Inoculation with plant growth-promoting bacteria and reduction of nitrogen fertilizer in herbage accumulation and nutritional value of Mavuno grass

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Abstract

Strategies that improve the use of nitrogen (N), by tropical forage grasses, can bring environmental and social gains. Thus, this study aimed to evaluate the forage productivity and the nutritional value (NV) of the hybrid Urochloa spp. cv. ´Mavuno`, under inoculation with plant growth-promoting bacteria (PGPB) and doses of N. The experiment was carried out in the field, for a period of 14 months, in a randomized block design, with nine treatments and four replications in plots of 9 m². Seed inoculations were tested with: Azospirillum brasilense Ab-V5 and Ab-V6 strains; Pseudomonas fluorescens CCTB 03 and co-inoculation with Rhizobium tropici CIAT 899 and A. brasilense Ab-V6, combined with 50 and 100 kg ha^{-1} of added mineral N; fertilization with N-mineral with these doses, without inoculation and the control treatment, without N and without inoculation. The bacteria were inoculated to the grass seeds. The forage was evaluated by determining the shoot dry weight yield (SDWY) and the levels of crude protein (CP), neutral detergent insoluble fiber (NDF), acid detergent insoluble fiber (ADF) and in vitro dry matter digestibility (IVDMD). The results were subjected to analysis of variance by the F test ($p \leq 0.05$) and compared to each other by the Scott-Knott test at 5% probability. Inoculation with PGPB resulted in greater SDWY when associated with a dose of 50 kg ha^{-1} of N. In plants inoculated with A. brasilense Ab-V5 + Ab-V6 and P. fluorescens CCTB 03, associated with a dose of 50 kg ha^{-1} of N-mineral, SDWY was similar to that obtained with double the dose of N in the absence of inoculation. The inoculation with PGPB resulted in an increase in the NV of Mavuno grass, with emphasis on A. brasilense Ab-V5 + Ab-V6 and P. fluorescens CCTB 03, associated with the dose of 100 kg ha^{-1} of N-mineral that improved the nutritional value in all evaluated items. The inoculation with A. brasilense Ab-V5 + Ab-V6 was the most promising, followed by P. fluorescens CCTB 03, mainly in association with 50 kg ha^{-1} of N.

Keywords: crude protein, tropical forage grass, diazotrophic bacteria, *Urochloa* spp.

1. Introduction

At the end of 2019, Brazil was home to 213.7 million cattle and 1.4 million buffalo [1] raised, in large part, on the 180.3 million hectares of pastures [2]. Part of this area has some degree of degradation. Nitrogen fertilization in pastures is little used, although several studies show its effectiveness [3]; [4]; [5]. Nitrogen (N) is a high-cost input in pasture production. The discovery and use of rational technologies are essential, since the increase in meat and milk production is achieved with the use of large quantities of mineral fertilizers.

The inoculation of forage grass seeds with plant growth-promoting bacteria (PGPB) has shown positive results in recent studies [6]; [7]; [8], this technique could reduce nitrogen fertilization costs in tropical pastures [9]; [10].

The industrial synthesis of ammonia, through the traditional Haber-Bosch process, is currently responsible for 1.2% of anthropogenic CO_2 emissions [11], therefore, using microorganisms that make biological nitrogen fixation (BNF) and promote plant growth by other mechanisms is a sustainable alternative [12] for the nutrition of forage plants.

Inoculation with PGPB has been studied in maize (*Zea mays*) [13]; [14], sorghum (*Sorghum vulgare*) [15], wheat (*Triticum aestivum*) [16], sugar cane (*Saccharum officinarum*) [17], soy (*Glycine max*) [18], beans (*Phaseolus vulgaris*) [19] and recently in forage plants [20]; [21], this technique can be used as a strategy to increase plant productivity [22]. The partial replacement of nitrogen fertilizers by inoculants containing *Azospirillum* can reduce CO_2 emissions [23].

Mavuno grass is a hybrid, resulting from the cross between (*Urochloa ruziziense*s x *U. brizantha*) x *U. brizantha* [7], launched in Brazil in 2013, has been used for pasture production in tropical regions [24], however, little is known about the response of this cultivar to inoculation with PGPB.

The hypothesis of this work is that the inoculation in the seeds of Mavuno grass with PGPB associated with N fertilization doses, allows to reduce the amount of N and obtain high productivity of forage dry mass with high nutritional value.

The aim of this study was to evaluate the effect of N doses and seed inoculation with *Azospirillum brasilense* Ab-V5 + Ab-V6, *P. fluorescens* CCTB 03 and co-inoculation with *R. tropici* CIAT 899 + Ab-V6 on productivity and the NV of Mavuno grass, grown in a dystrophic Red Yellow Argisol.

2. Material and methods

Site and experiment description

The experiment was carried out in the field, from November 2018 to January 2020, at the Animal Science Experimental area of the Faculty of Veterinary Medicine, São Paulo State University – UNESP, Araçatuba Campus, located in the northwest region of the State of São Paulo, Brazil, at 390 meters above sea level, latitude 21º 11 '12"S and longitude 50º 26' 20"W, the climate is Aw, according to the Köppen classification [25]. The data of monthly accumulated precipitation and average maximum and minimum temperature are shown in Figure 1.

