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EFFECT OF INOCULATION OF CORN SEEDS WITH *AZOSPIRILLUM BRASILENSE* ON GRAIN AND SILAGE PRODUCTION

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Abstract

The objective of this study was to determine the effects of inoculation of corn seeds with Azospirillum brasilense on grain and fodder production, nutritional value and fermentative characteristics of corn silage. The experimental design was completely randomized and consisted of two treatments with the inoculation of A. brasilense and control. The nutritive value of silages and grain yield were evaluated. The inoculation with A. brasilense did not increase the forage production (P> 0.05) however it resulted in an increase (P <0.05) in the silage material buffer power from pH 4.77 to pH 5.37 (meq HCl/100g MS). In silage the inoculation increased (P<0.05) crude protein, total digestible nutrients and degradability of organic matter. These results are associated with lower (P <0.05) concentration of lignin in the silages inoculated with A. brasilense. The grain yield and plant morphology were not altered (P>0.05) by inoculation. The inoculation with Azospirillum brasilense did not affect the production however it made possible an improvement in the nutritional value of the silage

Key words Fermentation, agronomic parameters, nutritional value, *Zea mays L.*

EFEITO DA INOCULAÇÃO DE SEMENTES DE MILHO COM AZOSPIRILLUM BRASILENSE SOBRE A PRODUÇÃO DE GRÃOS E SILAGEM

Resumo

Objetivou-se com este estudo determinar os efeitos da inoculação de sementes com Azospirillum brasilense, sobre a produção de grãos e de forragem, o valor nutricional e as características fermentativas das silagens de milho. O delineamento experimental foi inteiramente casualizado e consistiu de dois tratamentos, com a inoculação da A. brasilense e controle. Foram avaliados o valor nutritivo das silagens e a produção de grãos. A inoculação com A. brasilense não elevou (P>0,05) a produção de forragem, contudo resultou em aumento (P<0,05) do poder tampão do material ensilado de 4,77 para 5,37 (meq. HCl/100g MS). Na silagem, a inoculação elevou (P<0,05) os teores de proteína bruta, nutrientes digestíveis totais e da degradabilidade da matéria orgânica. Esses resultados estão associados a menor (P<0,05) concentração de lignina nas silagens inoculadas com A. brasilense. A produção de grãos e a morfologia das plantas não foram alteradas (P>0,05) pela inoculação. A inoculação com Azospirillum brasilense não afetou a produção, contudo possibilitou melhoria no valor nutricional da silagem.

Palavras-chave Fermentação, parâmetros agronômicos, valor nutricional, *Zea mays L.*

INTRODUCTION

Plant growth-promoting bacteria (PGPB) are microorganisms capable of fixing nitrogen from the atmosphere and making it available in an assimilable form in the roots, resulting in the increase of biomass and grain production in various crops of the agricultural sector especially corn and wheat (MORAIS et al. 2016). Among the growth-promoting bacteria those of the genus *Azospirillum* are found in different environments and population densities (CASSAN et al. 2014; ALMEIDA et al. 2021). Beneficial effects of inoculation with *Azospirillum* include a higher percentage of seed germination, increased vigor plant and higher development of the root system (BRASIL et al. 2021). These effects are directly linked to the availability of nitrogen as well as to the production of phytohormones, which are able to change the morphology of the plant providing a more efficient use of resources maximizing the absorption of nutrients due to the greater number of radicelles and the increase in diameter of absorbent roots (SKONIESKI et al. 2019).

The Increase in nutrient assimilation might determine the speed of development and size of plant morphological and reproductive structures, changing for instance the proportion of the stem the leaves and cobs/grains in maize crop (CÁSSAN and DIAZ-ZORITA. 2016). These changes which occur in the structure of the plant affect the nutrient levels such as the concentration of nitrogen in different tissues and organs levels and types of carbohydrates among other nutrients that are important for animal nutrition. In this regard, the effects of the inoculation with *Azospirillum brasilense* in maize hybrids whose plants are destined for the production of silages have been investigated (SKONIESKI et al. 2017).

