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EFFECT OF ARBUSCULAR MYCORRHIZAL INOCULATION ON THE REHABILITATION OF GUINEA GRASSLAND (*Megathyrsus maximus* cv. LIKONI)

Efecto de la inoculación micorrízica arbuscular en la rehabilitación de un pastizal cultivado de guinea (*Megathyrsus maximus* cv. Likoni)

Pedro R. Rosales Jenqui[1]✉, Pedro J. González Cañizares[1], Juan F. Ramirez Pedroso[2] and Joan Arzola Batista[3]

ABSTRACT. In order to evaluate the effect of arbuscular mycorrhizal inoculation on the rehabilitation of a guinea grassland (*Megathyrsus maximus* cv. Likoni), an experiment was carried out on Ferralitic Red Leachate soil. A completely randomized design was used with six treatments: control, mechanical rehabilitation with plow and harrow, alone and combined with the applications of 25 t ha^{-1} of cattle manure and 100 kg ha^{-1} of N; with the mycorrhizal inoculation of the *Glomus cubense* species plus the application of 50 and 70 % of the doses of cattle manure and N, respectively, and with mycorrhizal inoculation plus 100 % of the doses of both fertilizers.The dry mass yield, plant height and percentage of grass area, as well as variables related to mycorrhizal functioning (frequency and intensity of colonization and spore content in the rhizosphere) and the contents of Crude protein (PB), neutral detergent fiber (FND) and organic matter digestibility (BMD) were evaluated. Inoculation with *G. cubense* was successfully integrated into the rehabilitation work. The greatest benefits were obtained when combined with 50 and 70 % of the dose of cattle manure and nitrogen fertilizer that, in that order, produced the greatest benefits in the recovery of uninoculated pasture. The effect of *G. cubense* was maintained until at least 270 days after its application.

Key words: colonization, Glomus, crude protein, productivity, rhizosphere

RESUMEN. Con el objetivo de evaluar el efecto de la inoculación micorrízica arbuscular en la rehabilitación de un pastizal de guinea (*Megathyrsus maximus* cv. Likoni), se llevó a cabo un experimento sobre un suelo Ferralítico Rojo Lixiviado. Se utilizó un diseño completamente aleatorizado con seis tratamientos: control, la rehabilitación mecánica con arado y grada, sola y combinada con las aplicaciones de 25 t ha^{-1} de estiércol vacuno y 100 kg ha^{-1} de N; con la inoculación micorrízica de la especie *Glomus cubense* más la aplicación del 50 y el 70 % de las dosis de estiércol vacuno y N, respectivamente, y con la inoculación micorrízica más el 100 % de las dosis de ambos fertilizantes. Se evaluó el rendimiento de masa seca, altura de las plantas y el porcentaje de área cubierta por el pasto, así como las variables relacionadas con el funcionamiento micorrízico (frecuencia e intensidad de la colonización y contenido de esporas en la rizosfera) y los contenidos de proteína bruta (PB), fibra neutro detergente (FND) y digestibilidad de la materia orgánica (DMO). La inoculación con G. cubense se integró satisfactoriamente en las labores de rehabilitación. Los mayores beneficios se obtuvieron cuando se combinó con el 50 y el 70 % de la dosis del estiércol vacuno y fertilizante nitrogenado que, en ese orden, produjeron los mayores beneficios en la recuperación del pasto no inoculado. El efecto de *G. cubense* se mantuvo al menos hasta los 270 días después de su aplicación.

Palabras clave: colonización, Glomus, proteína bruta, rendimiento, rizosfera

INTRODUCTION

Rehabilitation is one of the ways to return degraded pastures to their productive capacity. This can be achieved through mechanical work together with the application of mineral or organic fertilizers, whose beneficial effects in the improvement of soil conditions,

[1]Instituto Nacional de Ciencias Agrícolas. Gaveta Postal 1, San José de las Lajas, Mayabeque, Cuba, CP 32700
[2]Instituto de Investigaciones de Pastos y Forrajes, Avenida Independencia, km 8 ½, Boyeros, La Habana, Cuba
[3]Empresa Pecuaria Genética Niña Bonita. Carretera 43 km 1½ Cangrejeras, Bauta, Artemisa, Cuba
✉ prafael@inca.edu.cu

as well as in the crop nutrition, promote the growth of the roots and the capacity of regrowth, contribute to increase the availability of biomass and consequently, to recover its productive capacity (1,2).

