

## BLACK OAT AND RYEGRASS INOCULATED WITH *Azospirillum brasilense* IN CROP-LIVESTOCK SYSTEM<sup>1</sup>

MARCOS DA SILVA BRUM<sup>2\*</sup>, VINICIUS DOS SANTOS CUNHA<sup>3</sup>, THOMAS NEWTON MARTIN<sup>3\*</sup>,  
PEDRO GARDIN ALESSIO<sup>3</sup>, GUILHERME ARISMENDI ALMEIDA<sup>3</sup>

**ABSTRACT** - The success of the integrated crop-livestock system depends on the correct pasture management, particularly regarding nitrogen fertilization and grazing intensity. The objective of this study was to evaluate the total production of forage and the proportion of the botanical and structural components of black oat and annual ryegrass mixture. The experimental design was randomized blocks with three replicates and the treatments were arranged in a three-factorial scheme: seed inoculation (with and without *Azospirillum brasilense* inoculation), grazing management (conventional grazing, where the animals were kept in pasture with no control of pasture height; 30, 20 and 10 cm at the end of grazing, and a control without grazing), and nitrogen fertilization (0, 50 and 100 kg nitrogen ha<sup>-1</sup>). In treatments with pasture heights where grazing ended at 30, 20 and 10 cm, the height was monitored and biomass was collected when the animals entered and exited the plots. Manual separation of the components was used to determine the dry mass of green leaves, stems, senescent material and inflorescence. Total dry mass production was obtained based on the initial forage mass plus the daily accumulation rates. The proportion of structural components varied according to the different grazing managements, nitrogen doses and inoculation with *Azospirillum brasilense*. Biomass production of black oat and ryegrass mixture was increased by nitrogen application and *Azospirillum brasilense* inoculation.

**Keywords:** Nitrogen fertilization. Grazing heights. Inoculation.

## AVEIA PRETA E AZEVÉM INOCULADAS COM *Azospirillum brasilense* EM SISTEMA LAVOURA-PECUÁRIA

**RESUMO** - O sucesso do sistema de integração lavoura-pecuária depende do correto manejo da pastagem, principalmente quanto às alturas de manejo do pastejo e adubação. Objetivou-se com esta pesquisa avaliar a produção de forragem e a proporção dos componentes botânicos e estruturais de uma pastagem de aveia preta e azevém. Os tratamentos foram organizados em um trifatorial: inoculação de sementes (com e sem inoculação com a bactéria *Azospirillum brasilense*), manejo de pastejo (pastejo convencional, onde os animais permaneciam na pastagem sem um controle da altura da pastagem, altura da pastagem na saída dos animais de 30, 20 e 10 cm e uma testemunha sem pastejo), e o fator adubação nitrogenada (0, 50 e 100 kg de nitrogênio ha<sup>-1</sup>). Foi coletada a massa de forragem presente em cada tratamento no momento da entrada e saída dos animais do pastejo e por meio de separação manual dos componentes determinou-se a massa seca de lâminas foliares verdes, massa seca de colmos, massa seca de material senescente, massa seca de inflorescência. A produção total de matéria seca foi obtida pela massa de forragem inicial acrescida das taxas de acúmulo diárias. As proporções dos componentes estruturais variaram em função dos diferentes manejos de pastejo, doses de nitrogênio e inoculação com *A. brasilense*. A produção de biomassa da pastagem de aveia preta e azevém foi incrementada pela aplicação de nitrogênio e com a inoculação de *A. brasilense*, sendo possível realizar a substituição do nitrogênio mineral pelo uso de *A. brasilense*.

**Palavras-chave:** Adubação nitrogenada. Alturas de pastejo. Inoculação.

\*Corresponding author

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<sup>2</sup>NPR Fertilizantes LTDA, Santa Maria, RS, Brazil, [marcostrum@yahoo.com.br](mailto:marcostrum@yahoo.com.br) -ORCID: 0000-0002-9950-7738.

<sup>3</sup>Crop Science Department, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil, [vinicius\\_scunha@hotmail.com](mailto:vinicius_scunha@hotmail.com) - ORCID: 0000-0002-8022-6821, [martin.ufsm@gmail.com](mailto:martin.ufsm@gmail.com) -ORCID: 0000-0003-4549-3980, [pedrogalessio98@gmail.com](mailto:pedrogalessio98@gmail.com) -ORCID: 0000-0003-0474-7884, [almeidaarismendi@gmail.com](mailto:almeidaarismendi@gmail.com) - ORCID: 0000-0002-4643-1717.

## INTRODUCTION

The integration between crop and livestock (CLI) is a system with greater sustainability (KUNRATH et al., 2014) and more intensive. In southern Brazil, black oat and ryegrass (winter) and soybean and corn (summer) crop are used. CLI improves soil quality by accumulating more organic matter and cycling nutrients; aggregating the soil (SOUZA et al., 2010); promoting efficiency in the use of fertilizers; increasing pasture production (CASSOL et al., 2011); interrupting the cycle of some diseases, pests and weeds; improving animal performance and reducing costs (BALBINOT et al., 2009). Excessive use of nitrogen fertilizers increases costs and causes damage to the environment through nitrate leaching in watercourses (WALKER et al., 2012). Biological nitrogen fixation aims to reduce costs, mitigate environmental impacts and promote greater plant growth and production gains (FILGUEIRAS; MENESES, 2015).

