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This issue is dedicated to the memory of **Myles J. Fisher** (10 August 1935 – 27 May 2020), Australian ecophysiologist who after working at CSIRO in the Northern Territory and Queensland, Australia, joined the CIAT Tropical Pastures Program in 1985. Upon his retirement he was appointed CIAT Emeritus Scientist and was a member of the Editorial Board of *Tropical Grasslands-Forrajes Tropicales* since the journal´s inception. His friends and colleagues in the tropical forages scientific community will not forget him for his visionary pioneer role in the area of carbon sequestration by tropical pastures, his contribution to our understanding of factors affecting persistence of legumes in pastures and his thorough scientific thinking and pleasant personality.

Principal Contacts

Rainer Schultze-Kraft
The Alliance of Bioversity International and CIAT
Colombia
Phone: +57 2 4450100 Ext. 3036
Email: CIAT-TGFT-Journal@cgiar.org

Technical Support
José Luis Urrea Benítez
The Alliance of Bioversity International and CIAT
Colombia
Phone: +57 2 4450100 Ext. 3354
Email: CIAT-TGFT-Journal@cgiar.org

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Research Paper

Seasonal herbage accumulation, plant-part composition and nutritive value of signal grass (*Urochloa decumbens*) pastures under simulated continuous stocking

Acumulación estacional de forraje, composición morfológica y valor nutritivo de Urochloa decumbens *bajo pastoreo continuo simulado*

GUSTAVO JOSÉ BRAGA[1], CARLOS GUILHERME SILVEIRA PEDREIRA[2], ALIEDSON SAMPAIO FERREIRA[2], ELIARA ANAÍ DE OLIVEIRA[3] AND VALDINEI TADEU PAULINO[3]

[1]*Embrapa Cerrados, Planaltina, DF, Brazil.* embrapa.br/cerrados
[2]*Departamento Zootecnia, ESALQ, Universidade de São Paulo, Piracicaba, SP, Brazil.* zootecnia.esalq.usp.br
[3]*Instituto de Zootecnia, APTA, Nova Odessa, SP, Brazil.* iz.sp.gov.br

Abstract

In order to optimize the regrowth and harvest of signal grass (*Urochloa decumbens*) cv. Basilisk pastures it is necessary to establish more precise grazing management guidelines. The objective of this study was to evaluate herbage accumulation, plant-part composition and nutritive value of signal grass managed under contrasting levels of steady-state canopy heights. Treatments included 3 canopy height targets, i.e. 10 (S-short), 17.5 (M-medium) and 25 cm (T-tall), in a completely randomized design with 4 replications. Experimental units were 144-m^2 plots which were grazed by groups of steers for short periods in an endeavor to keep canopy heights at the 3 desired targets. On average, herbage accumulation rate (HAR) in T pastures was greater than in M and S pastures, including the dry-wet season transition period in spring (September–November). The S pastures had higher crude protein and lower acid detergent fiber concentrations than M and T pastures, especially in the first half of the calendar year. However, in vitro organic matter digestibility was similar for all treatments (612 g/kg). As S and M pastures had lower HARs than T pastures in the spring, it appears advantageous to maintain the signal grass canopy at ~25 cm in order to ensure quick regrowth with the return of the wet season. However, longer-term studies are needed with recording of animal performance before these initial findings can be promoted widely.

Keywords: Crude protein, digestibility, grazing management, light interception, pasture height, tropical pastures.

Resumen

Para optimizar la producción de pasturas de *Urochloa* spp. es necesario establecer pautas de manejo del pastoreo más precisas. En Brotas, Estado de São Paulo, Brasil, se evaluaron la acumulación de forraje, la morfología de planta y el valor nutritivo de *Urochloa decumbens* cv. Basilisk en función de diferentes alturas de planta. Los tratamientos incluyeron 3 alturas del pasto: 10 cm (S - baja), 17.5 cm (M - mediana) y 25 cm (T - alta), en un diseño completamente al azar con 4 repeticiones en parcelas de 144 m^2 que fueron utilizadas por grupos de novillos durante períodos cortos con el fin de mantener las alturas de planta de acuerdo con los tratamientos. En promedio, la tasa de acumulación de forraje (TAF) en las pasturas del tratamiento T fue mayor que en las pasturas en los tratamientos M y S, incluyendo el período de transición de estación seca a lluviosa en la primavera (septiembre–noviembre). Las pasturas en el tratamiento S presentaron mayores concentraciones de proteína cruda y menores concentraciones de fibra detergente ácido que las pasturas en M y T, especialmente en el primer semestre del año calendario. Sin embargo, la digestibilidad in vitro de la materia orgánica fue similar para todos los tratamientos (612 g/kg). Las pasturas en los tratamientos S y M presentaron

Correspondence: Gustavo José Braga, Embrapa Cerrados, BR 020,
km 18, Planaltina, CEP 73310-970, DF, Brazil.
Email: gustavo.braga@embrapa.br

TAF más bajas en la primavera que las pasturas en T, lo que sugiere que mantener una altura de pastura de ~25 cm sobre el nivel del suelo permite un rápido crecimiento al comienzo de la época de lluvias. Sin embargo, se necesitan estudios de producción animal a largo plazo antes de que estos resultados iniciales puedan promoverse en forma amplia.

Palabras clave: Altura de pastura, digestibilidad, intercepción de luz, manejo de pastoreo, pastos tropicales, proteína cruda.

Introduction

Signal grass [*Urochloa decumbens* (Stapf) R.D. Webster, syn. *Brachiaria decumbens* Stapf] cv. Basilisk is a decumbent perennial tropical grass, that despite being susceptible to spittlebug (*Deois* spp. and *Zulia* spp.) is widely used in forage-livestock systems in central Brazil, owing to its great tolerance to acidic and infertile soils (Rao et al. 1996). With the increasing competition for land associated with expansion of cropping areas, i.e. maize (*Zea mays*), soybean (*Glycine max*) and sugarcane (*Saccharum officinarum*), pastures must be more productive and sustainable; improvement of grazing management is required to achieve this objective.

Plant growth and harvest efficiency in grazed pastures are closely related to canopy structure (Bircham and Hodgson 1983). The presence of photosynthetically active leaves supports fast regrowth, eventually followed by stem elongation, a process triggered by the complete or near-complete interception of light by the forage canopy (maximum leaf area index, LAI) (Korte et al. 1982). For tropical grasses, excessive elongation of stem has a negative effect on pasture utilization (Hodgson and Silva 2002) due to increasing proportion of rejected patches and plant lodging. Grazing management, based on the maintenance of specific canopy structure by controlling LAI, canopy height or herbage mass, may avoid excessive stem growth and allow more predictable levels of herbage accumulation and animal performance (Silva et al. 2013). In addition, plant-part composition is an important variable for cattle nutrition, as animals can select a higher quality diet from a leafy pasture than from one with lower leaf proportion. Canopy height ranges recommended for grazing management are associated with the canopy architecture, as well as the plant-part (leaf, stem and dead material) accumulation dynamics under grazing. For example, keeping canopy height of Marandu palisade grass (*Urochloa brizantha*) at 10 cm by heavy grazing intensity negatively affected herbage accumulation (Silva et al. 2013). For signal grass pastures, optimum canopy height seems to be around 15–25 cm under continuous stocking (Santos et al. 2013), with a pre-grazing target under rotational stocking of 18–30 cm (Pedreira et al. 2017).

Although some research-based recommendations regarding defoliation strategies for signal grass are available (Braga et al. 2009; Santos et al. 2010; Portela et al. 2011; Pedreira et al. 2017), a better comprehension of the effects of year-round grazing at steady-state (constant) canopy heights is still necessary rather than just average wet-season estimates. Forage growth in central Brazil is markedly seasonal, concentrated in the warm, wet months (November–April), and overgrazing during the dry season is common due to limited forage accumulation during this period. This raises the question of how canopy structure in the dry season affects plant regrowth at the onset of the following wet season. The objective of this study was to evaluate herbage accumulation, plant-part composition (leaf blade, stem and dead material) and nutritive value on a monthly basis in signal grass pastures managed under various steady-state canopy heights mimicking continuous stocking management.

Materials and Methods

Experimental site

The research was carried out at APTA (Agência Paulista de Tecnologia dos Agronegócios) in Brotas, State of São Paulo, Brazil (21°59′ S, 47°26′ W; 650 masl). The climate at the site is a subtropical Cwa, according to the Köppen-Geiger classification (Peel et al. 2007). The experimental area was a 25-years-old pasture of signal grass cv. Basilisk. The soil at the site is a Quartzipsamment with 9% clay, 33% fine sand, 57% coarse sand and 1% silt. Chemical analysis in the 0–20 cm layer showed 5 mg P/dm^3 (P_{resin}), 3 mmol$_c$ Ca/dm^3, 3 mmol$_c$ Mg/dm^3, 2 mmol$_c$ K/dm^3, 42 mmol$_c$ H+Al/dm^3, 22 g OM/dm^3, 16% base saturation and $pH_{(CaCl2)}$ 4.2. Rainfall data were recorded by the Department of Environment of the City of Brotas, and the maximum and minimum monthly average temperatures were recorded at a weather station 45 km from the experimental site (Figure 1).

Treatments, experimental design and grazing management

Treatments included 3 canopy height targets, 10 (S-short), 17.5 (M-medium) and 25 cm (T-tall), set in a completely randomized design with 4 replications. On 24 October

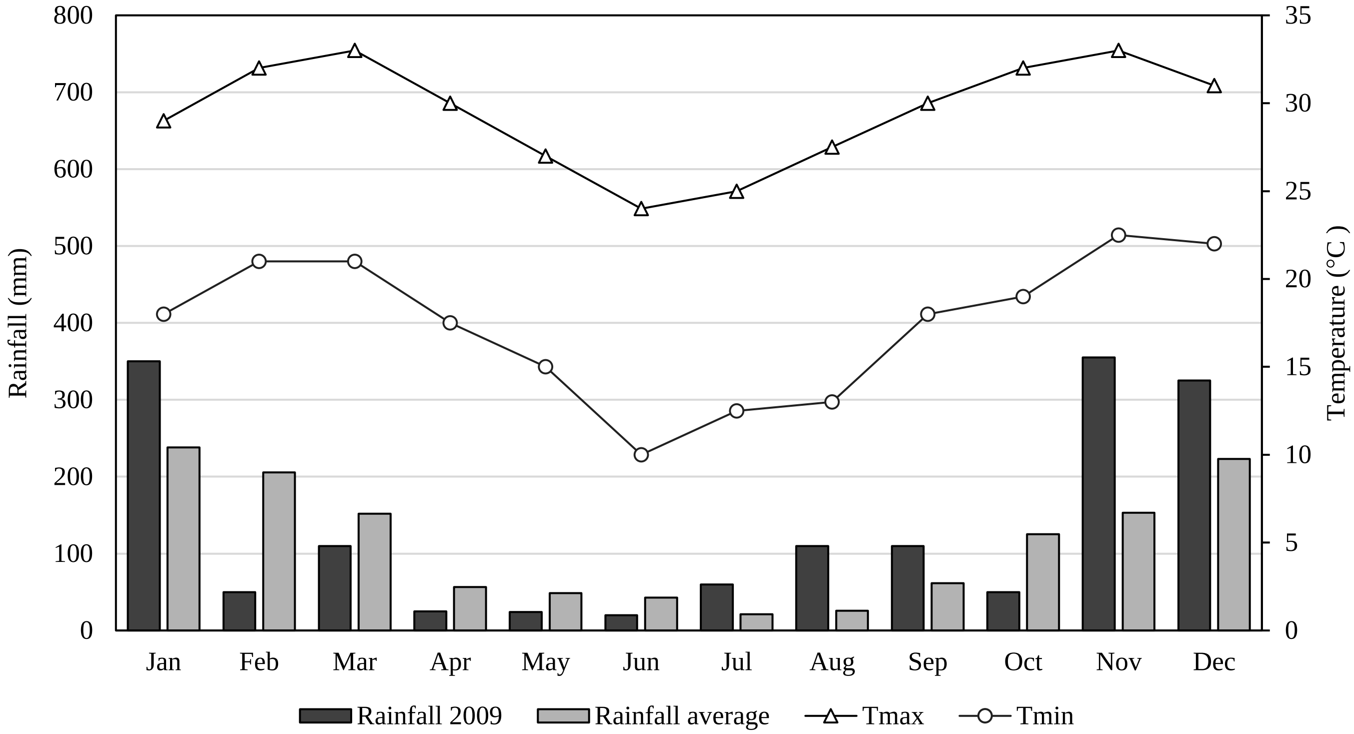

Figure 1. Monthly rainfall and temperatures during the experimental period (2009) and average long-term rainfall (1983–2006).

2008, the experimental area was mechanically clipped to reduce the canopy height to approximately 5 cm. In November 2008, dolomitic lime (0.98 t/ha) and single superphosphate (44 kg P/ha) were surface-applied to increase the base saturation to 40% and soil P to >15 mg/dm (P_{resin}), respectively. The area was divided by electric fence into 12 experimental plots measuring 144 m^2 (12 × 12 m) each. The experimental period was January 2009–January 2010. Mob stocking was used to impose defoliation on the experimental plots according to their respective treatments. Two crossbred steers, with mean live weight of 650 kg were used to graze each plot at each grazing event, always after overnight fast for solids. Defoliation mimicked continuous stocking, with animals assigned to pastures whenever canopy height was approximately 10% above target height. During the wet season, animals grazed pastures approximately once a week, while during the dry season the period between grazing events was 1–2 weeks. As paddocks were small, grazing events lasted for only 15 min to 2 h, and steers grazed non-experimental pastures when they were not grazing treatment pastures. Aiming not to decrease the height of pastures by more than 10% below the target, several visual observations and measurements of canopy height were made during grazing. Ammonium sulfate and potassium chloride were used to provide a total of 250 kg N + 210 kg K/ha during the course of the experiment. Fertilizers were split-applied in equal amounts on 24 December 2008, 7 February 2009, 10 March 2009, 4 May 2009 and 24 September 2009.

Measurements

Canopy height and herbage mass (HM) were evaluated using a rising plate meter (RPM). Periodic calibrations were necessary to correlate the RPM reading with canopy height and HM. The calibrations were made 6 times during the experimental period by selecting 2 sites (0.30 m^2) per plot, representing the extremes of height, i.e. the tallest and the shortest canopy areas. To measure canopy height a light-transparent acetate sheet (21 × 30 × 0.02 cm) was placed on top of the canopy and an RPM reading was taken. Herbage inside the quadrat (0.30 m^2) was then clipped at soil level (double sampling). The samples were dried in a forced-air oven for 72 h at 60 °C to estimate HM on a dry matter basis. Linear regression curves were established to estimate canopy height and HM as a function of the RPM readings. RPM readings were taken every 5 days in the wet season, and every 7 days in the dry season at 42 sites per plot in a systematic way following a grid-like pattern. All HM values were expressed on a dry matter basis and estimated from the RPM calibration derived from the double sampling sites. Light interception (LI) was evaluated monthly (except August) with an LAI-2000 canopy analyser (Li-COR, Lincoln, NE, USA) at 20 sites per plot, with a single reading above the canopy for every 5 readings near the base of the canopy.

Herbage accumulation rate (HAR) was estimated at 3 sites per plot which were protected from grazing using cylindrical exclusion cages (0.9 m diameter × 1.5 m in height) in 21-day cycles on average. Herbage accumulation inside the cages was estimated from RPM readings inside and outside the cages, and cages were repositioned at new sites in each plot for the following accumulation cycle. Herbage accumulation rate was estimated by the difference, as follows: HAR = ($HM_{last\ day}$ - $HM_{first\ day}$)/d, where $HM_{last\ day}$ = herbage mass inside the cages on the last day of exclusion, $HM_{first\ day}$ = herbage mass on the pasture on the first day of exclusion and d = number of days of the accumulation cycle. Tiller population density was measured every month at 3 random 0.2 × 0.5 m sites per plot inside a metal frame. Tillers were classified as basal, aerial or reproductive, allowing for the calculation of their participation on each evaluation date. Tillers were considered basal when originated from basal tissue buds, and aerial when originated from axillary buds of the main tiller. Tillers with visible inflorescence were classified as reproductive.

Forage samples were clipped monthly, except in August and September, at 3 sites per plot inside a 0.3 m^2 quadrat at soil level to evaluate plant-part composition. Sites that had been previously sampled were avoided in subsequent samplings. A subsample (~0.25 kg) was taken from each sample and separated into green leaf blades, green stems (true stems plus leaf sheaths) and dead material. Dead material was visually defined as senescent leaves and stems with >50% area of yellow or dry tissue. The samples were dried in a forced-air oven for 72 h at 60 °C to calculate the proportion of each plant-part component on a dry matter basis and then the amount of each based on HM estimates.

Forage nutritive value was estimated from hand-plucked samples taken monthly, except in September. The samples were collected from the top of the canopy after observation of the grazing behavior of the animals, and then dried in a forced-air oven for 72 h at 55 °C. They were then ground in a Wiley mill to pass a 1 mm screen and taken to the laboratory for chemical analyses. Ash concentration was determined by incineration at 600 °C. Crude protein (CP) concentration was calculated as N × 6.25, with N concentration determined using the Micro Kjeldahl method (AOAC 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were determined according to Van Soest et al. (1991). In vitro organic matter digestibility (IVOMD) was estimated according to the two-stage procedure of Tilley and Terry (1963) modified by Moore and Mott (1974).

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (Littell et al. 2006) using an auto-regressive first order covariance model. Canopy height, period and their interactions were considered fixed effects, and period was analyzed as a repeated measure (Littell et al. 2006). Treatment means were estimated using LS means and compared using the probability of the difference (PDIFF) by t-test (5%). Rising plate meter calibration curves were analyzed for their need for sorting by date or canopy height treatments using the covariance analysis of PROC GLM in SAS (SAS Institute 2002).

Results

Canopy height and herbage mass

The calibration curves between RPM and canopy height did not differ across treatments and dates of calibration (P>0.05). Consequently, canopy height was monitored with RPM readings using a single calibration curve (Figure 2). For HM prediction there was no effect of treatment (P>0.05), but there was an effect of calibration date (P<0.05), so results for HM (including HAR estimates) used 2 calibration curves covering 2 periods (January–February and March–December) (Figure 2). The response was positive and linear, and models were adjusted satisfactorily, both for canopy height (R^2 = 0.96) and HM, for the first 2 calibration dates (R^2 = 0.88) and for the last 4 dates (R^2 = 0.92). Monitoring canopy height throughout the year revealed some variation around the target heights, especially for M and T treatments (Figure 3). Canopy heights of S pastures were maintained near the target of 10 cm throughout the year, whereas M and T pastures tended to exceed the target heights (17.5 and 25 cm, respectively) during the months of greater plant growth such as January–March and November–December. The opposite happened during the dry season (May–September), when canopy height decreased to 20–25 cm in T pastures.

There was a height × month interaction effect on HM (P = 0.0024), although T canopies always had greater HM throughout the experimental period (2,590–4,010 kg/ha) than M (1,810–3,040 kg/ha) and S pastures (1,070–1,660 kg/ha) with smaller differences between M and T pastures (~700 kg/ha) in the dry season (May–September) (Figure 4A), similar to canopy height variation. Leaf mass also showed a height × month interaction (P<0.0001). Early in the year (January–April), leaf mass followed the order T>M>S pastures (Figure 4B) but there was no canopy height effect between May and October (P>0.05), followed by a greater leaf mass on M pastures, especially

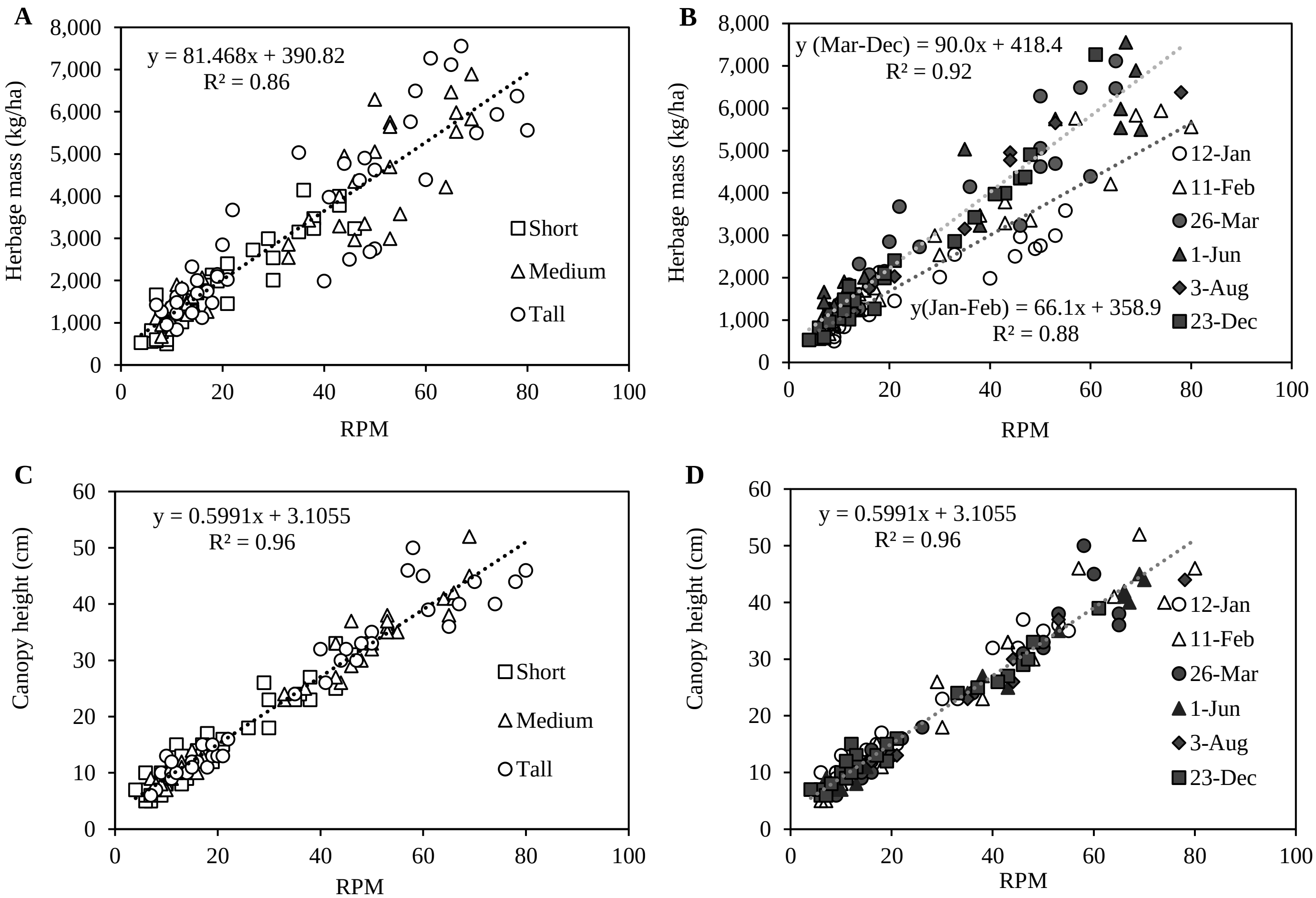

Figure 2. Prediction of herbage mass and canopy height as a function of rising plate meter (RPM) readings considering the effects of treatment (A and C) and date of calibration (B and D) in signal grass (*Urochloa decumbens*) cv. Basilisk pastures at 3 canopy heights in Brotas, SP, Brazil.

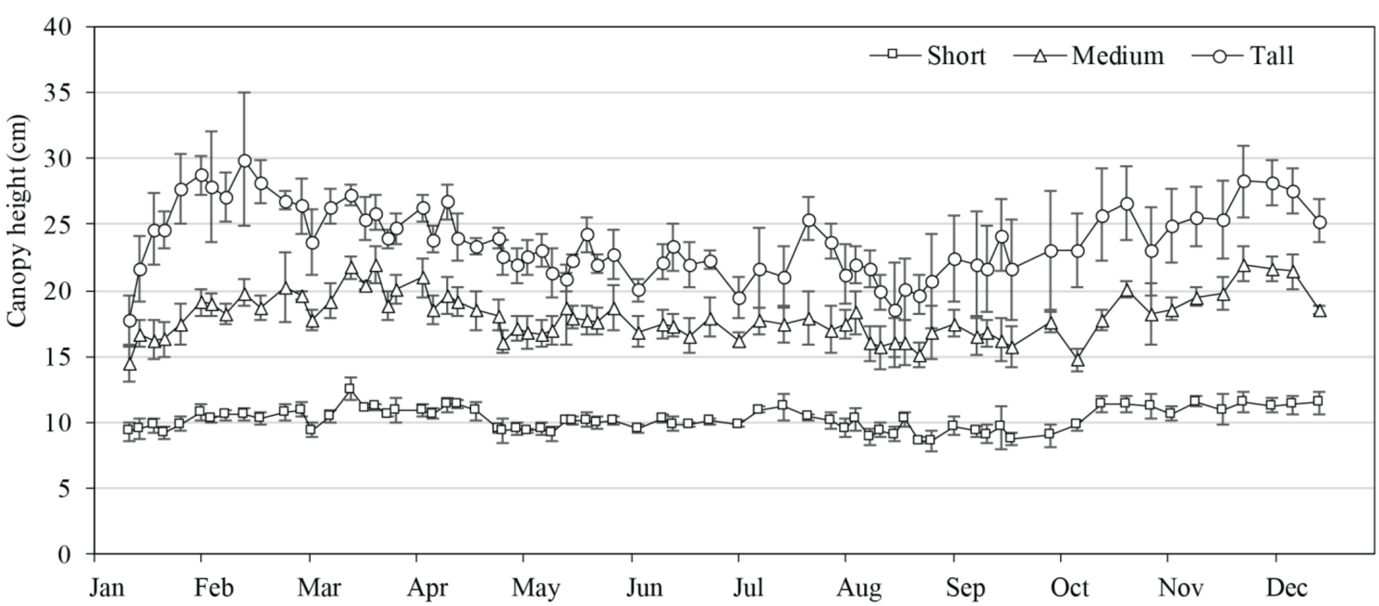

Figure 3. Actual canopy heights in signal grass (*Urochloa decumbens*) cv. Basilisk pastures while aiming at 3 target canopy heights (10 – short, 17.5 – medium and 25 – tall cm) in Brotas, SP, Brazil. Bars correspond to ± standard deviation.

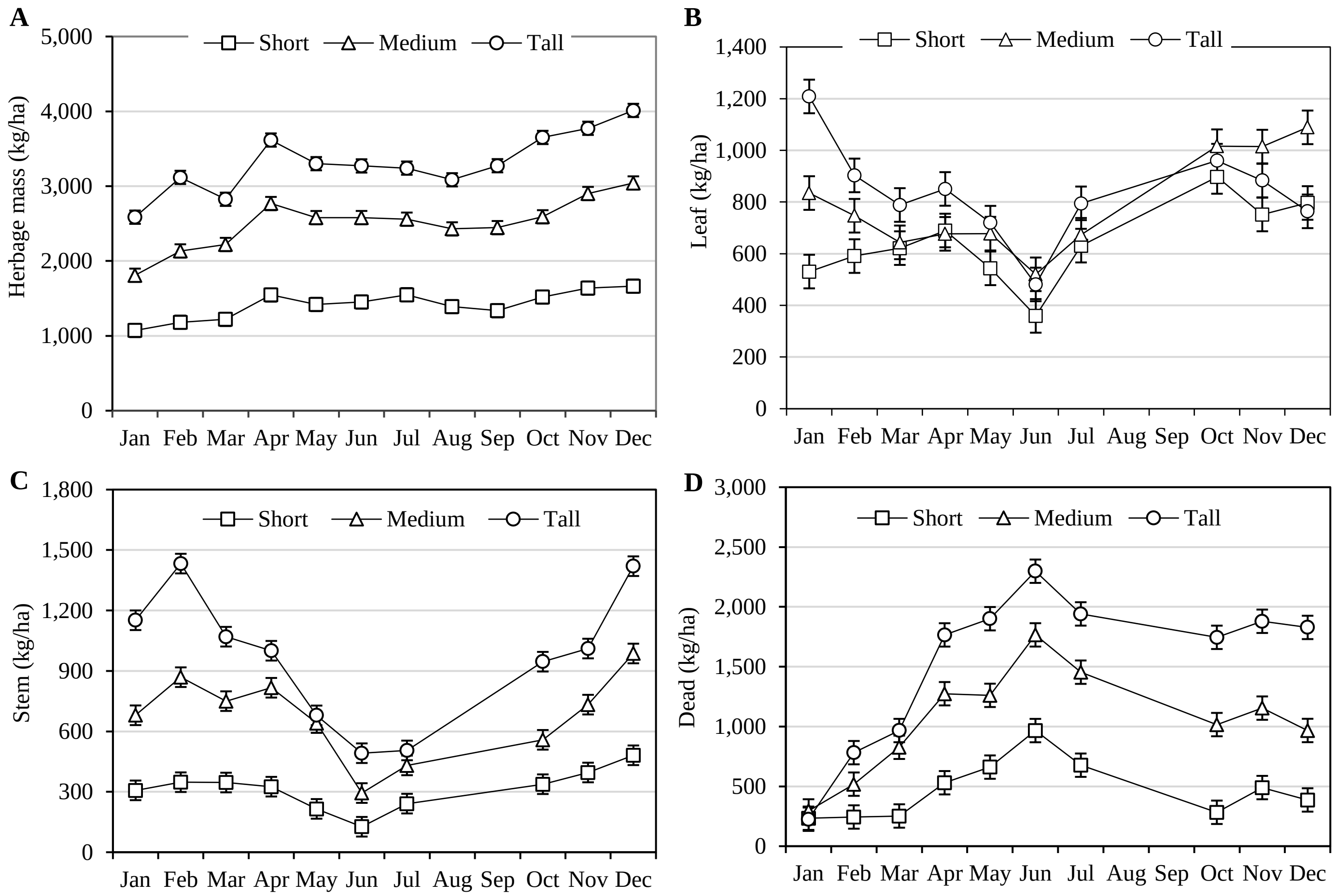

Figure 4. A) Herbage mass; B) leaf; C) stem; and D) dead material in signal grass (*Urochloa decumbens*) cv. Basilisk pastures maintained at 3 canopy heights in Brotas, SP, Brazil. Bars correspond to ± standard error of mean.

in December. A height × month interaction effect on stem mass (P<0.0001) was also observed. Tall pastures had the greatest stem mass, followed by M and S pastures (Figure 4C), with stem mass being greatest during January–April and October–December, especially in T and M pastures, but displaying much less variation in S pastures over the course of the year. Dead material mass displayed a height × month interaction (P<0.0001) but was greater in T pastures, followed by M and S pastures (Figure 4D). Mass of dead material increased during the first half of the year peaking in June, regardless of canopy height.

Herbage accumulation rate

Overall, HAR mirrored rainfall and temperature levels being high initially before dropping to near zero in May–June (Periods 4 and 5), then increasing to peak in December (Period 10) (Figure 5). Herbage accumulation rate was affected by a height × period interaction (P = 0.0005). In general, T pastures had greater HAR than M and S pastures, the advantage being most evident in Periods 2 and 3 (February–April) and Periods 8, 9 and 10 (September–December). During the dry season (May–August; Periods 4–7), there was no difference among treatments (P>0.05).

Tiller population density and light interception

Time of year was the only factor affecting total tiller population density (P<0.0001), and the increase in tiller numbers in February and March was largely an increase in aerial tillers (Figure 6). Aerial tiller numbers were affected by a height × month interaction (P<0.0045) as S pastures had fewer aerial tillers only in February and March (440 and 145 tillers/m^2, respectively) than M and T pastures (626 and 317 tillers/m^2, respectively). There were effects of month (P<0.0001) and height (P = 0.0474) on basal tiller population density as M pastures had more than T pastures (1,340 vs. 1,250 tillers/m^2, respectively). Reproductive tiller numbers displayed a height × month interaction (P<0.0001) and were present mainly between

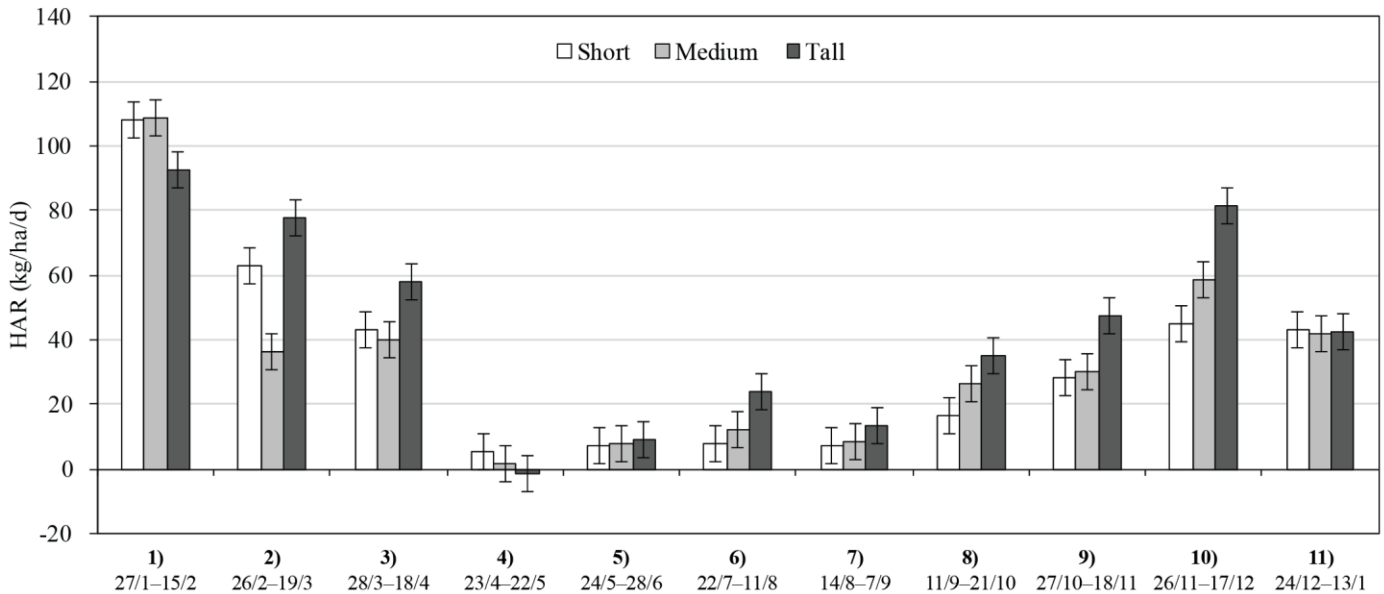

Figure 5. Daily herbage accumulation rates (HAR) during Periods 1–11 in signal grass (*Urochloa decumbens*) cv. Basilisk pastures maintained at 3 canopy heights in Brotas, SP, Brazil. Bars correspond to ± standard error of mean.

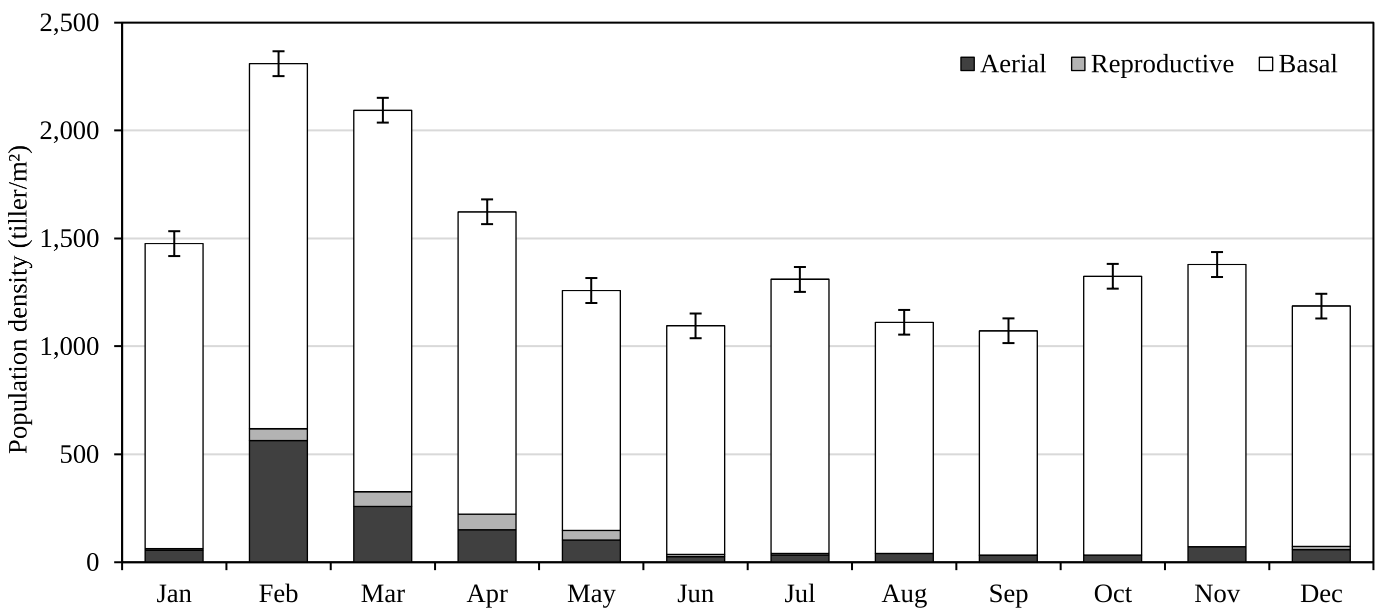

Figure 6. Population density of various tiller categories in signal grass (*Urochloa decumbens*) cv. Basilisk pastures maintained at 3 canopy heights in Brotas, SP, Brazil. Bars correspond to ± standard error of mean.

February and May, with S pastures having fewer of them in this period (38 tillers/m^2) followed by M (67 tillers/m^2) and T (74 tillers/m^2) pastures.

There was a height × month interaction effect (P<0.0001) on LI. Light interception was more variable in S pastures, peaking at 90% in June and decreasing to 50% in September (Figure 7). LI in M and T pastures was relatively stable throughout, fluctuating between 90 and 100%.

Forage nutritive value

Crude protein concentration was affected by a height × month interaction (P<0.0001). CP% in S pastures was greater than in M and T pastures in January, February, May, June and October (Figure 8A), with no differences in the other months. After peaking in October (130–170 g/kg), mean CP concentration decreased to approximately

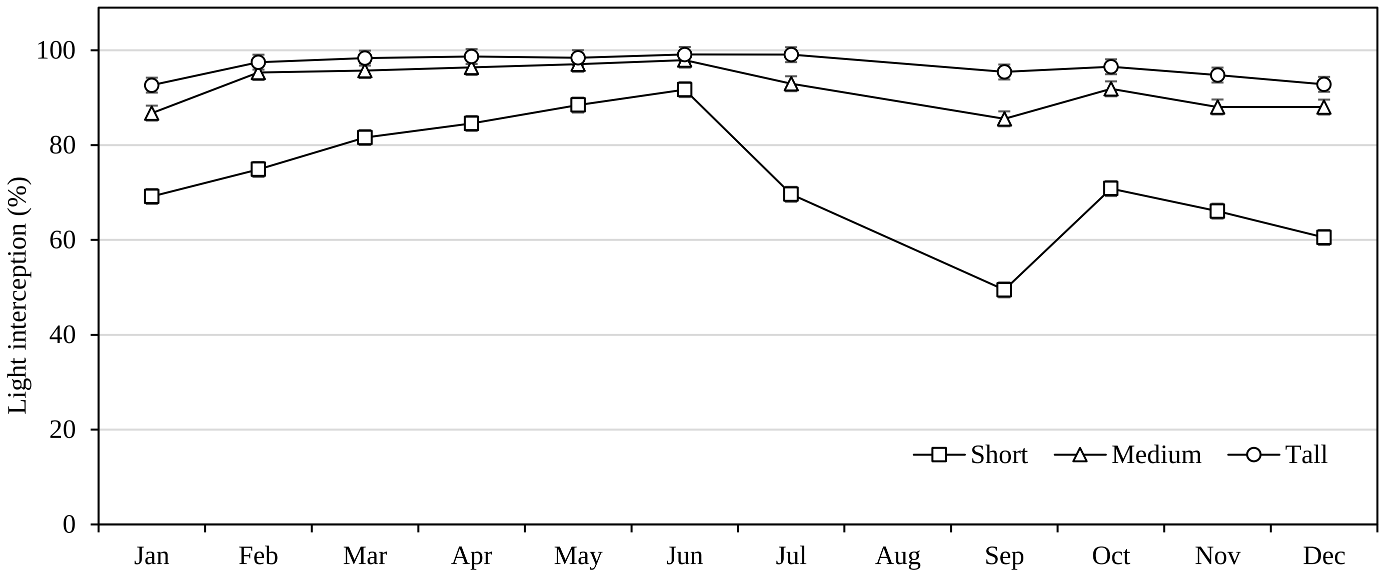

Figure 7. Light interception in signal grass (*Urochloa decumbens*) cv. Basilisk pastures maintained at 3 canopy heights in Brotas, SP, Brazil. Bars correspond to ± standard error of mean.

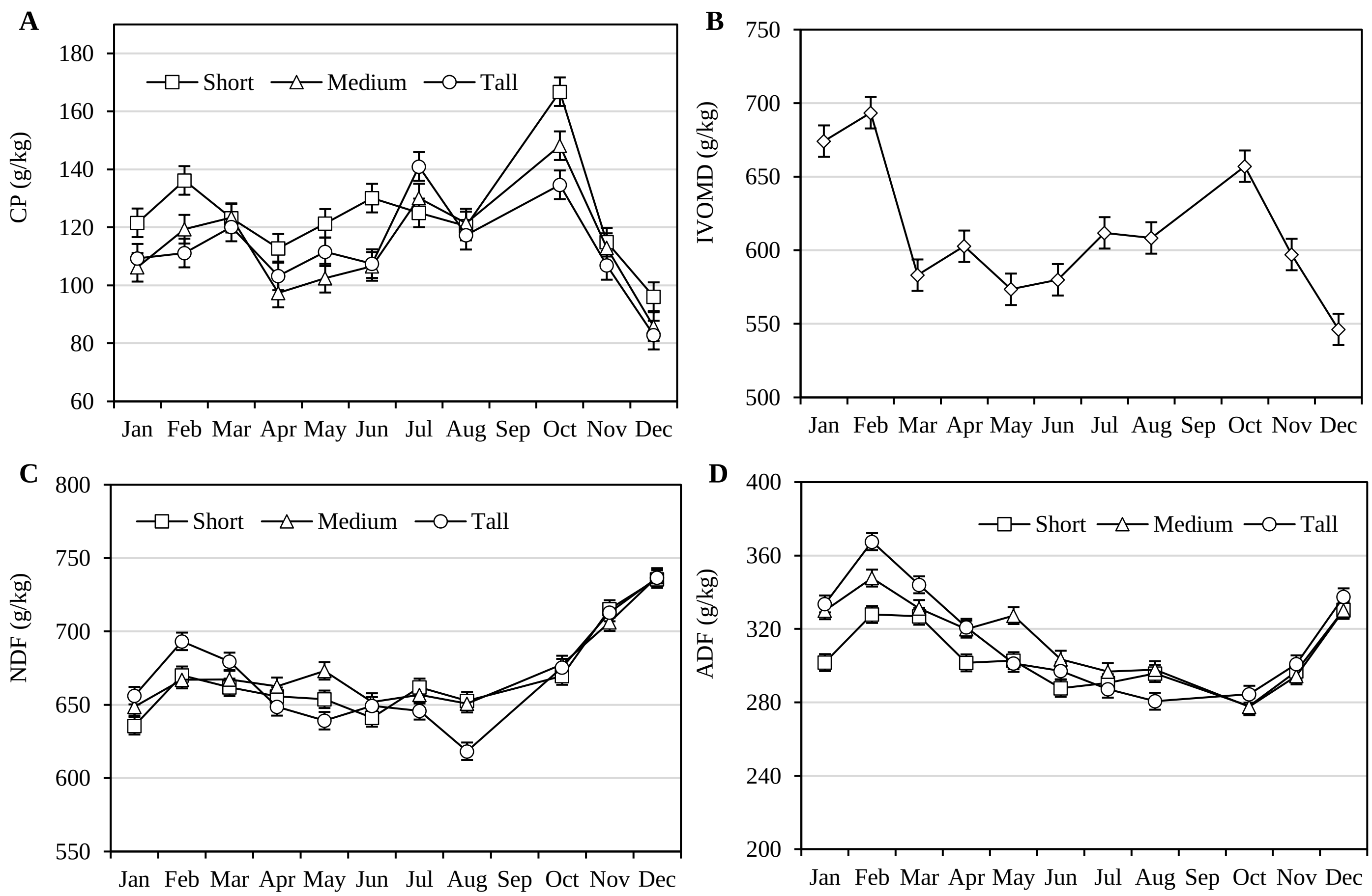

Figure 8. (A) Crude protein (CP) concentration; (B) In vitro organic matter digestibility (IVOMD); (C) Neutral detergent fiber (NDF) concentration; and (D) Acid detergent fiber (ADF) concentration of signal grass (*Urochloa decumbens*) cv. Basilisk maintained at 3 canopy heights in Brotas, SP, Brazil. Bars correspond to ± standard error of mean.

110 and 90 g/kg in November and December, respectively.

Time of year had the greatest effect on IVOMD (P<0.0001), which peaked in January-February and again in October, before declining sharply in November and December (Figure 8B). Both neutral detergent fiber and acid detergent fiber concentrations were affected by a height × month interaction (P<0.0001). Both tended to peak in February before declining slowly until August–October before increasing to December (Figures 8C and 8D).

Discussion

Steady-state canopy heights did not ensure a constant plant-part component mass within treatments throughout the year, regardless of canopy height. There was a decrease in leaf and stem mass from January to June, especially in M and T pastures, followed by an increase in dead material, even for the S pastures. This process peaked at the end of the wet season likely due to increased tissue senescence triggered by the imminent soil water deficit during this period (Figure 1). In the second half of the year, dead material mass remained relatively constant showing differences among treatments and greater senescence in T pastures, followed by M and S pastures. As expected, S pastures had less HM (leaf + stem + dead material) than M and T pastures and less stem and dead material, as also observed by Santos et al. (2010) in 4 areas of signal grass grazed at 10, 20, 30 and 40 cm canopy height during the wet season.

As expected, mean HAR varied seasonally and greater values were recorded during the warm and wet periods (January–April and September–December). During the cooler months (May–August; Periods 4–7), HAR was near zero because the daily minimum temperatures, i.e. under 15 °C (Figure 1), during this period are probably close to the threshold values that restrict the growth of C_4 species (Silva et al. 2012). The partially greater HAR in T pastures can be attributed to the additional amount of leaves, i.e. LAI, and plant reserves normally associated with the greater shoot and root plant structures (Donaghy and Fulkerson 1998). The advantage of T pastures was particularly significant in the transition between the dry and wet seasons (September–November). The positive effect of greater canopy height on HAR was similar to that reported for Xaraés palisade grass (*Urochloa brizantha*) pastures managed at 15, 30 and 45 cm (Euclides et al. 2010). On the other hand, Euclides et al. (2010) reported a negative effect of height on HAR of Marandu palisade grass, even though in both trials HAR differences were minimal for different canopy heights. This similarity is expected for a given range of canopy conditions, and sometimes the contrasting canopy heights do not result in contrasting HAR values (Bircham and Hodgson 1983). Since short canopies have small tillers with greater population density (Sbrissia and Silva 2008), the photosynthetic apparatus, i.e. leaf area index, could partially compensate. In the current study, however, differences in tiller population density were small and this compensation was not evident.

Considering the low proportion of reproductive tillers, all treatments inhibited flowering, a positive effect in terms of livestock performance considering the negative impact of reproductive tillers on stem proportion, food intake and diet quality (Benvenutti et al. 2008). Under our management system, production of reproductive tillers was concentrated between February and May, although signal grass typically concentrates its flowering between December and January at this latitude. There was a strong seasonal variation in total tiller population density, which reached a maximum of 2,300 tillers/m^2 in February and decreased to ~1,100 tillers/m^2 between June and September, although basal tiller numbers were less variable (1,000–1,700 tillers/m^2). According to Portela et al. (2011) in signal grass pastures managed under rotational stocking, there was less variation than in the current study in tiller density throughout the year and the range was 1,100–1,500 basal tillers/m^2, with pastures managed at heavier grazing intensity (5 cm post-grazing canopy height) having 10–20% more basal tillers.

Plants in T pastures were larger and probably invested more energy in maintenance respiration. Under environmental restrictions, this could result in negative HAR, as observed in Period 4 of the current study (Figure 5). This was reported by Silva et al. (2013) in palisade grass pastures grazed at >30 cm canopy height during the dry season. Santos et al. (2013) also reported negative HAR, i.e. death of plant parts was greater than the growth of new plant tissue, in signal grass pastures maintained at 25 cm canopy height in the dry season. In the current study, the onset of the wet season in spring resulted in greater HAR in T pastures (Periods 8–10; Figure 5), similar to the findings of Silva et al. (2013) at the same time of year. This greater herbage accumulation is important in forage-based systems in central Brazil because feed supply at the end of the dry season can become critically low and a rapid growth response by the pasture with the return of wet season conditions helps buffer the roughage demand on the farm. Using an alternative grazing scheme, Santos et al. (2013) temporarily lowered canopy height to 15 cm in the early dry season, which boosted HAR in the following spring compared with a pasture kept at 25 cm, in contrast with results of the current study.

There have been many studies on the effects of LI on herbage accumulation dynamics of tropical grasses, especially in rotationally stocked pastures, e.g. Pedreira et al. (2017) and Moura et al. (2017). In general, maximum canopy light interception (~95–100% LI) modifies plant-part growth, shifting the primary leaf accumulation to a less desirable stem elongation, especially in well-fertilized pastures. For this reason the 95% LI criterion has been proposed as the moment when the rest/regrowth period should be terminated in intermittent (rotational and its variations) grazing schemes (Carnevalli et al. 2006). By associating LI with canopy height it may be possible to recommend specific grazing management targets for different forage species and cultivars. However, the phenotypic plasticity of species such as signal grass can lead over time to a more prostrate plant architecture, which in turn can modify the established association between canopy LI and height (Braga et al. 2006; Pedreira et al. 2017). In the current study, LI values measured in M and T pastures were close to the ceiling (95–100%) in the first half of the year, whereas in the second half, LI approached 95% for T pastures and 90% for M pastures. These values fall within the range of canopy LIs and canopy heights observed by Pedreira et al. (2017) for signal grass pastures. The large LI values were probably related to the presence of dead leaves near the bottom of the canopy as observed by Braga et al. (2006), even for the M pastures kept at shorter canopy heights. Although T pastures displayed greater HAR than shorter pastures (M and S), the plant-part composition profile changed at the end of the experimental period (November and December) and M pastures showed more leaf than S and T pastures. At the same time, T pastures showed increasing stem mass and less leaf mass. As signal grass usually starts flowering at this time of the year, more lenient grazing could favor the development of stems as opposed to leaves. If excessive stem elongation occurs, additional effort may be needed to maintain canopy height, something we did not achieve in the current study, even in the taller canopies. The control of canopy structure is important because the presence of mature stems may lead to decreased harvest efficiency by livestock associated with rejected patches and lodging, a process that becomes more important as the intensity of use of the pasture, i.e. fertilizer application, irrigation, etc., increases.

Forage nutritive value in S pastures was greater than in M and T pastures, although no differences in IVOMD were recorded. On the other hand, higher CP concentration and lower ADF concentration, especially in the first half of the year, were recorded in S pastures. The higher grazing intensity in S pastures combined with greater defoliation frequency led to greater tissue renewal and predominance of younger plant tissues with lower proportion of cell wall components such as lignin, minimizing potential negative consequences for diet nutritive value. As observed by Hernández Garay et al. (2004) and Sollenberger and Vanzant (2011), CP% and digestibility increase with heavier grazing management as a consequence of forage utilization increase and its effect on forage maturity and leaf proportion. This greater forage nutritive value in S pastures would normally result in better animal performance. However, this might not occur, because performance depends on forage intake, also regulated by herbage allowance (Herling et al. 2011; Sollenberger and Vanzant 2011). Short canopies may be associated with smaller herbage allowance and, if taken to extreme levels, may lead to overgrazing, limiting forage intake by bite size restrictions and/or insufficient grazing time to satisfy appetite (Silva et al. 2013). At the end of the year (October–December) forage nutritive value declined, regardless of treatment. This may be associated with N fertilizer application, which in the second half of the year happened only in September, reducing grazing frequency and plant tissue renewal at the end of the experimental period, and consequently lowering overall forage nutritive value. Regardless of treatment, however, the nutritive value of signal grass can be considered satisfactory since CP concentrations in hand-plucked samples which mimicked animal selection, for example, remained above 100 g/kg for most of the year, and IVOMD above 600 g/kg, similar to levels observed by Silva et al. (2013) in palisade grass pastures grazed at 4 canopy heights (10, 20, 30 and 40 cm).

The steady-state canopy allows not only better harvest efficiency, but also results in forage of greater nutritive value compared with pastures that are managed without a canopy-based criterion (Nave et al. 2010). Conversely, adhering to a canopy condition and the intensification of harvest efficiency during the warm wet season does not allow for a build-up of forage of lower nutritive value to be consumed in the cool and dry season, when forage mass usually does not meet the livestock demand (stockpiling or deferred grazing). Producers often use lax grazing intensity in the wet season, especially in low-input forage-livestock systems in order to accomplish this. In contrast, in intensive, high-input grazing systems, such as the one represented in the current study, it may be advantageous to efficiently harvest forage of greater nutritive value, maximizing the animal output in terms of both performance, i.e. daily weight gain, milk production, etc., and pasture carrying capacity, to justify the high production costs, mainly where land is expensive. At the same time, to deal with a shortage of forage on offer during the dry season in central Brazil, feeding options such as stockpiling, protein and/or energy supplements or mixed grass-legume pastures may be required.

Despite expectations to the contrary, the short canopy management (~10 cm) in signal grass pastures was not detrimental to forage productive vigor (in terms of HAR) during the wet season. In addition, leaf mass was almost the same as in M and T pastures throughout the experimental period. However, this may not be true in the long term, especially without fertilizer application. The S and M pastures produced forage with greater nutritive value, i.e. higher CP and lower fiber concentrations, than T pastures. However, with the onset of the wet season, HAR of T pastures exceeded those of S and M pastures, suggesting an advantage in maintaining signal grass at ~25 cm in order to ensure rapid growth response in spring if pastures were continuously stocked.

The results of the present study have shown the phenotypic plasticity of signal grass under a mimicked continuous stocking condition with no clear evidence of stand decline or loss of vigor, i.e. severe reduction of yield and/or tiller density, in the S pastures. How this situation will apply under long-term grazing remains to be answered. Grazing target recommendations for optimal animal performance still need to be developed, but it is expected that keeping canopy height in signal grass pastures between 15 and 25 cm should ensure maximum animal production, as a result of quality of available forage being maintained at a high level. Testing of this hypothesis commercially and recording animal performance is warranted.

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References

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AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis. 15th Edn. AOAC Inc., Arlington, VA, USA.

Benvenutti MA; Gordon IJ; Poppi DP; Crowther R; Spinks W. 2008. Foraging mechanics and their outcomes for cattle grazing reproductive tropical swards. Applied Animal Behaviour Science 113:15–31. doi: 10.1016/j.applanim.2007.10.005

Bircham J; Hodgson J. 1983. The influence of sward condition on rates of herbage growth and senescence in mixed swards under continuous stocking management. Grass and Forage Science 38:323–331. doi: 10.1111/j.1365-2494.1983.tb01656.x

Braga GJ; Pedreira CGS; Herling VR; Luz PHC; Lima CG. 2006. Sward structure and herbage yield of rotationally stocked pastures of 'Marandu' palisadegrass [*Brachiaria brizantha* (A. Rich.) Stapf] as affected by herbage allowance. Scientia Agricola 63:121–129. doi: 10.1590/S0103-90162006000200003

Braga GJ; Portela JN; Pedreira CGS; Leite VBO; Oliveira EA. 2009. Herbage yield in Signalgrass pastures as affected by grazing management. South African Journal of Animal Science 39:130–132. doi: 10.4314/sajas.v39i1.61168

Carnevalli RA; Silva SC da; Bueno AAO; Uebele MC; Bueno FO; Hodgson J; Silva GN; Morais JPG. 2006. Herbage production and grazing losses in *Panicum maximum* cv. Mombaça under four grazing managements. Tropical Grasslands 40:165–176. goo.gl/EilPlN

Donaghy DJ; Fulkerson WJ. 1998. Priority for allocation of water-soluble carbohydrate reserves during regrowth of *Lolium perenne*. Grass and Forage Science 53:211–218. doi: 10.1046/j.1365-2494.1998.00129.x

Euclides VPB; Valle CB do; Macedo MCM; Almeida RG de; Montagner DB; Barbosa RA. 2010. Brazilian scientific progress in pasture research during the first decade of XXI century. Revista Brasileira de Zootecnia 39:151–168. doi: 10.1590/S1516-35982010001300018

Herling VR: Pedreira CGS; Luz PHC de; Braga GJ; Marchesin WA; Macedo FB; Lima CG de. 2011. Performance and productivity of Nellore steers on rotationally stocked palisadegrass (*Brachiaria brizantha*) pastures in response to herbage allowance. The Journal of Agricultural Science 149:761–768. doi: 10.1017/S0021859611000116

Hernández Garay A; Sollenberger LE; McDonald DC; Ruegsegger GJ; Kalmbacher RS; Mislevy P. 2004. Nitrogen fertilization and stocking rate affect stargrass pasture and cattle performance. Crop Science 44:1348–1354. doi: 10.2135/cropsci2004.1348

Hodgson J; Silva SC da. 2002. Options in tropical pasture management. Proceedings of Annual Meeting of the Brazilian Animal Science Society, Recife, Brazil, 29 July–1 August 2002. p. 180–202.

Korte CJ; Watkin BR; Harris W. 1982. Use of residual leaf area index and light interception as criteria for spring-grazing management of a ryegrass-dominant pasture. New Zealand Journal of Agricultural Research 25:309–319. 10.1080/00288233.1982.10417892

Littell RC; Milliken GA; Stroup WW; Wolfinger RD; Schabenberger O. 2006. SAS for mixed models. 2nd Edn. SAS Institute Publishing, Cary, NC, USA.

Moore JE; Mott GO. 1974. Recovery of residual organic matter from in vitro digestion of forages. Journal of Dairy Science 57:1258–1259. doi: 10.3168/jds.S0022-0302(74)85048-4

Moura AM; Tomich TR; Pereira LGR; Teixeira AM; Paciullo DSC; Jayme DG; Machado FS; Gomide CAM; Campos MM; Chaves AV; Gonçalves LC. 2017. Pasture productivity and quality of *Urochloa brizantha* cultivar Marandu evaluated at two grazing intervals and their impact on milk production. Animal Production Science 57:1384–1391. doi: 10.1071/AN16715

Nave RLG; Pedreira CGS; Pedreira BC. 2010. Nutritive value and physical characteristics of Xaraes palisadegrass as affected by grazing strategy. South African Journal of Animal Science 40:285–293. doi: 10.4314/sajas.v40i4.65236

Pedreira CGS; Braga GJ; Portela JN. 2017. Herbage accumulation, plant-part composition and nutritive value on grazed signal grass (*Brachiaria decumbens*) pastures in response to stubble height and rest period based on canopy light interception. Crop and Pasture Science 68:62–73. doi: 10.1071/CP16333

Peel MC; Finlayson BL; McMahon TA. 2007. Updated world map of the Köppen–Geiger climate classification. Hydrology and Earth System Sciences 11:1633–1644. doi: 10.5194/hess-11-1633-2007

Portela JN; Pedreira CGS; Braga GJ. 2011. Demografia e densidade de perfilhos de capim-braquiária sob pastejo em lotacão intermitente. Pesquisa Agropecuária Brasileira 46:315–322. doi: 10.1590/S0100-204X2011000300013

Rao IM; Kerridge PC; Macedo MCM. 1996. Nutritional requirements of *Brachiaria* and adaptation to acid soils. In: Miles JW; Maass BL; Valle CB do; Kumble V, eds. *Brachiaria*: Biology, agronomy, and improvement. CIAT Publication No. 259. International Center for Tropical Agriculture (CIAT), Cali, Colombia. p. 53–71. hdl.handle.net/10568/82025

Santos MER; Fonseca DM da; Silva GP; Pimentel RM; Carvalho VV de; Silva SP da. 2010. Estrutura do pasto de capim-braquiária com variação de alturas. Revista Brasileira de Zootecnia 39:2125–2131. doi: 10.1590/S1516-35982010001000004

Santos MER; Fonseca DM da; Gomes VM 2013. Forage accumulation in brachiaria grass under continuous grazing with single or variable height during the seasons of the year. Revista Brasileira de Zootecnia 42:312–318. doi: 10.1590/S1516-35982013000500002

SAS Institute. 2002. SAS, Version 9.0. SAS Institute Publishing, Cary, NC, USA.