Arbuscular mycorrhizal fungi (AMF) are essential components of the rhizosphere of grasslands, whose plants remain associated through a network of interconnected hyphae that increase the volume of soil that explore the roots and facilitate the absorption of nutrients and water, among other functions (3).

In this sense, some authors suggest that the inoculation of efficient AMF strains can be a desirable and even necessary management option to improve pasture nutrition and productivity, in cases where the resident AMF are not sufficiently effective or they are not found in adequate quantities to produce an important agronomic response in crops (4,5).

Based on the functions of the AMF in pasture agroecosystems, the negative impact that the processes of degradation can have on the communities of these microorganisms, and on the possibilities of achieving their effective management through the inoculation of efficient strains, their inclusion in Recovery programs could be an economically and ecologically viable option to improve pasture productivity and, at the same time, reduce the volumes of fertilizers used in this work (6).

On the basis of these premises, this experiment was carried out with the objective of evaluating the contribution of arbuscular mycorrhizal inoculation in the rehabilitation of degraded grassland of Guinea grass (*Megathyrsus maximus* cv. Likoni).

MATERIALS AND METHODS

The experiment was carried out at the microstation of pastures and forages of the Niña Bonita Genetic Livestock Company, located in the Bauta municipality, Artemisa province, on a leached red Ferralitic soil (7), whose main chemical characteristics are presented in Table I.

Table I. Chemical characteristics of the soils (depth: 0 - 20 cm)

pH H_2O	MO (%)	P_2O_5 (mg 100 g^{-1})	Ca^{2+}	Mg^{2+}	Na^+	K^+	CCB
			(cmol$_c$ kg^{-1})				
6,2	3,27	2,3	9,9	2,0	0,15	0,32	12,37

MO: organic matter, CCB: base exchange capacity

The soil possessed a slightly acidic pH, medium contents of interchangeable organic matter and potassium (K), very low contents of calcium ($Ca^{2+)}$ and sodium (Na^+) interchangeable, as well as low contents of assimilable phosphorus (P_2O_5) and interchangeable magnesium ($Mg^{2+)}$. Basis exchange capacity (CCB) was also low (8). Soil analyzes were carried out using the methods established in the soil, organic fertilizers and vegetable tissue laboratory of the Biofertilizers and Plant Nutrition Department of the National Institute of Agricultural Sciences (INCA) (9).

During the period in which the experiment was conducted, the average temperature of the locality was 27,5 ºC and the total precipitation of 1160,7 mm, of which 83,1 % was distributed between May and October and 16,9 %, between November and April (10).

This study was carried out from June 2012 to February 2013, under rainfed conditions, in a degraded pasture with more than 20 years of exploitation. Its botanical composition at the beginning of the experiment was 40 % of guinea Likoni, 24 % of jiribilla (*Dichanthium caricosum*), 15 % of mallow of pig (*Sida rhombifolia* L.), 11 % of espartillo (*Sporobolus indicus*), 6 % of *Centrosema* sp. and 4 % of other unidentified species.

It was done in a completely randomized design with ten replications, where six paddocks of 1 ha each was taken and six treatments were included, Table II.

Table II. Description of treatments

No.	Descripción
1	Control
2	Rehabilitación mecánica
3	Rehabilitación mecánica + 25 t ha^{-1} de estiércol vacuno + 100 kg ha^{-1} $año^{-1}$ de N
4	Rehabilitación mecánica + aplicación de inoculante micorrízico a razón de 4 x 10^5 esporas ha^{-1}
5	Rehabilitación mecánica + aplicación de inoculante micorrízico a razón de 4 x 10^5 esporas ha^{-1} + 12,5 t ha^{-1} de estiércol vacuno + 70 kg ha^{-1} año de N
6	Rehabilitación mecánica + aplicación de inoculante micorrízico a razón de 4 x 10^5 esporas ha^{-1} + 25 t ha^{-1} de estiércol vacuno + 100 kg ha^{-1} año de N

Before applying the treatments, 10 frames of 1 m^2 each were randomly distributed in each paddock, to evaluate the variability of the experimental area. In each frame the yield of dry mass (DM) of the grass and the number of tillers per m^2 was determined. The analysis of variance indicated that there were no significant differences between paddocks for any of the evaluated variables, which showed homogeneity in the selected paddocks (Table III).