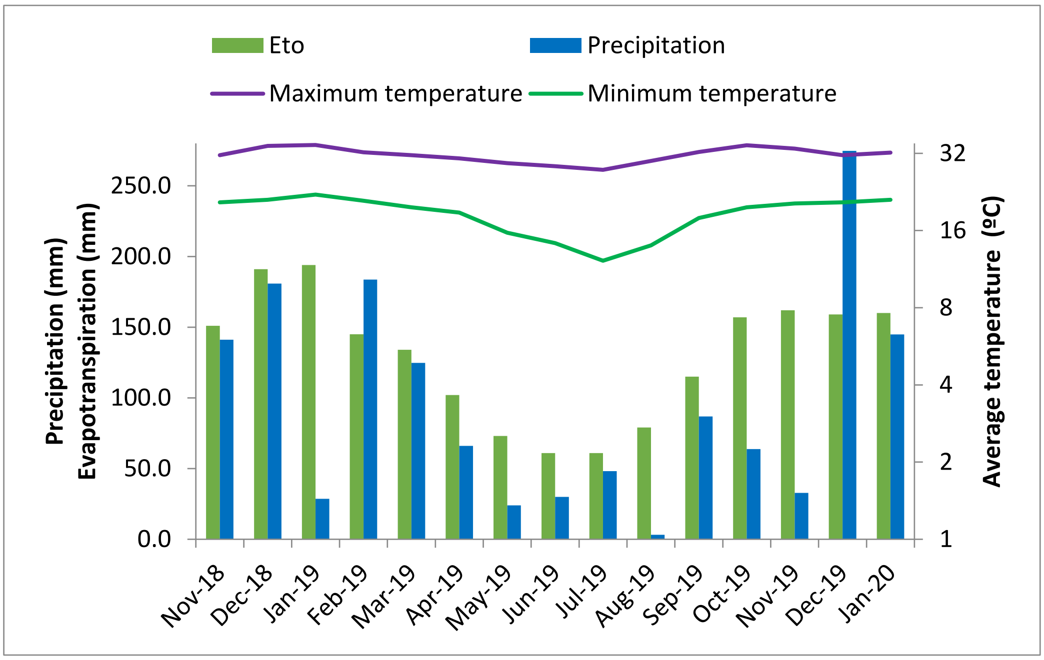

Figure 1. Monthly averages of air temperatures, maximum and minimum (ºC), accumulated precipitation and reference evapotranspiration (mm) during the experimental period. (2018/2020). Source: [26].

Before sowing, soil was collected for chemical analysis, evaluating the need for liming and fertilization with potassium and phosphorus. The sampled soil was classified as dystrophic Red Yellow Argisol, according to the Brazilian Soil Classification System [27].

Sampling was carried out at a depth of 0 to 0.20 m. Chemical determinations were made according to the methodology of [28]: for P, K, Ca and Mg using the ion exchange resin method; pH in $CaCl_2$; organic matter by colorimetry; H + Al with SMP buffer solution; Al in KCl. The results were: organic matter = 24 g dm^{-3}; pH = 4.9; P, S, B, Cu, Fe, Mn and Zn of 9; 1; 0.19; 1; 80; 9.8 and 1.2 mg dm^{-3}, respectively; K, Ca, Mg, H + Al, Al, SB and CEC of 1.9; 13; 11; 30; 1; 25.9; and 55.9 $mmol_c$ dm^{-3}, and base saturation (V) = 46.3%, respectively.

The population of diazotrophic microorganisms in the soil was estimated using the most probable number (MPN) technique in the N-Free NFbsemi-solid culture medium, according to [29] and [30], obtaining the value of $9.5x10^4$ bacteria g^{-1} of soil.

In order to increase the base saturation to 70%, the soil was corrected with the application of 1.3 t ha^{-1} of dolomitic limestone with 90% PRNT [31]. The application of the limestone was carried out 30 days before sowing, by haul, followed by incorporation with a plow of discs at a depth of 20 cm.

Before sowing, 60 kg ha^{-1} of K_2O were applied in the form of potassium chloride and 100 kg ha^{-1} of P_2 O_5 in the form of simple superphosphate. Sowing was done manually, in blocks divided into plots of 3.0 m x 3.0 m (9 m^2).

Statistical design and treatments

The bacteria used were supplied by Embrapa Soybean Soil Biotechnology laboratory, the inoculants were prepared in culture media suitable for each microorganism, in the final concentration of 2 x 10^8 cells

ml^{-1}. For each kilogram of seed, 15 ml of inoculum were mixed and allowed to dry in the shade for 30 minutes before sowing, in soil prepared for seeding. The cultural value of the seed was 76% and the sowing rate was equivalent to 9 kg ha^{-1} of pure viable seeds.

Sowing was carried out on November 13, 2018, in a randomized block design with nine treatments and four replications, totaling 36 experimental units. The treatments are described below: (1) without N and without inoculation, (2) 50 kg ha^{-1} of N without inoculation, (3) 100 kg ha^{-1} of N and without inoculation, (4) 50 kg ha^{-1} of N and inoculation with *Azospirillum brasilense* strains Ab-V5 (CNPSo 2083) and Ab-V6 (CNPSo 2084), (5) 100 kg ha^{-1} of N and inoculation with *A. brasilense* strains Ab-V5 and Ab-V6, (6) 50 kg ha^{-1} of N and inoculation with *Pseudomonas fluorescens* CCTB 03 (CNPSo 2719), (7) 100 kg ha^{-1} of N and inoculation with *P. fluorescens* CCTB 03, (8) 50 kg ha^{-1} of N and co-inoculation with *Rhizobium tropici* CIAT 899 (CNPSo 103, SEMIA 4077) and *A. brasilense* Ab-V6 and (9) 100 kg ha^{-1} of N and co inoculation with *R. tropici* CIAT 899 and *A. brasilense* Ab-V6.

Evaluation of productivity and nutritional value of Mavuno grass

During the experimental period, six cuts were made (01/17/2019; 03/06/2019; 04/12/2019; 05/31/2019; 12/06/2019 and 01/24/2020) to evaluate the Mavuno grass. The cuts were made when the average height of the plants reached 0.50 m, through sampling. A metallic square with an area of 1.0 m^2 was used, leaving a residue of 0.15 m high above the ground [24], with the use of cleavers. The squares were positioned at representative points of each plot, and through the forage contained inside it was possible to determine the weight of the green mass of forage.

The samples were dried in a forced ventilation oven at 65 ° C for 72 hours, according to [32] and the forage mass values were converted to kg of dry mass per hectare.

After the drying period, the samples were ground in a Willey knife mill with a 1 mm sieve and subjected to chemical analysis.

The levels of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method of [33] adapted for a Tecnal TE-149 fiber determiner. After determining the NDF, the residual fiber was incubated to determine the ADF.

The *in vitro* dry matter digestibility (IVDMD) was determined using the technique described by [34], modified by [35] with an ANKOM adaptation [36], in an ANKOM daisy^{II} incubator and a Tecnal TE-149 fiber analyzer. In summary, the samples were incubated for 48 hours in the ANKOM Daisy^{II} incubator, using ruminal fluid from cattle fed with tropical grasses. After incubation, the samples were subjected to the NDF analysis described above and the digestion residues were determined and used to calculate digestibility.

The determination of the N-total content occurred by sulfuric digestion followed by distillation by the micro-Kjeldahl method following the description of AOAC [37]. The crude protein content of the plant was calculated by multiplying the total N-content by 6.25 [32].

The data were tested for the normality of errors and homogeneity of variances. The results were subjected to analysis of variance by the F test ($p \leq 0.05$) and compared to each other by the Scott-Knott test at 5% probability, with the SISVAR statistical analysis program [38].

