South African Journal of Animal Science 2022, 52 (No. 1)

Evaluation of grass and legume tropical mixtures and performance of grazed sheep

R.L. Moura[1], M.E. Oliveira[2], W.F. Carvalho[3], M.M. Rodrigues[4], M.S. Santos[5], R.L. Edvan[2#], A.L. Abdalla[6], M.Z. Moreira[6] & E.M. Silva[1]

[1]Multiple Higher Education Institute, Animal Science, Timón, Maranhão, Brazil
[2]Federal University of Piauí, Agriculture Science Center, Teresina, Piauí, Brazil
[3]Animal Science, Federal Institute of Piauí, Valença, Piauí, Brazil
[4]Federal University of Maranhão, Animal Science, Bacabal, Maranhão, Brazil
[5]State University of Piauí, Animal Science, União, Piauí, Brazil
[6]University of São Paulo, Nuclear Energy Center in Agriculture, Piracicaba, São Paulo, Brazil

(Submitted 28 April 2021; Accepted 4 November 2021; Published 24 January 2022)

Abstract

The objective of this research was to determine the pasture structure, nutritional value, animal behaviour, intake, and performance of Santa Inês sheep grazing pastures with various mixtures of grass *(Andropogon gayanus)* and forage legume (*Stylosanthes* sp. and *Calopogonium mucunoides*). A randomized block design was adopted with the treatments arranged in a 3 x 2 factorial scheme, with the factors consisting of cropping systems and grazing cycles. Grass in the mixed species pastures had a greater leaf/stem ratio than in the monoculture. Total forage mass was greater in the mixed pastures, which had the highest concentrations of crude protein and total digestible nutrients, the lowest fibre concentration, and the highest in vitro dry matter digestibility. Legumes were grazed with the highest frequency, and biting rate was highest in *Andropogon gayanus* with *Stylosanthes* sp. The highest intake (kg/day) was found in the mixed swards and the highest animal weight gain (143 g/day) in the mixed pastures. The pastures of *Andropogon gayanus* mixed with *Stylosanthes* sp. and *C. mucunoides* showed improved forage nutritive value and intake compared with the grass monoculture.

Keywords: *Andropogon gayanus*, animal production, *Calopogonium mucunoides, Stylosanthes* spp., tropical grass
[#]Corresponding author: edvan@ufpi.edu.br

Introduction

Animal production in pastures of tropical regions is characterized by the use of grasses, mainly because of their high potential forage production (Poppi *et al.*, 2018). But tropical forage grasses grown in monoculture systems require many inputs, especially chemical fertilizers, which result in increased costs (Oliveira *et al.*, 2019).

The adoption of a mixture of grasses and legume species made it possible to overcome low nutritional quality problems in tropical pastures, providing greater economic viability of the production system (Gimenes *et al.*, 2017; Schultze-Kraft *et al.*, 2018; Gomes *et al.*, 2020), and reducing input costs, especially fertilizers. A mixture of grasses and forage legumes in pastures can increase animal performance (Gama *et al.*, 2014). Thus, new information on the mixture of grass and legume forage is important to help maintain sheep production systems.

Andropogon gayanus is a tropical grass that can be mixed with herbaceous legumes (Barcellos *et al.*, 2008; Carvalho *et al.*, 2017). It is used widely because it is persistent in tropical regions (Oliveira *et al.*, 2019). One of its limitations is its low nutritional quality in the dry season (Costa *et al.*, 2017), but this can be improved with the inclusion of forage legumes (Euclides *et al.*, 2010; Lista *et al.*, 2019) such as *Stylosanthes* spp. and *C. mucunoides,* which have high nutritional quality and are suited to the tropical environment (Araújo *et al.*, 2017; Gimenes *et al.*, 2017).

Because of differences in the biological interrelationship between form and function among species, plant growth and productivity are important factors in the cultivation of mixed pastures (Teixeira *et al.*, 2014).