Bacteria of the genus *Azospirillum* are classified as symbiont bacteria, contributing to the improvement of crop yield (AON et al., 2015; AAZADI et al., 2014). These bacteria are capable of fixing nitrogen and producing plant growth hormones such as gibberellins, auxins and cytokinins (HUNGRIA; NOGUEIRA; ARAUJO, 2015). It is an alternative to reduce or supplement fertilization with mineral N in grass crops. Kurepin, Zaman and Pharis (2014) report that inoculation with *A. brasilense* increases grass biomass by 25%. For corn, Schaefer et al. (2019) and Brum et al. (2016) verified that the production of shoot biomass, yield and thousand-grain weight were higher due to inoculation of seeds with *A. brasilense*. In wheat, it was found that *A. brasilense* improves the initial characteristics of plant development, but these effects are reduced over time (KAZI et al., 2016; PICCININ et al., 2013). Increments in grass yield have been reported in some studies with the use of *Azospirillum brasilense* (MULLER et al., 2016; DOMINGUES NETO et al., 2014). However, research using this bacterium in cold-season grasses is limited. For oats, Bilal et al. (2017) observed increases in dry mass and crude protein yield when using *A. brasilense* as a substitute for nitrogen.

The objective of this study was to evaluate the total production of forage and the structural components of black oat and ryegrass managed under different grazing heights, nitrogen doses and seed inoculation with *A. brasilense* in a crop-livestock integration system.

## MATERIAL AND METHODS

The experiment was conducted in 2012 and 2013, in Santa Maria, RS, Brazil, at the geographic

coordinates 29°41'51.07"S and 54°02'30.42"W, at an altitude of 195 meters. The climate is Cfa, subtropical humid, according to Köppen's classification. The experimental area was 0.36 ha, in a crop-livestock integration system, with pasture formed by an intercropping of black oats and ryegrass in the winter period and with corn and soybean in subsequent summers. In 2012, common cultivars (black oat and ryegrass) were used. In 2013, the black oat cultivar IAPAR 61 and the ryegrass cultivar BRS Ponteio were used. The soil of the experimental area is classified as *Argissolo Vermelho distrófico arênico* (Ultisol) (EMBRAPA, 2006). The area was cultivated with ryegrass, soybean and millet in the periods prior to sowing of the experiments (May 17, 2013, both years).

The number of suitable seeds per m<sup>2</sup> was 400 seeds (300 of black oats + 100 of ryegrass) in both years. The experimental design was randomized blocks with three replicates and the treatments were arranged in a trifactorial scheme (inoculation of seeds (2) x grazing management (5) x nitrogen application (3)). Black oat and ryegrass seeds were subjected or not to inoculation with *A. brasilense* (strains Abv5 and Abv6), using liquid inoculant (AZO TOTAL®) at a concentration of 2 x 10<sup>8</sup> colony-forming units per mL of product. The managements were carried out as follows: (I) - conventional grazing (CG), where the animals remained in the pasture throughout the experimental period with no control of pasture height, (II) - pasture height at the exit of the animals of 30 cm (H30), (III) - pasture height at the exit of the animals of 20 cm (H20), (IV) - pasture height at the exit of the animals of 10 cm (H10) and (V) - No grazing (NG). Fertilization management was as follows: without nitrogen fertilization; 50 kg ha<sup>-1</sup> nitrogen (N) and 100 kg ha<sup>-1</sup> of N. In treatments with dose of 50 kg ha<sup>-1</sup>, the application was carried out broadcast in a single time, whereas in treatments with dose of 100 kg ha<sup>-1</sup>, the dose was split into two applications. The N source used was urea (45% N). In 2012 and 2013, the first application of N occurred at the beginning of tillering, at the dose of 50 kg of N ha<sup>-1</sup>. At this time, N was applied to the plots that would receive doses of 50 and 100 kg of N ha<sup>-1</sup>. The second application of 50 kg of N ha<sup>-1</sup> was performed in the plots that would receive 100 kg of N ha<sup>-1</sup>. Each experimental unit had dimensions of 8 x 4 m, totaling an area of 32 m<sup>2</sup>.

The animals used for grazing were Corriedale sheep, with an average age of one year, with five sheep per experimental unit. For the heights of the exit of the animals of 10, 20 and 30 cm, the rotational grazing method was adopted, and the animals remained in the plots for the time necessary to reach the desired heights, ranging from two to five days. The interval between grazing events was 28 days. In 2012, grazing occurred twice because of a delay for the pasture to reach an initial forage mass

of around 1500 kg ha<sup>-1</sup>. In 2013, grazing was carried out three times. For the characterization of pasture height, it was monitored by the “sward stick” ruler method.

Forage mass was determined in each plot by means of cuts with scissors, close to the ground, in an area delimited by a 0.25 m<sup>2</sup> frame. Forage mass was determined in each experimental unit at the entry and exit of the animals in each grazing period. From the samples obtained from the cuts, the botanical and morphological composition of the pasture was determined by manually separating the structural components: leaf (blade), stem (leaf sheath + stem), senescent material and inflorescence. All samples were dried in an oven with forced air circulation at 60 °C, until reaching constant weight, and subsequently weighed. The following parameters were evaluated: dry mass of green leaves (DMGL), dry mass of stems (DMS), dry mass of senescent material (DMSM), dry mass of inflorescence (DMI) and total dry mass (TDM).