3. Results and discussion

Shoot dry mass yield (SDMY)

The SDMY values differed statistically from each other ($p \leq 0.05$) in the first, second, fourth and fifth assessment cuts (Figure 2).

The inoculation with *A. brasilense* Ab-V5 + Ab-6 in association with the dose of 50 kg ha^{-1} of N, increased SDMY in the first, second, fourth and fifth evaluation cuts by 6.6%, 9.9%, 8.7% and 13.7%, respectively, compared to treatment with the same dose of mineral N and without inoculation. The results agree with [8], who observed in *U. brizantha* cv. Marandu, under inoculation with *A. brasilense* Ab-V5 + Ab-6, an increase of 13% in SDMY in the first year of evaluation, in relation to the control treatment, when compared to those obtained in this experiment that presented, in the average of the evaluations, an increase of 9.7%.

The inoculation with *P. fluorescens* CCTB 03, in association with a dose of 50 kg ha^{-1} of N, resulted in an increase in SDMY in the first, second and fourth evaluation cuts by 14.2%, 7.5% and 6.6 %, respectively, when compared to treatment with the same dose of mineral N and without inoculation. A similar effect was observed by [20], in *U. ruziziensis*, with increased biomass productivity in response to a dose of 50 kg ha^{-1} of mineral N in association with inoculation with *P. fluorescens* CCTB 03 and also with *P. fluorescens* ET76.

In the first evaluation cut, co-inoculation with *R. tropici* CIAT 899 and *A. brasilense* Ab-V6 associated with a dose of 50 kg ha-1 of N, showed SDMY 9.3% more than that observed in the treatment with the same dose of N in the absence of inoculation. At a dose of 50 kg ha^{-1}, inoculations resulted in statistically similar SDMY.

No inoculation with PGPB associated with a dose of 100 kg ha^{-1} of N promoted an increase in productivity when compared to the same dose of N without inoculation, with a negative effect of inoculation on SDMY, as reported by [20], in *U. ruzizienseis*, under inoculation of *A. brasilense* Ab-V5, *A. brasilense* Ab-V6, *P. fluorescens* CCTB 03, *P. fluorescens* ET76 and *Pantoea ananatis* AMG 521.

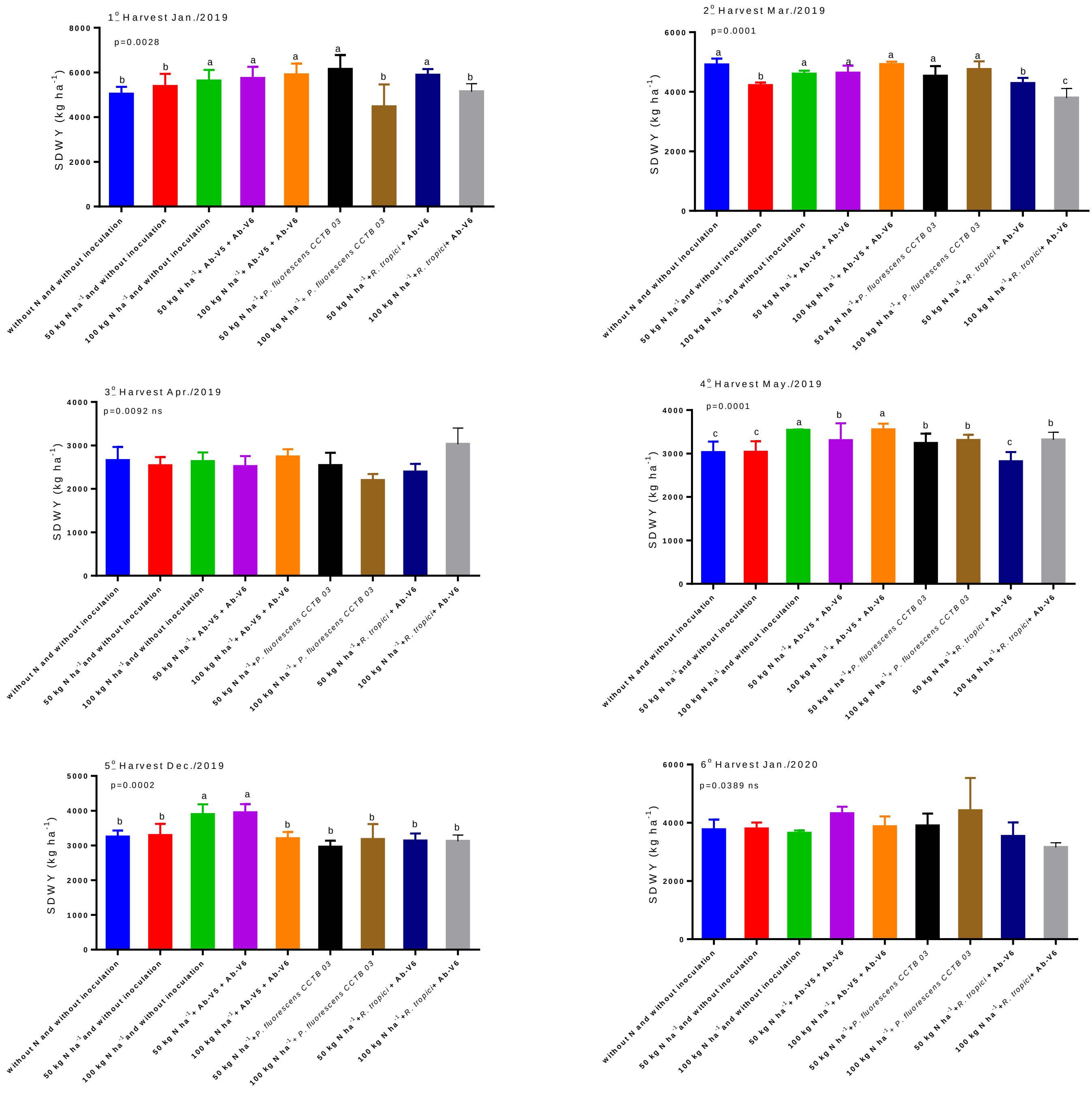

Figure 2 – Shoot dry mass yield (SDMY) (kg ha^{-1}) of Mavuno grass under inoculation with *A. brasilense* AB-V5 + Ab-V6, *P. fluorescens* CCTB 03 and co-inoculation with *R. tropici* + *A. brasilense* Ab-V6, in association with nitrogen fertilization at 50 and 100 kg ha^{-1} N doses and the control without N and without inoculation. The error bars represent the standard deviation. The averages followed by lowercase letters differ for treatments using the Scott-Knott test ($p \leq 0.05$). ^{ns} = not significant.

