of the year, especially regarding DMY, plant height, and canopy diameter. Gains in DMY and plant size were observed in both seasons, but they were notable in the rainy period. We could observe the gliricidia persistence in this 2-year trial, considering the good DMY found after four subsequent harvests and two dry seasons (2022 and 2023), with many months under negative water balance and no irrigation. Losing leaves is one of the survival mechanisms in gliricidia that helps tolerate water scarcity. In addition, gliricidia's good regrowth ability is a crucial feature for intensive cut-and-carry systems where the stubble height is often low (Silva *et al.*, 2021), such as in the present study (only 50 cm).

Leaf/stem ratio and canopy diameter results indicate that gliricidia exhibited robust canopies, featuring more leaves than stems per harvest, despite the bovine digesta levels reducing the leaf/stem ratio. A suitable leaf/stem ratio is essential in cut-and-carry systems composed of arboreal forage plants since animals ingest leaves while woody stems are discarded or rejected (Lee, 2018; Castro-Montoya & Dickhoefer, 2020).

Gliricidia showed a significant response to organic fertilization with bovine digesta, owing to its remarkable ability to remove soil nutrients and its long roots (Vennila *et al.,* 2016). Vides-Borrel (2011) evaluated the shoot biomass of gliricidia fertilized with different sources of NPK in a pot trial and also found higher DMY in plants fertilized with organic fertilizer (vermicomposting) (817 g/m$^2$) than in those non-fertilized (591 g/m$^2$).

The gliricidia response to organic fertilization was outstanding because the bovine digesta increased the organic matter concentration from 1.02% to 3.10%, and the soil base saturation from 48.95% to 70.80%. Organic fertilizers derived from animals can more effectively return C, N, P, zinc (Zn), manganese (Mn), and boron (Br) to soil than chemical salts. Moreover, they can increase soil aggregate cohesion, in addition to organic matter, and positively affect soil microbiota (Lin *et al.*, 2019).

Decreases in DM leaf content in gliricidia suggest that new leaves continued appearing as the bovine digesta level was increased and gliricidia plants were still growing during the rainy seasons. New leaves display more moisture and less dry matter content than old or senescent leaves. Conversely, older and drier leaves were harvested in the dry season because of the high evapotranspiration and negative water balance from the scarce rainfall (Camargo & Camargo, 2000; Sales-Silva *et al.*, 2023).

The increments in leaf ash content as the bovine digesta was increased, especially in the dry season, reveal that gliricidia was efficient in removing mineral nutrients from organic fertilizers applied on soil since leaves are a drain of such nutrients (Marschner, 2011; Lemaire *et al.*, 2021). Ahmed *et al*. (2018) also found the great potential of gliricidia in accumulating minerals in leaves. The authors found higher ash contents in gliricidia leaves (9.51%) than in other tree legumes such as *Leucena leucocephala* (7.30%) and *Kleinhovia hospita* (7.51%).

The higher NDF contents found in gliricidia leaves in the dry than in the rainy season suggest that older leaves were harvested in that season of the year. As plants age, cell content decreases, and structural carbohydrates accumulate in the cell wall (Wilson & Mertens, 1995; Silva *et al.*, 2017). However, ADF concentrations had no linear or quadratic patterns that caused the bovine digesta levels, which is positive for the gliricidia crop since ADF comprises fiber fractions like cellulose and lignin that decrease forage digestibility (Sales-Silva *et al.*, 2023). Gliricidia responses to the organic fertilizer levels indicate improvements in the nutrient composition and a possible enhancement in forage digestibility, as we could observe in the TDN concentration, especially in the rainy season. As mentioned before, such improvement likely occurred because new leaves still appeared as the bovine digesta levels increased.

The benefits of organic fertilization using bovine digesta became more evident in the CP concentration results. The increment was linear and consistent as the bovine digesta level was increased. The N from the soil and the organic fertilizer were efficiently converted into CP in leaves. Legume species, such as gliricidia, are $C_3$ plants characterized by a higher proportion of mesophyll and a higher concentration of Rubisco in the leaves when compared to $C_4$ plants (Wilson, 1997). Thus, they often have high CP content, especially in the leaves (Valente *et al.*, 2016). Guadayo *et al*. (2019) found considerably higher CP content in gliricidia leaves (19.27% of DM) than in Napier grass (*Cenchrus purpureus* Schum. cv. Napier) (8.92%) during an *in situ* incubation experiment.

There is an inverse correlation between CP and NFC in the feed chemical composition (Sniffen *et al.*, 1992), so if the CP content increased consistently in gliricidia fertilized with bovine digesta, the opposite occurred in the leaf NFC concentration, which could be observed especially in the dry season. NFC are carbohydrates that do not compose the vegetal cell wall (except the pectin), that is, carbohydrates like starch, saccharose, and water-soluble sugars (Sales-Silva *et al.*, 2023). The CP increment plus the NFC reduction due to the increasing bovine digesta levels characterizes the gliricidia as an excellent source of roughage protein supplementation but not an energetic one.

## CONCLUSION

Bovine digesta is an effective organic fertilizer for growing gliricidia in non-irrigated fodder banks since it boosts the gliricidia's desirable traits. As the gliricidia responds linearly to the bovine digesta fertilization regarding important agronomic and nutrient-composition traits, we recommend applying the top-recommended dose (12.50 t/ha) to combine maximum forage yield and great roughage nutrient composition. It is not worth saving the organic fertilizer using lower dosages.

## CONFLICT OF INTEREST

The authors state no conflict of interests.

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