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v52i1.4

Grazing sheep can impair forage plant growth and production (Glienke *et al.*, 2010), especially for herbaceous legumes. The search for tropical species that present high forage production in mixed species pastures (henceforth mixed pastures) is essential to provide more options for sheep farmers in tropical regions. Thus, a mixture of *Andropógon* with *Stylosanthes* spp. or *C. mucunoides* was assumed to be a good option for grazing sheep in these regions, instead of a monoculture of *Andropógon.* The objective of this research was to compare a monoculture of *Andropógon* with mixtures of *Andropógon* and the legume Campo Grande (*Stylosanthes capitata* × *Stylosanthes macrocephala*) and *C. mucunoides*. To this end, the structure of the pasture, the chemical composition of the forage, in vitro digestibility, and behaviour, intake and performance of growing sheep were evaluated.

Materials and methods

The experiment was carried out in the city of Teresina, Piauí, Brazil, located at 5°06'18" S and 42°48'12" W in 2013. The climate of the region, according to the Köppen climate classification scheme is a tropical rainy savannah with dry winters (June to November) and rainy summers (December to May). Most of the rainfall is concentrated from January to April (Alvares *et al.,* 2013). The soil of the experimental area was a red-yellow latosol of medium texture and dystrophic (Raij *et al.*, 2001) with these characteristics: pH in water 5.0, calcium 0.5 cmol/dm^3, magnesium 0.3 cmol/dm^3, potassium 0.07 cmol/dm^3, aluminium 0.4 cmol/dm^3, phosphorus 1.0 mg/dm^3, 1.5% organic matter, 3.8 cmol cation exchange capacity, and 20.8% base saturation. Rainfall, temperatures, and relative humidity during the experimental period were recorded at 500 m from the experimental area (Figure 1).

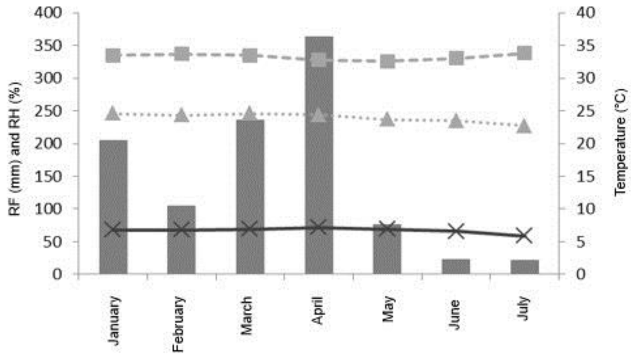

Figure 1 Rainfall, relative humidity, and maximum and minimum monthly temperature during 2013 when the experiment was conducted

█ :rainfall, X: relative humidity; --■--: maximum temperature, ··▲··: minimum temperature

The soil of the experimental area was ploughed and harrowed in early January 2013, and lime was applied at 1.2 tons of limestone per hectare to achieve the recommended 45% base saturation for the forage species. The area was fertilized with 45 kg/ha of phosphorus pentoxide and 60 kg/ha of potassium chloride, based on the soil analysis and interpretation of the results (Martha *et al.,* 2006).

A randomized block design was adopted with the treatments arranged in a 3 x 2 factorial scheme. The blocks were represented by paddocks. The factors consisted of cropping systems and grazing cycles, with 16 replications per treatment of pasture evaluation and 8 replications per treatment of animal evaluation. Three cropping systems were evaluated, which consisted of one monoculture of *Andropogon gayanus* genotype Planaltina and two mixtures, namely a mixture of *A. gayanus* and *Stylosanthes* sp. genotype Campo Grande and a mixture of A. gayanus and *Calopogonium mucunoides*. Subsequent to the preparation of the experimental area the paddocks were planted. In the intercropped pastures, two rows of *A. gayanus* and one row of the legume were planted, with spacing between furrows of 30 cm to reach the proportion of 66.7% grass and 33.3% legume, as proposed by Thomas (1992). The same spacing was adopted for the monoculture of *A. gayanus*, and the seeds were sown at a depth of 2 to 3 cm.