The inoculation with *A. brasilense* Ab-V5 + Ab-V6 associated with a dose of 50 kg ha^{-1} of N resulted in SDMY similar ($p \leq 0.05$) to that obtained with the dose of 100 kg ha^{-1} of N and without inoculation in the first, second and fifth evaluation cuts, the same occurred with inoculation with *P. fluorescens* CCTB 03 in the first and second evaluations. This result was also obtained with the co-inoculation with *R. tropici*

CIAT 899 and *A. brasilense* Ab-V6 in the first cut, showing an increase in the efficiency of nitrogen fertilization when the inoculation with PGPB is associated with smaller doses of mineral N.

Adding the SDMY of the six cuts, the accumulated productivity of the shoots was obtained. The inoculation with *A. brasilense* Ab-V5 + Ab-V6 and *P. fluorescens* CCTB 03, in association with the dose of 50 kg ha^{-1} of mineral N, did not differ and were higher in 9.9% and 4, 7%, respectively, when compared to the same dose of mineral N without inoculation (Figure 3).

In the absence of inoculation, fertilization with 50 kg ha^{-1} of N did not result in SDMP greater than that obtained with treatment without inoculation and without nitrogen fertilization. It is likely that in treatments with inoculation, greater root growth occurred, consequently, greater absorption of N in periods of water deficiency.

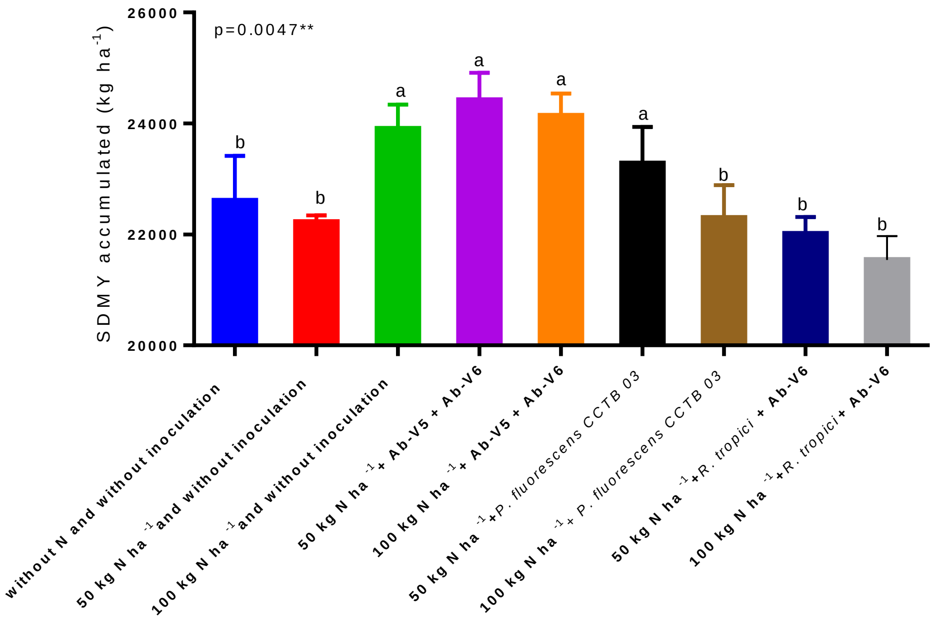

Figure 3 - Shoot accumulated dry mass yield (kg ha^{-1}) in Mavuno grass under inoculation with *A. brasilense* Ab-V5 + Ab-V6, *P. fluorescens* CCTB 03 and co-inoculation with *R. tropici* + *A. brasilense* Ab -V6, in association with nitrogen fertilization at doses 50 and 100 kg ha^{-1} of N and the control without N and without inoculation. The error bars represent the standard deviation. The averages followed by lowercase letters differ for treatments using the Scott-Knott test ($p \leq 0.05$). ** = Significant ($p \leq 0.01$).

Similar results were reported by [39], working with *U. brizantha* cv. Paiaguás under inoculation with *A. brasilense* Ab-V5 + Ab-V6, both in the absence and in association with fertilization with mineral N.

Similar results have been reported by [23], in *Urochloa brizantha* and *U. ruzizienses*, with the inoculation of the bacteria *A. brasilense* Ab-V5 + Ab-V6 in association with fertilization with 40 kg ha^{-1} of N, observed an average increase of 22.1% in productivity of forage in relation to treatment without inoculation and without N, while fertilization with N without inoculation increased the accumulation of forage by only 5.4%.

Co-inoculation with *R. tropici* CIAT 899 and *A. brasilense* Ab-V6, associated with a dose of 50 kg ha^{-1} of N, did not increase forage accumulation, being 11% lower than that obtained with *A. brasilense* Ab-V5 + Ab -V6 and 5.8% lower than that provided by inoculation with *P. fluorescens* CCTB 03. Under this co-inoculation, the accumulation of forage did not differ from that achieved by fertilization with 50 kg ha^{-1} of N and no inoculation.

With the application of 100 kg ha^{-1} of N, the inoculation of seeds with PGPB did not increase the accumulated SDMY, as observed by [9] in *Megathyrus maximus* cv. BRS Zuri.

Nutritional value of Mavuno grass

Crude protein (CP) in the shoot

Inoculation with PGPB increased the CP content of the biomass in the second, third, fourth and fifth evaluation cuts (Figure 4).

In association with the 50 kg ha^{-1} dose of N, inoculation with *A. brasilense* Ab-V5 and Ab-V6 increased the CP content by 11.9% and 17.8% in the fourth and fifth cuts, respectively, compared to the same fertilization in the absence of inoculation. A similar effect was reported by [20], in *U. ruziziensis* under inoculation of the two strains separately and doses of N.

Co-inoculation with *R. tropici* CIAT 899 and *A. brasilense* Ab-V6 associated with a dose of 50 kg ha^{-1} of N promoted an increase of 12.7% in the CP content, in the third cut, in comparison with the same dose of N and in the absence of inoculation.

The inoculation with *P. fluorescens* CCTB 03 associated with a dose of 50 kg ha^{-1} of N did not promote an increase in the CP content of the shoot, when compared to the same fertilization with N and absence of inoculation. Similar results were found by [7] in Mavuno grass, also by [6] and by [40] in *M. maximus* cv. BRS Zuri.