Corn crop is suitable for the production of grains and forage preserved in the form of silage. According to Kung et al. (2018), among the main fodder crops intended for silage process, corn has a prominent role for presenting high yield of dry mass and good nutrient concentration. Corn is one of the most nutrient-demanding crops especially nitrogen affecting the final production.

According to Cássan and Diaz-Zorita (2016) in corn crop the genus *Azospirillum* is the most studied among the *rhizobacteria* due to its ability to interact with different plant genotypes and environments resulting in increased production grain yield and efficiency of resources such as fertilization. Thus, the inoculation with *Azospirillum brasiliense* alters the composition of the plant, modifying the protein and carbohydrate levels and consequently the energy levels of the material influencing the silage process. Therefore, changes in the digestibility and quality of the ensiled material are expected.

Therefore, the aim of this study was to evaluate the use of *Azospirillum brasilense* in corn seeds and its effects on forage production grain yield and correlated agronomic variables as well as the effects on the fermentation and nutritional quality of silages.

METHODS AND MATERIAL

The experiment was conducted by the Study Group on Additives in Animal Production (GEAPA) at UFSM located in the region named Central Depression in the State of Rio Grande do Sul, Brazil. The experimental period ranged from October 2013 to April 2014. The weather in the region is of the Cfa type humid subtropical according to Köppen classification (1928). The soil is classified as Arrenic Red Argisol (EMBRAPA, 2006).

The commercial corn hybrid Agroceres AG 8025®RR was used in the present experiment. The sowing was performed on October 13^{th}, 2013 using a 5 row tractor-seeder spacing 45 cm between rows, with sowing density of 3.6 seeds/linear m. The base and top fertilization was performed according to the need expressed by the Manual for Fertilization and Liming and considering the expected production of 6 t/ha of grains, the productive potential of this hybrid in the region (ROLAS, 2004).

The experimental area was divided into eight plots of 4.50 m wide (10 lines) and 20 m long each, making 90 m^2 for each experimental unit and total area of 720 m^2. ~~In~~ Each experimental unit containing 8 lines. Two extreme lines on each side of the experimental units were considered as borders and discarded from any sampling. Three of the six central lines were used for sampling and collecting agronomic variables for grain production and another three for silage.

The trial design used in the phase of implantation of maize cultivation was a completely randomized (DIC) with 2 treatments (inoculated and non-inoculated) and 4 replications (steps) per treatment. For the silage 3 samples were ensiled from each replication or plot of maize cultivation totaling 12 replications (mini silos) per treatment. The two treatments consisted on the inoculation or not (control) of corn seeds with commercial inoculant *Azospirillum brasilense* Ab-V5 and Ab-V6 strains and at a concentration of 2.0 x 108 CFU ml^{-1}. A dosage of 200 ml/ha^{-1} of liquid inoculant (Aztotal – Total Biotechnology®) was used.

The silage was performed on February 4th, 2014 when the grains were in the phenological stages between ½ and ¾ of floury grain. The plants in the three central rows were harvested manually with a cut performed at 20cm above the ground. From each experimental unit, four plants were harvested randomly removed for morphological evaluation at the time of silage. The morphological separation divided the botanical components of maize plants into stalk ,inflorescence, leaves, ear (grain + cob + straw) which were weighed (g/kg DM).

The separated morphological components were coarsely ground and dried in a stove with air circulating at 55 °C until constant weight was reached for evaluation of the final participation in the total dry mass of the ensiled forage. Observed average levels of 161.2 and 209.5 for stalk; 4.5 and 6.4 for inflorescences; 82 and 95.3 for the leaves; 161.2 and 200.3 g/kg DM) for the ear of control and inoculated treatments, respectively. The remaining plants from each treatment were separately minced in a stationary grinder with an average particle size of 2 cm.

The ground material from each plot was mixed and three 8 kg samples from each treatment were manually ensiled in mini silos. Each mini silo was hermetically sealed inside 4 plastic bags. The 1st bag had holes made in the bottom for the drainage of effluents; the 2nd bag contained 2 kg of washed and oven-dried sand and a TNT fabric which were used to absorb the effluents from the 1st bag; the 3rd bag served for better sealing and protection of bags one and two and the 4th bag a dark one, for protection against light.

