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Effect of organic and inorganic fertilization on the production and quality of *Brachiaria brizantha*

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Abstract

The yield and bromatological composition of two cultivars of *Brachiaria brizantha* (Sinaí and Xaraés), in two soil types and three types of fertilizer, were evaluated by a completely randomized design with factorial arrangement (2x2x3) with four repetitions. The variables studied were yields of green matter, dry matter, number of leaves, as well as plant height, crude protein, the contents of calcium, phosphorus and crude fiber. The fertilizer factor had a positive effect on the variable plant height. With the application of bovine manure, the highest average height of 96.06 cm was obtained. At 21 days, the highest green matter yield of 756.2 kg ha⁻¹ was obtained with the mineral fertilizer. The dry matter content was significantly influenced by the triple interaction ($p \le 0.05$), where Sinaí achieved the highest average value of 132.5 kg ha⁻¹ at 21 days of regrowth. The highest average CP content was found in Sinaí (1.9%) and Xaraés (2.08%), which received organic fertilization. While the highest average content of P was found in Xaraés (0.42%) with the application of AMF. Finally, the Sinaí plant that received bovine manure in sterilized soil had a higher average CF content (27.27%). Organic and inorganic fertilization had a significant effect on grass yield and nutritional quality.

Keywords: Brachiaria brizantha, bromatological composition, yield.

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Introduction

Grasses are a source of essential, accessible and economical components in livestock production, which is generally used in the livestock sector for animal feed. However, to get the best benefit from them, it is necessary that they are of good nutritional quality. According to Lok *et al.* (2017), forage species have had a great impact in hot climates, for being a very low-cost food, as well as for their contribution in terms of protein requirements in the animal diet. *Brachiaria* species are important forage grasses in tropical and subtropical countries (Bogdan, 1977). These are perennial grasses from tropical Africa, recently introduced into the Ecuadorian Amazon.

These forage species have erected and semi-erect growths, with deep yellowish-white roots of a soft consistency. The inflorescence is a panicle 40 to 50 cm in length, usually with four racemes of 8 to 12 cm and a single row of spikelets on them; they grow in soils of medium to high fertility and require rainfall of 1 000 to 3 500 mm annually. According to Reyes *et al.* (2009), forage production of *Brachiaria* cultivars varies greatly between cultivars. A total average dry matter (DM) yield of 6.34 t ha⁻¹ has been reported at 10 weeks of regrowth in *Brachiaria* spp. (Garay *et al.*, 2017), it was also found that the digestibility of DM in *B. brizantha* cv. Toledo varied from 67%, 64% and 60% at 25, 35 and 45 days, respectively (Lascano *et al.*, 2002).

At those ages, annual production varied between 8 and 20 t of DM ha⁻¹. On the other hand, the cultivar *humidicola* presented a digestibility of DM of 55% (Da Silva-Souza *et al.*, 1992; Moura *et al.*, 2002). The *cv* Toledo reach crude protein (CP) concentrations in the leaves of 13%, 10% and 8% at regrowth ages at 25, 35 and 45 days respectively. The nutritional value of grasses depends on the age of the species and fertilization, especially the contribution of nitrogen (N). The N applied under favorable conditions for plant growth provides a higher production of DM and the production of proteins from carbohydrates (Havlin *et al.*, 2005).

The fertilization of *Brachiaria* grasses is one of the practices with the greatest impact on increases in biomass production and protein levels, which has also improved the quality of forage. Therefore, several types of fertilizers have been used, such as: bovine manure, mineral fertilizer (Jiménez *et al.*, 2010) and also arbuscular mycorrhizal fungi (AMF) (González *et al.*, 2011), to increase the yield of grasslands. The use of these fertilizers (organic and inorganic) increases the fertility of acidic soils (Jiménez *et al.*, 2010). To improve the quality of grasses, it is necessary to apply proper fertilization. The objective of this study was to evaluate the components, yield and bromatological composition of two cultivars of *B. brizantha* (Sinaí and Xaraés) by applying three types of fertilizers (arbuscular mycorrhizal fungus, bovine manure and mineral fertilizer).