Table III. Grass yield and number of tillers m^{-2} before applying the treatments

Tratamientos	MS (t ha^{-1})	No. macollas m^{-2}
1	1,76	3,4
2	1,82	3,2
3	1,77	3,5
4	1,81	3,3
5	1,78	3,1
6	1,80	3,0
ES	0,03	0,17

Mechanical rehabilitation was carried out by plowing at a depth of 20 cm, for which a three-disc ADI-3 plow was used, followed by the pass of a 3500-kg stand (11). In the treatments with 100 % organic and nitrogen fertilization, cow manure and urea were applied, at a rate of 25 t ha^{-1} and 100 kg ha^{-1} year of N, respectively. The N was distributed in 50 % split doses, at the beginning of the experiment (June 2012) and at the end of the rainy period (October 2012). The chemical characteristics of the manure are presented in Table IV.

The manure came from the dairy itself where the experiment was carried out and had time to deposit in the four-month old dunghill. This, together with the first fraction of the dose of N, was applied on the surface of the grass after the plowing; by means of a mechanical spreader of organic matter and they were incorporated to the ground with the step of harrow. The second fraction of the dose of N was also applied manually and broadcast, after grazing.

The effect of the treatments on the chemical characteristics of the soil was evaluated, through the pH and the contents of organic matter (OM), assimilable phosphorus and interchangeable soil cations.

For the application of the mycorrhizal inoculant, the INCAM-4 strain of the species *Glomus cubense* (12) was used, selected due to its high efficiency index shown in previous tests carried out under similar conditions to which this experiment was conducted (5), with a concentration of 20 spores g^{-1} of substrate. This was applied at a rate of 20 kg ha^{-1}.

The inoculant was added after the plowing, along with the manure and nitrogen fertilizer, so that it was also incorporated into the soil with the harrow.

The yield of dry mass was evaluated at 70 and 270 days after applying the treatments, coinciding with the rainy and dry season, respectively, and prior to the entry of the animals to grazing. In the area occupied by each treatment, 10 frames of 1 m^2 each were randomly distributed, which constituted the experimental unit and the MV was harvested. The MV was weighed and a 200 g sample was taken, which was taken to an air circulation oven at 70 ºC until reaching a constant mass, to determine the dry mass percentage (DM). The DM yield was calculated, from the green mass yield (MV) and the DM percentage (9). The height of the plants was measured at the time of each cut, for which 10 individuals were taken within the calculation area of each plot. The crude protein content (PB) = N x 6,25 (13), the organic matter digestibility (BMD) (14), and the neutral detergent fiber content (NDF) were determined to the dried samples in the stove (15).

The botanical composition was determined at the beginning of the experiment and the percentage of the pasture area covered by guinea at 70 and 270 days after the treatments were applied (16).

For the chemical characterization of cow manure, five random samples were taken at the time of their application to the grass, to which the pH was determined, and the total contents of OM, N, C: N, P, K, Ca and Mg. (9)

In the cut, five subsamples of roots and soil of the rhizosphere were taken from each plot at a depth of 0-20 cm, by using a metallic cylinder of 5 cm in diameter and 20 cm in height, they were washed with abundant common water, they were placed in an oven at 70 °C until constant weight was reached and later they were dyed (17). The samples were read in a stereoscope (Carl Zeiss, Stemi 2000-C/50 x) and then the indicators of mycorrhizal colonization and visual density (18) were estimated, which are expressed as frequency and intensity of colonization respectively, as well as the number of spores in the rhizosphere (19), with modifications (20).

The data were processed by analysis of variance, according to the experimental design used, and the Duncan test (21), $p < 0,05$ or the confidence interval at $\alpha = 0,05$ (22), to establish significant differences between the means of treatments. The statistical program SPSS 11.5 for Windows (23) was used. All the variables fulfilled the assumptions of normality and homogeneity of variance, so in all cases the original data were analyzed (24).

Table IV. Chemical characteristics of cow manure (% DM)

MO	N	Relación C:N	P	K	Ca	Mg	Na	pH	Humedad (%)
78,3	2,25	20,1	0,86	1,83	4,72	0,54	0,12	7,1	59,6

RESULTS AND DISCUSION

For a better understanding of the inclusion effect of the selected AMF strain in the rehabilitation of the pasture, it is convenient to evaluate, in the first place, the modifications that produced the treatments in the chemical characteristics of the soil, due to its possible influence on the behavior of the rest of the evaluated indicators.