At the time of the silage, a sample of fresh material of each treatment was removed by treatment to determine the forage's buffering capacity according to the methodology described by Playne and Mcdonald (1966). The density of the silages were determined by calculating the volume occupied by the forage mass inside each silo by measuring the height and upper and lower diameters of the silos with a ruler.

When the maize plants were at the stage of complete maturation of grains (reproductive 6) the evaluation of the agronomic parameters of grain production in the three remaining rows of the experimental units was done. Starting from the north-south direction of the rows, plants 4th, 8th and 12th in each of the rows of each experimental unit had their ear height measured from ground level and total plant height with a measuring tape (from the ground to the insertion of the flag sheet).

In the same plants, the diameter of the stalk was measured using a digital caliper between the first and second internodes of the plant from ground level. All ears of the three rows were harvested and the total number of plants per experimental unit counted. The prolificacy (%) was calculated from the ear ration harvested/ number of plants. From the total number of ears collected at each experimental unit five ears were randomly sampled to count the number of grains per row and the total number per ear. Also in each ear the measurement of the length and diameter was performed with a graduated ruler (mm) and a digital caliper.

For the evaluation of grain yield all harvested ears were threshed in an electric threshing machine and the grains resulting from the threshing were weighed. The final grain yield was corrected for 13% moisture and expressed in kg/ha.

The opening of the silos was performed on April, 26th of 2014, after 80 days of fermentation and the weight of the set composed from sand bag and silage was taken and separately from each of the components to determine the losses by effluents gases and dry matter recovery (RDM) according to Jobim et al. (2007) through the following equations:

$$E = (Pab - Pen)/(MVfe) \times 1000$$

Where: E = Effluent production (kg/t of green mass); Pab = Weight of the set (silo + sand + TNT) when opened (kg); Pen = Weight of the set (silo + sand + TNT) in the silage (kg); MVfe = Green mass of ensiled forage (kg).

$$G = [(PCen - Pen) * MSen] - [(PCab - Pen) * MSab] \times 100 \ [(PCen - Pen) * MSen]$$

Where: G = Loss by gas in % of DM; PCen = Weight of full silo upon ensilage (kg); Pen = Weight of the set (silo + lid + sand + TNT) upon ensilage (kg); MSen = content of DM of forage upon ensilage (%); PCab = Weight of full silo upon ensilage (kg); MSab = DM content of forage upon opening (%).

$$RDM = [(MFs \times MSs) / (MFf \times MSf)] * 100$$

Where: RDM: recovery of DM; MFs: forage mass silage, composed by the weight of the bag + silage; MFf: forage mass prior to silo, composed by the weight of the bag + forage; MSs: content of the silage DM; MSf: content of forage DM.

From each mini silo, 3 silage samples were taken. The first sample was taken to determine the pH according to SILVA E QUEIROZ (2002). The second sample was taken for the extraction by pressing of the silage liquid (Carver press) used for analysis of the ammoniacal Nitrogen content in relation to the total Nitrogen ($N\text{-}NH_3/NT$) by the method of colorimetry according to Weatherburn (1967).

The third sample was taken to the oven with forced air circulation at an average temperature of 55°C until it reached constant weight, for the determination of partially dry matter (PDM) and then ground in a Willey-type mill with a 1mm (chemical analysis) and 2mm sieve (degradability estimate). The total dry matter (TDM) was evaluated in an oven at 105º C for a minimum period of 8 hours the mineral matter (MM) by incineration in a muffle at 550º C for 4 hours.

Crude protein (CP) was determined by the micro Kjeldhal method according to AOAC (1995), where 0.5g of sample was weighed in 16µ polyester bags for the determination of neutral detergent fiber (NDF) using the thermostable α-amylase enzyme (Termamyl 120L Novozimes Latin America. LTDA) and an autoclave according to the technique of Senger et al. (2008). The acid detergent fiber content (ADF) and the lignin content (LDA) were performed according to the methodology of Van Soest et al. (1991), with LDA being extracted by the use of 72% sulfuric acid Hemicellulose (HEM) and cellulose contents were obtained from differences between the NDF and FDA and FDA and lignin contents, respectively.