Materials and methods

Location and description of the experimental area

The study was carried out for five months (March to July 2016) in the experimental nursery of the Faculty of Agronomic and Veterinary Sciences (AGROVET) of the Autonomous University of Santo Domingo (UASD, for its acronym in Spanish), located at latitude 18° 27' 27" and longitude

 70° 0' 25" and in an approximate area of 1 088 m². In this area there is a dry season of 6 months, predominantly a variable dry climate and a rainy season of 6 months in which a variable rainy climate predominates, with an average monthly rainfall of 1 444.1 mm, an average monthly temperature of 26.2 °C, an average monthly relative humidity of 78.4% and a monthly evapotranspiration of 99.9 mm (Gómez-Mena *et al.*, 2008).

Soil characteristics

Prior to the establishment of the crop, random soil samples were taken and sent to the Dominican Agribusiness Laboratory (LAD/JAD) in Higuey for analysis purposes. The physicochemical characteristics of the soil were as follows: pH of 4.91, electrical conductivity (EC) of 0.68 mmho cm⁻¹, calcium (Ca) of 13.73 meq 100 g⁻¹, Magnesium (Mg) of 4.58 meq 100 g⁻¹, potassium (K) of 0.06 meq 100 g⁻¹, phosphorus (P) of 13%, and organic matter (OM) of 1.15%.

Establishment of the crop and experimental design

Two cultivars of *Brachiaria brizantha* seeds (Sinaí and Xaraés) were used, to which, prior to the experiment, the percentage (%) of germination was determined at 8 and 15 days, where both cultivars had a germination percentage of 91% 'Sinaí' and 90% 'Xaraés', respectively. On the other hand, the soil was sterilized by placing it under direct heat in an oven (FAITHFUL 101-1AB, China) at a temperature of 80 °C for 30 min, to eliminate microorganisms not desirable in the study. Subsequently, for the establishment of the experiment, 24 pots (capacity of 3.18 kg) were filled with sterilized soil and another 24 with unsterilized soil.

For all treatments, sowing was carried out directly and well distributed, placing 10 seeds per pot. For treatment with the arbuscular mycorrhizal fungus (AMF) *Glomus fasciculatum*, it was previously subjected to a direct counting process carried out in the microbiology laboratory of the Faculty of Agronomic and Veterinary Sciences of the Autonomous University of Santo Domingo (UASD). The inoculant contained 11 spores per 100 g of substrate, as reported by other studies (Flores-Juárez *et al.*, 2020). Before sowing, the seeds were inoculated by applying a dose of 36 g of the fungus for one day, for a total dose of 15 kg ha⁻¹.

Inoculation was performed by coating the seeds. A mineral fertilizer (FERQUIDO 20-5-10, Dominican Republic) was used and a dose of 80 g pot⁻¹ (150 kg ha⁻¹), equivalent to 50-100-25 of N, P₂O₅ and K₂O respectively, was applied. The manure was obtained from the effluents of the cattle barn and a dose of 100 g pot⁻¹ (5 000 kg ha⁻¹) equivalent to 33-7-70 of N, P₂O₅ and K₂O was used. It was necessary a reinoculation of the mycorrhizae with a dose of 15 kg ha⁻¹ mixed with 150 liters of water (1:10) applied via spraying to the total area, after each cut and in the rainy season.

The experiment was established by a completely randomized design (CRD) with a 2x2x3 factor arrangement, with four repetitions: first factor cultivars [Sinaí and Xaraés], second factor soil [with sterilization (C) and without sterilization (S)] and third factor types of fertilization [mycorrhiza (M), bovine manure (E) and chemical fertilizer (Q)]. The description of the experiment is: plant area= 500 cm², experimental unit= 0.05 m²= 0.25 m x 0.2 m, planting frame= 20 plants m⁻² and total area of the experiment= $2.4 \text{ m}^2 = 0.05 \text{ m}^2 \times 48$.

Analysis of physical-morphological and bromatological data

For this experiment, a uniformity cut was made 45 days after the establishment of the cultivars. Then, three cuts were made: a) the first was on May 25, 21 days after the application of the treatments; b) the second was carried out after 42 days (15 June 2016); and c) the third at 63 days (06 July 2016). The harvest was carried out with cutting scissors at an average height of 5 centimeters from the ground, weighing each sample and storing it in plastic bags to then analyze them in the laboratory. Data on plant height with the support of a tape measure (Stanley[®] FatMax[®] H-1842, USA) (every 21 days after cutting), absolute rate of leaf emergence, number of leaves, fresh weight and dry weight were taken. Subsequently, the nutritional quality of the two cultivars was analyzed using the procedures explained below.