In treatments with different pasture heights (H10, H20 and H30), the evaluations of the dry mass accumulation rate (kg ha<sup>-1</sup> day<sup>-1</sup>) were performed in each plot, based on the difference in forage mass at the entry of the animals during the period (X) and forage mass at the exit of the animals during the period (X-1), divided by the number of days of the period. In treatments with conventional grazing, dry

mass accumulation rates were determined with the aid of grazing exclusion cages, using one cage in each experimental unit. The total dry mass production was obtained based on the initial forage mass plus the daily accumulation rates multiplied by the number of days of the period. The data were subjected to analysis of variance and, when significant, the means were compared by the Duncan test, with 5% probability of error.

## RESULTS AND DISCUSSION

The structural components of the forage mass were influenced by the application of nitrogen (N) in the first evaluation period (Table 1). Higher DMGL, DMS and TDM were observed in treatments that received 50 kg ha<sup>-1</sup> of N. The DMGL of the black oat-ryegrass intercropping showed an increase of 7.72 kg ha<sup>-1</sup> of DM for each kg of N applied. This result should be emphasized, because the application of 50 kg of N ha<sup>-1</sup> in the tillering stage led to increments of 386 kg ha<sup>-1</sup> for DMGL and 633 kg ha<sup>-1</sup> for TDM. In a practical way, N application is fundamental for pasture management, as it allows the pasture to produce the same amount of leaves in a shorter period of time when compared to pasture without N, being possible to reduce the sowing interval until the first grazing.

**Table 1.** Average values for the components dry mass of green leaf blades (DMGL, kg ha<sup>-1</sup>), dry mass of stems (DMS, kg ha<sup>-1</sup>), leaf/stem ratio (LSR), dry mass of senescent material (DMSM, kg ha<sup>-1</sup>), dry mass of inflorescence (DMI, kg ha<sup>-1</sup>), total dry mass (TDM, kg ha<sup>-1</sup>) and height (H, cm) of a pasture of black oat and ryegrass under different managements in a crop-livestock integration system.

Entry first grazing						
Factors	Treatments	DMGL	DMS	LSR	DMSM	TDM
N doses (kg ha <sup>-1</sup> )	0	809.92 b*	336.11 b	2.41	95.29	1231.07 b
	50	1196.02 a	562.47 a	2.13	105.84	1864.33 a
Inoculation <i>Azospirillum</i>	Without	928	400.57	2.32	96.57	1411.48
	With	949.25	422.54	2.25	101.04	1472.83
Exit first grazing						
		DMGL	DMS	DMSM	TDM	H
Grazing management	30 cm	797.02 a*	684.18 a	96.73	1577.93 a	23.55 a
	20 cm	522.51 b	474.00 b	101.04	1097.56 b	19.95 b
	10 cm	251.53 c	308.44 c	94.31	641.60 c	12.00 c
N doses (kg ha <sup>-1</sup> )	0	499.82 b	438.68 b	96.87	1029.02 b	18.08 b
	50	571.42 a	589.27 a	98.35	1259.04 a	19.34 a
Inoculation <i>Azospirillum</i>	Without	516.95	496.55	95.27	1100.31	18.82
	With	530.43	481.20	99.45	1111.08	18.18

\*Means not followed by the same letter differ at 5% probability of error by Duncan's test.

In ryegrass pasture, Pellegrini et al. (2010) verified that N increases the forage mass produced and DMGL shows an increasing linear behavior of 1.28 kg ha<sup>-1</sup> of DM for each kg of N applied in the pasture. The values found for DMGL ranged from 515.0 to 814.6 kg ha<sup>-1</sup> of DM for the N doses of 0 and 225 kg ha<sup>-1</sup>, respectively. When N is added, the plant increases the number of active meristems and

density of tillers and maintains the growth of leaves. At the beginning of the second grazing (Table 2), it was possible to observe the influence of grazing management on pasture structure, and the treatments with heights of 30 and 20 cm showed higher values of TDM. However, they had lower leaf/stem ratio (LSR) values in comparison to those with more intense grazing.

**Table 2.** Dry mass of green leaf blades (DMGL, kg ha<sup>-1</sup>), dry mass of stems (DMS, kg ha<sup>-1</sup>), dry mass of senescent material (DMSM, kg ha<sup>-1</sup>), dry mass of inflorescence (DMI, kg ha<sup>-1</sup>), total dry mass (TDM, kg ha<sup>-1</sup>) and height (H, cm) in post-grazing residue of a pasture of black oat and ryegrass under different managements in a crop-livestock integration system.