In association with a dose of 100 kg ha^{-1} of N, inoculation with *P. fluorescens* CCTB 03 promoted an increase of 13.4% and 21.7% in the CP content in the fourth and fifth cuts, respectively, compared to the same fertilization without inoculation.

In the second and fifth evaluation cut, co-inoculation with *R. tropici* CIAT 899 and *A. brasilense* Ab-V6, combined with 100 kg ha^{-1} of N, increased the CP content by 21% and 9.4%, respectively.

With fertilization of 100 kg ha^{-1} of N, inoculation with *A. brasilense* Ab-V5 and Ab-V6 increased the CP content by 6.7% in the fifth cut, but did not differ statistically from the other inoculations. Similar results were reported by [9] in *M. maximus* cv. BRS Zuri, in this same dose of N, and by [41], in *M. maximus* cv. Mombasa, with *A. brasilense* Ab-V5 + Ab-V6, regardless of the associated N dose.

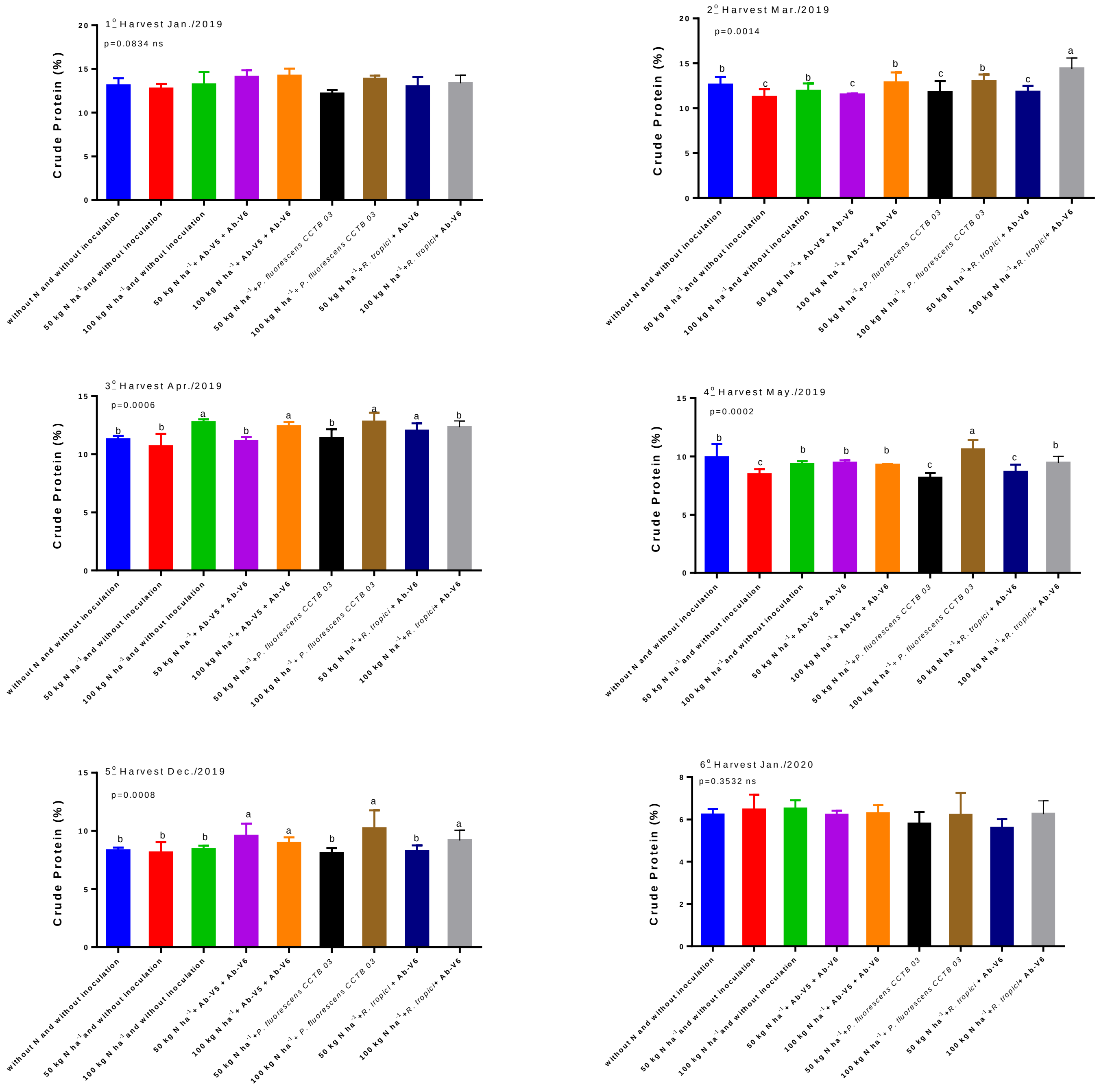

Figure 4 – Crude protein content (% CP) of the shoot in 'Mavuno' grass under inoculation with A. brasilense Ab-V5 + Ab-V6, *P. fluorescens* CCTB 03 and co-inoculation with *R. tropici* + *A. brasilense* Ab-V6, in association with nitrogen fertilization at doses 50 and 100 kg ha^{-1} of N and control, without N and without inoculation. The error bars represent the standard deviation. The averages followed by lowercase letters differ for treatments using the Scott-Knott test ($p \leq 0.05$). ^{ns} = not significant.

In vitro dry weight digestibility (IVDWD)

There was a significant difference between the means of IVDWD in the first four assessment cuts (Figure 5).