In situ organic matter degradability (OMD) was conducted according to the methodology of Mehrez and Orskov (1977) with 48 hours of incubation for all treatments. 1.0g of the sample was weighed in polyamide bags with 41µ porosity and subsequently incubated in fistulated bovine. The use of this technique was approved by the Ethics Committee on Animal Use of the Federal University of Santa Maria (CEUA) under protocol number 5439180417.

After the incubation period the bags were removed, washed and went through a residue analysis process to determine neutral detergent fiber (NDF). They were dried in an oven at 105°C for 8 hours and later burnt in a muffle at 500°C for 24h to evaluate the percentage of residual mineral matter and calculate the percentage of missing organic matter (OM). The percentage of total digestible nutrients (TDN) was inferred from the degradability of organic matter and the NDF level proposed by Cappelle et al. (2001) by the equation:

TDN= 3.71095-0.129014NDF +1.02278DMO

The statistical model used was the following: Yij= m + ti+ eij. Where: Yij: relative level of the response variable in the treatment of order "i" and repetition j; m: general average; ti: the processing effect of order "i"; eij: effect associated with experimental error.

Data were subjected to analysis of variance and the F test of the ANOVA analysis. Significance was considered as $P \leq 0.05$ (equal to or greater than 95% probability of occurrence) and all tests were performed using the SAS statistical program version SAS® University Edition.

RESULTS AND DISCUSSION

The vast majority of studies with *A. brasilense* in maize observed an increase in grain yield or total plant biomass as an effect. This occurs due to the production of plant hormones capable of changing the morphology of the plant allowing an increase in nutrient absorption as a result of greater number of radicelles and the diameter of absorbent roots (ALMEIDA et al. 2021; CASSÁN and DIAS-ZORITA, 2016). However, in this work no effects of inoculation with *Azospirillum brasilense* were observed (P>0.05) on yield variables and agronomic characteristics of the hybrid AG 8025 RR (Table 1).

Table 1. Influence of seed inoculation of the maize hybrid AG 8025 RR with the bacteria *Azospirillum brasilense* on the production characteristics.

Variables	*Azospirillum brasilense*		Standard error	Pr > F
	Inoculated	control		
Ear height (m)	1.03	0.96	0.05	0.45
Total height (m)	1.84	1.67	0.03	0.06
Prolificacy (%)	0.92	0.95	0.01	0.29
Stalk diameter (mm)	20.22	19.90	0.95	0.83
Ear diameter (mm)	42.63	42.46	0.70	0.87
Ear length (cm)	18.34	17.46	0.48	0.26
Number of grains per ear	472.7	438.3	21.6	0.32
Number of grains per ear row	34.60	33.26	0.86	0.33
Grain production (kgMS/ha)	4.358	4.721	119	0.09
Forage production (kgMS/ha)	17.180	17.519	5.60	0.77
Buffering capacity*	4.77^{b}	5.37^{a}	0.20	0.003

* meq. HCl in 100g DM; There is significant differences when P≤0.05 according to variance analysis.

The average grain production was 4.558 kg/ha. The absence of responses to inoculation is also observed in the literature, as in the work by Detoni et al. (2017). In contrast Kappes et al. (2013) observed a 9.4% increase in maize plant yield when maize seeds were inoculated. The authors reported that the stalk diameter is important to obtain good yield since the larger its diameter the greater the plant's capacity to store photo assimilates that will help the filling of the grains.

Likewise Dartora et al. (2013) studying seed inoculation with *A. brasilense* and *Herbaspirillum seropedicae* in maize at different stages also did not observe results on plants height. In contrast, Skoniski et al. (2019) observed that inoculation in different hybrids evaluated improves ear formation increasing its diameter and length which is related to the increase in grain yield.

In concerning forage mass yield, no effect (P>0.05) of seed inoculation with *A. brasilense* was observed with an average level of 17.349 (kg DM/ha). However, the efficiency of the inoculation response depends on many biotic and environmental factors, which include plant genotype, inoculated bacteria and soil properties such as pH humidity temperature and the availability of carbon and nitrogen source being such characteristics important to determine if the effect of inoculation with *Azospirillum* is positive, irrelevant or even negative (QUADROS et al. 2014; SKONIESKI et al. 2019).