As can be seen in Table V, cow manure significantly increased the pH, as well as the contents of organic matter (OM), assimilable P_2O_5, and interchangeable Ca, Mg and K, with the highest effects observed with the highest dose (25 t ha^{-1}). However, with the application of 12,5 t ha^{-1}, although no changes were observed in the pH or in the interchangeable Ca and Mg contents, values of OM, P_2O_5 assimilable and exchangeable K were significantly higher than in the treatments where the manure was not applied. The rest of the treatments had no effect on the chemical characteristics of the soil.

The effect of cow manure on the soil chemical characteristics corresponded to its contribution of MO and nutrients. According to its chemical composition, it can be inferred that it incorporated significant amounts of OM, P, Ca, Mg and K, which undoubtedly contributed to increase the contents of these elements in the soil, especially with the application of the most high (Table IV). Several authors agree that the good qualities of cow manure as an improver of the chemical characteristics of the soil, are fundamentally that it is constituted by organic substances whose rapid decomposition through microorganisms, together with its own contribution of mineral elements, makes Plant availability of significant quantities of macro and micronutrients, practically from the time of their incorporation (25,26).

Table V. Effect of organic fertilization on the chemical characteristics of the soil

Tratamientos	pH H_2O	MO (%)	P_2O_5 (mg $100g^1$)	Ca	Mg ($cmol_c$ kg^{-1})	K
1	6,4 b	3,25 c	2,1 c	9,3 b	2,0 b	0,32 c
2	6,3 b	3,19 c	2,2 c	9,1 b	1,9 b	0,33 c
3	6,9 a	3,82 a	3,4 a	10,5 a	2,9 a	0,55 a
4	6,3 b	3,21 c	2,0 c	9,4 b	2,1 b	0,35 c
5	6,7 ab	3,59 b	2,6 b	10,0 ab	2,5 ab	0,41 b
6	6,9 a	3,85 a	3,5 a	10,8 a	3,0 a	0,57 a
ES	0,1**	0,12**	0,2**	0,2**	0,1**	0,03**

Treatments: 1-Control, 2- Mechanical rehabilitation, 3- Mechanical rehabilitation + 25 t ha^{-1} of cow manure + 100 kg ha^{-1} year of N, 4- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 105 spores ha^{-1}, 5- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 105 spores ha-1 + 12,5 t ha^{-1} of cow manure + 70 kg ha^{-1} year of N, 6- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 105 spores ha-1 + 25 t ha^{-1} cow manure + 100 kg ha^{-1} year N.** Means with different letters in the same column differ significantly according to the Duncan test (p <0,05). ES- standard error

Table VI. Effect of the treatments on the% of covered area, the height of the plant and the yield of the biomass of the grass

Tratamientos	70 días Área cubierta (%)	Altura (cm)	Rendimiento (t MS ha^{-1})	270 días Área cubierta (%)	Altura (cm)	Rendimiento (t MS ha^{-1})
1	42,7 d	61,7 d	1,81 d	36,7 d	29,7 d	0,97 d
2	62,7 c	73,2 c	3,02 c	58,3 c	37,9 c	1,93 c
3	79,3 a	103,1 a	6,13 a	78,2 a	51,4 a	4,51 a
4	71,7 b	88,5 b	4,20 b	67,9 b	45,3 b	2,83 b
5	81,5 a	101,9 a	5,91 a	79,7 a	52,7 a	4,82 a
6	78,7 a	100,6 a	6,22 a	78,3 a	51,1 a	4,75 a
ES	1,57**	2,72**	0,38**	1,48**	1,58**	0,29**

Treatments: 1-Control, 2- Mechanical rehabilitation, 3- Mechanical rehabilitation + 25 t ha^{-1} of cow manure + 100 kg ha^{-1} year of N, 4- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 10^5 spores ha^{-1}, 5- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 105 spores ha^{-1} + 12,5 t ha^{-1} of cow manure + 70 kg ha^{-1} year of N, 6- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 105 spores ha^{-1} + 25 t ha^{-1} cow manure + 100 kg ha^{-1} year N. ** Means with different letters in the same column differ significantly according to the Duncan test (p <0,05). ES- standard error

These authors also observed that the use of cow manure as a source of organic fertilizer for pastures, increases the contents of organic matter, nitrogen, phosphorus and potassium assimilable; as well as, interchangeable soil cations, at least 90 days after application (25,26).