Sbrissia AF; Silva SC da. 2008. Compensação tamanho/densidade populacional de perfilhos em pastos de capim-marandu. Revista Brasileira de Zootecnia 37:35–47. doi: 10.1590/S1516-35982008000100005

Silva EA da; Silva WJ da; Barreto AC; Oliveira Jr. AB de; Paes JMV; Ruas JRM; Queiroz DS. 2012. Dry matter yield, thermal sum and base temperatures in irrigated tropical forage plants. Revista Brasileira de Zootecnia 41:574–582. doi: 10.1590/S1516-35982012000300014

Silva SC da; Gimenes FMA; Sarmento DOL; Sbrissia AF; Oliveira DE; Hernandez-Garay A; Pires AV. 2013. Grazing behaviour, herbage intake and animal performance of beef cattle heifers on Marandu palisade grass subjected to intensities of continuous stocking management. The Journal of Agricultural Science 151:727–739. doi: 10.1017/S0021859612000858

Sollenberger LE; Vanzant ES. 2011. Interrelationships among forage nutritive value and quantity and individual animal performance. Crop Science 51:420–432. doi: 10.2135/cropsci2010.07.0408

Tilley JMA; Terry RA. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass and Forage Science 18:104–111. doi: 10.1111/j.1365-2494.1963.tb00335.x

Van Soest PJ; Robertson JB; Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583–3597. doi: 10.3168/jds.S0022-0302(91)78551-2

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Research Paper

Spectral sensors prove beneficial in determining nitrogen fertilizer needs of *Urochloa brizantha* cv. Xaraés grass in Brazil

Evaluación del beneficio de los sensores espectrales para determinar los requerimientos de nitrógeno en pasturas de Urochloa brizantha *cv. Xaraés en Brasil*

HELIZANI C. BAZAME[1], FRANCISCO A.C. PINTO[2], DOMINGOS S. QUEIROZ[3], DANIEL M. DE QUEIROZ[2] AND DANIEL ALTHOFF[2]

[1]*Departamento de Engenharia de Biossistemas, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, SP, Brazil.* leb.esalq.usp.br
[2]*Departamento de Engenharia Agrícola, Universidade Federal de Viçosa, Viçosa, MG, Brazil.* dea.ufv.br
[3]*Empresa de Pesquisa Agropecuária de Minas Gerais, Viçosa, MG, Brazil.* epamig.br

Abstract

The objective of the present work was to evaluate the use of spectral sensors to determine nitrogen fertilizer requirements for pastures of *Urochloa brizantha* cv. Xaraés in Brazil. The experimental design was a randomized block design with 4 replications of 4 treatments: a control treatment (T_T) without application of N; a reference treatment (T_R) with N applied at a standard predetermined fixed rate (150 kg urea/ha/cycle); a treatment using GreenSeeker™ (T_G) to determine N requirement by the canopy normalized difference vegetation index (NDVI); and a treatment using SPAD 502 (T_S) to determine N requirement by foliar chlorophyll assessment. For treatments involving spectral sensors, N fertilizer was applied at half the rate of that in the reference treatment at the beginning of each cycle and further N was applied only when the nitrogen sufficiency index dropped below 0.85. The sensors used in the work indicated that no additional N fertilizer was required by these pastures above the half rates applied. Applying N at the reduced rates to the pastures was more efficient than the pre-determined fixed rate, as both sensor treatments and the fixed rate treatment produced similar total forage yields, with similar crude protein concentrations. All fertilized pastures supported similar stocking rates, while the sensor treatments used less N fertilizer, i.e. 75 kg urea/ha/cycle less than the reference plot. Longer-term studies to verify these findings are warranted followed by promotion of the technology to farmers to possibly reduce fertilizer application rates, improve profitability and provide environmental benefits.

Keywords: GreenSeeker™, pasture support capacity, precision agriculture, Spad 502, tropical grasses.

Resumen

El objetivo del trabajo fue evaluar el uso de sensores espectrales para determinar los requerimientos de fertilizantes nitrogenados en pasturas de *Urochloa brizantha* cv. Xaraés, Para el efecto en Leopoldina, Minas Gerais, Brasil, en un diseño experimental de bloques al azar con 4 repeticiones se evaluaron, durante 3 ciclos de rebrote del pasto y subsiguiente pastoreo, los tratamientos: (1) control (T_T) sin aplicación de N; (2) de referencia (T_R) con aplicación de N al inicio de cada ciclo en dosis fija predeterminada estándar (150 kg de urea/ha/ciclo); (3) uso del sensor GreenSeekerTM (T_G) para determinar el requerimiento de N por el índice de vegetación de diferencia normalizada (NDVI); y (4) uso del sensor SPAD 502 (T_S) para determinar el requerimiento de N por evaluación foliar de clorofila. Para los tratamientos con sensores espectrales, el

Correspondence: Helizani Couto Bazame, Departamento de Engenharia de Biossistemas, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Av. Pádua Dias, 11 – Piracicaba, CEP 13418-900, SP, Brazil.
E-mail: helizanicouto@gmail.com

fertilizante nitrogenado se aplicó usando la mitad de la tasa del tratamiento de referencia al comienzo de cada ciclo, con aplicación de N adicional solo cuando el índice de suficiencia de nitrógeno era menor que 0.85. Los sensores utilizados mostraron que las pasturas no requerían fertilizante adicional por encima de las tasas medias de N aplicadas. La aplicación de N a las pasturas usando las tasas reducidas fue más eficiente que la tasa fija predeterminada, ya que los tratamientos con sensores y el tratamiento de tasa fija produjeron rendimientos totales de forraje similares, con concentraciones de proteína cruda similares. Todas las pasturas fertilizadas soportaron cargas animal similares, mientras que los tratamientos con sensores demandaron menos fertilizante de N, i.e. 75 kg de urea/ha/ciclo menos que la parcela de referencia. Se justifican estudios a más largo plazo para verificar estos resultados, seguidos de la promoción de la tecnología a los agricultores para posiblemente reducir las tasas de aplicación de fertilizantes, mejorar la rentabilidad y proporcionar beneficios ambientales.

Palabras clave: Agricultura de precisión, capacidad de carga, GreenSeeker^{TM}, pastos tropicales, Spad 502.

Introduction

One important characteristic of Brazilian livestock systems is the raising of pasture-fed cattle (Ferraz and Felício 2010), which is regarded as one of the most economical ways to produce beef and milk (Carvalho et al. 2009; Deblitz 2009). In this scenario, factors such as climatic conditions and availability of nutrients in the soil must be considered for the adequate development of the pastures (Fernandes et al. 2015). Farmers must be aware that low availability of nutrients in soils can result in low production and quality of tropical forage (Hare et al. 2015). Determining optimal levels of fertilizer to apply to pastures is critical for maintaining a sustainable business both financially and biologically.

Among the essential nutrients, nitrogen (N) is considered the most important for plant growth and increasing crop yields (Subbarao et al. 2013; Cecato et al. 2014). Nitrogen is present in the amino acids that act in the synthesis of structural and functional proteins (Barbieri et al. 2017), and directly involved within the photosynthetic process due to its participation in the chlorophyll molecule. It also increases tillering and improves the nutritional value of pastures (Marques et al. 2016).

A number of precision farming techniques have been developed to address the spatial variability of nutrients in crop fields more effectively (Hedley 2015). These techniques consist of site-specific management of agricultural crops based on information from each location. Among the techniques of precision agriculture, spectral sensors have been used widely to obtain data which may be related to agronomic characteristics of the crops (Handcock et al. 2016; Wachendorf et al. 2018; Viana et al. 2019).

Minolta's indirect chlorophyll meter, SPAD (Soil-Plant Analyses Development) 502, is an example of such sensors. The SPAD 502 quantitatively evaluates the intensity of the green color in leaves by measuring light transmitted at 650 nm, where light absorption by the chlorophyll molecule occurs, and at 940 nm, where absorption ceases (Nogueira et al. 2018). Intensity of green color in leaves is an indication of the level of N taken up by the plant. Another example of use of sensors for site-specific N management is the GreenSeeker^{TM}. This sensor uses radiation emission diodes at the red (650 nm) and near infrared (770 nm) wavelengths over the vegetation canopy, providing the normalized difference vegetation index (NDVI) (Bredemeier et al. 2013).

The GreenSeeker^{TM} seems to present some advantages when compared with the SPAD 502. Since the GreenSeeker^{TM} is a canopy sensor, it has a wider field of vision and is able to cover a larger area of study, integrating information about the vegetation as a whole (Chapman and Barreto 1997).

The use of spectral sensors in the management of nitrogen fertilizer application has become a promising technique for farmers seeking practical, easily applied and reliable methods for pasture management. Research has shown that measurements with SPAD and the NDVI index correlate with plant nitrogen concentration in tissue and/or yield of various crops, including the osier (Daniel et al. 2016), Japanese cucumber (Pôrto et al. 2014), irrigated rice (Pocojeski et al. 2015), wheat (Theago et al. 2014), potato (Giletto and Echeverría 2016), cotton (Lee et al. 2009), forest species (Ribeiro et al. 2009) and forage (Bravin and Oliveira 2014; Villar et al. 2015; Corrêdo et al. 2019). We designed this study to evaluate the use of spectral sensors for determining desirable levels of nitrogen fertilizer application to *Urochloa brizantha* cv. Xaraés pastures.

Materials and Methods

The study was carried out in an experimental field, located in Leopoldina, Minas Gerais, Brazil (21º28'25'' S, 42º43'15'' W; 187 masl), during the period April–August 2017. According to the climatic classification of Köppen, the climate type of the region is Aw, tropical humid with dry winters and rainy summers, with average temperature of the coldest month being above 18 ºC. Soil chemical properties in the 0–20 cm layer of the experimental area are described in Table 1.

Table 1. Soil chemical properties and nutrients before mineral fertilizer application.

Property	Unit	Block 1	Block 2	Block 3	Block 4
Acidity (pH)	H_2O	6.1	5.8	5.8	6.0
P	mg/dm^3	4.6	5.3	6.8	7.3
K	mg/dm^3	64	37	75	53
Ca^{2+}	cmol$_c$/dm^3	2.3	2.3	2.4	2.1
Mg^{2+}	cmol$_c$/dm^3	1.0	1.0	0.9	0.8
H+Al	cmol$_c$/dm^3	2.6	3.1	3.1	2.5
SB	cmol$_c$/dm^3	3.5	3.4	3.5	3.0
CEC(t)	cmol$_c$/dm^3	3.5	3.4	3.5	3.0
CEC(T)	cmol$_c$/dm^3	6.1	6.5	6.6	5.5
BS	%	57.0	52.0	53.0	55.0

SB = sum of basic cations; CEC(t) = effective cation-exchange capacity; CEC(T) = potential cation-exchange capacity; BS = percent base saturation.

With the exception of N, soil nutrient levels were corrected prior to the implementation of the experiment, based on the recommendation of the 5th approximation (Ribeiro et al. 1999), where each plot received 80 kg P_2O_5/ha (35 kg P/ha) and 100 kg K_2O/ha (83 kg K/ha).

Experimental design and management

The experimental area consisted of 16 plots of 0.175 ha each, where *Urochloa brizantha* cv. Xaraés grass has been cultivated since its establishment in December 2008. The experimental design was a randomized block design, with 4 blocks, each containing 4 plots, i.e. a replicate for each of the 4 treatments: T_T – control plot, without application of nitrogen fertilizer; T_R – reference plot, with application at a fixed rate of 150 kg urea/ha/cycle (~46% N); T_G and T_S – experimental plots, with 75 kg urea/ha applied at the beginning of each cycle and further applications based on measurement of spectral variables by the sensors, i.e. the normalized difference vegetation index (NDVI) determined by the GreenSeeker™ (T_G) and the readings of the portable chlorophyll meter SPAD 502 (T_S).

Before the beginning of the experiment on 3 April the grass was cut with a brushcutter coupled to a tractor to a stubble height of 10 cm above soil level. The study continued for 3 cycles with each cycle including the regrowth period following the exit of animals (cows) from the pasture plus the subsequent grazing period until cows were again removed. As a result of limitations in labor availability, commencement of studies in the 4 blocks was staggered with a delay of 1 week between Blocks 1 and 2, Blocks 2 and 3 etc.

The full nitrogen fertilizer dose (150 kg urea/ha) was applied at the beginning of each cycle for reference plots, i.e. when animals were removed, while sensor treatments received only 75 kg urea/ha. Further applications of N fertilizer to sensor treatments were based on the nitrogen sufficiency index (NSI) as proposed by the Potash & Phosphate Institute (PPI). The Potash & Phosphate Institute published a bulletin (Francis and Piekielek 1999) with guidelines and recommendations for site-specific management of N fertilizer rates. The principle of the PPI methodology is that the plants in the reference plot point to the N absorption potential for a given edaphoclimatic condition (Villar et al. 2015). The NSI is determined based on the readings of given spectral variables, according to Equation 1 (Francis and Piekielek 1999):

$$NSI = \frac{VS_{ps}}{VS_{pr}} \qquad (1)$$

where:

NSI is nitrogen sufficiency index;

VS_{ps} is spectral variable in the sensor plots; and

VS_{pr} is spectral variable in the reference plot.

Whenever sensor plots presented NSI below 0.85, 25% of the N fertilizer dose of the reference plot, equivalent to 37.5 kg urea/ha, was applied. The spectral variables were determined based on readings when plants in the reference plot reached 20 and 25 cm in height. To determine the spectral variable for each plot by the chlorophyll meter SPAD 502, readings were performed in the middle third of the newest fully expanded leaf blade of 30 randomly identified plants. The average of readings represented the SPAD value of the plot. The determination of the NDVI spectral variable was performed by GreenSeeker™ (Trimble), where 40 readings were performed randomly throughout the plot with the apparatus positioned at a height of 1.0 m above the canopy. The average of the readings represented the NDVI value of the plot.

Production parameters

Forage yields were determined before grazing when pastures in each plot reached 30 cm in height and after grazing in each grazing cycle. Representative areas of 3 m^2 were selected and sampled using an iron frame of 1 m² at 3 different locations in the plot and weight of fresh forage recorded.

From the cut forage, 300 g subsamples were taken and separated into leaf blade, culm+leaf sheath and dead forage, before being weighed and heated to 65 °C for 72 hours to determine dry matter (DM) percentage for the calculation of forage DM. The growth rate was determined by the difference between amount of forage available when animals were removed from a block and that when they were reintroduced within each cycle divided by the length of the regrowth period, i.e. the amount of forage produced during the regrowth period divided by the length of the regrowth period. The crude protein (CP) concentration in pasture was determined by the Kjeldahl method (Rodrigues et al. 2017) on samples collected prior to the commencement of grazing in each cycle.

After pasture sampling was complete, lactating cows (crossbred Holstein × Zebu) were introduced according to pasture availability. The plots were grazed until the plants reached a mean of 15 cm in height, at which point the cows were removed and the average stocking rate (AU/ha) during each grazing cycle was calculated according to the methodology of Delevatti et al. (2019).

The yield response to fertilizer (Hare at al. 2015), also known as yield efficiency (Abassi et al. 2005), was then calculated, according to Equation 2, for each treatment at the end of each management cycle:

$$\text{Yield response} = \frac{DM_{fertilized} - DM_{control}}{N_{app}} \qquad (2)$$

where:

$DM_{fertilized}$ is dry matter yield of the N fertilized plots (T_S, T_G or T_R);

$DM_{control}$ is dry matter yield of the control plots (T_T); and

N_{app} is the amount of N applied during the management cycle.

Data on total forage yield, crude protein concentration, growth rate, stocking rate and leaf blade consumption were subjected to analysis of variance. The comparison of means was performed by the Tukey test (P<0.05), using the programming language and environment, R.

Results

Rest periods and weather details

The rest periods, i.e. regrowth periods, for each treatment for the 3 management cycles plus the duration of grazings are shown in Table 2. The length of each growth cycle of Xaraés grass for the different treatments varied according to prevailing weather conditions and whether or not N fertilizer was applied. Fertilized treatments presented similar rest periods in each cycle, while the Control plots required much longer rest periods for forage to reach the desired height. In Cycles 2 and 3, grazing periods were longer in fertilized treatments than in Control treatments as animals took longer to consume the available forage above 15 cm.

The temperature and rainfall observed during the study are presented in Figure 1. Total rainfall during the period was 74 mm, with the highest rainfall concentration occurring in late May. No rain fell in July-August and mean minimum temperatures fell below 15 °C on numerous occasions during this period.

Spectral index during growth cycles of Xaraés grass

Data for the NDVI and SPAD variables and the mean of the NSI variation are shown in Figure 2. The 3 cycles of crop management presented NSI values constantly above 0.85 for treatments where N fertilizer was applied and below 0.85 for the Control, which received no N fertilizer. During each cycle, none of the sensor treatment plots received additional fertilizer after the application of 75 kg urea/ha at the beginning of the cycle, i.e. they received 75 kg urea/ha/cycle less than the reference plots.

Pasture growth and quality

The average presentation yields of the different components of Xaraés grass for each treatment and for the 3 management cycles when the cows entered the pasture are presented in Table 3. During the experimental period, the availability of total forage and leaf blades in both reference plots and sensor plots were similar (P>0.05). Total forage available for the Control treatment was inferior to that of N-fertilized treatments during all cycles but differences were significant (P<0.05) only in Cycles 2 and 3. The amounts of leaf blade available were higher in fertilized treatments than in Controls in all cycles but differences were significant only in Cycles 1 and 2.

These differences understate the value of fertilizer as the Control treatment took longer to reach the necessary height for grazing so data for Controls represent a longer period than those for the N-fertilized treatments. To obtain a more valid comparison between treatments, their respective daily pasture growth rates, plus stocking rates and leaf blade consumptions are presented in Table 4. Growth rates of total forage during Cycle 2 and leaf blades during both Cycles 2 and 3 were significantly higher (P<0.05) for the fertilized than for the Control treatments. Only during Cycle 2 did the T_G treatment present growth rate of leaf blades significantly inferior to the growth rate of T_S. However, T_G growth rate was still similar to the T_R treatment and superior to the Control. As yield data were not collected when pastures reached 15 cm height and the study officially commenced following the initial cut on 3 April, growth rates for Cycle 1 are not presented in Table 4.

Table 2. Average length (days) of the 3 cycles (regrowth and grazing) of Xaraés grass.

Treatment	Cycle 1		Cycle 2		Cycle 3	
	Regrowth	Grazing	Regrowth	Grazing	Regrowth	Grazing
T_T	38	3	38	2.5	46	2.5
T_R	30	3	23	4	30	4
T_G	31	3	23	4	30	4
T_S	30	3	23	4	30	4

T_T = Control; T_R = Reference; T_G = GreenSeeker^{TM} sensor; T_S = SPAD 502 sensor.

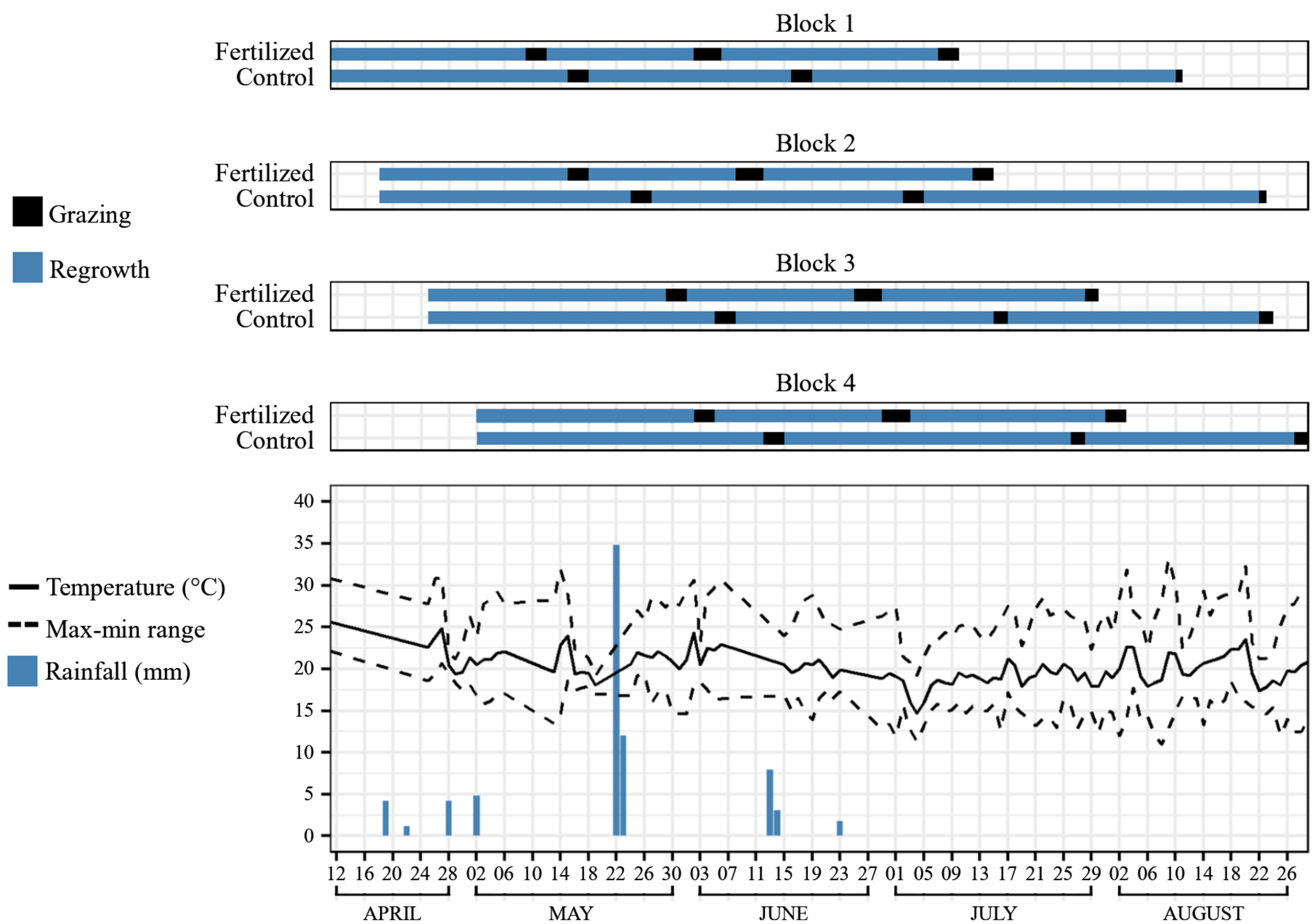

Figure 1. Length of regrowth and grazing periods and the climatic variables observed during the 3 management cycles.

The average stocking rates for treatments that received N fertilizer in the 3 crop management cycles (Table 4) were not significantly different (P>0.05), while stocking rates on the Control plots were significantly lower than on fertilized treatments in all management cycles (P<0.05). The difference in favor of the fertilized treatments increased in Cycles 2 and 3.

The estimated crude protein (CP) concentrations in available forage pre-grazing for each treatment in the 3 crop management cycles are presented in Table 5. All treatments receiving N fertilizer had higher CP percentages in forage than the Control treatment, but differences were significant only for Cycles 2 and 3. Despite differences in amounts of N applied to the various fertilized treatments, CP% did not differ between fertilized treatments (P>0.05), although the reference treatment always presented higher absolute values. While CP% in N treatments remained constant over the 3 cycles, CP% in the Control treatment declined from Cycle 1 to Cycle 3.

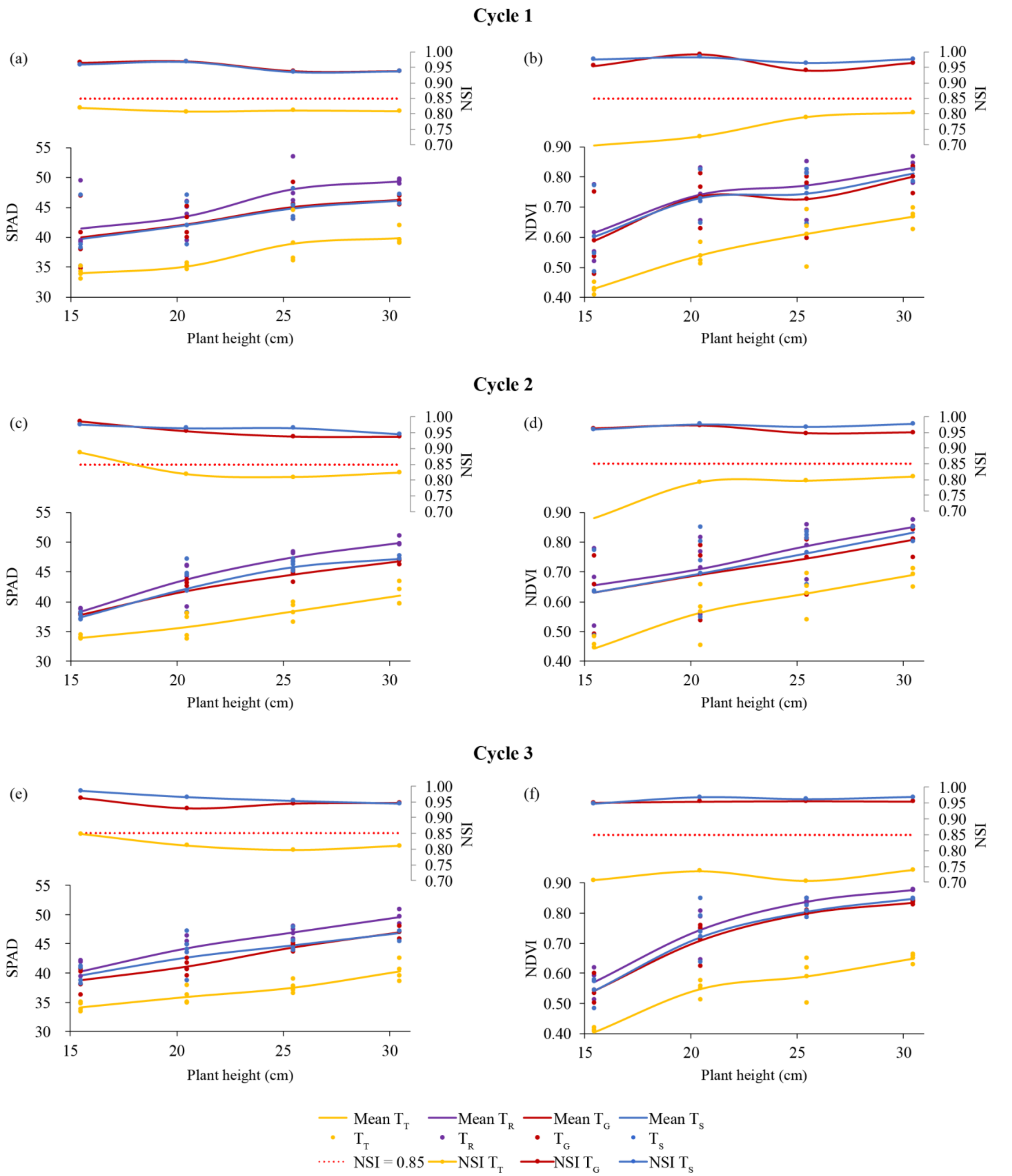

Figure 2. Spectral variables (NDVI and SPAD) and the mean of the Nitrogen Sufficiency Index (NSI) variation based on the GreenSeeker™ and SPAD 502 measurements for each treatment during the regrowth period for the 3 management cycles of Xaraés grass. T_T = Control; T_R = Reference; T_G = GreenSeeker™ sensor; and T_S = SPAD 502 sensor.

Table 3. Presentation yields of the different components of Xaraés grass for each treatment and cycle when the cows entered the pasture – mean per evaluation.

Parameter	T_T	T_R	T_G	T_S	P-value[1]	CV (%)
Cycle 1						
Total forage (kg DM/ha)	1,982	2,405	2,352	2,451	0.15	12.3
Leaf blade (kg DM/ha)	1,279b[2]	1,661a	1,596ab	1,737a	0.01	9.5
Culm (kg DM/ha)	578	604	618	544	0.84	20.6
Dead forage (kg DM/ha)	133	140	131	173	0.90	61.6
Cycle 2						
Total forage (kg DM/ha)	1,993b	2,725ab	2,635ab	3,069a	0.01	13.0
Leaf blade (kg DM/ha)	1,038b	1,708a	1,595a	1,918a	0.00	11.5
Culm (kg DM/ha)	771	767	821	902	0.74	29.2
Dead forage (kg DM/ha)	166	194	116	243	0.34	58.9
Cycle 3						
Total forage (kg DM/ha)	2,045b	2,759ab	2,969a	2,793ab	0.04	14.9
Leaf blade (kg DM/ha)	1,427	1,759	1,854	1,714	0.15	14.4
Culm (kg DM/ha)	491	899	969	979	0.09	32.2
Dead forage (kg DM/ha)	122	85	91	71	0.29	38,9

[1]Probability values by the F test of the analysis of variance. [2]Means followed by the same letter on the same line do not differ by the Tukey test (P>0.05), while means with different letters are significantly different (P<0.05). T_T = Control; T_R = Reference; T_G = GreenSeeker™ sensor; T_S = SPAD 502 sensor.

Table 4. Growth rates of the various components of Xaraés grass, stocking rate and leaf blade consumption for each treatment at the end of the 3 management cycles (mean per evaluation).

Parameter	T_T	T_R	T_G	T_S	P-value[1]	CV (%)
Cycle 1						
Stocking rate (AU/ha)	2.3b	3.5a	3.4a	3.3a	0.00	8.7
Leaf blade consumption (kg DM/ha)	792	949	922	1179	0.09	19.5
Cycle 2						
Total forage (kg DM/ha/d)	20.7b[2]	51.9a	56.6a	78.0a	0.00	25.9
Leaf blade (kg DM/ha/d)	15.0c	44.6ab	40.8b	61.1a	0.00	21.3
Culm (kg DM/ha/d)	7.0	6.2	17.3	16.8	0.26	80.8
Stocking rate (AU/ha)	2.1b	6.1a	5.7a	5.6a	0.00	14.1
Leaf blade consumption (kg DM/ha)	329b	931a	825ab	1,159a	0.00	28.0
Cycle 3						
Total forage (kg DM/ha/d)	9.7	23.6	29.0	26.8	0.10	46.7
Leaf blade (kg DM/ha/d)	12.9b	32.7a	33.9a	32.7a	0.00	23.9
Culm (kg DM/ha/d)	~0	~0	1.6	3.3	0.44	>100.0
Stocking rate (AU/ha)	1.5b	4.3a	4.1a	4.1a	0.00	7.3
Leaf blade consumption (kg DM/ha)	908	703	944	742	0.48	30.5

[1]Probability values by the F test of the analysis of variance. [2]Means followed by the same letter on the same line do not differ from each other by the Tukey test (P>0.05), while means followed by different letters are significantly different (P<0.05). T_T = Control; T_R = Reference; T_G = GreenSeeker™ sensor; T_S = SPAD 502 sensor.

Table 5. Crude protein concentration (% DM) in forage pre-grazing for each treatment in the 3 crop management cycles.

Management cycle	T_T	T_R	T_G	T_S	P-value[1]	CV (%)
Cycle 1	9.2	13.4	11.4	11.9	0.09	19.0
Cycle 2	8.0b[2]	13.6a	12.4a	12.0a	0.00	11.6
Cycle 3	6.5b	13.4a	11.6a	11.7a	0.00	11.5

[1]Probability values by the F test of the analysis of variance. [2]Means followed by the same letter on the same line do not differ by the Tukey test (P>0.05), while means followed by different letters are different (P<0.05). T_T = Control; T_R = Reference; T_G = GreenSeeker™ sensor; T_S = SPAD 502 sensor.

The yield responses to nitrogen fertilizer (kg DM/kg N applied) for all management cycles are presented in Table 6. As growth data were not available for Cycle 1, we based our calculations for Cycle 1 on the differences in presentation yields of forage when cows entered the pastures for grazing and the amounts of N applied (Table 3). We considered this approach was valid because the pastures received equal treatment prior to the commencement of the study and any differences between treatments at commencement of grazing were a function of the N applied. Yield response values were higher for the Sensor treatments (T_G and T_S) than for the reference treatment (T_R), although they were not statistically different for Cycles 1 and 3.

Table 6. Yield response to fertilizer (kg DM above Control/kg total N applied) for each treatment during the regrowth period for each management cycle.

Cycle	T_R	T_G	T_S
1	6.1	10.7	13.6
2	5.5c[1]	14.2b	27.9a
3	6.7	13.4	10.6

[1]Means followed by the same letter on the same line do not differ from each other by the Tukey test (P>0.05), while means with different letters are different (P<0.05). T_R = Reference; T_G = GreenSeeker™ sensor; T_S = SPAD 502 sensor.

Discussion

Despite its short duration, this study has shown the benefits of using sensors to determine the need for N fertilizer applications to *U. brizantha* cv. Xaraés pastures during the April–August period in the Southeast region of Brazil. Application of N according to readings made with the sensors produced as much forage with similar CP% as applying N at a set rate at pre-determined intervals, and resulted in a saving of 75 kg urea/ha/cycle. Since fertilizer costs are substantial this would result in significant savings to a farmer.

The absence of statistical differences between the 3 N fertilizer treatments confirms the benefits to be gained from the use of precision tools for determining N fertilizer requirements for Xaraés grass pastures rather than applying fertilizer at fixed rates at set times. The sensor treatments used half the amount of fertilizer applied to the fixed rate treatment, but produced similar amounts of total forage and leaf blade, allowing similar stocking rates on all fertilized treatments. Therefore, using this technology to apply N at variable rates in areas with spatial variability could contribute to higher efficiency of nitrogen use. Additionally, using this methodology would lead to a reduction in application of N in locations where the productive potential is high and plants are well supplied with N. In those cases, there would be no response to higher doses of nitrogen, and forage production would not suffer (Bredemeier et al. 2013).

The positive results of using the NSI methodology were made possible by the spectral responses evaluated in this experiment. For instance, the NDVI values presented a similar response in the 3 management cycles, tending to increase as the canopy height increased. Increases in NDVI values were expected, since the biomass of the plots increased with the development of the pasture. In addition, there was little difference in the amplitude of NDVI curves between treatments receiving nitrogen fertilizer. This explains why, in situations where available nitrogen levels are high, the maximum potential of the photosynthetic system is reached and the surplus of nitrogen is stored as other reserve compounds (Argenta et al. 2001; Amaral and Molin 2014). In this context, the value of using sensors is enhanced because, besides detecting plots which are deficient in nutrients, it is also possible to infer when fertilizer application exceeds the needs of the pasture, i.e. over-fertilizing. Over-fertilizing leads to an increase in nitrogen losses and a decrease in the efficiency of nutrient use by the plants.

Saturation in sensors that work with NDVI may occur when a high leaf area index is reached, where the linear relationship will no longer apply between sensor measurements and parameters such as biomass increase (Tremblay et al. 2009; Tian et al. 2016). NDVI saturation did not occur during the first 2 cycles since grazing began when height of the pasture reached 30 cm, corresponding to 95% light interception by the leaf canopy of Xaraés grass. This light interception condition prevents the crop canopy from reaching very dense levels, avoiding auto-shading and senescence of the lower leaves, which represents forage loss. There was only a small tendency for NDVI saturation during Cycle 3. This may be a response to cattle grazing and trampling on pasture, which helped cover small regions were soil had been exposed after the drastic cut at the beginning of the experiment.

We also observed a tendency for the SPAD index to increase with growth of the pasture. Supporting evidence for this increase was provided by Cancellier et al. (2013), who evaluated the dynamics of chlorophyll indices resulting from the application of N fertilizer to upland rice genotypes in the municipality of Gurupi, Tocantins, Brazil. The authors concluded that the younger leaves at the top caused an increase in the readings, which were carried out on the last fully expanded leaf of the plant.

As a consequence of the drastic initial brushcut, the rest period for the first growth cycle (30 days for the treatments with N applied) was the same as for the third cycle, when not only the days were shorter but also total precipitation and average temperatures were lower. There are limited studies on the benefits or setbacks from cutting tropical pastures too close to ground level, but Santos and Fonseca (2016) suggest that cutting pasture short to eliminate material of low acceptance to animals could compromise the speed of regrowth. One must remember that pasture had to regrow from 10 cm after the initial cut to the 30 cm target for Cycle 1, while regrowth in Cycles 2 and 3 was from 15 cm to 30 cm.

The higher forage yields in the second crop management cycle may have been a response to the timing of the rainfall, which occurred mostly at the beginning of the cycle (Figure 1). Another contributing factor could be the fact that defoliation of pasture during the study was carried out by animals and was not as severe or as rapid as defoliation by the brushcutter, so the residual pasture was in a more favorable condition to regenerate.

Only in Cycle 1 has the Control treatment yielded total forage values approaching those of the fertilized treatments. Before the implementation of the experiment, the area was fertilized every 60 days with N, according to the farmer's own criteria, for the maintenance of forage supply for cattle so there may have been residual N in all plots when the study commenced. However, the lack of N fertilizer application on the Control treatment during the study would have resulted in the depletion of N stocks in the soil and caused a reduction in green leaf color (Figure 2) and a decrease in forage yield when compared with the fertilized treatments (Table 3). Leaf blade production on the Control treatment was also statistically similar to those of fertilized treatments during Cycle 3. This may be only a reflection of significantly lower leaf blade consumption in the Control plots during Cycle 2, resulting in high leaf availability at the commencement of grazing in Cycle 3 (Table 4). In Cycle 3, there were clear signs of N deficiency in the Control pasture, including leaf chlorosis, the appearance of smaller leaves and growth restrictions that reflected the extended duration of the crop cycle (Table 2).

Consideration of individual parameters in isolation represents only part of the true differences between treatments. An important factor was the difference in the lengths of time to complete each cycle by the various treatments, i.e. fertilized versus Control. Table 3 and Figure 1 show clearly that not only did fertilized treatments produce more forage than the Control in each cycle, but also they did it in a much shorter time. A nitrogen deficiency was the most likely reason for the increase in length of regrowth periods and lower DM yields in the Control treatment.

Since *Urochloa brizantha* is a tropical species, variation in climatic factors could have contributed to the drop in productivity with time in the Control treatment. Tropical forage species have optimal growth within a temperature range of 25–35 °C and their growth is reduced at lower temperatures, ceasing at temperatures between 10 and 15 °C (Dantas et al. 2016). Minimum temperatures were below 15 ºC on only a few occasions, but more frequently during the third management cycle of the experiment. This could result in thermal limitation to the growth of pastures.

Total forage yield depends on factors including genetic composition of the species, availability of soil nutrients and climatic factors such as temperature, luminosity, soil moisture, etc. In the work carried out by Galzerano et al. (2011), the authors evaluated Xaraés grass during the wet season in the region of Jaboticabal, São Paulo, Brazil, using 95% interception of photosynthetically active radiation by the sward as a management criterion and applying 100 kg N/ha. The total forage yield obtained was 149 kg/ha/d, which was similar to the 118–138 kg/ha/d found in Cycle 2 for treatments that received N fertilizer, even though the current study was conducted in the dry season, when the climate was much less favorable for the growth of tropical forages.

Yields of leaf blade tended to exceed those of culm and dead forage in all 3 management cycles, which is a function of pasture being managed at lower heights and high grazing intensity, favoring greater control of stem elongation (Euclides et al. 2009; Carloto et al. 2011). The higher presence of leaf blades relative to culm and dead forage positively affects animal performance, as leaf has higher nutritional value than the other structures (Castro et al. 2013).

Euclides et al. (2009) evaluated animal production and its relationship with the characteristics of *Urochloa brizantha* cvv. Marandu, Xaraés and Piatã, in Campo Grande, Mato Grosso do Sul, Brazil. Mean stocking rates for Xaraés were 3.8 AU/ha in the wet season and 1.4 AU/ha during the dry season, which were generally lower than stocking rates obtained in our study. While many factors impact on stocking rates of pastures, e.g. age of the stand, soil fertility, climatic conditions, etc., those authors applied only 50 kg N/ha in November-December of each year, so there was probably insufficient N available to meet demands of the sward to produce higher forage yields.

In pastures of Marandu grass fertilized with 1,000 kg/ha/yr (20:05:20, N:P:K) and managed with 30 days rest in Valença, Rio de Janeiro, Brazil, Fukumoto et al. (2010) obtained an average stocking rate of 5 AU/ha in the period from January to June 2005. Similar stocking rate data were obtained in Cycle 2 in our study for the treatments that received N fertilizer, confirming that the application rates determined by the sensors were consistent with the actual needs of the crop.

It is interesting that crude protein concentration in available forage during Cycle 1 did not differ significantly between treatments, although absolute values were higher in fertilized treatments. One possible explanation for the lack of differences is the history of fertilizer application to the area. Before the experiment commenced, the area received nitrogen fertilizer every 60 days in an endeavor to maintain the forage supply for cattle. Residual N in the soil may have boosted CP% in the forage produced in the Control treatment. However, as the study advanced, CP% declined in the Control while it was maintained in the N treatments despite much higher forage yields in these treatments, which should have produced a dilution effect.

The lack of any significant difference in CP values between reference and sensor treatments in Cycles 2 and 3 suggests that there was a possible N loss in the reference treatment that received N fertilizer at a higher fixed rate. The key to optimizing the relationship between crop yield, profit and the preservation of the environment is to achieve a better synchrony between amount of N applied and the demands for N by the grass. Minimizing the environmental impact caused by the excessive use of nutrients is crucial for production systems worldwide if sustainable situations are to be maintained (Shanahan et al. 2008).

The optimization of available N levels in soil is crucial for plants to photosynthesize efficiently, increasing yield and reducing production costs (He et al. 2016). Many studies have shown that increasing doses of N applied can result in increased DM yields (Cecato et al. 2014; Hare et al. 2015; Delevatti et al. 2019), but yield responses can decline at higher N rates (Hare et al. 2015). Delevatti et al. (2019) argued that many countries have been overusing N fertilizer, resulting in harmful impacts on the environment, such as increased greenhouse gas emissions, increased nutrient levels in water runoff and loss of biodiversity. Efficient management of pasture is essential worldwide, as it enhances production while conserving land and resources (Delevatti et al. 2019). Our findings have shown that the use of spectral sensors could be a mechanism for managing pastures in a sustainable manner, by optimizing fertilizer use in individual situations and reducing negative impacts on the environment.

Conclusion

The GreenSeeker™ and SPAD 502 sensors used in this work were efficient in determining the need to apply N fertilizer to *Urochloa brizantha* cv. Xaraés. After 3 grazing cycles, applying N fertilizer at a lower rate determined by readings taken with sensors was more efficient than applying N at a fixed rate at preset intervals. Longer-term studies to confirm these findings seem warranted followed by promotion of this technology to make pasture production more cost-efficient with associated environmental benefits.

Acknowledgments

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References

(Note of the editors: All hyperlinks were verified 7 April 2020.)

Abassi MK; Kazmi M; Hussan Fu. 2005. Nitrogen use efficiency and herbage production of an established grass sward in relation to moisture and nitrogen fertilization. Journal of Plant Nutrition 28:1693–1708. doi: 10.1080/01904160500250698

Amaral LC do; Molin JP. 2014. The effectiveness of three vegetation indices obtained from a canopy sensor in identifying sugarcane response to nitrogen. Agronomy Journal 106:273–280. doi: 10.2134/agronj2012.0504

Argenta G; Silva PRF da; Bortolini CG; Forsthofer EL; Strieder ML. 2001. Relação da leitura do clorofilômetro com os teores de clorofila extraível e de nitrogênio na folha de milho. Revista Brasileira de Fisiologia Vegetal 13:158–167. doi: 10.1590/S0103-31312001000200005

Barbieri JD; Dallacort R; Faria Jr CA; Freitas PSL de; Carvalho MAC de. 2017. Peanut cultivars submitted to irrigation levels and nitrogen adubation in tropical climate. Journal of the Brazilian Association of Agricultural Engineering 37:1126–1136. doi: 10.1590/1809-4430-eng.agric.v37n6p1126-1136/2017

Bravin MP; Oliveira TK de. 2014. Nitrogen fertilization in maize and xaraés palisade grass under no-tillage and conventional tillage in an agrosilvopastoral system. Pesquisa Agropecuária Brasileira 49:762–770. (In Portuguese). doi: 10.1590/S0100-204X2014001000003

Bredemeier C; Variani C; Almeida D; Rosa AT. 2013. Wheat yield potential estimation using active optical sensor for site-specific nitrogen fertilization. Ciência Rural 43:1147–1154. (In Portuguese). doi: 10.1590/S0103-84782013005000080

Cancellier EL; Silva J; Santos MM dos; Siebeneichler SC; Fidelis RR. 2013. Chlorophyll index in parts of upland rice. Revista Verde de Agroecologia e Desenvolvimento Sustentável 8:199–206. (In Portuguese). bit.ly/2Rla203

Carloto MN; Euclides VPB; Montagner DB; Lempp B; Difante GS; Paula CCL de. 2011. Animal performance and sward characteristics of xaraés palisade grass pastures subjected to different grazing intensities, during rainy season. Pesquisa Agropecuária Brasileira 46:97–104. (In Portuguese). doi: 10.1590/S0100-204X2011000100013

Carvalho TB de; Zen S de; Tavares ECN. 2009. Comparação de custo de produção na atividade de pecuária de engorda nos principais países produtores de carne bovina. In: 47º Congresso da Sociedade Brasileira de Economia, Administração e Sociologia Rural (SOBER), Porto Alegre, 26–30 julho 2009. bit.ly/2xVAFSv

Castro LM; Barbosa MAAF; Barbero RP; Brito VC; Saad RM; Ribeiro ELA; Mizubuti IY; Bridi AM. 2013. Forage production and structural composition of pastures of Xaraés grass managed in different grazing heights. Semina: Ciências Agrárias 34:4145–4156. (In Portuguese). doi: 10.5433/1679-0359.2013v34n6Supl2p4145

Cecato U; Mari GC; Beloni T; Piotto VC; Lins TOA; Pinheiro AA. 2014. Accumulation of dry matter and morphological composition of irrigated Mombaça grass with and without nitrogen fertilizer under grazing. Tropical Grasslands-Forrajes Tropicales 2:27–28. doi: 10.17138/TGFT(2)27-28

Chapman SC; Barreto HJ. 1997. Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth. Agronomy Journal 89:557–562. doi: 10.2134/agronj1997.00021962008900040004x

Corrêdo LP; Pinto FAC; Queiroz DS; Valente DSM; Villar FMM. 2019. Nitrogen variable rate in pastures using optical sensors. Semina: Ciências Agrárias 40:2917–2932. doi: 10.5433/1679-0359.2019v40n6Supl2p2917

Daniel ES; Amarante CVT; Martin MS; Miquelluti DJ; Campos ML. 2016. Relationship between absolute and relative content of chlorophyll in leaves of osier. Ciência Florestal 26:307–312. (In Portuguese). doi: 10.5902/1980509821122

Dantas GF; Faria RT de; Santos GO; Dalri AB; Palaretti LF. 2016. Herbage yield and quality of irrigated *Brachiaria* in autumn and winter. Engenharia Agrícola 36:469–481. (In Portuguese). doi: 10.1590/1809-4430-Eng.Agric.v36n3p469-481/2016

Deblitz C. 2009. Agri benchmark beef report 2009: Benchmarking farming systems worldwide. Johann Heinrich von Thünen Institute, Braunschweig, Germany. bit.ly/34jTYAX

Delevatti LM; Cardoso AS; Barbero RP; Leite RG; Romanzini EP; Ruggieri AC; Reis RA. 2019. Effect of nitrogen application rate on yield, forage quality, and animal performance in a tropical pasture. Scientific Reports 9:7596. doi: 10.1038/s41598-019-44138-x

Euclides VPB; Macedo MCM; Valle CB do; Difante GS; Barbosa RA; Cacere ER. 2009. Forage nutritive value and animal production in *Brachiaria brizantha* pastures. Pesquisa Agropecuária Brasileira 44:98–106. (In Portuguese). doi: 10.1590/S0100-204X2009000100014

Fernandes LO; Reis RA; Paes JMV; Teixeira RMA; Queiroz DS; Paschoal JJ. 2015. Performance of Gir young bull maintained in "*Brachiaria brizantha*" pastures submitted to different management. Revista Brasileira de Saúde e Produção Animal 16:36–46. (In Portuguese). doi: 10.1590/S1519-99402015000100004

Ferraz JBS; Felício PE de. 2010. Production systems - an example from Brazil. Meat Science 84:238–243. doi: 10.1016/j.meatsci.2009.06.006

Francis DD; Piekielek WP. 1999. Assessing crop nitrogen needs with chlorophyll meters. Site-Specific-Management Guidelines No. 12. Potash & Phosphate Institute, Atlanta, GA, USA. bit.ly/39QJwSR

Fukumoto NM; Damasceno JC; Deresz F; Martins CE; Cóser AC; Santos GT dos. 2010. Milk yield and composition, feed intake and stocking rate of crossbreed cows in tropical grasses managed in a rotational grazing system. (In Portuguese). Revista Brasileira de Zootecnia 39:1548–1557. doi: 10.1590/S1516-35982010000700022

Galzerano L; Malheiros EB; Ruggieri AC; Raposo E; Morgado ES; Silva WL; Costa JPR; Caputti GP. 2011. Desenvolvimento das características do dossel do capim Xaraés e suas inter-relações. Scientia Plena 7:1–5. bit.ly/2JONXms

Giletto CM; Echeverría HE. 2016. Canopy indices to quantify the economic optimum nitrogen rate in processing potato. American Journal of Potato Research 93:253–263. doi: 10.1007/s12230-016-9501-0

Handcock RN; Gobbett DL; González LA; Bishop-Hurley GJ; McGavin SL. 2016. A pilot project combining multispectral proximal sensors and digital cameras for monitoring tropical pastures. Biogeosciences 13:4673–4695. doi: 10.5194/bg-13-4673-2016

Hare MD; Phengphet S; Songsiri T; Sutin N. 2015. Effect of nitrogen on yield and quality of *Panicum maximum* cvv. Mombasa and Tanzania in Northeast Thailand. Tropical Grasslands-Forrajes Tropicales 3:27−33. doi: 10.17138/TGFT(3)27-33

He L; Song X; Feng W; Guo BB; Zhang YS; Wang YH; Wang CY; Guo TC. 2016. Improved remote sensing of leaf nitrogen concentration in winter wheat using multiangular hyperspectral data. Remote Sensing Environment 174:122–133. doi: 10.1016/j.rse.2015.12.007

Hedley C. 2015. The role of precision agriculture for improved nutrient management on farms. Journal of the Science of Food and Agriculture 95:12–19. doi: 10.1002/jsfa.6734

Lee JH; Kang CS; Roh AS; Park KY; Lee HJ. 2009. Assessment of N topdressing rate at panicle initiation stage with chlorophyll meter-based diagnosis in rice. Journal of Crop Science and Biotechnology 12:195–200. doi: 10.1007/s12892-009-0133-5

Marques MF; Romualdo LM; Martinez JF; Lima CG; Lunardi LJ; Luz PHC; Herling VR. 2016. Time of nitrogen application and some structural and bromatologic variables of massaigrass. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 68:776–784. (In Portuguese). doi: 10.1590/1678-4162-8500

Nogueira BB; Iglesias L; Mesquita JV; Nakatani MC; Putti FF. 2018. SPAD index in tomatoes plants cultivated in coconut fiber and submitted to fertirrigation pulses. Brazilian

Journal of Biosystems Engineering 12:1–6. (In Portuguese). doi: 10.18011/bioeng2018v12n1p1-6

Pocojeski E; Silva LS da; Kaefer S; Moro VJ; Griebeler G. 2015. Monitoring nutritional status of flooded rice cultivars by chlorophyll meter in nitrogen fertilization management. Revista Ceres 62:310–318. (In Portuguese). doi: 10.1590/0034-737X201562030011

Pôrto MLA; Puiatti M; Fontes PCR; Cecon PR; Alves JC. 2014. SPAD index for diagnosis of nitrogen status in the Japanese cucumber plants under protected cultivation. Horticultura Brasileira 32:292–296. (In Portuguese). doi: 10.1590/S0102-05362014000300009

Ribeiro AC; Guimarães PTG; Alvarez VVH. 1999. Recomendações para o uso de corretivos e fertilizantes em Minas Gerais - 5ª Aproximação. Comissão de Fertilidade do Solo do Estado de Minas Gerais, Viçosa, MG, Brazil.

Ribeiro HM; Vasconcelos E; Ramos A; Coutinho J. 2009. Evaluation of nitrogen status of *Eucalyptus globulus* Labill. ssp. *globulus* mother plants with a portable chlorophyll meter. Revista de Ciências Agrárias 32:40–49. (In Portuguese). bit.ly/34oaD6v

Rodrigues JPP; Ramin M; Huhtanen P; Aru F; Detmann E; Marcondes MI. 2017. Effect of soya bean oil supplementation and forage type on methane production and fibre digestibility using the in vitro gas production system. Grass and Forage Science 73:368–380. doi: 10.1111/gfs.12326

Santos MER; Fonseca DM da. 2016. Adubação de pastagens em sistemas de produção animal. Editora UFV, Viçosa, MG, Brazil.

Shanahan JF; Kitchen NR; Raun WR; Schepers JS. 2008. Responsive in-season nitrogen management for cereals. Computers and Electronics in Agriculture 61:51–62. doi: 10.1016/j.compag.2007.06.006

Subbarao GV; Rao IM; Nakahara K; Ando Y; Sahrawat KL; Tesfamariam T; Lata JC; Boudsocq S; Miles JW; Ishitani M; Peters M. 2013. Nitrogen management in grasslands and forage-based production systems – Role of biological nitrification inhibition (BNI). Tropical Grasslands-Forrajes Tropicales 1:168–174. doi: 10.17138/TGFT(1)168-174

Theago EQ; Buzetti S; Teixeira Filho MCM; Andreotti M; Megda MM; Benett CGS. 2014. Nitrogen application rates, sources, and times affecting chlorophyll content and wheat yield. Revista Brasileira de Ciência do Solo 38:1826–1835. (In Portuguese). doi: 10.1590/S0100-06832014000600017

Tian F; Brand M; Liu YY; Verger A; Tagesson T; Diouf AA; Rasmussen K; Mbow C; Wang Y; Fensholt R. 2016. Remote sensing of vegetation dynamics in drylands: Evaluating vegetation optical depth (VOD) using AVHRR NDVI and in situ green biomass data over West African Sahel. Remote Sensing of Environment 177:265–276. doi: 10.1016/j.rse.2016.02.056

Tremblay N; Wang Z; Ma BL; Belec C; Vigneault P. 2009. A comparison of crop data measured by two commercial sensors for variable-rate nitrogen application. Precision Agriculture 10:145. doi: 10.1007/s11119-008-9080-2

Viana LA; Tomaz DC; Martins RN; Rosas JTF; Santos FFL dos; Portes MF. 2019. Optical sensors for precision agriculture: An outlook. Journal of Experimental Agriculture International 35:1–9. doi: 10.9734/jeai/2019/v35i230203

Villar FMM; Pinto FAC; Fonseca DM da; Queiroz DM de; Alcântara GR de. 2015. Sufficiency Index for defining nitrogen recommendation in *Brachiaria* grass pasture. Bioscience Journal 31:1333–1340. doi: 10.14393/BJ-v31n5a2015-26338

Wachendorf M; Fricke T; Möckel T. 2018. Remote sensing as a tool to assess botanical composition, structure, quantity and quality of temperate grasslands. Grass and Forage Science 73:1–14. doi: 10.1111/gfs.12312

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Research Paper

Evaluation of forage quantity and quality in the semi-arid Borana Lowlands, Southern Oromia, Ethiopia

Evaluación de la cantidad y calidad del forraje natural en la zona semi-árida de Borana Lowlands, Southern Oromia, Etiopía

GEMEDO DALLE

Center for Environmental Science, Addis Ababa University, Ethiopia. aau.edu.et

Abstract

This study was conducted with the aim of determining herbaceous biomass during different seasons, plus nutritive value of herbaceous species and forage on selected woody plants and documenting pastoralists' perceptions of the value of various forage species in Borana Zone, Oromia, Ethiopia. Data were collected from a total of 92 main plots of 500 m^2 during rainy and dry seasons located across different functional Land Use Units called Kalo (enclosed areas), Worra (grazed by lactating stock) and Foora (more remote and grazed by dry and non-lactating stock). Total herbage and leaves of woody plants were analyzed for concentrations of crude protein (CP), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and ash. Perceptions of farmers were determined through group discussions. Herbage biomass plus chemical composition of both herbaceous and woody forage species varied significantly across seasons and Land Use Units. Mean herbaceous biomass in all Land Use Units was poor (876–1,469 kg DM/ha). Mean CP, NDF and ADF concentrations of the herbaceous samples were 62, 749 and 444 g/kg DM, respectively. Mean CP% of leaves from woody plants was higher (11%) than from herbage (6%). In both groups, crude protein concentrations were highest during the wet season and lowest during the dry season, whereas fiber concentrations were highest in the dry season. Mean CP% of herbaceous forage species was below the critical level recommended for both beef cattle (7%) and small ruminants (9%) but forage from woody species should provide a reliable supply of supplementary nitrogen. Management of rangelands should be designed to ensure that desirable herbaceous species are preserved, while desirable woody species are also a valuable asset. Determination of management strategies to ensure that the desirable mix of species is maintained is imperative if sustainable production is to continue.

Keywords: Borana pastoralists, herbaceous biomass, nutritive value, pasture management, tropical rangelands.

Resumen

En la zona semi-árida de Borana, Oromia, Etiopía, se midió la biomasa herbácea disponible en pasturas naturales durante diferentes estaciones del año y se determinó el valor nutritivo de las especies herbáceas y del follaje de plantas leñosas seleccionadas. Además se documentó la percepción de los productores pastoriles tradicionales sobre el valor de varias especies forrajeras. Los datos se obtuvieron durante las épocas lluviosas y secas en un total de 92 parcelas de 500 m^2 cada una, ubicadas en diferentes unidades funcionales de uso pastoril: *Kalo* (áreas encerradas), *Worra* (pasturas utilizadas con hembras lactantes) y *Foora* (áreas remotas utilizadas con animales no lactantes). De las plantas herbáceas enteras y del follaje de las especies leñosas se analizaron las concentraciones de proteína cruda (PC), materia orgánica (MO), fibra detergente neutra (FDN), fibra detergente ácida (FDA), lignina detergente ácida y cenizas. Las percepciones de los pastores fueron registradas con base en reuniones grupales. La biomasa disponible y la composición química de las especies herbáceas y del follaje de las leñosas variaron significativamente según las estaciones y las unidades de uso pastoril. En promedio de las unidades de uso pastoril, la biomasa herbácea disponible fue baja (876–1,469 kg MS/ha).

Correspondence: Gemedo Dalle, Center for Environmental Science, Addis Ababa University, P. O. Box 80119, Addis Ababa, Ethiopia.
E-mail: gemedo.dalle@aau.edu.et

Las concentraciones, promedio, de PC, FDN y FDA de las muestras herbáceas fueron de 62, 749 y 444 g/kg de MS, respectivamente. La concentración, promedio, de PC en el follaje de las plantas leñosas fue mayor (11%) que la las herbáceas (6%). En ambos grupos, las concentraciones de PC fueron más altas durante la estación lluviosa y más bajas durante la estación seca, mientras que las concentraciones de fibra fueron más altas en la estación seca. El porcentaje de PC promedio de las especies herbáceas fue menor que el nivel crítico recomendado tanto para ganado bovino (7%) como para pequeños rumiantes (9%), mientras que se espera que el follaje de las especies leñosas proporcione un suministro confiable de nitrógeno complementario. Los resultados demuestran que el manejo de las pasturas naturales debe ser diseñado para asegurar la conservación de las especies herbáceas deseables, considerando que las especies leñosas deseables son un activo valioso. La identificación y aplicación de estrategias de manejo, tendientes a mantener una combinación deseable de especies en las pasturas, son imprescindibles para asegurar una producción ganadera sostenible en la región.

Palabras clave: Biomasa herbácea, manejo pastoril, pasturas naturales tropicales, productores pastoriles, valor nutritivo.

Introduction

The Borana Lowlands occupy 95,000 km^2 (Alemayehu Mengistu 2004) in Ethiopia and are populated by pastoralists who represent a vital part of Ethiopia's population, contributing significantly to the nation's GDP. Review of different studies, e.g. Shapiro et al. (2017), estimated direct contribution of livestock production in lowland pastoral systems of Ethiopia to agricultural GDP and national GDP to be 39 and 17%, respectively. The area supports 480,000 families with an annual population growth rate of 2.5–3% (Homan et al. 2004). Livestock production dominated by the Boran breed has been the major source of livelihood for Borana pastoralists. According to CSA (2008), in 2007 there were 1,771,589 cattle, 1,991,196 goats, 699,887 camels and 52,578 donkeys in the Borana zone. The Boran breed remains one of the most productive breeds as it is fast-growing and fertile with good milk production compared with other indigenous cattle breeds in Ethiopia (Aynalem Haile et al. 2011).