The pasture area was divided into 48 paddocks (plots) of 250 m² each. A rotational stocking grazing was adopted, with four days of occupation and 28 days of rest, with forage supply corresponding to 10% of the sheep's live weight (LW) (Gomez *et al.,* 2010). Grazing occurred in two cycles, namely cycle I from March to April and cycle II from May to June.

The animals in the research were treated in accordance with the guidelines of the Ethics Committee for the Use of Animals (CEUA) of the Centre for Nuclear Energy in Agriculture of the University of São Paulo (protocol number 011-2016). Three- to four-month-old Santa Inês male sheep were used with average weight of 21.7 ± 3 kg. Eight sheep were allotted to each pasture type and the forage supply was maintained at 10%

The structure of the pasture canopy was characterized in four paddocks identified by treatment when the sheep entered to start grazing in each cycle. The height of the canopy was measured with a ruler graduated in centimetres, with 20 readings per plot. Thus, there were 80 measurements per treatment. The forage mass, leaf blades and canopy stems were evaluated using frames of 1.0 m × 0.5 m (0.5 m² of area), with four samples collected at 20 cm from the ground per paddock (Gardner, 1986), which was the height defined for the post-grazing residue. The samples were weighed and divided into two fractions: one to assess the morphological composition (leaf, stems, and stalks) and the other to measure the forage mass. The components were weighed and dried in a forced air circulation oven at 60 ± 5 °C for 72 hours, allowing forage mass and leaf/stem and leaf/stalk ratios to be identified for grass and legumes. The pasture height, total forage mass (TFM), grass forage mass (GFM) and legume forage mass (LFM) were recorded. In this same area, the chemical composition of the forage was established from samples that were obtained by observing the animals, identifying the types of material they consumed, and grazing was simulated by collecting similar samples.

The samples were packed in paper bags and pre-dried in a forced air circulation oven at 60 ± 5 °C for 72 hours. The concentrations of dry matter (DM) (method No. 934.01) and crude protein (CP) (method No. 981.10) were assessed according to AOAC (2012). To determine the concentration of neutral detergent fibre (NDF) and acid detergent fibre (ADF) method INCT-CAF-002/1 was applied (Detmann *et al*., 2012).

An in vitro digestibility trial was carried out in an incubator model TE-150 (TECNAL Equipment for Laboratory, Piracicaba, Sao Paulo, Brazil), using the forage samples from the simulated grazing. The trial was divided into six inoculations, three inoculations in duplicate. Twelve rumen-cannulated Santa Inês wethers weighing approximately 65 ± 2 kg were used to provide the rumen contents. The liquid and solid phases of the rumen content were separated, with four donor animals providing the material for each inoculum (Bueno *et al.,* 2005).

In vitro dry matter digestibility (IVDMD) was assessed from the difference between the forage material recovered after incubation – after drying in an oven at 105 °C for 12 hours – and the material in the initial sample. To estimate in vitro organic matter digestibility (IVOMD), the material was incinerated in a muffle oven at 600 °C for four hours and re-weighed. IVDMD and IVOMD (D) (g/kg) were calculated as:

$$D = \{[M - (R - B)] \div M\} \times 100$$

where: M = mass of DM or OM incubated (g),
R = DM or OM residue from the incubation (g), and
B = DM or NDF residue obtained in 'blanks' (g).

The concentration of total digestible nutrients (TDN) was estimated with the equation proposed by Cappelle *et al.* (2001):

$$\%TDN = -2.49 + 1.0167*IVOMD$$

The IVDMD and IVOMD digestibility were determined by the technique of Tilley and Terry (1963) as adapted by Van Soest (1994).

Animal behaviour was assessed at 10-minute intervals between 08h00 and 17h00 as time spent grazing, ruminating, idling, and moving over four days in each of the grazing cycles. The biting rate was determined every two hours by observing each animal and recording the time spent to make 20 bites. All behaviour analyses were carried out by the same trained observers. The species of grass or legume that the animals were consuming was recorded at this time. Thus, the frequency of ingestion of the forage species was determined (Forbes & Hodgson, 1985).