For the crude protein (CP) content, the Kjeldahl method (Horwitz, 2000) was used, calculating the nitrogen content by the following formula: $\% N = \frac{Volume used of H_2SO_4 \times 0.1408}{Sample weight}$. Then, from the nitrogen content determined by the Kjeldahl method multiplying by the factor 6.25, according to the following equation: $\% Cp = N(\%) \times 6.25$.

The % of Ca was determined from the ashes, where it precipitates in the form of calcium oxalate by neutralization with ammonia. The formula used is as follows (Cuello *et al.*, 2017): %Ca= $\frac{V \times N \text{ Meq Ca x 100}}{\text{Sample grams per aliquot}}$. Regarding the percentage of phosphorus (%), this was determined by calorimetry (MacNaughtan and Farhat, 2008). The crude fiber content (% CF) was determined from the dry matter, using the method of digestion, drying and incineration (Cuello *et al.*, 2017).

Statistical analysis

Data were analyzed using the Minitab statistical package (version 17). An analysis of variance (Anova) was performed for yield composition and bromatological composition. Subsequently, the Tukey test ($p \le 0.05$) was applied for comparison of means. The statistical model described below was used: $Y_{ijk} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + \varepsilon_{ijk}$. Where: i= 1, 2 (factor A= cultivars); j = 1, 2 (factor B= soils); k= 1, 2, 3 (factor C= fertilization).

Results and discussion

Plant height

The fertilization factor generated statistically significant results on the plant height in the first cut, where the plants that received bovine manure achieved the highest height (average value of 96.06 cm) compared to those that had mycorrhizae (average value of 84 cm) mineral fertilization (average value of 82.43 cm) (Figure 1a). However, there were no significant differences for the factors soil, cultivar, cultivar*soil; cultivar*fertilization; soil*fertilization, cultivar*soil*fertilization.

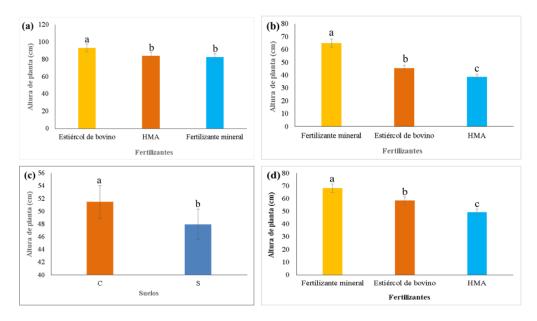


Figure 1. Effect of fertilization on the height of two cultivars of *Brachiaria brizantha* in a) and b) first cut; c) second cut; and d) third cut. Means with the same letter for each figure are statistically equal (Tukey, $p \le 0.05$).

The results of the second cut showed that there were significant differences for the type of fertilization, being the mineral fertilizer with which the highest average plant height (64.93 cm) was obtained, and the inoculant AMF presented the lowest plant height (38.75 cm) (Figure 1b). The soil factor also generated statistically significant results on plant height (Figure 1c). On the other hand, there were no statistically significant differences for the triple interaction (cultivar*soil*fertilization), for the double interactions (cultivar*soil; cultivar*fertilization; soil*fertilization) and the factor and cultivar.

For the third cut, with the application of mineral fertilizer, the highest height of the plants (an average of 68.18 cm) was obtained, compared to the inoculant AMF and bovine manure (Figure 1d). On the other hand, there were no statistically significant differences for the triple interaction (cultivar*soil*fertilizer), nor for the double interactions (cultivar*soil; soil*fertilizer; cultivar*fertilizer), nor for the cultivar factor, in this phase of the experiment. The results found in this study, for the variable plant height, were greater than those reported by González Muñoz *et al.* (2020), who applied a mineral fertilizer on the Insurgente and Urochloa hybrid grasses, they found the highest average height in plants of the cultivar Cobra (30.9 cm), followed by the cultivar *Mulato* II (28 cm).

The cultivar Insurgente presented the lowest height of plants (24 cm). On the other hand, Flores-Juárez *et al.* (2020), when inoculating the forage oat crop with AMF, found a plant height of 25.9 cm. AMFs took a while to infect plant roots (Uc-Ku *et al.*, 2019). The application of bovine manure significantly increased pH, and organic matter content (MO), as well as the assimilable phosphorus (P) and exchangeable potassium (K) of the soil (González *et al.*, 2011). So, the yield of the variable plant height was influenced by manure in this study.