Livestock play a crucial role in the subsistence economy, culture and religion of pastoralists in Ethiopia, and represent both social capital and an insurance against disaster (Herlocker 1999). Borana pastoralists are known for their strong tradition of livestock production through using their indigenous rangeland and water management strategies. The herbage on offer in the rangelands, however, is highly variable, both in quantity and quality. Vázquez-de-Aldana et al. (2000) reported that the botanical composition of available forage was highly variable as was the nutritional quality, which was further exacerbated by topographic relief, soil characteristics, climate, season and management. The semi-sedentary Borana pastoralists have developed strategies to exploit this highly variable resource, and are known for sustainably using the Borana land in southern Ethiopia for livestock production.

Rangeland management markedly affects botanical composition and, consequently, herbage quantity and quality. In order for the grazing system to be sustainable, better understanding of the characteristics of the forage available is needed. However, little or no data are currently available on the quality of plant resources in the study area.

Therefore, this study was conducted in the Borana Lowlands to determine both quality and quantity of forage resources in this semi-arid pastoral production system throughout the year. The specific objectives were to determine herbaceous biomass and nutritive value of forage species and document pastoralists' perceptions on forage species.

Materials and Methods

Study area

The study was conducted in Arero and Yaballo Districts of Borana Zone, Oromia, Ethiopia (Figure 1). This study was part of a larger project of the Borana Lowland Development Program (BLPDP)/Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) aimed at developing a pastoral-oriented self-help concept for sustainable natural resource management under changing ecological and socio-economic conditions. Field data were collected from 2001 to 2003 in different seasons.

The main study sites were Dida Hara Pastoral Association (PA) in Yaballo and Web PA in Arero. In addition, a government ranch called Dida Tuyura and Foora (an area used for dry or non-lactating livestock) were selected randomly for the forage resource assessment. The government ranch was reputed to be in relatively good rangeland condition and was included as a benchmark for comparing the other Land Use Units. Yaballo town is 570 km south of Addis Ababa (9°0'19" N, 38°45'49" E; 2,355 masl). Dida Hara and Web are located about 30 km northeast and 85 km southeast of Yaballo town, respectively. Foora is located in Dida Hara PA, about 48 km southeast of Yaballo town. Dida Tuyura Ranch is in Dida Yaballo PA (Yaballo district), about 25 km northeast of Yaballo town.

Livestock populations in the Borana Lowlands are predominantly cattle, while small ruminants and camels are also important in the production system. Rearing dromedaries has expanded since the 1990s. Estimates have shown that herd composition in Tropical Livestock Units (TLU) was 90% cattle, 5% small ruminants and 4% dromedaries (Homann 2004). Borana cattle are a *Bos indicus* breed that belong to the Large East African Zebu breed group (Homann 2004).

Figure 1. Location of Borana Zone, in Oromia, Southern Ethiopia. (Source: Google Earth).

Climatic characteristics

The elevation of the study area ranges from 750 to about 2,000 m above sea level. Rainfall is bimodal, with the long rains during March–May and short rains during October–November (Haugen 1992; Coppock 1994). Mean annual rainfall is 412 mm in Web (Web weather station; data from Southern Range Development Unit) and 566 mm in Dida Hara (Yaballo town as the nearest station; data from the National Meteorological Services Agency of Ethiopia). While mean annual temperature varies from 19 to 24 °C (Alemayehu Mengistu 1998), the mean maximum temperatures for Yaballo stations ranged from 24.4 to 26.4 °C and minimums from 13.8 to 14.8 °C (1989–2001 raw data from the National Meteorological Services Agency of Ethiopia). In general, December–February is the hot dry season, March–May is the long rainy season, June–August is the cool dry season and September–November is the short rainy season. The difference between the long rains and short rains is the amount of rain that the area receives.

Soil characteristics

The soils in the study area are granitic and volcanic soils and their mixtures (Coppock 1994). Valley bottomlands of the Borana rangelands are dominated by vertisols. Review of studies that described upland rangeland soils in the study area showed that the soils vary in color (yellow, brown, grey or red) and have almost equal proportions of sand, silt and clay (Alemayehu Mengistu 2004). In general, Dida Hara soils are the lightest, containing the highest proportion of sand, whereas Web has soils with higher levels of available P, Ca, Mg, CEC and pH. Mean available P ranged from 2.0 ppm in Foora to 30 ppm in Web Worra. Concentrations of P and Ca and CEC are highly variable in both Dida Hara and Web (Gemedo Dalle 2004).

Sampling strategy

Borana pastoralists classify their grazing lands into enclosed grazing lands for calves (Kalo), grazing lands for lactating livestock (Worra) and grazing lands for dry livestock (Foora). Based on suitability for different classes of livestock (i.e. availability of forage and watering points), the pastoralists establish their villages (pastoral camps) locally called Olla. Classification and demarcation of the grazing land into Kalo, Worra and Foora is based on distance from the villages and accessibility of watering points: Kalo is adjacent to the villages, Worra the next removed and Foora the most remote. Similarly, Kalo and Worra are located within walking distance (distance from water covered by grazing livestock in a single day, which is about 12 km) from watering points, whereas Foora are remote from the watering points (having no permanent watering point within the grazing area) and dry livestock utilizing this area depend on surface rainwater or must walk long distances to access watering points. Kalo was fenced and protected from grazing from early wet season to hot dry season, and was accessible for grazing only during the hot dry season. Worra and Foora were open to livestock throughout the year. A stratification sampling technique was used to collect samples from these functional Land Use Units. Within each Land Use Unit, the initial sampling point was established randomly, but subsequent units were established at 200 m intervals on a linear transect.

Samples of both herbaceous and woody forage species were gathered from different Land Use Units. Herbaceous samples were collected during cool dry (June–August), short rains (September–November) and long rains (March–May) seasons, whereas woody samples were taken during short rains, hot dry (December–February) and long rains seasons. Within the various districts, an effort was made to sample from different sites in all seasons, in an attempt to ensure that the samples were representative for the specific study sites.

Forage sampling

Forage samples were collected from a total of 92 main plots of 50 × 10 m (500 m^2) each (Table 1). Within each 500 m^2 plot, 5 subplots of 0.5 × 0.5 m (0.25 m^2), 4 at the corners and 1 in the center, were established and herbaceous samples were collected for both biomass and forage nutritive value determination. Samples from the 5 subplots were pooled and assumed to represent the main plot. To demarcate these subplots, a 3-sided frame of welded metal (0.5 × 0.5 m), left open at one side as recommended by Whalley and Hardy (2000), was used. All grasses, herbaceous forbs and sedges rooted within the marked area of 0.25 m^2 were cut at 2 cm above ground following the method of Vázquez-de-Aldana et al. (2000). Immediately after harvesting, the material sampled in each plot was sorted manually into species and weighed in the field using a portable digital balance to determine contributions of individual species to total fresh biomass. Because of logistical issues sorted samples could not be dried individually and were pooled again, dried at 60 °C for about 48 hours in a well-ventilated oven (Adesogan et al. 2000) and weighed to determine both total herbaceous biomass and the contribution of individual species to total dry biomass.

For woody plants, samples of green leaves (including young and old) of each plant and each species were collected at random. For *Vachellia tortilis* (syn. *Acacia tortilis*), fruits (pods) were also collected as they were preferred by animals. A total of 75 samples (25 samples for each of the 3 seasons) were collected and analyzed. An effort was made to sample from the same species in all 3 seasons. However, during the hot dry season, some species had shed leaves, so samples were taken from other drought-resistant forage species as indicated by the pastoralists. In each season, only 1 sample per species was taken from the first site where the identified woody plant was encountered. In other words, only a single sample of each browse species was collected and only from a single tree/shrub.

Nomenclature of plant species follows published volumes of Flora of Ethiopia and Eritrea (Hedberg and Edwards 1995; Edwards et al. 1995, 1997) and was updated according to the taxonomy of the Genetic Resources Information Network GRIN (npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch.aspx).

Chemical analyses

After oven-drying of samples at 105 °C, dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), ash, ADF-Ash and in vitro digestibility of dry matter (IVDMD) were determined in the laboratory of the International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia. IVDMD was analyzed only during the cool dry season.

CP was determined using the Kjeldahl method (N × 6.25), IVDMD by the in vitro rumen digestibility procedure (Van Soest and Robertson 1985) and NDF, ADF and ADL by the detergent system of analysis (Van Soest and Robertson 1985). Ash was determined by igniting samples at 500 °C (AOAC 1990).

Pastoralists' perceptions

Eight community-level group discussions were held in 4 places: Dikale (DIK), Dambala Abba Chana (DAC) (both in Dida Hara), Tesso Qallo (TSQ) and Dhibu Kolocho (DBK) (both in Web PA). One hundred and eight pastoralists (52 men and 56 women) participated in the group discussion. Pair-wise preference ranking was used to identify the most preferred forage species. According to their palatability to livestock, grass species were classified as highly desirable (decreasers), intermediate (increasers) and least desirable (pioneers), based on pastoralists' perceptions and field observations. Decreasers were defined as desirable grass species that are likely to decrease with heavy grazing pressure (Baars et al. 1996).

Table 1. Descriptions of Land Use Units and sampling details in the Borana Lowlands, Ethiopia.

Land Use Unit	Explanation	Sampling intensity		
		No. of samples	No. of seasons	Total
Dida Hara Kalo (DHK)	Dida Hara grazing land for calves	21	3	63
Dida Hara Worra (DHW)	Dida Hara grazing land for lactating livestock	14	3	42
Web Kalo (WBK)	Web grazing land for calves	19	3	57
Web Worra (WBW)	Web grazing land for lactating livestock	14	3	42
Dida Tuyura Ranch (DTR)	Government ranch used for conserving Borana breeds	10	2	20
Foora (FOR)	Grazing land for dry livestock between Dida Hara and Web	14	3	42

Data analysis

Descriptive statistics were used in organizing, summarizing and describing the data. Comparison of mean values was performed using two-tailed t-test following Fowler and Cohen (1996). ANOVA was applied to investigate variability across Land Use Units and seasons. Effects of season × land use interactions were determined using GLM (General Linear Model).

Results

Rainfall

Rainfall data for Dida Hara were taken from Yaballo town, which was the nearest station to Dida Hara and that of Web was from Web station, which was collected by Southern Range Development Unit. The mean annual rainfall in Dida Hara (Yaballo) and Web is presented in Table 2.

While mean monthly rainfall ranged from 5.9 to 144.1 mm in Dida Hara and 2.0 to 113.9 mm in Web (Table 2), variation within individual months was great as observed from the CV%.

Two rainfall peaks are conspicuous, demonstrating the bimodal nature of rainfall in the Borana Lowlands. Annual totals were highly variable, ranging from 188 to 803 mm with mean of 545 mm in Dida Hara, and from 211 to 638 mm with mean of 412 mm in Web. This difference in annual rainfall between the two sites was statistically significant ($t = 2.196$, $df = 22$ at $P=0.05$), indicating that Web is more arid than Dida Hara.

Herbaceous biomass

The above-ground herbaceous presentation yields (standing crop) were highly variable both spatially and temporally (Tables 3 and 4), with significant differences across seasons ($P = 0.026$) and Land Use Units ($P = 0.000$). Presentation yields for Dida Hara Kalo and Dida Tuyura Ranch, which were relatively protected sites, were higher than those in other Land Use Units, which were communally grazed, during the main rainy season.

Mean herbage biomass over all Land Use Units ranged from 921 kg DM/ha in the early wet season to 1,241 kg DM/ha in the cool dry season following the long rains. Available herbage biomass during the cool dry season was higher than in the other seasons.

Biomass contribution of herbaceous species

The contribution to available biomass by various herbaceous species varied among sites and seasons. After short rains, *Chrysopogon aucheri*, *Digitaria milanjiana* and *Eragrostis papposa* were the main contributors in Dida Hara, *C. aucheri* alone contributing almost half of the herbaceous biomass. In Web, most of the contribution

Table 2. Mean monthly rainfall in mm (1988–2001) and the coefficient of variation at the main study sites in the Borana lowlands, Ethiopia (raw data for Yaballo were taken from the National Meteorological Services Agency of Ethiopia).

Month	Dida Hara		Web	
	Mean	CV (%)	Mean	CV (%)
January	24.5	125.7	17.1	193.6
February	33.6	129.5	9.2	208.7
March	56.4	76.4	68.6	66.9
April	144.1	49.2	113.9	60.9
May	77.1	69.8	57.9	68.6
June	14.5	65.5	3.2	146.9
July	12.0	106.7	3.1	151.6
August	5.9	105.1	2.0	170
September	35.2	98.3	15.7	135.0
October	87.4	59.3	58.6	93.7
November	37.1	83.6	51.7	98.1
December	17.4	69.5	11.6	171.5
Overall total	545		413	
SD	40.2		35.3	

Table 3. Effects of season on mean herbaceous presentation yields (kg/ha) on different Land Use Units in the Borana Lowlands, Ethiopia.

Land Use Unit	Cool dry (Jun-Jul 2001)		Short rains (Nov-Dec 2001)		Long rains (Mar-Apr 2002)	
	Mean	SD	Mean	SD	Mean	SD
Dida Hara Kalo	1,285	119	1,841	676	1,093	540
Dida Hara Worra	1,220	68	680	297	850	320
Web Kalo	1,269	93	1,393	662	712	210
Web Worra	1,162	174	983	496	576	658
Dida Tuyura Ranch	-[1]	-	1,542	251	1,396	740
Foora	1,270	129	458	152	901	635
Mean	1,241		1,150		921	

[1]Logistical issues prevented data collection at Dida Tuyura Ranch in June-July 2001.

Table 4. Herbaceous biomass contribution by each species (% DM basis) on different Land Use Units in the short and long rainy seasons, and their forage value (FV) as perceived by pastoralists in the Borana Lowlands, Ethiopia.

Plant family	Species	Land Use Unit[1] (%)						FV[2]
		DHK	DHW	WBK	WBW	FOR	DTR	
		Short rains (November)						
Acanthaceae	*Barleria spinisepala*	0.8	0.2	0.0	0.0	0.1	0.5	H
Asparagaceae	*Chlorophytum gallabatense*	6.0	0.5	0.1	0.0	0.0	1.8	H
Commelinaceae	*Commelina africana*	1.8	1.0	0.1	0.0	0.2	0.3	H
Cyperaceae	*Cyperus bulbosus*	0.0	0.3	0.0	0.0	1.9	0.0	I
	Cyperus sp.	2.7	1.6	0.2	0.4	0.4	0.4	I
Fabaceae	*Indigofera volkensii*	0.3	0.0	0.0	1.4	0.0	0.0	I
Poaceae	*Cenchrus ciliaris*	0.0	0.0	6.2	2.4	0.0	0.0	H
	Tetrapogon roxburghiana (syn. *Chloris roxburghiana*)	0.0	1.4	5.5	4.4	13.4	0.0	I
	Chrysopogon aucheri	41.3	50.2	4.5	2.5	57.1	37.5	I
	Cynodon dactylon	0.0	0.0	10.9	0.0	0.0	0.0	H
	Digitaria milanjiana	12.5	10.7	0.0	0.0	16.1	1.7	H
	Eleusine intermedia	3.1	0.0	0.0	0.0	0.0	0.9	L
	Eragrostis papposa	13.1	21.1	0.3	1.0	10.2	2.1	I
	Harpachne schimperi	4.4	1.0	0.0	0.0	0.6	0.7	I
	Heteropogon contortus	2.4	7.3	0.0	0.0	0.0	33.0	I
	Ischaemum afrum	0.0	0.0	5.3	0.0	0.0	0.0	L
	Leptothrium senegalense	0.0	0.0	0.1	0.7	0.0	0.0	I
	Megathyrsus maximus (syn. *Panicum maximum*)	1.5	0.0	0.0	5.9	0.0	0.0	H
	Cenchrus mezianus (syn. *Pennisetum mezianum*)	4.7	0.0	66.9	81.6	0.0	0.0	L
	Themeda triandra	4.1	3.2	0.0	0.0	0.0	21.0	I
Velloziaceae	*Xerophyta humilis*	0.8	1.2	0.0	0.0	0.0	0.2	I
		Long rains (April)						
Acanthaceae	*Barleria spinisepala*	0.0	0.0	0.0	0.0	0.7	0.0	H
Asparagaceae	*Chlorophytum gallabatense*	3.4	0.8	0.0	0.0	0.3	0.1	H
Commelinaceae	*Commelina africana*	3.1	1.2	0.4	3.9	1.5	0.0	H
Cyperaceae	*Cyperus bulbosus*	0.0	0.0	2.7	0.4	0.0	0.0	I
	Cyperus sp.	5.9	0.8	4.9	1.8	0.0	0.4	I
Poaceae	*Andropogon chinensis*	0.0	0.0	0.0	0.0	0.0	29.7	L
	Bothriochloa radicans	0.0	0.0	0.0	0.0	0.0	3.2	L
	Cenchrus ciliaris	0.0	2.4	42.8	26.1	0.0	0.0	H
	Tetrapogon roxburghiana (syn. *Chloris roxburghiana*)	1.3	0.0	0.4	8.2	0.0	0.4	I
	Chrysopogon aucheri	40.4	52.1	8.5	13.4	57.8	14.2	I
	Digitaria milanjiana	11.7	14.3	19.5	7.6	7.5	0.0	H
	Digitaria neghellensis	0.0	0.0	0.0	0.8	0.0	0.0	H
	Eleusine intermedia	3.3	0.0	0.0	0.0	0.0	0.0	L
	Eragrostis papposa	11.3	12.1	0.0	0.0	1.6	0.0	I
	Harpachne schimperi	0.0	0.0	0.0	0.0	0.0	0.4	I
	Heteropogon contortus	10.4	5.6	0.0	0.0	0.0	34.9	I
	Leptothrium senegalense	0.0	3.4	0.0	0.0	0.0	0.0	I
	Megathyrsus maximus (syn. *Panicum maximum*)	0.0	0.0	0.0	6.2	0.0	0.0	H
	Cenchrus mezianus (syn. *Pennisetum mezianum*)	3.2	1.6	0.0	18.9	30.6	0.0	L
	Setaria verticillata	0.0	0.0	0.2	0.1	0.0	0.0	H
	Sporobolus pellucidus	0.0	0.0	20.3	12.7	0.0	0.0	I
	Themeda triandra	6.1	0.8	0.0	0.0	0.0	16.7	I
Velloziaceae	*Xerophyta humilis*	0.0	4.7	0.0	0.0	0.0	0.0	I

[1]Land Use Unit: DHK = Dida Hara Kalo; DHW = Dida Hara Worra; WBK = Web Kalo; WBW = Web Worra; FOR = Foora; and DTR = Dida Tuyura Ranch; [2]Forage value: H = highly palatable; I = intermediate; and L = least palatable, as assessed by pastoralists from the main study sites, Dida Hara and Web.

to biomass (74%) was from the least palatable grass, *Cenchrus mezianus* (syn. *Pennisetum mezianum*). In Foora, most of the biomass (57%) was from a single species *C. aucheri*, although *Tetrapogon roxburghiana* (syn. *Chloris roxburghiana*) (13%), *D. milanjiana* (16%) and *E. papposa* (10%) made marked contributions. On Dida Tuyura Ranch, *C. aucheri* (37%), *Heteropogon contortus* (33%) and *Themeda triandra* (21%) were major contributors (Table 4).

In the early wet season (Long rains; Table 4), *C. aucheri* contributed nearly half (46%) to available herbaceous biomass at Dida Hara, and about 58% at Foora sites. *C. mezianus* made a large contribution (31%) at Foora site. In Web, *Cenchrus ciliaris* contributed the highest proportion (34%), while at Dida Tuyura Ranch, the dominant contributor was *H. contortus* (35%). Overall, *C. aucheri* was the main contributor to herbaceous biomass at most sites during both seasons. At Web sites, the dominant species were *C. mezianus* in November (short rains) and *C. ciliaris* in April (long rains).

Estimation of contributions to available biomass by highly desirable, intermediate and least desirable forage grasses showed that intermediate grasses were most prominent in Dida Hara, Dida Tuyura and Foora areas but not in Web zones (Table 5).

Nutritive quality of herbaceous species

Chemical composition of herbaceous samples was compared across both Land Use Units and seasons (Table 6). In general, there was a strong trend for an increase in CP% from the cool dry season to the long rainy season. Mean CP% was 48 g/kg DM in cool dry season, 62 g/kg DM after short rains and 76 g/kg DM during the long rainy season. There was variation in CP% across the seasons ($P = 0.000$) and Land Use Units ($P = 0.013$).

However, there were no differences in CP levels across various Land Use Units during the cool dry season. During the short rains, Dida Hara and Foora sites had higher CP than Web sites and Dida Tuyura Ranch. During the long rains, Web sites showed higher CP levels than Dida Hara sites with Dida Tuyura Ranch showing lowest levels (Table 6).

Nutritive value of woody plants

Mean chemical composition of leaves from woody plants is summarized in Table 7. CP concentration ranged from 47 to 168 g/kg DM. *Senegalia brevispica* (syn. *Acacia brevispica*), *Balanites aegyptiaca, Chionothrix latifolia, Combretum hereroense, Cordia sinensis* (syn. *Cordia gharaf*), *Dalbergia microphylla, Ficus sycomorus, Maerua triphylla* and *Ziziphus* sp. were the top species with higher CP%. CP% for most species reported in Table 6 was an average for 3 seasons. However, *C. hereroense, C. sinensis, D. microphylla, F. sycomorus* and *Ziziphus* sp. were sampled only during the hot dry season when other highly ranked species had shed their leaves. *Salvadora persica, Kirkia burgeri, F. sycomorus, Commiphora kataf* (syn. *Commiphora erythraea*) and *Terminalia prunioides* had high amounts of ADF, NDF and ADL.

CP% in woody plants was highest in the wet season (169 g/kg DM) and lowest in the dry season (139 g/kg DM; Table 8).

Table 5. Mean contribution (% DM basis) to available biomass by highly desirable, intermediate and least desirable species in the Borana Lowlands, Ethiopia for the various Land Use Units.

	Land Use Unit[1]					
	DHK	DHW	WBK	WBW	DTR	FOR
	Short rains (November)					
Grasses						
Highly desirable	14.0	10.7	17.1	8.3	1.7	16.1
Intermediate	65.3	84.2	10.4	8.6	94.3	81.3
Least desirable	7.8	0.0	72.2	81.6	0.9	0.0
Sedges	2.7	2.0	0.2	0.4	0.4	2.3
Forbs	10.2	3.0	0.2	1.4	2.8	0.3
	Long rains (April)					
Grasses						
Highly desirable	11.7	16.9	62.5	40.8	0.0	7.5
Intermediate	69.5	74.1	29.2	34.3	66.6	59.5
Least desirable	6.5	1.6	0.0	18.9	32.9	30.6
Sedges	5.9	0.8	7.6	2.2	0.4	0.0
Forbs	6.5	6.7	0.5	3.9	0.1	2.5
	Mean of the two seasons					
Grasses						
Highly desirable	12.8	13.8	39.8	24.5	0.8	11.8
Intermediate	67.4	79.1	19.8	21.4	80.4	70.4
Least desirable	7.2	0.8	36.1	50.2	16.9	15.3
Sedges	4.3	1.4	3.9	1.3	0.4	1.1
Forbs	8.3	4.9	0.4	2.6	1.5	1.4

[1]Land Use Units: DHK = Dida Hara Kalo; DHW = Dida Hara Worra; WBK = Web Kalo; WBW = Web Worra; FOR = Foora; and DTR = Dida Tuyura Ranch.

Pastoralists' perceptions

Ranking of the forage value of plant species was performed through group discussions with pastoralists. According to the perceptions of Borana pastoralists, *Cenchrus ciliaris* and *Digitaria milanjiana* were grasses with the highest nutritive value (Tables 4 and 9). Similarly, *Senegalia brevispica, Grewia tembensis* and *Maeroa triphylla* were the most highly appreciated woody species (Table 8). Some forbs, such as *Commelina africana*, have high nutritional quality. All sedges (*Cyperus* spp.) were perceived to have intermediate nutritional value (Table 4).

Table 6. Mean chemical composition of the herbaceous biomass sampled in three seasons across different Land Use Units (LUU) in the Borana Lowlands, Ethiopia.

LUU[1]	Ash (%)	OM (%)	NDF (g/kg)	ADF (g/kg)	ADL (g/kg)	ADF-Ash (g/kg)	CP (g/kg)	IVDMD (%)
	Cool dry season (June-July)							
DHK	11	89	761	429	91	68	45	38
DHW	13	87	742	434	89	82	45	32
WBK	11	90	754	439	144	59	52	37
WBW	9	91	780	491	277	42	47	33
DTR[2]	-	-	-	-	-	-	-	-
FOR	10	90	768	468	95	67	49	36
	Short rains (November-December)							
DHK	10	90	768	481	101	51	67	
DHW	11	89	763	418	87	69	79	
WBK	9	91	798	504	111	52	49	
WBW	9	91	803	508	126	52	52	
DTR	9	91	768	479	89	51	50	
FOR	11	89	730	407	80	68	76	
	Long rains (March-April)							
DHK	12	88	717	413	91	57	78	
DHW	15	86	696	383	78	77	84	
WBK	21	79	638	368	78	134	96	
WBW	23	77	653	375	112	143	101	
DTR	9	91	779	468	90	46	40	
FOR	11	89	778	450	114	65	58	

[1]Land Use Units: DHK = Dida Hara Kalo; DHW = Dida Hara Worra; WBK = Web Kalo; WBW = Web Worra; FOR = Foora; and DTR = Dida Tuyura Ranch.
[2]During the cool dry season, samples were not collected from DTR.
OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein; IVDMD = in vitro dry matter digestibility.

Table 7. Mean nutritive values of leaves from woody plants across 3 seasons and frequency of appreciation (FAP) of the forage species by pastoralists in the Borana Lowlands, Ethiopia.

Family	Species	DM (%)	Ash (%)	NDF (g/kg)	ADF (g/kg)	ADL (g/kg)	CP (g/kg)	FAP (%)
Amaranthaceae	*Chionothrix latifolia*[1]	91	19	378	251	36	136	38[3]
Anacardiaceae	*Rhus natalensis*[1]	91	10	538	422	246	90	75
Asteraceae	*Vernonia phillipsiae*	91	12	480	328	119	76	50
Apocynaceae	*Cynanchum viminale* (syn. *Sarcostemma viminale*)[1]	89	11	498	482	93	47	38
Burseraceae	*Commiphora kataf* (syn. *Commiphora erythraea*)	88	10	307	296	127	93	13
	Commiphora habessinica	89	14	473	484	291	114	25
Capparaceae	*Boscia mossambicensis*[1,3]	92	8	616	414	143	100	63[3]
	Cadaba farinosa[1]	91	24	379	290	156	113	25
	Maerua triphylla[1]	92	14	451	294	107	141	63
Combretaceae	*Combretum hereroense*[2]	91	11	314	268	72	140	13
	Terminalia prunioides[1]	89	9	290	286	68	100	25
Convolvulaceae	*Cladostigma hildebrandtioides*	92	9	546	390	79	117	25[3]
Cordiaceae	*Cordia sinensis* (syn. *Cordia gharaf*)[1,2]	91	18	365	407	89	141	13
Ebenaceae	*Euclea divinorum*[1,2]	92	8	245	329	151	80	38[3]
Fabaceae	*Senegalia brevispica* (syn. *Acacia brevispica*)[1]	91	7	421	278	132	154	88
	Senegalia goetzei (syn. *Acacia goetzei*)[1]	91	7	511	458	250	109	13[3]
	Vachellia tortilis (syn. *Acacia tortilis*)[1]	91	7	490	440	208	113	63[3]
	Dalbergia microphylla[1,2]	92	8	293	217	65	150	25

Continued

Family	Species	DM (%)	Ash (%)	NDF (g/kg)	ADF (g/kg)	ADL (g/kg)	CP (g/kg)	FAP (%)
Kirkiaceae	*Kirkia burger*	88	8	280	217	68	109	13
Lamiaceae	*Plectranthus igniarius*	90	18	502	540	250	116	38
Malvaceae	*Grewia damine* (syn. *Grewia bicolor*)[1,3]	91	9	484	367	128	104	100[3]
	Grewia tembensis[1]	90	13	407	303	67	130	88
Moraceae	*Ficus sycomorus*[1,2]	89	15	250	234	54	134	13[3]
Oleaceae	*Olea europaea* subsp. *cuspidata*[1]	93	6	384	297	127	73	38[3]
	Schrebera alata[1,2]	92	6	306	301	79	97	-
Phyllanthaceae	*Phyllanthus sepialis*	91	12	432	298	97	99	38[3]
Rhamnaceae	*Ziziphus* sp.[2]	92	5	177	153	41	168	-
Rutaceae	*Vepris glomerata*[1]	93	10	465	380	173	144	38
Salvadoraceae	*Salvadora persica*[1]	88	36	252	162	39	89	13
Sapindaceae	*Haplocoelum foliolosum*[1]	93	7	473	359	153	77	25[3]
	Pappea capensis[1,3]	92	6	526	423	163	83	75[3]
Zygophyllaceae	*Balanites aegyptiaca*[1,3]	92	11	408	287	132	120	38

[1]Drought-resistant forage species (according to the pastoralists' perceptions).
[2]Species sampled only once during hot dry season (December–February) but were not appreciated as highly desirable forage species by the pastoralists (Gemedo Dalle 2004).
[3]Woody forage species appreciated by the pastoralists for grazers (cattle).
DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein; FAP = frequency of appreciation.

Table 8. Mean seasonal chemical composition of forage from woody plants in the Borana Lowlands, Ethiopia.

Season	Ash (%)	NDF (g/kg)	ADF (g/kg)	ADL (g/kg)	CP (g/kg)
Hot dry (Jan-Feb)	10.9	326	300	96	139
Short rains (Nov-Dec)	12.5	506	391	161	167
Long rains (Mar-Apr)	11.6	444	332	138	169

NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein.

Table 9. Preference ranking (1 = highest rank) of the top 5 grass and woody forage species by men (M) and women (W) pastoralists (number of participants in parenthesis) during group discussions in the Borana Lowlands, Ethiopia.

	Site[1] and gender group							
	DIK		DAC		TSQ		DBK	
	M (11)	W(13)	M (13)	W (10)	M (13)	W(21)	M (15)	W (12)
Forage grasses								
Cenchrus ciliaris	1	2	1	2	1	2	1	2
Chrysopogon aucheri	4	4	3	3	-	4	3	5
Digitaria milanjiana	2	1	2	1	3	1	2	4
Digitaria neghellensis	3	-[2]	-	-	2	-	-	-
Eleusine intermedia	-	-	5	-	-	-	5	-
Halchisoo (botanical name not determined)	-	-	-	-	4	-	-	1
Megathyrsus maximus (syn. *Panicum maximum*)	-	-	4	5	-	-	4	-
Cenchrus mezianus (syn. *Pennisetum mezianum*)	5	3				3		3
Themeda triandra	-	5	-	4	5	5	-	-
Woody plants for browsers								
Senegalia brevispica (syn. *Acacia brevispica*)	1	-	1	1	2	-	2	1
Vachellia nilotica subsp. *nilotica* (syn. *Acacia nilotica*)	-	5	-	-	-	-	-	-
Vachellia tortilis (syn. *Acacia tortilis*)	4	-	-	-	-	-	-	-
Balanites aegyptiaca	-	4	2	-	-	-	-	-
Blepharispermum pubescens[3]	-	-	-	-	-	2	-	-
Cadaba farinosa	-	-	-	-	4	1	-	-

Continued

	Site[1] and gender group							
	DIK		DAC		TSQ		DBK	
	M (11)	W(13)	M (13)	W (10)	M (13)	W(21)	M (15)	W (12)
Commiphora kataf (syn. *Commiphora erythraea*)	-	-	-	-	-	-	3	-
Dalbergia microphylla	-	-	-	-	-	-	-	5
Grewia damine (syn. *Grewia bicolor*)	-	-	-	-	-	5	-	-
Grewia tembensis	5	2	3	3	1	-	1	4
Kirkia burgeri	-	-	-	-	-	-	4	-
Maerua triphylla	3	-	5	4	-	4	-	3
Phyllanthus sepialis	-	-	4	-	-	-	-	-
Plectranthus barbatus (syn. *Plectranthus comosus*)[3]	-	-	-	-	5	-	-	-
Rhus natalensis						3		2
Cynanchum viminale (syn. *Sarcostemma viminale*)	2	1	-	2	-	-	-	-
Vepris glomerata	-	-	-	-	3	-	5	-
Vernonia phillipsiae		3		5				

[1]DIK = Dikale, DAC = Dambala Abba Chana, TSQ = Tesso Qallo and DBK = Dhibu Kolocho.
[2]Empty cell (-) means the species was ranked below rank 5.
[3]Species not listed in Table 7 (Table 7 contains species that were sampled, whereas this table contains species mentioned by local communities during free listing).

Discussion

Herbaceous standing crop

The greater availability of forage on the government ranch and Kalos (enclosures) than on open-grazed areas such as the Worra and Foora showed the importance of exclosures for conserving and sustainably using forage resources during the dry season. This was in agreement with previous reports (Oba et al. 2001; Ayana Angassa et al. 2010). The pattern for highest mean herbaceous biomass to occur in the cool dry season (Jun-Jul) and lowest in the main rainy season (Mar-Apr), after the hot dry season, was according to expectations. In the cool dry season growth of vegetation has been stimulated by the long rains and herbage is mature. It is traditional for Borana pastoralists to protect Kalos from grazing during the wet season and open them for general grazing in the hot dry season (Dec-Jan), when there is relatively high accumulation of herbage mass. Grazing land management practices may be the main reasons for the significant differences in presentation yields of herbaceous biomass across the Land Use Units in this study rather than the actual productive ability of these areas. The different presentation yields of forage on the government ranch from those on the communal grazing lands may reflect grazing management imposed. There were only few Borana cattle on the government ranch during the study years and it was therefore subjected to only low stocking pressure during all seasons. Old growth accumulated and rank grass dominated the new growth of desirable species. Furthermore, the ranch was highly encroached by woody species that might have contributed to low forage quality. Although the author of this manuscript agrees with protection of the ranch area, proper management following standard range management techniques should be followed. It should serve as a demonstration farm and learning laboratory for surrounding pastoralists. Ayana Angassa et al. (2010) also reported that there was variation as a result of rangeland management that affected biomass of most herbaceous species, plus grass basal cover and herbaceous species richness and diversity.

Quantifying the contributions of various species to forage mass allows useful comparisons of the productivity of different species and different management practices, and provides a basis for appropriate stocking rates to be developed (Sollenberger and Cherney 1995). Rainfall (precipitation), soil moisture, radiation, temperature, soil nitrogen and phosphorus are important environmental factors that affect herbage production (Gutman et al. 1990). The maximum presentation yield of herbage recorded in this study was 1,840 kg DM/ha during the short rains, while studies in similar semi-arid ecosystems reported much higher values. In similar arid areas of northern Kenya, mean herbaceous biomass inside exclosures was 4,180 kg DM/ha and that of continuously grazed open rangelands 1,802 kg DM/ha (Oba et al. 2001). Our results for herbaceous biomass yields from all sites in the Borana Lowlands fall within the category of poor, and such low herbaceous yields would directly affect livestock production and ecosystem stability.

Unlike the study of Ayana Angassa and Baars (2000), very low percentages of highly palatable grasses were found. Grasses considered of intermediate nutritive value by pastoralists were dominant at most sites, while highly palatable species were present at much lower levels, in agreement with the rangeland condition assessment report by Gemedo Dalle et al. (2006). This aspect is discussed further in a subsequent section.

Seasonal changes in forage value

Both herbage biomass and chemical composition of the herbaceous forage samples varied significantly across the seasons. Physiological age of forage species, time of grazing, species and botanical fraction are some of the factors that cause variability in chemical composition of forages (Adesogan et al. 2000). The decline in CP% in herbaceous forage from 7.6% in the wet season to 4.8% in the dry season, and that of woody plants from 16.9 to 13.9%, respectively, is in agreement with previous findings (Pérez Corona et al. 1998; Hussain and Durrani 2009; Habtamu Teka et al. 2013; Zhai et al. 2018). According to the report by Habtamu Teka et al. (2013), chemical composition of all grass species showed significant ($P<0.05$) variation between sites, seasons and species in agreement with our results.

In contrast, nutrient concentrations of browse from woody plants is subject to relatively less seasonal variation (Crowder and Chheda 1982) and this particularly enhances their value as dry season feeds for livestock (Dicko and Sikena 1992). About three-quarters of the woody forage plants studied were perceived as drought-resistant species by pastoralists. Several studies from arid ecosystems, e.g. Dicko and Sikena (1992), have shown that perennial shrubs retained high CP% for a longer period than herbaceous species, with a range of 7% in winter to 14% in spring. During the hot dry season when herbaceous species were almost dry and in limited supply for livestock, mean CP% in leaf material on woody plants was 13.9%, which would provide a valuable N supply to rumen microflora provided the nitrogenous components were digestible. Mean in vitro dry matter digestibility of the herbaceous forage was very low (35%) with a range from 32 to 38% indicating its limited potential to contribute energy. Conservation of these drought-resistant species is an important strategy for sustaining livestock production, especially during dry periods.

Comparing pastoralists' perceptions and scientific knowledge on forage nutritive value

The pastoralists identified *Cenchrus ciliaris*, *Digitaria milanjiana*, *Megathyrsus maximus* and *Themeda triandra* as highly palatable grasses. Similar perceptions of Borana pastoralists were reported by Habtamu Teka et al. (2013) and in an earlier report by Skerman and Riveros (1989). Furthermore, the pastoralists identified *Chrysopogon aucheri* and *Digitaria milanjiana* as drought-resistant forage grasses, concurring with the report by Skerman and Riveros (1989). Among the woody species *Vachellia tortilis*, *Boscia mossambicensis*, *Chionothrix latifolia*, *Grewia damine* and *Pappea capensis* for grazers (cattle) and *Senegalia brevispica*, *Grewia tembensis* and *Maerua triphylla* for browsers, e.g. camels and goats, were ranked as the top forage species. Le Houérou and Corra (1980) also reported that most of the woody plants identified during this study were considered palatable for animals, being preferred over other species.

Comparing pastoralists' indigenous knowledge with laboratory results showed that Borana pastoralists have an accurate perception of the nutritive value of the various forage species. In general, the pastoralists' knowledge of forage species growth and quality indicated that they know which species should be retained in the pasture and which were least useful to ensure sustainable animal production in the area.

Comparison of the Land Use Units

The high fluctuation of species composition on Web sites might be due to presence of permanent watering points (Web deep wells or Ela) in the area, which allowed high numbers of livestock to remain in the area resulting in overgrazing and consumption of desirable species. During the long rains, most livestock are taken away from the deep wells as water is more readily available and highly desirable species get the opportunity to regrow during this time.

When comparing Land Use Units, CP% varied across the Land Use Units as reported by Habtamu Teka et al. (2013). Overall, forage on the Dida Tuyura Ranch had the lowest CP levels and the highest concentrations of NDF and ADF reflecting the under-utilization of this area and accumulation of a bulk of mature fibrous material. This suggests that 'over-protection' of rangelands is not necessarily a desirable management strategy and significant defoliation by grazing animals at certain times might be needed to stimulate pastures and ensure a sustainable system.

Status of forage nutritive value in relation to livestock production

Herbaceous species had lower forage quality than the woody browse species in agreement with previous reports (Hussain and Durrani 2009; Gete Zewudu and Gemedo Dalle 2019). CP concentrations in standing forage exceeded the threshold level of 7% (Humphreys 1991; Pérez Corona et al. 1998; Ganskopp and Bohnert 2001) only during the long rains. This and other studies, e.g. Habtamu Teka et al. (2013), showed that quality of standing herbaceous forage in the Borana Lowlands was largely below the threshold level for good livestock production. The minimum recommended CP concentration in diets for small ruminants is even higher (9%) (Araújo Filho et al. 1998). While animals would select a higher quality diet from herbaceous forage than the whole plant data indicate, as the seasons progressed CP concentrations in the selected diet would decline. Access to some browse from woody species would alleviate this N deficiency as time progressed, while feeding of N supplements would also increase intake of the low quality forage. The study highlighted the importance of restoring degraded rangelands and also the need to improve forage quality through focused interventions aimed at increasing CP concentration in the herbaceous forage.

Tree leaves are nutritionally desirable, mainly as a source of CP (Forwood and Owensby 1985). The mean CP concentration in foliage of woody plants determined by this study was 11%. This was significantly higher than the CP concentration in herbaceous forage in agreement with reports from other areas, e.g. Musco et al. (2016). Furthermore, NDF, ADF and ADL concentrations in forage from woody plants were lower than those of herbaceous species. Trees and shrubs represent an integral part of diets for domestic ruminants in Africa and may constitute an important source of proteins, minerals and vitamins, especially during the dry season (Dicko and Sikena 1992). Borana pastoralists recognized the importance of woody species with higher CP concentration in this semi-arid environment. For long-term sustainability of the system, grazing management must be designed to retain the desirable woody plants, while restricting encroachment by undesirable trees and shrubs.

In conclusion, this study provides additional information on quantity (herbaceous) and nutritive value (both herbaceous and woody plants) of available forage in different seasons across different functional Land Use Units in a semi-arid rangeland of the Borana Lowlands, Ethiopia, where the main production system is livestock production, predominantly cattle breeding. Mean nutritive value of available forage from herbaceous species was below the critical level recommended for maintenance of both beef cattle and small ruminants for much of the time, while forage from woody species contained moderate levels of crude protein. This indicates the importance of maintaining a mixture of herbaceous species and desirable woody species in these rangelands. In the Dida Hara rangelands, very few highly desirable herbaceous species were present, even in areas where grazing was restricted. There is an urgent need to restore these rangelands to a condition where desirable species are more prevalent. Surprisingly, in Web areas desirable species were dominant during the long rains. Further studies are warranted to determine why these differences occurred between the Land Use Units and whether strategies can be developed to improve the situation in the Dida Hara area. It seems that 'over-protection' as has been practiced on the Dida Tuyura Farm is not the solution and more intensive study of the Web sites may yield possible solutions for testing. Furthermore, herd diversification for effective utilization of browse species was recommended as a result of this study.

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References

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Adesogan AT; Givens DI; Owen E. 2000. Measuring chemical composition and nutritive value in forages. In: Mannetje L't; Jones RM, eds. Field and laboratory methods for grassland and animal production research. CAB International, Wallingford, UK. p. 263–278. doi: 10.1079/9780851993515.0263

Alemayehu Mengistu. 1998. The Borana and the 1991–92 drought. A rangeland and livestock resource study. Institute for Sustainable Development and French Catholic Committee against Hunger and for Development, Addis Ababa, Ethiopia.

Alemayehu Mengistu. 2004. Rangelands biodiversity: Concepts, approaches and the way forward. Addis Ababa University, Addis Ababa, Ethiopia.

Ayana Angassa; Baars RMT. 2000. Ecological condition of encroached and non-encroached rangelands in Borana, Ethiopia. African Journal of Ecology 38:321–328. doi: 10.1046/j.1365-2028.2000.00250.x

Ayana Angassa ; Oba G; Treydte AC; Weladji RB. 2010. Role of traditional enclosures on the diversity of herbaceous vegetation in a semi-arid rangeland, southern Ethiopia. Livestock Research for Rural Development 22, Article #163. lrrd.org/lrrd22/9/anga22163.htm

AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis. 15th Edn. AOAC, Arlington, VA, USA.

Araújo Filho JA de; Leite ER; Silva NL da. 1998. Contribution of woody species to the diet composition of goat and sheep in Caatinga vegetation. Pasturas Tropicales 20(2):41–45. bit.ly/3eNCXnB

Aynalem Haile; Workneh Ayalew; Noah Kebede; Tadelle Dessie; Azage Tegegne. 2011. Breeding strategy to improve Ethiopian Boran cattle for meat and milk production. Improving Productivity and Market Success of Ethiopian Farmers Project Working Paper No. 26. ILRI, Nairobi, Kenya. hdl.handle.net/10568/3141

Baars RMT; Chileshe EC; Kalokoni DM. 1996. Rangeland condition in high cattle density areas. In: Baars RMT, ed. Condition and management of the rangelands in the western province of Zambia. Ph.D. Thesis. Wageningen Agricultural University, Wageningen, The Netherlands. p. 71–82. edepot.wur.nl/493462

Crowder LV; Chheda HR. 1982. Tropical grassland husbandry. Longman, London, UK.

Coppock DL. 1994. The Borana Plateau of Southern Ethiopia: Synthesis of pastoral research, development and change, 1980–1991. ILCA, Addis Ababa, Ethiopia. hdl.handle.net/10568/4660

CSA (Central Statistical Authority). 2008. Ethiopian Statistical Abstract. CSA, Addis Ababa, Ethiopia. bit.ly/3eJgKXZ

Dicko MS; Sikena LK. 1992. Feeding behavior, quantitative and qualitative intake of browse by domestic ruminants. In: Speedy A; Pugliese PL, eds. Legume trees and other fodder trees as protein sources for livestock. FAO Animal Production and Health Paper 102:129–144. bit.ly/2RGI1Aq

Edwards S; Mesfin Taddese; Hedberg I. 1995. Flora of Ethiopia and Eritrea Vol. 2, Part 2. Uppsala University, Uppsala, Sweden.

Edwards S; Sebsebe Demissew; Hedberg I. 1997. Flora of Ethiopia and Eritrea. Vol. 6. Uppsala University, Uppsala, Sweden.

Forwood JR; Owensby CE. 1985. Nutritive value of tree leaves in the Kansas Flint Hills. Journal of Range Management 38:61–64. bit.ly/3bdqrM6

Fowler J; Cohen L. 1996. Practical statistics for field biology. John Wiley and Sons, Chichester, UK.

Ganskopp D; Bohnert D. 2001. Nutritional dynamics of seven northern Great Basin grasses. Journal of Range Management 54:640–647. doi: 10.2458/azu_jrm_v54i6_ganskopp

Gemedo Dalle 2004. Vegetation ecology, rangeland condition and forage resources evaluation in the Borana Lowlands, Southern Oromia, Ethiopia. Ph.D. Thesis. Georg-August Universität Göttingen, Germany. bit.ly/3eoCdFy

Gemedo Dalle; Maass BL; Isselstein J. 2006. Rangeland condition and trend in the semi-arid Borana Lowlands, Southern Oromia, Ethiopia. African Journal of Range and Forage Science 23:49–58. doi: 10.2989/10220110609485886

Gete Zewudu; Gemedo Dalle. 2019. Evaluation of nutritive value of some native forage species in Tikur Incinni District, Oromia, Ethiopia. Forage Research 45:103–110. bit.ly/34D5MP3

Gutman M; Seligman NG; Noy-Meir I. 1990. Herbage production of Mediterranean grassland under seasonal and yearlong grazing systems. Journal of Range Management 43:64–68. bit.ly/2XDcSle

Habtamu Teka; Madakadze IC; Ayana Angassa; Hassen A. 2013. Nutritive value of grasses in semi-arid rangelands of Ethiopia: Local experience based herbage preference evaluation versus laboratory analysis. Asian-Australasian Journal of Animal Science 26:366–377. doi: 10.5713/ajas.2012.12551

Haugen T. 1992. Woody vegetation of Borana, south Ethiopia. A study on the main vegetation types of the area. SINET: Ethiopian Journal of Science 15:117–130.

Hedberg I; Edwards S. 1995. Flora of Ethiopia and Eritrea. Volume 7. Uppsala University, Uppsala, Sweden.

Herlocker DJ. 1999. Rangeland ecology and resource development in eastern Africa. GTZ, Nairobi, Kenya.

Homann S. 2004. Indigenous knowledge of Borana pastoralists in natural resource management: A case study from Southern Ethiopia. Ph.D. Thesis. Justus Liebig University, Giessen, Germany. core.ac.uk/display/56347787?recSetID=

Humphreys LR. 1991. Tropical pasture utilisation. Cambridge University Press, Cambridge, UK. doi: 10.1017/CBO9780511525810

Hussain F; Durrani MJ. 2009. Nutritional evaluation of some forage plants from Harboi rangeland, Kalat, Pakistan. Pakistan Journal of Botany 41(3):1137–1154. bit.ly/3eikcse

Le Houérou HN; Corra M. 1980. Some browse plants of Ethiopia. In: Le Houérou HN, ed. Browse in Africa. The current state of knowledge. Proceedings of the International Symposium on Browse in Africa, Addis Ababa, 8–12 April 1980. p. 109–114. hdl.handle.net/10568/67392

Musco N; Koura IB; Tudisco R; Awadjihè G; Adjolohoun S; Cutrignelli MI; Mollica MP; Houinato M; Infascelli F; Calabrò S. 2016. Nutritional characteristics of forage grown in South of Benin. Asian-Australasian Journal of Animal Sciences 29:51–61. doi: 10.5713/ajas.15.0200

Oba G; Vetaas OR; Stenseth NC. 2001. Relationships between biomass and plant species richness in arid-zone grazing lands. Journal of Applied Ecology 38:836–845. doi: 10.1046/j.1365-2664.2001.00638.x

Pérez Corona ME; Vázquez de Aldana BR; García Criado B; García Ciudad A. 1998. Variations in nutritional quality and biomass production of semi-arid grasslands. Journal of Range Management 51:570–576. bit.ly/2ykYLWR

Shapiro BI; Gebru G; Desta S; Negassa A; Nigussie K; Aboset G; Mechale H. 2017. Ethiopian livestock sector analysis: A 15 year livestock sector strategy. ILRI Project Report. ILRI, Nairobi, Kenya. hdl.handle.net/10568/92057

Skerman PJ; Riveros F. 1989. Tropical grasses. FAO Plant Production and Protection Series No. 23. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. bit.ly/3cfqUxk

Sollenberger LE; Cherney DJR. 1995. Evaluating forage production and quality. In: Barnes RF; Miller DA; Nelson CJ, eds. Forages. Volume II: The science of grassland agriculture. 5th Edn. Iowa State University Press, Ames, IA, USA. p. 97–120.

Van Soest PJ; Robertson JB. 1985. Analysis of forage and fibrous foods. Laboratory manual. Volume 613. Cornell University, Ithaca, NY, USA.

Vázquez-de-Aldana BR; García-Ciudad B; Pérez-Corona ME; García-Criado A. 2000. Nutritional quality of semi-arid grassland in western Spain over a 10-year period: Changes in chemical composition of grasses, legumes and forbs. Grass and Forage Science 55:209–220. doi: [10.1046/j.1365-2494.2000.00217.x](https://doi.org/10.1046/j.1365-2494.2000.00217.x)

Whalley RDB; Hardy MB. 2000. Measuring botanical composition of grasslands. In: Mannetje L't; Jones RM, eds. Field and laboratory methods for grassland and animal production research. CAB International, Wallingford, UK. p. 263–278. doi: [10.1079/9780851993515.0067](https://doi.org/10.1079/9780851993515.0067)

Zhai X; Zhang Y; Wang K; Chen Q; Li S; Huang D. 2018. Grazing effects on the nutritive value of dominant species in steppe grasslands of northern China. BMC Ecology 18:30. doi: [10.1186/s12898-018-0186-8](https://doi.org/10.1186/s12898-018-0186-8)

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Research Paper

Ammonium sulfate enhances the effectiveness of reactive natural phosphate for fertilizing tropical grasses

Sulfato de amonio como mejorador de la efectividad del fosfato natural reactivo en la fertilización de gramíneas tropicales

CARLOS E.A. CABRAL[1], CARLA H.A. CABRAL[1], ALYCE R.M. SANTOS[2], KÁSSIO S. CARVALHO[3], EDNA M. BONFIM-SILVA[1], LUIZ J.M. MOTTA[1], JENIFER S. MATTOS[1], LETÍCIA B. ALVES[1] AND ANA P. BAYS[2]

[1]*Instituto de Ciências Agrárias e Tecnológicas (ICAT), Universidade Federal de Mato Grosso, Rondonópolis, MT, Brazil.* ufmt.br
[2]*Faculdade de Agronomia e Zootecnia (FAAZ), Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil.* ufmt.br
[3]*Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso, Sorriso, MT, Brazil.* srs.ifmt.edu.br

Abstract

Reactive natural phosphate is a slow and gradual solubilizing fertilizer, which makes it difficult to use in neutral to alkaline soils. Nitrogen fertilizers which acidify the soil may increase the possibility of using this phosphate fertilizer commercially. Two greenhouse experiments were conducted to compare responses of Xaraés palisadegrass (*Urochloa brizantha* syn. *Brachiaria brizantha* cv. Xaraés) and Mombasa guineagrass (*Megathyrsus maximus* syn. *Panicum maximum* cv. Mombasa), when different combinations of P and N fertilizers were applied during the establishment phase in non-acidic soils or with corrected acidity. The experiments were carried out in a completely randomized design with 3 fertilizer combinations (simple superphosphate plus urea, SSU; natural reactive phosphate plus urea, RPU; and natural reactive phosphate plus ammonium sulfate, RPAS). There was no difference in tiller density, leaf numbers, forage mass, leaf mass and stem mass for either forage on SSU and RPAS treatments but they exceeded those on RPU. Soil pH was lower in soil fertilized with ammonium sulfate than in soil fertilized with urea. Applying natural reactive phosphate plus ammonium sulfate seems as effective as simple superphosphate plus urea in promoting increased growth in tropical grasses on low-P soils. Longer-term and more extensive field studies are needed to determine if these results can be reproduced in the long term, and the level of soil acidification over time.

Keywords: Ammonium sulfate, establishment fertilization, *Megathyrsus maximus,* phosphorus, soil acidification, urea, *Urochloa brizantha*.

Resumen

El fosfato natural reactivo es un fertilizante que se solubiliza en forma lenta y gradual, lo que dificulta su uso en suelos de reacción neutra a alcalina. Los fertilizantes nitrogenados que acidifican el suelo pueden favorecer el uso del fosfato natural reactivo. Por tanto, el objetivo de este trabajo fue verificar cuál fertilizante nitrogenado favorece el uso de fosfato natural reactivo durante la fase de establecimiento de 2 gramíneas tropicales, en suelo no ácido o con acidez corregida. Para el efecto en condiciones de invernadero se realizaron sendos experimentos con un diseño completamente al azar, con los pastos *Urochloa brizantha* (syn. *Brachiaria brizantha*) cv. Xaraés y *Megathyrsus maximus* (syn. *Panicum maximum*) cv. Mombasa. Los tratamientos consistieron en fertilización con superfosfato simple y urea (SSU), fosfato reactivo natural y urea (RPU) y fosfato reactivo natural y sulfato de amonio (RPAS). No se encontraron diferencias en la densidad de rebrotes, número de hojas, biomasa forrajera, biomasa de hoja y tallo en ambos pastos entre los

Correspondence: Carlos Eduardo Avelino Cabral, Instituto de Ciências Agrárias e Tecnológicas (ICAT), Universidade Federal de Mato Grosso, Rondonópolis, CEP 78735-901, MT, Brazil.
Email: carlos.eduardocabral@hotmail.com

tratamientos de SSU y RPAS, aunque estos parámetros presentaron valores más bajos en el tratamiento RPU. El pH fue más bajo en el suelo fertilizado con sulfato de amonio, en comparación con la urea. El sulfato de amonio, al acidificar el suelo más que la urea, favorece el uso de fosfato natural reactivo en la fertilización de pastos en suelos bajos en fósforo. Se necesitan estudios de campo a plazo más largo y más extensivos para determinar el nivel de acidificación del suelo a lo largo del tiempo y si los resultados de este estudio pueden ser reproducidos a largo plazo.

Palabras clave: Acidificación del suelo, fertilización de establecimiento, fósforo, *Megathyrsus maximus,* sulfato de amonio, urea, *Urochloa brizantha*.

Introduction

Phosphorus plays an important role in development of root systems of plants, and concentration of this nutrient in tropical soils is often low (Marcante et al. 2016; Zambrosi et al. 2017). Deficiency of P during pasture establishment reduces forage photosynthetic activity (Ghannoum et al. 2008), which has an impact on leaf elongation (Kavanova et al. 2006) plus forage mass and root production (Rezende et al. 2011; Waddell et al. 2017; Ros et al. 2018). In P-deficient soils applying P fertilizer is essential to get satisfactory pasture growth and animal performance, which represents a significant cost for cattle farmers.

While water-soluble phosphate fertilizers, such as triple and single superphosphate, are most commonly used, a lower-cost alternative is reactive natural phosphate. Reactive natural phosphates come from sedimentary rocks and differ from those from igneous and metamorphic rocks, which have low reactivity and are commonly called 'rock' phosphate (Corrêa et al. 2005). The lower cost of reactive natural phosphate results from physical processing being all that is involved in manufacture, as opposed to water-soluble fertilizers, which are both milled and chemically (Ivanova et al. 2006) or thermally solubilized.

Despite its low cost, an obstacle to the use of natural reactive phosphate is that it requires low soil pH, which enables phosphorus in the fertilizer to be converted to a soluble form and enter the soil solution (Guedes et al. 2009). However, soil acidity also has a negative effect on availability of P in tropical soils, as it increases the adsorption of P by oxides and promotes the precipitation of this nutrient with free aluminum and cationic micro-nutrients (Souza et al. 2006). Therefore, for productive pastures, correction of soil acidity is an important practice.

Most grass pastures will respond to N fertilizer application and P fertilizer is rarely applied without added N fertilizer. During the nitrification process release of ammonia from nitrogen fertilizers also releases hydrogen in the soil solution, which acidifies the soil by lowering pH (Schroeder et al. 2011; Bortoluzzi et al. 2017; Cabral et al. 2018).

Urea is the most popular choice of nitrogen fertilizer, due to its high nitrogen concentration and relatively low cost. However, high losses of N can occur through volatilization. Ammonium sulfate is an alternative source of N and is less susceptible to volatilization, but is more expensive than urea per unit of N and has a lower concentration of this nutrient which increases freight costs (Werneck et al. 2012). Both urea and ammonium sulfate can be applied to enhance the viability of applying natural phosphate to forages (Nascimento et al. 2002).

This work was designed to determine which nitrogen fertilizer promoted better responses in tropical grasses grown in non-acid or corrected acid soils when applied with reactive natural phosphate.

Materials and Methods

The experiments were performed in a greenhouse at Federal University of Mato Grosso, Cuiabá, MT, in a completely randomized design with 3 treatments.

Treatments consisted of different combinations of nitrogen and phosphorus fertilizers, i.e. simple super-phosphate plus urea (SSU), reactive natural phosphate plus urea (RPU) and reactive natural phosphate plus ammonium sulfate (RPAS). The SSU treatment was considered the Control treatment, because it combined a readily available source of P with the N fertilizer most commonly used.

Experiment 1

Experiment 1 was conducted with Xaraés palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R.D. Webster cv. Xaraés] with 6 replicates of the fertilizer treatments. The soil used was the top 20 cm of an Oxisol, collected in native Cerrado areas, with texture characterized by 57.5% sand, 5.0% silt and 37.5% clay. Chemical composition of the soil was: P = 1.1 mg/dm^3; potassium (K) = 47 mg/dm^3; calcium (Ca) = 0.2 cmol$_c$/dm^3; magnesium (Mg) = 0.1 cmol$_c$/dm^3; hydrogen and aluminum (H+Al) = 5.7 cmol$_c$/dm^3; cation exchange capacity = 6.1 cmol$_c$/dm^3; base saturation = 6.9%; aluminum saturation = 70.4%; and pH (in calcium chloride) = 4.1. After collection, the soil was sieved through a 4.0 mm mesh and transferred to pots with 3.5 dm^3 volume. Dolomitic limestone was applied to raise base saturation to 50%. After the incorporation, the soil was left for 30 days with soil moisture kept at 80% field capacity for limestone reaction. Thirty days after limestone incorporation, P fertilizer was applied at the rate of 300 mg P_2O_5/dm^3 (131 mg P/dm^3). Seed was sown after P fertilizer application, and after seedling emergence seedlings were thinned to leave 3 plants per pot. The criteria for thinning were based on seedling vigor and uniformity. After seedling emergence, soil moisture was maintained near field capacity, estimated according to the methodology described by Cabral et al. (2016).

Experiment 2

Experiment 2 was conducted with Mombasa guineagrass [*Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs cv. Mombasa] and involved 5 replicates of the fertilizer treatments. The soil used was the top 20 cm of an Inceptisol from a degraded pasture with texture characterized by 80% sand, 12% silt and 8% clay. Chemical composition of the soil was: P = 22 mg/dm^3; potassium (K) = 152 mg/dm^3; calcium (Ca) = 7.4 $cmol_c/dm^3$; magnesium (Mg) = 2.0 $cmol_c/dm^3$; hydrogen and aluminum (H+Al) = 1.4 $cmol_c/dm^3$; cation exchange capacity = 11.2 $cmol_c/dm^3$; base saturation = 87%; aluminum saturation = 0%; and pH (in calcium chloride) = 5.9. After collection, the soil was sieved through a 4.0 mm mesh and transferred to 5.0 dm^3 pots. Phosphorus fertilizer was applied on the day of sowing at rates of 300 mg P_2O_5/dm^3 (131 mg P/dm^3). After seedling emergence excess seedlings were removed leaving 3 plants per pot. Criteria for thinning and maintenance of soil moisture were as described in Experiment 1.