Inoculation with *A. brasilense* increased (P<0.05) 12.6% the buffering capacity of the material to be ensiled (Table 2). Related to the capacity of the ensiled mass to resist a decrease in pH. It basically depends on the composition of the plant in what refers to crude protein content, inorganic ions (Ca, K, Na) and the presence of organic acids (KUNG et al. 2018).

Low rates/levels of buffering capacity allow a faster decrease in pH during anaerobic fermentation reaching the fermentative stability of the silage more quickly, which can result in a reduction of losses and gain in the final quality of the silage. This increase in the level of buffering capacity can be explained by the increase in the concentration of crude protein (P<0.05) in the plant due to the inoculation of the *A. brasilense* bacteria. However, the increase in the buffering capacity was not enough to affect the fermentation process of the ensiled material since the silages presented adequate pH levels (Table 3).

The inoculation with *A. brasilense* increased (P<0.05) DM content of corn silage

Table 2 - Chemical composition of corn silage made from plants with or without inoculation with *Azospirillum brasilense*

Variables (% DM)	*Azospirillum brasilense*		Standard error	Pr > F
	Control	Inoculated		
Dry matter (%)	32.09[b]	34.13[a]	0.28	0.0001
Mineral matter	3.83	3.73	0.08	0.41
Organic matter	96.17	96.27	0.08	0.41
Neutral-detergent fiber	47.35	45.25	1.42	0.30
Acid-detergent fiber	25.39	23.93	0.78	0.20
Lignin	1.88[a]	1.34[b]	0.09	0.001
Hemicellulose	21.99	21.13	0.65	0.35
Cellulose	23.61	22.28	0.77	0.23
Crude Protein	8.29[b]	8.75[a]	0.13	0.03
Ether extract	3.00	2.96	0.05	0.56
Non-fiber carbohydrates	37.07	39.77	1.35	0.17
Degradability of OM	68.22[b]	72.56[a]	1.32	0.03
Total Digestive Nutrients	65.60[b]	69.86[a]	1.29	0.03

There are significant differences when P≤0.05 according to variance analysis

(Table 2). This increase may have occurred because this genus of bacteria has mechanisms to facilitate the plant growth such as hormonal induction by means of the production of auxins, cytokinins, gibberellins among other substances (CÁSSAN and DIAZ-ZORITA. 2016).

According to Fukami et al. (2018) these hormones enable the development of tissues and organs, including the roots increasing the root surface and providing greater capacity to absorb water and nutrients. favoring greater accumulation of dry matter in inoculated plants. which can impact the quality of forage nutrition and biomass yield for ensilage. Quadros et al. (2014) also observed an increase in DM content in maize plants inoculated with *Azospirillum spp.*

Table 3. Evaluation of fermentative characteristics and physical losses of corn silages inoculated or not with *Azospirillum brasilense*

Parameters	*Azospirillum brasiliense*		Standard error	Pr > F
	Control	Inoculated		
DM Recovery (%)	82.5	82.5	14.0	0.97
Effluents loss (kg/t MV)	12.8	13.5	7.30	0.54
Gas losses (% da MS)	3.37	3.33	0.49	0.90
Ammoniacal nitrogen (% NT)	3.22	2.94	0.50	0.36
pH	3.84	3.86	0.03	0.35
Density (kg MV/M^3)	685	640	7.26	0.22

There are significant diferences when P≤0.05 according to variance analysis.

The levels of organic matter (OM) mineral matter (MM) and non-fiber carbohydrates (NFC) in corn silage were similar (P>0.05) between treatments with and without seed inoculation (Table 2). Schumacher et al. (2021) studied the effects of *A. brasiliense* inoculation on the fractionation of carbohydrates and nitrogen in sorghum silage and did not observe any effect on NCF, while the crude protein (CP) content increased (P<0.05) with the inoculation of the bacteria in the seeds. This increase in CP must be related to the greater absorption of nutrients including Nitrogen (N) which increases as a result of inoculation of the *A. brasilense* strain (ALVES et al. 2020).