These results confirm the potential of manure as an improver of chemical properties and as a source of nutrients for plants (Obour *et al.*, 2009; De Souza *et al.*, 2010). In previous studies, it was observed that B. *brizantha* reached greater height compared to other species of the same genus (Pérez *et al.*, 1997). These differences observed between the two cultivars (Sinaí and Xaraés) under study are due to the growth habit of each one.

Number of leaves

The results of the first cut showed that there were no statistically significant differences for any of the factors, nor for the triple and double interactions with respect to the number of leaves. This means that neither the soil factor nor the fertilizer factor influenced the leaf yield of the two cultivars during this cut. In the second cut, the fertilization factor generated statistically significant results on the number of leaves, being mineral fertilizer the one that generated the highest number of leaves (the average was 26.69), applying (Figure 2a), compared to the leaves generated by manure and AMF, which were 20.06 and 18.93, respectively.

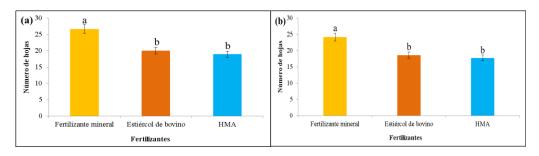


Figure 2. Effect of fertilization on the number of leaves of two cultivars of *Brachiaria brizantha* in a) second cut; and b) third cut. Means with the same letter for each figure are statistically equal (Tukey, $p \le 0.05$).

For the second cut, there were also no significant differences between the interactions; nor did the main effects (soil and cultivar) show significant differences. The results of the third cut showed that the mineral fertilizer had a significant effect on the number of leaves (Figure 2b), reaching an average value of 24.13 in the third cut. This meant that mineral fertilizer was the best option to reach the highest rate of leaf emergence in the two cultivars of *Brachiaria brizantha*. While for the factor's cultivar, soil; interactions cultivar*soil, cultivar*fertilization, soil*fertilizer, cultivar*soil*fertilization, there were no statistically significant differences.

Luna *et al.* (2015), who evaluated the agronomic response of three varieties of *Brachiaria brizantha*, found that cultivar *B. mulatto* achieved the highest production of foliage compared to the other variables. This cultivar reached a value of 774.85 for the number of leaves at 68 days. This high yield is due to the establishment of the experiment in the open field (in 52 plots), compared to this study that was carried out in pots. For Ramírez *et al.* (2009), the age of regrowth greatly influences both the growth and the quality of the grasses.

GM yield

The results of the first cut showed that there were no statistically significant differences for triple and double interactions on GM yield. The fertilization factor generated statistically significant results on factors soil and cultivar. The results also indicated that the best yield of GM was obtained with the mineral fertilizer during the three cuts; reaching values of 75.62 g m⁻² (756.2 kg ha⁻¹) for the first cut (Figure 3a), 50.1 g m⁻² (501 kg ha⁻¹) for the second cut (Figure 3b) and 25.44 g m⁻² (254.4 kg ha⁻¹) for the third cut (Figure 3c), respectively, compared to treatments with bovine manure and the inoculant AMF (*Glomus fasciculatum*) that turned out to be the same in this study.

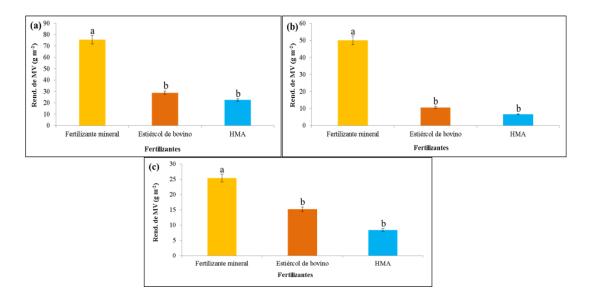


Figure 3. Effect of fertilization on the GM yield of two cultivars of *Brachiaria brizantha* in a) first cut; b) second cut; and c) third cut. Means with the same letter for each figure are statistically equal (Tukey, $p \le 0.05$). Rend= yield.

Fagundes *et al.* (2006), who evaluated the structural characteristics of *Brachiaria* grass in nitrogenfertilized grasslands in all four seasons, reported a GM yield higher than found in this study. According to these authors, the application of nutrients in adequate proportions can be a crucial practice when it comes to increasing forage production. GM production decreased considerably in the third cut, this is due to the age of regrowth (Vega *et al.*, 2006; Ramírez *et al.*, 2009).

