Fermentation profile of signal grass silages intercropped with calopo or fertilized with nitrogen and treated with microbial inoculant

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Abstract

Tropical grasses have characteristics that allow the occurrence of undesirable fermentations resulting in low quality silage. Inoculants can improve silage quality. Fermentation profile and microbial population of signal grass silages under four management (M), with and without microbial inoculant, were evaluated. The experiment was carried out in a 4 × 2 factorial scheme, with silages of signal grass managed without N application (control, 0N), fertilized with 50 kg ha^{-1} of N (50N), fertilized with 100 kg ha^{-1} of N (100N), or signal grass intercropped with calopo (*Calopogonium mucunoides* D.) (LEG), with inoculant (I) and without inoculant (WI), in a randomized block design, with two replications of area and two replications of treatment per block. Analysis of pH, organic acids, ethanol, ammonia nitrogen, and microbial populations were performed in the silages. The use of inoculant reduced the acetic acid concentration in the 100N treatment silages (20.1 to 12.8 g kg^{-1} of DM). The lowest concentration of butyric acid (BA) (2.33 g kg^{-1} DM) was observed in signal grass silage fertilized with 100 kg of N, without inoculant. In inoculated silages, the treatments 50N and LEG provided lower concentrations of BA in relation to the control. Silage from the uninoculated 50N treatment had the lowest concentration of N-NH_3 in relation to the other silages. The lactic acid bacteria (LAB) population was not affected by the factors studied. The inoculant provided a reduction in the population of enterobacteria. No mold and yeast populations were detected in the silages investigated. Signal grass silage fertilized with N or intercropped with calopo, with microbial inoculant, had better fermentative characteristics and could be an option for strategic management of pastures in tropical areas.

Introduction

Species of the genus *Urochloa* stand out for their adaptability to acidic and low fertility soils, in addition to their high productivity of dry biomass, which shows marked seasonality. Grass silage produced in the rainy season could be a reasonable alternative to supplement the low productivity of pastures in the dry season (Negrão et al., 2016; Li et al., 2019). However, the high moisture content and the low content of water-soluble carbohydrates are limiting factors for the satisfactory fermentation process of perennial grass silage, which makes the rapid decline of pH difficult (Negrão et al., 2016; Bernardes et al., 2018). Thus, the addition of inoculants composed of homofermentative bacteria aims to add beneficial microorganisms to dominate the fermentation, inhibit the growth of undesirable microorganisms, such as enterobacteria and clostridia, form final products that stimulate consumption and animal production, and/or improve dry matter recovery from preserved forage (Kung Jr., 2018). Sufficient N supply maximizes productivity of forages, as well as increases the proportion of more nutritious leaves (Berça et al., 2021). Nitrogen can be supplied through mineral and organic fertilizers and biological fixation, which occurs through the symbiosis between N-fixing bacteria and the legume root system (Feitoza et al., 2018). Therefore, the objective of this study was to evaluate the effects of signal grass silage without N fertilizer, fertilized with two doses of N or intercropped with calopo, with and without microbial inoculant. Thus, we hypothesized that N via mineral fertilization or biological N fixation, in combination with a microbial inoculant, would produce signal grass silage with a better fermentative profile.

Methods

The experiment was carried out at the Unit of Teaching, Research and Extension in Forages of the Animal Science Department at the Federal University of Viçosa, *Campus* of Viçosa (20º45’20’’S, 42º52’40’’W), Brazil. The fermentative profile of signal grass silage intercropped with calopo (sown in December 2017) or fertilized with two doses of N (on 6 March 2018), using a microbial inoculant was evaluated. The experimental area consisted of 16 paddocks, with approximately 1,300 m^2 each. Grazing management in the rainy season was carried out with Nelore cows and pre- and post-grazing heights were 25 and 15 cm, respectively. After N fertilization, the pastures were deferred (for 70 days), grazed (for 43 days) and remained at rest during the dry period, after which the forage was ensiled (on 14 November 2018). The experiment was carried out in a 4 × 2 factorial scheme, with signal grass managed without N application (control, 0N), fertilized with 50 kg ha^{-1} of N (50N), fertilized with 100 kg ha^{-1} of N (100N) or intercropped with calopo (LEG), with inoculant (I) and without inoculant (WI), in a randomized block design, with two replications of area and two replications of treatment per block. The forage was harvested at 5 cm from the ground and chopped in an average particle size of 1.5 cm. Two piles of 8 kg of forage were prepared from each paddock, totaling 32 piles, and the inoculant (Sil-All 4x4) was applied to half of them. Before ensilage, the water-soluble carbohydrates were analyzed (Nelson, 1944). The forage was ensiled in plastic buckets with a capacity of 12 L, at a density of 600 kg of FM/m^3, which were sealed and stored for 60 days. After opening the silos, analysis of pH, organic acid and ethanol (Siegfried et al.,1984), and ammonia N (Okuda et al., 1965) were performed in the silages. The microbial populations were counted in the fresh material and in the silages.