No significant effects were observed (P>0.05) on NDF, ADF, hemicellulose, cellulose and non-fiber carbohydrates in the silages (Table 2). However, when inoculated with *A. brasilense*, there was an average increase of 6% in the degradability of organic matter and in the total digestible nutrient content (TDN) of the silage. This increase in degradability and energetic values of silage was mainly due to the 28% reduction in lignin contents. Skoniski et al. (2017) evaluating the effects of inoculation with *A brasilense* on the production and quality of silage, found an increase of approximately 1% in TDN contents and a decrease in ADF contents around 2%.

The authors reported that the effects of inoculation with *A. brasilense* on the cell wall content of plants which qualitatively alter the ensiled materials, was probably due to changes in the anatomical histology of plant tissues such as the deposition of mesophyll cells parenchyma and sclerenchyma, which have different cell walls and content concentrations. This causes changes in the levels of crude protein, structural and soluble carbohydrates, in addition to affecting the energy content. This set of factors alter the degradability and quality of the ensiled material.

The results of the evaluation of the fermentative profile of the silages (Table 3) showed that the inoculation with *A. brasilense* did not change (P>0.05) the variables studied. On average, effluent losses were 13.21 kg/t MV, while gas losses produced during the active phase of fermentation were 3.35% of the total ensiled DM. Losses by effluents and gases can be high for some tropical forages (BORREANI et al. 2018).

The average rates obtained for the density and pH of the silages were 662 Kg MV/m^3 and 3.85, respectively. This pH rate is considered ideal for forage conservation (KUNG et al. 2018). On the other hand, Borreani et al. (2018) reported that corn silage with densities greater than 705 kg.m^3 are desired to obtain a reduction in losses and greater conservation of nutrients, as a result of the amount of oxygen available at the beginning of fermentation stage. The adequate content of dry matter of 30 to 35%, associated with good compaction leads to suitable densities together with the low buffering capacity of the forage at the time of ensiling are essential to mitigate measured losses by effluents and gases and achieve DM satisfactory recovery rates (SUCU et al. 2016).

The average ammoniacal nitrogen ($N-NH_3$) content observed was 3.08% of $N-NH_3$/NT. Skoniski et al. (2017) studying the influence of inoculation with *Azospirillum spp*. in corn seed, on the production and quality of silage, obtained average levels of 4.48% of $N-NH_3$/NT, which are higher than the ones in the present work. However, in both works the levels can be considered adequate for quality silages, considering that $N-NH_3$ is the result of unwanted fermentations and that rates/levels lower than 11 or 12% of the total nitrogen in grass silages indicate good fermentation (MONTEIRO et al. 2011).

CONCLUSION

The inoculation of corn seeds with *Azospirillum brasilense* does not affect the production of corn forage for silage and the final grain yield. However, it increases the nutritional quality of the silage.

REFERENCES

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS – AOAC. **Official methods of analysis**. 16th ed. Arlington: AOAC International, 1995. 2000p.

ALMEIDA, L.S.; SOBRINHO, S.P.; LUZ, P.B.; CALDEIRA, D.S.A.; OLIVEIRA, A.J.; VILARINHO. M.K.C.. VIEIRA. C.L.. SILVA. A.S.P.. SILVA. G.V.B. Uso de inoculante *Azospirillum brasilense* na produção de mudas de Tento Carolina. **Research. Society and Development**, v.10, p.1-9, 2021. https://doi.org/10.33448/rsd-v10i1.11469

ALVES, G.C.; SOBRAL, L.F.; REIS, V.M. Grain yield of maize inoculated with diazotrophic bacteria with the applicationof nitrogen fertilizer. **Revista Caatinga,** v.33, p.644-652, 2020. https://doi.org/10.1590/1983-21252020v33n307rc

BORREANI, G.; TABACCO, E.; SCHMIDT, R. J.; HOLMES, B. J.; MUCK, R. E. *Silage review:* Factors affecting dry matter and quality losses in silages. **Journal Dairy Science,** v.101, p.3952–3979, 2018. https://doi.org/10.3168/jds.2017-13837

BRASIL, M.S.; SOUZA, M.S.T.; GUIMARÃES, S.L.; JUNIOR. S.L.K.; BATISTELA, M.W.A. Initial development of upland rice plants inoculated with the MAY12 strain of *Azospirillum* spp. Ciência rural, v.51, p.1-9, 2021. http://doi.org/10.1590/0103-8478cr20200824