For both experiments, fertilizer application after thinning consisted of N fertilizer application (200 mg N/dm^3) as urea or ammonium sulfate and potassium fertilizer application (100 mg K_2O/dm^3 or 83 mg K/dm^3) as potassium chloride.

Measurements

Measurements commenced 30 days after seedling emergence. Estimation of the chlorophyll index of the youngest adult leaf of a representative tiller was performed with a non-destructive method using a Clorofilog (CLF 1030 Falker, Brazil) for Experiment 1 only.

Aerial parts were then cut at 10 and 25 cm above the soil for Xaraés and Mombasa, respectively. Forages were allowed to regrow and evaluated at intervals of 20 days. Four regrowth cycles were evaluated for Xaraés palisadegrass and 2 regrowth cycles for Mombasa guineagrass.

Plant height was measured with a graduated rule and the numbers of tillers per pot were recorded at the end of each regrowth cycle, before harvest. All leaves present in each pot above the residue height were counted, to obtain leaf numbers (LN). Leaf appearance rate (LAR) was estimated by the ratio of number of leaves per tiller and the interval between cuts. Phyllochron (PHY) was calculated as the inverse of LAR.

At the end of each regrowth period the harvested material was separated into morphological components, i.e. leaf and stem (stem + sheath). These fractions were conditioned in paper bags, then subjected to drying in an air circulation oven at 65 ºC for 72 hours and weighed. Forage accumulation rate (FAR) was calculated by dividing forage mass (FM) at each cut by the interval between cuts.

At the final evaluation, plants were harvested at ground level and the stem residue and roots were collected. The root system was washed with running water using a 4 mm mesh sieve. Afterwards, residue and roots were dried in a forced air circulation oven under the same conditions mentioned for FM to obtain dry matter data. Soil pH (in calcium chloride) was determined at this time.

Statistical analysis

Data were submitted to analysis of variance using the general linear mixed model method, using the PROC MIXED command (SAS® Institute Inc., Cary, NC, USA). Least squares means of treatments were compared by Tukey test ($P \leq 0.05$).

The model was as follows:

$y_{ijk} = \mu + T_i + e_{ij} + C_k + \varepsilon_{ijk}$;

where:

y_{ijk} = expected response;

μ = average/constant, associated with the experiment;

T_i = treatment effect (different nitrogen fertilizers) i;

e_{ij} = treatment error i, in repetition j, normally and independently distributed;

C_k = random effect associated with regrowth cycle k, normally distributed; and

ε_{ijk} = experimental error associated with treatment i, in repetition j, in cycle k, normally distributed.

Results

Experiment 1

Fertilizer type had no significant effect ($P>0.05$) on height, leaf appearance rate, phyllochron or root mass of Xaraés

(Tables 1 and 2). However, forage mass, leaf mass, stem mass, leaf number, tiller density, forage accumulation rate and stem residue for RPAS and SSU treatments were greater (P<0.05; Table 1) than for RPU.

Table 1. Effects of N and P fertilizer combinations on productive and structural characteristics and chlorophyll index of *Urochloa brizantha* cv. Xaraés plus soil pH during establishment.

Variable	SSU	RPU	RPAS	s.e.m.	P-value
Height (cm)	48	50	49	1.14	0.53
FM (g DM/pot)	19.5a	11.1b	19.1a	0.59	<0.01
FAR (g/d)	0.89a	0.52b	0.89a	0.02	<0.01
LM (g DM/pot)	15.9a	9.0b	15.6a	0.49	<0.01
SM (g DM/pot)	3.6a	2.1b	3.5a	0.19	<0.01
LN (No./pot)	83a	50b	80a	2.66	<0.01
TD (No./pot)	32a	19b	33a	0.84	<0.01
LAR (No./tiller/d)	0.12	0.12	0.11	0.003	0.38
PHY (No. of days/leaf)	8.6	8.8	9.0	0.27	0.50
Soil pH	4.14b	4.66a	3.56c	0.04	<0.01
Chlorophyll index	52.7a	45.7b	53.0a	0.82	<0.01

Means within rows with different letters differ (P<0.05) by Tukey's test.
SSU = simple superphosphate plus urea; RPU = reactive natural phosphate plus urea; RPAS = reactive natural phosphate plus ammonium sulfate.
FM = forage mass; FAR = forage accumulation rate; LM = leaf mass; SM = stem mass; LN = leaf number; TD = tiller density; LAR = leaf appearance rate; PHY = phyllochron.

Table 2. Effects of N and P fertilizer combinations on stem residue and root mass (g DM/pot) of *Urochloa brizantha* cv. Xaraés (Experiment 1) and *Megathyrsus maximus* (syn. *Panicum maximum*) cv. Mombasa (Experiment 2) during establishment.

Treatment	Xaraés		Mombasa	
	Residue mass	Root mass	Residue mass	Root mass
SSU	17.6a	67.1	13.0	4.8
RPU	9.8b	49.7	7.1	2.9
RPAS	18.2a	58.6	9.6	4.5
P-value	<0.01	0.15	0.05	0.43
s.e.m.	0.83	5.88	1.68	1.21

Means within columns with different letters differ (P<0.05) by Tukey's test.
SSU = simple superphosphate plus urea; RPU = reactive natural phosphate plus urea; RPAS = reactive natural phosphate plus ammonium sulfate.

Chlorophyll index for RPAS and SSU was greater (P<0.05) than for RPU (Table 1).

Final soil pH had the pattern RPU>SSU>RPAS (P<0.05) with RPU being greater than the original pH and RPAS lower than the original.

Experiment 2

As for Experiment 1, fertilizer combination had no significant effect (P>0.05) on leaf appearance rate, phyllochron, stem residue or root mass of Mombasa guineagrass (Tables 3 and 2). However, plant height, forage mass, leaf mass, stem mass and forage accumulation rate were greater (P<0.05) for RPAS and SSU than for RPU. Leaf number followed the pattern SSU>RPAS>RPU, while tiller density for SSU exceeded that for RPAS and RPU (P<0.05).

Table 3. Effects of N and P fertilizer combinations on productive and structural characteristics of *Megathyrsus maximus* (syn. *Panicum maximum*) cv. Mombasa plus soil pH during establishment.

Variable	SSU	RPU	RPAS	s.e.m.	P-value
Height (cm)	62a	56b	64a	1.04	<0.01
FM (g DM/pot)	9.00a	5.23b	8.44a	0.62	<0.01
FAR (g/d)	0.30a	0.17b	0.28a	0.02	<0.01
LM (g DM/pot)	8.68a	5.13b	8.14a	0.59	<0.01
SM (g DM/pot)	0.32a	0.09b	0.29a	0.04	<0.01
LN (No./pot)	35a	22c	28b	5.26	<0.01
TD (No./pot)	11a	7b	9b	0.57	<0.01
LAR (No./tiller/d)	0.10	0.11	0.11	0.005	0.80
PHY (No. of days/leaf)	9.7	10.0	9.5	0.44	0.71
Soil pH	7.89a	7.91a	6.12b	0.10	<0.01

Means within rows with different letters differ (P<0.05) by Tukey's test.
SSU = simple superphosphate plus urea; RPU = reactive natural phosphate plus urea; RPAS = reactive natural phosphate plus ammonium sulfate.
FM = forage mass; FAR = forage accumulation rate; LM = leaf mass; SM = stem mass; LN = leaf number; TD = tiller density; LAR = leaf appearance rate; PHY = phyllochron.

Final pH for SSU and RPU exceeded that for RPAS (P<0.05) with that for RPAS being similar to the original pH while those for SSU and RPU were higher than the original.

Discussion

The similar results found for the productive and structural variables for the RPAS and SSU treatments suggest that reactions between natural phosphate and ammonium sulfate produced sufficient P in a soluble form to meet the growth requirements of a high nutrient extraction grass (Galindo et al. 2018). This synergistic effect between natural reactive phosphate and ammonium sulfate is due to the reduction in soil pH, which promotes the conversion of phosphorus present in natural phosphate into a soluble form (Degryse et al. 2017). Costa et al. (2008) and Vitti et al. (2002) showed reduction in soil pH of up to 1.1 units when ammonium sulfate was applied as fertilizer. An additional factor which could have contributed to forage growth was the sulfur content of ammonium sulfate, which can increase growth of tropical forages (Miranda et al. 2017; Santos et al. 2019), when available sulfur levels in soil are limiting. According to Artur and Monteiro (2014) sulfur has a greater impact on regrowth than on establishment.

In the case of natural phosphate plus urea, growth of forage was restricted relative to that with SSU and RPAS (Tables 1 and 3). This is probably a function of the lesser effect of urea in lowering soil pH as observed in Tables 1 and 3 to promote solubilization of natural reactive phosphate. Lower acidification of the soil by urea compared with ammonium sulfate is due to hydrolysis that occurs soon after the application of urea to the soil, which consumes protons from the soil (Fageria et al. 2010). In the hydrolysis of urea, hydrogen ions in soil are combined with N to produce ammonia, which temporarily increases soil pH around the urea granule. This increase in pH promotes ammonia volatilization, which reduces the amount of ammonia to be nitrified and/or supplied to plants (Lara Cabezas and Souza 2008). In addition to this increase in pH around the urea granule, when part of the nitrogen is lost by volatilization, less nitrogen remains in soil to be oxidized to nitrate in the nitrification process, which is one of the factors that contributes to soil acidification (Isobe et al. 2011), the main factor that promotes the solubilization of the phosphorus present in natural phosphate. Plants on this treatment would have less available N and P for growth. With SSU, it is important to note that most of the phosphorus in simple superphosphate is water soluble, and is readily available to be absorbed by the plant as well as to be adsorbed on soil. In very adsorptive soils, P can become bound and not available to seedlings, which may impair forage establishment.

Oxidized soils in the Brazilian Cerrado have a high capacity for P fixation and precipitation, which can rapidly reduce availability of this nutrient in a short period. In the case of Latosols, a soil class that predominates in the Cerrado, as much as 30–97% of the soluble P added to soil as fertilizer in readily soluble form can be withdrawn from the soil solution within 24 hours of application (Santos et al. 2011). Therefore, it is important to apply soluble phosphate fertilizers to soil as close to sowing as possible.

Phosphorus is essential to the initial establishment of forages, because it enhances root development (Merlin et al. 2016; Waddell et al. 2017). While root weight in RPU was lower than in the other treatments, differences were not large enough to be considered significant. On the other hand residue weights (residual stem weights) were higher in the SSU and RPAS treatments than in RPU. The combination of lower root development in RPU combined with lower levels of available N and P in the soil solution plus lower residual stem weight from which to regrow would have contributed to reduced growth of forage on this treatment. Benot et al. (2019) indicated that the stem base, which is present in the residue mass, accumulates non-structural total carbohydrates, which are important for regrowth of forages under conditions of lower photosynthetic activity, such as water deficit, shading and after defoliation.

Commonly, chlorophyll index is a variable used to verify the nitrogen fertilizer efficiency of crops (Cardoso et al. 2011). However, in this study, a phosphate fertilizer effect was observed, as chlorophyll index for Xaraés grass fertilized with RPAS and SSU exceeded that for RPU.

Conclusions

Applying ammonium sulfate as N fertilizer in conjunction with natural reactive phosphate on low-P soils should give similar growth responses in grass pastures as simple superphosphate and urea. Field studies are needed to verify these greenhouse results and determine rate of reactive natural phosphate solubilization, as well as cost-of-production, since ammonium sulfate is more expensive per unit of N than urea, and requires correction of soil acidity, which may increase pasture fertilization costs.

References

(Note of the editors: All hyperlinks were verified 21 April 2020.)

Artur AG; Monteiro FA. 2014. Marandu palisadegrass growth and nutrient accumulation as affected by nitrogen and sulfur fertilizations. Australian Journal of Crop Science 8:422–429. bit.ly/3eBg4no

Benot ML; Morvan-Bertrand A; Mony C; Huet J; Sulmon C; Decau ML; Prud'homme MP; Bonis A. 2019. Grazing intensity modulates carbohydrate storage pattern in five grass species from temperate grasslands. Acta Oecologica 95:108–115. doi: 10.1016/j.actao.2018.11.005

Bortoluzzi G; Romeo T; La Cono V; La Spada G; Smedile F; Esposito V; Sabatino G; Di Bella M; Canese S; Scotti G; Bo M; Giuliano L; Jones D; Golyshin PN; Yakimov MM; Andaloro F. 2017. Ferrous iron- and ammonium-rich diffuse vents support habitat-specific communities in a shallow hydrothermal field off the Basiluzzo Islet (Aeolian Volcanic Archipelago). Geobiology 15:664–677. doi: 10.1111/gbi.12237

Cabral CEA; Cabral LS; Bonfim-Silva EM; Carvalho KS; Kroth BE; Cabral CHA. 2016. *Brachiaria brizantha* cv. Marandu response to nitrogen fertilizers associated to natural reactive phosphate. Comunicata Scientiae 7:66–72. (In Portuguese). doi: 10.14295/cs.v7i1.964

Cabral CEA; Cabral LS; Bonfim-Silva EM; Carvalho KS; Abreu JG de; Cabral CHA. 2018. Reactive natural phosphate and nitrogen fertilizers in Marandu grass fertilization. Comunicata Scientiae 9:729–736. (In Portuguese). doi: 10.14295/cs.v9i4.1170

Cardoso AD; Alvarenga MAR; Melo TL; Viana AES; Matsumoto SN. 2011. SPAD index in the leaves of potato under splitting and doses of nitrogen and potassium. Revista Ciência Agronômica 42:159–167. (In Portuguese). doi: 10.1590/S1806-66902011000100020

Corrêa RM; Nascimento CWA do; Souza SKS; Freire FJ; Silva GB da. 2005. Gafsa rock phosphate and triple superphosphate for dry matter production and P uptake by corn. Scientia Agricola 62:159–164. doi: 10.1590/S0103-90162005000200011

Costa KAP; Faquin V; Oliveira IP de; Rodrigues C; Severiano EC. 2008. Nitrogen doses and sources in marandu pasture. I - Changes in soil chemical properties. Revista Brasileira de Ciência do Solo 32:1591–1599. (In Portuguese). doi: 10.1590/S0100-06832008000400023

Degryse F; Baird R; Silva RC da; McLaughlin MJ. 2017. Dissolution rate and agronomic effectiveness of struvite fertilizers – effect of soil pH, granulation and base excess. Plant and Soil 410:139–152. doi: 10.1007/s11104-016-2990-2

Fageria NK; Santos AB dos; Moraes MF. 2010. Influence of urea and ammonium sulfate on soil acidity indices in lowland rice production. Communications in Soil Science and Plant Analysis 41:1565–1575. doi: 10.1080/00103624.2010.485237

Galindo FS; Buzetti S; Teixeira Filho MCM; Dupas E; Ludkiewicz MGZ. 2018. Dry matter and nutrients accumulation in mombasa guineagrass in function of nitrogen fertilization management. Revista de Agricultura Neotropical 5(3):1–9. (In Portuguese). doi: 10.32404/rean.v5i3.2132

Ghannoum O; Paul MJ; Ward JL; Beale MH; Corol DI; Conroy JP. 2008. The sensitivity of photosynthesis to phosphorus deficiency differs between C_3 and C_4 tropical grasses. Functional Plant Biology 35:213–221. doi: 10.1071/FP07256

Guedes EMS; Fernandes AR; Lima EV; Gama MAP; Silva ALP da. 2009. Arad natural phosphate and lime application and *Brachiaria brizanta* growth in oxisol under degraded pastures of the Amazon. Revista de Ciências Agrárias 52:117–129. (In Portuguese). bit.ly/3auPWau

Isobe K; Koba K; Otsuka S; Senoo K. 2011. Nitrification and nitrifying microbial communities in forest soils. Journal of Forest Research 16:351–362. doi: 10.1007/s10310-011-0266-5

Ivanova RP; Bojinova DY; Gruncharov IN; Damgaliev DL. 2006. The solubilization of rock phosphate by organic acids. Phosphorus, Sulfur and Silicon and the Related Elements 181:2541–2554. doi: 10.1080/10426500600758399

Kavanova M; Lattanzi FA; Grimoldi AA; Schnyder H. 2006. Phosphorus deficiency decreases cell division and elongation in grass leaves. Plant Physiology 141:766–775. doi: 10.1104/pp.106.079699

Lara Cabezas WAR; Souza MA. 2008. Ammonia volatilization, leaching of nitrogen and corn yield in response to the application of mix of urea and ammonium sulphate or gypsum. Revista Brasileira de Ciência do Solo 32:2331–2342. (In Portuguese). doi: 10.1590/S0100-06832008000600012

Marcante NC; Muroaka T; Bruno IP; Camacho MA. 2016. Phosphorus uptake and use efficiency of different cotton cultivars in savannah soil (Acrisol). Acta Scientiarum. Agronomy 38:239–247. doi: 10.4025/actasciagron.v38i2.26551

Merlin A; Rosolem CA; He ZL. 2016. Non-labile phosphorus acquisition by *Brachiaria*. Journal of Plant Nutrition 39:1319–1327. doi: 10.1080/01904167.2015.1109117

Miranda RP; Pietroski M; Matos FB; Seben Junior GFS; Caione G. 2017. Nitrogen, potassium, calcium and sulfur omission in grass Convert. Comunicata Scientiae 8:452–458. doi: 10.14295/cs.v8i3.2257

Nascimento JL do; Almeida RA; Silva RSM; Magalhães LAF. 2002. Effects of liming levels and phosphorus sources on Tanzânia grass yields (*Panicum maximum* Jacq. cv. Tanzânia). Pesquisa Agropecuária Tropical 32:7–11. (In Portuguese). bit.ly/3aunZ2u

Rezende AV de; Lima JF de; Rabelo CHS; Rabelo FHS; Nogueira DA; Carvalho M; Faria Junior DCNA; Barbosa LA. 2011. Morpho-physiological characteristics of *Brachiaria brizantha* cv. Marandu in response to phosphate fertilization. Revista Agrarian 1:335–343. (In Portuguese). bit.ly/3aqXpYb

Ros MBH; De Deyn GB; Koopmans GF; Oenema O; van Groenigen JW. 2018. What root traits determine grass resistance to phosphorus deficiency in production grassland? Journal of Plant Nutrition and Soil Science 181:323–335. doi: 10.1002/jpln.201700093

Santos HC; Oliveira FHT de; Salcedo IH; Souza AP de; Silva VDM. 2011. Kinetics of phosphorus sorption in soils in the state of Paraíba. Revista Brasileira de Ciência do Solo 35:1301–1310. doi: 10.1590/S0100-06832011000400024

Santos LFM; Lapaz AM; Tomaz RS; Lira MVS; Moreira A; Reis AR dos; Heinrichs R. 2019. Evaluation of sulfur source and dose on the nutritional state and production of piatã forage. Semina: Ciências Agrárias 40:1237–1248. doi: 10.5433/1679-0359.2019v40n3p1237

Schroeder JP; Croot PL; von Dewitz B; Waller U; Hanel R. 2011. Potential and limitations of ozone for the removal of ammonia, nitrite, and yellow substances in marine re-circulating aquaculture systems. Aquacultural Engineering 45:35–41. doi: 10.1016/j.aquaeng.2011.06.001

Souza RF de; Faquin V; Torres PRF; Baliza DP. 2006. Liming and organic fertilizer: influence on phosphorus adsorption in soils. Revista Brasileira de Ciência do Solo 30:975–983. (In Portuguese). doi: 10.1590/S0100-06832006000600007

Vitti GC; Tavares Jr. JE; Luz PHC; Favarin JL; Costa MCG. 2002. Influence of ammonium sulfate in mixture with urea on the volatilization of NH_3-N. Revista Brasileira de Ciência

do Solo 26:663–671. (In Portuguese). doi: 10.1590/S0100-06832002000300011
Waddell HA; Simpson RJ; Ryan MH; Lambers H; Garden DL; Richardson AE. 2017. Root morphology and its contribution to a large root system for phosphorus uptake by *Rytidosperma* species (wallaby grass). Plant and Soil 412: 7–19. doi: 10.1007/s11104-016-2933-y
Werneck CG; Breda FA; Zonta E; Lima E; Polidoro JC; Balieiro FC; Bernardi ACC. 2012. Ammonia volatilization from urea with natural zeolite. Pesquisa Agropecuária Brasileira 47:466–470. (In Portuguese). doi: 10.1590/S0100-204X2012000300020
Zambrosi FCB; Ribeiro RV; Machado EC; Garcia JC. 2017. Phosphorus deficiency impairs shoot regrowth of sugarcane varieties. Experimental Agriculture 53:1–11. doi: 10.1017/S0014479715000290

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Research Paper

Vertical distribution, nutrient concentration and seasonal changes of fine root mass in a semi-deciduous tropical dry forest and in two adjacent pastures in the Western Llanos of Venezuela

Distribución vertical, concentración de nutrientes y cambios estacionales en la masa de raíces finas en un bosque seco tropical semicaducifolio y dos pastizales adyacentes en los Llanos Occidentales de Venezuela

ANA FRANCISCA GONZÁLEZ-PEDRAZA[1,2,3] AND NELDA DEZZEO[3]

[1]*Universidad de Pamplona, Facultad de Ciencias Agrarias, Pamplona, Colombia.* unipamplona.edu.co/fagrarias
[2]*Universidad Nacional Experimental Sur del Lago "Jesús María Semprum", Santa Bárbara, Venezuela.* unesur.edu.ve
[3]*Instituto Venezolano de Investigaciones Científicas (IVIC), Altos de Pipe, Venezuela.* ivic.gob.ve

Abstract

With the objective to contribute to a better understanding of ecological consequences of deforestation on the below-ground system in the Western Llanos of Venezuela, we evaluated the vertical distribution, nutrient concentration and seasonal changes of total fine root mass (FRM) (<2 mm diameter) in a semi-deciduous tropical dry forest and in 2 adjacent pastures of *Cynodon nlemfuensis*: a young pasture (YP, 5 years old) and an old pasture (OP, 18 years old) in the Obispo municipality, Barinas State. This evaluation included measurements at the end of the rainy season, during the dry season and during the subsequent early rainy season in 2005/2006. Highest FRM was recorded during the dry season, which probably indicates a plant water-stress response mechanism. The highest proportion (63–88%) of FRM was concentrated in the 10–20 cm soil layer at all studied sites, probably due to a higher nutrient and moisture content at that depth. Non-significant differences (P>0.05) were observed in the total concentrations of organic carbon, nitrogen, phosphorus, calcium and magnesium in the FRM in soils supporting forest, OP and YP at the evaluated depths. Non-significant changes in the total FRM and nutrient concentrations were observed between the sampling periods and the 3 study sites. YP soils showed a slight increase in FRM that could be associated with the root growth of secondary vegetation, which is considered a weed and is periodically removed. Our results suggest that the land use change from tropical forest to pastures has not significantly affected the mass of fine roots and their carbon and nutrient concentrations. Further studies are needed to determine if these findings apply to other ecosystems.

Keywords: *Cynodon nlemfuensis*, root distribution, soil C, soil N, tropical pastures.

Resumen

Con el objetivo de contribuir a entender mejor las consecuencias ecológicas de la deforestación en el sistema suelo-raíces en los Llanos Occidentales de Venezuela, evaluamos la distribución vertical, concentración de nutrientes y los cambios estacionales en la masa total de raíces finas (MRF) (<2 mm de diámetro) en un bosque seco tropical semicaducifolio y en dos pasturas adyacentes de *Cynodon nlemfuensis*: una pastura de 5 años (PJ) y una de 18 años (PV). Esta evaluación se realizó en 2005/2006 en el municipio Obispo, estado Barinas, e incluyó mediciones al final del período de lluvias, durante el período seco y al inicio del período de lluvias subsiguiente. La mayor MRF se registró durante el período seco, lo que probablemente indica un mecanismo de respuesta al estrés hídrico de la planta. La mayor proporción (63–88%) de MRF se concentró en la capa de suelo de 10–20 cm en todos los sitios estudiados, probablemente debido a una mayor concentración de nutrientes y humedad a esa profundidad. No se observaron diferencias significativas (P>0.05) en las concentraciones totales de carbono orgánico, nitrógeno, fósforo, calcio y magnesio en la MRF en suelos de bosque, PJ y PV a las profundidades evaluadas. No se observaron cambios significativos en la MRF total y en las concentraciones de nutrientes entre los períodos de muestreo en

Correspondence: Ana Francisca González-Pedraza, Universidad de Pamplona, Facultad de Ciencias Agrarias, Pamplona, Norte de Santander, Colombia. Email: anagonzalez11@gmail.com

los 3 sitios de estudio. Los suelos en PJ mostraron un ligero aumento en la MRF que podría estar asociado con el crecimiento de raíces de la vegetación secundaria, que se considera una maleza y se elimina periódicamente. Nuestros resultados indican que la conversión del bosque a pasturas no afectó significativamente la masa de raíces finas y sus concentraciones de carbono y nutrientes. Se requieren estudios adicionales para determinar si los resultados son aplicables a otros ecosistemas.

Palabras clave: C en el suelo, *Cynodon nlemfuensis*, distribución de raíces, N en el suelo, pastos tropicales.

Introduction

Fine root mass in tropical forests is the most important component of below-ground C dynamics and can contribute significantly to global net primary production (Malhi et al. 2011). In tropical dry forests, fine root production is high, constituting an important source of carbon (C) and nutrients in the soil (Fiala et al. 2017). It has been suggested that the roots in these forests provide more N, P and K than the above-ground biomass (Singh et al. 1989).

Several environmental factors affect production of fine roots in tropical dry forests, such as the marked climatic seasonality (Murphy and Lugo 1986; Singh et al. 1989), soil nutrients (Blair and Perfecto 2001) and forest disturbances and land use history (Castellanos et al. 2001; Jaramillo et al. 2003). The clearing of dry forests for the establishment of pastures can have important effects on the production and distribution of roots within the soil profile and on the contributions of C and nutrients (Jaramillo et al. 2003).

In Venezuela, large areas of tropical dry forests have been cleared and replaced by pastures. It is likely that this conversion has had impacts on the mass and distribution of fine roots within the soil profile. Studies on the replacement of tropical deciduous forests with pastures have shown important effects on C and nutrients in the soil profile (Crespo 2015), and pastures have the potential to store significant amounts of C in soils (Rodríguez et al. 2013; Crespo 2015). The dense and deep root system of grasses contributes to the formation of soil aggregates and provides protection to the soil C, making it least susceptible to oxidation and eventual loss to the atmosphere (Cambardella and Elliot 1993).

Considering that the rate of deforestation of tropical dry forests is increasing rapidly, it is important to improve the understanding of the ecological consequences of forest disturbance on the below-ground system. Detailed information on the changes that have occurred in fine root production once forests have been cleared and converted into pastures can be very useful in predicting the consequences of deforestation and to design effective pasture management strategies. The objective of this study was to evaluate the fine root mass and its nutrient composition in a tropical dry forest and in 2 adjacent pastures (5- and 18-years-old) planted following the logging and burning of forest in the Western Llanos of Venezuela.

Materials and Methods

Study site

The study area was in the El Mangón farm, located in the Obispo municipality, Hurtado sector of Barinas State in the Western Llanos of Venezuela (40º01'10"–40º59'10" N, 91º57'30"–91º25'18" W; 120 masl) (Figure 1). Average annual rainfall is 1,244 mm, with a rainy season from April to December and a dry season from January to March. Average annual temperature is 26.8 °C, and the relief is flat with a slope between 0 and 2% (Ewel et al. 1976; SIRA-INIA-CENIAP 2010).

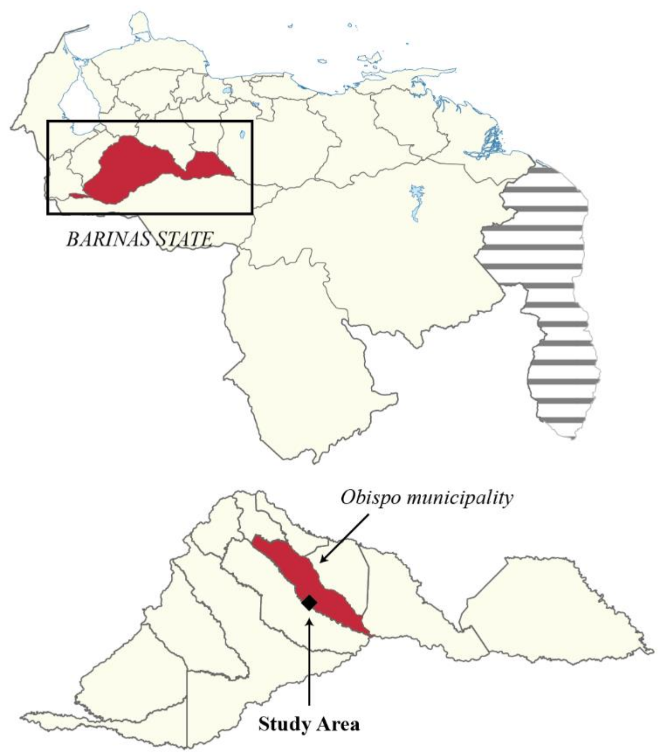

Figure 1. Study area in the Western Llanos of Venezuela (SIRA-INIA-CENIAP 2010).

The landscape corresponds to an alluvial overflow plain, influenced by the fluvial dynamics of an old bed of the Santo Domingo River (Schargel and Strebin 1970).

Table 1. Soil characteristics of the study sites (mean ± SD) (Source: González-Pedraza and Dezzeo 2011, 2014a, 2014b).

Characteristic	Depth (cm)	Forest	5-years-old pasture (YP)	18-years-old pasture (OP)
pH (H_2O)	0–5	5.4±0.4Ba	5.0±0.5Cab	4.8±0.5Db
	5–10	5.8±0.4Ba	5.5±0.2BCa	5.5±0.4Ca
	10–20	6.0±0.4ABa	5.9±0.2Aa	6.1±0.3Ba
	20–30	5.7±0.7Bab	5.3±0.1BCb	5.9±0.4BCa
	30–40	6.6±1.1Aab	5.9±0.2Ab	6.9±0.7Aa
Total organic carbon (g/m^2)	0–5	1,390±251.9Aa	1,473±400.2Ba	1,517±249.9Ba
	5–10	1,379±228.5Ab	2,232±287.9Aa	1,904±436.1Aa
	10–20	916±143.5Bc	1,675±196.4Ba	1,364±229.4BCb
	20–30	1,002±133.6Bb	1,018±118.6Cb	1,123±117.3Ca
	30–40	493±59.9Ca	529±145.5Da	494±84.8Da
Total nitrogen (g/m^2)	0–5	173±59.6Ba	209±99.8Ba	184±55.2Ba
	5–10	184±33.2Ba	173±35.7Ba	205±28.3Ba
	10–20	641±465.5Ab	838±710.2Aab	1,404±811.1Aa
	20–30	204±89.2Ba	147±12.5Ca	215±114.8Ba
	30–40	137±43Ba	104±25.7Ca	126±23.3Ba
Total phosphorus (μg/g)	0–5	588.5±206.2Aa	926.3±329.9Aa	656.5±67.2Aa
	5–10	442.8±85.8Bb	776.1±177.3Ba	512.3±70.6Bb
	10–20	59.0±36.9Db	163.4±76.8Ca	99.0±68.4Dab
	20–30	295.5±115.4Cb	594.4±120.1Ba	309.9±68.5Cb
	30–40	321.4±65.7BCb	487.3±101.4Ba	298.3±15.4Cb
Calcium (meq/100 g)	0–5	11.0±3.9Aa	12.5±4.6Aa	9.1±1.8Aa
	5–10	6.1±3.0Cc	14.9±3.1Aa	11.4±3.0Ab
	10–20	9.2±1.6ABb	12.7±3.7Aa	10.6±2.1Aab
	20–30	9.0±1.3ABab	8.2±1.4Bb	10.3±1.8Aa
	30–40	7.3±0.7BCb	7.3±1.0Bb	9.2±2.7Aa
Magnesium (meq/100 g)	0–5	4.1±1.1ABa	5.0±1.2Aa	5.2±1.8Aa
	5–10	3.4±1.1Ba	4.5±0.9ABa	4.5±2.2Aa
	10–20	3.9±1.2ABa	3.9±0.9ABa	4.8±2.1Aa
	20–30	4.7±1.0Aab	3.4±1.0Bb	5.3±1.8Aa
	30–40	4.8±1.1Aa	3.4±0.8Bb	4.7±1.2Aa
Potassium (meq/100 g)	0–5	0.5±0.2Ab	1.2±0.7Aa	0.6±0.3Ab
	5–10	0.2±0.2Bb	0.8±0.4ABa	0.3±0.2Bb
	10–20	0.2±0.1Bb	0.5±0.3BCa	0.2±0.1Bb
	20–30	0.1±0.0Bb	0.4±0.2BCa	0.2±0.0Bb
	30–40	0.2±0.2Ba	0.3±0.2Ca	0.2±0.1Ba
Sodium (meq/100 g)	0–5	0.1±0.0Ca	0.1±0.0Ca	0.1±0.1Ba
	5–10	0.1±0.1BCa	0.1±0.0Ca	0.1±0.1Ba
	10–20	0.1±0.1BCa	0.1±0.0Ca	0.2±0.3Ba
	20–30	0.3±0.2Ba	0.2±0.1Ba	0.4±0.3Ba
	30–40	1.2±0.4Aa	0.8±0.1Ab	1.1±0.3Aab
Aluminum (meq/100 g)	0–5	1.7±0.1Aa	2.0±0.2Aa	1.8±0.6Aa
	5–10	0.5±0.1CDb	0.6±0.0Ba	0.5±0.0Bab
	10–20	0.6±0.1Ca	0.6±0.1Ba	0.6±0.1Ba
	20–30	0.7±0.1Ba	0.6±0.1Bb	0.6±0.1Bb
	30–40	0.5±0.1Da	0.4±0.1Ca	0.3±0.1Cb
Cation exchange capacity (meq/100 g)	0–5	17.2±3.8Aa	20.3±5.5Aa	16.8±3.3Aa
	5–10	10.3±2.4Cc	20.8±3.9Aa	16.8±3.1Ab
	10–20	13.9±1.3Bb	17.7±4.3Aa	16.2±3.7Aab
	20–30	14.7±1.7ABab	12.7±1.9Bb	16.5±2.9Aa
	30–40	13.5±1.7Bab	12.3±1.1Bb	15.2±2.9Aa
Base saturation (%)	0–5	90.5±3.8Ba	90.8±5.4Ba	88.9±4.7Ba
	5–10	95.4±2.1Bb	97.1±0.6Aa	96.7±0.7Aa
	10–20	96.2±1.4Ba	96.5±1.0Aa	96.2±1.1Aa
	20–30	95.7±1.4Ba	95.7±1.6Aa	96.5±1.2Aa
	30–40	96.9±1.2Aab	96.6±1.3Ab	98.1±0.9Aa

Different lower-case letters indicate significant differences between sites for the same depth ($P<0.05$). Different upper-case letters indicate significant differences between depths for the same site ($P<0.05$).

The soil parent material is of alluvial origin, a sandy clay loam with kaolinite as dominant clay mineral (Ewel et al. 1976). In general terms, the soils have been described as deep, moderately fertile, with moderate concentrations of organic matter, nitrogen and phosphorus, high cation exchange capacity and base saturation (Table 1), with clay loam and silty clay loam textures (Table 2) (Schargel and Strebin 1970; González-Pedraza and Dezzeo 2011, 2014a, 2014b). The main soil orders found in this area correspond to Inceptisols and Vertisols, among them Tropaquepts, Usterts and Aquerts (Mogollón and Comerma 1994).

According to González-Pedraza and Dezzeo (2011), soils at the study site are fine-textured with a predominance of silt. Forest and OP soils show similar clay and sand contents, while YP soils have significantly higher clay content (up to 18% more than forest and OP soils). In general, the soil properties at the studied sites tended to be similar (Table 2).

The predominant vegetation in the region is semi-deciduous, periodically flooded forest (Ewel et al. 1976). These forests consist of 2 strata, the upper one consisting of deciduous and semi-deciduous trees with heights of 15–20 m, and the lower one consisting of deciduous and evergreen trees with heights less than 15 m (Table 3) (Aymard 2015).

Table 2. Particle size distribution (mean ± SD) in the 0–40 cm of forest and pasture soils (Source: González-Pedraza and Dezzeo (2011).

Particle size distribution	Depth (cm)	Forest	5-years-old pasture	18-years-old pasture
% Sand	0–5	19.8±5.9Aa	12.5±7.8ABb	22.3±5.6Aa
	5–10	20.8±7.6Aa	10.0±7.4ABb	22.7±7.6Aa
	10–20	15.6±4.4Aa	5.6±1.8Bb	18.3±8.0Aa
	20–30	15.8±2.6Aa	15.4±6.8Aa	13.5±5.5Aa
	30–40	17.1±2.5Aa	12.9±3.3ABb	15.4±4.3Ab
% Silt	0–5	49.7±6.2Ba	44.8±6.1Ba	47.1±4.5Ca
	5–10	53.9±5.6ABa	46.8±5.7Bb	47.1±3.0Cb
	10–20	57.5±4.1Aa	60.0±3.5Aa	52.7±3.6Bb
	20–30	57.5±4.2Aa	60.4±5.3Aa	57.0±4.1ABa
	30–40	58.3±2.0Ab	66.3±4.4Aa	60.0±2.7Ab
% Clay	0–5	30.5±9.6Ab	42.7±10.6Aa	30.6±6.4Ab
	5–10	25.2±6.9Ab	43.2±8.3Aa	30.2±6.2Ab
	10–20	26.9±1.6 Ab	34.4±3.2ABa	28.9±5.1Ab
	20–30	26.7±2.0Aab	24.2±3.4BCb	29.5±3.3Aa
	30–40	24.6±1.0Aa	20.8±2.0Cb	24.6±2.5Aa

Different lower-case letters indicate significant differences between sites (P<0.05). Different upper-case letters indicate significant differences between depths for the same site (P<0.05).

Table 3. Main species of trees present in the semi-deciduous forest. (Source: Adapted from Figueroa 2006).

Family	Botanical name	Common name	Phenology
Acanthaceae	*Trichanthera gigantea* (Bonpl.) Nees	Naranjillo	Evergreen
Anacardiaceae	*Spondias mombin* L.	Jobo	Deciduous
Annonaceae	*Rollinia exsucca* (DC.) A. DC.	Anoncillo	Semi-deciduous
	Annona jahnii Saff.	Manidito	Semi-deciduous
Bixaceae	*Cochlospermum vitifolium* (Willd.) Spreng.	Bototo	Deciduous
Cordiaceae	*Cordia collococca* L.	Caujaro	Semi-deciduous
Euphorbiaceae	*Sapium glandulosum* (L.) Morong	Lechero	Semi-deciduous
Fabaceae	*Samanea saman* (Jacq.) Merr.	Samán	Deciduous
	Inga sp.	Guamo	Evergreen
	Albizia sp.	Carabali	Semi-deciduous
	Pterocarpus acapulcensis Rose	Drago	Facultatively deciduous
Malvaceae	*Ceiba pentandra* (L.) Gaertn.	Ceiba	Facultatively deciduous
	Sterculia apetala (Jacq.) H. Karst.	Camuruco	Deciduous
	Luehea candida (DC.) Mart.	Guácimo cimarrón	Facultatively deciduous
	Guazuma ulmifolia Lam.	Guácimo	Evergreen
Moraceae	*Maclura tinctoria* (L.) D. Don ex Steud. subsp. *tinctoria*	Mora	Deciduous
	Brosimum alicastrum Sw.	Charo	Evergreen
Rubiaceae	*Genipa americana* L.	Caruto	Deciduous
Sapindaceae	*Sapindus saponaria* L.	Paraparo	Deciduous
Urticaceae	*Cecropia peltata* L.	Yagrumo	Evergreen

In this region in the last 50 years, large areas of natural forest have been converted into pasture using the slash-and-burn method. Estrella grass (*Cynodon nlemfuensis* Vanderyst) is grown for grazing by cattle (Truter et al. 2015).

Field work was carried out in an area of tropical dry forest with dominant deciduous vegetation and in 2 adjacent areas, where the original forest had been cut down manually and burnt, and Estrella grass had been planted (5- and 18-years-old, YP and OP, respectively). The pastures have never been fertilized, but have been mowed annually to control weeds and to promote grass growth. At sampling time, species from the original forest that could not be cut by hand, as well as vegetation of secondary growth, like palms and some species of legumes, were observed in the pasture. Cattle were introduced into YP for grazing during the dry season and early and late in the rainy season. On each occasion the cattle remained in this pasture until they consumed all available grass. Every 1–2 months cattle were introduced to the old pasture (OP) and remained for 3–7 days, while consuming all available grass.

Fine root sampling

At each selected site (forest, YP and OP), soil samples were taken from a 600 m^2 plot (20 × 30 m). The distance between the 3 sites was 1–3 km. At each plot, soil samples for determining the vertical distribution of the fine root mass (<2 mm) and their nutrient concentrations were collected at 12 points arranged along 3 transects with 4 sampling points per transect. The samples were collected with a soil core at 0–5, 5–10, 10–20, 20–30 and 30–40 cm depths at the end of the rainy season 2005 (November). For determining the seasonal changes in mass of fine roots, soil samples at 0–5 and 5–10 cm depths were collected at 12 points in each study plot along 3 transects on 3 occasions: end of rainy season 2005 (November), during the dry season (March 2006) and early rainy season (May 2006) (Figure 2).

Fine root mass

All collected soil samples were dried and passed through a 2 mm soil sieve. The fine roots were extracted from the soil fraction which passed through the 2 mm sieve. From each sieved soil sample, 2 × 200 g subsamples were taken. In these subsamples all fine roots (both living and dead) were manually extracted and weighed. Mean weight of fine root mass from the 2 subsamples was used to calculate the dry matter (DM) of fine root mass from each soil sample. The data were converted to t DM/m^2 considering the area of the core, before being converted to a hectare basis according to the following equation:

Fine root mass (t DM/ha) = weight of fine root mass (t DM/m^2) × area of one hectare of soil (10,000 m^2/ha).

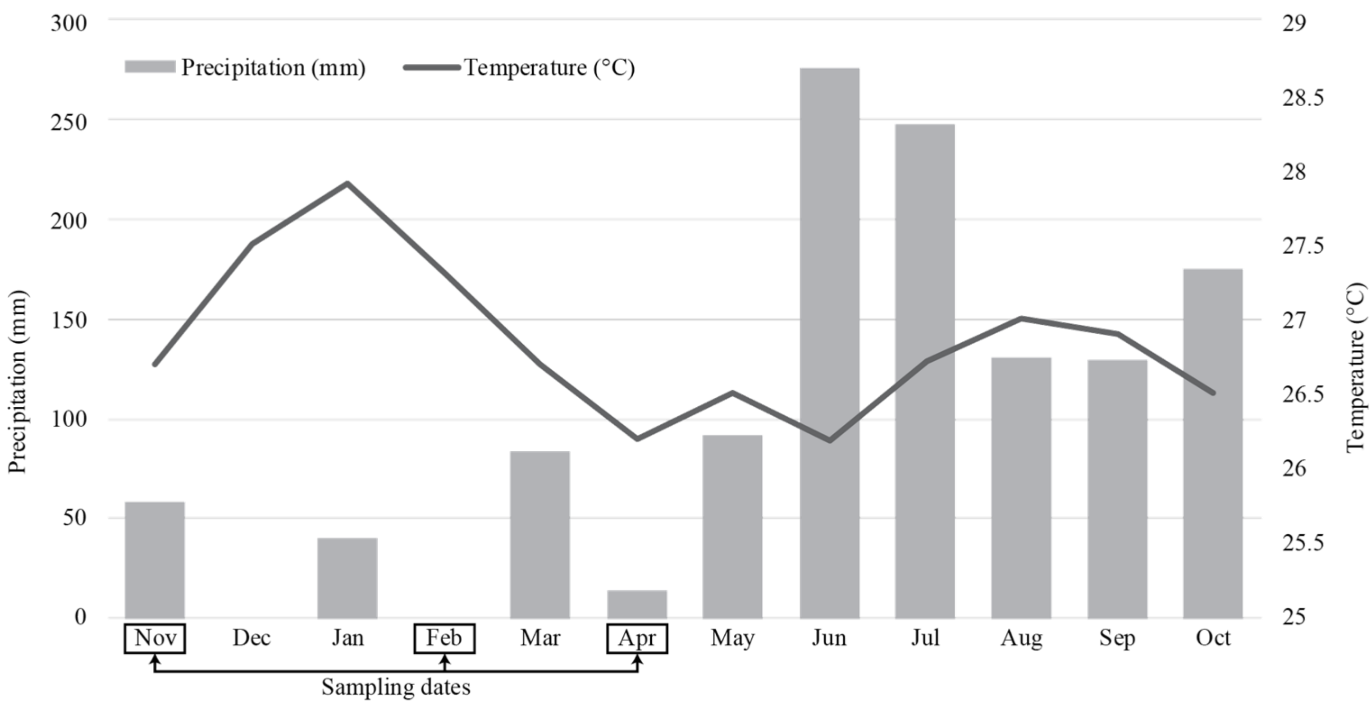

Figure 2. Distribution of precipitation and temperature during the sampling period. [Source: Barinas Agrometeorological Station (SIRA-INIA-CENIAP 2010)].

Laboratory analyses

Total organic carbon in fine roots (TOCr) was determined using the Loss on Ignition (LOI) method (Schulte and Hopkins 1996). It was assumed that 58% of the organic matter (OM) was organic carbon (Nelson and Sommers 1996) and %TOCr was calculated according to the following equation:

%TOCr = [pre-ignition weight (g) – post-ignition weight (g)] × 0.58 × 100/pre-ignition weight (g).

Concentration of total organic carbon in the fine roots was multiplied by the respective root mass to give TOCr and the results were expressed in kg/ha based on dry weight and bulk density of the soil.

Total nitrogen (N), phosphorus (P), calcium (Ca) and magnesium (Mg) in the fine root mass was extracted by digestion with concentrated sulfuric acid and oxidation with hydrogen peroxide (Tiessen and Moir 1993). Total nitrogen in the roots (TNr) was determined by the colorimetric method of Keeney and Nelson (1982) on a Technicon Autoanalyzer II. Total phosphorus (TPr) was measured by the molybdenum blue method of Murphy and Riley (1962). Total Ca and total Mg were measured on an atomic absorption spectrophotometer (AA). Total N, P, Ca and Mg concentrations obtained in the fine root mass were multiplied by their respective root masses, and the results were expressed in kg/ha based on dry weight and bulk density of the soil.

Statistical analysis

Statistical analysis of data was carried out using one-way analysis of variance (ANOVA). Means were separated with Tukey's test when statistical differences ($P<0.05$) were observed. When necessary, the data were transformed to homogenize variances, and when that did not meet this assumption ($P>0.05$) according to Levenne's test, a non-parametric Mann-Whitney test was also applied. To determine relationships between variables at sites of interest, a simple linear regression analysis was used. All statistics were computed using STATISTICA for Windows 6.0 (Statistica 2001).

Results

Vertical distribution of fine roots

No significant differences ($P>0.05$) between study sites (forest, YP and OP) were detected in the vertical distribution of the fine root mass. However, total mass of fine roots in the top 40 cm of soil was somewhat higher in YP (5.95 ± 0.57 t DM/ha) than in forest (5.27 ± 0.68 t DM/ha) and OP (4.57 ± 0.54 t DM/ha). Distribution of fine root mass was not uniform down the soil profile as root mass in the 10–20 cm horizon was generally greater than in the other horizons at all study sites ($P<0.05$; Figure 3).

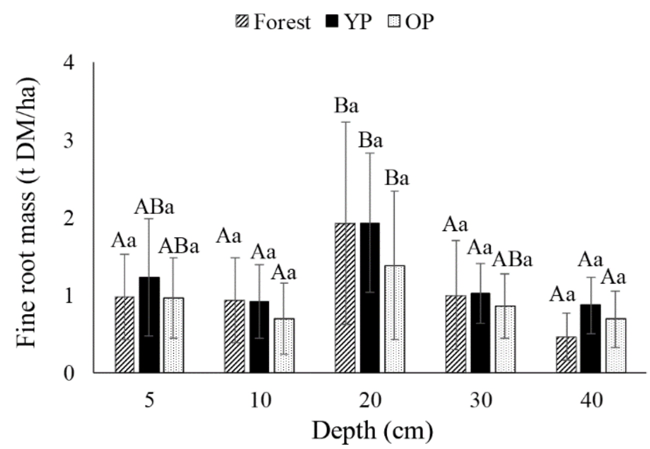

Figure 3. Vertical distribution of fine root mass in soils supporting dry semi-deciduous forest and 5-years-old (YP) and 18-years-old (OP) pasture. All points are mean values with standard error bars across forest and pastures. Different lower-case letters indicate significant differences between sites ($P<0.05$). Different upper-case letters indicate significant differences between depths ($P<0.05$).

Seasonal changes in fine root mass

Within each sampling period, no significant differences ($P>0.05$) between forest, YP and OP were observed in the mass of fine roots in both 0–5 cm and 5–10 cm horizons. Differences between seasons in mass of fine roots occurred in the 0–5 cm horizon, with root mass increasing during the dry season to values exceeding those in the early or late rainy season for all sites ($P<0.05$; Table 4).

Table 4. Seasonal variation in mass of fine roots (mean ± SD) in the top 10 cm of soils supporting forest and pastures of 2 ages.

Season	Depth (cm)	Fine root mass (t DM/ha) Forest	YP[1]	OP
End rainy season	0–5	0.9±0.5ABa	1.0±0.4Ba	1.0±0.5Ba
	5–10	0.7±0.3Aa	0.9±0.4Aa	0.5±0.5Ba
Dry season	0–5	1.4±0.7Aa	2.1±0.8Aa	1.6±0.7Aa
	5–10	0.8±0.6Aa	1.1±0.5Aa	1.0±0.4Aa
Early rainy season	0–5	0.7±0.4Ba	1.1±0.6Ba	1.1±0.4Ba
	5–10	0.7±0.4Aa	0.9±0.4Aa	0.6±0.3ABb

[1]YP: 5-years-old pasture; OP: 18-years-old pasture.
Different lower-case letters indicate significant differences between depths ($P<0.05$). Different upper-case letters indicate significant differences between seasons ($P<0.05$).

Total carbon and nutrients in the fine root mass

The total concentrations of carbon and nutrients in fine roots in the top 40 cm of soil was determined only in soil samples collected at the end of the rainy season. No significant differences (P>0.05) in total organic carbon (TOCr) and total nitrogen (TNr) in fine roots were found between Forest, YP and OP except for the 30–40 cm soil depth, where YP contained significantly (P<0.05) more TOCr than forest soil (Table 5). TOCr was highest (P<0.05) in the 10–20 cm horizon in all soils. In contrast, TNr tended to decrease steadily with increasing soil depth, with levels in the top 5 cm exceeding those in the 30–40 cm horizon (Table 5).

No significant differences (P>0.05) between forest and pasture soils were found in total phosphorus, calcium and magnesium in the mass of fine roots in the top 40 cm of soil (Table 6). However, there were changes in concentrations down the profile. A significant increase (P<0.05) in total phosphorus in roots in the forest soil in the 10–20 cm horizon was observed. Total calcium and magnesium in fine roots tended to decline with depth but differences were inconsistent and rarely significant.

Significant positive relationships existed between fine root mass and nutrient content in 0–40 cm soil horizons in forest and pasture (Table 7).

Table 5. Total organic carbon and total nitrogen (mean ± SD) in fine roots (<2 mm) in the top 40 cm of soils supporting forest and 5-years-old and 18-years-old pastures.

Nutrient (kg/ha)	Depth (cm)	Forest	5-years-old pasture	18-years-old pasture
Total organic carbon in the fine root mass (TOCr)	0–5	455±246.3Ba	466±227.9Ba	381±204.9Ba
	5–10	403±234.4Ba	360±177.5Ba	305±196.5Ba
	10–20	864±542.8Aa	894±421.9Aa	706±480.9Aa
	20–30	424±309.9Ba	390±130.5Ba	430±228.5Ba
	30–40	182±148.7Bb	357±160.3Ba	304±147.6Bab
Total nitrogen in the fine root mass (TNr)	0–5	7.2±3.5Aa	9.0±3.7Aa	8.0±4.4Aa
	5–10	4.8±1.2Aa	5.9±3.2ABa	4.2±2.5Ba
	10–20	5.9±4.3Aa	6.8±4.2Aa	4.8±1.9Ba
	20–30	3.5±2.5Ba	2.4±0.6BCa	3.2±1.7Ba
	30–40	2.6±2.5Ba	1.8±1.4Ca	2.9±2.5Ba

Different lower-case letters indicate significant differences between sites (P<0.05). Different upper-case letters indicate significant differences between depths (P<0.05).

Table 6. Vertical distribution of total nutrients (mean ± SD) in fine root mass in soils supporting forest and 5-years-old and 18-years-old pastures.

Nutrient (kg/ha)	Depth (cm)	Forest	5-years-old pasture	18-years-old pasture
Phosphorus	0–5	0.7±0.4Ba	1.2±0.6Aa	0.8±0.5Aa
	5–10	0.4±0.2Ba	0.6±0.4Aa	0.4±0.2Aa
	10–20	1.5±1.4Aa	1.4±0.7Aa	0.8±0.6Aa
	20–30	0.5±0.4Ba	0.7±0.4Aa	0.6±0.3Aa
	30–40	0.5±0.3Ba	0.4±0.1Aa	0.3±0.1Aa
Calcium	0–5	8.3±4.9Aa	10.0±6.6Aa	7.0±4.9Aa
	5–10	6.5±3.1Aa	9.4±6.5Aa	5.6±42.2Aa
	10–20	8.2±6.2Aa	4.2±2.6ABab	1.9±0.8Bb
	20–30	7.3±2.6Aa	6.6±4.7Aab	5.7±2.8Aa
	30–40	4.2±2.3Aa	3.2±2.8Ba	3.9±2.4ABa
Magnesium	0–5	4.6±1.9Aa	6.1±3.1Aa	5.9±2.6Aa
	5–10	4.1±1.8Aa	4.9±3.3ABa	3.9±1.8Aa
	10–20	4.7±2.5Aa	6.5±3.4Aa	4.4±2.3Aa
	20–30	4.7±2.1Aa	3.3±1.3ABa	4.9±3.9Aa
	30–40	2.5±1.8Aa	1.8±1.2Ba	2.6±2.0Aa

Different lower-case letters indicate significant differences between sites (P<0.05). Different upper-case letters indicate significant differences between depths (P<0.05).

Table 7. Linear correlation coefficients (Pearson; *r*) for relationships between fine root mass (<2 mm) and nutrient concentrations in fine roots in the top 40 cm of soils supporting forest and 5-years-old (YP) and 18-years-old (OP) pasture.

Nutrient	Forest	YP	OP
TOCr	0.99	0.96	0.93
TNr	0.94	0.94	0.92
P	0.77	0.82	0.76
Ca	0.80	0.76	0.68
Mg	0.96	0.82	0.88

TOCr: total organic carbon; TNr: total nitrogen; P: phosphorus; Ca: calcium; Mg: magnesium.

Discussion

The total fine root mass in the top 40 cm of soil was higher than those reported by Jaramillo et al. (2003) for semi-deciduous dry forests (3.6–4.3 t DM/ha in the top 60 cm) and for pastures of *Panicum maximum*, *Cenchrus ciliaris* and *Andropogon gayanus* (3.1–3.7 t DM/ha in the top 60 cm). However, the values for YP and OP can be considered low compared with that reported by Crespo and Lazo (2001) for a pasture of *Cynodon nlemfuensis* (10 t DM/ha in the top 15 cm of soil).

While no significant differences in total fine root mass were found between sites, the top 20 cm of soil accounted for 72.5, 68.4 and 66.2% of the total in Forest, YP and OP, respectively. Accordingly the fine root mass declined with depth as reported by Du et al. (2019) in 4 vegetation types (grassland, shrubland, secondary forest and primary forest) in a karst area, Southwest China during vegetation restoration. In that study the fine roots were concentrated in the top 10 cm of soil, which accounted for more than 57% of the root biomass, and decreased with increasing soil depth (soil samples from 0 to 30 cm deep). In karst ecosystems, fine roots contribute to the regulation of nutrient cycling in terrestrial ecosystems and the high density of fine roots within the top few centimeters of soil is crucial for acquiring nutrients.

According to Du et al. (2019), although fine root biomass of all vegetation types decreased with soil depth, the decrease was more rapid in forests (especially in secondary forests) than in other vegetation types. They suggested that the vertical distribution patterns of fine roots showed a more rapid decline in species-rich communities than in species-poor communities, which probably reflected changes in soil water content, nutrient concentration and bulk density in the soil profile.

Fiala et al. (2017) also measured a reduction in fine root mass (diameter <1 mm) with increasing soil depth in 6 Cuban forests (submontanous evergreen broad-leaved forest, submontanous evergreen narrow-leaved forest, semi-deciduous narrow-leaved forest, semi-deciduous broad-leaved forest and 2 species of mangroves, *Rhizophora mangle* and *Avicennia germinans*). Fifty-seven percent of the dry mass of fine live roots was concentrated in the upper 0–5 cm soil layer in the semi-deciduous forests. Semi-deciduous narrow-leaved forest had live (87 g DM/m^2) and dead (284 g DM/m^2) fine roots and semi-deciduous broad-leaved forest had live (200 g DM/m^2) and dead (210 g DM/m^2) (range 371–410 g DM/m^2) fine roots in the top 15 cm of soil, which exceeded those in evergreen forests. The authors concluded that fine root biomass is better predicted by nutrients in litterfall. The mangrove stands had 554 g live fine roots DM/m^2 (*A. germinans*) and 758 g DM/m^2 (*R. mangle*) in the top 15 cm of soil.

Variations in root dry mass and percentage distribution of roots of Florico grass (*Cynodon nlemfuensis*) in the 0–40 cm soil layer under 4 different grazing strategies and seasons were evaluated by Barros et al. (2017). High concentrations of roots were observed in the 0–10 cm layer (51.8–65.6%) for all grazing strategies in all seasons. This concentration of roots near the soil surface was explained by the branched architecture of the root system, common in forage grasses and the ability of the plant to acquire water and nutrients. According to Barros et al. (2017) the root system has less need to go deeper to acquire water and nutrients when grazing is less severe.

Similarly, Rodríguez et al. (2013) reported more than 80% of the below-ground root biomass was present in the 0–5 cm soil layer in different grasslands of Mayabeque province, Cuba.

Despite *C. nlemfuensis* being easy to establish, persistent, highly productive and adapted to different climate and soil conditions, this species does not withstand high-intensity grazing for long periods. After defoliation by grazing the plants consume organic reserves for restoration of tissues lost and then physiological activity is adjusted as the stocks of reserves are gradually restored (Sollenberger 2008; Truter et al. 2015).

The significant increase in root mass detected in the 10–20 cm soil horizon at all study sites could be associated with the highest nutrient concentrations in soil at that depth (González-Pedraza and Dezzeo 2011; 2014a; 2014b). A further hypothesis may be that plant roots explore deeper soil in search of water, particularly during the dry season, in order to survive during periods of water stress, as shown by Snyman (2009) in pastures under different drying conditions in semi-arid regions of southern Africa. A high concentration of roots at this same depth of soil was also reported for savannas in tropical dry forest areas of India (Tripathi et al. 1999).

In this region the soils have been described as deep, moderately fertile, with moderate concentrations of organic matter, nitrogen and phosphorus, high capacity for cation exchange (CEC) and saturation with bases, with clay loam and silty clay loam textures (Schargel and Strebin 1970; González-Pedraza and Dezzeo 2011). Schargel and Strebin (1970) described a buried horizon at a depth of close to 50 cm, where increases in the percentages of clay, CEC, calcium, potassium, carbon and nitrogen were observed.

Fine roots are usually quick to respond to changes in vegetation type, soil temperature, moisture and nutrient content (Du et al. 2019). The increase in fine root mass during the dry period at 0–5 cm soil depth (Table 4) is contrary to the results found by Singh et al. (1989), Kummerow et al. (1990) and Trujillo et al. (2006). However, other studies have reported small increases in root biomass with decreased water availability in the soil (Castellanos et al. 2001; Snyman 2009; Barros et al. 2017; Du et al. 2019), and this has been considered as an adaptive strategy of plants under significant water stress (Snyman 2009).

For example in temperate grasslands, Walter et al. (2012) demonstrated that root length was highest when rainfall variability was intermediate and was 43 and 24% shorter with extreme and low rainfall variability. According to this author, although enhanced root growth under drought conditions is viewed as an adaptive feature of many species, sometimes grassland roots may not respond to dryness with enhanced root growth.