		Entry second grazing					
Factors	Treatments	DMGL	DMS	LSR	DMSM	DMI	TDM
Grazing management	CG	585.60 c	757.11 c	0.77 a	338.95 b	158.44 c	1840.11 c
	30 cm	716.64 b	2283.73 a	0.31 b	464.00 a	523.31 a	3987.68 a
	20 cm	714.53 b	1646.00 b	0.43 b	273.97 b	319.11 b	2953.62 b
	10 cm	878.18 a	1063.93 c	0.83 a	155.42 c	127.44 c	2224.97 c
N doses (kg ha <sup>-1</sup> )	0	543.27 b	1218.65 b	0.45 b	310.767 ab	253.38 ab	2326.06 b
	50	828.28 a	1451.41 ab	0.57 a	237.867 b	209.61 b	2727.18 b
	100	799.67 a	1643.01 a	0.49 b	375.633 a	383.23 a	3201.55 a
Inoculation <i>Azospirillum</i>	Without	684.07	1407.78	0.49	281.71	294.43	2668.00
	With	763.41	1467.60	0.52	334.46	269.72	2835.20
		Exit second grazing					
		Dry mass of stems (DMS)		Grazing Management			
				30 cm	20 cm	10 cm	
Inoculation <i>Azospirillum</i>	Without			1682.93 a A	1331.73 a B	713.46 a C	
	With			1871.20 a A	991.68 b B	483.86 a C	
		Total dry mass (TDM)					
Inoculation <i>Azospirillum</i>	Without			2362.17 a A	1986.62 a B	1171.95 a C	
	With			2614.57 a A	1623.33 b B	911.77 a C	
		Dry mass of senescent material (DMSM)					
		N doses		0 kg of N ha <sup>-1</sup>	50 kg of N ha <sup>-1</sup>	100 kg of N ha <sup>-1</sup>	
Inoculation <i>Azospirillum</i>	Without			296.57 a B	279.60 a B	404.17 a A	
	With			308.53 a A	336.88 a A	272.53 b A	
		Grazing management		DMGL	DMI	H	
Grazing management	30 cm			134.07	147.04 a	25.93 a	
	20 cm			176.22	143.49 a	20.45 b	
	10 cm			172.58	75.22 b	11.07 c	
N doses (kg ha <sup>-1</sup> )	0			130.27	129.93	19.03	
	50			177.18	111.18	18.57	
	100			175.42	124.64	19.86	
Inoculation <i>Azospirillum</i>	Without			159.85	110.90	19.44	
	With			162.06	132.93	18.86	

\*Different uppercase letters in the row and different lowercase letters in the column indicate difference between treatments at 5% probability level by the Duncan test.

As verified in the first evaluation, the DMGL values were higher for the treatments with the use of 100 and 50 kg ha<sup>-1</sup> of N. On the other hand, TDM was superior in the treatment with 100 kg ha<sup>-1</sup> of N. In the second grazing, according to the regression equation ( $Y = 8.759X + 2313.9$ ), there was an increase in forage mass (TDM) of 8.75 kg ha<sup>-1</sup> of DM for each kg of N applied.

There was an increase in residual TDM from the first to the second grazing for the three grazing management heights. Such increase results from the increase in DMS, decrease in DMGL and decrease in water content in plant tissues. After the second grazing (Table 2), differences were verified in DMS for the different grazing heights, which did not occur for DMGL. Due to the increased participation of black oat stems from the first to the second grazing, this was the component that most contributed to TDM. For leaves, there were no differences between grazing heights because of their low participation in TDM.

The total dry mass production (DMP, kg ha<sup>-1</sup>) was higher when 100 kg of N ha<sup>-1</sup> was applied, followed by the application of 50 kg of N ha<sup>-1</sup> and the control. With the application of 100 kg of N ha<sup>-1</sup>, there was an increase of 1,665.18 kg of DM ha<sup>-1</sup> compared to the control without nitrogen fertilization. This corresponds to an increase of 16.65 kg of DM ha<sup>-1</sup> for each kg of N applied in the pasture (regression equation:  $Y = 16.652X + 3497.2$ ).

Pellegrini et al. (2010) obtained similar results in which each kg of N applied caused an increase of 15.83 kg ha<sup>-1</sup> in the dry mass of ryegrass. Cassol et al. (2011) state that the use of nitrogen fertilization promotes an increase in the forage production of black oat and ryegrass pasture, which increases the support capacity of the pasture.

The production with inoculation was 4582.30 kg ha<sup>-1</sup>, higher than that obtained without inoculation (4077.20 kg ha<sup>-1</sup>). There was an increase of 505 kg ha<sup>-1</sup> in DMP under inoculation of *A. brasilense*. Considering that there is an increase of 16.65 kg of DM ha<sup>-1</sup> for each 1 kg of mineral N applied in the pasture, the inoculation of *A. brasilense* replaced 30.33 kg ha<sup>-1</sup> of mineral N (67.4 kg of urea). Due to the low cost of the inoculant, and the DMP response observed in this study, it can be inferred that the use of inoculation with *Azospirillum brasilense* in black oats and ryegrass can replace part of the fertilization with mineral nitrogen.

The values of DMGL and TDM showed a

distinct behavior as a function of inoculation, N doses and grazing management practiced in the previous year. The values of DMGL and TDM were higher when 50 kg of N ha<sup>-1</sup> was used and the seeds were inoculated, regardless of the uniform grazing management of the previous year when nitrogen application was used. The response varied according to the grazing management applied in the previous year. Similar behavior was verified for DMGL and TDM, prior to the third grazing (Table 5).