The values of stable carbon isotope ($\delta^{13}C$) in the forage and faeces of the sheep were estimated in each cropping system, adopting the principle of isotopic dilution of C-13 by measuring the isotopic ratio of carbon between C_4 plants and C_3 plants. Because of the difference in C-13 discrimination, C3 plants (legumes) had $\delta^{13}C$ ranging from -24 to -32 parts per thousand (‰), and C4 plants (tropical grasses) had $\delta^{13}C$ ranging from -8 to -12 ‰ Vienna Pee Dee Belemnite (VPDB) (Gilbert *et al.*, 2012).

The samples of faeces and forage were dried in a forced air circulation oven at 50 ± 5 °C and 60 ± 5 °C, respectively, for 72 hours, ground to 1.0 mm, and subjected to isotopic analysis. The carbon isotopic composition of the samples was determined by combustion, under continuous helium flow, in an elementary analyser (Carlo Erba, CH-1110, Thermo Fisher Scientific, Waltham, Massachusetts, USA) coupled to a mass spectrometer (Thermo Finnigan Delta Plus, Thermo Fisher Scientific, Waltham, Massachusetts, USA). The carbon dioxide gas from the combustion of the samples was analysed with an analytical error of 0.3 ‰. The isotopic ratios were expressed by the delta (δ) notation, compared with the international standard VPDB, and calculated with the formula:

$$\delta \text{ sample (‰)} = (\text{R sample - R standard/R standard}) \times 1000$$

Dry matter intake (DMI) was estimated by faecal excretion, using the formula:

$$\text{DMI (g/day)} = \text{faecal excretion}/(1 - \text{IVDMD})$$

Faecal excretion was obtained using the external indicator titanium dioxide (TiO_2) as indicated in Titgemeyer, (1997) and Ferreira *et al.*, (2009) with the formula:

$$\text{Faecal dry matter (g DM day}^{-1}) = \text{DMI} \times (TiO_2 \text{ supplied})/(TiO_2 \text{ in faeces})$$

The TiO_2 indicator was administered orally as capsules, at a dosage of four grams/sheep/day, with an adaptation period of eight days to obtain a more consistent excretion plateau, followed by four days of collection (Titgemeyer, 1997; Ferreira *et al.*, 2009). The faeces were collected directly from the animals' rectal ampoule, at 07h00, and kept in a freezer at -5 to -10 °C. Subsequently, they were homogenized to form a composite sample for each animal, dried in a forced air ventilation oven at 50 ± 5 °C, and ground to determine the DM concentration (AOAC, 2012).

The analyses of TiO_2 were carried out according to Myers *et al.* (2004). A sample of 0.25 g faeces was digested in 15 mL sulfuric acid and 1.0 g protein digesting mixture (Micro-Kjeldahl) for two hours at 400 °C, in tubes of 25 × 250 mm. After digestion, 15 mL of 30% hydrogen peroxide was added slowly and the contents of the tube were transferred to a beaker, which was filled with distilled water to 100 mL. Then, contents of the beaker were transferred to flasks. A standard curve was prepared with 2, 4, 6, 8, and 10 mg TiO_2 and readings were taken with a spectrophotometer at a wavelength of 410 nm.

The sheep were weighed every 14 days, after fasting (feed) for 12 hours. Each day, the animals remained in the paddocks from 08h00 to 15h00, at which time they were gathered and returned to the pen for overnight. Anthelmintic was applied to the sheep one week before the beginning of the experiment and, subsequently, whenever necessary, according to their faecal egg count, which was monitored at 15-day intervals. The animals had mineral supplementation in the pen and water at will in the paddocks.

The data were analysed with analysis of variance using PROC MIXED of SAS (SAS Institute Inc., Cary, North Carolina, USA) and effects were declared significant at *P* =0.05. When the interaction between cropping systems and grazing cycles was significant, the SNK mean comparison test was used. The residual values were plotted against the predicted values to verify the assumptions of the model of homoscedasticity, error independence, and normality. The statistical model was:

$$Y_{ijkl} = \mu + B_i + F_j + E_k + FE_{jk} + \varepsilon_{ijkl}$$

where: Y_{ijkl} = an observed value,
μ = the mean of the observations,
B_i = the effect of the ith block (I = 1 to 4),
F_j = the effect of the jth pasture system (j = 1 to 3),
E_k = the effect of the kth grazing cycle (k = 1 to 2),
FE_{jk} = the interaction effect of the jth pasture system with the kth grazing cycle, and
ε_{ijkl} = the residual error associated with the lth observation.