During the dry season, Barros et al. (2017) reported a higher concentration (65.6%) of fine roots of *C. nlemfuensis* near the surface (0–10 cm) in more severe grazing treatments than in less severe ones. This may be associated with the renewal of the root system, also known as “turnover”, during the favorable dry season growth period and with the accumulation of organic reserves. The lower densities of root dry mass in winter, relative to the other seasons, would reflect the fast recovery of the aerial parts of plants in the rainy season and redirection of organic reserves to restore the root system reserves.

Pastures under grazing tend to accumulate more reserves in their roots, as a mechanism of adaptation to defoliation (Barros et al. 2017). This aspect, combined with the water shortage during the dry season, could be contributing to the increased mass of fine roots during this time of year. On the other hand, the significant reduction in the mass of fine roots with increasing soil depth that occurred during the dry season could be related to a higher concentration of nutrients in the first 5 cm of soil, as was noted by Castellanos et al. (2001) for tropical dry forests and pastures.

The TOCr and TNr results obtained in the forest and pastures of this study are similar to those reported by Trujillo et al. (2006), Jaramillo et al. (2003) and Crespo and Lazo (2001) for other tropical dry forests and pastures. The similarity between the studied forest and pastures in the C and N concentrations in the mass of fine roots is also consistent with the results found by Jaramillo et al. (2003). The high values for TOCr and TNr in fine roots within the 10–20 cm soil horizon are closely related to the high root mass present at that depth of soil. A positive and significant correlation ($P<0.05$) was found between the mass of fine roots and the amounts of TOCr and TNr throughout the soil profile (Table 4).

While the sites were not contiguous, soils on which the various sites were located were very similar. Our data suggest that conversion of forests to pasture did not change carbon and nutrient levels in the soil to any significant degree. Several factors can influence these outcomes such as the land cover, post-conversion land management, climate and soil type and texture (Dengiz et al. 2019).

Dengiz et al. (2019) analyzed the spatial variability of soil organic carbon density under different types of land cover within different soil types in a subhumid terrestrial ecosystem and found that soil type and land cover were critical factors influencing spatial variation of soil organic carbon (SOC) density. Land cover was the primary factor affecting variation in SOC density, while soil properties like texture, genetic horizons and soil depth also had an important influence. The observation that organic carbon concentration decreases with increasing soil depth under all land cover types has been generally observed in most situations.

Soil fertility may have a direct influence on fine root mass production. In this study, soil fertility affected the quantities of fine roots in these soils (high correlation between fine root mass and nutrient content in the top 40 cm of forest and pasture soils). Reynolds and D´Antonio (1996) indicated the possibility that nutrient availability has direct effects on root mass because fine roots are generally plastic organs and plants can deploy photosynthate below-ground to gather growth-limiting resources. Similarly, Hutchings and de Kroom (1994) said that root proliferation in fertile soils promotes extensive exploration of the soil and increases root surface area for greater water and nutrient uptake.

Tropical pastures can accumulate large stores of C in the soil. Chaplot et al. (2016) evaluated the potential of grassland rehabilitation through high density-short duration grazing to sequester atmospheric carbon and found a significant increase in SOC stocks with increasing grass biomass and grass cover to rates as high as 12%/year. Amongst the proposed explanations were increased root biomass production, greater soil aggregate stability and associated greater organic matter protection from decomposers.

Our findings suggest that the quantities of fine roots, soil carbon and other nutrients in soils supporting tropical pastures can be equal to or exceed those in soils supporting tropical forest trees. However, the values reported here were recorded at 3 specific times of the year from 3 relatively similar sites but not strictly the same soils. Nevertheless, the data do add to the increasing database of soil C storage information under various types of vegetation.

Conclusions

According to the results of this study, the land use change from forest to pastures has not significantly affected the mass of fine roots (<2 mm) and their carbon and nutrient concentrations in the soil. Additionally, the changes in distribution of fine root mass down the soil profile were closely related to the changes in the nutrient content of the soil at the considered depths.

The moderate increase in the mass of fine roots in the young pasture (YP) may be associated with additional root inputs from the growth of secondary forest vegetation, which is considered a weed and therefore removed periodically. Further studies in other regions are needed to determine if these findings apply to other ecosystems.

Further studies are needed to clarify the effects of climate seasonality and soil nutrients on magnitude and distribution of fine root mass within the soil profile and its contribution to C storage in soil under both forest and pastures.

References

(Note of the editors: All hyperlinks were verified 4 April 2020.)

Aymard G. 2015. Bosques de los Llanos de Venezuela: Estructura, composición florística, diversidad y estado actual de conservación. En: López R; Hétier JM; López D; Schargel R; Zinck A, eds. Tierras Llaneras de Venezuela. 2nd Edn. p. 241–268. IRD-CIDIAT, Mérida, Venezuela.

Barros ACC de; Almeida JCC; Camargo Filho ST; Carvalho CAB de; Campana LL; Morais LF de. 2017. Root dry matter mass and distribution of Florico grass under different grazing strategies. Pesquisa Agropecuária Brasileira 52:1276–1285. doi: 10.1590/s0100-204x2017001200017

Blair BC; Perfecto I. 2001. Nutrient content and substrate effect on fine root density and size distribution in a Nicaraguan rain forest. Biotropica 33:697–701. doi: 10.1111/j.1744-7429.2001.tb00227.x

Cambardella CA; Elliot ET. 1993. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Science Society of America Journal 57:1071–1076. doi: 10.2136/sssaj1993.03615995005700040032x

Castellanos J; Jaramillo VJ; Sanford RL; Kauffman JB. 2001. Slash-and-burn effects on fine root biomass and productivity in a tropical dry forest ecosystem in Mexico. Forest Ecology and Management 148:41–50. doi: 10.1016/S0378-1127(00)00523-5

Chaplot V; Dlamini P: Chivenge P. 2016. Potential of grassland rehabilitation through high density-short duration grazing to sequester atmospheric carbon. Geoderma 271:10–17. doi: 10.1016/j.geoderma.2016.02.010

Crespo G. 2015. Factores que influyen en el reciclaje de nutrientes en pastizales permanentes, avances en el desarrollo de su modelación. Revista Cubana de Ciencia Agrícola 49:1–10. bit.ly/2yBUrCY

Crespo G; Lazo J. 2001. A study on the root biomass of *C. nlemfuensis* cv. Panamanian, *P. maximum* cv. Likoni and *D. annulatum* and their nutrient contribution. Cuban Journal of Agricultural Science 35(3):259–263.

Dengiz O; Saygın F; İmamoğlu A. 2019. Spatial variability of soil organic carbon density under different land covers and soil types in a sub-humid terrestrial ecosystem. Eurasian Journal of Soil Science 8:35–43. doi: 10.18393/ejss.486582

Du H; Liu L; Su L; Zeng F; Wang K; Peng W; Zhang H; Song T. 2019. Seasonal changes and vertical distribution of fine root biomass during vegetation restoration in a karst area, Southwest China. Frontiers in Plant Science 9, article 2001. doi: 10.3389/fpls.2018.02001

Ewel JJ; Madriz A; Tosi Jr JA. 1976. Zonas de vida de Venezuela: Memoria explicativa sobre el mapa ecológico. Segunda Edn. Ministerio de Agricultura y Cría, Fondo Nacional de Investigaciones Agropecuarias, Caracas, Venezuela.

Fiala K; Hernández L; Holub P. 2017. Comparison of vertical distribution of live and dead fine root biomass in six types of Cuban forests. Journal of Tropical Forest Science 29:275–281. jstor.org/stable/44272905

Figueroa C. 2006. Plan de manejo simplificado y de impacto ambiental, aprovechamiento de productos forestales. Fundo Agropecuario El Mangón, C.A., superficie 1821 hectáreas, Parroquia La Luz, municipio Obispos, Estado Barinas. Venezuela. Informe técnico. (Unpublished.)

González-Pedraza AF; Dezzeo N. 2011. Efecto del cambio de bosque a pastizal sobre las características de algunos suelos en los Llanos Occidentales de Venezuela. Interciencia 36:135–141. bit.ly/2xP8Nzp

González-Pedraza AF; Dezzeo N. 2014a. Changes in the labile and recalcitrant organic matter fractions due to transformation of semi-deciduous dry tropical forest to pasture in the Western Llanos, Venezuela. In: Greer FE, ed. Dry forests: Ecology, species diversity and sustainable management. Nova Science Publishers Inc., New York, USA. p. 105–132.

González-Pedraza AF; Dezzeo N. 2014b. Effects of land use change and seasonality of precipitation on soil nitrogen in a dry tropical forest area in the Western Llanos of Venezuela.

The Scientific World Journal 2014, Article 514204. doi: 10.1155/2014/514204

Hutchings MJ; de Kroom H. 1994. Foraging in plants: The role of morphological plasticity in resource acquisition. Advances in Ecological Research 25:159–238. doi: 10.1016/S0065-2504(08)60215-9

Jaramillo VJ; Ahedo-Hérnandez R; Kauffman JB. 2003. Root biomass and carbon in a tropical evergreen forest of Mexico: changes with secondary succession and forest conversion to pasture. Journal of Tropical Ecology 19:457–464. doi: 10.1017/S0266467403003493

Keeney DR; Nelson DW. 1982. Nitrogen-Inorganic forms. In: Page AL, ed. Methods of soil analysis: Part 2 Chemical and microbiological properties. Agronomy Monograph No. 9. ASA and SSSA, Madison, WI, USA. p. 643–698. doi: 10.2134/agronmonogr9.2.2ed.c33

Kummerow J; Castellanos J; Mass M; Larigauderie A. 1990. Production of fine roots and the seasonality of their growth in a Mexican deciduous dry forest. Vegetatio 90:73–80. doi: doi.org/10.1007/BF00045590

Malhi Y; Doughty C; Galbraith D. 2011. The allocation of ecosystem net primary productivity in tropical forests. Philosophical Transactions of the Royal Society B: Biological Sciences 366:3225–3245. doi: 10.1098/rstb.2011.0062

Mogollón L; Comerma J. 1994. Suelos de Venezuela. Editorial Ex Libris C.A., Caracas, Venezuela.

Murphy J; Riley JP. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chemica Acta 27:31–36. doi: 10.1016/S0003-2670(00)88444-5

Murphy PG; Lugo AE. 1986. Ecology of tropical dry forest. Annual Review of Ecology and Systematics 17:67–88. doi: 10.1146/annurev.es.17.110186.000435

Nelson DW; Sommers LE. 1996. Total carbon, organic carbon, and organic matter. In: Sparks DL; Page AL; Helmke PA; Loeppert RH; Soltanpour PN; Tabatabai MA; Johnston CT; Sumner ME, eds. Methods of soil analysis. Part 3. Chemical methods. SSSA Book Series No. 5. SSSA, Madison, WI, USA. p. 961–1010. doi: 10.2136/sssabookser5.3.c34

Reynolds HL; D´Antonio C. 1996. The ecological significance of plasticity in root weight ratio in response to nitrogen: Opinion. Plant and Soil 185:75–97. doi: 10.1007/BF02257566

Rodríguez I; Hernández L; Crespo G; Sandrino B; Fraga S. 2013. Performance of the below ground root biomass in different grasslands of Mayabeque province, Cuba. Cuban Journal of Agricultural Science 47:201–207. bit.ly/2JJ07gA

Schargel R; Strebin S. 1970. Estudio agrológico preliminar. Sector Obispos, Estado Barinas. Ministerio de Obras Públicas, Guanare, Estado Barinas, Venezuela.

Schulte EE; Hopkins BG. 1996. Estimation of soil organic matter by weight loss-on-ignition. In: Magdoff FR; Tabatabai MA; Hanlon Jr. EA, eds. Soil organic matter: Analysis and interpretation. SSSA Special Publication No. 46. SSSA, Madison, WI, USA. p. 21–31. doi: 10.2136/sssaspecpub46.c3

Singh JS; Raghubanshi AS; Singh RS; Srivastava SC. 1989. Microbial biomass acts as a source of plant nutrients in dry tropical forest and savanna. Nature 338:499–500. doi: 10.1038/338499a0

SIRA-INIA-CENIAP. 2010. Sistemas de información en recursos agroecológicos, estación meteorológica Barinas. Instituto Nacional de Investigaciones Agrícolas, Ministerio del Poder Popular para la Agricultura y Tierras, Maracay, Estado Aragua, Venezuela.

Snyman HA. 2009. Root studies on grass species in a semi-arid South Africa along a soil-water gradient. Agriculture, Ecosystems & Environment 131:247–254. doi: 10.1016/j.agee.2009.01.023

Sollenberger LE. 2008. Sustainable production systems for *Cynodon* species in the subtropics and tropics. Revista Brasileira de Zootecnia 37:85–100. doi: 10.1590/S1516-35982008001300011

Statistica. 2001. Basic statistical analysis methods. Version 6.0. StatSoft, Tulsa, OK, USA.

Tiessen H; Moir JO. 1993. Characterization of available P by sequential extraction. In: Carter MR; Gregorich EG, eds. Soil sampling and methods of analysis. Taylor & Francis Group, Boca Raton, FL, USA. p. 75–86.

Tripathi SK; Singh KP; Singh PK. 1999. Temporal changes in spatial pattern of fine-root mass and nutrient concentrations in Indian bamboo savanna. Applied Vegetation Science 2:229–238. doi: 10.2307/1478986

Trujillo W; Fisher MJ; Lal R. 2006. Root dynamics of native savanna and introduced pastures in the Eastern Plains of Colombia. Soil and Tillage Research 87:28–38. doi: 10.1016/j.still.2005.02.038

Truter WF; Botha PR; Dannhauser CS; Maasdorp BV; Miles N; Smith A; Snyman HA; Tainton NM. 2015. Southern African pasture and forage science entering the 21st century: Past to present. African Journal of Range & Forage Science 32:73–89. doi: 10.2989/10220119.2015.1054429

Walter J; Grant K; Beierkuhnlein C; Kreyling J; Weber M; Jentsch A. 2012. Increased rainfall variability reduces biomass and forage quality of temperate grassland largely independent of mowing frequency. Agriculture, Ecosystems & Environment 148:1–10. doi: [10.1016/j.agee.2011.11.015](https://doi.org/10.1016/j.agee.2011.11.015)

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Research Paper

The effects of bovine urine application on two soil nitrogen compounds and growth of three forage grasses in the Colombian Piedmont plains

Efecto de la aplicación de orina bovina en dos compuestos nitrogenados del suelo y el crecimiento de tres pastos en el piedemonte de los Llanos Orientales de Colombia

JAIME E. GARZÓN[1], OSCAR PARDO[2] AND EDGAR A. CÁRDENAS[1]

[1]*Departamento de Producción Animal, Universidad Nacional de Colombia, Bogotá, Colombia.* unal.edu.co
[2]*Corporación Colombiana de Investigación Agropecuaria (Agrosavia), La Libertad, Colombia.* agrosavia.co

Abstract

The effects of application of bovine urine on biomass and nitrogen (N) accumulation in 3 tropical grasses (*Urochloa decumbens* cv. Basilisk, *U. humidicola* cv. Humidicola and *Megathyrsus maximus* cv. Mombasa), and on available N concentrations in soil (NH_4^+-N, NO_3^--N) were studied using a randomized complete block design with 3 replicates. There were significant interactions between species and urine application over time in terms of herbage accumulation and N concentration ($P<0.01$), with significant differences in the concentrations of N available in the soil ($P<0.01$). Soil temperature and precipitation had important effects on the concentrations of both soil ions. Application of bovine urine increased dry matter accumulation of all grasses in the short term and of *U. decumbens* over the whole year. Application of urine increased soil N levels, but for *U. humidicola* and *M. maximus* the effects were transient. It is necessary to continue with longer-term studies in the Piedmont plains to determine the effects of livestock grazing on the biogeochemical cycles, environmental impacts and natural mitigation options that the ecosystem offers, e.g. CO_2 sequestration, biological nitrification inhibitors and organic matter decomposition.

Keywords: Ammonium, herbage accumulation, *Megathyrsus maximus*, nitrates, nitrogen, *Urochloa* spp.

Resumen

En un Oxisol del piedemonte llanero colombiano se estudiaron los efectos de la aplicación de orina de bovinos en la acumulación de biomasa aérea y nitrógeno (N) en las gramíneas tropicales *Urochloa decumbens* cv. Basilisk, *U. humidicola* cv. Humidicola and *Megathyrsus maximus* cv. Mombasa, así como en las concentraciones de N disponible en el suelo (N-NH_4^+, N-NO_3^-). Para el efecto se dispuso de un diseño de bloques al azar con tres repeticiones. Para la producción de materia seca y concentración de N se observaron, a través del tiempo, interacciones significativas ($P<0.01$) entre especies y la aplicación de orina, con significancia estadística en las concentraciones de N disponible en el suelo ($P<0.01$). La temperatura del suelo y la precipitación fueron factores importantes asociados con las concentraciones de ambos iones de N en el suelo. La aplicación de orina bovina incrementó la acumulación de materia seca en los tres pastos a corto término y en *U. decumbens* a través del año. Igualmente se incrementaron los niveles de N en el suelo, pero para *U. humidicola* and *M. maximus* el efecto fue transitorio. Se sugiere continuar con estudios a largo plazo para determinar efectos del pastoreo de bovinos sobre los ciclos biogeoquímicos, su impacto ambiental y opciones de mitigación naturales que el ecosistema ofrece, como son secuestro de CO_2, los inhibidores biológicos de la nitrificación y la decomposición de materia orgánica.

Palabras clave: Amonio, *Megathyrsus maximus*, nitratos, nitrógeno, producción de biomasa, *Urochloa* spp.

Correspondence: Jaime E. Garzón, Universidad Nacional de Colombia, Departamento de Producción Animal, Facultad de Medicina Veterinaria y de Zootecnia, Carrera 30 # 45-03, Bogotá, Colombia. Email: jegarzona@unal.edu.co

Introduction

The Colombian Piedmont plains are a transition zone located between the slopes of the eastern Andes mountain range and the eastern plains (Llanos Orientales) in Colombia. With an area of 2,010,000 ha (7.6% of the Colombian Orinoquia region), they occupy a strip of land parallel to the mountain range. In general, the plains are known for their low soil fertility, aluminum toxicity and predominant acidity with high clay content, aluminum saturation and low concentrations of available phosphorus and interchangeable bases (IGAC 2004). These properties limit plant growth and only species with physiological adaptations can survive (Rao 2001).

The warm temperatures and humid environment enable the whole territory to be used for cropping and livestock production, mainly under grazing with Zebu (*Bos indicus*) cattle. Pastures are composed of native and increasingly planted species, such as *Urochloa decumbens* (Stapf) R.D. Webster (syn. *Brachiaria decumbens* Stapf), *U. humidicola* (Rendle) Morrone & Zuloaga (syn. *B. humidicola* (Rendle) Schweick., including cv. Llanero, previously considered as *B. dictyoneura*) and *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs (syn. *Panicum maximum* Jacq.) plus some legumes, all of which are adapted to the edaphoclimatic conditions of the area (Bernal 1994). Organic matter concentration in soils is low and producers do not usually apply chemical fertilizers to their grasslands, mainly due to costs. Cattle are relied upon to disperse nutrients to the soil through their excreta. Di and Cameron (2012) reported that "under a dairy cow urine patch, the N-loading rate from a single urination can be as high as 1,000 kg N/ha", which should benefit grass production.

Nitrogen (N) is the highest nutrient requirement for grasses and is absorbed through the roots in the form of nitrate (NO_3^-) and ammonium (NH_4^+) ions. In general, the plants prefer the former over the latter, mainly due to its greater solubility in water and because NH_4^+ ions in high concentrations can be toxic (Whitehead 2000). Many factors can alter the concentration of the ions mentioned above; precipitation, temperature and humidity are among the abiotic factors that influence immobilization and mineralization of N, mediated by microbial populations (Saggar et al. 2004; Dubeux Jr et al. 2007; Cameron et al. 2013).

The objective of the present investigation was to evaluate the effects of bovine urine on biomass and N production in 3 regionally important grasses, together with the concentrations of available N in the soil.

Materials and Methods

Study site

The study was performed at AGROSAVIA "La Libertad" Research Center (4°03'33.2" N, 74°27'27.1" W; 412 masl). The average temperature is 26.5 °C and mean annual precipitation is 2,800 mm (Rincón et al. 2010). The climate classification corresponds to Tropical Humid Forest (Holdridge 1978; IGAC 2004).

Environmental variables

The soil within the studied area was classified as an oxisol (Mejia 1996) with the following physico-chemical characteristics: bulk density (cylinder method) – 1.23 g/cm^3; pH (suspension soil:water, 1:1 v/v) – 4.4; organic carbon (Walkley-Black) – 1.95%; total nitrogen (organic carbon × 0.0862) – 0.14%; phosphorus (Bray II) – 8.2 mg/kg; potassium, calcium and magnesium (extraction with NH_4^+ 1 M acetate, pH 7) – 0.08, 1.43 and 0.29 meq/100 g, respectively; exchangeable acidity (extraction with 1 M KCl) – 2 meq/100 g; effective cation exchange capacity (exchangeable bases + exchangeable acidity) – 3.83 meq/100 g; and 32, 25 and 43% sand, silt and clay, respectively (McKean 1993).

Temperature and water volume in the soil were measured daily with a Decagon® datalogger, to calculate the water-filled pore space (WFPS), using the formula recommended by USDA (2014). Particle density was assumed as 2.65 g/cm^3 (Lin et al. 2013). Atmospheric temperature, precipitation and evaporation were recorded daily at the meteorological station of the research center.

Experimental methodology

A randomized complete block design was adopted with 18 plots of 2 × 2.5 m, in a factorial arrangement with repeated measures in time. The factors were 3 regional grass species: signalgrass (*Urochloa decumbens*) cv. Basilisk (CIAT 606), koroniviagrass (*U. humidicola*) cv. Humidicola (CIAT 679) and guineagrass (*M. maximus*) cv. Mombasa (BRA-006645) × 2 levels of bovine urine application (with and without) with 3 replicates. Urine was applied at the beginning of the rainy and dry seasons, respectively (May 2014 and February 2015). The grasses were established 2 years prior to the application of the urine treatments, without any management prior to the experiment.

The N applications were separated into dry and rainy seasons (DS and RS, respectively). However, the split data did not comply with the statistical assumptions for analysis of variance. In consequence, the data were analyzed together to strengthen the model, containing the experimental variation within the grass species to reach normality and homoscedasticity.

Estimation of the amount of bovine urine to apply

In RS and DS separately, urine was collected from 10 Zebu cows [418 ± 17.4 kg live weight (LW)], grazing a *U. decumbens* pasture.

To determine the amount of urine to be applied, a small amount of urine was collected at the beginning of each season and preserved with 5 mL of 5% H_2SO_4 per 100 mL urine. Thereafter, concentrations of N (Kjeldahl) and creatinine (commercial kit) in the urine were determined. The amount of urine excreted per animal/day was calculated using the following formula (Valadares et al. 1999):

VU = W × (Ccreatinine/Mcreatinine)

where:

VU = Urine volume (L/d);

W = Live weight (kg);

Ccreatinine = Creatinine coefficient (mg/kg LW/d); and

Mcreatinine = Creatinine concentration in the sample (mg/L).

Creatinine coefficient value was assumed as 17.3 mg/kg LW/d (Rennó et al. 2000).

Assuming a theoretical stocking rate of 2 animals/ha in *Urochloa* pastures, plus 9 months for RS and 3 months for DS and a standard area of 1 ha, the total volume of urine voided in each season was calculated using the following formula:

U = (V × SR × Period)/A

where:

U = Amount of urine/season (L/m^2);

V = Volume of urine/animal/d (L);

SR = Stocking rate (animals/ha);

Period = Season length (days); and

A = Standard area (m^2).

Urine was collected from cows by massaging the vulva and stored at 2 °C without acid preservation, to obtain the necessary volumes needed for the plots.

Urine was sprinkled on the pasture at the start of each season (13 May 2014 and 2 February 2015) in the plots randomly allocated to the urine treatment. Application rates were 1.37 and 0.77 L urine/m^2 for RS and DS, corresponding to 7.8 and 6.1 g N/m^2 (78 and 61 kg N/ha). The lower amounts of urine/nitrogen applied in the DS were a combination of a higher concentration of N in the urine (0.57 and 0.8% N in RS and DS, respectively) and the shorter duration of DS.

Control plots were established to simulate an area without animal effects; they received no N application, i.e. no urine.

Herbage accumulation and N content

Every 28 days, herbage mass was determined by harvesting 2 samples per plot, using a 0.5 × 0.5 m quadrat and leaving 10 cm stubble height. Each sample was dried at 60 °C for 48 h to calculate dry matter (DM) yield. Samples were then ground in a Wiley mill to pass a 1 mm stainless steel screen for determination of N concentration by the Kjeldahl method. N content was calculated by multiplying the DM yield by the N concentration in each sample. Following sampling, additional material within each experimental unit was cut and removed from the area, leaving the same stubble height in all plots.

Due to logistical issues in management of the research center, sampling periods were restricted to May–December 2014 (RS) and February–April 2015 (DS).

Available nitrogen in soil

During the first month after urine was applied, soils in each experimental plot were sampled weekly by collecting and pooling 8 soil cores to 20 cm depth. Samples were dried at 50 °C and passed through a 2 mm sieve. Concentrations of ammonium (NH_4^+-N) and nitrate (NO_3^--N) nitrogen were determined by extraction using 1 M KCl, with reduction of ammonium for the calculation of NO_3^--N (AOAC 1998). The analyses were performed in the Laboratory of Soil and Water of the Universidad Nacional de Colombia (Bogotá).

Statistical analyses

The data did not comply with the statistical assumptions for linear models due to the presence of outliers, so outliers were eliminated by confidence intervals with one standard deviation. Significant effects of the treatments were determined by analysis of variance and post-hoc HSD Tukey test.

To determine the level of association between the climatic variables and N available in soil, a linear regression model was used, after verification of statistical assumptions. For the construction of the model, all variables were evaluated through backward elimination, forward selection and stepwise regression, selecting those factors with lower scores of Akaike Information Criterion (AIC) (Winner 2018).This criterion uses model fit and the number of parameters as criteria. In comparing models, the model with the smallest AIC is considered optimal, computing the value with the following regression equation (Kaps and Lamberson 2017):

$AIC = n \log(SS_{RES}/n) + 2 \times p$

where:

SS_{RES} = Residual sum of squares;

n = Number of observations; and

p = Number of parameters in the model.

Finally, Pearson's correlation test was performed among those predictors with the greatest adjustment to their respective response variable.

All data were analyzed using the RStudio® software, with a level of significance of P<0.05.

Results

Environmental variables

Water balance (precipitation and evaporation) and air temperature are presented in Figure 1. These revealed a high rainfall incidence during April–June 2014 and November–December 2014, with a relatively dry period in January–March 2015. Average relative humidity was 86%.

During both sampling periods, average soil temperatures were 26.5 ± 0.7 and 28.6 ± 0.7 °C for RS and DS, respectively. Similarly, average water-filled pore space (WFPS) values were 51.8 ± 4.3 and 49.5 ± 6.0% in the same periods of time (Figure 2).

Herbage accumulation and N content

The growth of pasture followed a seasonal pattern with highest yields during the rainy season and lowest production in the dry season (Table 1). The response in forage accumulation to application of urine was not uniform across species over the total period, with *U. decumbens* showing a 33% increase (P<0.05) in growth compared with its Control, while the remaining species showed no responses (Table 1). However, during the periods immediately following urine application, i.e. harvests in June 2014 and April 2015, DM accumulation averaged over the 3 species increased by 20.8% (June) and 80.8% (April). In the intervening period DM yields each month did not vary significantly between urine and Control treatments.

Similarly, application of bovine urine significantly increased N content overall in *U. decumbens* (P<0.05), with no significant responses in *U. humidicola* and *M. maximus* (Table 2). As for DM accumulation, during the month following application of urine to the grasses, N uptake by plants treated with urine was 79% higher than by Control in June 2014 and 58% higher than by Control in April 2015 (Table 2).

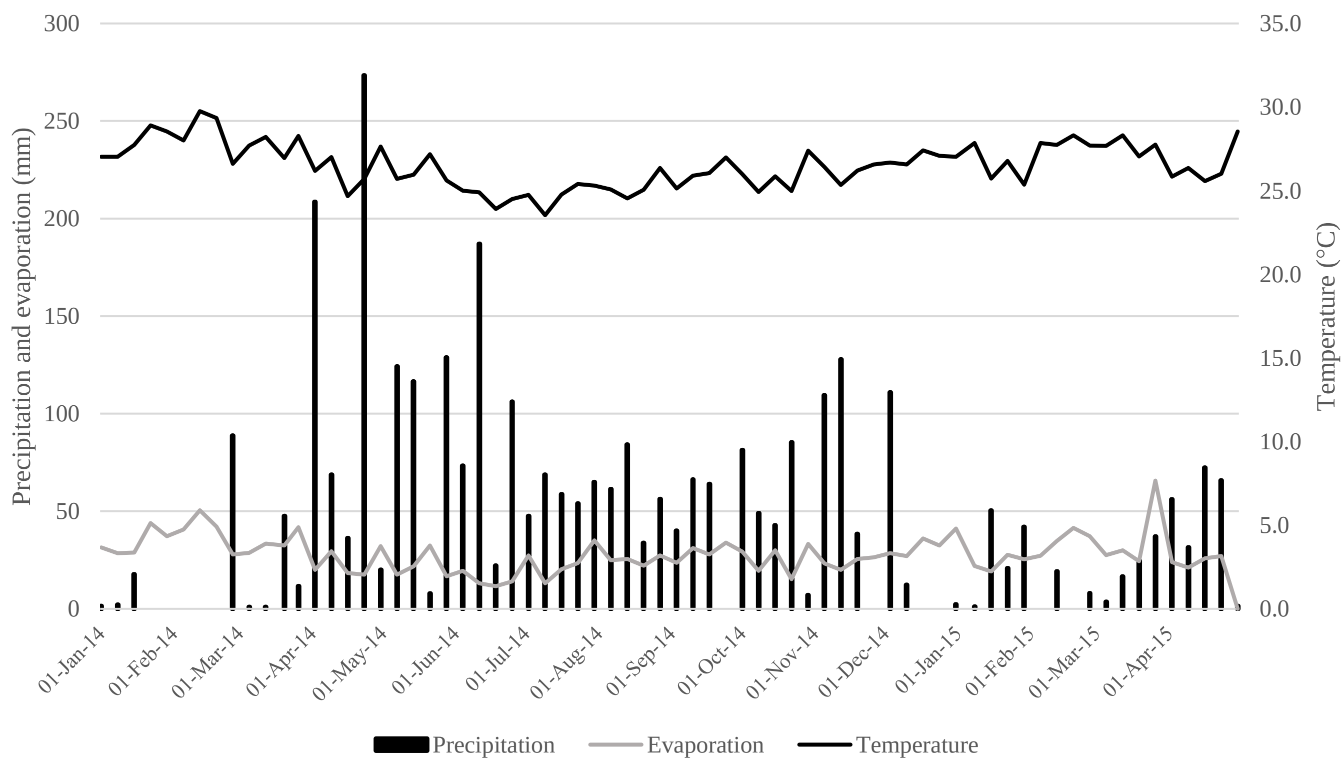

Figure 1. Accumulated precipitation, evaporation and average atmospheric temperature in AGROSAVIA "La Libertad" during the study period.

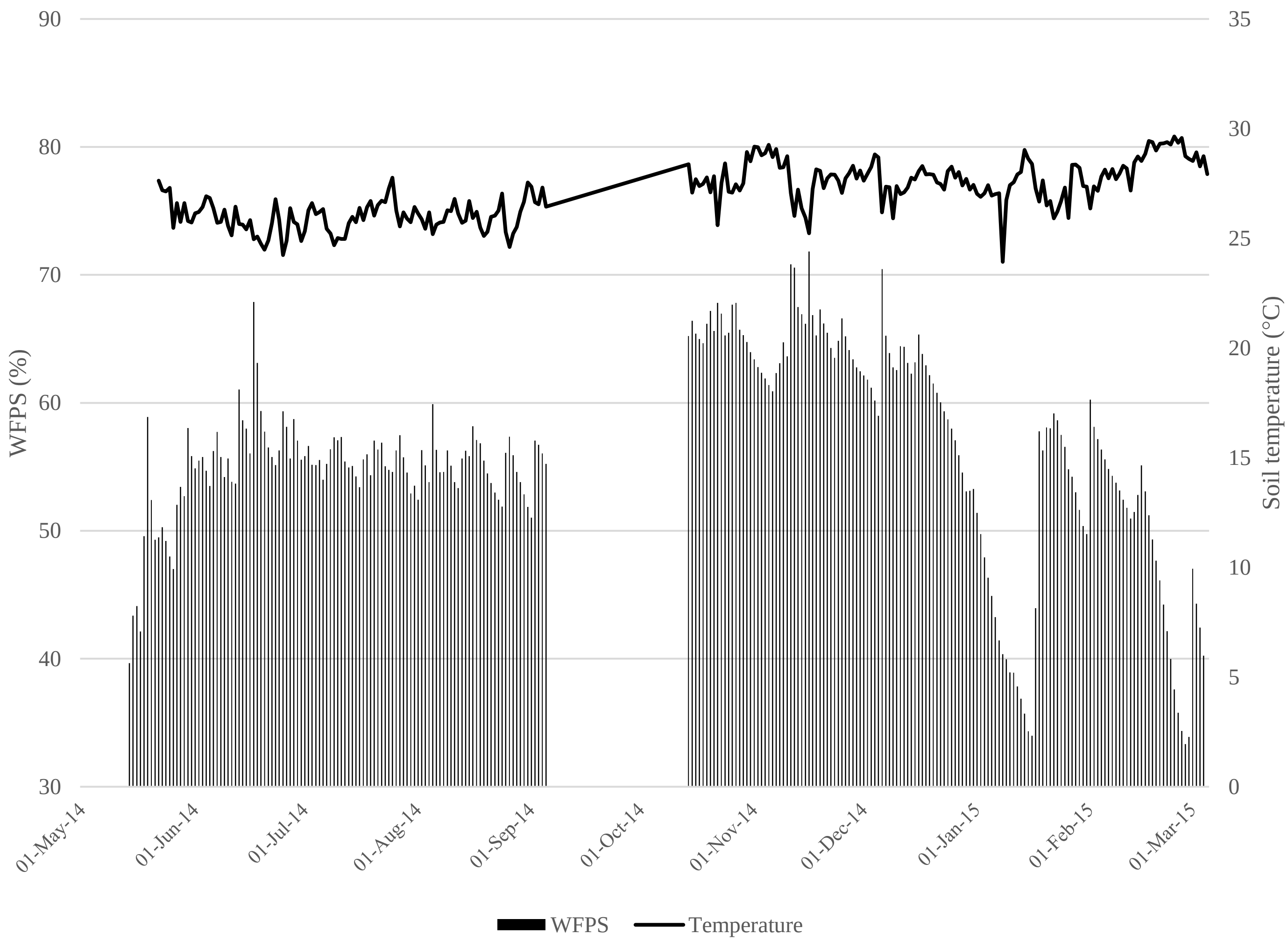

Figure 2. Changes in soil moisture level (WFPS) and temperature during sampling period for ammonium and nitrate concentrations. Data from September to October 2014 were not recorded because of datalogger damage.

Table 1. Effects of species and urine application on monthly herbage accumulation (t DM/ha) during 2014–2015.

Month	*Urochloa decumbens*		*Urochloa humidicola*		*Megathyrsus maximus*	
	Control	Urine	Control	Urine	Control	Urine
Jun	1.03	1.20	1.43	1.67	1.09	1.42
Jul	0.96	1.75	1.27	1.35	1.90	1.68
Aug	1.45	1.78	1.88	1.68	1.89	1.46
Sep	1.49	1.09	1.67	1.59	0.84	1.05
Oct[1]	1.65	1.98	2.42	2.45	2.15	1.37
Nov	1.20	1.54	1.43	1.57	1.26	1.02
Dec	1.00	1.36	2.02	1.36	1.10	0.73
Feb	0.30	0.53	1.40	1.29	0.61	0.67
Apr	1.23	2.49	0.99	1.59	0.95	1.65
Mean ± s.e.	1.14 ± 0.1a	1.52 ± 0.1b	1.61 ± 0.1a	1.60 ± 0.1a	1.31± 0.1a	1.23 ± 0.1a
Mean ± s.e.	1.3 ± 0.01B		1.6 ± 0.07A		1.2 ± 0.08B	

Means within species followed by the same lower-case letters are not different (P≥0.05).
Overall means for species followed by the same upper-case letters are not different (P≥0.05).
[1]The harvest in October 2014 was made 36 days after the previous defoliation event, due to logistical issues at the research center.

Table 2. Effects of urine application in May 2014 and February 2015 on N content (kg N/ha) of 3 tropical grasses.

Month	*Urochloa decumbens*		*Urochloa humidicola*		*Megathyrsus maximus*	
	Control	Urine	Control	Urine	Control	Urine
Jun 14	14.6	22.3	13.5	25.7	22.3	41.9
Jul 14	17.4	33.8	21.8	20.2	25.6	35.5
Aug 14	23.1	30.8	21.5	23.4	37.1	30.5
Sep 14	30.1	20.8	28.6	26.7	21.0	25.6
Oct 14	26.9	35.9	26.6	25.9	42.9	28.7
Nov 14	21.6	27.0	20.7	21.4	26.9	22.5
Dec 14	16.5	22.2	22.1	19.7	27.8	17.1
Feb 15	5.8	11.3	14.5	24.9	12.4	13.5
Apr 15	17.2	29.9	11.1	20.5	20.6	26.6
Mean ± s.e.	19.2 ± 1.9a	26.0 ± 2.3b	19.8 ± 2.0a	22.9 ± 1.5a	26.3 ± 2.9a	26.3 ± 3.0a
Mean ± s.e.	22.6 ± 1.3AB		21.3 ± 1.0B		26.3 ± 1.7A	

Means within species followed by the same lower-case letter are not different ($P \geq 0.05$).
Overall means for species followed by the same upper-case letter are not different ($P \geq 0.05$).

Soil concentrations of NH_4^+-N and NO_3^--N

Table 3 shows the concentrations of the two N forms during the month following treatment application. A peak in concentration of NH_4^+-N was registered following urine application in May 2014 with declining levels during the following month. The second application showed a lesser effect on concentration during DS.

In the first week after urine application in RS, the NO_3^--N concentrations were minimal, and displayed a substantial increase later in the season. However, during the DS, concentrations remained relatively stable during all samplings regardless of treatment (Table 3).

Seven variables were evaluated in building the explanatory model: rainfall, air and soil temperatures, relative humidity, WFPS and concentrations of NO_3^--N and NH_4^+-N. All initial models presented Akaike Information Criterion (AIC) scores greater than 30, which were reduced once different methodologies were applied (Table 4). Soil temperature and NO_3^--N concentration had the greatest effects on NH_4^+-N concentration in soil, while rainfall and soil temperature were the factors that best predicted changes in NO_3^--N concentration in the soil.

Table 4. Best adjusted models for soil NH_4^+-N and NO_3^--N concentrations as response variables.

Method	Best model[1]	AIC[2] score	
		Initial model	Final model
Backward	NH_4^+ = ST + NO_3^-	32.0	25.0
	NO_3^- = Rain + AT + RH + ST	35.1	32.2
Forward	NH_4^+ = ST + NO_3^-	30.7	25.0
	NO_3^- = Rain + ST	38.2	30.9
Stepwise	NH_4^+ = ST + NO_3^-	30.7	25.0
	NO_3^- = Rain + ST	38.2	30.9

[1]AT = Air temperature; ST = Soil temperature; RH = Relative humidity; Rain = Rainfall;
[2]AIC = Akaike Information Criterion.

Table 3. Effects of urine application on concentrations of NH_4^+-N and NO_3^--N (mg/kg) in soil, during the month following application (2014–2015).

Day	NH_4^+-N			NO_3^--N		
	Control	Urine	Mean ± s.e.	Control	Urine	Mean ± s.e.
May 16	17.7	33.3	23.9 ± 3.5a	0.6	1.3	0.9 ± 0.6b
May 22	8.0	16.0	11.2 ± 2.2de	20.9	21.2	21.1 ± 6.0a
May 29	6.2	9.2	7.7 ± 1.5de	23.4	8.3	17.4 ± 9.5ab
Jun 5	6.6	7.5	7.1 ± 0.7e	25.9	10.3	18.1 ± 7.8ab
Feb 4	14.7	17.7	16.2 ± 1.0bc	7.6	6.8	7.2 ± 2.1ab
Feb 12	16.0	18.6	17.3 ± 1.3b	8.1	8.2	8.1 ± 3.2ab
Feb 19	8.9	12.2	10.5 ± 0.9de	6.3	4.0	5.2 ± 2.2ab
Feb 25	10.6	14.0	12.3 ± 0.9cd	0.5	7.7	4.1 ± 2.4ab
Mean ± s.e.	11.7 ± 1.0A	15.3 ± 1.0B		11.6 ± 3.3A	8.5 ± 1.8A	

Overall means within columns followed by the same lower-case letter are not different ($P \geq 0.05$).
Means within N soil ions followed by the same upper-case letter are not different ($P \geq 0.05$).

Discussion

Herbage accumulation and N content

The highest herbage accumulation in all pastures was registered in October 2014. During this time, humidity, precipitation and temperature favored DM production, as opposed to the following month, when temporary waterlogging in some plots reduced growth rates. Conversely, the lowest herbage accumulation was registered in all plots during February 2015. This month is part of the DS in the Piedmont plains, when little rainfall is received (Figure 1), resulting in lower soil moisture levels (Figure 2) but elevated temperature. Water is vital in plant metabolic processes and supplies electrons for the reduction of CO_2 in photosynthesis. Likewise, its elimination through transpiration is linked to the stomatic conductance of the leaves; to avoid water loss, a lower moisture content within the plant decreases the stomatal opening, reducing the amount of CO_2 taken in by the leaf for photosynthesis. All this is reflected in reduced plant growth (Berlyn and Cho 1999).

Observing the total N content for the study period, Table 2 shows important differences between plant species, with the highest values for *M. maximus* and *U. decumbens*. Due to its larger foliar area, type of growth and genetic characteristics, *M. maximus* normally presents a higher rate of photosynthesis (Silva 2004) and efficiency in N use (Pérez 2014) than other tropical grasses, meaning a high herbage accumulation and crude protein concentration (Fernández et al. 2004). In this study, *U. humidicola* produced more DM than *M. maximus* (Table 1), which may be explained by its growth habit. *U. humidicola* grows by stolons, with a higher DM compared with their leaves but lower digestibility (Vergara and Araujo 2006). Moreover, *Urochloa* grasses have morphological adaptations to promote better utilization of N available in soils with low fertility, such as increased amount and length of roots or lower leaf expansion per unit dry weight (Rao et al. 1995). These factors decrease the dependence of forages on high N concentrations in the soil, presenting lower concentrations of N in their tissues in consequence (Rao 2001). The effect is more pronounced in *U. humidicola*, probably through its ability to synthesize biological nitrification inhibitors from its roots (Subbarao et al. 2009).

When analyzing responses to treatments over time, significant differences in N content were observed between the urine treatments and their controls in the first month after urine application (Table 2). The regional recommendation is to apply 50–70 kg N/ha to *Urochloa* pastures for grazing, as a compromise between biomass production and nutritional value of the forage (Rincón et al. 2010). During our experiment, we applied the equivalent of 61 and 78 kg N/ha in DS and RS, respectively, which would have generated the significant response in N concentration during these first samplings.

While herbage accumulation also increased, the responses were not large enough to detect significant differences (Table 1). The absence of differences in the following months was possibly due to the transformation and use of the N applied within the soil through ammonification and nitrification or by its escape from the system by volatilization or denitrification (Orozco 1999). Although the second application of urine occurred in February 2015, none of the pastures showed effects in their productive variables in the following month, and responses were delayed until April 2015. This could happen due to weather conditions during the DS, with few precipitation events (Figure 1) and decreased soil moisture (Figure 2) in January and February 2015. Reduced levels of water in the soil solution suppress activity of the microbial populations responsible for making N available for plants (Cameron et al. 2013), which could result in loss of N by ammonia volatilization (Jantalia et al. 2012) or its inorganic preservation by the low soil pH and presence of clays. The immobilization of N by the soil during the DS could preserve this element until the return of the rains, when it is mineralized by the microbial populations, making it available for the plants (Baggs et al. 2010).

Concentrations of ammonium and nitrate N in the soil

According to Table 3, NH_4^+-N was always present in the soil, since both treatments showed concentrations greater than 6 mg/kg. With total N concentration of 0.14%, this soil could be considered as low in organic matter. However, it is known that soil microorganisms present adaptive capacities in such situations, fulfilling their function as regulators of the N cycle in the soil (Dubeux Jr et al. 2007; Chirinda 2015). Additionally, the application of urine could increase the concentrations of NH_4^+-N in the soil during the RS, decreasing over time as the N source was transformed into other compounds.

The marked increase in NO_3^--N observed in the RS after treatment application (Table 3) was possibly due to the interaction of the biochemical processes of ammonification and nitrification (Orozco 1999). Likewise, Baggs et al. (2010) suggested that lower moisture levels in the soil could stimulate nitrate ammonification, which would also explain the lower nitrate levels during the DS.

It is noticeable how the concentrations of NH_4^+-N and NO_3^--N differed from the baseline levels. Due to their reductive conditions, oxisol soils could stimulate ammonification over nitrification, favoring the production of ammonia and its subsequent loss due to volatilization (Cameron et al. 2013).

Urochloa grasses showed a higher N content (Table 2) during the RS, which could be explained by a delay of nitrification until the second month after application of bovine urine. Although tropical grasses are capable of absorbing small amounts of N in NH_4^+-N form without exhibiting intoxication (Moser et al. 2004), they prefer to absorb this element as NO_3^--N, which is more soluble and easily transportable in the roots (Azcón-Bieto and Talon 2008). Its higher concentration in the soil would then imply greater absorption, resulting in a greater deposition of N components within the plant.

Variation in NH_4^+-N and NO_3^--N concentrations was noted in the control plots, despite urine not being applied on these experimental units. This phenomenon requires further investigation, but Day and Detling (1990) suggested that forage responses on urine patches relative to unaffected areas may be associated with increased soil N availability and root N concentration. The impact of urine beyond the area of application is aided by the large proportion of water in the urine and the mobility and availability of the nutrients it contains (White-Leech et al. 2013), reaching areas 3 times or more the area to which it was deposited (Haynes and Williams 1993).

Available N values reported in this study differ from those reported from other investigations. Conducting an experiment to determine ammonifying microbial populations, Verhamme et al. (2011) reported concentrations of approximately 190 and 70 g/kg of NH_4^+-N and NO_3^--N, respectively, in a soil in Scotland, while Salazar-Sosa et al. (2009) published values of 50–100 mg/kg nitrates in soils cultivated with a mixture of corn and soy in Mexico. Similar results were shown by Trejo-Escareño et al. (2013), evaluating the effects of application of bovine feces in a corn crop. Those studies suggest that the values reported here can be considered low, although this greatly depends on the management given to the soil and the external application of nutrients.

As fundamental components of many biochemical processes in the soil, the concentrations of NH_4^+-N and NO_3^--N are very variable, being affected by several environmental factors (Whitehead 2000). According to linear model building in this study, the environmental variables that had greatest influence on NH_4^+-N were soil temperature and NO_3^--N (Table 4), with high correlations between the response variables (Table 5). The high negative correlation between NO_3^--N and NH_4^+-N may be due to the inverse behavior shown by both compounds during the sampling period (Table 3), where ammonium is an initial component for nitrification, resulting in nitrate as the final product (Ardakani et al. 1974; Whitehead 1995).

Table 5. Correlation coefficients between response variables and predictors of the best-fitted models (%).

Response	Factor	ρ_{xy}
NH_4^+-N	ST	-77.4
	NO_3^-	-57.8
NO_3^--N	ST	67.0
	Rain	36.2

ST = Soil temperature; Rain = Rainfall.

We conclude that applying urine from cows fed with tropical forages increased herbage accumulation in *U. decumbens* but not in *U. humidicola* and *M. maximus* in the Colombian Piedmont plains. However, it did produce temporary increases in N content in the grasses immediately following application. This supports the concept that grazing animals play a beneficial role in dispersing nutrients in pastures. However, the effects seem to be limited in duration possibly because of the loss of N through volatilization. Likewise, the edaphoclimatic conditions and regional agricultural management limit the concentrations of N available in the soil, showing the validity of using forages adapted to these areas.

Finally, it is necessary to determine the relationships between the concentrations of available N in the soil and in the grasses in the region, to understand better the impact of agricultural practices on the N cycle in pastoral systems.

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References

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AOAC. 1998. Official methods of analysis. 16th Edn. AOAC International, Gaithersburg, MD, USA.

Ardakani MS; Rehbock JT; McLaren AD. 1974. Oxidation of ammonium to nitrate in a soil column. Soil Science Society of America Journal 38:96–99. doi: 10.2136/sssaj1974.03615995003800010030x

Azcón-Bieto J; Talon M, eds. 2008. Fundamentos de fisiología vegetal. McGraw-Hill, Barcelona, Spain. bit.ly/3cArINz

Baggs EM; Streminska M; Barret G; Morley N. 2010. Nitrous oxide production in soil: Microbial source partitioning to inform management options for mitigation. 19th World Congress of Soil Science, Brisbane, Australia, 1–6 August 2010. p. 191–194. bit.ly/2VlWJ1Q

Berlyn G; Cho J. 1999. Light, moisture and nutrient use by plants. In: Ashton M; Montagnini F, eds. The silvicultural basis for agroforestry systems. CRC Press, Washington, DC, USA. p. 9–40.

Bernal J. 1994. Pastos y forrajes tropicales: Producción y manejo. Banco Ganadero, Bogotá, Colombia.

Cameron KC; Di HJ; Moir JL. 2013. Nitrogen losses from the soil/plant system: A review. Annals of Applied Biology 162:145–173. doi: 10.1111/aab.12014

Chirinda N. 2015. Just how sustainable is sustainable tropical agriculture?. III Congreso Colombiano de Silvopastoreo y II Conferencia de Gases de Efecto Invernadero en Sistemas Agropecuarios en Latinoamérica (GALA). Universidad Nacional de Colombia, Medellín, Colombia.

Day TA; Detling JK. 1990. Grassland patch dynamics and herbivore grazing preference following urine deposition. Ecology 71:180–188. doi: 10.2307/1940258

Di HJ; Cameron KC. 2012. How does the application of different nitrification inhibitors affect nitrous oxide emissions and nitrate leaching from cow urine in grazed pastures? Soil Use and Management 28:54–61. doi: 10.1111/j.1475-2743.2011.00373.x

Dubeux Jr JCB; Sollenberger LE; Mathews BW; Scholberg JM; Santos HQ. 2007. Nutrient cycling in warm-climate grasslands. Crop Science 47:915–928. doi: 10.2135/cropsci 2006.09.0581

Fernández JL; Benítez DE; Gómez I; Souza A de; Espinoza R. 2004. Rendimiento de materia seca y contenido de proteína bruta del pasto *Panicum maximum* cv. Likoni en un suelo vertisol de la provincia Granma. Revista Cubana de Ciencia Agrícola 38:417–421. bit.ly/2UPYzs6

Haynes RJ; Williams PH. 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy 49:119–199. doi: 10.1016/S0065-2113(08)60794-4

Holdridge LR, ed. 1978. Ecología basada en zonas de vida. Instituto Ineramericano de Ciencias Agrícolas (IICA), San José, Costa Rica. repositorio.iica.int/handle/11324/7936

IGAC. 2004. Estudio general de suelos y zonificación de tierras: Departamento de Meta. Instituto Geográfico Agustín Codazzi (IGAC), Bogotá, Colombia.

Jantalia CP; Halvorson AD; Follet RF; Alves BJR; Polidoro JC; Urquiaga S. 2012. Nitrogen source effects on ammonia volatilization as measured with semi-static chambers. Agronomy Journal 104:1595–1603. doi: 10.2134/agronj 2012.0210

Kaps M; Lamberson W. 2017. Biostatistics for animal science. 3rd Edn. CABI Publishing, Wallingford, Oxon, UK.

Lin S; Iqbal J; Hu R; Shaaban M; Cai J; Chen X. 2013. Nitrous oxide emissions from yellow brown soil as affected by incorporation of crop residues with different carbon-to-nitrogen ratios: A case study in central China. Archives of Environmental Contamination and Toxicology 65:183–192. doi: 10.1007/s00244-013-9903-7

McKean S. 1993. Manual de análisis de suelos y tejido vegetal: Una guía teórica y práctica de metodologías. Documento de trabajo No. 129. Centro Internacional de Agricultura Tropical (CIAT), Palmira, Colombia. hdl.handle.net/10568/70025

Mejia L. 1996. Génesis y características de los oxisoles y suelos óxicos de los Llanos Orientales de Colombia y su relación con la fertilidad. Suelos Ecuatoriales 26:7–34.

Moser LE; Burson BL; Sollenberger LE, eds. 2004. Warm-season (C_4) grasses. Agronomy Monograph No. 45. ASA, CSSA, SSSA, Madison, WI, USA. doi: 10.2134/agronmonogr45

Orozco FH, ed. 1999. La biología del nitrógeno: Conceptos básicos sobre sus transformaciones biológicas. Universidad Nacional de Colombia, Medellín, Colombia. bdigital.unal.edu.co/3666

Pérez O. 2014. Eficiencia de uso de nitrogeno en pasturas de *Panicum maximum* y *Brachiaria* sp. solas y asociadas con *Pueraria phaseoloides* en la altillanura colombiana. M.Sc. Thesis. Universidad Nacional de Colombia, Bogotá, Colombia. bdigital.unal.edu.co/46432

Rao IM. 2001. Adapting tropical forages to low-fertility soils. In: Gomide JA; Mattos WRS; Silva SC da, eds. Proceedings of the XIX International Grassland Congress, São Pedro, SP, Brazil. p. 247–254. hdl.handle.net/10568/56023

Rao IM; Ayarza MA; García R. 1995. Adaptive attributes of tropical forage species to acid soils I. Differences in plant growth, nutrient acquisition and nutrient utilization among C_4 grasses and C_3 legumes. Journal of Plant Nutrition 18:2135–2155. doi: 10.1080/01904169509365052

Rennó LN; Valadares RFD; Valadares Filho SD; Leão MI; Silva JFC da; Cecon PR; Gonçalves LC; Dias HLC; Linhares RS. 2000. Plasma urea concentration and urea and creatinine excretions in steers. Revista Brasileira de Zootecnia 29:1235–1243. (In Portuguese). doi: 10.1590/S1516-35982000000400038

Rincón A; Bueno GA; Álvarez M; Pardo O; Pérez O; Caicedo S. 2010. Establecimiento, manejo y utilización de recursos forrajeros en sistemas ganaderos de suelos ácidos. Corporación Colombiana de Investigación Agropecuaria (Corpoica), Villavicencio, Colombia. hdl.handle.net/20.500.12324/12703

Saggar S; Bolan NS; Bhandral RB; Hedley CB; Luo J. 2004. A review of emissions of methane, ammonia and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. New Zealand Journal of Agricultural Reseach 47:513–544. doi: 10.1080/00288233.2004.9513618

Salazar-Sosa E; Trejo-Escareño HI; Vázquez-Vázquez C; López-Martínez JD; Fortis-Hernández M; Zuñiga-Tarango R; Amado-Álvarez JP. 2009. Distribution of available nitrogen in the soil profile after applying bovine manure in corn forage. Terra Latinoamericana 27:373–382. (In Spanish). bit.ly/2RV6zWu

Silva S da. 2004. Fundamentos para o manejo do pastejo de plantas forrageiras dos gêneros *Brachiaria* e *Panicum*. Simpósio sobre manejo estratégico da pastagem, Viçosa, Brazil. p. 347–385. bit.ly/3elAHE9

Subbarao GV; Nakahara K; Hurtado MP; Ono H; Moreta DE; Salcedo AF; Yoshihashi AT; Ishikawa T; Ishitani M; Ohnishi-Kameyama M; Yoshida M; Rondon M; Rao IM; Lascano CE; Berry WL; Ito O. 2009. Evidence for biological nitrification inhibition in *Brachiaria* pastures. Proceedings of the National Academy of Sciences (PNAS) 106:17302–17307. doi: 10.1073/pnas.0903694106

Trejo-Escareño HI; Salazar-Sosa E; López-Martínez JD; Vázquez-Vázquez C. 2013. Impact of cow manure in soil and corn forage production. Revista Mexicana de Ciencias Agrícolas 4:727–738. (In Spanish). bit.ly/3anbExY

USDA (United States Department of Agriculture). 2014. Soil bulk density/moisture/aeration. Soil Health for Educators. Natural Resources Conservation Service, Washington, DC, USA. bit.ly/2lmxai4

Valadares RFD; Broderick GA; Valadares Filho SC; Clayton MK. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. Journal of Dairy Science 82:2686–2696. doi: 10.3168/jds.S0022-0302(99)75525-6

Vergara-López J; Araujo-Febres O. 2006. Yield, chemical composition and ruminal degradability of the *Brachiaria humidicola* (Rendle) Schweick in tropical dry forest. Revista Científica 16:239–248. (In Spanish). bit.ly/2Sx7jl6

Verhamme DT; Prosser JI; Nicol GW. 2011. Ammonia concentration determines differential growth of ammonia-oxidizing archaea and bacteria in soil microcosms. Multidisciplinary Journal of Microbial Ecology 5:1067–1071. doi: 10.1038/ismej.2010.191

White-Leech R; Liu K; Sollenberger LE; Woodard KR; Interrante SM. 2013. Excreta deposition on grassland patches. II. Spatial pattern and duration of forage responses. Crop Science 53:696–703. doi: 10.2135/cropsci2012.07.0408

Whitehead DC, ed. 1995. Grassland nitrogen. CAB International, Wallingford, Oxon, UK.

Whitehead DC, ed. 2000. Nutrient elements in grasslands. Soil-plant-animal relationships. CABI Publishing, Wallingford, Oxon, UK.

Winner L. 2018. Applied Statistical Methods II. University of Florida, Gainesville, FL, USA.

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Research Paper

Effects of swine manure application and row spacing on growth of pearl millet (*Cenchrus americanus*) during the establishment period and quality of silage produced in Southwest Nigeria

Efectos de la aplicación de estiércol de porcinos y de la distancia de siembra en el establecimiento y la calidad del ensilaje de Cenchrus americanus *en el suroeste de Nigeria*

V.O.A. OJO[1], F.T. ADESHINA[1], G.A. ADETOKUNBO[1], S.O. JIMOH[1,2,3], T.A. ADEYEMI[1], J.L. NJIE[4] AND O.S. ONIFADE[1]

[1]*Department of Pasture and Range Management, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria.* unaab.edu.ng
[2]*Institute of Grassland Research, Chinese Academy of Agricultural Sciences, Hohhot, Inner Mongolia, China.* caas.cn
[3]*Sustainable Environment Food and Agriculture Initiative (SEFAAI), Lagos, Nigeria.* sefaai.org
[4]*School of Agriculture and Environment Sciences, University of The Gambia, Serrekunda, The Gambia.* utg.edu.gm

Abstract

The effects of swine manure application and row spacing on dry matter yields of *Cenchrus americanus* (pearl millet) at 6 weeks after sowing and chemical composition, fermentative characteristics and in vitro gas production of silage produced from the forage were studied. The design was a 2 × 2 factorial with 2 row spacings (0.5 and 1.0 m) and 2 levels of manure application [no manure (Control) and swine manure at 5 t/ha (22% DM; 0.34% N on DM basis)] replicated 3 times. Swine manure application had no effect (P>0.05) on dry matter yield but a row spacing of 0.5 m produced higher (P<0.05) dry matter yields than 1.0 m spacing (mean 7.05 vs. 5.57 t DM/ha). Fresh forage from manured treatments had significantly higher crude protein concentration (114.9–124.2 g/kg DM) than from unfertilized plots (86.2–95.1 g/kg DM). After being ensiled for 42 days, CP% in the forage had declined by 16–18% but relative differences remained. Quality measurements indicated that silages from the various treatments were all of acceptable standard although CP% of silage from Control plots was barely high enough to provide a maintenance diet. This study suggests that, under the experimental conditions, planting of pearl millet at a spacing of 0.5 m rather than 1.0 m would increase DM yields obtained in the first 6 weeks of growth, while application of swine manure would not affect yields but would increase CP% of forage produced. The laboratory study indicates that the forage produced could be ensiled successfully although there was significant loss of crude protein during the process. Since there were no significant increases in DM yields of forage, other benefits, e.g. increase in N concentration, improved soil organic matter, etc., would need to be considered in justifying the additional cost of drying and applying the manure.

Keywords: Fertilization, forage conservation, nutritive value, tropical forages.