In relation to the effect of the treatments in the rehabilitation of the grass, it was observed that the cultural works favored, as much the percentage of the area covered by the guinea as the height of the plants and the yield of biomass of the aerial part (Table VI). The use of the plow and the harrow only (treatment 2), significantly increased these three indicators in relation to the control that was not rehabilitated (treatment 1), which coincided with the result of other work done using both mechanical tasks for rehabilitation of Likoni guinea grassland and indicated its contribution to the recovery of degraded pastures (27).

The beneficial effect of the plow and the harrow in the rehabilitation of the grass can be attributed to the improvement of the soil physical properties, which due to the inadequate handling of the grazing and the non-application of cultural maintenance tasks throughout its life cycle, they could deteriorate over time, which led to compaction due to the excessive trampling of the animals, and consequently, the decrease of the yield and the useful life of the pasture (28).

It cannot be ruled out that the improvement of the area covered by the guinea and the increase in the yield of the grass has also been the result of the indirect effect of the mechanical work on the increase of the population of this species, since when the superficial layer of the soil is removed the necessary conditions are created for the emergence of the seeds of this species that over time are incorporated into the soil with the trampling of the animals themselves. Also, the mechanical work itself may have contributed to reducing weed infestation and, in fact, to increasing the presence of guinea grass in the pasture, both by the physical removal of the invasive plants and by the elimination of their propagules or seeds (27).

The work of plow and harrow help to section the clusters of the guinea and this makes the growth points of both the roots and stems multiply, contributing significantly to the repopulation of the pasture with the improved species (29, 30). This is very important because after grazing or cutting, when a large part of the aerial biomass is eliminated, the grass must emit new roots in order to access the nutrients of the soil to recover its aerial biomass again and with it its photosynthetic activity. So, any work such as the plow and harrow that contributes to increase the growth points of the root system can increase the contact surface of the roots with the soil, favor the absorption of nutrients, water and consequently, stimulate the growth of aerial biomass (2).

The mechanical works accompanied by the applications of 25 t of manure ha^{-1} and of 100 kg N ha^{-1} increased significantly the percentage of guinea in the botanical composition of the pastureland; as well as, the height of the plants and the yield of the biomass in relation to the mechanical works only (treatment 3), which confirmed the beneficial effect of the manure on the soil characteristics observed in the previous table, and evidenced the need of an adequate supply of nutrients for the recovery of degraded pastures (26,31). In other words, the rehabilitation was more effective when the aforementioned benefits of the removal of the superficial layer of the soil by means of mechanical work, added the effect of the nutrients addition whose influence on the growth of the guinea undoubtedly contributed to its quick recovery.

The use of cattle manure and mineral fertilizers alone or combined, as part of the cultural work for the rehabilitation of pastures has been studied by many authors, and all recognize their benefits in improving the nutritional status of plants and consequently, in the improvement of covered area percentage and improved grass productivity (32).

The inclusion of the mycorrhizal inoculant formulated with an AMF efficient strain in the work for the pasture rehabilitation improved the evaluated indicators (treatment 4), whose values were significantly higher than those reached with the mechanical work alone (treatment 2); however, the greatest effects were obtained when combined with the addition of 50 and 70 % of the doses of cattle manure and nitrogen fertilizer, respectively (treatment 5), which did not differ from those achieved with the application of 100b % both fertilizers (treatment 3).

With the mycorrhizal inoculant combined with the addition of 100 % of the doses of cow manure and nitrogenous fertilizer (treatment 6), the height of the improved pasture; as well as, their percentage of area covered within the grassland botanical composition and their biomass yield did not differ from those obtained with the treatment 3.

The beneficial effect of the mycorrhizal inoculation on the increase of plant height and on the improvement of the area covered percentage by the improved grass; as well as, in the increase of the yield of the biomass, judging from the results of the previous experiment, it could be related to the influence of the strain introduced in the improvement of the nutritional status of the plants. The addition of AMF efficient strains can increase the absorption effectiveness of soil nutrients and fertilizers, and this translates into an increase in grass biomass production (26, 33).