Thus, inoculation with *A. brasilense* may have provided an additional amount of N, promoting higher TDM. On the other hand, DMS values varied according to nitrogen doses and grazing management. Regarding inoculation, the structural components of the pasture varied according to grazing management and nitrogen doses, and there was no standard response regarding the use of inoculation. For DMSM, in the treatments CG, 20 cm and NG and without nitrogen, the use of inoculation allowed lower values of senescent material in the pasture (Table 3).

DMGL was higher in the treatment of 30 cm; however, there was lower LSR due to the greater accumulation of stems (DMS) in this treatment. This result is in accordance with Baggio et al. (2008), who report that in situations of moderate grazing (lower frequency and intensity of defoliation), greater grazing management heights generate greater participation of stems in black oat and ryegrass pasture. DMGL was higher when 100 kg of N ha<sup>-1</sup> were applied, compared to doses of 0 and 50 kg of N ha<sup>-1</sup>, with no effect of inoculation on DMGL. The highest values of DMS, DMSM, DMI and TDM were found in treatments with 30 cm grazing management height, a consequence of the higher residual dry mass left after the first grazing.

There was a triple interaction for pasture height before the entry of the animals, for the first, second and third grazing. For the first grazing, an increase in pasture height was observed when 50 kg ha<sup>-1</sup> of N were applied, in most of the grazing managements of the previous year (Table 4). Before the second and third grazing, the values of entry heights were influenced by the management of exit heights of the previous grazing (Tables 4 and 5). The greatest pasture heights were verified when 100 kg ha<sup>-1</sup> of N were applied in most treatments. The heights did not respond uniformly to inoculation, varying according to grazing management and N doses applied. In this case, it is verified that pasture height did not increase with the inoculation of *A. brasilense*.

**Table 3.** Dry mass of green leaf blades (DMGL, kg ha<sup>-1</sup>), dry mass of stems (DMS, kg ha<sup>-1</sup>), dry mass of senescent material (DMSM, kg ha<sup>-1</sup>), total dry mass (TDM, kg ha<sup>-1</sup>) and height (H, cm) of a pasture of black oats and ryegrass under different managements in a crop-livestock integration system (entry of the first grazing, year 2013).

DMGL						
Inoculation	N doses (kg of N ha <sup>-1</sup> )	Grazing Management				
		CG	30 cm	20 cm	10 cm	No grazing
Without <i>Azospirillum</i>	0	**β 521.99 BC	*β 323.34 C	α 846.29 a A	α 657.79 AB	β 605.30 B
	50	α 742.73 a A	α 761.23 A	β 687.90 AB	β 537.25 b B	α 693.49 b AB
With <i>Azospirillum</i>	0	β 362.70 B	β 351.43 B	β 540.71 b AB	706.99 A	β 477.22 AB
	50	α 498.29 b C	α 674.60 B	α 829.01 AB	726.64 a B	α 908.85 a A
DMS						
	0	β 142.22 AB	β 106.90 B	β 151.90 AB	α 255.76 A	α 233.55 A
	50	α 191.48 A	α 214.64 A	α 234.03 A	β 188.03 A	β 190.08 A
DMSM						
Without <i>Azospirillum</i>	0	α 184.10 a A	60.97 C	α 104.06 a B	β 39.70 C	α 215.78 a A
	50	β 41.57 B	50.98 B	β 67.48 B	α 55.04 B	β 119.42 A
With <i>Azospirillum</i>	0	66.08 b AB	α 85.47 A	β 35.26 b BC	β 12.68 C	89.86 b A
	50	61.65 B	β 58.19 B	α 64.97 B	α 51.56 B	94.95 A
TDM						
Without <i>Azospirillum</i>	0	β 878.00 a A	β 456.12 B	1098.26 a A	α 1163.66 A	α 1110.34 a A
	50	α 1095.37 a A	α 981.63 A	1122.93 A	β 720.04 b B	β 961.23 b A
With <i>Azospirillum</i>	0	β 541.33 b B	β 578.90 B	β 731.87 b AB	β 937.22 A	β 744.93 b AB
	50	α 710.67 b D	α 950.60 C	α 1184.45 AB	α 1026.54 a BC	α 1281.00 a A
H						
Without <i>Azospirillum</i>	0	β 22.11 BC	β 22.33 BC	β 24.5 AB	α 27 A	β 19.44 C
	50	α 26.61 A	α 27.72 A	α 29.5 a A	β 22.08 b B	α 23.16 b B
With <i>Azospirillum</i>	0	β 18.77 B	β 22.22 B	26.78 A	β 23.11 AB	β 21.44 B
	50	α 26.41 A	α 26.27 A	26.41 b A	α 25.61 a A	α 26.27 a A

\*Different uppercase letters in the row and different lowercase letters in the column indicate difference between treatments at 5% probability level by the Duncan test. \*\*Different Greek letters in the column represent the difference between nitrogen doses for each level of inoculation and grazing management.

**Table 4.** Dry mass of green leaf blades (DMGL, kg ha<sup>-1</sup>), dry mass of stems (DMS, kg ha<sup>-1</sup>), dry mass of senescent material (DMSM, kg ha<sup>-1</sup>), dry mass of inflorescence (DMI, kg ha<sup>-1</sup>), total dry mass (TDM), leaf/stem ratio (LSR), and height (H, cm) of a pasture of black oat and ryegrass under different managements in a crop-livestock integration system (entry of the second grazing, year 2013).