Results and Discussion

There were no significant interaction effects between cropping systems and grazing cycles on structural characteristics and pasture forage mass (*P* <0.05) (Table 1). The cropping systems affected (*P* <0.01) only the leaf/stem ratio, which had the lowest value in the monoculture, whereas it was not affected for legumes (*P* >0.05). The total forage mass in the systems was affected (*P* =0.03), with the mixed pastures producing more forage than the monoculture, which produced the greatest mass of grass (*P* <0.01), whereas the mixed pastures produced similar amounts of the legumes. In the May to June cycle, the rainfall was 40% of that recorded during the March to April cycle. However, even with this decrease in precipitation, forage production was unaffected (*P* >0.05). Only the height of the forage was affected by the grazing cycle (*P* <0.01).

Table 1 Pasture structure and accumulated forage mass in monoculture and mixture of grass and legume in various grazing cycles of sheep

	Height, cm	L/S	L/ST	Forage mass		
				Total	Grass	Legume
Cropping systems						
Andropogon gayanus monoculture	78.7	0.9[b]	-	1.7[b]	1.7[a]	-
A. gayanus + *Stylosanthes* mixture	91.4	1.2[a]	0.8	2.6[a]	1.0[b]	1.5
A. gayanus + *Calopogonio mixture*	87.8	1.3[a]	0.9	2.3[a]	0.9[b]	1.3
Grazing cycles						
March to April	102.7[a]	1.1	0.9	2.3	1.3	1.49
May to June	69.3[b]	1.1	0.8	2.0	1.1	1.42
SE	6.6	0.1	0.08	0.2	0.1	0.1
P-value cropping systems	0.13	<0.01	0.06	0.03	<0.01	0.27
P-value grazing cycles	<0.01	0.54	0.62	0.12	0.06	0.76
P-value interaction	0.08	0.07	0.89	0.84	0.60	0.72

L/S: grass leaf/stem ratio. L/ST: legume leaf/stalk ratio
[a,b] Within an effect, treatment means followed by the superscript were not different with probability *P* >0.05

The monoculture produced less DM per kg of forage than the mixture of *Andropogon gayanus* and *Calopogonio*, with *Andropogon gayanus* and *Stylosanthes* being intermediate (Table 2). The forage produced in the May to June cycle was slightly drier than that produced in the March to April cycle. In general, forage produced by the monoculture had lower nutritional values and was more fibrous than that produced by the mixed pastures. The IVDMD was greater for the forages in the mixed pastures than the monoculture. However, there were no significant effects of cropping system, grazing cycle and their interaction on IVOMD.

Table 2 Chemical composition (g/kg dry matter) and in vitro digestibility of forage in monoculture and mixture in various grazing cycles of sheep

Cropping system	Cycle	DM, g/kg	CP	NDF	ADF	TDN	IVDMD	IVOMD
Mono	I	219	119	719	524	648	659	648
	II	222	127	719	558	617	632	617
Mix I	I	224	148	669	494	695	705	695
	II	230	152	663	507	686	700	686
Mix II	I	236	136	711	452	687	678	665
	II	238	139	702	492	665	691	616
SE		9.4	6.0	8.3	12	12.2	8.4	9.7
P-values								
Cropping systems		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.10
Grazing cycles		<0.01	0.22	<0.01	0.03	<0.01	0.78	0.70
Interaction		0.98	<0.01	<0.01	<0.01	<0.01	0.19	0.17

Mono: *Andropogon gayanus* monoculture, Mix I: *A. gayanus* and *Stylosanthes*, Mix II: *A. gayanus* and *Calopogonio*, DM: dry matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, TDN: total digestible nutrients, IVDMD: in vitro dry matter digestibility, IVOMD: in vitro organic matter digestibility