Such benefits could also explain the fact that with mycorrhizal inoculation, together with the addition of smaller amounts of mineral and organic fertilizers, similar results can be obtained to those achieved with the application of higher doses of both fertilizers in the absence of inoculation which agrees with that found in other studies, by including the application of mycorrhizal inoculants and mineral or organic fertilizers in the work for the establishment and rehabilitation of pastures, respectively (34,35).

The effect of the rehabilitation work on plant height, the percentage of the area covered by guinea and the yield of the grass was observed both at 70 and 270 days after the application of the treatments, and this indicated not only that the cultural methods used for pasture recovery were effective over time, but also that the management to which the pasture was subjected once recovered (resting times of 28 and 37 days in the rainy and dry season, respectively, and global load of 1,5 UGM ha^{-1}) was adequate to maintain its productivity, at least during the time evaluated.

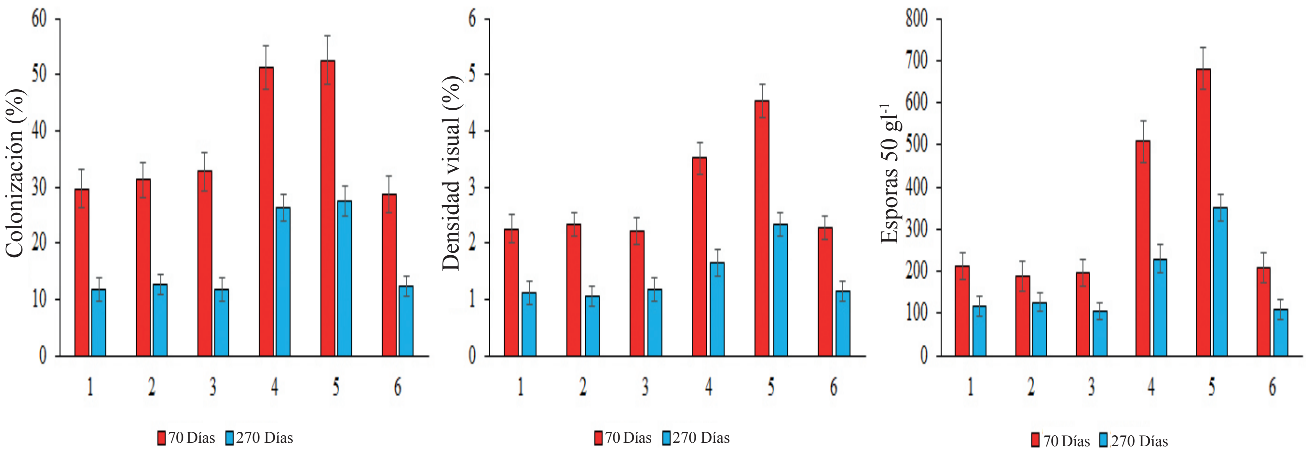
The lowest absolute values that reached the height of the plants, the percentage of the guinea in the grassland and the yield of the biomass at 270 days after the rehabilitation, in relation to the first sampling (at 70 days) can be attributed to the different meteorological conditions that existed in both seasons, since the first was carried out in the rainy period and the second, in the rainy season.

It is known that the biomass production of most of the tropical forage grasses, among which the guinea is included has a seasonal character, since the highest growth rates reach it during the time where the highest levels of precipitation, temperatures and luminosity (35).

In relation to the behavior of the fungal variables (Figure 1), it was observed that the application of the mycorrhizal inoculant increased the colonization percentages, visual density and the number of spores in the rhizosphere with respect to the treatments that were not inoculated; however, these variables reached the highest values with the joint application of the mycorrhizal inoculant plus 12,5 t ha^{-1} of cow manure and 70 kg ha^{-1} year^{-1} of N. When the AMF strain was inoculated and the 100 % of the doses of both fertilizers, such indicators decreased until reaching values similar to those observed in the non-inoculated treatments, which evidenced the depressive effect of the highest doses of both fertilizers in the mycorrhizal variables.

The percentages of colonization, visual density and number of spores were significantly higher at 70 days after the treatments were applied than at 270 days.