CAPPELLE. E. R.; VALADARES FILHO. S.C.; SILVA. J.F.C; CECON. P.R. Estimativas do valor energético partir de características químicas e bromatológicas dos alimentos. **Revista Brasileira de Zootecnia,** v.30, p.1837-1856, 2001. https://doi.org/10.1590/s1516- 35982001000700022

CASSÁN. F.; DIAZ-ZORITA. M. *Azospirillum sp*. In current agriculture: From the laboratory to the field. **Soil Biology & Biochemistry,** v.103, p.117-130, 2016. http://dx.doi.org/10.1016/j.soilbio.2016.08.020

CASSAN. F.; VANDELEYDEN. J.; SPAEPEN. S. Physiological and agronomical aspects of phytohormone production by model plant-growth-earting rhizobacteria PGPR belonging to the genus *Azospirillum*. **Journal of Plant Growth Regulation,** v.33, p.440-459, 2014. https://doi.org/10.1007/s00344-013-9362-4

DARTORA, J.; GUIMARÃES, V. F.; MARINI, D.; SANDER, G. Adubação nitrogenada associada à inoculação com *Azospirillum brasilense* e *Herbaspirillum seropedicae* na cultura do milho. **Revista Brasileira de Engenharia Agrícola e Ambiental,** v.17, p.1023–1029, 2013. https://doi.org/10.1590/S1415-43662013001000001

DETONI, M. J.; SARTOR, L. R.; GASPERINI, A. M.; OLOGINI, K. F.; FRIGOTTO, T. Inoculação de *Azospirillum brasilense* e doses de nitrogênio em milho para produção de grãos. **Científica,** v.45, p.321-324, 2017. http://dx.doi.org/10.15361/1984-5529.2017v45n3p321-324

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA- CNPS. **Sistema Brasileiro de Classificação de Solos**. Embrapa - Produção de informação, 2006. 412p.

FUKAMI, J.; CEREZINI, P.; HUNGRIA, M. *Azospirillum*: benefits that go far beyond biological nitrogen fixation. **AMB Express**, v.8, p.2-12, 2018. https://doi.org/10.1186/s13568-018-0608-1

JOBIM, C.C.; NUSSIO, L.G.; REIS, R. A.; SCHMIDT, P. Avanços metodológicos na avaliação da qualidade da forragem conservada. **Revista Brasileira de Zootecnia,** v.36, p.101-119, 2007. http://dx.doi.org/10.1590/S1516-35982007001000013

KAPPES, C.; ARF, O.; ARF, M. V.; FERREIRA, J. P.; BEM DAL, E. A.; PORTUGUAL, J. R.; VILELA, R. G. Inoculação de sementes com bactéria diazotrófica e aplicação de nitrogênio em cobertura e foliar em milho. **Semina: Ciências Agrárias,** v. 34, p. 527-538, 2013. http://dx.doi.org/10.5433/1679-0359.2013v34n2p527

KÖPPEN. W; GEIGER. R. **Klimate der Erde.** Gotha: Verlag Justus Perthes, 1928.

KUNG JR, L.; SHAVER, R.D.; GRANT, R.J.; SCHIMIDT, R.J. Silage review: Interpretation of chemical. microbial. and organoleptic components of silages. **Journal of Dairy Science,** v.101, p.4020–4033, 2018. https://doi.org/10.3168/jds.2017-13909

MEHREZ, A. Z.; ORSKOV, E. R. A study of the artificial fibre bag technique for determining the digestibility of feed in the rumen. **Journal of Agricultural Science,** v. 88, p.645-650, 1977. https://doi.org/10.1017/s0021859600037321

MONTEIRO, I. J. G.; ABREU, J. G.; CABRAL, L.S.; RIBEIRO, M.D.; REIS, R. H. P. Silagem de capim-elefante aditivada com produtos alternativos. **Acta Scientiarum Animal Sciences,** v. 33, p. 347-352, 2011. http://dx.doi.org/10.4025/actascianimsci.v33i4.12629

MORAIS, T. P.; BRITO, C. H.; BRANDÃO, A. M.; REZENDE, W. S. Inoculation of maize with *Azospirillum brasilense* in the seed furrow. **Revista Ciência Agronômica,**

v. 47, p. 290-298, 2016. http://dx.doi.org/10.1007/s11104-009-0262-0

PLAYNE, M.J.; MCDONALD, P. The buffering constituents of herbage and of silage. **Journal of the Science of Food and Agriculture,** v. 17, p. 264-268, 1966.