There was no significant effect of the interaction between cropping system and grazing cycle on the behaviour characteristics of the grazing sheep (*P* >0.05) (Table 3). Nor was there was an individual effect of cropping system (P >0.05) or grazing cycle (*P* > 0.05) on grazing, rumination, rest and idle times. The frequency of grazing grass was higher in *A. gayanus* and *Calopogonio* than *A. gayanus* and *Stylosanthes*. Conversely, the frequency of grazing legumes was higher in *A. gayanus* and *Stylosanthes*. In both mixed species treatments, grass was grazed more frequently in the March to April cycle, whereas legumes were preferred in May to June. The biting rate was higher in *A. gayanus* and *Stylosanthes* than in the other two treatments, in which biting rates were similar.

Table 3 Grazing behaviour of sheep in monoculture and mixtures of grass and legumes in various grazing cycles

	GT, %	RT, %	MT, %	IT, %	GFG, %	GFL, %	BR/minute
Cropping system							
Andropogon gayanus monoculture	7.58	1.06	0.05	0.45	-	-	19.2^{b}
A. gayanus & *Stylosanthes* mixture	7.42	1.12	0.04	0.57	46.0^{b}	53.9^{a}	21.9^{a}
A. gayanus & *Calopogonio mixture*	7.35	0.94	0.05	0.80	77.3^{a}	22.6^{b}	17.9^{b}
Grazing cycle							
March to April	7.38	0.96	0.06	0.76	56.4^{B}	43.5^{A}	19.2
May to June	7.51	1.12	0.04	0.48	66.8^{A}	33.1^{B}	20.2
SE	0.15	0.09	0.02	0.13	2.1	2.1	0.6
P-value cropping systems	0.32	0.17	0.87	0.16	<0.01	<0.01	<0.01
P-value grazing cycles	0.30	0.30	0.09	0.09	<0.01	<0.01	0.11
P-value interaction	0.18	0.06	0.10	0.25	0.10	0.11	0.29

GT: grazing time, RT: rumination time, MT: movement time, IT: idle time, GFG: grazing frequency for grass, GFL: grazing frequency for legumes BR: biting rate per minute
[a,b] Within a column, treatment means followed by a common superscript were not different with probability *P* >0.05

The $\delta^{13}C$ values did not show an interaction of cropping system with grazing cycle or an effect of grazing cycle (Table 4). The isotopic composition of carbon (C) varied among the forages in each cropping system, with lower values $\delta^{13}C$ observed for legumes than grass. The highest $\delta^{13}C$ value of faeces was found in the monoculture (P <0.01). The inferred ratio of carbon to nitrogen was lower in the legumes than in the grass.

Table 4 Values of $\delta^{13}C$ in the forage and sheep faeces in the grass and legumes in various cropping systems

	$\delta^{13}C$ Grass	$\delta^{13}C$ Legume	$\delta^{13}C$ Faeces	C/N Grass	C/N Legume
Cropping system					
Andropogon gayanus monoculture	-11.94^{a}		-17.52^{a}	22.24^{a}	
A. gayanus & *Stylosanthes* mixture	-11.75^{a}	-28.32^{b}	-22.60^{c}	21.35^{a}	11.68^{b}
A. gayanus & *Calopogonio mixture*	-12.18^{a}	-28.30^{b}	-19.90^{b}	21.41^{a}	12.22^{b}
SE	0.07		0.37	0.58	
P-value cropping systems	<0.01		<0.01	<0.01	
P-value grazing cycles	0.35		0.91	0.48	
P-value interaction	0.22		0.35	0.45	

C/N: carbon nitrogen ratio
[a,b,c]Within a column, treatment means followed by a common superscript were not different with probability *P* >0.05

No significant effect of the interaction between cropping system and grazing cycle was found for forage intake expressed as a percentage of live weight or as mass, or ADG of the sheep (Table 5). Higher intake was observed for sheep grazing the mixed pastures than the monoculture and in the May to June cycle compared with March to April. Average daily gain was higher for sheep that grazed *A. gayanus* and *Stylosanthes* compared with the other two systems.

