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Signal Grass Deferred Pastures Fertilized with Nitrogen or Intercropped with Calopo

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Abstract: This study aimed to evaluate the accumulation, structural characteristics, and chemical composition of deferred signal-grass pastures that were subjected to four treatments: without nitrogen fertilization, intercropped with calopo (*Calopogonium mucunoides*), and fertilized with urea N (50 kg ha^{-1} and 100 kg ha^{-1}) for 2 years. The design was in randomized blocks, with two blocks and two repetitions of each treatment per block. There were effects of the interaction between treatment and year on green dry mass, forage accumulation, density of vegetative tillers, and crude protein content (simulated grazing). The effects of the treatments on the height, falling index, green dry mass/dead dry mass ratio, number of dead, live and total tillers, and crude protein content (direct cutting) were also observed. Signal-grass–calopo-intercropping ensured adequate mass and forage accumulation and crude protein content equivalent to those of fertilized pastures. In addition, the intercropped pasture showed a higher percentage of leaves and a higher crude protein content compared with those for the other treatments (simulated grazing). The green dry mass/dead dry mass ratio was highest in the intercropped pasture and was equivalent to only that of the pasture fertilized with a low dose of nitrogen. Therefore, signal-grass–calopo-intercropping may be recommended for deferment.

Keywords: chemical composition; falling index; forage accumulation; morphological characteristics; structural characteristics

1. Introduction

Seasonal variations have a significant impact on forage production, especially during dry periods, in tropical regions and when climatic limitations compromise the development and chemical composition of pastures and affect animal performance. Deferment is an option to minimize this problem and consists of postponing or delaying grazing to achieve a specific management objective [1]. The proper choice of plant species to be used in the deferment is essential, and characteristics, such as good growth potential in the beginning of autumn and a high leaf/stem ratio, are sought after to provide improved nutritional value to the accumulated forage. Therefore, species of the genus *Urochloa* have been recommended for deferment [2,3].

Deferment management strategies can influence the forage mass, chemical composition, and structural characteristics of pastures, which affects animal production [3–5]. Nitrogen fertilization before pasture deferment can improve the forage mass and its chemical composition. In a study of deferred signal grass [5], the authors observed an increase in forage mass and an improvement in structural characteristics as the nitrogen dose increased, while increases in mass and allowance forage were identified [6]. However, nitrogen fertilization considerably increases the costs of production, as the synthesis of fertilizers requires sources of fossil fuel, and most nitrogen inputs are currently imported [7].

Therefore, nitrogen from biological fixation by legumes constitutes an important contribution to agricultural and pasture systems, as it benefits the environment and supports resource efficiency, although it becomes available slowly [8,9].

Among many legumes used in intercropping systems, calopo (*Calopogonium mucunoides* Desv.) stands out because of its ability to adapt to conditions of high acidity and low soil fertility, in addition to its high tolerance to Al [10]. In reference [11], the researchers observed that calopo intercropped with *Urochloa decumbens* cv. Basilisk (Stapf) R.D. Webster) recycles 84 kg ha^{-1} year^{-1} of N during continuous stocking in the rainy season, resulting in increased nitrogen availability for grasses and crude protein (CP) content in forage.