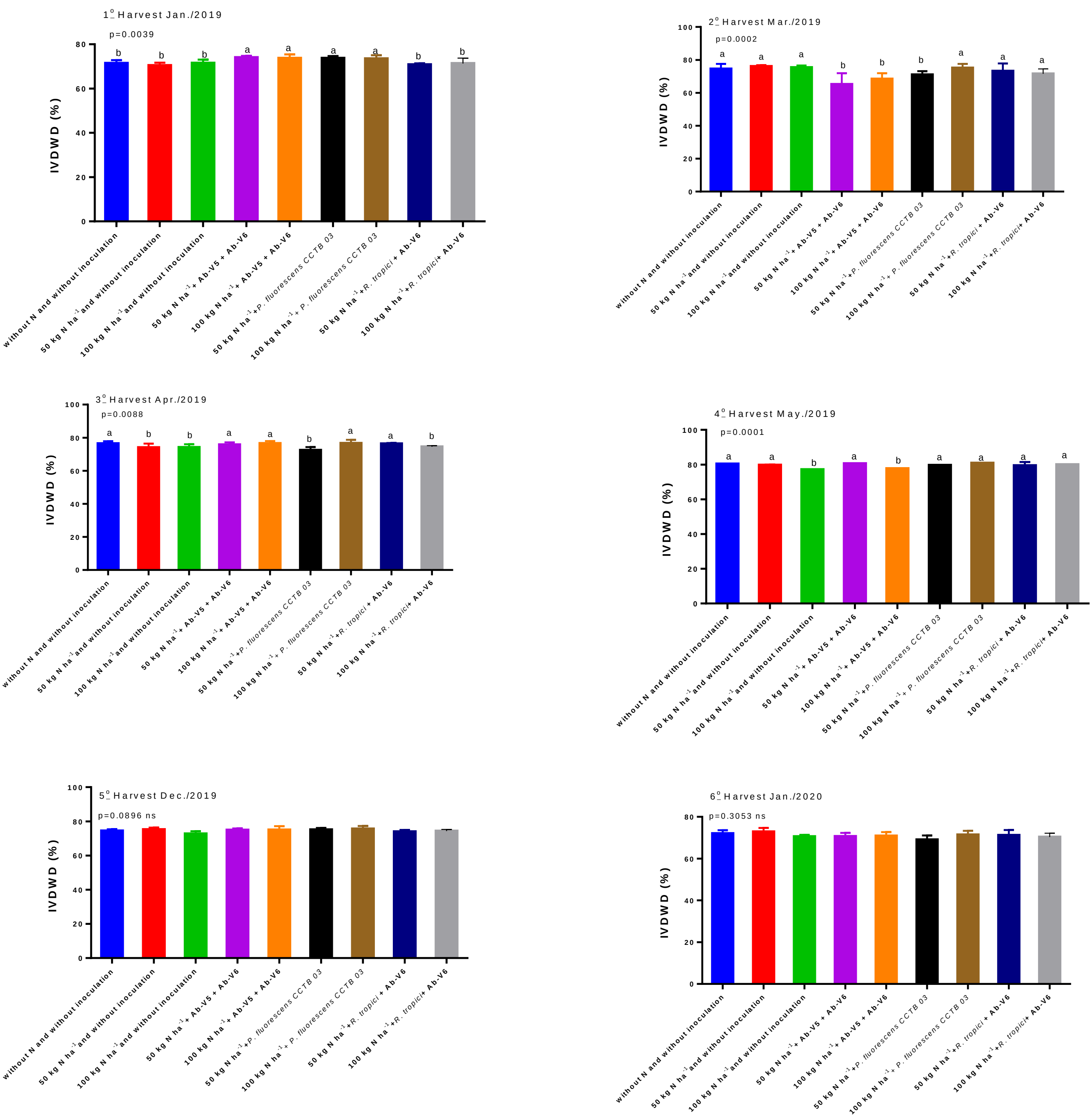

Figure 5 - *In vitro* dry weight digestibility (% IVDWD) of the shoot of Mavuno grass, under inoculation with *A. brasilense* Ab-V5 + Ab-V6, *P. fluorescens* CCTB 03 and co-inoculation with *R. tropici* CIAT 899 + *A. brasilense* Ab-V6, in association with nitrogen fertilization at doses 50 and 100 kg ha^{-1} of N and the control, without N and without inoculation. The error bars represent the standard deviation. The averages followed by lowercase letters differ for treatments using the Scott-Knott test ($p \leq 0.05$). ^{ns} = not significant.

The increase in IVDWD is due to the increase in the nitrogen fraction, with consequent proportional reduction of the cell wall [42]. This is a desirable characteristic in forage plants intended for feeding ruminants for better nutrition.

Studies showing the effect of PGPB inoculation on IVDWD in tropical forages are rare [9] and [42].

The inoculation with *A. brasilense* Ab-V5 + Ab-V6, in association with a dose of 50 kg ha-1 of mineral N, increased the IVDWD by 5.1% and 2.4% in the first and third cuts, respectively.

For the dose of 100 kg ha^{-1} of N, inoculation with *A. brasilense* Ab-V5 + Ab-V6, provided an increase in IVDWD of 3.0% and 3.3% in the first and third cuts, respectively, while in the second cut, there was a 10.1% reduction in IVDWD (Figure 5). The observed increments agree with those obtained by [42], with *U. ruziziensis*, under inoculation with *A. brasiliense* Ab-V5 and Ab-V6 separately, the authors reported an increase in IVDWD in three cuts of *U. ruziziensis* and a reduction in one cut.

The inoculation with *P. fluorescens* CCTB 03 associated with a dose of 50 kg ha^{-1} of N promoted a 4.7% increase in IVDWD in the first cut, not differing from inoculation with *A. brasilense* Ab-V5 and Ab-V6. For the dose of 100 kg ha^{-1} of N, such inoculation increased the IVDWD by 2.7%, 3.4% and 4.8% in the first, third and fourth cuts, respectively. In the first and third cuts, at the highest dose, there was no statistical difference between inoculation with *A. brasilense* Ab-V5 + Ab-V6 and *P. fluorescens* CCTB 03.

Similar results were obtained by [42], who observed in *U. brizantha* cv. Paiaguás, under inoculation with PGPB and N fertilization, in seven evaluations performed, the bacterium *P. fluorescens* CCTB 03 promoted an increase in IVDWD in three cuts.

Co-inoculation with *R. tropici* CIAT 899 and *A. brasilense* Ab-V6, in association with doses of 50 kg ha^{-1} of N, promoted an increase of 3.1% in the third cut, a result statistically similar to that obtained with *A. brasilense* Ab-V5 and Ab-V6. While at the dose of 100 kg ha^{-1} of N, co-inoculation increased IVDWD by 3.8%, not differing from inoculation with *P. fluorescens* CCTB 03.

Increased IVDWD was also observed by [9], in a study with *M. maximus* cv. BRS Zuri under inoculation with the same bacteria studied here, associated with a dose of 100 kg ha^{-1} of N.

Neutral detergent fiber (NDF)

The lower the NDF content, the better the quality of the forage, that is, the greater the cellular content in relation to the cell wall. NDF consists of cellulose, fiber-bound N, hemicellulose and lignin [43]. There was a statistical difference in NDF levels in the first, second and sixth assessment cuts (Figure 6).