Table 5 Intake and performance of sheep grazing monoculture or mixed species pastures in various grazing cycles

	Intake, %LW	Intake, kg/day	ADG, g/day
Cropping systems			
Andropogon gayanus monoculture	2.82[b]	0.68[b]	88[b]
A. gayanus & *Stylosanthes* mixture	3.43[a]	0.95[a]	143[a]
A. gayanus + Calopogonio mixture	3.54[a]	0.87[a]	76[b]
Grazing cycles			
March to April	3.06[b]	0.74[b]	110[a]
May to June	3.47[a]	0.93[a]	94[a]
SE	0.16	0.04	0.18
P-value cropping systems	<0.01	<0.01	<0.01
P-value grazing cycles	<0.01	<0.01	0.15
P-value interaction	0.20	0.38	0.49

[a,b] Within an effect, treatment means followed by the superscript were not different with probability $P > 0.05$

Forage production was greater in the mixed pastures and lower in the monoculture. As well as improving soil, chemical composition of the plant, and weed control (Machado *et al.*, 2017; Stern, 1993), legume forages can also increase the forage mass in pastures composed of mixed species (Gulwa *et al.*, 2018). Although the evaluation period was short, the higher production of legumes in the mixtures could indicate their adaptability to mixed pastures despite tropical grasses (C_4) growing faster than tropical legumes (C_3) (Medina *et al.*, 1999). However, legumes may not persist in mixed pastures owing to the competitiveness of the grass.

Nitrogen fixation is one reason for the increase in forage mass when legumes and grasses are grown together. In mixtures, nitrogen fixation in the soil by legumes can be beneficial to plant growth (Mendonça *et al.*, 2017). The proportion of legumes in mixed swards should vary between 10% and 40% to maintain the nitrogen balance (Thomas, 1992). In this study, despite the lower proportion of legume seed that was planted a higher forage mass was observed in the mixed pastures, demonstrating the importance of the legumes in these systems.

The mixture produced a higher leaf to stem ratio of Andropógon grass, which provides better quality feed for the animals. Almeida *et al.* (2017) also found a higher leaf to stem ratio in *Panicum maximum* grown in an intercropping system. In the monoculture, although the plants had similar height, a greater proportion was stems, which had the lowest nutritional value and influenced the digestibility and intake of forage negatively (Lee, 2018).

The difference in the amount of rainfall between the grazing cycles did not affect forage production because of the regularity of the rains, despite the smaller total amount in the May to June cycle. This may be because the plants were well adapted to the tropical environment in which they were grown, and thus maintained productivity as the amount of rain decreased. Mixed pastures tended to be more resilient to adverse climatic factors in tropical regions, especially variations in rainfall during the production period (Korres *et al.*, 2016).

The *Andropógon* grass monoculture had a lower concentration of CP and TDN, which was associated with a higher concentration of NDF and ADF, compared with *A. gayanus* and *Stylosanthes*. Thus, it was of lower nutritional quality, which might compromise performance. Similarly, Chaudhry (2008) found mixed systems had greater nutritional quality for grazing animals.

The simulated sheep diets were composed of approximately 40 ± 2% *Andropógon* grass and 60 ± 2% legumes (*Stylosanthes* sp. and *C. mucunoides*), which improved IVDMD compared with the monoculture, because of the better chemical composition and digestibility of the legumes. Paris *et al*. (2012) found that in some mixtures the legume improved the nutritional value of the feed for animals. The difference in the botanical composition observed in the monoculture and in the mixed systems did not influence the recorded behaviour of the sheep. In the mixed pastures the animals had two plants from which to choose. Sheep prefer herbaceous forage (Ngwa *et al.*, 2000), which was readily available in these cropping systems and thus diet selection was limited.