The use of a nitrogen fertilizer has been evaluated in several deferment studies; however, studies with grass–legume-intercropping systems under deferment are not available. Therefore, the aim of this study was to evaluate the total mass, accumulation, structural characteristics, and chemical composition of signal-grass pastures under deferment, intercropping with calopo or fertilization with nitrogen for two years. We hypothesized that signal grass–calopo intercropping or signal-grass pastures fertilized with nitrogen under deferment would result in the improved mass, accumulation, structural characteristics, and chemical composition of forage during the dry season.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted in 2017 and 2018 at the Teaching, Research, and Extension Unit of the Animal Science Department of the Universidade Federal de Viçosa (UFV), located in Viçosa, Minas Gerais State, Brazil (20°45″ S, 52°55″ W; elevation of 649 m a.s.l.). Accumulated precipitation and average temperature data during the experimental period were obtained from the Viçosa Meteorological Station and were 846 mm and 21.8 °C, respectively, in 2017 and 1133 mm and 22.2 °C, respectively, in 2018 (Figure 1).
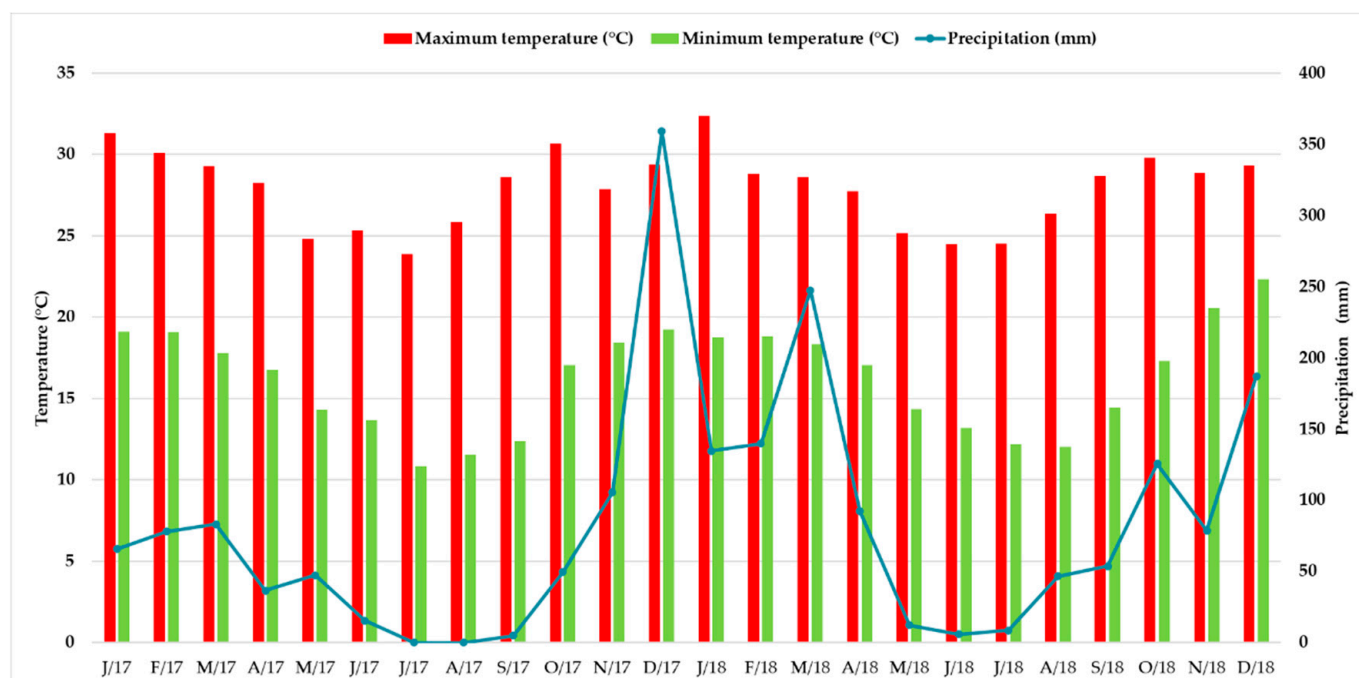
Figure 1. Weather data from January (J) 2017 to December (D) 2018, in Viçosa, Minas Gerais, Brazil. Source: INMET, 2019.

The soil was classified as red–yellow dystrophic latosol [12]. Soil samples were collected with a probe from 0 to 10 cm layers in August 2016, and the chemical composition was analyzed (Table 1). Given the results, soil fertilization was carried out following the recommendations of [13]. There was no need for soil correction or potassium fertilization.

In January 2017, 70 kg ha^{-1} P_2O_5 was applied to all paddocks using simple superphosphate as the source.

Table 1. Chemical analysis of soil samples collected in the 0–10 cm layers in the experimental area.

pH	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}	H + Al	EB	(t)	(T)	V	m	OM	P-rem
H$_2$O	mg dm^{-3}					cmol$_c$ dm^{-3}				%		dag kg^{-1}	mg L^{-1}
5.51	7.4	177	2.61	0.99	0.1	6.9	4.05	4.15	10.95	37	2.4	3.33	29.6

Abbreviations: pH, in water, 1:2.5; EB, sum of exchangeable bases; t, capacity of effective cation exchange; T, cation exchange capacity at pH 7.0; V, base saturation index; m, aluminum saturation; OM, organic matter; P-rem, remaining phosphorus.

The experiment was conducted in a signal-grass pasture containing 16 paddocks (~1300 m^2 each) with a water trough and a mineral mixture feeder in each paddock. The pastures were established ~23 years ago and have been properly managed and fertilized for other studies. At the beginning of this experiment, the signal grass had good soil coverage.

In January 2017, 50 cm spaced lines of signal grass were desiccated with glyphosate (200 mL/20 L water) using a costal pump in the pastures, in which calopo was sown. After desiccating the signal grass, the calopo was sown by animal traction using a seeder with cutting discs at a sowing rate of 4.8 kg ha^{-1} of viable pure seeds. In December 2017, due to the low density of the calopo plants, reseeding was carried out by direct broadcasting at the same sowing rate. Before reseeding, the pasture height was lowered by cow grazing, and the animals were again introduced into the paddocks for a few hours after reseeding to promote the contact between the seeds and soil.

2.2. Treatments and Experimental Design

Deferred signal-grass pastures were subjected to the following treatments: B (without nitrogen fertilization as a control), BC (intercropped with calopo), B50 (fertilized with a low dose of nitrogen of 50 kg ha^{-1}), or B100 (fertilized with a high dose of nitrogen of 100 kg ha^{-1}). The experiment was carried out under a two-way factorial assay in a randomized complete block design. The four treatments were distributed in two randomized blocks with two repetitions of the treatment per block in two experimental years (2017 and 2018).