Dry mass of green leaves (DMGL)					
Grazing management	N doses		Inoculation		
CG	408.29 c*	0 kg of N ha <sup>-1</sup>	441.23 b	With <i>Azospirillum</i>	504.23
30	690.47 a	50 kg of N ha <sup>-1</sup>	438.14 b	Without <i>Azospirillum</i>	514.90
20	490.38 b	100 kg of N ha <sup>-1</sup>	649.33 a		
10	449.11 bc				

\*Different uppercase letters in the row and different lowercase letters in the column indicate difference between treatments at 5% probability level by the Duncan test. \*\*Greek letters in the column represent the difference between nitrogen doses for each level of inoculation and grazing management.

Table 4. Continuation.

Dry mass of green leaves (DMGL)					
Grazing management		N doses		Inoculation	
DMS					
Inoculation	N doses (kg of N ha <sup>-1</sup> )	CG	Grazing management		
			30 cm	20 cm	10 cm
Without <i>Azospirillum</i>	0	**β 182.52 D	β 961.62 A	β 538.65 B	354.40 C
	50	β 220.59 C	α 1637.01 a A	α 813.25 a B	366.66 C
	100	α 426.17 C	α 1529.32 a A	α 820.41 B	306.15 C
With <i>Azospirillum</i>	0	β 178.97 C	α 1037.09 A	α 674.59 B	β 223.09 C
	50	β 223.50 C	β 812.65 b A	β 409.88 b B	αβ 375.23 BC
	100	α 448.64 C	β 834.07 b A	α 668.53 B	α 442.98 C
DMSM					
Without <i>Azospirillum</i>	0	37.91 BC	β 102.12 A	αβ 95.30 AB	β 19.33 C
	50	11.81 B	αβ 142.34 b A	α 58.77 B	α 125.39 a A
	100	29.09 B	α 190.13 A	α 146.32 a A	β 17.54 B
With <i>Azospirillum</i>	0	19.15 B	β 134.09 A	α 146.30 A	13.40 B
	50	58.01 B	α 307.01 a A	β 77.42 B	38.96 b B
	100	14.32 B	β 158.14 A	β 41.41 b B	17.94 B
DMI					
Without <i>Azospirillum</i>	0	11.48 A	π 53.18 b A	β 31.34 A	31.52 A
	50	11.32 C	α 299.08 a A	α 112.64 a B	18.86 C
	100	5.45 B	β 208.42 a A	β 35.57 B	11.45 B
With <i>Azospirillum</i>	0	9.35 B	α 195.83 a A	20.92 B	12.26 B
	50	26.38	β 44.51 b	30.52 b	27.12
	100	31.10	β 50.03 b	54.29	41.80
TDM					
Without <i>Azospirillum</i>	0	β 446.87 C	β 1805.48 b A	π 943.67 b B	880.16 B
	50	β 558.47 C	α 2731.24 a A	β 1453.89 a B	731.76 C
	100	α 1055.35 C	α 2782.08 a A	α 1721.33 a B	892.00 C
With <i>Azospirillum</i>	0	β 570.00 C	α 2058.65 a A	α 1322.56 a B	β 649.96 b C
	50	β 746.00 B	β 1738.21 b A	β 875.43 b B	α 897.47 a B
	100	α 1114.66 BC	α 1987.66 b A	α 1332.11 b B	α 966.66 a C
H					
Without <i>Azospirillum</i>	0	18.00 a C	47.00 A	β 43.5 A	β 29.77 B
	50	18.00 D	51.22 b A	αβ 46.5 B	αβ 31.55 C
	100	19.89 C	48.66 b A	α 49.5 A	α 35.89 B
With <i>Azospirillum</i>	0	β 13.33 b D	π 50.44 A	β 41.83 B	32.33 C
	50	β 16.88 D	β 59.5 a A	β 43.16 B	31.00 C
	100	α 22.11 D	α 66.67 a A	α 48.66 B	34.11 C
LSR					
Grazing management		N doses (kg of N ha <sup>-1</sup> )		Inoculation	
CG	1.51 a	0	1.16	With <i>Azospirillum</i>	1.12
30	0.77 b	50	0.95	Without <i>Azospirillum</i>	1.02
20	0.65 b	100	1.11		
10	1.37 a				

\*Different uppercase letters in the row and different lowercase letters in the column indicate difference between treatments at 5% probability level by the Duncan test. \*\*Greek letters in the column represent the difference between nitrogen doses for each level of inoculation and grazing management.

**Table 5.** Dry mass of green leaf blades (DMGL, kg ha<sup>-1</sup>), dry mass of stems (DMS, kg ha<sup>-1</sup>), dry mass of senescent material (DMSM, kg ha<sup>-1</sup>), dry mass of inflorescence (DMI, kg ha<sup>-1</sup>), total dry mass (TDM), leaf/stem ratio (LSR), and height (H, cm) of a pasture of black oat and ryegrass under different managements in a crop-livestock integration system (entry of the third grazing, year 2013).