In association with a dose of 50 kg ha^{-1} of N, inoculation with PGPB did not reduce the levels of NDF, in the sixth cut, caused an increase in the levels of NDF, causing a reduction in NV. [7] did not observe a reduction of the NDF contents in Mavuno grass and in *M. maximus* cv. BRS Zuri [40] under inoculation with these same PGPB.

At the dose of 100 kg ha^{-1} of N, inoculation with *A. brasilense* Ab-V5 + Ab-V6 resulted in a reduction of the NDF content by 2.6% in the first cut and 5.3% in the second cut, while inoculation with *P. fluorescens* CCTB 03 resulted in a reduction of 3.5% and 7% in the NDF content in the first and second cuts, respectively. There was no statistical difference between the two inoculations above, while co-inoculation with *R. tropici* CIAT 899 and *A. brasilense* Ab-V6 did not affect the NDF content.

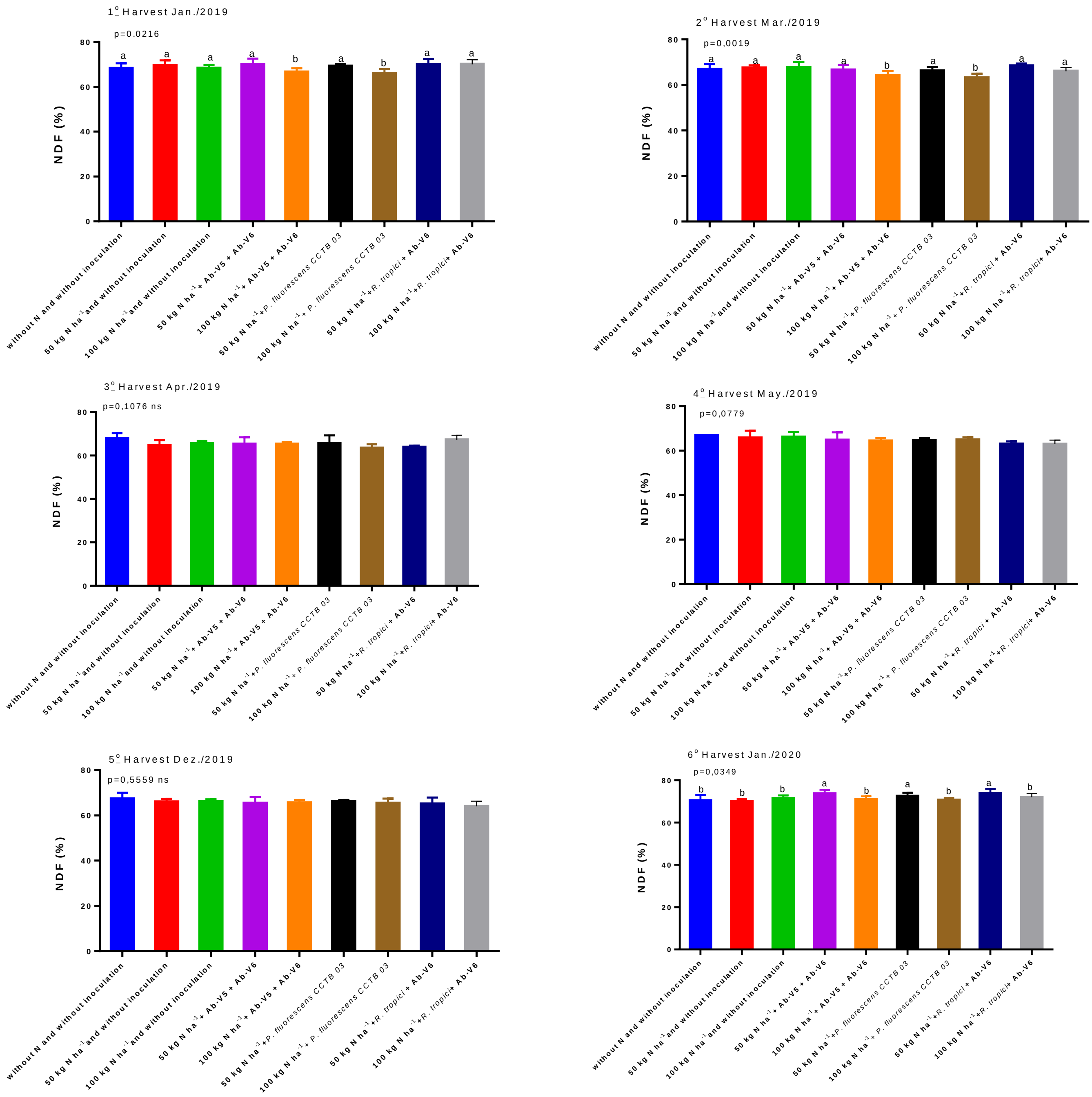

Figure 6 - Neutral detergent fiber (NDF) content of the shoot of Mavuno grass under inoculation with *A. brasilense* Ab-V5 + Ab-V6, *P. fluorescens* CCTB 03 and co-inoculation with *R. tropici* + *A. brasilense* Ab-V6, in association with nitrogen fertilization at doses 50 and 100 kg ha^{-1} of N and control, without N and without inoculation. The error bars represent the standard deviation. The averages followed by lowercase letters differ for treatments using the Scott-Knott test ($p \leq 0.05$). ^{ns} = not significant.

Acid detergent fiber (ADF)

The ADF is the fraction of the forage that is least digestible for ruminants. It is composed almost entirely of lignocellulose [43]. The ADF contents of the forage differed in the first, third, fourth and sixth cuts (Figure 7). Smaller values indicate a higher forage NV.