2.3. Grazing Management

During the predeferral periods, grazing with Nellore heifers was carried out under intermittent stocking conditions with variable stocking rates. The pre- and postgrazing heights were 25 cm and 15 cm, respectively, in both experimental years. The pastures were deferred on 7 March in 2017 and 2018 and remained closed to grazing (without animals) until 13 June 2017 and until 15 May 2018, respectively, when the animals returned to the paddocks. In 2017 and 2018, the deferral periods for the pastures were 98 days and 70 days, respectively, and the periods during which the animals remained grazing after the deferral period were 58 days and 43 days, respectively. The protocol for the research project has been approved by the Ethics Commission on the use of farm animals of UFV (protocol no. 020/2018).

2.4. Mass, Forage Accumulation Rate (FAR), and Structural Characteristics and Chemical Composition of Pastures

Prior to the start of the deferral period, the postgrazing residue heights were measured at 20 points per experimental unit with a graduated ruler, and four samples of forage were collected (2 cm above the ground level) at random in each paddock using frames (0.50 m × 0.50 m). Subsequently, nitrogen fertilization was carried out in the experimental units that received these treatments, and the pastures of some paddocks were fertilized with nitrogen doses of 50 kg ha^{-1} (low dose) and 100 kg ha^{-1} (high dose) using urea as a source.

The forage samples were taken to the laboratory where they were weighed and separated into a green material (G) and a senescent and dead material (DE), separately weighed and dried in an oven with forced air circulation at 55 °C until reaching a constant mass. Subsequently, the samples were ground in a knife mill with a 1 mm sieve, and subsamples were dried in an oven at 105 °C to determine the dry matter (method 930.15; [14]). In addition, analyses of the CP content (method 990.03; [14]) and the insoluble neutral detergent fiber corrected for ash and protein (NDFap) contents [15,16] were performed.

The falling index (FI) was obtained by dividing the extended tiller height (ETH) by sward height (SH), according to the methodology proposed by [17]. Four samples of forage were also collected (2 cm above the ground) at random in each paddock using a frame (0.5 m × 0.5 m) to quantify the total population density of tillers. Live tillers (LTs) with inflorescences were classified as reproductive (RT), whereas LTs without inflorescences were classified as vegetative (VT) and those of which the stems were necrotic were classified as dead tillers (DTs). The sum of the number of vegetative and reproductive tillers (tillers/m^2) corresponded to LTs, and that of LTs and DTs corresponded to total tillers (TTs).

After counting the tillers, each sample was weighed and divided into two subsamples. The first subsample was separated into green leaf blades (L), green stems (S), and DEs. The L and S portions with either yellowing or necrotizing characteristics were incorporated into the DE fraction. After separation, the components were weighed and dried in an oven with forced air circulation, the samples were ground, and the subsamples were dried in an oven to determine the dry matter content (method 930.15; [14]). From these data, leaf dry mass (LDM), stem dry mass (SDM), dead dry mass (DEM), green dry mass (GDM), and total dry mass (TDM) were obtained, in addition to the relative proportion (%) of each morphological component in the available forage. The GDM/DEM and L/S ratios were also estimated. The second subsample was subsequently analyzed for chemical composition, as previously described.

Forage accumulation (FA) was calculated as the difference between the forage mass harvested after pasture deferment and the forage mass before deferment for each experimental year. The FAR was estimated as the ratio between the FA and the number of days of deferment for each experimental year.

On the first, second, and third days of grazing, forage samples were collected in each paddock, simulating the forage that was removed by heifers, according to the methodology described by [18]. Each sample was separated into the L, S, and DE components and subsequently dried and weighed according to the same previously described criteria. Subsequently, all morphological components were joined together to recompose their original samples, which were analyzed for chemical composition, as previously described.

2.5. Statistical Analysis

For each characteristic, an analysis of variance (ANOVA) was performed under a two-way factorial assay in a randomized complete block design. The treatments (B, BC, B50, and B100) and the experimental year (2017 and 2018), as well as their interaction, were tested as fixed effects. The analysis of the experimental data was performed using the System for Statistical Analysis software (SAEG v. 8.1; [19]). The ANOVA assumptions given by the normality of residuals and homogeneity of variances were evaluated using the Shapiro–Wilk and O'Neill-Matthews tests, respectively. The comparisons between the means of treatments were performed through the Tukey's test, considering a 5% probability of type I error.

3. Results

The SH was affected ($p = 0.0043$) by treatment (Table 2) and by experimental year ($p = 0.0031$), and the SH was greater in 2018 (58.8 cm) than in 2017 (50.6 cm). The BC treatment showed a higher SH than treatment B, whereas the other treatments presented intermediate values. The highest ($p = 0.0241$) FI was also observed in the intercropped

pasture compared with that of the signal-grass pasture without a nitrogen fertilizer, with intermediate values in the other treatments (Table 2).