Resumen

En Abeokuta, Oguna State, Nigeria se estudiaron los efectos de la aplicación de estiércol porcino y el espaciado entre hileras sobre los rendimientos de materia seca de *Cenchrus americanus* a las seis semanas después de la siembra, la composición

Correspondence: S.O. Jimoh, Institute of Grassland Research, Key Laboratory of Grassland Ecology and Restoration, Chinese Academy of Agricultural Sciences, 120 East Wulanchabu Street, Hohhot, Inner Mongolia 010010, China.
E-mail: sahjim05@gmail.com

química, las características fermentativas y la producción de gas in vitro por el forraje ensilado de esta gramínea. Se empleó un diseño factorial 2 × 2 con dos distancias de siembra (0.5 y 1.0 m entre hileras) y dos niveles de aplicación de estiércol [sin y con 5 t/ha (22% MS; 0.34% N con base en MS)] y tres repeticiones. La aplicación de estiércol porcino no mostró ningún efecto (P>0.05) en el rendimiento de materia seca, pero la distancia entre hileras de 0.5 m produjo rendimientos de materia seca más altos (P<0.05) que la distancia de 1.0 m (media 7.05 vs. 5.57 t MS/ha). El forraje fresco en los tratamientos con estiércol presentó mayor (P<0.05) concentración de proteína cruda (114.9–124.2 g/kg de MS) que el de las parcelas no fertilizadas (86.2–95.1 g/kg de MS). Después de estar ensilado durante 42 días, el porcentaje de PC en el forraje se redujo en 16–18%, pero las diferencias relativas se mantuvieron. Las mediciones de calidad indicaron que los ensilajes en los tratamientos eran aceptables; no obstante en el testigo (tratamiento sin estiércol) el porcentaje de PC del ensilaje solo llenaba los requerimientos de mantenimiento para bovinos. Este estudio mostró que en las condiciones experimentales, la siembra de *C. americanus* a una distancia de 0.5 m aumenta los rendimientos de MS obtenidos en las primeras 6 semanas de crecimiento, mientras que la aplicación de estiércol porcino no afecta los rendimientos, pero aumenta la concentración de CP en el forraje.. El estudio de laboratorio indica que el forraje producido puede ser ensilado con éxito, pero con una pérdida significativa de PC durante el proceso. En vista de que la aplicación de estiércol no mostró aumentos significativos en el rendimiento de forraje, otros beneficios, p.ej. el aumento de la concentración de N y el incremento de la materia orgánica en el suelo, etc., deberían considerarse para justificar el costo adicional del secado y la aplicación del estiércol.

Palabras clave: Conservación de forraje, fertilización, forrajes tropicales, valor nutritivo.

Introduction

Forages are the primary diets for ruminants in many tropical regions and grasses constitute the bulk of the energy sources for grazing ruminants (Olanite et al. 2010). High-yielding forages of high quality are the most economical feed for ruminants and result in acceptable liveweight gains and animal performance. However, prolonged annual dry seasons negatively affect plant performance, resulting in limited quantity and poor quality of available forage at this time, leading to a reduction in voluntary feed intake and nutrient utilization, with reduced overall performance of ruminant animals.

Ojo et al. (2015a) suggested that the challenge of feed shortages could be solved through the cultivation of forage crops with better nutritional values than the existing native feed resources. Similarly, Bamikole et al. (2004) reported that high concentrations of protein, vitamins and minerals in sown forages could markedly improve animal performance. Conservation and preservation of these cultivated species, combined with improved management practices, is a possible solution to the limitations posed by poor quality and quantity of native forages in the dry season (Ojo et al. 2015b). Babayemi (2009) recommended the ensiling of forages, at a growth stage when there is a balance between yield and quality of the available crop, for feeding to animals during times of nutritional stress.

Amodu et al. (2005) indicated that pearl millet [*Cenchrus americanus* (L.) Morrone syn. *Pennisetum americanum* (L.) Leeke] is a promising forage crop with high potential for integration into Nigerian livestock production systems. The crop is well adapted to a wide range of agronomic conditions varying from the semi-arid to the subhumid zone, where annual rainfall varies between 600 and 1,100 mm, with forage yields of 2.7–7.7 t DM/ha (Agishi 1985). Preliminary studies with pearl millet confirmed that it could be harvested and conserved for feeding to ruminant animals during prolonged dry seasons (Nuru 1989).

To ensure both adequate yields and acceptable quality of forage, application of fertilizer and optimal row spacing are essential (Miah et al. 1990; Olanite et al. 2010). In the past, application of inorganic fertilizer has been the normal practice, but increased costs of these products, combined with the attendant nutrient depletion and negative residual effects of inorganic fertilizers on the environment (Malhi et al. 2002), have resulted in increased interest in using organic manures on soils. Applying organic manures to soils/crops would have multiple benefits by overcoming the problem of manure management and disposal, improving the nutrient status of the soils and possibly improving both yields and nutrient value of the forage produced (Ojo et al. 2013).

The present study investigated the effects on DM yields and nutritive value of the resulting forage of applying swine manure to a crop of pearl millet sown at 2 different row spacings, and quality of silage produced from the forage.

Materials and Methods

Location

The experiment was conducted at the Pasture Unit of Federal University of Agriculture, Abeokuta (FUNAAB)

Farm, Ogun State, Nigeria (7°58' N, 3°20' E; 75 masl). The site is situated in the derived savannah agro-ecological zone of Southwest Nigeria with average annual rainfall of 1,037 mm. Mean monthly temperature ranges from 25.7 ºC in July to 30.2 ºC in February (earth.google.com/). Data on rainfall, temperature and humidity covering the period when the study was conducted are shown in Table 1.

Table 1. Meteorological data for the experimental area during June–December 2015.

Month	Rainfall (mm)	Mean temp. (°C)	Mean rel. humidity (%)
Jun	165	26.9	79.4
Jul	66	26.6	80.9
Aug	29	26.3	79.3
Sep	165	26.5	81.3
Oct	159	27.4	81.9
Nov	17	28.6	72.5
Dec	0	26.1	39.7

Source: Agrometeorology Department, FUNAAB, Nigeria.

Land preparation

A total land area of 598 m^2 was cleared, plowed and allowed to rest for a period of 2 weeks before harrowing, following conventional tillage operations to provide a fine seed bed. Prior to sowing, soil samples were collected at random from the area to a depth of 15 cm using a soil auger to determine the pre-planting nutrient status of the soil. Analysis of these soil samples indicated that it was a sandy silt with the following parameters: pH 6.75, organic carbon 1.27%, available phosphorus (P) 30.4 mg/kg, potassium (K) 0.79 cmol/kg, calcium (Ca) 3.36 cmol/kg, magnesium (Mg) 2.42 cmol/kg, sodium (Na) 1.51 cmol/kg and total nitrogen (N) 0.11%.

Swine manure was collected from the Piggery Section of the Directorate of University Farms, FUNAAB, 14 days before application in bi-axially oriented polypropylene bags. Following collection, the manure was spread to dry and stored under a barn to allow for normal decomposition. The pigs had been fed a standard "Pigs finisher diet". Chemical analysis of the manure revealed that it contained: 4.76% Ca, 2.40% Mg, 1.92% K, 2.07% Na, 0.34% N and 1.2% P on a DM basis.

Experimental design

The experiment was laid out as a 2 × 2 factorial design comprised of 2 row spacings (drilled at 0.5 and 1.0 m inter-row intervals) and 2 levels of swine manure application (0 and 5 t/ha at 22% DM). A randomized complete block design was used with a total land area measuring 19 × 17 m divided into 3 equal blocks. Each block measured 5 × 17 m with a buffer zone of 1 m between blocks, while each plot measured 4 × 5 m, with a buffer zone of 1 m between plots. Variability in soil was blocked across the slope and each treatment was replicated 3 times.

The manure was broadcast onto individual plots according to treatment in a single application and subsequently raked into the soil manually and the plots were left for 2 weeks before sowing the grass. Seeds of *C. americanus* were drilled in rows at the predetermined row spacings at a seeding rate of 20 kg/ha in August 2015. No herbicides were applied.

Forage harvest and ensiling process

Millet forage was harvested at 6 weeks after sowing by cutting the entire forage in each plot at 15 cm above ground level. Fresh weight of the forage was determined immediately after harvesting, following which 500 g subsamples of the fresh forage from each treatment were collected to determine DM% and chemical composition. Thereafter, the harvested forage was bulked within treatments and allowed to wilt for 4 hours. Wilting helped reduce the moisture content of the forage to give a DM concentration of 33%, following which the material was manually chopped to lengths of about 2 cm. The chopped forage was rapidly compressed and sealed into laboratory glass silos of 960 mL capacity with 3 silos per treatment. Small glass silos were used as this was an exploratory study and no animal feeding trials were planned. A total of 500 g of forage was manually compressed into the silo bottles to a density of 0.52 g/mL. The silos were tightly sealed with 6 layers of duct tape to prevent re-entry of air into the silos, and the ensiled materials were allowed to stand for a period of 6 weeks, at an ambient room temperature of 26 ºC.

Quality analyses

Fresh forage. Analysis of the fresh forage commenced immediately after sample collection. Subsamples (500 g) were oven-dried to a constant weight at 65 ºC. The dried samples were then milled through a 1 mm sieve and crude protein (CP) and ash concentrations were determined according to the standard methods of AOAC (2000). Fiber fraction concentrations [neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL)] were determined according to Van Soest et al. (1991). Cellulose concentration was estimated as the difference between ADF and ADL concentrations, while hemicellulose concentration was estimated as the difference between NDF and ADF concentrations.

Silage. At the expiration of the ensiling period, the silos were opened and assessed for physical and sensory properties, including color, odor, moisture and presence of mold (Appendix 1). Six trained silage experts assessed all treatments using a scoring sheet (Bates 1998). After opening the silos, about 200 g of silage was weighed into 500 ml beakers. Color and moldiness scores were awarded based on the visual appearance of the silage. Odor score was based on how assessors felt when smelling a sample of silage. For moistness, the assessors – wearing latex gloves – squeezed moisture from about 30 g of the silage and allocated a score for the level of free water in the silage. For the scoring schemes, see Appendix 1. Ammonia and volatile fatty acid concentrations (acetic, propionic, butyric and lactic acid concentrations) in the silages were determined according to the procedures of AOAC (1990) and Mathew et al. (1997), respectively. Immediately after opening of the silos, 10 g samples of silage were taken from each silo and soaked in 100 mL of distilled water for 12 hours. The mixtures were then filtered and the supernatant divided into 4 aliquots each for pH determination using a pH meter (Hanna instruments, pH 211, microprocessor pH meter, K012818, Portugal) (Wilson and Wilkins 1972).

Subsamples of 300 g silage were oven-dried to a constant weight at 65 ºC. Concentrations of CP, ash and fiber fractions were determined following the same procedures outlined for the fresh samples. In addition, mineral concentrations (Ca, P, K, Mg, iron and copper) of the silage samples were determined according to the standard methods of AOAC (2000).

To evaluate the degradability of the ensiled forage materials, in vitro gas production was determined according to the procedure of Menke and Steingass (1988).

Statistical analysis

Data collected were subjected to a 2-way analysis of variance and treatment means were separated using Duncan's Multiple Range Test (SAS 2003) and analyzed using the PROC GLM procedure.

Results

Fresh forage

The narrower row spacing produced significantly ($P<0.05$) higher forage yields (6.85 and 7.25 t DM/ha for no manure and manured, respectively) than the wider row spacing (5.48 and 5.65 t DM/ha for no manure and manured, respectively) (Figure 1). There was no significant ($P>0.05$) response to manure application at either row spacing.

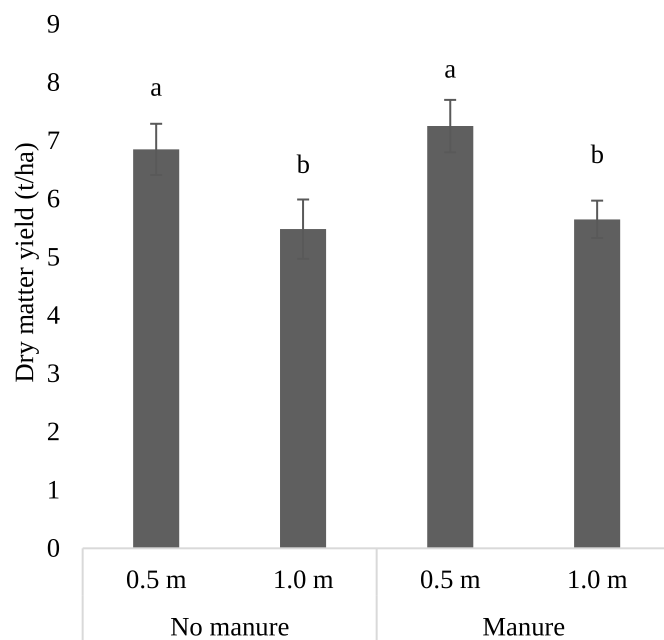

Figure 1. Yields of *Cenchrus americanus* as affected by row spacing and swine manure application. Error bars indicate standard error of the mean. Values for columns with different letters are significantly ($P<0.05$) different.

At both row spacings, fertilized pearl millet had higher ($P<0.05$) CP percentage than the unfertilized grass (Table 2). There were no consistent patterns between treatments in NDF, ADF, ADL, hemicellulose and cellulose levels but unfertilized forage at the wider row spacing tended to have the lower levels of NDF, ADF and cellulose.

Table 2. Chemical composition (g/kg DM) of fresh *Cenchrus americanus* forage as affected by row spacing and swine manure application.

Row spacing (m)	Manure application	CP	Ash	NDF	ADF	ADL	Hemi-cellulose	Cellulose
0.5	Manure	114.9a[1]	163	645a	386a	87.7	199b	297b
	No manure	95.1b	179	668a	390a	82.0	287a	305b
1.0	Manure	124.2a	151	626ab	409a	89.9	203b	321a
	No manure	86.2b	200	586b	365b	80.3	227b	277c
s.e.m.		5.76	8.3	22.7	6.2	4.39	30.8	6.3

[1]Means in the same column followed by different letters are significantly (P<0.05) different.

Silage

Irrespective of row spacing, silage made from unfertilized pearl millet recorded higher (P<0.05) silage color scores than silage from fertilized forage, with the silage having desirable green to yellowish-green color (Table 3; Appendix 1). All silages except that from the fertilized plots at wide row spacing had quite acceptable odors.

Fermentative characteristics of the silage were significantly (P<0.05) affected by both row spacing and manure application (Table 4). Fertilized forage from the narrow row spacing had lower VFA (volatile fatty acid) concentrations but higher ammonia N concentrations and pH values than other silages. Meanwhile, silage from unfertilized treatments had the lowest pH regardless of row spacing.

Silage from the narrow row spacing had significantly (P<0.05) higher DM percentage than that from the wider row spacing irrespective of manure application (Table 5), while manure application had no effect on DM% (P>0.05). Manure application at both row spacings increased (P<0.05) CP% of silage. There were no consistent patterns in the effects of both manure application and row spacing on ADF, ADL, hemi-cellulose and cellulose concentrations.

All mineral concentrations in silage followed the same pattern except for P, in the order Narrow + manure > Wider + manure > Wider + no manure > Narrow + no manure (P<0.05) (Table 6). Concentration of P in silage showed an increase from manure application at narrow row spacing but a reduction from manure application at the wider spacing.

In general, patterns of gas production from silage over time were inconsistent and not significantly affected by either manure application or plant spacing (P>0.05) (Table 7). Fermentation of substrates was ongoing beyond 24 hours of incubation.

Table 3. Physical characteristics of *Cenchrus americanus* silage as affected by row spacing and swine manure application.

Row spacing (m)	Manure application	Color	Odor	Moistness	Moldiness
0.5	Manure	7.8b[1]	23.6b	9.0a	8.0b
	No manure	9.8a	26.0a	9.0a	9.0a
1.0	Manure	6.7c	14.9d	6.0c	5.0d
	No manure	9.6a	22.0c	8.0b	6.0c
s.e.m.		0.16	0.13	0.06	0.07

For the scoring schemes, see Appendix 1.
[1]Means in the same column followed by different letters are significantly (P<0.05) different.

Table 4. Fermentative characteristics of *Cenchrus americanus* silage as affected by row spacing and swine manure application.

Row spacing (m)	Manure application	VFA[1] (%)	Ammonia N (%)	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)	Lactic acid (%)	pH
0.5	Manure	10.8c[2]	3.0a	1.30bc	0.87c	0.13b	1.95c	5.17a
	No manure	12.0a	1.7c	1.44a	0.96a	0.14a	2.16a	4.15c
1.0	Manure	11.7ab	2.2b	1.25c	0.94ab	0.14a	2.11ab	4.70b
	No manure	11.3b	1.4d	1.36ab	0.90c	0.14a	2.03b	4.27c
s.e.m.		0.09	0.02	0.05	0.01	0.00	0.02	0.02
P-value		0.0001	<0.0001	0.0001	0.0001	0.0001	0.0001	<0.0001

[1]Volatile fatty acids. [2]Means in the same column followed by different letters are significantly (P<0.05) different.

Table 5. Chemical composition of *Cenchrus americanus* silage as affected by row spacing and swine manure application.

Row spacing (m)	Manure application	DM (%)	CP	Ash	NDF	ADF	ADL	Hemicellulose	Cellulose
			(g/kg DM)						
0.5	Manure	68.0a[1]	94.6a	137ab	537ab	320	73.3	167	247
	No manure	64.1a	79.9b	147ab	557a	320	66.7	237	253
1.0	Manure	59.1b	101.3a	127b	510ab	340	73.3	170	267
	No manure	55.1b	71.2b	167a	490b	300	66.7	190	233
s.e.m.		4.22	2.64	6.37	13.5	11.55	6.67	14.7	11.7

[1]Means in the same column followed by different letters are significantly ($P<0.05$) different.

Table 6. Mineral concentrations in *Cenchrus americanus* silage as affected by row spacing and swine manure application.

Row spacing (m)	Manure application	Calcium	Phosphorus	Potassium	Magnesium	Iron	Copper
		(g/kg DM)				(mg/kg DM)	
0.5	Manure	9.83a[1]	5.22a	20.3a	6.25a	74.3a	7.44a
	No manure	3.83d	3.65c	18.1d	4.58d	36.7d	2.34d
1.0	Manure	8.09b	3.77c	20.2b	5.45b	62.4b	6.69b
	No manure	7.37c	4.33b	19.7c	5.31c	41.9c	4.13c
s.e.m.		0.00	0.08	0.00	0.00	0.01	0.02

[1]Means in the same column followed by different letters are significantly ($P<0.05$) different.

Table 7. In vitro gas production (mL/h) of *Cenchrus americanus* silage as affected by row spacing and swine manure application.

Row spacing (m)	Manure application	Time (h)						
		3	6	9	12	24	36	48
0.5	Manure	1.3	3.3	6.7	8.7	20.7	30.0	31.3
	No manure	2.0	4.0	8.0	10.7	20.0	24.0	24.0
1.0	Manure	1.3	4.7	8.0	10.7	19.3	24.0	25.3
	No manure	1.0	4.7	8.7	9.3	22.0	28.7	30.7
s.e.m.		0.77	1.35	2.09	2.48	3.85	4.09	4.10

Discussion

This study revealed that planting *Cenchrus americanus* with narrow row spacing under the conditions existing in this study resulted in about 25–28% increase in DM yield compared with the wider row spacing. This higher DM yield recorded for plants on the narrow-spaced plots may be due to higher nutrient use efficiency (Jiang et al. 2013) as a result of more effective distribution of plants over the available surface, as opposed to the wider-spaced plots with a higher plant density within rows.

Interestingly DM yields for plants on both manured and control plots were similar. A lack of response to manure application may be due to the slow nutrient release rate associated with swine manure (Chastain et al. 1999; Silva et al. 2016), implying that perhaps much of the nutrients in the applied manure may not have been converted to plant usable forms, and the quantities released to the plant may have been insufficient to induce higher dry matter yields. Since the quantity of manure applied supplied only about 3.7 kg N/ha it is scarcely surprising that no growth response occurred.

In contrast, the higher CP% in fertilized forage was surprising given the low N rates in the manure but corroborates an earlier study by Jimoh et al. (2019) who reported a 20.1% increase in CP% when swine manure was applied to *Panicum maximum* cvv. Local and Ntchisi relative to the control in southwestern Nigeria. It is possible that P in the manure may have influenced the increased N uptake by the plants since N uptake has been reported to be influenced by higher P availability (Cleveland et al. 2011; Alkhader and Abu Rayyan 2015). The quality of grasses remains comparatively stable from vegetative to early stages of stem elongation (Jones and Wilson 1987) and declines as the plant matures (Ojo et al. 2016a, 2018; Ali et al. 2019). In this study, forages were harvested at 6 weeks of growth, when forage should have ample CP% to meet ruminant demand, allowing multiple harvests within the growing season. CP concentrations in the fresh forage were quite satisfactory for ruminant feeding at 8.6–12.4%. However, there was a marked reduction in CP% in the corresponding silages with a range of 7.1–10.1%, which agrees with the findings of Naeini et al. (2014), who reported a reduction in CP%

from 6.2% in fresh sweet sorghum to 5.4% in the silage. The critical limit required by rumen microbes to build their body protein for effective digestion of forages in ruminants is 7.0% (Van Soest 1994). The lower values for silage in our study are barely high enough to provide maintenance levels of protein for ruminants and values below this could result in reduction in DM intakes.

The color of the silages in this study fell within the range of 5–8 (green to yellowish-green) and 9–12 (yellow to brownish) which are regarded as good to acceptable ranges for silage (Bates 1998; Babayemi 2009). Further, the odor of the silage also fell within the range of 11–23 (acceptable) and 24–28 (desirable) reported by Bates (1998), although better scores were obtained from silage made from the narrowly spaced plots which could be due to the higher dry matter percentage that has been reported to influence the stability of ensiled forages (Wilkinson and Davies 2013).

The pH values recorded for the silages were within the range of 4.5–5.5 classified as indicative of good silage (Meneses et al. 2007). However, concentrations of desirable lactic acid in silage (1.95–2.16%) were below the range of 2.37–5.89% reported by Kung and Shaver (2001). High concentrations of lactic acid in silage are a clear indication of good preservation, which invariably results in lower loss of DM and energy during storage. Ammonia nitrogen is an important indicator of proteolytic activity during the fermentation process. Ammonia concentrations in the silage were very low and well below 12%, which is considered the indicator of an excellent and well preserved forage (Silveira 1975; Kung and Shaver 2001). Acetic acid concentrations were within the range (0.5–3.0% DM) classified as normal for grass silage (Kung and Shaver 2001) and also fell in line with the range of 0.74–1.53% DM for *Pennisetum* hybrid silage reported in another experiment (Ojo et al. 2016b).

The NDF values of *C. americanus* silage recorded in this study were well below the 65% threshold suggested as the level at which intake and degradability of tropical feeds by ruminants would be limited (Eastridge 2006). This was not surprising given the growth stage at which the forage was harvested. Higher NDF concentration in silage made from the forage planted at 0.5 m intervals could be a function of competition for light owing to the close spacing, resulting in continuous elongation in the height of the plants, thereby leading to increased fiber concentration.

There were general improvements in the Ca, K, Mg, Fe and Cu concentrations of silage made from the manured plants. This was expected as the manure may have made more nutrients available to the plants and corroborates the report by Christophe et al. (2019) that organic amendments tend to improve plant mineral composition. In addition, mean concentration values for major limiting nutrients (Ca, P, Mg and Cu) were higher than the minimal concentration levels of 2.3–2.9, 2.0–3.5, 0.4–0.5 g/kg and 4 mg/kg, respectively, suggested for dairy cows (NRC 1984). This implies that there would be no need for supplements if the silage were fed to animals. Usually, deficiencies and imbalances in nutrients are neutralized by mineral supplements which may be costly for poor resource farmers (Tiemann et al. 2009); however, the mineral concentration values recorded for the silages in this study, especially those from the manured treatments, were at favorable levels.

The observed similarities in the in vitro gas volumes implies that despite the differences in the NDF concentrations of the ensiled forages, this may not be sufficient to hinder the degradability of the forages if fed to ruminants. Anassori et al. (2012) had asserted that gas volume readings could be used to estimate the degradability of forages in vitro. This finding further supports the initial proposition that the higher NDF values recorded for the narrow spaced plants falls within the threshold of what can be efficiently degraded by rumen microbes.

Conclusion

While application of swine manure to pearl millet at 5 t/ha increased CP% of forage and N recovery rate when harvested at 42 days, it failed to increase forage yields. However, decreasing row spacing from 1.0 to 0.5 m increased DM yield by 25–28%, indicating significant gains to be made by planting pearl millet at a closer row spacing at the sowing rates used in that environment. Plant populations, soil fertility and rainfall levels could have significant impacts on these findings. While silage made from the forage was of a good standard, CP% in the forage had declined by 16–18%. It seems that applying swine manure at 1.1 t DM/ha to pearl millet planted at 0.5 m spacing and cutting at 6 weeks after planting could produce high yields of forage. Ensiling this material would give a product of sufficient quality to be fed as supplements or a complete diet to ruminants especially during the dry season. Better responses in yield might be expected from other manure sources with higher N content. Cutting at more mature stages of growth would be expected to increase DM yields but quality of forage should decline, especially N concentration, and ability to compress the forage during ensiling would be expected to decline as well. Longer-term studies to determine if yield advantages from the narrower row spacing could be maintained as the stand matured as well as the quality outcomes of repeated cutting at close intervals with those from less frequent cutting seem warranted.

References

(Note of the editors: All hyperlinks were verified 4 May 2020.)

Agishi EG. 1985. Forage resources of Nigerian rangelands. In: Adu IE; Taiwo BBA; Osinowo OA; Alhassan WS, eds. Small Ruminant Production in Nigeria. Proceedings of the National Conference on Small Ruminant Production, Zaria, Nigeria, 6–10 October 1985. p. 115–140.

Amodu JT; Adamu AM; Adeyinka IA; Alawa JP; Jegede JO. 2005. The nutritive value of laboratory ensiled lablab (*Lablab purpureus*) and pearl millet (*Pennisetum americanum*). Tropical Grasslands 39:117–123. bit.ly/34CzQKz

Ali W; Nadeem M; Ashiq W; Zaeem M; Thomas R; Kavanagh V; Cheema M. 2019. Forage yield and quality indices of silage-corn following oganic and inorganic phosphorus amendments in podzol soil under Boreal climate. Agronomy 9:489. doi: 10.3390/agronomy9090489

Alkhader AM; Abu Rayyan AM. 2015. The impact of phosphorus fertilizers on nutrients content and uptake in lettuce and on selected chemical properties of a calcareous soil. Jordan Journal of Agricultural Sciences 11:1021–1035. bit.ly/3fmuLLm

Anassori E; Dalir-Naghadeh B; Pirmohammadi R; Taghizadeh A; Asri-Rezaei S; Farahmand-Azar S; Besharati M; Tahmoozi M. 2012. *In vitro* assessment of the digestibility of forage based sheep diet, supplemented with raw garlic, garlic oil and monensin. Veterinary Research Forum 3: 5–11. bit.ly/3c437AJ

AOAC. 1990. Official methods of analysis. 15th Edn. Association of Official Analytical Chemists, Arlington, VA, USA.

AOAC. 2000. Official methods of analysis. 16th Edn. AOAC International, Arlington, VA, USA.

Babayemi OJ. 2009. Silage quality, dry matter intake and digestibility by West African dwarf sheep of Guinea grass (*Panicum maximum* cv. Ntchisi) harvested at 4 and 12 week regrowths. African Journal of Biotechnology 8:3983–3988. bit.ly/2W6hUFM

Bamikole MA; Akinsoyinu AO; Ezenwa I; Babayemi OJ; Akinlade J; Adewumi MK. 2004. Effect of six-weekly harvests on the yield, chemical composition and dry matter degradability of *Panicum maximum* and *Stylosanthes hamata* in Nigeria. Grass and Forage Science 59:357–363. doi: 10.1111/j.1365-2494.2004.00437.x

Bates G. 1998. Corn silage. SP 434-D. The University of Tennessee Agricultural Extension Service, Knoxville, TN, USA. bit.ly/3fmomA0

Chastain JP; Camberato JJ; Albrecht JE; Adams J. 1999. Swine manure production and nutrient content. CAMM Swine Training Manual. Clemson University, Clemson, SC, USA. bit.ly/34xHQwt

Christophe HL; Albert N; Martin Y; Mbaiguinam M. 2019. Effect of organic fertilizers rate on plant survival and mineral properties of *Moringa oleifera* under greenhouse conditions. International Journal of Recycling of Organic Waste in Agriculture 8:123–130. doi: 10.1007/s40093-019-0282-6

Cleveland CC; Townsend AR; Taylor P; Alvarez-Clare S; Bustamante MMC; Chuyong G; Dobrowski SZ; Grierson P; Harms KE; Houlton BZ; Marklein A; Parton W; Porder S; Reed SC; Sierra CA; Silver WL; Tanner EVJ; Wieder WR. 2011. Relationships among net primary productivity, nutrients and climate in tropical rain forest: A pan-tropical analysis. Ecology Letters 14:939–947. doi: 10.1111/j.1461-0248.2011.01658.x

Eastridge ML. 2006. Major advances in applied dairy cattle nutrition. Journal of Dairy Science 89:1311–1323. doi: 10.3168/jds.S0022-0302(06)72199-3

Jiang W; Wang K; Wu Q; Dong S; Liu P; Zhang J. 2013. Effects of narrow plant spacing on root distribution and physiological nitrogen use efficiency in summer maize. The Crop Journal 1:77–83. doi: 10.1016/j.cj.2013.07.011

Jimoh SO; Amisu AA; Dele PA; Ojo VOA; Aderemi TA; Olanite JA. 2019. Effects of animal manures and cutting height on the chemical composition of two *Panicum maximum* varieties (Local and Ntchisi) harvested at different stages of growth. Pertanika Journal of Tropical Agricultural Science 42:359–376. bit.ly/3a5p14Q

Jones DIH; Wilson A. 1987. Nutritive quality of forages. In: Hacker JB; Ternouth JH, eds. The nutrition of herbivores. Academic Press, Sydney, Australia. p. 65–90.

Kung L; Shaver R. 2001. Interpretation and use of silage fermentation analysis reports. Focus on Forage, Vol. 3, No. 13. Extension Team Forage, University of Wisconsin, Madison, WI, USA. bit.ly/2XzfzUW

Malhi SS; Harapiak JT; Gill KS; Flore N. 2002. Long term N rates and subsequent lime application effects on macroelements concentration in soil and bromegrass hay. Journal of Sustainable Agriculture 21:79–97. doi: 10.1300/J064v21n01_07

Mathew S; Sagathewan S; Thomas J; Mathen G. 1997. An HPLC method for estimation of volatile fatty acids of ruminal fluid. Indian Journal of Animal Sciences 67:805–807. bit.ly/2Rz6APK

Meneses MD; Megías J; Madrid A; Martínez-Teruel F; Hernández J; Oliva J. 2007. Evaluation of the phytosanitary, fermentative and nutritive characteristics of the silage made from crude artichoke (*Cynara scolymus* L.) by-product feeding for ruminants. Small Ruminant Research 70:292–296. doi: 10.1016/j.smallrumres.2006.05.008

Menke KH; Steingass H. 1988. Estimation of the energetic feed value from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research and Development 28:7–55.

Miah MNH; Karim MA; Rahman MS; Islam MS. 1990. Performance of Nizersail mutants under different row spacings. Bangladesh Journal of Training Development 3(2):31–34.

Naeini SZ; Khodambashi N; Rowghani E; Bayat A. 2014. Influence of ensiling time on chemical compositon, fermentation characteristics, gas production, and protein fractions of sweet sorghum silage. Research Opinions in Animal and Veterinary Sciences 4:286–293. bit.ly/3dloeyV

NRC (National Research Council). 1984. Nutrient requirements of beef cattle. National Academy Press, Washington, DC, USA.

Nuru S. 1989. Research and development in pastoral production system in Nigeria: Past, present and future. In: Gefu JO, ed. Pastoralism in Nigeria: Past, present and future. Proceedings of the National Conference on Pastoralism in Nigeria, NAPRI, Zaria, Nigeria, 26–29 June 1988.

Ojo VOA; Dele PA; Olanite JA; Adeoye SA; Ajayi SO; Idowu OMO. 2013. Influence of manure type on the productivity of *Pennisetum purpureum* under different planting method and age. Journal of Organic Agriculture and Environment 1:71–77.

Ojo VOA; Badaru SO; Ogunsakin AO; Onifade OS; Arigbede OM; Dele PA; Odunaye BT; Nukasi IG; Ogundele SO; Amodu JT. 2015a. Effects of animal manure on the growth and dry matter yield of *Pennisetum purpureum* varieties. In: Moorby JM; Jewkes EC, eds. Grassland and forage management. British Grassland Society 12th Research Conference Proceedings, Aberystwyth, Ceredigion, Wales, UK, 7– 9 September 2015. p. 103–104.

Ojo VOA; Ogunsakin AO; Dele PA; Olanite JA; Adeoye SA; Amole TA; Onifade OS. 2015b. Yield and chemical composition of *Pennisetum* hybrid fertilized with animal manures and harvested at different intervals. Malaysian Journal of Animal Science 18(2):65–80. bit.ly/3fn8aya

Ojo VOA; Popoola KO; Omisore KO; Adelusi OO; Yusuf KO; Ogunsakin AO; Amole TA; Adeyemi TA; Jolaosho AO. 2016a. Fermentative characteristics and *in vitro* gas production of *Pennisetum purpureum* hybrid grass silage as influenced by manure type and age at harvest. Nigerian Journal of Animal Science 18(1):230–241. bit.ly/2YBaKdZ

Ojo VOA; Oyebanjo ED; Opayemi OT; Mustapha SO; Adelusi OO; Adeoye SA; Anele UY; Ogunsakin AO; Jolaosho AO; Ofinade OS. 2016b. Effects of seasonal changes on the nutritive quality of *Moringa oleifera* and *Panicum maximum* silage. Slovak Journal of Animal Science 49:76–84. sjas.ojs.sk/sjas/article/view/164

Ojo VOA; Adelusi OO; Jimoh SO; Yusuf KO; Dele PA; Akinyemi BT. 2018. Effects of proportion and ensiling duration on nutrient digestibility and fermentation characteristics of *Pennisetum purpureum* and *Enterolobium cyclocarpum* seeds silage using in vitro gas production technique. Bulletin of Animal Health and Production in Africa 66:163–175. bit.ly/3b4XaSW

Olanite JA; Anele UY; Arigbede OM; Jolaosho AO; Onifade OS. 2010. Effect of plant spacing and nitrogen fertilizer levels on the growth, dry-matter yield and nutritive quality of Columbus grass (*Sorghum almum* Stapf) in southwest Nigeria. Grass and Forage Science 65:369–375. doi: 10.1111/j.1365-2494.2010.00755.x

SAS. 2003. SAS User's Guide. Version 9.1. SAS Institute Inc., Cary, NC, USA.

Silva AN da; Basso CJ; Muraro DS; Ortigara C; Pansera E. 2016. Pig slurry composting as a nitrogen source in proso millet crop. Pesquisa Agropecuária Tropical 46:80–88. doi: 10.1590/1983-40632016v4638457

Silveira AC. 1975. Técnica para produção de silage. In: Anais, 2º Simpósio sobre manejo de pastagem. ESALQ, Piracicaba, Brazil. p. 156–180.

Tiemann TT; Franco LH; Peters M; Frossard E; Kreuzer M; Lascano CE; Hess HD. 2009. Effect of season, soil type and fertilizer on the biomass production and chemical composition of five tropical shrub legumes with forage potential. Grass and Forage Science 64:255–265. doi: 10.1111/j.1365-2494.2009.00691.x

Van Soest PJ. 1994. Nutritional ecology of the ruminant. Cornell University Press, Ithaca, NY, USA.

Van Soest PJ; Robertson JB; Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583–3597. doi: 10.3168/jds.S0022-0302(91)78551-2

Wilkinson JM; Davies DR. 2013. The aerobic stability of silage: Key findings and recent developments. Grass and Forage Science 68:1–19. doi: 10.1111/j.1365-2494.2012.00891.x

Wilson RF; Wilkins RJ. 1972. An evaluation of laboratory ensiling techniques. Journal of Science of Food and Agriculture 23:377–385. doi: 10.1002/jsfa.2740230315

Appendix 1. Silage physical and sensory evaluation sheet.

Factor	Description	Score range
Color	Desirable: Green to yellowish-green	9–12
	Acceptable: Yellow to brownish	5–8
	Undesirable: Deep brown or black indicating excessive heating or putrefaction	0–4
Odor	Desirable: Light, pleasant odor with no indication of putrefaction	24–28
	Acceptable: Fruity, yeasty, musty which indicate a slightly improper fermentation; slight burnt odor, sharp vinegar odor	11–23
	Undesirable: strong burnt odor indicating excessive heating; putrid, indicating improper fermentation	0–10
Moistness	No free water when squeezed in hand; well preserved	9–10
	Some moisture can be squeezed from silage or silage dry or musty	5–8
	Silage wet, slimy or soggy, water easily squeezed from sample; silage too dry with a strong burnt odor	0–4
Moldiness	No mold	9–10
	Slightly moldy	5–8
	Highly moldy	0–4

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Research Paper

Effect of seed storage on seed germination and seedling quality of *Festulolium* in comparison with related forage grasses

Efecto del almacenamiento de la semilla de Festulolium *y especies relacionadas en su germinación y la calidad de plántulas*

RADE STANISAVLJEVIĆ[1], DOBRIVOJ POŠTIĆ[1], RATIBOR ŠTRBANOVIĆ[1], MARIJENKA TABAKOVIĆ[2], SNEŽANA JOVANOVIĆ[2], JASMINA MILENKOVIĆ[3], DRAGOSLAV ĐOKIĆ[3] AND DRAGAN TERZIĆ[3]

[1]*Institute for Plant Protection and Environment (IZBIS), Belgrade, Serbia.* izbis.com
[2]*Maize Research Institute, Belgrade, Serbia.* mrizp.rs
[3]*Institute of Forage Crops (IKBKS), Krusevac, Serbia.* ikbks.com

Abstract

Tests of seed germination, seed dormancy and seedling growth were performed on 0-, 6-, 20- and 30-months-old seed lots of *Festulolium* in comparison with Italian ryegrass (*Lolium multiflorum*) and meadow fescue (*Festuca pratensis*). Tests were performed on seeds harvested in 2 different years (2014 and 2015) resulting in no major difference between the years. Seed storage affected seed viability and dormancy and seedling growth in all 3 grasses. The maximum germination of *Festulolium* seeds was achieved 6 months after harvest (95% normal seedlings); germination decreased significantly thereafter. While maximum germination of *L. multiflorum* and *F. pratensis* seeds was also achieved following storage for 6 months, these germination rates (93 and 90%, respectively) were retained until at least 20 months in storage. After storage for 30 months, seed germination of *Festulolium*, *L. multiflorum* and *F. pratensis* had declined to 72, 79 and 83%, respectively. High germination in all species was associated with higher rates of seedling growth. In an artificial seed ageing test, a temperature of 41 °C (during 48 and 72 hours) was found to effectively rank seed lots for germination performance in all 3 grasses. This test seems to have application for use in the seed trade to identify seed lots which could deteriorate more rapidly in storage. Further studies are needed to verify this hypothesis.

Keywords: Ageing of seed, ageing test, dormancy, embryonic stem and radicle, forage grasses.

Resumen

Se realizaron pruebas de germinación y de crecimiento de plántulas provenientes de lotes de semillas de *Festulolium* almacenadas durante 0, 6, 20 y 30 meses, en comparación con raigrás italiano (*Lolium multiflorum*) y festuca de pradera (*Festuca pratensis*). En lotes de semillas cosechadas en 2014 y 2015 no se encontraron diferencias entre los años. El almacenamiento afectó la viabilidad y la latencia de las semillas y el crecimiento de las plántulas en las tres especies. La germinación máxima de las semillas de *Festulolium* se presentó 6 meses después de la cosecha (95% de plántulas normales), a partir de los cuales disminuyó significativamente. También las semillas de *L. multiflorum* y *F. pratensis* presentaron máxima germinación después de 6 meses (93 y 90%, respectivamente); estas tasas, sin embargo, se mantuvieron hasta al menos 20 meses de almacenamiento. Después de 30 meses, la germinación de las semillas disminuyó a 72, 79 y 83% para *Festulolium*, raigrás y festuca, respectivamente. La alta germinación en todas las especies se asoció con mayores tasas de crecimiento de plántulas. En una prueba rápida de envejecimiento artificial de semillas (temperatura de 41 °C durante 48 y 72 horas) fue posible predecir el comportamiento de germinación de las semillas de las tres especies. Esta prueba parece tener aplicación en el comercio para identificar lotes de semillas que podrían deteriorarse más rápidamente durante el almacenamiento. Se necesitan más estudios para verificar esta hipótesis.

Palabras clave: Dormancia, envejecimiento de semilla, gramíneas forrajeras, prueba de envejecimiento, radícula y tallo embrionarios.

Correspondence: Rade Stanisavljević, Institute for Plant Protection and Environment, Teodora Drajzera 9, 11000 Belgrade, Serbia.
E-mail: stanisavljevicrade@gmail.com

Introduction

Lolium multiflorum, *L. perenne*, *Festuca pratensis* and *F. arundinacea* are widely cultivated forage grasses and have been studied from the aspects of genetics and breeding, seed production, growing practices and utilization. Work on interspecies hybridization between *Lolium* and *Festuca* was conducted in the 1970s, and a novel hybrid named *Festulolium* was recorded in Europe after 2010 (Østrem et al. 2013). According to Humphreys et al. (2013; 2014), the hybrid grass *Festulolium* was a result of crosses of *F. pratensis* or *F. arundinacea* with *L. perenne* or *L. multiflorum.* As stated by Akgun et al. (2008), *Festulolium* is characterized by higher yields of green fodder and dry matter than those of *L. multiflorum* and *F. pratensis*, improved resistance to environmental stresses (Abdelhalim et al. 2016) and improved fodder quality (Skládanka et al. 2010). In addition, it displays a different pattern of plant growth in the autumn-early winter period; e.g. × *Festulolium braunii* expands its root apical meristem (RAM) and continues to grow, while cellular growth in the RAM of *F. pratensis* declines and reduces vegetative growth (Pašakinskienė and Švėgždienė 2018). Furthermore, seed yields recorded for *Festulolium* have been superior to those of *L. multiflorum* and *F. pratensis* (Akgun et al. 2008). Optimum growing systems have been developed for the cultivation of *Festulolium* for seed (Deleuran et al. 2010).

While *Festulolium, Festuca arundinacea* and *L. perenne* are considered temperate grasses, in a subtropical climate (central Mexico) analyses of forage quality and milk production in feeding studies with dairy cows showed these species compared favorably with kikuyu grass (*Cenchrus clandestinus* syn. *Pennisetum clandestinum*) (Plata-Reyes et al. 2018). Likewise Mwendia et al. (2019) concluded that perennial ryegrass and festulolium have the potential to contribute to improving the forage resource base in the highlands of Central Kenya and similar areas.

Fodder manufacturers and seed companies have enquired about the relationship between seed quality and establishment of *Festulolium* in combination with legumes or as a monocrop. Moreover, seed companies and seed trade companies are concerned about maintenance of seed quality during storage. Literature searches revealed no studies on changes in *Festulolium* seed quality during the storage period (seed ageing).

In the absence of previous research, studies reported in this paper were conducted to observe changes in seed germination, dormancy and seedling vigor in *Festulolium*, *L. multiflorum* and *F. pratensis* over 30 months of storage.

Materials and Methods

Seeds were sampled from seed crops grown near the city of Smederevo, Serbia (44°40'–44°66' N, 20°56'–20°93' E; 66–98 masl). Three seed production lots (I, II and III) were used for each species tested. All seed lots were harvested with a small combine in June 2014 and in June 2015. After harvest, the cleaned seeds were dried to moisture content below 14% and stored under ambient conditions for 30 months (Table 1). Seeds of the following species were collected: 'Perun' *Festulolium* (*Festulolium*), 'Kruševački 21' meadow fescue (*Festuca pratensis*) and 'Kruševački 13' Italian ryegrass (*Lolium multiflorum*). Storage conditions during the investigation period are shown in Table 1.

Kruševački 21 was produced by breeding genotypes originating from indigenous populations of eastern Serbia and Resava. Plant height is about 105 cm and tillering is strong, while resistance to rust disease (*Puccinia* sp.) is enhanced. The semi-erect shoots are characterized by a clear pattern of light green leaves and a dark green fine stem. The seed is medium-sized and uniform, with 1,000-seed weight of approximately 1.96 g. Under conditions of continuous cropping, it produces 9–10 t DM/ha with 160 g CP/kg at a sowing rate of 20–25 kg/ha. It is recommended for all types of long-lasting grass and clover-grass mowing mixtures.

Table 1. Average monthly temperatures (°C) and relative humidity (%) during storage of up to 30 months of *Festulolium*, *Festuca pratensis* and *Lolium multiflorum* seeds harvested in June 2014 (period of seed storage: June 2014–November 2016) and June 2015 (period of seed storage: June 2015–November 2017).

Year of storage	Variable	Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec
2014	T						20.6	22.4	22.2	12.4	11.0	6.0	3.0
	RH						64.0	64.3	68.5	74.5	76.4	77.7	79.5
2015	T	2.1	6.2	9.2	11.3	13.8	20.5	23.1	24.1	13.0	11.2	6.2	3.2
	RH	81.0	79.2	78.5	66.1	65.6	63.2	64.8	68.6	74.7	76.7	77.4	79.6
2016	T	2.3	6.3	9.3	11.4	13.7	20.5	22.7	23.6	12.7	11.1	6.0	3.3
	RH	81.8	79.4	78.6	66.3	65.9	63.6	64.7	68.5	74.6	76.6	77.5	79.6
2017	T	2.3	6.3	9.3	11.4	13.8	20.4	22.5	23.1	12.5	11.2	6.2	
	RH	81.5	79.3	78.5	66.3	65.8	63.7	64.5	68.3	74.4	76.8	77.3	

T - Temperature; RH - Relative humidity.

Kruševački 13 was developed by breeding and selection of genotypes of introduced populations. The height of regenerative shoots in the first cutting reaches 1 m, while vegetative shoots are about 80 cm tall. The shoots are covered with many broad, distinctly glossy leaves of clear green color. The plants tiller strongly and produce medium tender, greyish green stems. The spikes are medium in length with 6–8 spikelets, while seeds are easily shed and have a 1,000-seed weight of 1.99 g. The crop is high-yielding and under favorable conditions can produce in excess of 14–15 t hay/ha with 16% CP. It is an excellent variety for sowing in mixtures with red clover for 3-year leys, is very productive under irrigation and is also suitable for early spring and late autumn utilization.

Seed testing was performed with 2 experiments: Experiment 1: testing of germination and seedling growth (embryonic stem, radicle, seedling weight); and Experiment 2: seed ageing test. Both experiments were performed on each seed lot (I, II and III) according to standard methodology. Given that there were no significant differences in germination and seedling growth between seed lots, results for individual seed lots are not shown. However, in the ageing test, differences in germination between seed lots were significant. This is significant in practice when deciding which seed lot can stay longer in a warehouse, and which needs to be packaged for market earlier.

Experiment 1: Seed germination and dormancy; seedling growth

Germination tests were performed after 0, 6, 18 and 30 months of storage. After being chilled at 5 °C for 5 days, seeds were sown in boxes filled with sand (20 × 14 × 4 cm). The boxes were then placed into germination cabinets (temperature 20/30 °C; 8 h light at 1,520 lux and 16 h dark; 50 seeds for each of 3 replications). Multiple incubators were employed and species were uniformly distributed. Seed germination and dormancy were determined on day 14 after sowing. Seed was considered to have germinated when a radicle and embryonic stem up to 1 cm had developed. The tetrazolium test was applied to distinguish dormant seeds from dead seeds (ISTA 2019) after 14 days. In addition, primary root length, shoot length and fresh seedling biomass (root + shoot) were measured after the final count. Seedling length was measured using a ruler (Stanisavljević et al. 2011).

Experiment 2: Ageing test

A seed ageing test was applied to a subsample of 6-months-old seeds for each species and each seed lot using 4 replications of 100 seeds that were evenly arranged on the top of a box screen (11 × 11 × 3.5 cm) containing 40 mL of distilled water, which provided a relative humidity of 98–100%. Grid boxes were used for this test to keep the seeds moist but not submerged in water. The boxes were placed in a water bath at 41 °C for 48 and 72 h. Seed moisture contents before the test and after 48 and 72 h were determined with values of 12.0–12.7% before placing in the water bath and 36.6–37.5% after being in the water bath, which is in accordance with ISTA recommendations (Hampton and TeKrony 1995). This test was performed according to the method applied for the evaluation of seed lots of *L. multiflorum* (Tunes et al. 2011) and *Festuca pratensis* (Stanisavljević et al. 2013). We were unable to locate any data in the available literature regarding the application of the ageing test to *Festulolium* seeds – data are lacking on appropriate temperature and duration of seed subjection and seed moisture prior to testing. Following exposure to this temperature (41 °C) and duration (48 and 72 h), a germination test was performed as described above.

Statistical analysis

The experimental data were analyzed using 3-way factorial analysis of variance (ANOVA). Tukey's multiple range F test and coefficient of variation were used to test for the effects of the treatments. Standard error of the mean was calculated to indicate variation around the mean. Data for germination and dormancy percentages were arcsine transformed [sqr(x/100)] before being subjected to analysis of variance. The program Minitab, version 16.1.0 (Minitab Inc., State College, PA, USA) was used to process data (free version).

Results

Since there were no significant differences for seed germination and seedling growth between the seed lots tested or for the harvest years 2014 and 2015, data for both years were combined and means for each species and treatment are presented (Tables 2 and 3). Seed age (length of time in storage) was the only parameter which affected germination percentages of seed of the 3 species and development of seedlings. Seed age significantly affected germination and initial growth of seedlings for *Festulolium* ($P \leq 0.001$), plus *L. multiflorum* and *F. pratensis* ($P \leq 0.01$ or $P \leq 0.05$) (Table 4).

Experiment 1 (seed germination and dormancy; seedling growth)

Seed germination and dormancy. Immediately after seed drying (0 months), germination of *Festulolium* seeds was higher by 7 and 4% than that of *F. pratensis* and

L. multiflorum seeds, respectively (Table 2). During the first 6 months of storage, germination of seeds of all species increased, reaching peak levels of 95, 93 and 90% for *Festulolium*, *L. multiflorum* and *F. pratensis*, respectively, while numbers of dormant seeds declined. Subsequently, germination of *Festulolium* declined, while those of *L. multiflorum* and *F. pratensis* remained constant until 20 months of storage. By 30 months of storage germination percentages had declined to 72, 79 and 83% for *Festulolium*, *L. multiflorum* and *F. pratensis*, respectively (P<0.05) and there were no dormant seeds.

Seedling growth. There were differences between species in growth of embryonic stems and radicles, which were reflected in seedling weights (Table 3). These followed similar patterns with *Festulolium* > *L. multiflorum* > *F. pratensis* for seeds up to 20 months of age and species differences generally disappeared for 30-months-old seeds. For all parameters peak performance was reached after 6 months storage and this level was maintained at 20 months. After 30 months storage, stem and radicle growth and seedling weight of *Festulolium* and *L. multiflorum* had declined (P<0.05) but not for *F. pratensis* (P>0.05) (Table 3).

Table 2. Effect of storage time (seed age) on germination and dormancy (± s.e.m.) of seeds of *Festulolium, Lolium multiflorum* and *Festuca pratensis*.

Species	Seed age 0 months	Seed age 6 months	Seed age 20 months	Seed age 30 months
Seed germination (%)				
Festulolium	88 ± 0.69aB	95 ± 0.91aA	89 ± 0.71bB	72 ± 0.66cC
Lolium multiflorum	84 ± 0.75bB	93 ± 0.88abA	90 ± 0.70abA	79 ± 0.70bC
Festuca pratensis	81 ± 0.79cC	90 ± 0.77bA	92 ± 0.59aA	83 ± 0.58aB
Mean	84	93	90	78
CV%	4.16	2.72	1.69	7.14
Dormant seeds (%)				
Festulolium	10 ± 0.33cA	3 ± 0.21cB	0 ± 0.00cC	0 ± 0.00aC
L. multiflorum	13 ± 0.41bA	6 ± 0.35bB	5 ± 0.71bB	0 ± 0.00aC
F. pratensis	17 ± 0.42aA	9 ± 0.33aB	7 ± 0.71aB	0 ± 0.00aC
Mean	13	6	4	0
CV%	26.3	44.4	90.1	–

Within parameters, means within rows with different upper-case letters and means within columns with different lower-case letters are significantly different at P<0.05 by Tukey's test.

Table 3. Effect of storage time (seed age) on growth of embryonic stems (cm), radicles (cm) and seedling weight (mg) of seeds of *Festulolium, Lolium multiflorum* and *Festuca pratensis*.

Trait	Seed age 0 months	Seed age 6 months	Seed age 20 months	Seed age 30 months
Embryonic stem				
Festulolium	6.26 ± 0.43aB	7.79 ± 0.54aA	7.68 ± 0.21aA	5.07 ± 0.36aC
L. multiflorum	4.49 ± 0.39bB	5.56 ± 0.39bA	5.51 ± 0.34abA	4.35 ± 0.29aB
F. pratensis	3.45 ± 0.33cB	4.45 ± 0.30cA	4.99 ± 0.53bA	4.29 ± 0.39aA
Mean	4.73	5.93	6.06	4.57
CV%	30.0	28.7	23.5	9.5
Radicle				
Festulolium	4.05 ± 0.29aB	4.98 ± 0.53aA	4.88 ± 0.36aA	3.75 ± 0.34aB
L. multiflorum	3.42 ± 0.34abB	4.12 ± 0.29bA	4.10 ± 0.71bA	3.46 ± 0.51aB
F. pratensis	2.85 ± 0.41bB	3.22 ± 0.61cA	3.39 ± 0.63cA	3.21 ± 0.63aA
Mean	3.44	4.11	4.12	3.47
CV%	17.4	21.4	18.1	7.8
Seedling weight				
Festulolium	14.9 ± 0.53aB	17.5 ± 0.61aA	17.2 ± 0.29aA	13.9 ± 0.53aC
L. multiflorum	12.1 ± 0.61bB	14.6 ± 0.39bA	14.0 ± 0.37bA	12.4 ± 0.61bB
F. pratensis	10.9 ± 0.48cB	12.3 ± 0.35cA	12.1 ± 0.31cA	11.8 ± 0.35bA
Mean	12.6	14.8	14.4	12.7
CV%	16.2	17.6	17.9	8.5

Within parameters, means within rows with different upper-case letters and means within columns with different lower-case letters are significantly different at P<0.05 by Tukey's test.

Experiment 2 (ageing test)

Application of the seed ageing test to the 6-months-old seeds showed differences in seed germination (%) between seed lots for all 3 species tested. Whereas the classical germination test did not show a significant difference (F test) between the seed lots of any of the tested species, the aging test was able to detect differences between seed lots in germination of all tested species (Table 4). The test also showed a faster decline in germination percentage (average for all 3 seed lots) of *Festulolium* seed (from 55% after 48 h to 17% after 72 h) than of *L. multiflorum* seed (from 76 to 53%) and *F. pratensis* seed (from 83 to 73%) (Table 4).

Table 4. Germination percentages (± s.e.m.) of 6-months-old seed lots of *Festulolium*, *Lolium multiflorum* and *Festuca pratensis* after an ageing test by placing in a water bath at 41 °C for 48 and 72 hours.

Species	Seed lot	Test duration 48 h	72 h
Festulolium	I	52 ± 0.45b	18 ± 0.27b
	II	68 ± 0.39a	21 ± 0.29a
	III	45 ± 0.37c	13 ± 0.51c
	Mean	55	17
L. multiflorum	I	77 ± 0.29b	53 ± 0.32b
	II	81 ± 0.63a	59 ± 0.39a
	III	71 ± 0.43c	48 ± 0.77c
	Mean	76	53
F. pratensis	I	86 ± 0.37a	79 ± 0.28a
	II	79 ± 0.44 b	66 ± 0.36c
	III	83 ± 0.51ab	74 ± 0.41b
	Mean	83	73

Values within columns and species with different letters are significantly different (P<0.05) by Tukey's multiple range test.

Discussion

Experiment 1

Observed seed lots were from regions geographically close and harvested in a similar way, which may be a reason for their uniformity in terms of tested traits of seed and seedling quality.

While germination rates of seed of all species was quite acceptable following harvest, the declining germination after storage is potentially an issue for the seed trade, because the minimum legislated germinations of *F. pratensis* and *L. multiflorum* seeds at sale are 75 and 70%, respectively. Following 30 months of storage, germination rates for *Festulolium* and *L. multiflorum* seeds were 72 and 79%, respectively, so seed ageing under ambient conditions was an important issue. Seed dormancy immediately after harvest or dispersal under natural conditions is a biological trait that prevents germination of some seeds as a survival mechanism in the event that seedlings cannot cope with environmental conditions following germination (Bewley 1997). This trait is influenced by multiple factors including genetics, physiology, biochemistry and histology (Graeber et al. 2012; Long et al. 2015; Sah et al. 2016).

Dormant seeds of plants can reduce germination percentage at any one time (Stanisavljević et al. 2014). For farming systems, where synchronized germination is desired, farmers sometimes compensate for dormancy by increasing sowing rates (an additional cost) to achieve optimum populations for high yields. An alternative approach is to treat seed prior to sowing, e.g. by scarification or heat treatment, to break dormancy and ensure more uniform germination. This is particularly important for species with high percentages of dormant seeds at harvest, e.g. legume seeds.

While seedlings developed from dormant seeds would normally be healthy, the delay in their emergence and development can reduce their capacity to compete with already developed seedlings (seedlings developed from immediately germinated seeds) (Bass et al. 1988). According to Adkins et al. (2002), seed dormancy is common in bred forage grasses, especially soon after harvest, unlike many cereal crops, where breeding has been used to reduce seed dormancy. When studying dormancy in *F. pratensis*, Stanisavljević et al. (2012) found low variability (CV = 3.6%) among 3 varieties and 3 populations immediately after harvest but further studies showed that, depending on seed moisture content at harvest, seed dormancy varied from 33 to 18% (Stanisavljević et al. 2013). Dormancy percentages in our current study following harvest ranged from 10% (*Festulolium*) to 17% (*F. pratensis*).

After the maturation period, the dormant seeds become germinable, total germination increases and at that stage the utilizable value of seeds is at its peak, i.e. the desired number of plants can be obtained from sowing lower amounts of seeds – the most economic establishment of crops. According to Stanisavljević et al. (2011), a storage period of 500 days under uncontrolled temperature and humidity conditions is necessary for *L. multiflorum* seeds to mature and achieve maximum germination. The current study suggested that storage for 180 days was sufficient to reach maximum germination percentage. Being able to market and plant seed at 180 days following harvest rather than 500 days after harvest is much more cost-efficient. While germination of *Festulolium* declined from this time, *L. multiflorum* (92–90%) and *F. pratensis* (90–92%)

remained at peak levels until 20 months of storage (Table 2), showing a small advantage for these species.

A significant advantage of sowing non-dormant seeds is faster and more uniform initial growth of the established crop. In our studies, an additional outcome with high germination was stronger growth of the embryonic stem and radicle, as well as increased weight of seedlings (Table 3). These factors are very important because mixtures of forage grasses are commonly grown (Wyszkowska et al. 2019), sometimes with forage legumes as a mixed crop (Neuberg et al. 2011).

As demonstrated in this study, the stage of maximum germination is followed by ageing of the seed, which involves irreversible changes to seeds, ultimately resulting in decreased capacity to germinate and grow as a seedling. In these studies, the most pronounced ageing was observed in *Festulolium* (germination was 72% after 30 months). Long-term storage of this species is not recommended.

Seed ageing is specific for each plant species (Nagel and Borner 2010). The consequence of seed ageing is not just a germination reduction but also a reduction in the initial growth of the radicle, embryonic stem and seedling weight (Rajjou et al. 2008; Stanisavljevic et al. 2011). A seed ageing test measures the ability of a seed lot to resist degradation changes and protection mechanisms that are present in the seed (Balešević-Tubić and Tatić 2012). According to Hare et al. (2018) germination percentage under laboratory conditions declined to below 50% after 3 years of storage of seed for 2 guinea grasses (*Megathyrsus maximus* syn. *Panicum maximum*) cvv. Mombasa and Tanzania, 4 years for Ubon paspalum (*Paspalum atratum*) and 4–5 years for *Urochloa* syn. *Brachiaria* hybrid cv. Mulato II seed.