Results and Discussion

The average pH of the silages was 4.9, which is above the value considered adequate for tropical grasses (4.3 - 4.7), according to Kung Jr. et al. (2018). In the present study, the inoculant did not affect the pH of the silages, as also observed by Rigueira et al. (2017). The high pH values can be attributed to the low lactic acid content (LA) (Table 1) observed in the silage, since, among the organic acids, LA is primarily responsible for lowering pH and improving silage fermentation (Kung Jr., 2018; Soundharrajan et al., 2021), while acetic and butyric acids, and NH_3-N, make the silage less acidic, and, consequently, increase the pH (Rabelo et al., 2017; Kung Jr. et al., 2018). Despite the low concentrations of dry matter and the water-soluble carbohydrates (22.7 g kg^{-1} of DM), before ensiling, and the high pH value of the silages, the concentrations of butyric acid (average value of 5.69 g kg^{-1} of DM) were within an adequate range (<0.5 - 1.0%), suggested by Kung Jr. et al. (2018) for tropical grass silages. In the uninoculated silages, the 0N treatment had greater concentration of butyric acid, compared to the 50N and LEG treatments. The use of inoculant reduced BA concentrations, in treatments 50N and LEG, and the concentrations of ethanol (ET), in treatments 0N, 50N and LEG, in relation to uninoculated silages (Table 1). The inoculant did not affect the LAB population in the silages, which can be attributed to the low concentrations of water-soluble carbohydrates observed in signal grass before ensilage, since the rapid growth and fermentation dominated by LAB in the inoculant depends on the amount of substrate present in the forage (Muck, 2013). No mold and yeast populations were detected in the silages ($p>0.05$). Reductions in enterobacteria populations in all inoculated silages and reduction of BA concentrations, in treatments 50N and LEG, and of AA, in the treatment 100N, demonstrated the suppressive effect of the inoculant on the growth of spoilage microorganisms, which improved the quality of the silage during the storage period in the silo.

Table 1 - Means of fermentation profile (g kg^{-1} DM) and microbial population (log cfu g^{-1} of forage) of signal grass silages under different managements, with and without microbial inoculant

Inoculant[1]	Management[2]	Item[3]							
		pH	LA	AA	BA	ET	NH_3-N (%TN)	LAB	ENT
WI	0N	4.90	21.5	13.5^{bA}	7.90^{aA}	10.38^{aA}	13.7^{bcA}	7.63	3.17
	50N	4.85	26.9	14.8^{abA}	5.97^{aA}	9.41^{abA}	12.5^{cB}	8.08	3.91
	100N	4.77	20.9	20.0^{aA}	2.33^{bB}	6.47^{bA}	18.1^{aA}	7.98	ND
	LEG	4.90	24.3	17.8^{abA}	7.60^{aA}	10.75^{aA}	16.0^{abA}	7.54	3.04
I	0N	4.91	27.9	13.4^{bA}	7.77^{aA}	7.85^{aB}	13.4^{bA}	8.00	2.58
	50N	4.86	22.9	15.5^{abA}	2.77^{bB}	4.88^{bB}	16.7^{aA}	7.88	2.84
	100N	4.95	24.8	12.8^{bB}	4.87^{abA}	7.65^{abA}	16.9^{aA}	7.62	3.41
	LEG	4.76	27.6	20.3^{aA}	2.55^{bB}	7.50^{abB}	16.9^{aA}	8.03	2.98
SEM		0.0646	1.331	1.0998	0.6616	0.5945	0.4499	0.127	0.204
General average for inoculant (I)									
WI		4.85	23.10	16.46	5.94	9.25	15.06	7.81	3.37^{a}
I		4.87	26.00	15.33	4.48	6.97	15.96	7.87	2.91^{b}
General average for management (M)									
0N		4.90	24.69	13.44	7.83	9.11	13.51	7.81	2.82
50N		4.86	24.90	15.07	4.37	7.14	14.56	7.99	3.30
100N		4.86	22.57	16.96	3.59	7.06	17.50	7.80	3.41
LEG		4.83	25.93	19.08	5.07	9.12	16.46	7.79	3.01
P-value									
I		0.8384	0.1762	0.3672	0.0188	0.0005	0.1319	0.613	0.007
M		0.9199	0.5868	0.0130	0.0004	0.0127	0.0002	0.780	0.102
M × I		0.5875	0.1563	0.0275	0.0008	0.0096	0.0215	0.166	0.127

[1]WI= without inoculant; I= with inoculant. SEM= mean standard error. M= management and M × I= interaction management vs inoculant
[2]0N= without nitrogen fertilization; 50N= 50 kg ha^{-1} per year of N; 100N= 100 kg ha^{-1} per year of N e LEG= signal grass and calopo intercropping
[3]LA= lactic acid; AA= acetic acid; BA= butiric acid; ET= ethanol; NH_3-N= ammonia nitrogen; LAB= lactic acid bacteria; ENT= enterobacteria
Means followed by the same lowercase letter in the column, management split in each inoculant, did not differ (p>0.05) from each other by Tukey test; and capital letters in the column, inoculant splitting in each management did not differ (p>0.05) from each other by F test.

Conclusions

Signal grass silage fertilized with N or intercropped with calopo, with microbial inoculant, had better fermentative characteristics and can be an option for strategic management of pastures in tropical areas.

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