Table 2. Averages of sward heights (SHs) and falling indices (FIs) for the different treatments at the end of the deferral period.

Treatment	SH (cm)	FI
B	47.6B	1.5B
BC	62.1A	2.0A
B50	55.1AB	1.8AB
B100	53.9AB	1.9AB
Mean standard error	1.63	0.007

Means followed by at least the same letters in the column do not differ at the level of a 5% probability by the Tukey's test. Abbreviations: B, signal grass which was not fertilized with nitrogen (control); BC, signal grass intercropped with calopo; B50, signal grass fertilized with a dose of 50 kg ha^{-1} N; B100, signal grass fertilized with a dose of 100 kg ha^{-1} N.

The LDM did not differ ($p = 0.7358$) between treatments and experimental years, with an average of 833.52 kg ha^{-1}. On the other hand, the TDM was influenced by the experimental year ($p = 0.0220$), with a higher value detected in 2018 (6622.2 kg ha^{-1}) than in 2017 (5688.9 kg ha^{-1}). In addition, the GDM was influenced by the interaction between treatment and experimental year ($p = 0.0307$; Table 3). In 2017, the GDM was higher for the BC treatment than for the B treatment. However, in 2018, there was no difference in GDM between treatments. When comparing years within each treatment, there was an increase in GDM from 2017 to 2018 for the B100 and B (control) treatments, while the GDM decreased for the BC treatment and remained constant for the B50 treatment.

Table 3. Averages of green dry mass (GDM), stem dry mass (SDM), and forage accumulation (FA) for the different treatments and experimental years at the end of the deferral period.

Treatment	2017	2018	Mean Standard Error
GDM (kg ha^{-1} DM)			
B	3140.6Bb	4625.5Aa	
BC	5110.4Aa	3793.8Ab	202.02
B50	4253.9ABa	5068.2Aa	
B100	3593.8ABb	5204.9Aa	
SDM (kg ha^{-1} DM)			
B	884.3Ba	1346.6Aa	
BC	1673.0Aa	1169.9Ab	201.30
B50	1332.3ABa	1444.1Aa	
B100	1072.5ABb	1584.7Aa	
FA (kg ha^{-1} DM)			
B	1592.6Bb	3556.3Aa	
BC	3992.6Aa	1861.2Ab	246.45
B50	2919.1ABa	3858.7Aa	
B100	2279.8AB	3164.3Aa	

Means followed by at least one capital letter in the column and lower case in the line do not differ at the level of 5% probability by the Tukey's test. Abbreviations: B, signal grass which was not fertilized with nitrogen (control); BC, signal grass intercropped with calopo; B50, signal grass fertilized with a dose of 50 kg ha^{-1} N; B100, signal grass fertilized with a dose of 100 kg ha^{-1} N.

Furthermore, the SDM was also affected ($p = 0.0703$) by the interaction between treatment and experimental year (Table 3). In 2017, the BC treatment had a higher SDM than the pasture without nitrogen fertilization (B), with intermediate values for the B50 and B100 treatments. However, in 2018, there was no difference in this variable among treatments. When we compared the experimental year within each treatment, it was observed that the SDM decreased from 2017 to 2018 for the BC treatment; however, the SDM increased during the same period for the B100 treatment.

The FA was also influenced ($p = 0.0156$) by the interaction between treatment and experimental year (Table 3). In 2017, there was a greater accumulation of forage for the BC treatment than for the B treatment. In contrast, in 2018, this variable showed similar values among treatments. When comparing years within each treatment, an increase in FA was observed from 2017 to 2018 for the B treatment, while the FA decreased for the BC treatment and remained unchanged in both years for the other two treatments. The FAR was affected only by experimental year ($p = 0.0001$), with higher values in 2018 than in 2017 (50.6 kg ha^{-1} day^{-1} DM vs. 27.5 kg ha^{-1} day^{-1} DM; mean standard error, SE = 3.19).

Although the DEM was affected ($p = 0.0035$) by all treatments, it was noted that the intercropped pasture had lower DEM values than those of the other treatments (Table 4). There was also an effect ($p = 0.0137$) of treatment on the GDM/DEM ratio, which was higher for the BC treatment than for the B and B100 treatments (Table 4).

Table 4. Averages of dead dry mass (DEM), the green dry mass/dead dry mass ratio (GDM/DEM), and percentages of stem (%S) and dead material (%DE) for the different treatments at the end of the deferral.