Experiment 2

The difference between seed germination in Experiment 1 and germination in the seed ageing test is noteworthy as are the differences between the 3 seed lots in the seed ageing test. This should be considered as evidence of the sensitivity of the ageing test. According to Marcos Filho (2015), the seed ageing test is one of the most applicable tests for determining how well seeds survive under storage. Its application in forage seed testing is less significant than in vegetable seeds (Wang et al. 2004). The reason for this is probably that expensive hybrid seeds and individual seed sowing are used in the production of vegetable and some field crops. It is important to ensure that expensive seeds do not decay before sale, so this quick test is a good way to evaluate longevity. In our case, the ageing test proved useful as a rapid test which provided us with information about how seed lots might perform after lengthy storage and estimates of the deadlines for use of certain seed lots to prevent serious deterioration in germination percentage. Prolonged exposure to adverse conditions during the ageing test inevitably leads to loss of quality and reduced germination (Marcos Filho 2015). In our experiment, by applying the 2 seed ageing test durations, germination of *Festulolium* decreased from 42 to 72 h test duration by 38%, *L. multiflorum* by 23%, and *F. pratensis* by 10% (Table 4). Tunes et al. (2011) reported that a seed ageing test performed on 4 seed lots of *L. multiflorum* with an increased duration of 72 vs. 48 h reduced germination by an average of 38%. The recommended temperatures for the ageing test and the length of seed exposure vary by forage species: for *Brachiaria brizantha*, Hernández et al. (2017) recommended 40 ºC during 48 or 60 h. For *L. multiflorum*, the recommended temperature is 41 ºC and exposure time 24, 48 or 72 h, depending on whether a traditional test or an alternative test with NaCl is performed (Tunes et al. 2011). In *F. pratensis* the recommended temperature is 41 °C and exposure time 48 or 72 h (Stanisavljević et al. 2013).

Conclusions

i) According to our results *Festulolium* seeds stored under ambient conditions with seeds of *Lolium multiflorum* and *Festuca pratensis* generally had lower seed dormancy and a shorter period was necessary for post-harvest maturation, release from dormancy and for achieving maximum germination. However, *Festulolium* seeds were less resistant to the ageing process, and germination percentage deteriorated more rapidly with time in storage than for seeds of *L. multiflorum* and *F. pratensis*. Whether this pattern would be changed with control of storage conditions remains unanswered.

ii) High germination performance was accompanied by vigorous growth of seedlings of all species, providing an added benefit with good quality seed.

iii) The seed ageing test proved a simple and rapid test to provide estimates of how germination of seed samples would change over extended periods of storage. More widespread use of this simple test commercially should identify seed lots which could deteriorate rapidly in storage. Further testing is needed to verify this hypothesis.

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References

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Abdelhalim M; Rognli OA; Hofgaard IS; Østrem L; Tronsmo AM. 2016. Snow mould resistance under controlled conditions and winter survival in the field in populations of perennial ryegrass, meadow fescue and *Festulolium* are partly dependent on ploidy level and degree of northern adaptation. Canadian Journal of Plant Science 96:579–589. doi: 10.1139/cjps-2015-0259

Adkins SW; Bellairs SM; Loch DS. 2002. Seed dormancy mechanisms in warm season grass species. Euphytica 126:13–20. doi: 10.1023/A:1019623706427

Akgun I; Tosun M; Sengul S. 2008. Comparison of agronomic characters of *Festulolium*, *Festuca pratensis* Huds. and *Lolium multiflorum* Lam. genotypes under high elevation conditions in Turkey. Bangladesh Journal of Botany 37:1–6. doi: 10.3329/bjb.v37i1.1556

Balešević-Tubić S; Tatić M. 2012. Seed ageing. Proceedings of International Conference on Bioscience, Biotechnology and Biodiversity. Institute of Field and Vegetable Crops Novi Sad and Seeds Association of Serbia, Serbia. p. 87–96. bit.ly/2S8YwFI

Bass LN; Gunn CR; Hesterman OB; Roos EE. 1988. Seed physiology, seedling performance and seed sprouting. In: Hanson AA; Barnes DK; Hill Jr RR, eds. Alfalfa and alfalfa improvement. Agronomy Monograph No. 29. ASA, CSSA, SSSA, Madison, WI, USA. p. 961–983. doi: 10.2134/agronmonogr29.c31

Bewley JD. 1997. Seed germination and dormancy. The Plant Cell 9:1055–1066. doi: 10.1105/tpc.9.7.1055

Deleuran LC; Gislum R; Boelt B. 2010. Effect of seed rate and row spacing in seed production of *Festulolium*. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science 60:152–156. doi: 10.1080/09064710902744463

Graeber K; Nakabayashi K; Miatton E; Leubner-Metzger G; Soppe WJJ. 2012. Molecular mechanisms of seed dormancy. Plant, Cell & Environment 35:1769–1786. doi: 10.1111/j.1365-3040.2012.02542.x

Hampton JG; TeKrony DM. 1995. Handbook of vigour test methods. 3rd Edn. The International Seed Testing Association (ISTA), Zurich, Switzerland.

Hare MD; Sutin N; Phengphet S; Songsiri T. 2018. Germination of tropical forage seeds stored for six years in ambient and controlled temperature and humidity conditions in Thailand. Tropical Grasslands-Forrajes Tropicales 6:26–33. doi: 10.17138/TGFT(6)26-33

Hernández Flores E; Joaquín Torres BM; Quero Carrillo AR; Hernández Garay A; Hernández Guzmán FJ. 2017. Envejecimiento acelerado y vigor de semillas del pasto *Brachiaria brizantha* cv Insurgente. Revista Mexicana de Ciencias Agrícolas 8:705–711. doi: 10.29312/remexca.v8i3.43

Humphreys MW; Farrell MS; Detheridge A; Scullion SJ; Kingston-Smith A; O'Donovan AS. 2013. Resilient and multifunctional grasslands for agriculture and environmental service during a time of climate change. Proceedings of the 17th Symposium of European Grassland Federation, Akureyri, Iceland, 24–26 June 2013. p. 335–337.

Humphreys MW; O'Donovan SA; Farrell MS; Gay AP; Kingston-Smith AH. 2014. The potential of novel *Festulolium* (2n = 4x = 28) hybrids as productive, nutrient use efficient fodder for ruminants. Food and Energy Security 3:98–110. doi: 10.1002/fes3.50

ISTA (International Seed Testing Authority). 2019. Biochemical test for viability: the topographical tetrazolium test. International Rules for Seed Testing. ISTA, Bassersdorf, Switzerland.

Long RL; Gorecki MJ; Renton M; Scott JK; Colville L; Goggin DE; Commander LE; Westcott DA; Cherry H; Finch-Savage WE. 2015. The ecophysiology of seed persistence: A mechanistic view of the journey to germination or demise. Biological Reviews 90:31–59. doi: 10.1111/brv.12095

Marcos Filho J. 2015. Seed vigor testing: An overview of the past, present and future perspective. Scientia Agricola 72:363–374. doi: 10.1590/0103-9016-2015-0007

Mwendia SW; Maass BL; Njenga D; Notenbaert AMO. 2019. Perennial ryegrass and novel festulolium forage grasses in the tropical highlands of Central Kenya: Preliminary assessment. Tropical Grasslands-Forrajes Tropicales 7:234–243. doi: 10.17138/TGFT(7)234-243

Nagel M; Borner A. 2010. The longevity of crop seeds stored under ambient conditions. Seed Science Research 20:1–12. doi: 10.1017/S0960258509990213

Neuberg M; Pavlíková D; Žižková E; Motyka V; Pavlík M. 2011. Different types of N nutrition and their impact on endogenous cytokinin levels in *Festulolium* and *Trifolium pratense*. Plant, Soil and Environment 57:381–387. doi: 10.17221/152/2011-PSE

Østrem L; Volden B; Larsen A. 2013. Morphology, dry matter yield and phenological characters at different maturity stages of ×*Festulolium* compared with other grass species. Acta Agriculturae Scandinavica, Section B - Soil and Plant Science 63:531–542. doi: 10.1080/09064710.2013.819440

Pašakinskienė I; Švėgždienė D. 2018. A comparative lowered temperature response in root apex cellular growth of *Festuca pratensis* and × *Festulolium braunii*. Zemdirbyste-Agriculture 105:349–356. doi: 10.13080/z-a.2018.105.044

Plata-Reyes DA; Morales-Almaraz E; Martínez-García CG; Flores-Calvete G; López-González F; Prospero-Bernal F; Valdez-Ruiz CL; Zamora-Juárez YG; Arriaga-Jordán CM. 2018. Milk production and fatty acid profile of dairy cows grazing four grass species pastures during the rainy season in small-scale dairy systems in the highlands of Mexico. Tropical Animal Health and Production 50:1797–1805. doi: 10.1007/s11250-018-1621-8

Rajjou L; Lovigny Y; Groot SPC; Belghazi M; Job C; Job D. 2008. Proteome-wide characterization of seed aging in *Arabidopsis*: A comparison between artificial and natural aging protocols. Plant Physiology 148:620–641. doi: 10.1104/pp.108.123141

Sah SK; Reddy KR; Li J. 2016. Abscisic acid and abiotic stress tolerance in crop plants. Frontiers in Plant Science 7:571. doi: 10.3389/fpls.2016.00571

Skládanka J; Adam V; Ryant P; Doležal P; Havlíček Z. 2010. Can *Festulolium*, *Dactylis glomerata* and *Arrhenatherum elatius* be used for extension of the autumn grazing season in Central Europe? Plant, Soil and Environment 56:488–498. doi: [10.17221/91/2010-PSE](https://doi.org/10.17221/91/2010-PSE)

Stanisavljević R; Đokic D; Milenkovic J; Đukanovic L; Stevovic V; Simic A; Dodig D. 2011. Seed germination and seedling vigour of Italian ryegrass, cocksfoot and timothy following harvest and storage. Ciência e Agrotecnologia 35:1141–1148. doi: 10.1590/S1413-70542011000600014

Stanisavljević RS; Vučković SM; Simić AS; Marković JP; Lakić ŽP; Terzić DV; Đokić DJ. 2012. Acid and temperature treatments result in increased germination of seeds of three fescue species. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 40:220–226. doi: 10.15835/nbha4027898

Stanisavljević R; Milenković J; Đokić D; Terzić D; Petrović M; Đukanović L; Dodig D. 2013. Drying of meadow fescue seeds of different moisture contents: Changes in dormancy and germination. Plant, Soil and Environment 59:37–43. doi: 10.17221/551/2012-PSE

Stanisavljević R; Đokić D; Milenković J; Terzić D; Stevović V; Tomić D; Dodig D. 2014. Drying of forage grass seed harvested at different times and its utility value in sowing periods. Zemdirbyste-Agriculture 101:169–176. doi: 10.13080/z-a.2014.101.022

Tunes LM de; Pedroso DC; Badinelli PG; Tavares LC; Rufino CA; Barros ACSA; Muniz MFB. 2011. Accelerated aging of ryegrass seeds submitted to saturated salt solution. Ciência Rural 41:33–37. (In Portuguese). doi: 10.1590/S0103-84782011000100006

Wang YR; Yu L; Nan ZB; Liu YL. 2004. Vigor tests used to rank seed lot quality and predict field emergence in four forage species. Crop Science 44:535–541. doi: 10.2135/cropsci2004.5350

Wyszkowska J; Borowik A; Kucharski J. 2019. Resistance of *Lolium perenne* L. × *hybridum*, *Poa pratensis*, *Festuca rubra*, *F. arundinacea*, *Phleum pratense* and *Dactylis glomerata* to soil pollution by diesel oil and petroleum. Plant, Soil and Environment 65:307–312. doi: 10.17221/42/2019-PSE

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Research Paper

Selection based on meiotic behavior in *Urochloa decumbens* hybrids from non-shattered seed

Selección con base en el comportamiento meiótico de híbridos procedentes de semillas no dehiscentes de Urochloa decumbens

JOANA NERES DA CRUZ BALDISSERA[1], ANDRÉA BEATRIZ DIVERIO MENDES[2], MARLON MATHIAS DACAL COAN[2], CLAUDETE APARECIDA MANGOLIN[2], CACILDA BORGES DO VALLE[3] AND MARIA SUELY PAGLIARINI[2]

[1]*Colegiado de Biologia, Instituto Federal do Paraná, Palmas, PR, Brazil.* palmas.ifpr.edu.br
[2]*Departamento de Agronomia, Universidade Estadual de Maringá, Maringá, PR, Brazil.* dag.uem.br
[3]*Empresa Brasileira de Pesquisa Agropecuária, Embrapa Gado de Corte, Campo Grande, MS, Brazil.* cnpgc.embrapa.br

Abstract

This study aimed to evaluate the end-products of meiosis in sexual and apomictic hybrids of *Urochloa decumbens*, so as to identify genotypes with good production of viable pollen for use in breeding programs to increase yields of pure viable seed and reduce degree of seed shattering. From 457 intraspecific hybrids of *U. decumbens* arising from crosses between 3 artificially tetraploidized sexual plants and the apomictic cultivar Basilisk, 27 hybrids from non-shattered seed were selected. Slides were prepared by smearing anthers and staining to determine the presence of abnormalities. The abnormalities found were micronuclei, microcytes and polyads. The data were compared by the Scott-Knott test at P<0.05. Data obtained enabled separation of hybrids into 4 groups depending on the presence of micronuclei and formation of polyads and into 6 groups based on the presence of microcytes in the tetrads. Among the analyzed apomictic hybrids, R179 has the attributes for viable seed production to proceed to cultivar development. Among the sexual hybrids, R161, R181, R193 and S47 are recommended as female parents for use in crossing programs.

Keywords: Abnormalities, breeding, cytogenetics, forages, intraspecific crosses.

Resumen

El estudio tuvo como objetivo evaluar los productos finales de la meiosis en híbridos sexuales y apomícticos de *Urochloa decumbens*, para identificar genotipos con buena producción de polen viable que puedan ser usados en un programa de mejoramiento genético y aumentar así los rendimientos de semilla pura viable y reducir su grado de dehiscencia. De un total de 457 híbridos intraespecíficos de *U. decumbens* que resultaron de cruzamientos entre tres plantas sexuales tetraploidizadas artificialmente y el cultivar apomíctico 'Basilisk', se seleccionaron 27 híbridos procedentes de semillas no desprendidas. Para el efecto se prepararon portaobjetos con anteras que fueron teñidas para determinar la presencia de anomalías. Las anomalías encontradas fueron micronúcleos, microcitos y políadas. Los datos se compararon mediante la prueba de Scott-Knott (P<0.05). Los resultados permitieron separar los híbridos en cuatro grupos dependiendo de la presencia de micronúcleos y la formación de políadas, y en seis grupos basados en la presencia de microcitos en las tétradas. El híbrido R179, entre los híbridos apomícticos analizados, presentó los atributos necesarios para el desarrollo de cultivares con potencial de producción de semillas viables. Entre los híbridos sexuales, se recomiendan R161, R181, R193 y S47 como progenitores femeninos en programas de cruzamiento.

Palabras clave: Anomalías, citogenética, cruces intraespecificos, fitomejoramiento, forrajes tropicales.

Correspondence: Joana Neres da Cruz Baldissera**,** Colegiado de Biologia, Instituto Federal do Paraná, Campus Palmas**,** Av. Bento Munhoz da Rocha Neto s/nº - PRT-280, Trevo da Codapar, Palmas, CEP 85555-000, PR, Brazil. Email: jondcb@gmail.com

Introduction

Brazil has 221.8 million head of cattle and produced 9.71 million tonnes of meat, worth R$ 523.25 billion in 2017 (ABIEC 2018), making the country one of the main producers of beef in the world. This is a result of the adoption of new technologies relating to genetics and the management and feeding of beef herds (Gomes et al. 2017).

Approximately 95% of the animals are raised on pasture (Araújo et al. 2017), so high quality forages and improvement of existing pastures are a prerequisite to efficient livestock production (Ribeiro-Junior et al. 2017). In addition, Brazil occupies a prominent position in the world as a producer and exporter of tropical forage seed (Pereira et al. 2011). In 2015, 50 thousand tonnes of certified seed was produced, with 75% destined for the domestic market and 25% for export (Rodrigues 2017).

Brachiaria (now: *Urochloa*) breeding in Brazil began when CIAT and EMBRAPA achieved compatibility between species with different ploidy levels in the late 1980s (Triviño et al. 2017). While a number of cultivars have been released, most have some limitations, so further genetic improvement is warranted. Low seed yields and seed quality are significant issues for cultivars which have been released most recently, especially the hybrids.

If a cultivar is to be adopted widely and have a significant impact on animal production, adequate supplies of good quality seed are essential (Valle et al. 2008). One factor affecting seed quality is the occurrence of natural shattering, i.e. seeds detaching from the raceme on reaching maturity. Seed must be retrieved from the ground, resulting in some being lost plus contamination by pathogens and impurities. Development of genotypes resistant to seed shattering would lead to increases in both seed yields and quality.

Another factor affecting seed quality and quantity is polyploidy because, when there is more than a chromosomal set in a cell, the organization of the same can be difficult at the time of pairing and segregation causing meiotic abnormalities. In the genus *Brachiaria* (now: *Urochloa*), most ecotypes studied are polyploids (Valle and Savidan 1996; Utsunomiya et al. 2005) and the vast majority reproduce by apomixis (Valle and Savidan 1996; Fuzinatto et al. 2007, 2008; Mendes-Bonato et al. 2007). Adamowski et al. (2008) revealed many important meiotic and post-meiotic abnormalities that compromise, sometimes seriously, the end-product of meiosis, causing pollen sterility. Apomixis in *Urochloa* is a pseudogamous apospory, where, despite the fact that the egg cell circumvents fertilization, the central cell requires it for endosperm formation. Therefore quality of pollen is very important to ensure the formation of viable seeds. Furthermore, hybridization is successful only if crossings are performed between parents with the same ploidy level, using a sexual genotype as a mother-plant and the apomictic or another sexual as pollen donor(s) (Barrios et al. 2013; Alves et al. 2014).

Considering the importance of selecting genotypes that combine high levels of seed retention and quantity/quality of viable seeds, the present study had as its objective evaluation of the final products of meiosis and pollen viability in hybrids of *U. decumbens*, in order to identify stable genotypes and good pollen producers for use in the *Urochloa* breeding program at Embrapa Beef Cattle Research Center (Embrapa Gado de Corte).

Materials and Methods

A base population of 457 intraspecific hybrids of *U. decumbens* was produced from crosses between 3 artificially tetraploidized sexual plants and the apomictic cultivar Basilisk. This population is maintained in an experimental field at Embrapa Beef Cattle Center, in Campo Grande, Mato Grosso do Sul (20°25'03" S, 54°42'20" W) in an allic Red Latosol type soil. From that population 27 hybrids from non-shattered seed were selected (Table 1) and were cytogenetically analyzed in the Laboratory of Cytogenetics from the Universidade Estadual de Maringá, Paraná.

The inflorescences of the hybrids were collected and fixed in a mixture of ethanol:chloroform:propionic acid (6:3:2) for 24 hours and stored in 70% alcohol at 4 °C. Three anthers from the same hermaphrodite flower, chosen randomly on the raceme, were used per slide, for the analysis of the final products of meiosis and pollen viability. Each slide was considered a replication, with 5 replications per hybrid. One hundred cells were counted per slide.

Tetrads of microspores and pollen viability were evaluated after squashing, then staining with 1% propionic carmine and analyzing under light microscopy. The pollen grains were classified into 2 groups: 1) viable pollen grains, with the exine intact and the protoplasm well stained; and 2) unviable pollen, with weak staining or shriveled and not stained.

The data on anomalous tetrads of microspores and non-viable pollen grains obtained in percentage (%) were transformed into arcsine function using the square root ($\sqrt{x}$) of the proportion of abnormal tetrads. Data were then submitted to analysis of variance using the SAS 9.2 program (SAS Institute 2009). The mean percentages were compared using the Scott-Knott test at the 5% probability level using GENES (Cruz 2001).

Table 1. Hybrids of *Urochloa decumbens* analyzed.

Hybrid	Reproduction mode	Female parent	Male parent
R158	Apomictic	D24/27	cv. Basilisk
R168	Apomictic	D24/27	cv. Basilisk
R169	Apomictic	D24/27	cv. Basilisk
R176	Apomictic	D24/27	cv. Basilisk
R177	Apomictic	D24/27	cv. Basilisk
R179	Apomictic	D24/27	cv. Basilisk
R184	Apomictic	D24/27	cv. Basilisk
R187	Apomictic	D24/27	cv. Basilisk
R189	Apomictic	D24/27	cv. Basilisk
S48	Apomictic	D24/27	cv. Basilisk
T87	Apomictic	D24/27	cv. Basilisk
X113	Apomictic	D24/45	cv. Basilisk
Y22	Apomictic	D24/45	cv. Basilisk
Y23	Apomictic	D24/45	cv. Basilisk
Z8	Apomictic	D24/45	cv. Basilisk
R 161	Sexual	D24/27	cv. Basilisk
R163	Sexual	D24/27	cv. Basilisk
R165	Sexual	D24/27	cv. Basilisk
R167	Sexual	D24/27	cv. Basilisk
R171	Sexual	D24/27	cv. Basilisk
R181	Sexual	D24/27	cv. Basilisk
R193	Sexual	D24/27	cv. Basilisk
S47	Sexual	D24/27	cv. Basilisk
Y21	Sexual	D24/45	cv. Basilisk
Z9	Sexual	D24/45	cv. Basilisk
X119	-	D24/45	cv. Basilisk
X122	Sexual- sterile	D24/45	cv. Basilisk

The most representative abnormalities in the tetrads and pollen grains were photographed under an OLYMPUS CX 31 capture microscope with attached SC 30 camera, using the AnalySIS getIT software, with 400× magnification.

Results

Many abnormalities were observed in the final products of meiosis of hybrids of *U. decumbens* analyzed, the main ones being 1, 2, 3 and 4 micronuclei in the microspores (Figures 1a–1d), microcytes (Figures 1e–1f) and polyads (Figures 1g–1i).

Cytogenetic analysis revealed the presence of micronuclei and microcytes in the same tetrad (Figures 1e–1f) and polyads with micronuclei (Figures 1g–1i).

The analyses of the tetrads of microspores from these hybrids are presented in Table 2.

The meiotic abnormalities in tetrads were expressed as percentages of abnormal cells and the significant differences between the irregularities of the hybrids were tested by the Scott-Knott test. In the analysis of variance for meiotic abnormalities, the mean square for the hybrid effect was significant by the F-test with 5% probability of error; therefore, there are differences between hybrids in the frequencies of chromosomal irregularities in the tetrads of microspores (Table 2). For the variables related to abnormalities in tetrads, the estimated coefficient of variation (CV) was high for the presence of micronucleus in 1 microspore (38.3%), microcytes (41.9%) and polyads (86.7%). These high values for CV can be explained by differences in the numbers of cells found with these abnormalities in each slide (replication).

Based on the Scott-Knott test it was possible to separate the hybrids into 4 groups (A, B, C and D) concerning the presence of micronuclei in 1, 2 and 3 microspores and 3 groups concerning the presence in 4 microspores. The groups differ on the basis of minimum significant difference while the hybrids within the groups are similar.

Table 2. Analysis of variance of the meiotic abnormalities observed in the 27 hybrids of *Urochloa decumbens.*

Source	DF	Mean Square of meiotic abnormalities					
		1	2	3	4	5	6
Hybrid	26	0.109*	0.123*	0.072*	0.156*	0.208*	0.73*
Error	108	0.008*	0.007*	0.007*	0.018*	0.007*	0.006*
Total	134						
CV%		38.3	22.4	16.5	21.6	41.9	86.7

1 = micronuclei in 1 microspore; 2 = micronuclei in 2 microspores; 3 = micronuclei in 3 microspores; 4 = micronuclei in 4 microspores; 5 = microcyte; 6 = polyad.
*Significant by the F-test (P<0.05).

Regarding the presence of micronuclei in just 1 microspore, hybrids of Group D (Table 3) presented the lowest frequencies of this abnormality. However, based on the parameters established by Love (1951), this group should be considered unstable, since more than 10% of abnormal tetrads were detected, with the presence of micronuclei in all 4 microspores (Table 3). For the presence of micronuclei in 2 microspores of the tetrad, Groups C and D (Table 3) were those with fewer than 10% of abnormal tetrads, while also presenting high frequency of micronuclei in the tetrad. The same is true for micronuclei in 3 microspores where hybrids R187 and R189, despite having fewer micronuclei in 3 microspores, showed 33 and 68% of micronuclei in the tetrads, with high frequency of microcytes and polyads. The only hybrid that presented fewer than 10% of tetrads with micronuclei in the 4 microspores was R181, although this hybrid did not differ statistically from other hybrids of Group C.

Hybrids have been classified into 6 groups from A to F on the basis of the presence of microcytes in tetrads (Table 3).

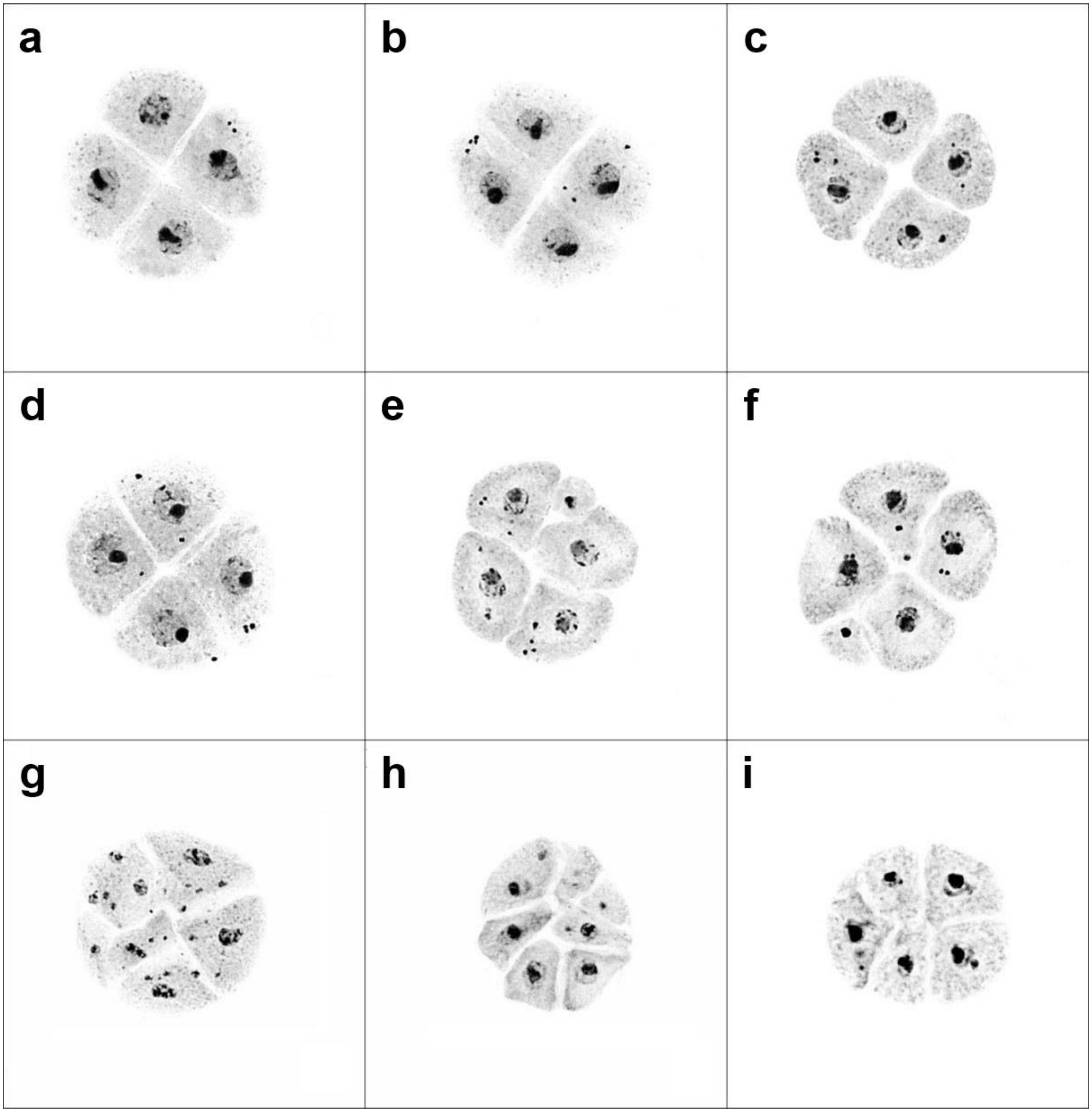

Figure 1. Meiotic abnormalities observed in tetrads of microspores, due to irregular segregation of chromosomes and genome asynchrony in tetraploid hybrids of *Urochloa decumbens*: a) micronuclei in 1 microspore; b) micronuclei in 2 microspores; c) micronuclei in 3 microspores; d) micronuclei in 4 microspores; e-f) tetrads with micronuclei in the microspores and microcytes; and g-h-i) polyads with microspores of different sizes and with micronuclei (400× magnification).

Hybrids in Groups A and B are expected to have higher frequency of unbalanced gametes and thus higher pollen infertility. According to the parameters established by Love (1951), hybrids of Groups D, E and F can be considered stable cytogenetically.

Hybrids were separated into 4 groups, from A to D, on the basis of the frequency of polyads (Table 3). Except for hybrid R187 with 25% of polyads, these occurred in much lower frequencies, probably not compromising pollen fertility.

Pollen viability of *U. decumbens* hybrids was tested using propionic carmine at 1% (Figures 2a–2c). Pollen grains of different sizes and staining patterns were observed in the hybrids analyzed, but in many cases it was not possible to accurately determine whether pollen grains were viable or non-viable.

Table 3. Grouping of the 27 *Urochloa decumbens* hybrids evaluated based on similar behavior regarding mean percentages of incidence of abnormal cells observed at the end of meiosis.

Micronuclei in microspores												Microcytes			Polyads		
1			2			3			4								
Hybrid	%	Group	Hybrid	%	Group	Hybrid	%	Group	Hybrid	%	Group	Hybrid	%	Group	Hybrid	%	Group
R181	28.0	A	R193	33.8	A	Y22	38.6	A	R189	68.0	A	S48	42.0	A	R187	25.0	A
R179	23.0	A	R179	30.1	A	X113	36.5	A	R163	64.5	A	R176	28.0	B	X122	7.8	B
R 161	19.0	A	X119	28.9	A	R177	32.9	A	R169	56.2	A	R187	33.0	B	S48	6.6	B
R193	19.0	A	R181	26.8	A	R158	30.4	A	R167	53.8	A	X122	23.0	B	R189	5.5	B
Y23	14.0	B	R171	26.5	A	Z9	30.0	A	Y22	50.0	A	R 161	15.0	C	R167	2.8	C
R171	11.0	B	Z9	24.8	A	X119	29.7	A	S47	48.2	A	R189	17.0	C	R163	2.3	C
X119	11.0	B	T87	24.5	A	R184	29.6	A	X122	43.5	B	R167	10.0	D	Y23	2.0	C
R165	10.0	B	R 161	24.4	A	Z8	28.6	A	Z8	43.3	B	Y21	8.6	D	R169	1.3	C
R168	8.3	B	R158	23.8	A	R171	28.0	A	R184	42.0	B	R163	3.3	E	Y21	1.3	C
R176	7.8	B	Y23	21.6	A	R165	27.7	A	R177	39.5	B	R165	1.1	E	R168	1.2	C
R158	7.7	B	R165	21.3	A	R193	27.1	A	S48	37.9	B	R168	3.1	E	R176	0.5	D
Z9	6.2	C	R184	19.8	B	T87	27.0	A	X113	37.1	B	R169	6.1	E	R 161	0.2	D
Z8	6.1	C	X113	18.7	B	S47	26.2	A	Y21	37.0	B	R171	1.3	E	R165	0.2	D
R184	5.4	C	S47	18.2	B	Y21	25.5	B	T87	34.5	B	T87	2.0	E	R171	0.2	D
T87	5.3	C	R177	17.9	B	Y23	24.6	B	R187	33.3	B	Y23	2.5	E	R181	0.2	D
Y21	4.5	C	R176	16.9	B	R179	24.1	B	Z9	33.1	B	Z8	2.0	E	Z9	0.2	D
R177	4.2	C	Y21	16.5	B	R167	22.9	B	R158	30.2	B	R158	0.6	F	Z8	0.1	D
S47	4.0	C	R168	14.8	B	R169	21.3	B	R168	29.5	B	R177	0.8	F	R158	0.0	D
X113	3.7	C	Z8	13.8	B	R176	20.9	B	R165	28.1	B	R179	0.1	F	R177	0.0	D
R167	2.6	C	Y22	10.3	B	X122	19.4	B	R171	25.7	B	R181	0.0	F	R179	0.0	D
R169	1.6	D	R169	8.0	C	R168	19.1	B	X119	24.8	B	R184	0.0	F	R184	0.0	D
R163	0.7	D	R163	7.8	C	R163	19.0	B	Y23	24.3	B	R193	0.0	F	R193	0.0	D
X122	0.4	D	R167	6.1	C	R 161	15.6	C	R176	18.6	C	S47	0.0	F	S47	0.0	D
S48	0.2	D	X122	3.6	C	R181	13.2	C	R193	12.1	C	X113	0.2	F	T87	0.0	D
Y22	0.2	D	S48	1.6	D	S48	10.2	C	R179	11.7	C	X119	0.5	F	X113	0.0	D
R187	0.0	D	R187	0.3	D	R189	5.1	D	R 161	10.9	C	Y22	0.0	F	X119	0.0	D
R189	0.0	D	R189	0.3	D	R187	1.8	D	R181	7.1	C	Z9	0.7	F	Y22	0.0	D

1 = micronuclei in 1 microspore; 2 = micronuclei in 2 microspores; 3 = micronuclei in 3 microspores; 4 = micronuclei in 4 microspores. Grouping based on significance by the Scott-Knott test ($P<0.05$).

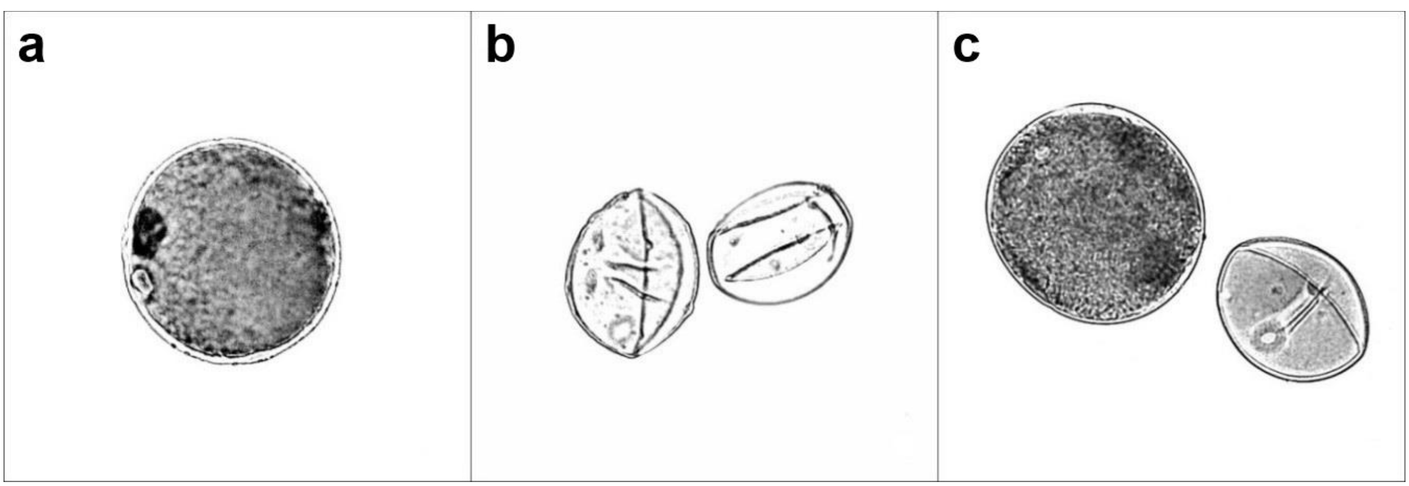

Figure 2. Pollen viability of the 27 *Urochloa decumbens* tetraploid hybrids determined by staining with 1% propionic carmine: a) viable pollen grain strongly stained; b) non-viable pollen grains unstained; c) viable and non-viable pollen grains (400× magnifications).

Discussion

Micronuclei are a consequence of segregation irregularities occurring in different phases of meiosis. As reported by Risso-Pascotto et al. (2004), micronuclei in 1 or more microspores are the most common cytological abnormality resulting from irregular chromosome segregation in higher plants. When formed, the micronuclei can remain in tetrads of microspores even after the dissolution of the callose wall and the release of microspores impairing normal gamete formation (Valle and Pagliarini 2009). Micronuclei can also be eliminated from the tetrads as microcytes by cytokinesis. In the hybrids analyzed, the elimination of micronuclei by additional cytokinesis gave rise to microcytes in tetrads and polyads.

Micronuclei in microspores of tetrads, tetrads with microcytes and polyads have often been reported in meiotic studies of interspecific hybrids of *Urochloa* (Risso-Pascotto et al. 2004; Mendes-Bonato et al. 2007), which, depending on the frequency of occurrence, results in the formation of unbalanced gametes. We expected that intraspecific hybridizations would produce fewer anomalies in meiosis than with interspecific hybrids, since chromosome sets were supposedly homologous. The occurrence of abnormalities in these intraspecific hybrids of *U. decumbens* could be due to the recent artificial replication of the chromosomes of their female parent. Artificial chromosome duplication using colchicine can cause loss of chromosomes or chromosomal rearrangements such as deletions or inversions, as well as sterility and abnormal growth (Luckett 1989).

The analysis of meiotic behavior of artificially tetraploidized accessions of *U. decumbens*, *U. brizantha* and *U. ruziziensis* has shown a rate of meiotic abnormalities varying from 5 to 60%, and a high rate of abnormalities in interspecific hybrids using tetraploidized parents (Fuzinatto et al. 2007; Souza et al. 2015). These culminated in abnormal tetrads and in the formation of a high rate of unviable pollen grains.

Love (1951) indicated that the analysis of tetrads easily proved the degree of stability of the meiotic process, since it demonstrated the pattern of chromosome behavior during the phases of meiosis. According to this author, a plant with 90–100% of normal tetrads is considered stable, whereas plants with fewer than 90% of normal meiotic products limit breeding, because this hampers production of viable seeds.

Although Love´s meiotic index is widely used to determine the meiotic stability and consequently the fertility of a plant, a more detailed analysis of the final products of meiosis may result in much more accurate information, especially for polyploid plants, which have a high rate of abnormalities in tetrads of microspores. This can be explained by the fact that a tetrad with micronuclei in 1 microspore can theoretically have 3 other normal microspores. These hybrids would thus produce viable pollen in the ratio of 3:1 (viable:unviable pollen). According to Souza et al. (2015), genotypes with a high frequency of micronuclei in only 1 microspore would be more promising, since the other 3 microspores of the tetrad may contain balanced genetic material.

Using this basis for selection, the best hybrids would be those with no micronuclei or a high frequency of tetrads with micronuclei in only 1 microspore. That was not the case in the hybrids studied, where the important criteria were to select hybrids with fewer micronuclei throughout and also absence of microcytes and polyads.

The formation of microcytes in tetrads and polyads is much more serious than the presence of micronuclei in microspores of the tetrad. When additional cytokinesis forms microcytes and polyads, all microspores are abnormal due to uneven division of the genomes. Tetrads with microcytes and polyads generate unbalanced pollen grains of different sizes.

Pollen viability is an accepted measure of male fertility and can be estimated by staining methods using mature pollen grains. Although several authors, e.g. Ricci et al. (2010); Simioni and Valle (2011); Souza et al. (2015), have already tested pollen viability in *Urochloa* using this staining method and were able to discriminate between viable and non-viable pollen grains, the method is often unreliable, because in addition to meiotic irregularities, pollen viability can be affected by failures in the microgametogenesis process (Twel 1995), natural water loss that occurs during the collection and storage of inflorescences (Tecchio et al. 2006) and the storage time of the inflorescences (Stanley and Linskens 1974). According to Souza et al. (2002), pollen grain is fully viable at the opening of the flower, and as time progresses, the viability decreases, reducing its efficiency.

Hybridizations performed in the *Urochloa* breeding program of Embrapa Beef Cattle use sexual genotypes as mother plants and apomictic ones as pollen donors (Mendes-Bonato et al. 2004). According to Souza et al. (2015), sexual hybrids that have a low frequency of abnormalities in tetrads and good viable pollen production may be included in polycross blocks with other sexual hybrids for the recombination of alleles or used in crosses with other elite apomictic genotypes to generate new populations from which to select future apomictic cultivars. Superior apomictic hybrids can be evaluated agronomically to select new cultivars or can be used as pollen donors in new crosses.

Among the apomictic hybrids analyzed, R179 could be regarded as a good pollen donor, since it had a high percentage of tetrads with micronuclei in only 1 or 2 microspores (Group A), and a low percentage of tetrads with micronuclei in the 4 microspores (Group C), tetrads with microcytes (Group F) and polyads (Group D). Apomictic hybrids R187, R189 and S48, however, with high rates of tetrads with micronuclei in the 4 microspores, microcytes and polyads must be discarded as parents for crossing. Among the sexual hybrids R161, R181, R193 and S47 may be considered for crossing blocks and the next generation evaluated to confirm potential fertility.

Absence of seed shattering was a key factor in the domestication of major grasses because humans could collect seed throughout the long summer season, making them preadapted candidates for domestication (Kislev et al. 2004). The inheritance of non-shattering behavior, which in some grasses seems to be controlled by few genes or transcription factors (Konishi et al. 2006; Li et al. 2006), is an important trait to be a focus in evaluation of the intraspecific hybrids of *U. decumbens* analyzed. Given the importance of this character for the improvement of this forage, the detailed analysis of the tetrads of microspores and pollen viability is essential in selecting hybrids that could produce larger quantities of fertile seeds that could be harvested conventionally. Hybrids resistant to shattering would improve significantly the harvesting of viable seed for either the breeding program or commercial purposes. Furthermore, selection of future cultivars with better potential production of directly harvested seed should reduce cost of seed, resulting in greater adoption rates and contributing to pasture diversification and sustainability (Fonseca and Martuscello 2011).

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References

(Note of the editors: All hyperlinks were verified 27 April 2020.)

ABIEC (Associação Brasileira das Indústrias Exportadoras de Carnes). 2018. Perfil da pecuária no Brasil. Relatório anual. AIBEC, São Paulo, SP, Brazil. bit.ly/3cTFBq8

Adamowski EV; Pagliarini MS; Valle CB do. 2008. Meiotic behavior in three interspecific three way hybrids between *Brachiaria ruziziensis* and *B. brizantha* (Poaceae: Paniceae). Journal of Genetics 87:33–38. doi: 10.1007/s12041-008-0005-7

Alves GF; Figueiredo UJ de; Pandolfi Filho AD; Barrios SCL; Valle CB do. 2014. Breeding strategies for *Brachiaria* spp. to improve productivity – an ongoing project. Tropical Grasslands-Forrajes Tropicales 2:1–3. doi: 10.17138/tgft(2)1-3

Araújo FR de; Rosinha GMS; Bier D; Chiari L; Feijó GLD; Gomes RC. 2017. Segurança do alimento carne. Nota Técnica. Embrapa Gado de Corte, Campo Grande, MS, Brazil. bit.ly/2yKXitt

Barrios SCL; Valle CB do; Alves GF; Simeão RM; Jank L. 2013. Reciprocal recurrent selection in the breeding of *Brachiaria decumbens*. Tropical Grasslands-Forrajes Tropicales 1:52–54. doi: 10.17138/tgft(1)52-54

Cruz CD. 2001. Programa Genes: Aplicativo computacional em genética e estatística. Editora UFV, Viçosa, MG, Brazil.

Fonseca DM da; Martuscello JÁ. 2011. Plantas forrageiras. Editora UFV, Viçosa, MG, Brazil.

Fuzinatto VA; Pagliarini MS; Valle CB do. 2007. Evidence of programmed cell death during microsporogenesis in an interspecific *Brachiaria* (Poaceae: Panicoideae: Paniceae) hybrid. Genetics and Molecular Research 6:308–315. geneticsmr.com/articles/356

Fuzinatto VA; Pagliarini MS; Valle CB do. 2008. Evaluation of microsporogenesis in an interspecific *Brachiaria* hybrid (Poaceae) collected in distinct years. Genetics and Molecular Research 7:424–432. geneticsmr.com/articles/490

Gomes RC; Feijó GLD; Chiari L. 2017. Evolução e qualidade da pecuária brasileira. Nota Técnica. Embrapa Gado de Corte, Campo Grande, MS, Brazil. bit.ly/3bKWa7z

Kislev ME; Weiss E; Hartmann A. 2004. Impetus for sowing and the beginning of agriculture: Ground collecting of wild cereals. Proceedings of the National Academy of Sciences of the USA 101:2692–2695. doi: 10.1073/pnas.0308739101

Konishi S; Izawa T; Lin SY; Ebana K; Fukuta Y; Sasaki T; Yano M. 2006. An SNP caused loss of seed shattering during rice domestication. Science 312:1392–1396. doi: 10.1126/science.1126410

Li C; Zhou A; Sang T. 2006. Rice domestication by reducing shattering. Science 311:1936–1939. doi: 10.1126/science.1123604

Love RM. 1951. Varietal differences in meiotic behavior of Brazilian wheats. Agronomy Journal 43:72–76. doi: 10.2134/agronj1951.00021962004300020005x

Luckett DJ. 1989. Colchicine mutagenesis is associated with substantial heritable variation in cotton. Euphytica 42:177–182. doi: 10.1007/BF00042630

Mendes-Bonato AB; Pagliarini MS; Valle CB do. 2004. Abnormal pollen mitoses (PM I and PM II) in an interspecific hybrid of *Brachiaria ruziziensis* and *Brachiaria decumbens* (Gramineae). Journal of Genetics 83:279–283. doi: 10.1007/bf02717897

Mendes-Bonato AB; Pagliarini MS; Valle CB do. 2007. Meiotic arrest compromises pollen fertility in an interspecific hybrid between *Brachiaria ruziziensis* × *Brachiaria decumbens* (Poaceae: Paniceae). Brazilian Archives of Biology and Technology 50:831–837. doi: 10.1590/S1516-89132007000500011

Pereira CE; Oliveira JÁ; Rosa MCM; Kikuti ALP. 2011. *Brachiaria* coated seed storage treated with fungicide and insecticide. Ciência Rural 41:2060–2065. (In Portuguese). doi: 10.1590/S0103-84782011001200004

Ribeiro-Júnior NG; Ariano APR; Silva IV. 2017. Death of pastures syndrome: Tissue changes in *Urochloa* hybrid cv. Mulato II and *Urochloa brizantha* cv. Marandu. Brazilian Journal of Biology 77:97–107. 10.1590/1519-6984.10715

Ricci GCL; Pagliarini MS; Valle CB do. 2010. Genome elimination during microsporogenesis in two pentaploid accessions of *Brachiaria decumbens* (Poaceae). Genetics and Molecular Research 9:2364–2371. doi: 10.4238/vol9-4gmr919

Risso-Pascotto C; Pagliarini MS; Valle CB do; Jank L. 2004. Asynchronous meiotic rhythm as the cause of selective chromosome elimination in an interspecific *Brachiaria* hybrid. Plant Cell Reports 22:945–950. doi: 10.1007/s00299-004-0784-0

Rodrigues M. 2017. Mercado de sementes forrageiras está promissor. Revista Safra, Goiânia, GO, Brazil. bit.ly/2Sd0ZyO

SAS Institute. 2009. SAS/STAT 9.2. User's Guide. SAS Institute, Cary, NC, USA.

Simioni C; Valle CB do. 2011. Meiotic analysis in induced tetraploids of *Brachiaria decumbens* Stapf. Crop Breeding and Applied Biotechnology 11:43–49. doi: 10.1590/S1984-70332011000100006

Souza MM de; Pereira TNS; Martins ER. 2002. Microsporogênese e microgametogênese associadas ao tamanho do botão floral e da antera e viabilidade polínica em maracujazeiro-amarelo (*Passiflora edulis* Sims f. *flavicarpa* Degener). Ciência e Agrotecnologia 26:1209–1217. bit.ly/35a1UW5

Souza VF; Pagliarini MS; Valle CB do; Bione NCP; Menon MU; Mendes-Bonato AB. 2015. Meiotic behavior of *Brachiaria decumbens* hybrids. Genetics and Molecular Research 14:12855–12865. doi: 10.4238/2015.October.21.5

Stanley RG; Linskens HF. 1974. Pollen: Biology biochemistry management. Springer-Verlag, Berlin, Germany. doi: 10.1007/978-3-642-65905-8

Tecchio VH; Davide LC; Pedrozo CA; Pereira AV. 2006. Viabilidade do grão de pólen de acessos de capim-elefante, milheto e híbridos interespecíficos (capim-elefante × milheto). Acta Scientiarum: Biological Sciences 28:7–12. doi: 10.4025/actascibiolsci.v28i1.1052

Triviño NJ; Perez JG; Recio ME; Ebina M; Yamanaka N; Tsuruta S; Ishitani M; Worthington M. 2017. Genetic diversity and population structure of *Brachiaria* species and breeding populations. Crop Science 57:2633–2644. doi: 10.2135/cropsci2017.01.0045

Twel D. 1995. Diphtheria toxin-mediated cell ablation in developing pollen: Vegetative cell ablation blocks generative cell migration. Protoplasma 187:144–154. doi: 10.1007/BF01280243

Utsunomiya KS; Pagliarini MS; Valle CB do. 2005. Microsporogenesis in tetraploid accessions of *Brachiaria nigropedata* (Ficalho & Hiern) Stapf (Gramineae). Biocell 29:295–301. techscience.com/biocell/v29n3/37678

Valle CB do; Savidan YH. 1996. Genética, citogenética y biología reproductiva de *Brachiaria*. In: Miles JW; Maass BL; Valle CB do; Kumble V, eds. *Brachiaria*: Biología, agronomía y mejoramiento. Centro Internacional de Agricultura Tropical (CIAT); Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Cali, Colombia. p. 163–180. bit.ly/359jyJD

Valle CB do; Simioni C; Resende RMS; Jank L. 2008. Melhoramento genético de *Brachiaria*. In: Simeão RM; Jank L; Valle CB do, eds. Melhoramento de forrageiras tropicais. Embrapa Gado de Corte, Campo Grande, MS, Brazil. p. 13–47.

Valle CB do; Pagliarini MS. 2009. Biology, cytogenetics, and breeding of *Brachiaria*. In: Singh RJ, ed. Genetic resources, chromosome engineering, and crop improvement. Vol. 5, Forage crops. CRC Press, New York, USA. p. 103–151

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Short Communication

The effect of stage of regrowth on the physical composition and nutritive value of the various vertical strata of kikuyu (*Cenchrus clandestinus*) pastures

Efecto de la edad de rebrote en la composición física y el valor nutritivo de los diferentes estratos verticales de pasturas de kikuyo (Cenchrus clandestinus)

MARCELO A. BENVENUTTI[1], CRAIG FINDSEN[1], JEAN V. SAVIAN[2,3], DAVID G. MAYER[1] AND DAVID G. BARBER[1]

[1]*Queensland Department of Agriculture and Fisheries, Gatton Campus, Lawes, QLD, Australia.* daf.qld.gov.au
[2]*Instituto Nacional de Investigación Agropecuaria (INIA), Treinta y Tres, Uruguay.* inia.uy
[3]*Grupo de Pesquisa Ecologia do Pastejo, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.* ufrgs.br/gpep

Abstract

A plot study was conducted at the Gatton Research Dairy, Queensland, Australia, to quantify the effects of 5 regrowth periods (9, 11, 14, 16 and 18 days) and 4 vertical strata on the composition and nutritive value of kikuyu (*Cenchrus clandestinus*) pastures using a block factorial design with 4 replicates. Pasture samples were analyzed for crude protein (CP), ethanol-soluble carbohydrates (ESC), acid detergent fiber (ADF), neutral detergent fiber (aNDFom), in vitro indigestible neutral detergent fibre (iNDF240) and minerals. Metabolizable energy (ME) was then calculated from the concentrations of other nutrients. Regardless of the stage of regrowth, stems were located mainly in the bottom 1 or 2 strata, while leaves were present mainly in the top 2 or 3 strata. CP, ESC and ME declined, but aNDFom, ADF and iNDF240 increased with stage of regrowth and from top to bottom of the swards ($P<0.05$). While herbage quality variables were affected by both factors, vertical stratum had a much larger impact on quality than stage of regrowth. These results indicate that grazing management of kikuyu pastures should be based not only on stage of regrowth but also on level of defoliation, as both have strong impacts on the nutritive value of the consumed forage.

Keywords: Chemical composition, grazing management, leaf stage, sward structure, tropical pastures.

Resumen

En Gatton Research Dairy, Queensland, Australia, en un diseño factorial en bloques con cuatro repeticiones, se evaluó el efecto de cinco periodos de rebrote (9, 11, 14, 16 y 18 días) y cuatro estratos verticales en el valor nutritivo de pasturas de kikuyo (*Cenchrus clandestinus*). En muestras de pasturas se determinaron la concentración de proteína cruda (PC), carbohidratos solubles en etanol (CSE), fibra detergente acido (FDA) y neutro (FDN), FDN indigestible (FDNi), energía metabolizable (EM) y minerales. Los resultados mostraron que los tallos estuvieron localizados principalmente en los dos estratos inferiores y las hojas en los dos o tres estratos superiores de las pasturas. Consecuentemente, la PC, CSE y EM se redujeron, y FDA, FDN y FDNi se incrementaron con el estado de rebrote y desde la parte superior a la inferior de la pastura. A pesar de que ambos factores experimentales afectaron la calidad del forraje, los estratos verticales afectaron más a la calidad que el estado de rebrote. Estos resultados indican que los dos factores deben ser considerados para el manejo del kikuyo ya que ambos afectan significativamente a la calidad del forraje ingerido.

Palabras clave: Composición química, estructura de pasto, manejo de pastoreo, pasturas tropicales.

Correspondence: M.A. Benvenutti, Queensland Department of Agriculture and Fisheries, Gatton Campus, Lawes, QLD 4343, Australia. Email: Marcelo.Benvenutti@daf.qld.gov.au

Introduction

Kikuyu (*Cenchrus clandestinus* syn. *Pennisetum clandestinum*) is one of the main improved pasture species used by the grazing industries in the northeast of Australia and other tropical and subtropical areas of the world. Previous studies examined the effects of stage of regrowth on herbage quality of leaves, stems and whole plants of kikuyu pastures (Reeves et al. 1996). These early studies provided essential data for the understanding of factors driving herbage quality in kikuyu, such as differences in quality between plant parts at different stages of regrowth; however, these herbage quality results may not necessarily represent the quality of herbage actually consumed by animals grazing these pastures.

It is well known from previous studies that beef and dairy cattle graze pastures in strata, and herbage quality declines from the top to the bottom of the swards (Ungar and Ravid 1999; Benvenutti et al. 2016, 2017; Ison et al. 2018). These studies found that, when grazing pressure increased, cattle were forced to graze forage in the lower strata which was of lower quality and as a result diet quality and animal performance declined. It is important to quantify the herbage quality of different strata of kikuyu pastures to assist members of the grazing industry in making informed decisions on grazing management, based on a sound understanding of the effects of sward structure on diet quality. The aim of this study was to quantify the effects of stage of regrowth and vertical distribution in the sward on physical composition and nutritive value of kikuyu pastures.

Materials and Methods

Experimental design and pasture assessment

The study was conducted during 18 days on established kikuyu pastures at the Gatton Research Dairy, Queensland, Australia (27°32'45" S, 152°19'44" E; 104 masl) in January–February 2016. The kikuyu pasture was planted in 2010 into a black alluvial soil. The effects of 5 regrowth periods (9, 11, 14, 16 and 18 days) and 4 vertical strata on physical structure and nutritive value of the pasture were assessed using a blocked factorial design with 4 replicates. On 18 January 2016 the experimental area (0.2 ha) of kikuyu pasture was divided into 4 blocks of 0.05 ha each and mown to 5 cm in height before 100 kg urea/ha was applied. Plots of 9 m^2 within blocks were randomly allocated to differing regrowth periods. The pasture was irrigated regularly to replace evapotranspiration losses. During the 3 weeks of the experimental period 44 mm of rainfall was received and 65 mm irrigation was applied. A single 1 m^2 sampling area was used on each occasion to assess physical attributes and perform harvests to determine yield and quality attributes of pasture in each of the 4 blocks for the 5 regrowth periods.

Prior to harvesting the 1 m^2 sampling area, 30 random tillers were measured for total non-extended height, stem height and number of fully-expanded leaves (Figure 1). Stem height was defined as the height from ground level to the base of the lamina (ligula) of the top fully-expanded leaf. The averages of total sward height, stem height and number of leaves were then calculated.

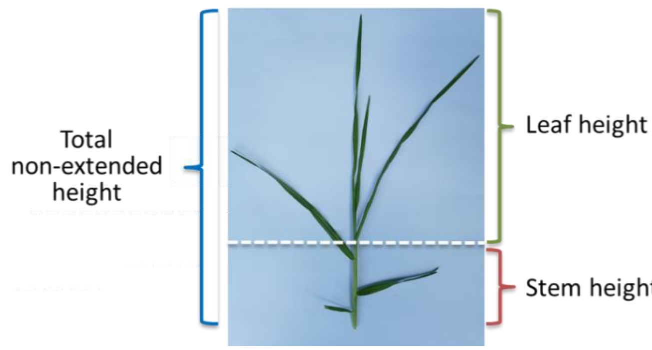

Figure 1. Heights of tillers and plant parts.

Pasture inside the sampling quadrat was then cut in 4 vertical strata. The strata were numbered from top to bottom of the sward so that stratum 1 was the top stratum. The bottom stratum consisted of plant material collected from ground level to 5 cm high. The top 3 strata were equal vertical proportions of pasture above 5 cm. Average total sward height minus 5 cm was divided by 3 to form the 3 strata, indicating that the depth of each stratum varied with the total height of the sward. Within each stratum, harvested material was dried at 60 °C to determine dry matter (DM) yield. Samples from each stratum were combined in pairs of replicates (blocks 1 and 2 and blocks 3 and 4). Combined subsamples from each stratum were then sent for analysis to the Dairy One Forage Lab (Ithaca, NY, USA) to determine crude protein (CP), ethanol-soluble carbohydrates (ESC), acid detergent fiber (ADF), amylase, sodium sulphite-treated neutral detergent fiber expressed on an ash-free basis (aNDFom) and minerals. Subsamples of each stratum were also analyzed for in vitro indigestible NDF from 240-hour incubations with rumen fluid (iNDF240). Metabolizable energy (ME) values were calculated using equations and relationships with other nutrients. The Dairy One Forage Lab uses a multiple component summative approach for its ruminant energy prediction system:

ME (MJ/kg DM) = (1.01 × 0.04409 × TDN – 0.45) × 4.184,

where: TDN is total digestible nutrients (%).

Chemical analysis and in vitro rumen fermentation

The ADF and aNDFom concentrations were determined using the ANKOM Model 200 and the fiber bag technique developed by ANKOM (ANKOM Technology, Macedon, NY, USA). The acid and neutral solutions for these analyses were prepared as per AOAC 973.18 (AOAC 1984) and Van Soest et al. (1991), respectively. ESC was determined by the method of Hall et al. (1999). Samples were analyzed for CP using the AOAC procedure 990.03 (AOAC 1984). To determine mineral concentrations, samples were digested using CEM Microwave Accelerated Reaction System (MARS6) with MarsXpress Temperature Control using 50 ml calibrated Xpress Teflon PFA vessels with Kevlar/fiberglass insulating sleeves then analyzed by ICP using a Thermo iCAP 6300 Inductively Coupled Plasma Radial Spectrometer.

The iNDF240 concentration was determined using 240-hour in vitro fermentation in Daisy Incubators (ANKOM Technology, Macedon, NY, USA) set at 39 °C. Each Daisy incubation cupboard can incubate 4 bottles (24 bags of samples per bottle) at a time with 1,520 mL buffer solution added to each bottle and then combined with 400 mL rumen fluid taken from a fistulated steer. After 120 h fresh rumen fluid was collected and solutions were replenished. After 240 h, bags were removed and rinsed until clear. NDF concentration in the residue was analyzed using the Ankom Fiber Analyzer.

Statistical analysis

Nutritive values for herbage components were analyzed according to a blocked 5 × 4 factorial design using analysis of variance. Here the fixed effects were stage of regrowth at the main-plot level, and vertical stratum as a split-plot effect. GenStat (2016) was used for the analyses.