DM yield

In the first cut, the triple factor cultivar*soil*fertilizer generated statistically significant results on DM. The best combination (Figure 4a) to obtain the highest yield of DM (13.5 g m⁻² (132.5 kg ha⁻¹)) was presented by 'Sinaí' with mineral fertilizer and unsterilized soil (Sinaí-Q-S). While the lowest yield (4.95 g m⁻² (49.5 kg ha⁻¹)) was obtained with the combination of the cultivar 'Sinaí' with the inoculant AMF and the unsterilized soil (Sinaí-M-S). Fertilization with manure + sterilized soil showed a significant effect on the DM yield (11.2 g m⁻² (112 kg ha⁻¹)) of the cultivar Sinaí.

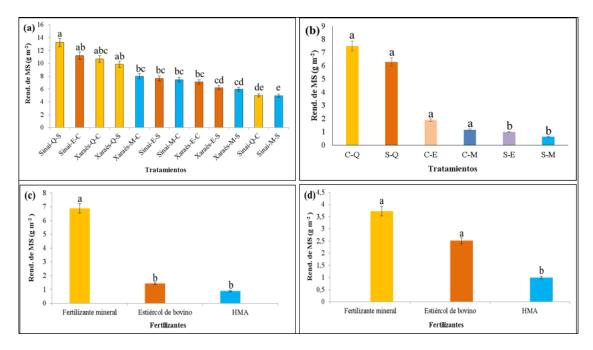


Figure 4. Effect of the interaction cultivar*soil*fertilizer on the a) and b) first cut and effect of fertilization on c) second cut; and d) third cut on the DM yield of two cultivars of *Brachiaria brizantha*. Means with the same letter for each figure are statistically equal (Tukey, p≤0.05). Q= mineral fertilizer; S= unsterilized soil; C= sterilized soil; M= arbuscular mycorrhizal fungus; Rend= yield.

Due to the combination of mineral fertilizer + sterilized soil, 'Xaraés' achieved a yield of 10.67 g m⁻² (106.7 kg ha⁻¹) of DM. For the double factor soil*fertilizer, there were statistically significant differences between the treatments. The two treatments that had the best yields (Figure 4b) were C-Q (average of 7.5 g m⁻²) and S-Q (average of 6.28 g m⁻²). It can be seen that the mineral fertilizer had a very significant effect on the DM yield for the cultivars, compared to the other sources of fertilization. In the second cut, the fertilizer factor produced statistically significant results on the DM content. Figure 4c shows that the application of mineral fertilizer achieved the best DM yield with a value of 6.88 g m⁻² (68.8 kg ha⁻¹), followed by manure (1.43 g m⁻²; 14.3 kg ha⁻¹) and the inoculant AMF (0.89 g m⁻²; 8.9 kg ha¹), respectively. In contrast, triple and double interactions, the main factor cultivar and soil generated statistically non-significant results on DM content.

In the third cut, the fertilizer factor produced statistically significant results on the DM content. The application of the mineral fertilizer showed the best DM yield, achieving a production of 3.73 g m⁻² (37.3 kg ha⁻¹) (Figure 4d). This decrease in DM yield, recorded in this study (during cuts 1 and 2), was due to the lack of rain during regrowth and to the age of the grasses.

The results obtained in the two grass cultivars analyzed in relation to the yield of DM were lower with respect to those reported by Luna *et al.* (2015), who found values of 480 kg ha⁻¹ (0.48 t ha⁻¹) at 21 days (first cut), 690 kg ha⁻¹ (0.69 t ha⁻¹) at 42 days (second cut) and 1 240 kg ha⁻¹ (1.24 t ha⁻¹) at 63 days (third cut), respectively. These authors reported values similar to those

found by Fernández *et al.* (2004); Reyes *et al.* (2009), who evaluated different cultivars of *Brachiaria* and *Panicum* under different environmental conditions. On the other hand, Garay *et al.* (2017) reported a higher yield (5.06 t ha⁻¹) in the *cv* Xaraés; compared to what was reported in this study.

Bromatological composition

CP Content

Table 1 shows the average contents of CP, Ca, P, CF of two cultivars of *Brachiaria brizantha*, where the highest content of CP was found in the plants of both varieties that received chemical fertilization regardless of whether they had a sterilized soil or not and the lowest concentrations existed in both cultivars that received mycorrhizae regardless of whether the soil presented sterilization or not from the beginning. The results of factors cultivar, soil and fertilization, as well as the double interactions cultivar*soil, cultivar*fertilizer, soil*fertilizer were statistically not significant.