According to the behavior of the fungal variables, the influence of mycorrhizal inoculation in the reduction of fertilizer doses necessary to obtain the greatest effects in the grass rehabilitation, confirms the effect of the AMF strain introduced in the improvement of the use of the nutrients, by virtue of the greater volume of soil that the roots could explore. This is inferred from the influence of the mycorrhizal inoculant in the increase of such variables especially in the treatment where 50 and 70 % of the organic and nitrogenous fertilization were applied, respectively, and with which the guinea reached the highest height and presence among the plant species that populated the pasture; as well as, the higher yield of aerial biomass.



Treatments: 1-Control, 2- Mechanical rehabilitation, 3- Mechanical rehabilitation + 25 t ha^{-1} of cow manure + 100 kg ha^{-1} year of N, 4- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 105 spores ha^{-1}, 5- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 105 spores ha^{-1} + 12,5 t ha^{-1} of cow manure + 70 kg ha^{-1} year of N, 6- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 105 spores ha-1 + 25 t ha^{-1} cow manure + 100 kg ha^{-1} year N. The vertical bars show the confidence interval of the mean. Intervals of confidence that overlap each other do not differ significantly (α = 0,05)

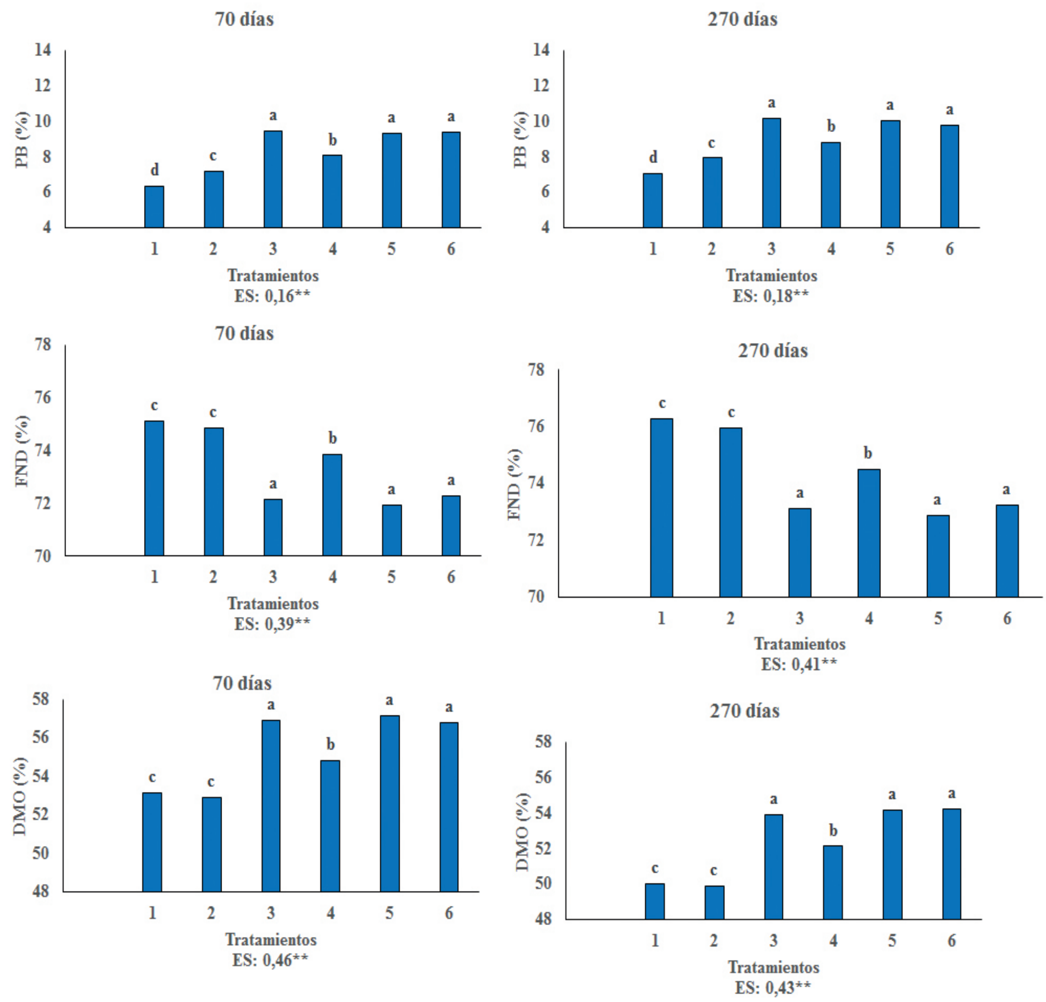
Figure 1. Inoculation effect of the *Glomus cubense* strain on the fungal variables of the grass

In addition to the AMF physical effect on the extension of the plant uptake system, other mechanisms related to its ability to access, as in the case of P, less available forms of soil nutrients (36-38), also could explain the influence of the strain introduced in the reduction of fertilizer doses to apply to pastures.