As they grow, grasses increase the cell wall component, and lignin increases ADF and reduces hemicellulose (Tilahun *et al.*, 2017). The mixture of *A. gayanus* and *Calopogonio* contained lower concentrations of CP and ADF in the May to June grazing, because of the formation of seed pods in the reproductive phase. Legume pods are rich in soluble carbohydrates (Obendorf & Górecki, 2012) and influence the nutritional value of the forage, mainly by increasing its digestibility.

The higher frequency with which sheep grazed *Stylosanthes* sp. may be related to their preference for *Stylosanthes* sp. as opposed to Andropógon grass, because of its structural characteristic and chemical composition. In a mixture of Andropógon grass with *C. mucunoides* the preference for the legume was low, which could be attributed to its chemical characteristics. The presence of flavonoids, alkaloids, phenolic acids, and carboxylic acids in *Calopogium mucunoides* could compromise the intake and performance of animals (Santos *et al.*, 2011). However, low acceptability may be desirable as it could contribute to the persistence of the legume in mixed swards, providing for fixation of atmospheric nitrogen and increasing the amount of litter (Barcellos *et al.*, 2008). Nitrogen fixation can benefit grazing animals directly (Silva *et al.*, (2012) through the increased CP content of the diet. In addition, it can provide a fertilization effect for other plants (Stagnari *et al.*, 2017).

Forage legumes are preferred by animals when rainfall is low (Hassen *et al.*, 2017). However, in this study, legumes were grazed at higher frequency in the March to April cycle than in the drier May to June cycle, probably because the legume plants were younger in the earlier cycle.

Biting rate was highest in *A. gayanus* and *Stylosanthes,* because of the morphology of the plant, which had leaves and leaflets (Caboco *et al.*, 2012). The sheep therefore consumed a smaller amount of forage per bite compared with a grass sward, which resulted in a higher biting rate (Glienke *et al.*, 2016). The similarity in the biting rate in the monoculture and *A. gayanus* and *Calopogonio* could be related to the higher frequency of grass consumption in this sward.

The distinct isotopic discrimination values between *Andropógon* grass and the legumes *Stylosanthes* sp. and *C. mucunoides* made it possible to use $\delta^{13}C$ to estimate the botanical composition of the diet consumed by the sheep, which is important in establishing correct pasture management strategies (Santos *et al.*, 2012). The higher $\delta^{13}C$ observed in *A. gayanus* and *Calopogonio* could be attributed to the higher nitrogen input from this legume. The lower carbon to nitrogen ratio in legumes results from their higher nitrogen content, which also improves digestibility (Waghorn & Clark, 2004).

Forage intake was higher in the May to June cycle, presumably because of the lower height of the pasture and the higher frequency of grass consumption. The highest forage intake was observed in mixed pastures, which was related to the better pasture structure and the higher L/S of the grass. The greater quantity of leaf tissue facilitates grazing (Chapman *et al.*, 2014). The amount of forage mass produced in the mixtures was greater than 1 tonne of DM/ha. Smaller values are considered limiting for the consumption of sheep (Roman *et al.*, 2007). Thus, the sheep in the mixed pastures had sufficient feed and had higher intake than that observed by Santos *et al.* (2012) in monocultures of Tanzania grass *(Panicum maximum)* and Marandu grass *(Brachiaria brizantha).*

Conclusion

The structure, production, chemical composition and forage digestibility indicated that pastures of mixed species could be successful in the short term with adequate management of forage crops and animals. The mixture of *A. gayanus* and *Stylosanthes* sp. was preferable to that of *A. gayanus* with *C. mucunoides* because the sheep grew more rapidly. Further studies with these species are recommended to verify the plant development and sheep grazing of these mixed systems over longer periods.

Acknowledgements

The Federal University of Piauí and the University of São Paulo, institutions are acknowledged for their support of Brazilian research.

Authors' Contributions

RLM: original draft preparation, methodology, visualization, investigation, reviewing, and editing. MEO: conceptualization, validation, resources, and supervision. WFC, MMR, MSS, and EMS: methodology, visualization, and investigation. RLE: reviewing, editing, and compiling final manuscript. ALA and MZM: conceptualization, validation, and supervision.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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