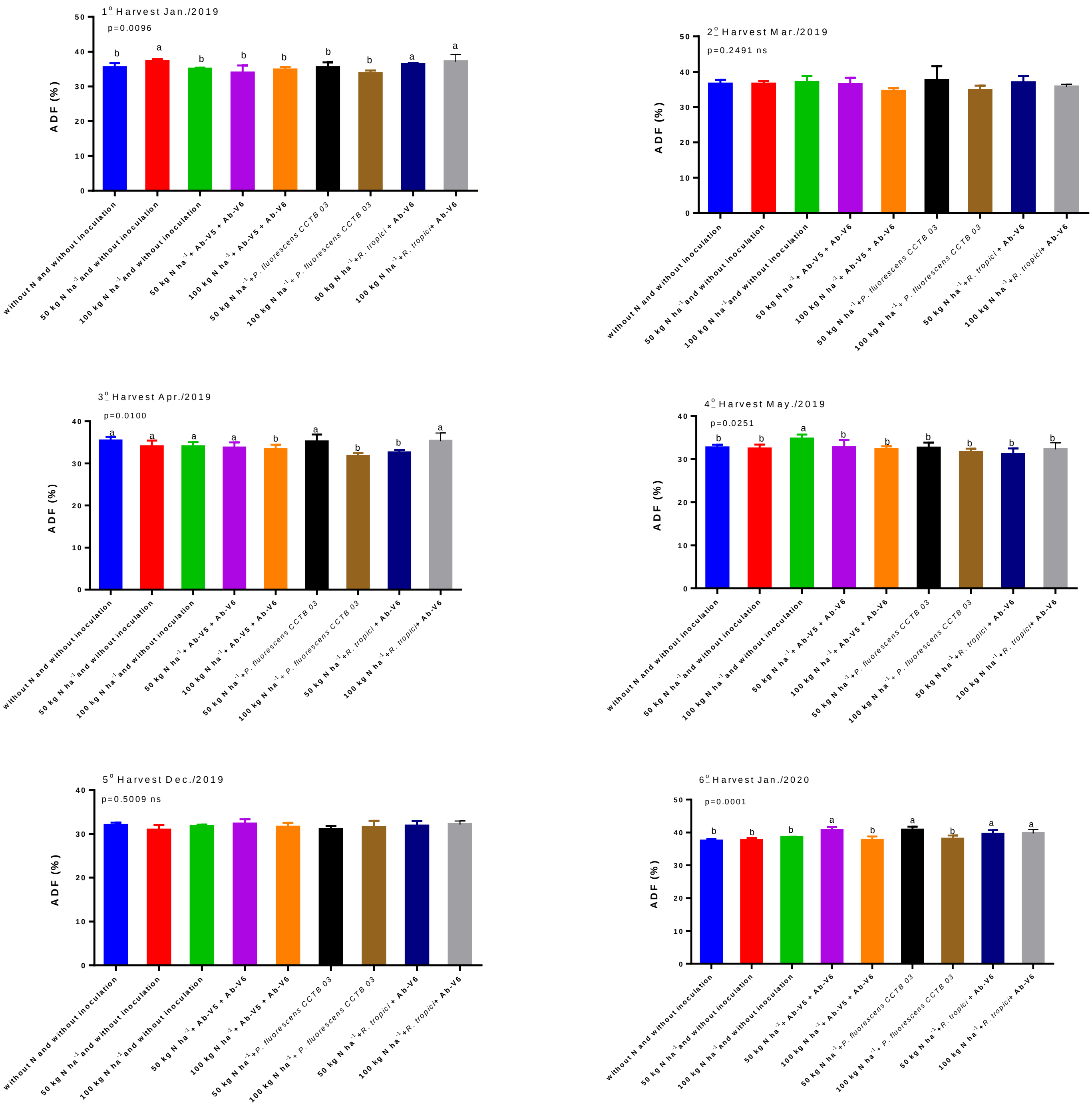

Figure 7 - Content of acid detergent fiber (ADF) in the shoot of Mavuno grass under inoculation with *A. brasilense* Ab-V5 + Ab-V6, *P. fluorescens* CCTB 03 and co-inoculation with *R. tropici* + *A. brasilense* Ab-V6, in association with nitrogen fertilization at doses 50 and 100 kg ha^{-1} of N and control, without N and without inoculation. The error bars represent the standard deviation. The averages followed by lowercase letters differ for treatments using the Scott-Knott test ($p \leq 0.05$). ^{ns} = not significant.

In association with the dose of 50 kg ha^{-1} of mineral N, in the first cut, inoculations with *A. brasilense* Ab-V5 + Ab-V6 and with *P. fluoresens* CCTB 03 reduced the levels of ADF by 9.7% and 5, 0%, respectively, but without statistical difference between them. In this same dose of N, co-inoculation with

R. tropici CIAT 899 and *A. brasilense* Ab-V6 reduced the ADF content by 4.5% in the third cut, improving the nutritional value of the forage, in agreement with [40], who observed similar results in Mavuno grass, under nitrogen fertilization and inoculation with the same PGPB studied here.

In the sixth cut, for the dose of 50 kg ha^{-1} of mineral N, inoculation with PGPB resulted in higher levels of ADF, consequently reducing the NV of the forage.

In the fertilization with 100 kg ha^{-1} of N, inoculation with *A. brasilense* Ab-V5 + Ab-V6 reduced the ADF content by 2.0% and 7.3% in the third and fourth cuts, respectively, and the inoculation with *P. fluorescens* CCTB 03 reduced ADF content by 7.3% and 9.9% in the third and fourth cuts, respectively, but without statistical difference between the two inoculations.

In combination with 100 kg ha^{-1} of N, co-inoculation with *R. tropici* CIAT 899 and *A. brasilense* Ab-V6 resulted in a 7.5% lower ADF content in the fourth cut, in agreement with [9], who observed a reduction in ADF levels in *M. maximus* cv. BRS Zuri, under inoculation with the same PGPB tested here.

4. Conclusions

In association with a dose of 50 kg ha^{-1} of N, inoculation with *A. brasilense* Ab-V5 + Ab-V6 increased the forage yield in 4 cuts, *P. fluorescens* CCTB 03 increased in 3 cuts and the co-inoculation with *A. brasilense* Ab-V6 + *R. tropici* CIAT 899 increased in one cut, such benefit of PGPB was not observed in the dose of 100 kg ha^{-1} of N.

Mavuno grass inoculated with *A. brasilense* Ab-V5 + Ab-V6 and *P. fluorescens* CCTB 03, fertilized with 50 kg ha^{-1} of N, accumulated dry forage mass similar to the application of 100 kg ha^{-1} of N in the absence of inoculation.

Inoculation with PGPB, at a dose of 50 kg ha^{-1} of N, improved the NV in the IVDDM and ADF items, but did not influence the NDF content. The bacteria, except *P. fluorescens* CCTB 03, also increased the CP content.

At a dose of 100 kg ha^{-1} of N, PGPB increased NV in a more pronounced way, in all variables analyzed, except NDF that was not affected by co-inoculation with *R. tropici* CIAT 899 and *A. brasilense* Ab-V6.

5. Acknowledgement

We would like to thank the EMBRAPA Soybean Biotechnology Laboratory (Londrina, PR, Brazil) for providing the bacteria, the Foundation for Research Support of the São Paulo State, FAPESP Process, Research Aid 2017 / 17573-4, for the financial assistance for support this research and the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001.

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