Treatment	DEM (kg ha^{-1} DM)	GDM/DEM	Percentages of Stem (%S)	Percentages of Dead Material (%DE)
B	999.5A	4.1B	37.9B	34.9A
BC	517.6B	9.3A	50.9A	20.4B
B50	904.2A	5.6AB	43.8AB	28.8AB
B100	978.5A	5.2B	40.6B	31.2A
Mean standard error	91.2	1.1	1.43	1.62

Means followed by at least the same letters in the column do not differ at the level of a 5% probability by the Tukey's test. Abbreviations: B, signal grass which was not fertilized with nitrogen (control); BC, signal grass intercropped with calopo; B50, signal grass fertilized with a dose of 50 kg ha^{-1} N; B100, signal grass fertilized with a dose of 100 kg ha^{-1} N.

The L percentage ($p = 0.9187$) and the L/S ratio ($p = 0.2091$) values were not affected by either treatment or experimental year, with averages of 27.85% and 0.66, respectively. The S percentage ($p = 0.0044$) and the DE ($p = 0.0075$) content were affected by treatment (Table 4). The BC treatment had a higher S percentage and lower DE values than the B and B100 treatments.

The number of RTs was not affected ($p = 0.1983$) by the factors studied, with an average of 292 tillers/m^2. The number of VTs was affected ($p = 0.0137$) by the interaction between treatment and experimental year (Table 5). When comparing the treatments within each year, it was noted that in 2017, the highest dose of the nitrogen fertilizer contributed to a greater number of VTs compared with that of the intercropped pasture. However, in 2018, all the other treatments provided higher numbers of VTs than those of the intercropped pasture. Moreover, when comparing years within each treatment, it was observed that the number of VTs increased from 2017 to 2018 for only the B and B50 treatments.

Table 5. Averages of the number of vegetative tillers (VTs) for different treatments and experimental years at the end of the deferral period.

Treatment	VT (tillers/m^2)	
	2017	2018
B	666ABb	922Aa
BC	559Ba	545Ba
B50	688ABb	996Aa
B100	867Aa	909Aa
Mean standard error	34.02	

Means followed by at least one capital letter in the column and lower case in the line, for each variable, do not differ at the level of a 5% probability by the Tukey's test. Abbreviations: B, signal grass which was not fertilized with nitrogen (control); BC, signal grass intercropped with calopo; B50, signal grass fertilized with a dose of 50 kg ha^{-1} N; B100, signal grass fertilized with a dose of 100 kg ha^{-1} N.

The numbers of DTs ($p = 0.0247$), LTs ($p = 0.0001$), and TTs ($p = 0.0001$) were affected by treatment (Table 6). The number of DTs was lower for the BC treatment than for the B and B100 treatments. However, the numbers of LTs and TTs were lower for the BC treatment than for the other treatments. The number of DTs was also affected ($p = 0.0465$) by experimental year, with a higher value observed in 2018 (355 tillers/m^2) than in 2017 (238 tillers/m^2). However, the values of LTs ($p = 0.2972$) and TTs ($p = 0.4230$) were not affected by experimental year, with average values of 1035 LT/m^2 and 1088 LT/m^2 in 2017 and 2018, respectively, and 1327 TT/m^2 and 1390 TT/m^2 in 2017 and 2018, respectively.

Table 6. Averages of the numbers of dead tillers (DTs), live tillers (LTs), and total tillers (TTs) for the different treatments at the end of the deferral period.

Treatment	DT (tillers/m^2)	LT (tillers/m^2)	TT (tillers/m^2)
B	374A	1046A	1420A
BC	150B	819B	969B
B50	281AB	1224A	1506A
B100	383A	1157A	1539A
Mean standard error	33.47	36.01	55.96

Means followed by at least the same letter in the column do not differ at the level of a 5% probability by the Tukey's test. Abbreviations: B, signal grass which was not fertilized with nitrogen (control); BC, signal grass intercropped with calopo; B50, signal grass fertilized with a dose of 50 kg ha^{-1} N; B100, signal grass fertilized with a dose of 100 kg ha^{-1} N.

In the samples collected via simulated grazing, it was found that the L/S ($p = 0.0042$) and the percentages of L ($p = 0.0013$), S ($p = 0.0355$) and DE ($p = 0.0084$) were affected by the interaction between treatment and experimental year (Table 7). When comparing treatments within years, in 2017, it was found that the BC treatment resulted in the higher L/S and %L values and lower values of %S and %DE compared with the other treatments. In 2018, the averages were similar between the different treatments for signal grass. When comparing the years within each treatment, there was a higher L/S ratio and a higher %L value and the lower values of %S and %DE in 2017 than in 2018 for the BC treatment (Table 7).

The proportions of calopo in the samples collected via direct cutting at the end of the deferral period in the intercropped pasture were 3.0% and 9.5% with regard to TDM in 2017 and 2018, respectively. The chemical composition of the calopo was also only evaluated in the samples via direct cutting, which was used to verify the DM contents of 15.9% and 26.5% in 2017 and 2018, respectively, the CP contents of 17.3% and 13.2% in 2017 and 2018, respectively, and the NDFap contents of 48.6% and 54.2% in 2017 and 2018, respectively.