Likewise, the lower percentages of colonization and visual density, and the smaller number of spores that were observed in the treatment where the inoculant was applied, together with the higher doses of both fertilizers may be a consequence of the decrease in the role of mycorrhizae in the absorption of nutrients in the presence of a high amount of fertilizers (39,40).

The mycorrhizal variables showed seasonal variations as well as pasture performance, (41) and can be explained by the fact that during the rainy season there is a rapid growth of the grass, for the reasons already explained, which implies the absorption of a greater amount of nutrients for the formation of biomass and consequently, the formation of larger amounts of mycorrhizal structures to guarantee the access of plants to such resources.

Regarding the nutritional value of the grass (Figure 2), although there were no significant differences between the control and the treatment where mechanical work was applied for the neutral detergent fiber (NDF) and digestibility of organic matter (BMD) indicators, it was found a beneficial effect of these tasks on the crude protein content (PB), in correspondence with the increase of the concentrations of N in the biomass of the aerial part that was observed in this treatment.



Treatments: 1-Control, 2- Mechanical rehabilitation, 3- Mechanical rehabilitation + 25 t ha^{-1} of cow manure + 100 kg ha^{-1} year of N, 4- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 105 spores ha^{-1}, 5- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 105 spores ha^{-1} + 12,5 t ha^{-1} of cow manure + 70 kg ha^{-1} year of N, 6- Mechanical rehabilitation + application of mycorrhizal inoculant at a rate of 4 x 105 spores ha-1 + 25 t ha^{-1} cow manure + 100 kg ha^{-1} year N.** Means with different letters in the same column differ significantly according to the Duncan test (p <0,05). ES- standard error

Figure 2. Effect of the treatments on the nutritional value of the grass, PB (crude protein), FND (neutral detergent fiber), DMO (digestibility of organic matter)

However, the best results were obtained when the mechanical work was combined with the application of the highest doses of manure and nitrogen fertilizer, with the addition of the mycorrhizal inoculant plus the application of 50 and 70 % of the dose of cow manure and fertilizer Nitrogen, respectively, or with the addition of 100 % of both fertilizers, since with these treatments the grass reached the highest levels of PB and BMD; as well as the lower contents of FND. The effect of the treatments on the nutritional value of the grass was observed in the two sampling moments.

The improvement of the grass nutritive value also was in correspondence with the improvement that in its nutritional state caused the additions of the highest doses of manure and nitrogen fertilizer, or from the mycorrhizal inoculant with smaller doses of both fertilizers. Fertilization, mainly nitrogen one either from mineral or organic sources increases the concentrations of N in the aerial part and, in fact, its crude protein contents (42).

N, by stimulating growth, increases the use of carbohydrates available for the formation of cells and protoplasm, instead of increasing the thickness of the cell wall, thereby reducing fiber and lignin levels, increasing the digestibility and the nutritional value of the grass (32).

However, the most interesting thing was that with the addition of the mycorrhizal inoculant plus the addition of lower doses of mineral fertilizer and organic manure, the indicators evaluated reached values similar to those obtained with the additions of higher doses of both fertilizers in absence of inoculation, so that the decrease in fertilization that was obtained with the use of the inoculant did not imply a reduction of the nutritive value of the grass biomass.

CONCLUSIONS

- ♦ The inoculation of *G. cubense* was successfully integrated into the work for the rehabilitation of the pasture. The grass improved the percentage of covered area when the AMF strain was combined with 50 and 70 % of the dose of organic fertilizer and nitrogen fertilizer, reaching biomass yields with a nutritional value similar to those obtained with the application 100 % of the doses of both fertilizers in the absence of mycorrhizal inoculation.
- ♦ The effect of *G, cubense* was maintained at least until 270 days after its application.

RECOMMENDATIONS

It is recommended to evaluate the inclusion effect of the inoculation of AMF in the work for the rehabilitation of pastures cultivated in other types of soils, previously selecting the most efficient strains for each edaphic condition and grass species.

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