		DMGL				
Inoculation	N doses (kg of N ha <sup>-1</sup> )	Grazing Management				
		CG	30 cm	20 cm	10 cm	No grazing
Without <i>Azospirillum</i>	0	89.33 B	**β 61.77 b B	*α 207.50 A	αβ 140.53 AB	β 66.50 B
	50	58.66 B	β 117.87 B	β 92.22 B	α 213.27 A	αβ 123.75 B
	100	67.63 b C	α 206.67 A	α 221.78 A	β 119.13 b BC	α 151.10 b AB
With <i>Azospirillum</i>	0	β 34.00 C	155.47 a AB	194.47 A	π 68.02 C	β 91.94 BC
	50	β 75.18 C	190.72 B	150.03 CB	β 282.32 A	β 147.88 CB
	100	α 150.62 a B	171.44 B	213.20 B	α 436.20 a A	α 431.47 a A
		DMS				
Without <i>Azospirillum</i>	0	158.00 D	β 916.75 b B	640.36 BC	αβ 379.39 CD	β 1521.38 A
	50	20.00 C	β 625.86 B	817.96 B	α 511.18 B	β 1794.50 b A
	100	51.63 D	α 1373.44 B	725.44 C	α 385.76 CD	α 2840.52 A
With <i>Azospirillum</i>	0	30.00 C	747.38 a B	526.90 B	435.55 BC	β 1550.11 A
	50	178.10 C	975.74 B	849.44 B	331.78 C	α 3016.18 a A
	100	360.45 C	1036.33 B	831.02 B	626.23 BC	α 2817.10 A
		DMSM				
Without <i>Azospirillum</i>	0	142.66 B	180.84 B	β 72.48 b B	109.87 B	β 406.46 A
	50	156.00 CB	212.14 B	β 86.43 b CD	16.79 D	β 413.84 a A
	100	71.09 b C	116.66 b C	α 384.78 B	109.99 C	α 674.00 A
With <i>Azospirillum</i>	0	β 108 C	256.46 B	β 244.96 a B	32.51 C	α 498.34 A
	50	β 167.54 B	259.29 AB	αβ 328.22 a A	16.17 C	β 281.01 b A
	100	α 471.47 a A	248.78 a C	α 368.11 B	22.26 D	α 567.44 A
		DMI				
Without <i>Azospirillum</i>	0	14.00 D	548.41 B	β 321.81 C	157.94 CD	β 965.89 A
	50	4.00 D	487.16 B	β 330.38 CB	215.11 C	β 1048.44 b A
	100	13.82 C	879.25 B	α 723.07 B	173.90 C	α 1133.11 b A
With <i>Azospirillum</i>	0	β 14.00 C	601.34 A	β 320.93 B	250.07 B	α 797.95 A
	50	β 53.18 C	506.24 B	αβ 219.67 C	149.25 C	β 1542.92 a A
	100	α 117.27 C	684.87 B	α 702.93 B	184.05 C	α 2490.00 a A
		TDM				
Without <i>Azospirillum</i>	0	404.00 D	β 1707.78 B	β 1242.16 C	687.41 D	π 2960.24 A
	50	238.66 D	β 1443.05 b B	β 1327.00 B	822.00 C	β 3380.54 b A
	100	204.18 b E	α 2576.03 a B	α 2055.08 C	788.79 b D	α 5660.34 b A
With <i>Azospirillum</i>	0	β 186.00 D	β 1472.99 B	β 1287.28 B	β 833.62 C	π 2938.36 A
	50	β 474.00 E	α 1932.00 a B	β 1547.38 C	β 779.54 D	β 4988.00 a A
	100	α 1099.82 a C	α 1898.00 b B	α 2115.26 B	α 1268.74 a C	α 6306.01 a A
		LSR				
Without <i>Azospirillum</i>	0	π 0.58 b A	0.06 A	0.32 A	0.36 A	0.04 A
	50	α 3.51 a A	0.20 b B	0.11 B	0.45 B	0.07 B
	100	β 1.37 a A	0.15 B	0.31 B	0.32 B	0.06 B
With <i>Azospirillum</i>	0	α 1.16 a A	0.22 B	0.37 B	β 0.16 B	0.06 B
	50	β 0.41 b AB	0.19 a B	0.18 B	α 0.88 A	0.05 B
	100	β 0.55 b A	0.16 A	0.27 A	α 0.69 A	0.15 A
		H				
Without <i>Azospirillum</i>	0	αβ 23.77 D	β 32.83 b B	αβ 38.33 b A	β 28.00 b C	
	50	β 23.33 b C	α 46.83 a A	β 36.00 B	β 24.33 b C	
	100	α 27.66 D	α 47.89 a A	α 40.89 a B	α 36.55 C	
With <i>Azospirillum</i>	0	27.55 C	42.5 a A	α 43.66 a A	33.00 a B	
	50	27.50 a C	41.16 b A	β 36.55 B	30.89 a C	
	100	30.33 B	40.50 b A	α 43.55 b A	32.55 B	

\*Different uppercase letters in the row and different lowercase letters in the column indicate difference between treatments at 5% probability level by the Duncan test. \*\*Greek letters in the column represent the difference between nitrogen doses for each level of inoculation and grazing management.