The chemical composition of the signal grass pastures at the end of the deferral period was evaluated in the samples collected via direct cutting and those obtained via simulated grazing. The CP contents in the samples that were directly cut differed ($p = 0.0001$) only among treatments (Figure 2), verifying that the B treatment presented a lower CP content compared with those of other treatments. However, the CP contents of the samples collected via simulated grazing were affected ($p = 0.0007$) by the interaction between treatment and experimental year, with a higher CP content ($p < 0.05$) found for the BC treatment compared with those of other treatments in 2017. However, in 2018, the means were similar among all treatments. When comparing years within treatments, the highest CP content in 2017 was found for the BC treatment.

Table 7. Averages of the leaf blade/stem (L/S) ratios and percentages of leaf blade (L), stem, and dead material, via simulated grazing, for different treatments and experimental years at the end of the deferral period.

Treatment	2017	2018	Mean Standard Error
	L/S		
B	1.24Ba	1.47Aa	
BC	2.20Aa	1.16Ab	0.08
B50	1.09Ba	1.42Aa	
B100	1.23Ba	1.10Aa	
	L (%)		
B	51.81Ba	54.79Aa	
BC	67.27Aa	49.95Ab	1.31
B50	48.98Ba	56.31Aa	
B100	52.33Ba	50.45Aa	
	S (%)		
B	42.26ABa	40.00Aa	
BC	31.61Bb	44.34Aa	1.22
B50	45.25Aa	40.42Aa	
B100	43.48Aa	46.18Aa	
	DE (%)		
B	5.92Aa	5.19Aa	
BC	1.10Bb	5.70Aa	0.41
B50	5.76Aa	3.25Aa	
B100	4.18ABa	3.36Aa	

Means followed by at least one capital letter in the column and lower case in the line, for each variable, do not differ at the level of a 5% probability by the Tukey's test. Abbreviations: B, signal grass which was not fertilized with nitrogen (control); BC, signal grass intercropped with calopo; B50, signal grass fertilized with a dose of 50 kg ha^{-1} N; B100 = signal grass fertilized with a dose of 100 kg ha^{-1} N.
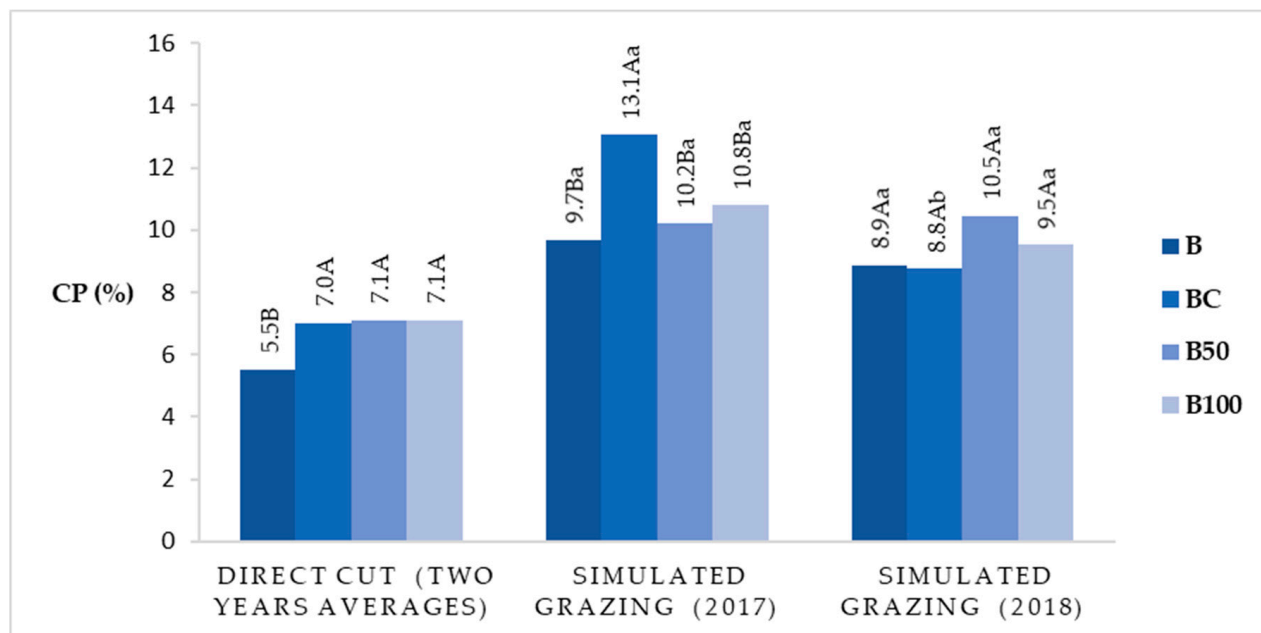

Figure 2. Average values of crude protein (CP) content in signal grass pastures, via direct cutting (among treatments) and via simulated grazing samples (interaction between treatments and years), at the end of the deferral period. Means followed by at least one capital letter (compares treatments) and one lower case (compares years) do not differ at the level of a 5% probability by the Tukey's test. Abbreviations: B, signal grass which was not fertilized with nitrogen (control); BC, signal grass intercropped with calopo; B50, signal grass fertilized with a dose of 50 kg ha^{-1} N; B100, signal grass fertilized with a dose of 100 kg ha^{-1} N.