QUADROS, P. D.; ROESCH, L. F. W.; SILVA, P. R. F.; VIEIRA, V. M.; ROEHRS, D. D.; CAMARGO, F.A.O. Desempenho agronômico a campo de híbrido de milho inoculados com *Azospirillum*. **Revista Ceres,** v. 61, p. 209-218, 2014. https://doi.org/10.1590/S0034-737X2014000200008

ROLAS - REDE OFICIAL DE LABORATÓRIOS DE ANÁLISE DE SOLO E DE TECIDO VEGETAL. **Manual de adubação e calagem para os estados do Rio Grande do Sul e Santa Catarina**. Ed.10. Sociedade Brasileira de Ciência do Solo, 2004. 400 p.

SAS INSTITUTE Inc. **SAS/STAT.** User's guide. version 9.1. Cary: SAS Institute, 2005.

SCHUMACHER, L.L.; VIÉGAS, J.; TONIN, T.J.; PEREIRA, S.N.; SKONIESKI, F.R.; CARDOSO, G.S.; HORA, A.L.V.C.; TEIXEIRA, J.V.; NEMOTO, B.S. Efeito da inoculação de sementes com *Azospirillum brasilense* sobre o fracionamento nitrogenado e glicídico em silagens de sorgo. **Research. Society and Development**, v. 10, p. 1-11, 2021. http://dx.doi.org/10.33448/rsd-v10i2.12321

SENGER, C. C. D.; MÜHLBACH, P. R. F.; SÁNCHEZ, L. M. B.; NETTO, D. P.; LIMA, L. D. Evaluation of autoclave procedures for fibre analysis in forage and concentrate feed stuffs. **Animal Feed Science and Technology**, v. 146, p. 169-174, 2008. https://doi.org/10.1016/j.anifeedsci.2007.12.008

SILVA, D. J; QUEIROZ, A. C. **Análise de alimentos:** Métodos químicos e biológicos. 3ª ed. Viçosa, MG: Editora UFV, 2002. 165 p.

SKONIESKI, F.R.; VIÉGAS, J.; MARTIN, T.N.; NÖRNBERG, J.L.; MEINERZ, G.R.; TONIN, T.J.; BERNHARD, P.; FRATA, M.T. Effect of seed inoculation with *Azospirillum brasilense* and nitrogen fertilization rates on maize plant yield and silage quality. **Revista Brasileira Zootecnia**, v.46, p.722-730, 2017. http://dx.doi.org/10.1590/s1806-92902017000900003

SKONIESKI, F.R.; VIÉGAS, J.; MARTIN, T.N.; MINGOTTI, C.C.A.; NAETZOLD, S.; TONIN, T.J.; DOTTO, L.R.; MEINERZ, G.R. Effect of nitrogen topdressing fertilization and inoculation of seeds with azospirillum brasilense on corn yield and agronomic characteristics. **Agronomy**, v. 9, p. 1-11, 2019. https://doi.org/10.3390/agronomy9120812

SUCU, E.; KALKAM, H.; CANBOLAT, O.; FILYA, I. Effects of ensiling density on nutritive value of maize and sorghum silages. **Revista Brasileira de Zootecnia,** v.45, p.596-603, 2016. https://doi.org/10.1590/S1806-92902016001000003

VAN SOEST, P.J.; ROBERTSON, J.B.; LEWIS, B.A. Methods for dietary fiber, neutral detergent fiber. and nonstarch polysaccharide in relation to animal nutrition. **Journal of Dairy Science,** v.74, p.3583- 3597, 1991.

WEATHERBURN. M. W. Phenol-hypochlorite reaction for determination of ammonia. **Analytical Chemistry,** v.39, p.971-974, 1967.