Treatments	СР	Ca	Р	CF
Sinaí-E-C	10.67 b	1.62 c	0.26 c	27.27 a
Sinaí-Q-C	20.81 a	1.69 bc	0.27 b	20.59 c
Sinaí-M-C	8.63 c	1.89 a	0.34 a	25.15 bc
Sinaí-E-S	9.87 b	1.9 a	0.35 b	21 c
Sinaí-Q-S	18.19 a	1.67 b	0.3 c	21.8 b
Sinaí-M-S	10.75 b	1.63 b	0.37 a	24.61 a
Xaraés-E-C	11.29 bc	1.72 a	0.3 b	23.64 bc
Xaraés-Q-C	19.85 a	1.37 b	0.34 b	22.39 с
Xaraés-M-C	10.31 b	1.62 a	0.37 a	27.1 a
Xaraés-E-S	10.08 b	1.72 b	0.28 c	23.74 bc
Xaraés-Q-S	18.81 a	1.47 b	0.35 b	21.69 c
Xaraés-M-S	9.79 c	2.08 a	0.42 a	25.33 a
Adequate range	4-18	0.3	0.3	<50

Table 1. Average chemical composition of two cultivars of <i>Brachiaria brizantha</i> studied in
relation to the effect of the interaction cultivar*soil*fertilization.

Averages with the same letter in the same column are statistically equal (Tukey, $p \le 0.05$). CP= crude protein; Ca= calcium; P= phosphorus; CF= crude fiber; E= bovine manure; M= mycorrhizae; Q= mineral fertilizer; S= unsterilized soil; C= sterilized soil.

The CP content obtained in both cultivars was higher than that reported in other studies, using *Brachiaria brizantha cv* Mulato II. CP values of 14.5% (Garay *et al.*, 2017; Gonzales Muñoz *et al.*, 2020) and 15% (Castillo *et al.*, 2006) were found, respectively. In this study, the highest values of CP were slightly higher than the appropriate ranges (Vargas González, 1989).

However, the lowest values found were within or equal to the appropriate ranges (Table 1). Jimenez *et al.* (2010), who reported that any type of fertilization influences CP. Which was confirmed in this study. Bernal (1994) reported that as the maturity of forages increases, the protein value decreases. Then, Garay *et al.* (2017) reported that the time factor influences the decrease in CP content.

Ca and P content

The highest Ca content (Table 1) was found in the Sinaí and Xaraés plants that received unsterilized organic fertilization and the lowest concentrations occurred in Xaraés plants that received inorganic fertilizer regardless of whether the soil was sterilized or not from the beginning. The highest content of P was found in the Xaraés plant that received mycorrhizae in unsterilized soil, and the lowest concentration occurred in the Sinaí plant that received manure with sterilized soil from the beginning.

In general, forages are a good source of Ca. According to Minson (1990), the Ca content of forages is influenced by the species, consumed portion of the plant; as well as, maturity, amount of exchangeable calcium in the soil and climate. The Ca content reported in both cultivars was higher than the adequate ranges (Vargas-González, 1989).

CF content

The highest CF content (Table 1) was found in the Sinaí plant that received bovine manure in sterilized soil, as well as in the Xaraés plant that received mycorrhizae in sterilized soil and the lowest concentration occurred in the Sinaí plant that received chemical fertilizer with sterilized soil from the beginning. The results obtained in this study suggest that regardless of the statistical differences in bromatological composition, the cultivars showed adequate nutritional characteristics and constitute an alternative for silvopastoral systems (Bugarín *et al.*, 2009).

Conclusions

The dry matter content was influenced by the three factors studied, where these turned out to be different. Thus, the highest average dry matter production (132.5 kg ha⁻¹) was achieved in the cv. Sinaí at 21 days. The best combination to obtain this yield was mineral fertilizer with unsterilized soil. On the other hand, the type of fertilization influenced the nutritional quality and foliage yield of the two cultivars. The cutting time was one of the most important factors, it was observed that, the shorter the cutting time, the amount of nutrients in the grasses increases frequently, since 21 days after regrowth, a greater concentration of nutritive elements was found.

On the other hand, the highest average CP content was found in plants of both varieties that received chemical fertilization. The highest average Ca content was found in Sinaí and Xaraés that received organic fertilization. While the highest average P content was found in Xaraés with the application of AMF. Finally, the Sinaí plant that received bovine manure in sterilized soil had a higher average CF content. This study affirms that any fertilization has a significant effect on the production of green forage and dry matter.

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