The NDFap content, as determined from the samples obtained via direct cutting, was not affected ($p > 0.05$) by any factors and presented an overall average of 66.5%. Only year affected the NDFap contents of the samples obtained via simulated grazing ($p = 0.0012$), in addition to the %DM of samples obtained via direct cutting ($p = 0.0001$) and those obtained via simulated grazing ($p = 0.0001$; Table 8). Lower levels of NDFap in the samples obtained via simulated grazing and higher levels of DM in samples obtained by both simulated grazing and direct cutting were observed in 2018 than in 2017 (Table 8).

Table 8. Averages of the neutral detergent fiber corrected for ash and protein (NDFap), in samples from simulated grazing and percentages of dry matter (DM), via direct cutting and simulated grazing, at the end of the deferral period in two experimental years.

Year	NDFap (%) (Simulated Grazing)	DM (%) (Direct Cutting)	DM (%) (Simulated Grazing)
2017	60.9A	26.9B	27.9B
2018	57.9B	32.4A	33.6A
Mean standard error	0.52	0.70	0.82

Means followed by at least the same letter in the column do not differ at the level of a 5% probability by the F test; 2017: deferral period of 98 days, starting from 03/07/2017; 2018: deferral period of 70 days, starting from 03/07/2018.

4. Discussion

The highest SH and FI (Table 2) at the end of the deferral period for the BC treatment did not differ from those of the treatments that received nitrogen doses, which may be explained by the greater availability of nitrogen in the soil [11], as well as by the quantity and quality of incident light in the forage canopy. According to [20], in intercropped pastures, the proximity of different plants impairs the quantity and quality of light that penetrates the forage canopy, in which the competition between grasses and legumes can result in stalk elongation and increased plant tipping, as was observed in the present study (Tables 2 and 4).

The deferment of a signal grass pasture generally results in an FI greater than 1.0 because of its decumbent growth form [5]. A high FI reflects prostrate growth in a pasture, while values close to one indicate that the pasture grows vertically [21]. In the present study, the FI ranged from 1.51 (control) to 2.07 (consortium), which may be attributed to the thin and flexible stems of signal grass, which promote falling during growth. Among the management strategies that could be used to improve the values of the FI in deferment pastures to reduce the possible forage losses associated with falling, the implementation of shorter deferral periods, lower pasture heights at the start of the deferral period, and reduced nitrogen doses applied at the beginning of the deferral period stand out [22].

As the GDM consists of the sum of the masses of L and S, greater GDM values were observed for the BC treatment compared with those for the B treatment in 2017, as the intercropped pasture presented approximately 50% S while that of the control was 38% (Table 4). The GDM value for the intercropped pasture was lower in 2018 than in 2017, which may be attributed to a reduction in SDM. In 2018, the highest rainfall during the study period was recorded, but only the B and B100 treatments responded with high GDM values (Table 3).

The greater FA for the BC treatment compared with that for the B treatment during the first year (Table 3) was probably the result of a greater availability of nitrogen in the soil due to the N fixation carried out by the legume; however, the amount of FA did not differ between treatments with a nitrogen fertilizer. In the second year, the FAs were similar among the treatments, which may be due to the greater amount of rainfall recorded during that period, as has already been reported. According to [23], higher pastures may reduce FA due to greater leaf senescence, a fact that was not observed in the present study. The intercropped pasture had a lower proportion of DE than the other treatments.

According to [24], the GDM/DEM ratio is important, because it allows for the balance between the masses of living and dead plant tissue in a pasture to be determined. In our

study, the intercropped pasture had the highest GDM/DEM ratio, without a difference from that for the B50 treatment (Table 4). This finding may favor animal performance, as it is a characteristic of pastures which is more favorable for consumption. However, it should be noted that under deferred pasture conditions, the senescence of plant tissues is generally more accentuated due to the extensive deferral period that is normally adopted to guarantee a reasonable forage stock for the winter period [24].

The highest percentage of S for the BC treatment, which did not differ from that for the B50 treatment, at the end of the deferral period (Table 4) in the samples obtained via direct cutting may be attributed to competition for light between plants, which favors stem elongation [21]. The highest percentages of DE for the B and B100 treatments, which differed from those for the B50 treatment (Table 7), may have been due to the high abundance of DT in these treatments (Table 6).