There was no triple interaction for the pasture management heights, at the exit of the animals from the first grazing (Data not presented). These heights were different only according to grazing management, but the values were below those desired. This occurred because of the delay of the pasture, under corn residues, in reaching a forage mass close to 1,500 kg of DM ha<sup>-1</sup>. For the pasture heights at the exit of the animals in the second grazing, there was triple interaction and the heights were closer to the desired heights.

It was verified that the differences of DMGL and residual TDM were more pronounced between the grazing management heights (10, 20 and 30 cm) when 50 kg of N ha<sup>-1</sup> were used, since the DMGL and TDM of the pasture before the entry of the grazing animals were higher due to nitrogen fertilization, allowing greater differences to be

observed at different heights after grazing (Data not presented), which was also verified for DMGL and residual TDM after the second grazing (Data not presented), when more significant differences between the grazing management heights were obtained when the pasture received fertilization of 100 kg ha<sup>-1</sup> of N for residual DMGL and fertilization of 50 and 100 kg ha<sup>-1</sup> of N for TDM.

There was a triple interaction for the DMP of the pasture under corn residues (Table 6). As for grazing management, there was higher pasture production in the treatment without grazing and in the treatment 30 cm. It is possible to infer that the increase in pasture height results in an increase in forage mass. As a consequence, a larger leaf area is maintained in these treatments, increasing the ability of plants to intercept radiation and accumulate biomass.

**Table 6.** Total dry mass production (DMP, kg ha<sup>-1</sup>) in black oat and ryegrass pasture under corn residues in a crop-livestock integration system (Year - 2013).

Total dry mass production (DMP)					
Inoculation	N doses (kg of N ha <sup>-1</sup> )	Grazing Management			
		30 cm	20 cm	10 cm	No grazing
Without <i>Azospirillum</i>	0	3089.46 A	**β 1917.46 B	2469.45 AB	β 2960.24 A
	50	3117.22 A	β 2291.11 B	2098.34 b B	β 3380.54 b A
	100	3578.62 B	α 3557.39 B	2194.71 b C	α 5660.34 A
With <i>Azospirillum</i>	0	αβ 3205.81 A	*π 1680.50 B	π 2072.46 B	π 2938.36 A
	50	β 2633.08 B	β 2340.66 B	β 2764.58 a B	β 4988.00 a A
	100	α 3404.47 B	α 3764.06 B	α 3455.98 a B	α 6306.01 A

\*Different uppercase letters in the row and different lowercase letters in the column indicate difference between treatments at 5% probability level by the Duncan test. \*\*Greek letters in the column represent the difference between nitrogen doses for each level of inoculation and grazing management.

N doses had a distinct effect as a function of inoculation and grazing management. Without inoculation, at heights of 10 and 30 cm, nitrogen application did not increase the total pasture production, and in grazing managements at 20 cm and without grazing, the pasture produced more when 100 kg ha<sup>-1</sup> of N were added. When considering *Brachiaria plantaginea*, the use of *A. brasilense* increased nitrogen accumulation in plant biomass in such a way to be equivalent to 40 kg of N ha<sup>-1</sup>, which contributes to the recovery of degraded pastures and the addition of carbon in the soil (HUNGRIA; NOGUEIRA; ARAUJO, 2016).

Corn is a crop that has high demand for nitrogen and, after harvesting, little of this nitrogen remains in the soil. The C/N ratio of corn is high, causing nitrogen release to be slow, which makes

this nutrient less available at the beginning of the subsequent crop development (SILVA et al., 2009). Thus, the association of inoculation with 100 kg ha<sup>-1</sup> of N may have met the need for this nutrient, resulting in significant differences. Schaefer et al. (2019) and Brum et al. (2016) attributed the increase in grain yield, grain mass and shoot dry mass to the use of *A. brasilense*.

For inoculation, the total pasture production did not respond or responded positively according to grazing management and N doses. At height of 10 cm with 50 and 100 kg of N ha<sup>-1</sup> and in the absence of grazing (NG) with 50 kg of N ha<sup>-1</sup>, the pasture produced more biomass when the seeds were inoculated. A probable explanation is the fact that diazotrophic bacteria do not contribute with sufficient amounts of fixed N to ensure maximum

yield in non-legume plants (BALDANI; BALDANI, 2005). Furthermore, the absence of N may represent a high cost of the association for the plant, under a condition of low N level in the soil.

The production of growth-promoting substances and an increase in the rate of nutrient absorption due to inoculation with *Azospirillum* may explain the increase in yield. After inoculation with *Azospirillum*, there is a change in the morphology of plant roots, which has been attributed to the production of plant growth-regulating substances. There is also an increase in the root surface due to the increase in the number of lateral roots and root hairs. This results in greater absorption of water and nutrients, which is the main factor for increasing plant growth.

## CONCLUSIONS

The biomass production of black oat and ryegrass pasture increased with nitrogen application and inoculation of *Azospirillum brasilense*. The proportions of structural components varied as a function of different grazing managements, nitrogen doses and inoculation with *Azospirillum brasilense*. *Azospirillum brasilense* can be used as substitute of nitrogen fertilization in black oat-ryegrass intercropping in a crop-livestock integration system.

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