The intercropped pasture at the end of the deferral period showed lower VT abundance compared with that for the B100 treatment in the first year, and this abundance did not increase during the second year, which was also observed for the B100 treatment (Table 5). In 2018, all treatments had larger VT populations than those of the intercropped pasture. According to [25], nitrogen fertilization is related to improved soil fertility, which promotes a significant increase in the flow of tissues from forage plants, including a greater appearance of tillers. In a study in which signal grass was deferred for 95 days, an increase in the abundance of VTs ($744 \ \text{m}^{-2}$ to $1.320 \ \text{m}^{-2}$) was observed, as the N dose increased ($0 \ \text{kg ha}^{-1}$, $50 \ \text{kg ha}^{-1}$, and $100 \ \text{kg ha}^{-1}$) [26]. However, in our study, the responses were not consistent for this characteristic, reflecting a similar abundance of VTs among the signal grass pastured (either fertilized or not (control)). In addition, it was observed that there was no difference in the number of DTs, LTs, and TTs in the fertilized pastures, while the BC treatment presented lower values for these variables (Table 6). The lower densities of LTs and TTs for the BC treatment may be attributed to its greater height (Table 2), with a subsequent compensation in the density/size ratio of tillers [27].

At the end of the deferral period, in the samples obtained by simulated grazing, the values of the L/S ratio and %L were higher, while the percentages of %S and %DE were lower (Table 7) than those of the samples obtained via direct cutting, regardless of either the treatment or year. These results demonstrated that the characteristics of the pasture did not represent the characteristics of the forage ingested by the animals due to selective grazing behavior. Animals prefer certain parts of plants, and thus, the composition of their diet often differs from that of the pasture [28].

With regard to the chemical composition of the samples obtained by direct cutting, the highest levels of CP, which were registered for the BC, B50, and B100 treatments (Figure 2), were probably the result of a greater availability of nitrogen in the soil and consequently high nitrogen content in the forage. In signal grass samples, which underwent three different periods of deferment (73 days, 95 days, and 116 days) and four doses of nitrogen ($0 \ \text{kg ha}^{-1}$, $40 \ \text{kg ha}^{-1}$, $80 \ \text{kg ha}^{-1}$, and $120 \ \text{kg ha}^{-1}$) in an experiment that was previously conducted in the same experimental area, CP levels ranging from 2.86% to 5.97% were found [22], which are values lower than those found in the present study.

The highest CP content for the BC treatment in the samples collected via simulated grazing in 2017 (Figure 2) may be attributed to the greater percentage of leaves of signal grass (Table 7), morphological component with the highest nitrogen content, and due to the calopo source. However, calopo was not obtained from the samples collected via simulated grazing, possibly because it was only performed in the initial three grazing days when the heifers were still acclimating to grazing and selected more grass than legumes. In addition, it has been recognized that calopo has low acceptability, which limits its consumption by grazing animals [29].

In a study carried out by [3], a CP content of 8.1% was found in the samples obtained through simulated grazing in a signal grass monoculture fertilized with a dose of $50 \ \text{kg ha}^{-1}$ N and 126 days of deferral. In the present study, higher CP levels were found, ranging from 8.7% (BC treatment in 2018) to 13.1% (BC treatment in 2017; Figure 2). The higher CP

levels found in samples collected via simulated grazing compared with those obtained via direct cutting may be attributable to the higher proportion of L than that of S in the upper grazing horizon of the canopy [30], with the leaf fraction being most selected by grazing animals [31].

The simulated grazing technique is important for adjusting possible nutritional imbalances in the available forage [32], as samples of tropical pastures obtained near the ground level underestimate the nutritional value of the pasture, which will be harvested by grazing animals [30]. However, if we assume that the forage obtained by simulated grazing is similar to that of the forage selected by animals, it can be inferred that the nitrogen content in the present study was not a limiting factor for dry matter intake by grazing animals, considering that the critical level of CP in the diet was estimated to be between 6% and 7% [33].

The higher NDFap content in the samples obtained via simulated grazing in 2017 compared with that in 2018 (Table 8) may be attributed to the longer deferral period of the pasture in the first year, as a greater deposition of the NDF fraction in plants with longer periods of growth has been reported [34]. Reference [3] recorded an average NDFap content of 69.2% in samples that were obtained via the simulated grazing of signal grass that was deferred for 126 days, which exceeds the values obtained from the samples obtained via simulated grazing and direct cutting in the present study.

In deferred pastures, it is known that the use of nitrogen fertilization improves the accumulation, structural characteristics, and chemical composition of forage, but the advance we achieved in this study is of great importance for agriculture, as we were able to confirm our hypothesis that the use of mixed signal grass–calopo pastures provides similar benefits to those of monoculture pastures fertilized with nitrogen.

The intercropping of signal grass and calopo may be recommended for deferment to guarantee the accumulation of forage, structural characteristics, and CP content equivalent to those of fertilized pastures. In addition, there are the benefits of biological nitrogen fixation and the lower cost of intercropping.

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