Results and Discussion

Pasture structure

Leaf numbers in the pastures ranged from 2.2 to 4.7 leaves per tiller (Table 1). This range included the currently recommended stage of regrowth of 4.5 leaves per tiller for grazing kikuyu pastures (Reeves et al. 1996). Regardless of stage of regrowth, all pastures consisted of the same general structure, with stems predominantly located at the bottom of the sward and leaves at the top of the sward (Table 1). The average height of the stems, as a proportion of total sward height, increased from 0.32 to 0.44 as stage of growth advanced from 1 to 5 (Table 1). This indicated that the proportion of stems increased from top to bottom of the sward as well as with the stage of regrowth. A large proportion of the bottom 1 or 2 vertical strata consisted of stems and the top 2 or 3 strata consisted mainly of leaves (Table 2). Similarly, Benvenutti et al. (2016) found that pastures of *Axonopus catarinensis* had a similar sward structure, where the stems were located in the bottom one-third of the sward for a range of regrowth stages. The results are also consistent with the study by Reeves et al. (1996), who found that the proportion of stems increased with the stage of regrowth of kikuyu pastures.

Table 1. Physical structure of kikuyu swards at different stages of regrowth. See also Figure 1.

Stage of regrowth (no. of days)	Pasture height (cm)	Stem height (cm)	Stem height (proportion of pasture height)	Leaf height (cm)	Fully expanded leaves (no.)
1 (9)	13.9	4.4	0.32	9.5	2.2
2 (11)	16.3	5.3	0.32	11.0	2.5
3 (14)	23.7	9.1	0.38	14.6	3.3
4 (16)	29.1	12.5	0.43	16.5	4.1
5 (18)	35.1	15.6	0.44	19.5	4.7
Probability	<0.001	<0.001	<0.001	<0.001	<0.001
s.e.m.	0.798	0.563	0.011	0.423	0.068
LSD (P<0.05)	2.46	1.74	0.033	1.30	0.21

Table 2. The effects of stage of regrowth (SoR) on stem proportion and herbage quality (DM basis) of vertical strata (S1–S4) of kikuyu pastures. S1 to S4: top to bottom stratum.

Regrowth stage and stratum	Stem height (proportion of stratum height)	Crude protein (g/kg)	ADF (g/kg)	aNDFom (g/kg)	iNDF240 (g/kg)	ESC (g/kg)	ME (MJ/kg)	Calcium (g/kg)	Phosphorus (g/kg)	Potassium (g/kg)	Sodium (g/kg)
SoR 1 (9 days)											
S1	0.0	340	212	371	0.05	68.5	11.8	4.60	4.60	29.9	1.35
S2	0.0	338	252	375	0.05	54.5	10.8	4.35	4.90	35.6	1.58
S3	0.0	310	277	445	0.08	46.0	9.8	4.85	5.10	38.3	1.86
S4	0.8	255	333	561	0.21	37.5	8.7	6.90	4.55	36.7	2.46
SoR 2 (11 days)											
S1	0.0	357	228	417	0.07	32.5	11.9	4.35	5.20	39.7	1.25
S2	0.0	335	300	462	0.08	30.0	9.5	4.10	5.70	46.6	1.61
S3	0.0	302	352	512	0.10	26.0	8.6	4.90	5.90	49.5	1.95
S4	0.9	247	366	595	0.24	34.0	8.0	7.50	4.65	37.2	2.29
SoR 3 (14 days)											
S1	0.0	350	294	422	0.06	39.0	10.7	3.90	5.25	40.7	0.94
S2	0.0	314	319	462	0.09	22.0	9.4	3.35	6.00	51.4	1.16
S3	0.5	295	309	495	0.13	18.0	9.5	3.85	6.05	54.9	1.52
S4	1.0	242	352	600	0.28	28.0	8.4	6.80	5.00	41.5	2.20
SoR 4 (16 days)											
S1	0.0	329	287	446	0.07	39.5	9.6	3.90	4.60	45.1	0.75
S2	0.0	303	280	491	0.08	31.5	9.8	3.05	5.15	55.3	0.98
S3	0.9	279	322	510	0.13	26.0	8.9	3.90	5.40	59.3	1.47
S4	1.0	228	362	602	0.28	19.0	8.0	6.75	3.95	40.7	2.14
SoR 5 (18 days)											
S1	0.0	314	285	451	0.07	42.5	10.3	3.95	4.60	43.2	1.13
S2	0.0	293	284	497	0.08	37.5	10.0	3.35	5.25	54.5	1.23
S3	0.9	268	329	522	0.13	27.5	8.8	4.15	5.05	59.2	1.60
S4	1.0	226	369	600	0.26	28.0	7.5	6.70	4.20	45.8	2.29
P SoR	0.002	0.049	0.072	0.014	<0.001	0.15	0.009	0.008	0.029	0.01	<0.001
P Stratum	<0.001	<0.001	<0.001	<0.001	<0.001	0.01	<0.001	<0.001	<0.001	<0.001	<0.001
P SoR × Stratum	<0.001	0.046	0.068	0.032	0.104	0.735	0.004	0.389	<0.001	<0.001	0.053
s.e.m.	0.04	7.18	15.14	11.49	0.007	8.29	0.25	0.20	0.14	1.70	0.05
LSD (P<0.05)	0.12	24.1	45.3	36	0.022	25.2	0.74	0.59	0.50	6.09	0.16

ESC – ethanol-soluble carbohydrates; ADF – acid detergent fiber; aNDFom – amylase and sodium sulphite-treated neutral detergent fiber expressed on an ash-free basis; iNDF240 – in vitro indigestible neutral detergent fiber from 240-hour incubations with rumen fluid; ME – metabolizable energy; stratum numbering from top (S1) to bottom (S4).

Nutritive value

Since there was a significant interaction (P<0.05) between experimental factors (stages of regrowth and vertical strata) for most herbage quality variables, results are shown for all combinations of factors in Table 2. While herbage quality was significantly affected by both factors, vertical stratum had a much larger impact on quality than stage of regrowth for most quality variables. This is explained in detail below for each herbage quality variable.

It is likely that the observed differences in herbage quality between vertical strata and between stages of regrowth were due to the differences in the proportion of different plant parts or to their changes in quality as the pasture matured (Table 2). The differences in herbage quality between strata were largely due to the differences in the proportions of leaves and stems in the different strata (Table 2). Consistently, Fulkerson et al. (2010) reported a significant difference in herbage quality between leaves and stems in kikuyu pastures. Since herbage quality differed between stages of regrowth for all strata, the observed differences in quality between stages of regrowth could be attributed to changes in quality of individual plant parts as the pasture matured. Reeves et al. (1996) found that the CP and mineral concentrations of leaves changed significantly with the stage of regrowth of kikuyu pastures.

CP and ESC concentrations declined significantly as stage of regrowth increased and from top to bottom of the swards (P<0.05). These quality variables were more affected by the vertical stratum than the stage of regrowth. While the average decreases in CP and ESC concentrations from stage 1 to stage 5 of regrowth were 11 and 34%, respectively, the average declines from the top to the bottom stratum were 25 and 44%, respectively. Previous studies also found that CP% declined with the stage of regrowth in kikuyu pastures (Reeves et al. 1996) and from top to bottom in pastures of *Axonopus catarinensis* (Benvenutti et al. 2016).

In contrast, concentrations of aNDFom, ADF and iNDF240 increased significantly with stage of regrowth and from top to bottom of the sward (P<0.05). These variables were also more affected by the vertical stratum than the stage of regrowth. While the average increases in concentrations of aNDFom, ADF and iNDF240 from stage 1 of regrowth to stage 5 were 20, 19 and 48%, the average increases from the top to the bottom stratum were 40, 36 and 300%, respectively. These results indicate that the digestibility of the plant material would decrease at later stages of regrowth and from top to bottom of the sward. This confirms the work of Reeves et al. (1996), who found that organic matter digestibility decreased as stage of regrowth increased in kikuyu pastures.

As might be expected, ME decreased consistently when ESC decreased and aNDFom, ADF and iNDF240 increased. Therefore, ME significantly declined with stage of regrowth and from top to bottom of the sward (P<0.05). This quality variable was also more affected by the vertical stratum than stage of regrowth. While the average decrease of ME from stage 1 of regrowth to stage 5 was 11%, the average decline from the top to the bottom stratum was 25%.

The effects of stage of regrowth and vertical stratum on mineral concentration in the plant material differed between minerals (Table 2). Concentrations of calcium and sodium increased from top to bottom of the sward, while concentrations of potassium and phosphorus increased from stratum 1 to stratum 3 and then decreased for the bottom stratum. Unlike potassium, concentrations of calcium and sodium decreased with stage of regrowth. In turn, the concentration of phosphorus increased from stage 1 of regrowth to stage 3 and then decreased. On the contrary, Reeves et al. (1996) found that, as leaves of kikuyu pastures matured, calcium concentration increased while those of potassium and phosphorus decreased, but sodium concentration did not change with maturity.

Conclusion

This study has shown that, regardless of the stage of regrowth, stems were located mainly in the bottom one or two strata, while leaves were present mainly in the top two or three strata. This indicates that herbage quality declines significantly from top to bottom of the sward as it is grazed. In addition, herbage quality declines significantly with the stage of regrowth for all vertical strata, probably as a result of changes in nutritive value within plant parts as the pasture matures. While herbage quality was affected by both factors, the vertical stratum had a much larger impact on nutritive value than stage of regrowth.

We conclude that grazing management of kikuyu pastures should be based not only on the stage of regrowth but also on the level of defoliation, as both have strong impacts on the herbage quality of forage consumed by the animals. By considering these issues, members of the grazing industry can now exert greater control over the nutritive value of the forage consumed by the animals by controlling the level of defoliation of the pasture according to its stage of regrowth. Similar studies on other pasture species, e.g. erect species, would verify if these concepts are relevant to species with different growth habits.

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References

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AOAC (Association of Official Analytical Chemists). 1984. Official methods of analysis. AOAC Inc., Arlington, VA, USA.

Benvenutti MA; Pavetti DR; Poppi DP; Gordon IJ; Cangiano CA. 2016. Defoliation patterns and their implications for the management of vegetative tropical pastures to control intake and diet quality by cattle. Grass and Forage Science 71:424–436. doi: 10.1111/gfs.12186

Benvenutti MA; Pavetti DR; Poppi DP; Mayer DG; Gordon IJ. 2017. Ingestive behaviour and forage intake responses of young and mature steers to the vertical differentiation of sugarcane in pen and grazing studies. The Journal of Agricultural Science 155:1677–1688. doi: 10.1017/S0021859617000673

Fulkerson WJ; Griffiths N; Sinclair K; Beale PN. 2010. Milk production from kikuyu grass based pastures. Primefact No. 1068. NSW Department of Primary Industries, Orange, NSW, Australia. bit.ly/39VFjOF

GenStat. 2016. GenStat Statistical Package for Windows. 2016 Edn. Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, UK.

Hall MB; Hoover WH; Jennings JP; Webster TKM. 1999. A method for partitioning neutral detergent-soluble carbohydrates. Journal of the Science of Food and Agriculture 79:2079–2086. DOI: 10.1002/(sici)1097-0010(199912)79:15<2079::aid-jsfa502>3.3.co;2-q

Ison K; Barber DG; Benvenutti MA; Mayer DG; Findsen C. 2018. The effect of grazing intensity of lucerne (*Medicago* *sativa*) pasture on defoliation dynamics and pasture intake in dairy cows. Proceedings of the 32nd Biennial Conference of the Australian Society of Animal Production, Wagga Wagga, New South Wales, Australia, 2–6 July 2018. p. 87. doi: 10.1071/ANv58n8abs

Reeves M; Fulkerson WJ; Kellaway RC. 1996. Forage quality of kikuyu (*Pennisetum clandestinum*): The effect of time of defoliation and nitrogen fertiliser application and in comparison with perennial ryegrass (*Lolium perenne*). Australian Journal of Agricultural Research 47:1349–1359. doi: 10.1071/AR9961349

Ungar ED; Ravid N. 1999. Bite horizons and dimensions for cattle grazing herbage to high levels of depletion. Grass and Forage Science 54:357–364. doi: 10.1046/j.1365-2494.1999.00188.x

Van Soest PJ; Robertson JB; Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583–3597. doi: 10.3168/jds.S0022-0302(91)78551-2

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Short Communication

In vitro digestion characteristics of various combinations of elephant grass hay, gliricidia hay or silage, soybean meal and corn meal in rations for sheep

Características de la digestión in vitro de varias combinaciones de heno del pasto elefante, heno o ensilaje de gliricidia, harina de soya y harina de maíz en raciones para ovinos

JULIANA CAROLINE SANTOS SANTANA[1], JUCILEIA APARECIDA DA SILVA MORAIS[2], GELSON DOS SANTOS DIFANTE[1], LUÍS CARLOS VINHAS ÍTAVO[1], ANTONIO LEANDRO CHAVES GURGEL[1], VINICIUS DA SILVA OLIVEIRA[2] AND MARIA JUCIARA SILVA TELES RODRIGUES[2]

[1]*Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.* famez.ufms.br
[2]*Departamento de Zootecnia, Universidade Federal de Sergipe, Aracaju, SE, Brazil.* ufs.br

Abstract

This study examined fermentation rates and kinetics of sheep rations based on combinations of elephant grass hay, gliricidia (*Gliricidia sepium*) hay or silage, soybean meal and corn meal using in vitro techniques. Three rations were prepared, namely: Control (elephant grass hay + soybean meal + corn meal); gliricidia hay (elephant grass hay + soybean meal + corn meal + gliricidia hay); and gliricidia silage (elephant grass hay + soybean meal + corn meal + gliricidia silage). A fixed ratio of roughage:concentrate of 55:45 was maintained for all rations, which were isocaloric and designed to support sheep gains of 200 g/day. The gliricidia replaced 57.6% of the soybean meal in the rations containing gliricidia and 81.8% of the elephant grass hay. Fermentation rates and kinetics, in vitro dry matter digestibility (IVDMD) and degradability of the rations were evaluated. Rations containing gliricidia as both hay and silage had higher ($P<0.05$) IVDMD than the Control ration (67.8 and 66.2 vs. 59.8%). The degradability of the ration containing gliricidia hay was higher ($P<0.05$) than that of the gliricidia silage ration (57.8 vs. 50.5%), whereas the Control showed an intermediate value (54.4%). The ration containing gliricidia hay and the Control produced more gas in the first 24 h than the ration containing gliricidia silage, and the gliricidia hay ration showed the shortest colonization time. Peak gas production occurred for the ration with gliricidia silage later than for the other rations. The study showed that substituting soybean meal with preserved gliricidia can result in higher digestibility of sheep rations. Feeding studies with animals are now warranted to verify these laboratory findings.

Keywords: Degradability, digestibility, gas production, *Gliricidia sepium*, sheep farming, tropical legumes.

Resumen

En el estudio se determinaron las tasas y la cinética de fermentación ruminal de raciones para ovinos basadas en combinaciones de heno de pasto elefante, heno o ensilaje de gliricidia (*Gliricidia sepium*), harina de soya y harina de maíz, utilizando técnicas in vitro. Se prepararon tres raciones: Control (heno de pasto elefante + harina de soya + harina de maíz); heno de gliricidia (heno de pasto elefante + harina de soya + harina de maíz + heno de gliricidia); y ensilaje

Correspondence: Juliana Caroline Santos Santana, Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Avenida Senador Filinto Müller 2.443, Campo Grande, CEP 79074-460, MS, Brazil.
Email: jukrol_@hotmail.com

de gliricidia (heno de pasto elefante + harina de soya + harina de maíz + ensilaje de gliricidia). Se mantuvo una proporción fija de forraje:concentrado de 55:45 para todas las raciones las cuales fueron isocalóricas y diseñadas para producir ganancias diarias de 200 g peso vivo de ovinos. En ambos tratamientos con gliricidia el 57.6% de la harina de soya fue reemplazado y el 81.8% del heno de pasto elefante. Se evaluaron las tasas y cinética de la fermentación ruminal, la digestibilidad in vitro de la materia seca (IVDMD) y la degradabilidad de las dietas. Las raciones que contenían gliricidia como heno y ensilaje tuvieron una IVDMD más alta (P<0.05) que el Control (67.8 y 66.2 vs. 59.8%). La degradabilidad de la ración con heno de gliricidia fue mayor (P<0.05) que la de la ración con ensilaje de gliricidia (57.8 vs. 50.5%), mientras que el Control presentó un valor intermedio (54.4%). La ración con heno de gliricidia y el Control produjeron más gas en las primeras 24 horas que la ración con ensilaje de gliricidia, y la ración con heno de gliricidia mostró el menor tiempo de colonización. La producción máxima de gas ocurrió en la ración con ensilaje de gliricidia más tarde que en las otras raciones. El estudio mostró que sustituyendo la harina de soya por forraje preservado de gliricidia puede resultar en una digestibilidad más alta de raciones para ovinos. Para corroborar estos resultados obtenidos a nivel de laboratorio se requieren estudios de alimentación con animales.

Palabras clave: Degradabilidad, digestibilidad, ganadería ovina, *Gliricidia sepium,* producción de gas.

Introduction

Pasture-based production systems are limited primarily by variations in climatic conditions, which directly interfere with plant growth (Euclides et al. 2019), resulting in negative impacts on animal performance, especially during the dry season (Emerenciano Neto 2018; Braga et al. 2019). One possible solution is to conserve forage in periods of high availability to be used during times of scarcity (Bueno et al. 2018), along with feeding of protein supplements.

The use of high-protein feedstuffs in sheep rations is a common practice worldwide and soybean meal ranks highly as a protein source. Since soybeans are also used for human consumption, costs of this product for feeding livestock are high. Identifying a less expensive plant-derived protein would be of great benefit to ruminant production worldwide.

Shrub legumes are possible options as alternative sources of fodder which are high in protein, e.g. gliricidia (*Gliricidia sepium*), which grows well in tropical climates and is relatively drought-tolerant. Since its chemical and productive characteristics are similar to those of other leguminous species, it is a viable option for animal feeding, especially in regions where water deficit is a constant problem (Fernandes et al. 2017; Santana et al. 2019; Fernandes et al. 2020). Incorporating it in rations should allow a reduction in the level of soybean meal required to supply the protein needs of the sheep.

As a prelude to conducting feeding trials with animals, which also are expensive, in vitro studies in the laboratory can provide preliminary data on likely outcomes from feeding various rations. The in vitro gas production method described by Theodorou et al. (1994) consists of incubating samples of feedstuffs in bottles attached to a gas meter. According to Tagliapietra et al. (2010), the gas released from feedstuffs inoculated with rumen fluid reflects microbial activity, as gas is a product of fermentation.

We conducted this study to obtain preliminary data on the fermentation rates and kinetics, dry matter digestibility and degradability of 3 sheep rations made up of mixtures of hay of elephant grass (*Cenchrus purpureus* syn. *Pennisetum purpureum*), gliricidia as hay or silage, soybean meal and corn meal using in vitro techniques.

Materials and Methods

The experiment was conducted at the Laboratories of Animal Nutrition and Rumen Fermentation at the Department of Animal Science (DZO), Federal University of Sergipe (UFS), Aracaju, Sergipe. It was set up as a completely randomized design with 3 treatments, i.e. 3 rations formulated for sheep, namely: Control – basal ration of elephant grass hay-soybean meal-corn meal; gliricidia hay – elephant grass hay-gliricidia hay-soybean meal-corn meal with only 42.6% of the soybean meal supplement contained in Control; and gliricidia silage – elephant grass hay-gliricidia silage-soybean meal-corn meal with 42.6% of the soybean meal supplement contained in Control. These rations were formulated for sheep to achieve an estimated dry matter intake of 3.5% of body weight and 200 g/day liveweight gain (NRC 2007) and are described in Table 1. Since the roughage:concentrate ratio was fixed at 55:45, including gliricidia hay or silage reduced percentage of elephant grass hay and soybean meal in the ration, while percentage of corn meal was increased. The concentrations of crude protein in elephant grass hay, gliricidia hay and gliricidia silage, soybean meal and corn meal were, respectively: 12.9; 15.7; 17.5; 42.5 and 8.4% on a dry matter basis.

Table 1. Proportions of ingredients and nutrient concentrations in the experimental rations.

	Ration		
	Control	GH	GS
Ingredient (% DM basis)			
Elephant grass hay	55.0	10.0	10.0
Gliricidia hay	-	45.0	-
Gliricidia silage	-	-	45.0
Soybean meal	23.5	10.0	10.0
Corn meal	21.5	35.0	35.0
Nutrient concentration (% DM)			
Organic matter	93.9	94.7	93.9
Crude protein	21.7	18.3	18.3
Hemicellulose	28.2	30.3	27.0
Cellulose	28.6	22.0	21.2
Lignin	2.6	6.6	8.2

GH = Gliricida hay; GS = Gliricidia silage.

The gliricidia used to produce the hay and silage was obtained from trees at approximately 12 months after planting, by cutting and selecting tender branches (≤8 mm thick) with leaves, from the EVA (Interdisciplinary Space of Agroecological Experience) area adjacent to the Department of Animal Science (DZO) at UFS. The forage was then chopped to produce an average particle size of 2 cm, spread on plastic sheeting in the sun and turned every 30 min. After 2 days, the hay was bagged. The elephant grass was cut approximately 5 cm above ground level at 45 days growth and the hay-making process was the same as that for gliricidia.

Three experimental PVC silos 10 cm in diameter and 30 cm long with PVC caps at each end were used, and were sealed with metal clips. The fresh chopped gliricidia was compacted in these silos to a specific mass of 600 kg/m^3. To ensure anaerobic conditions in the silos, adhesive tape was used to promote better sealing than with the metal clips alone. The silos were weighed before and just after sealing. There was a layer of sand at the bottom of the silo to retain effluents and prevent contamination of the silage; a permeable mesh between the silage and the sand layer prevented contact between the sand and the silage.

In vitro gas production from the various rations was determined in accordance with the methodology described by Theodorou et al. (1994). To this end, after being dried in a forced-air oven, ration samples were ground through a Wiley mill with a 5-mm sieve.

Rumen fluid was collected from 3 sheep maintained on a diet of corn, soybean meal and fresh gliricidia and samples were mixed/bulked to form the rumen inoculum, which was filtered through gauze and stored in a thermos previously heated in water at 38 ºC. A constant flow of CO_2 was maintained during the preparation process.

For each ration 5 samples were incubated in penicillin-type glass bottles with a capacity of 100 mL. Each bottle contained 670 mg of sample in 67 mL of incubation solution. The incubation solution was prepared as described by Theodorou et al. (1994) using cysteine-HLC as a reducing agent (Mould et al. 2005).

Rumen fluid formed 20% of the incubation solution. A constant flow of CO_2 was maintained. Each bottle was inoculated manually by using a graduated syringe, and bottles were closed with rubber corks (14 mm), sealed with an aluminum seal and kept in a water bath at 39 ºC. In addition to the bottles with the samples, an additional 4 bottles containing incubation medium without samples (blanks) were evaluated.

Gas production was measured for a period of 48 h, and the pressure within the bottle was recorded with a digital manometer coupled to a 3-way valve. Immediately after the pressure readings, gas volume was measured using a graduated syringe attached to the valve. The syringe plunger was extracted until the transducer pressure returned to zero.

Gas volume and pressure data were tabulated to obtain the linear, quadratic and cubic statistical models and then determine the correlation between gas volume and pressure reading using Excel software. The model considered satisfactory was that which showed the highest R^2 value ($R^2 = 0.95$).

The following equation was obtained:

$y = -0.382x^2 + 6.087x - 0.772$,

where:

y is the final gas volume in mL; and

x is the gas pressure in kilopascal at the respective times.

Gas production on each occasion was corrected for the average gas production from bottles containing the incubation medium without diet samples. Mean gas production volumes for each treatment at the respective incubation times were adjusted to the 2-compartment logistic model proposed by Pell and Schofield (1993), as follows:

$TV = Vf1/(1 + \exp(2 - 4*c1*(T - L))) + Vf2/(1 + \exp(2 - 4*c2*(T - L)))$,

where:

TV = total gas volume (mL/100 mg DM) accumulated by time T;

Vf1 = gas volume (mL) of the rapidly digested fraction;

c1 = degradation rate of the rapidly digested fraction (L/h);

L = lag time, or fiber colonization time (h);

Vf2 = gas volume (mL) of the slowly digested fraction; and

c2 = degradation rate of the slowly digested fraction (L/h).

After 48 hours of incubation, the contents of the bottles (which had been tared previously) were filtered through non-woven fabric ('TNT') of known dimensions. Dry matter degradability was determined based on the difference between the constant weight obtained by drying at 105 ºC and the weight of the incubated material. To determine the in vitro dry matter digestibility (IVDMD), the residue was washed with neutral detergent and weighed after drying at 105 ºC in a forced-air oven for 24 h and expressed as a percentage of the initial weight of the sample. The ash concentration in the residue was determined by method 942.05 of AOAC (1990).

The parameters estimated by the mathematical model were obtained using iterative non-linear methods. Results were adjusted by least-squares estimates, adopting the Marquardt method, by the PROC NLIN procedure of SAS statistical package (SAS Institute Inc., Cary, NC, USA). Data pertaining to degradability, digestibility and residual ash were subjected to analysis of variance and means were compared by Tukey's test at the 5% significance level.

Results

Rations containing gliricidia had higher IVDMD levels than the Control (P<0.001; Table 2). While degradability of the ration containing gliricidia hay was higher than that of the ration containing gliricidia silage (P = 0.015), degradability of the Control was intermediate.

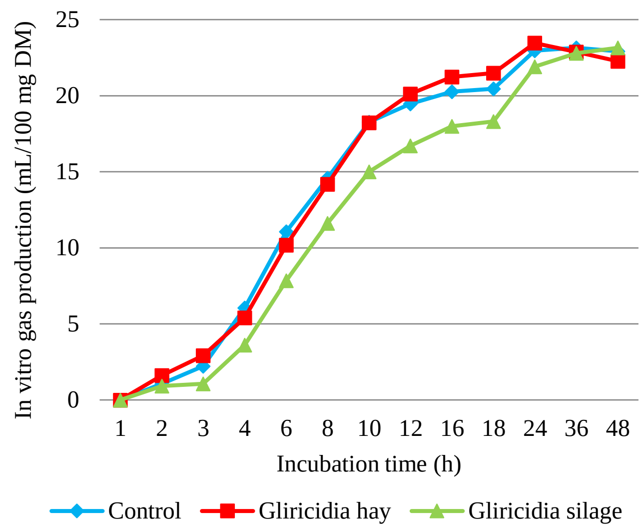
Table 2. In vitro dry matter digestibility (IVDMD) and degradability of rations comprised of varying combinations of elephant grass hay, gliricidia hay or silage, soybean meal and corn meal designed to produce 200 g/d gain in sheep.

	Ration			P value	s.e.m.
	Control	GH	GS		
IVDMD (%)	59.8b	67.8a	66.2a	<0.001	0.70
Degradability (%)	54.4ab	57.8a	50.5b	0.015	1.59

Means within rows followed by different letters differ (P<0.05) according to Tukey's test. Control (elephant grass hay + soybean meal + corn meal); GH: gliricidia hay treatment (elephant grass hay + soybean meal + corn meal + gliricidia hay); GS: gliricidia silage treatment (elephant grass hay + soybean meal + corn meal + gliricidia silage).

Significant (P<0.05) differences in accumulation of gas over time were observed for the different rations. The Control treatment and the ration containing gliricidia hay produced more gas in the first 24 h than the ration containing gliricidia silage, while the ration containing gliricidia hay showed the shortest colonization time. Peak gas production occurred later for the ration containing gliricidia silage (Figure 1).

The ration containing gliricidia silage produced the smallest volume of gas from the highly soluble fraction (12.4 mL/100 mg of incubated DM) and showed the longest fiber colonization (lag) time (3.8 h). This ration also produced the largest volume of gas from the lowly soluble fraction (10.7 mL/100 mg of incubated DM).



Control: $GP = 15.6185/\{1 + \exp^{[2+4*0.1925*(2.8873-Time)]}\} + 7.4599/\{1 + \exp^{[2+4*0.05*(2.8873-Time)]}\}$, $R^2 = 0.99$;
Hay: $GP = 15.5116/\{1 + \exp^{[2+4*0.1608*(2.7619-Time)]}\} + 7.2305/\{1 + \exp^{[2+4*0.0677*(2.7619-Time)]}\}$, $R^2 = 0.99$;
Silage: $GP = 12.4415/\{1 + \exp^{[2+4*0.2037*(3.8231-Time)]}\} + 10.6961/\{1 + \exp^{[2+4*0.0438*(3.8231-Time)]}\}$, $R^2 = 0.99$.

Figure 1. In vitro gas production for Control (elephant grass hay + soybean meal + corn meal), gliricidia hay treatment (elephant grass hay + soybean meal + corn meal + gliricidia hay) and gliricidia silage treatment (elephant grass hay + soybean meal + corn meal + gliricidia silage) following incubation in buffered rumen fluid.

Discussion

This study suggests that using gliricidia hay or silage to replace some of the soybean meal and elephant grass hay components of an elephant grass-soybean meal-corn meal ration (Control) for growing sheep could result in an increase in IVDMD of the ration, while degradability of the ration might be improved only in the case of gliricidia hay.

The lower IVDMD of the Control ration could be a function of a higher proportion of cellulose provided by the elephant grass hay in this ration (Table 1), since this component reduces both the level and rate of fiber degradation (Díaz et al. 2018). In general, tropical forages are poorly digestible (Gerdes et al. 2000; Oliveira et al. 2017), which is due mainly to the high concentration of cell wall components such as cellulose and low concentration of potentially digestible compounds such as non-fibrous carbohydrates, proteins, ether extract, vitamins and minerals (Oliveira et al. 2017). In the case of elephant grass hay the material used was whole plant material cut at 45 days of age, while the gliricidia was comprised of only fine stems and leaves. Inclusion of gliricidia in the ration allowed the proportion of soybean meal to be reduced, while the proportion of corn increased, increasing the supply of soluble carbohydrates to the population of microorganisms, which may have increased microbial activity.

The higher degradability of the ration including gliricidia hay could be a consequence of longer degradation time for the gliricidia hay, given the shorter colonization time on the particles by the incubated microorganisms. On the other hand, the lower degradability of the ration containing gliricidia silage may be a reflection of fermentation of carbohydrates during the ensiling process, since soluble carbohydrates serve as substrates for the growth of anaerobic bacteria. This, in turn, prompts a decline in the pH of the medium, resulting in the preservation of the material (Gomes et al. 2018; Santana et al. 2019). As a consequence, the levels of non-fibrous carbohydrates (NFC) (a dietary component with higher rumen degradation rates; Oliveira et al. 2017) decrease during the ensiling process (Ribeiro et al. 2014). Conversely, the higher gas production during the initial 24 h of fermentation from both the Control ration and that containing gliricidia hay was likely a consequence of their higher NFC concentration in comparison with the gliricidia silage diet, resulting in increased IVDMD. Oliveira et al. (2017) measured in vitro gas production from a range of forage plants and observed that higher fermentation rates within the first hours of incubation were detected in plants with higher soluble carbohydrate concentrations.

The different results for digestibility found in this study between the Control ration and rations containing gliricidia (hay or silage) demonstrate the beneficial effects of this plant, since incorporating gliricidia in the ration increased the amount of digested material without affecting gas production.

Since NFCs are considered the main substrate for lactic fermentation within the silo, their concentration decreases throughout the ensiling process (Zardin et al. 2017). The ration containing gliricidia silage showed longer fermentation times and higher gas production from the fibrous material, which is considered a slowly degraded component.

The time taken by dietary microorganisms to colonize the material influences gas production, since this parameter determines how long the rumen microorganisms will act on the substrate. Thus, peak production may be achieved sooner or later (Díaz et al. 2018). The shorter colonization time observed in the ration containing gliricidia hay allowed the microorganisms to act for a longer period, with peak production occurring sooner in this treatment.

The ration containing gliricidia silage showed the lowest values for fermentation parameters, which may be explained by its higher lignin and lower hemicellulose concentrations (Table 1). Lignin present in the cell wall complexes itself to carbohydrates (mainly hemicellulose) through covalent bonds, forming a mechanical barrier to rumen microorganisms, thus reducing the fermentation of carbohydrates (Oliveira et al. 2017; Díaz et al. 2018). This fact also explains why the Control and gliricidia-hay rations presented shorter lag phases, higher gas volumes and higher rates of gas production from rapidly digested fractions.

Conclusions

Incorporating gliricidia as hay or silage in traditional sheep rations based on elephant grass hay, soybean meal and corn meal should allow a reduction in amounts of elephant grass and more importantly soybean meal in the ration. These rations should also be more digestible than the traditional ones, which should result in better animal performance. Added to this, reducing the proportion of expensive soybean meal should lower the cost, while increasing the corn meal component would cancel out some cost advantages.

Further studies with sheep to determine feed intakes and animal performance on these or similar rations are needed to confirm if these laboratory findings can be reflected in improved production. Other shrub legumes might also be used depending on availability. If feeding studies are successful, the likely financial benefits to farmers would depend on the relative costs of the ration components, i.e. elephant grass hay, gliricidia hay or silage and soybean meal and corn meal.

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References

(Note of the editors: All hyperlinks were verified 7 April 2020.)

AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis. 15th Edn. AOAC Inc., Arlington, VA, USA

Braga GJ; Maciel GA; Guimarães Jr R; Ramos AKB; Carvalho MA; Fernandes FD; Fonseca CEL; Jank L. 2019. Performance of young Nellore bulls on guineagrass pastures under rotational stocking in the Brazilian Cerrado. Tropical Grasslands-Forrajes Tropicales 7:214–222. doi: 10.17138/TGFT(7)214-222

Bueno AVI; Jobim CC; Rossi RM; Gritti VC; Leão GFM; Tres TT. 2018. Wilting whole crop black oat with glyphosate for ensiling: Effects on nutritive, fermentative, and aerobic stability characteristics. Revista Brasileira de Zootecnia 47:1–7. doi. 10.1590/rbz4720170142

Díaz TG; Branco AF; Ítavo LCV; Santos GT dos; Carvalho ST; Teodoro AL; Oliveira RL. 2018. In vitro gas production kinetics and digestibility in ruminant diets with different levels of cashew nut shell liquid. Semina: Ciências Agrárias 39:1669–1682. doi: 10.5433/1679-0359.2018v39n4p1669

Emerenciano Neto JV; Difante GS; Lana ÂMQ; Medeiros HR; Aguiar EM; Montagner DB; Souza JS. 2018. Forage quality and performance of sheep in Massai grass pastures managed at pre-grazing canopy heights. South African Journal of Animal Science 48:1073–1081. doi: 10.4314/sajas.v48i6.10

Euclides VPB; Montagner DB; Macedo MCM; Araújo AR de; Difante GS; Barbosa RA. 2019. Grazing intensity affects forage accumulation and persistence of Marandu palisade grass in the Brazilian savannah. Grass and Forage Science 75:1–13. doi: 10.1111/gfs.12422

Fernandes LS; Difante GS; Montagner DB; Emerenciano Neto JV; Araújo IMM de; Campos NRF. 2017. Structure of massai grass pasture grazed on by sheep supplemented in the dry season. Grassland Science 63:177–183. doi: 10.1111/grs.12165

Fernandes LS; Difante GS; Emerenciano Neto JV; Araújo IMM de; Veras ELL; Costa MG. 2020. Performance of sheep grazing *Panicum maximum* cv. Massai and supplemented with protein sources during the dry season. South African Journal of Animal Science 50:1–8. bit.ly/34RzH6j

Gerdes L; Werner JC; Colozza MT; Possenti RA; Schammass EA. 2000. Avaliação de características de valor nutritivo das gramíneas forrageiras Marandu, Setária e Tanzânia nas estações do ano. Revista Brasileira de Zootecnia 29:955–963. doi: 10.1590/S1516-35982000000400003

Gomes ALM; Jacovaci FA; Bolson DC; Nussio LG; Jobin CC; Daniel JLP. 2018. Effects of light wilting and heterolactic inoculant on the formation of volatile organic compounds, fermentative losses and aerobic stability of oat silage. Animal Feed Science and Technology 247:194–198. doi: 10.1016/j.anifeedsci.2018.11.016

Mould FL; Morgan R; Kliem KE; Krystallidou E. 2005. A review and simplification of the in vitro incubation medium. Animal Feed Science and Technology 123–124:155–172. doi: 10.1016/j.anifeedsci.2005.05.002

NRC (National Research Council). 2007. Nutrient requirements of sheep. National Academies Press, Washington, DC, USA.

Oliveira VS; Morais JAS; Muniz EM; Fagundes JL; Lima IGS; Santana JCS; Santos CB. 2017. Cinética ruminal de forrageiras tropicais submetidas ou não a sistema de irrigação. Boletim de Indústria Animal 74:195–204. doi: 10.17523/bia.v74n3p195

Pell A; Schofield P. 1993. Computerized monitoring of gas production to measure forage digestion in vitro. Journal of Dairy Science 76:1063–1073. doi: 10.3168/jds.S0022-0302(93)77435-4

Ribeiro LSO; Pires AJV; Carvalho GGP de; Pereira MLA; Santos AB dos; Rocha LC. 2014. Fermentation characteristics, chemical composition and fractionation of carbohydrates and crude protein of silage of elephant grass wilted or with addition of castor bean meal. Semina: Ciências Agrárias 35:1447–1462. doi: 10.5433/1679-0359.2014v35n3p1447

Santana JCS; Morais JAS; Santos MSAA; Gurgel ALC; Muniz EN; Oliveira VS. 2019. Características fermentativas, composição química e fracionamento da proteína da silagem de gliricídia submetida a diferentes períodos de fermentação. Boletim de Indústria Animal 76:1–9. doi: 10.17523/bia.2019.v76.e1436

Tagliapietra F; Cattani M; Bailoni L; Schaivon S. 2010. *In vitro* rumen fermentation: Effect of headspace pressure on the gas production kinetics of corn meal and meadow hay. Animal Feed Science and Technology 158:197–201. doi: 10.1016/j.anifeedsci.2010.04.003

Theodorou MK; Williams BA; Dhanoa MS; McAllan AB; France J. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Animal Feed Science and Technology 48:185–197. doi: 10.1016/0377-8401(94)90171-6

Zardin PB; Velho JP; Jobim CC; Alessio DRM; Haygert-Velho IMP; Conceição GM da; Almeida PSG. 2017. Chemical composition of corn silage produced by scientific studies in Brazil – A meta-analysis. Semina: Ciências Agrárias 38:503–512. doi: 10.5433/1679-0359.2017v38n1p503

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Short Communication

Evaluation of *Asystasia gangetica* as a potential forage in terms of growth, yield and nutrient concentration at different harvest ages

Evaluación del potencial forrajero de Asystasia gangetica *a diferentes edades de cosecha*

N.R. KUMALASARI, L. ABDULLAH, L. KHOTIJAH, L. WAHYUNI, INDRIYANI, N. ILMAN AND F. JANATO

Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor, Indonesia. www.intp.fapet.ipb.ac.id

Abstract

The objective of this experiment was to analyze growth dynamics, yield and nutrient concentration of *Asystasia gangetica* (L.) T. Anderson at different harvest ages. A pot experiment was conducted at Green House Laboratory of Agrostology, Faculty of Animal Science, Bogor Agricultural University, Indonesia, during the growing season of 2018. Seedlings were transplanted into 115 polybags arranged in a completely randomized design with 23 replications. Plant height, number of leaves, number of branches, dry matter (DM) yields and nutrient concentrations at 30, 40, 50, 70 and 90 days after transplanting (DAT) were determined. Whereas plant height, number of leaves, number of branches and DM yields increased with age, nutrient concentrations followed different patterns. Crude protein % in leaf peaked at 24.2% at 40 DAT then decreased progressively to 8.4% at 90 DAT, while corresponding figures for stem were 10.6 and 2.8%, respectively. Crude fiber concentrations in leaf increased from 10.6% at 30 days to 17.3% at 90 days; corresponding figures for stem were 23.2 and 39.2%. From this pot study, cutting between 40 and 50 days after planting seemed to represent a suitable compromise between DM yield and protein percentage. Studies are needed to determine the repeatability of these results under field conditions and the regrowth potential of plants following harvesting.

Keywords: Growth dynamics, nutritive value, forage production.

Resumen

En condiciones de invernadero, en el Green House Laboratory of Agrostology, Faculty of Animal Science, Bogor Agricultural University, Indonesia, se analizaron la dinámica de crecimiento, el rendimiento y la concentración de nutrientes de *Asystasia gangetica* (L.) T. Anderson a diferentes edades de las plantas. Las plantas crecieron en 115 bolsas de plástico que fueron dispuestas en un diseño completamente al azar con 23 repeticiones. Se evaluaron la altura de la planta, el número de hojas y de ramas, el rendimiento de materia seca (MS) y las concentraciones de nutrientes a los 30, 40, 50, 70 y 90 días después de la siembra. Mientras que la altura de planta, el número de hojas y de ramas, y el rendimiento de MS aumentaron con la edad de las plantas, las concentraciones de nutrientes mostraron tendencias diferentes. El porcentaje de proteína cruda en la hoja alcanzó un valor máximo (24.3%) a los 40 días y luego disminuyó progresivamente a 8.4% (90 días), mientras que los valores correspondientes para el tallo fueron 10.6 y 2.8%, respectivamente. La concentración de fibra cruda en la hoja aumentó de 10.6% (30 días) a 17.3% (90 días), mientras que la del tallo fue de 23.2 y 39.2%, respectivamente. De este estudio a nivel de invernadero se puede concluir que una cosecha entre 40 y 50 días después de la siembra representa un compromiso aceptable entre el rendimiento de MS y el valor nutritivo. Se sugieren estudios complementarios para determinar la repetibilidad de estos resultados en condiciones de campo y el potential de rebrote de las plantas después de un corte o pastoreo.

Palabras clave: Dinámica de crecimiento, valor nutritivo, producción.

Correspondence: N.R. Kumalasari, Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Jl. Agatis, Kampus IPB Darmaga, Bogor 16680, Indonesia. Email: nurrkumala@gmail.com

Introduction

Asystasia gangetica (L.) T. Anderson (Acanthaceae) is an attractive herbaceous ground cover that grows from 30 to 60 cm in height. It is widely distributed from tropical Asia to Africa including Nigeria (Lithudzha 2004; GRIN 2007) and in Indonesia is commonly known as 'ara sungsang' in Sumatra Island or 'bayaman' in Java. It is a fast-growing, spreading, perennial herb, with usually erect, branched, square stems up to 2 m long, often rooting at the lower nodes (Shu et al. 2011). It is a soft weed species that is widely grown as ground cover in Indonesian palm plantations (Ramdani et al. 2017).

This plant can dominate over huge areas because it is highly tolerant of low soil fertility and shade (Samedani et al. 2013). It has potential for use as a commercial forage plant due to its ability to reliably grow from seed (Kumalasari et al. 2018). The plant can grow rapidly as cover crop (Asbur et al. 2018a) and minimize erosion (Asbur et al. 2018b).

It has many medicinal, nutritional and local values including its use as forage (Adetula 2004). Norlindawati et al. (2019) reported it has high production and crude protein concentration, which can reach 23.5% and can be higher in the dry season than in the rainy season (Adjorlolo et al. 2014). High mineral concentrations (Khalil et al. 2018) and high palatability for animals (Sobayo et al. 2012) are other desirable attributes. Considerable research has been conducted on its benefit as feed for animals, e.g. broilers (Sobayo et al. 2012) and ruminants (Wigati et al. 2016). However, there is a lack of information on its morphological characteristics and quality as forage at different growth stages.

Research was carried out with *A. gangetica* to assess plant growth dynamics, dry matter (DM) yields and nutrient concentrations at different ages as a guide to identifying optimal times for harvesting.

Materials and Methods

The research was conducted at Green House Laboratory of Agrostology, Faculty of Animal Science, Bogor Agricultural University, during the growing season of 2018. Forage quality was analyzed at Laboratory of University Center (PAU).

Seedlings were prepared in trays for 21 days in a nursery until they reached the 4-leaf stage, before being transplanted into 115 polybags with capacity of 5 kg filled with latosol. The basal fertilizer was fresh cattle manure (with 11.2% organic C, 0.46% total N, 0.24% P_2O_5 and 0.29% K_2O), at the rate of 250 g/polybag, and inorganic fertilizer (Mutiara - 16% N, 16% P_2O_5, 16% K_2O, 0.5% MgO and 6% CaO) at the rate of 2.5 g/polybag, which was mixed with soil 2 weeks before transplanting. The polybags were arranged in a completely randomized design with 23 replications. Treatments consisted of 5 different harvesting ages, i.e. 30, 40, 50, 70 and 90 days after transplanting (DAT).

Growth attributes were measured on all plants during the growth period as follows: plant height (cm) from the base of the plant to the tip of the central spike tassel; and numbers of leaves and branches. Branches were categorized into 3 types, i.e. primary, secondary and tertiary branches. At the predetermined ages, plants were cut approximately 5 cm from the ground and weighed to determine fresh yields. Plants were then separated into branches (stems) and leaves, and weighed to obtain the relevant contributions to total yield. Samples were selected, air dried and weighed to calculate DM yields.

Fresh herbage samples from each treatment were selected, air dried under sunlight for 2 × 12 h, before drying in an air-forced oven at 60 °C for 48 h, and ground to pass through a 1 mm sieve for chemical analyses. Dry matter, crude protein, crude fat, crude fiber and ash concentrations were determined according to AOAC International (2005) procedures.

Data were analyzed statistically as a completely randomized design with R i386 3.6.1 using Analysis of Variance Test (ANOVA); if there was a significant difference, the analyses were continued with the Tukey Honest Significant Difference Test (HSD).

Results

Plant height

Plant height of *A. gangetica* increased progressively with age according to a quadratic relationship ($P<0.001$; Table 1), reaching a mean of 131 cm at 90 DAT.

Number of leaves per plant

Number of leaves per plant of *A. gangetica* increased progressively with age ($P<0.001$; Table 1) reaching a mean of 635 leaves at 90 DAT.

Number of branches per plant

Total number of branches per plant increased progressively with time after transplanting (Table 1; $P<0.001$). Number of primary branches increased until 50 days after transplanting then decreased with time, while numbers of both secondary and tertiary branches increased progressively over time.

Table 1. Effects of plant age on *Asystasia gangetica* growth indicators.

Parameter	Age (days after transplanting)					s.e.m.	P
	30	40	50	70	90		
Plant height (cm)	27.9d	72.1c	95.6b	97.9b	131a	0.55	<0.001
Number of leaves	66.4d	306c	372bc	423b	635a	3.19	<0.001
Number of branches							
Primary	2.3b	2.6a	2.8a	2.3b	2.1c	0.06	<0.001
Secondary	3.8c	7.2bc	13.8b	23.7a	24.1a	0.18	<0.001
Tertiary	2.7e	6.0d	11.2c	31.1b	36.1a	0.27	<0.001
Dry weight (g/plant)							
Leaf	1.3c	5.5c	8.0b	8.6b	11.6a	0.07	<0.001
Stem	0.6d	5.9c	9.5b	10.4b	19.0a	0.11	<0.001
Total	1.8d	11.4c	17.6bc	19.0b	30.6a	0.09	<0.001
Leaf:stem ratio (dry weight basis)	2.3:1	0.95:1	0.84:1	0.82:1	0.61:1		

Means in the same row without common letters are different at P<0.001.

Forage yield

Dry matter (DM) yields of both leaf and stem increased (P<0.001) progressively with age and reached 11.6 g leaf DM and 19.0 g stem DM/plant at 90 DAT (Table 1). Leaf:stem ratio (DM basis) declined progressively with age from 2.3:1 at 30 DAT to 0.6:1 at 90 DAT. Figure 1 demonstrates changes in appearance of plants as they aged, with changes in leaf:stem ratio and senescence of leaves by 90 DAT being quite noticeable.

Forage quality

Leaf crude protein (CP) concentration peaked at 24.2% at 40 DAT, then declined progressively to 8.4% at 90 DAT (P<0.01; Table 2). The pattern for stem CP was similar but concentrations were much lower (peak of 10.6% at 40 DAT, declining to 2.8% at 90 DAT) (P<0.01).

Crude fiber (CF) concentrations increased with age (P<0.01; Table 2) for both leaf and stem; stem CF concentrations, however, were much higher than in leaf.

30 DAT 40 DAT 50 DAT 70 DAT 90 DAT

Figure 1. *Asystasia gangetica* plants of different ages (DAT, days after transplanting).

Table 2. Effects of plant age on nutrient concentrations (% DM) in leaf and stem of *Asystasia gangetica* forage.

Plant part/Nutrient	Age (days after transplanting)					s.e.m.	P
	30	40	50	70	90		
Leaf							
Crude protein	16.6b	24.2a	20.7b	10.4c	8.4c	0.59	<0.01
Crude fat	2.1	2.7	1.8	3.4	4.7	0.11	<0.01
Crude fiber	10.6c	9.7c	12.0b	11.9b	17.3a	0.21	<0.01
Ash	15.5	14.7	12.3	13.3	15.4	0.14	<0.01
Stem							
Crude protein	7.6b	10.6a	7.7b	3.6c	2.8c	0.28	<0.01
Crude fat	1.2	0.8	4.1	1.8	0.9	0.11	<0.01
Crude fiber	23.2b	31.3ab	39.0a	39.4a	39.2a	0.46	<0.01
Ash	17.3a	11.0ab	7.7b	9.8b	14.0a	0.27	<0.01

Means in the same row without a common letter are different at P<0.01.

Discussion

This study has provided useful information on changes in nutrient concentrations with age in *A. gangetica* forage. As was expected, increasing age had positive effects on plant height, number of leaves and numbers of secondary and tertiary branches, resulting in marked increases in dry matter yields. Plant height and number of branches in this study followed a different pattern from that reported by Asbur et al. (2018b). Plants in our study were taller and displayed much greater branching due to being grown in full sunlight (Samedani et al. 2013) as opposed to former research where plants were grown under palm plantation shading (Asbur et al. 2018b). While biomass yields of both leaf and stem increased progressively with age, leaf:stem ratio declined progressively. As can be observed in Table 1, the number of primary branches declined from 50 to 70 DAT as a result of senescence.

As expected, nutrient concentration in *A. gangetica* leaves was better than in stems (Table 2). While crude protein concentrations (CP%) in both plant parts declined progressively from 40 DAT, at 50 DAT CP% still exceeded the critical level of 7% for satisfactory functioning of rumen microflora (Ansah et al. 2018). Peak protein concentrations (24.2%) in leaf at 40 DAT was higher than 17.5% reported by Herilimiansyah (2019) for 50 DAT but DM yields were still relatively low at this stage. Growth for the next 10 days was not rapid and protein concentration of forage declined to a greater degree. While marked increases in DM yields of both components, especially stem, occurred after 70 DAT, CP% had dropped to low levels by this time with CP% of stems (3.6%) being well below maintenance levels.

As can be seen in Figure 2, decisions on when to utilize the forage would be a compromise between DM yields of both leaf and stem and CP% of these 2 components. While this is a pot study, it would seem that harvesting between 40 and 50 DAT would give a reasonable compromise between DM yield and CP% in the available forage, as leaf:stem ratio still exceeded 0.8 at 50 DAT. The leaf material produced would be of high CP% and should provide an excellent supplement to low quality roughage for ruminants. However, feeding studies with animals are needed to determine responses of animals when this forage is fed as supplements with other forage sources or as a complete ration.

It must be stressed that these data are for single plants grown in polybags and competition between plants would not have been expressed as would occur in swards in the field. Further studies under field situations are needed to verify that the results obtained in our study represent those obtained in commercial situations and how the situation might change when grown under shade in palm plantations.

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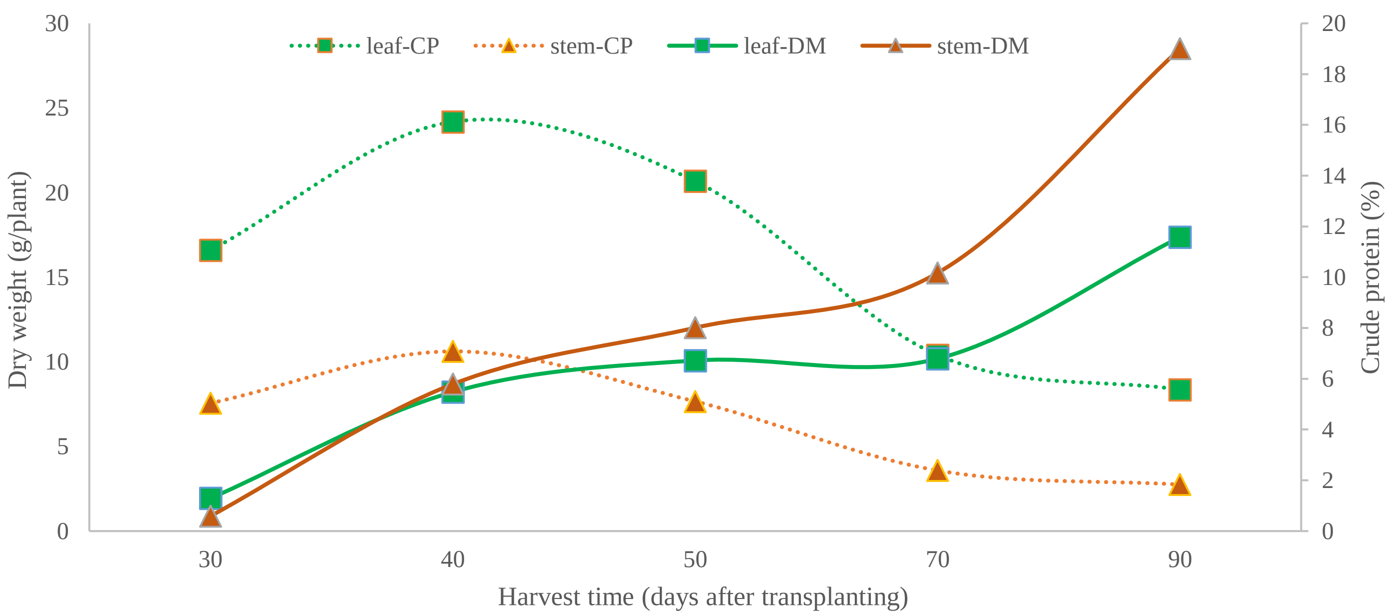

Figure 2. Effects of harvest time (days after transplanting) on DM yields and crude protein concentrations (% DM) in leaf and stem of *Asystasia gangetica*.

References

(Note of the editors: All hyperlinks were verified 28 April 2020.)

Adetula OA. 2004. *Asystasia gangetica* (L.) Anderson. In: Grubben GJH; Denton OA, eds. PROTA (Plant Resources of Tropical Africa). Wageningen, the Netherlands. bit.ly/2SB5J1n

Adjorlolo LK; Adogla-Bessa T; Amaning-Kwarteng K; Ahunu BK. 2014. Effect of season on the quality of forages selected by sheep in citrus plantations in Ghana. Tropical Grasslands-Forrajes Tropicales 2:271−277. doi: 10.17138/tgft(2)271-277

Ansah T; Wilkinson RG; Huntington J; Dei HK. 2018. Effects of tropical browse plants on *in vitro* rumen protein degradability. Livestock Research for Rural Development 30, Article #46. lrrd.org/lrrd30/3/tans30046.html

AOAC International. 2005. Official methods of analysis. 18th Edn. AOAC Inc., Gaithersburg, MD, USA.

Asbur Y; Purwaningrum Y; Aryanti M. 2018a. Growth and nutrient balance of *Asystasia gangetica* (L.) T. Anderson as cover crop for mature oil palm (*Elaeis guineensis* Jacq.) plantations. Chilean Journal of Agricultural Research 78:486−494. doi: 10.4067/S0718-58392018000400486

Asbur Y; Yahya S; Murtilaksono K; Sudradjat; Sutarta ESSS. 2018b. The roles of *Asystasia gangetica* (L.) T. Anderson and ridge terrace in reducing soil erosion and nutrient losses in oil palm plantation in South Lampung, Indonesia. Journal of Tropical Crop Science 3:49−55. doi: 10.29244/jtcs.3.2.49-55

GRIN (Germplasm Resources Information Network). 2007. GRIN Taxonomy. U.S. National Plant Germplasm System, Agricultural Research Service, US Department of Agriculture, Beltsville, MD, USA. ars-grin.gov

Herilimiansyah; Kumalasari NR; Abdullah L. 2019. Evaluation of *Asystasia gangetica* T. Anderson cultivation system on different plant spacing under palm plantation shade. Jurnal Ilmu Nutrisi dan Teknologi Pakan 17:6–10. (In Indonesian). doi: 10.29244/jintp.17.1.6-10

Khalil; Suyitman; Montesqrit. 2018. Crude nutrient and mineral composition of *Asystasia gangetica* (L) derived from different growing areas. Proceeding of the 4th International Seminar on Animal Industry, Bogor, Indonesia, 28–30 August 2018. p. 189–192. repo.unand.ac.id/29178

Kumalasari NR; Wahyuni L; Abdullah L. 2018. Germination of *Asystasia gangetica* seeds exposed to different source, color, size, storage duration and pre-germinative treatments. Proceeding of the 4th International Seminar on Animal Industry, Bogor, Indonesia, 28−30 August 2018. p. 130−134. bit.ly/3bP3386

Lithudzha E. 2004. *Asystasia gangetica* (L) T. Anderson subsp. *micrantha* (Nees) Ensermu. South Africa National Biodiversity Institute, Silverton, South Africa. pza.sanbi.org/asystasia-gangetica

Norlindawati AP; Haryani H; Sabariah B; Noor MI; Samijah A; Supie MJ; Edham AZW. 2019. Chemical composition of weeds as potential forage in integrated farming. Malaysian Journal of Veterinary Research 10(2):19–24. bit.ly/2ytvw4C

Ramdani D; Abdullah L; Kumalasari NR. 2016. Analysis of local forage potential under ruminant - palm plantation integration system in Mandau District, Bengkalis Regency of Riau Province. Buletin Makanan Ternak 104:1−8. (In Indonesian). bit.ly/3eZknJg

Samedani B; Juraimi AS; Anwar MP; Rafii MY; Awadz SHS; Anuar AR. 2013. Competitive interaction of *Axonopus compressus* and *Asystasia gangetica* under contrasting sunlight intensity. The Scientific World Journal 2013, Article 308646. doi: 10.1155/2013/308646

Shu WCS; Jiaqi H; Yunfei D; Daniel TF. 2011. *Asystasia* Blume; Bijdr. 796. 1826. Flora of China 19:437–438. bit.ly/2KEqqFi

Sobayo RA; Adeyemi OA; Sopide OG; Oso OA; Fafiolu AO; Ogunade IM; Iyasere OS; Omoniyi LA. 2012. Growth response of broiler birds fed *Asystasia gangetica* leaf meal in hot humid environment. Journal of Agricultural Science and Environment 12:53−59. bit.ly/3eZkQv0

Wigati S; Maksudi; Wiyanto E. 2016. The use of rubber leaves (*Hevea brasiliensis*) as forage in supporting the development of goats. Proceedings of International Seminar on Livestock Production and Veterinary Technology, Bogor, Indonesia. p. 284–290. doi: 10.14334/Proc.Intsem.LPVT-2016-p.284-290

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Short Communication

Pest insects in natural and sown pastures of Paraguay
Insectos plagas en pasturas naturales y cultivadas de Paraguay

HUMBERTO J. SARUBBI AND MARÍA B. RAMÍREZ

Facultad de Ciencias Agrarias, Universidad Nacional de Asunción, San Lorenzo, Paraguay. agr.una.py

Abstract

Paraguayan livestock production is based mainly on the use of natural and sown pastures as basic cattle feed. Several genera of harmful insects reported in forage grasses can cause damage to both yield and quality of forage. A review of the insect collection of the Plant Protection Area of the Faculty of Agrarian Sciences, National University of Asunción was carried out, in order to prepare a list of insects with incidence in grasses. Then random sampling of different species of Poaceae showing insect damage in open areas of paddocks grazed by cattle was carried out during 2014–2017 in all Regions of Paraguay. Thirteen different genera and species of pastures were collected and 20 species of insects were identified in the following orders: Hymenoptera (Formicidae family: 5 species); Isoptera (Termitidae: 3 species); Hemiptera (Cercopidae: 6 species; Lygaeidae: 1 species); Lepidoptera (Noctuidae: 2 species); and Orthoptera (Acrididae: 3 species). The most common forms of damage observed in pastures were: leaf consumption (25%), leaf cutting (25%) and leaf yellowing-drying (35%).

Keywords: Families, identification, predation, sampling.

Resumen

En Paraguay la producción pecuaria se basa principalmente en el uso de pasturas naturales y cultivadas como alimento base para ganado vacuno. En el país se han identificado varios géneros de insectos dañinos que pueden ocasionar daños tanto en la cantidad como la calidad de las gramíneas forrajeras. Inicialmente se realizó una revisión de la colección de insectos del Área de Protección Vegetal de la Facultad de Ciencias Agrarias, Universidad Nacional de Asunción, con el objeto de elaborar un listado de insectos con incidencia en pasturas. Posteriormente, durante los años 2014–2017, se realizaron muestreos al azar en diferentes especies de gramíneas que presentaban daños por insectos en áreas abiertas destinadas al pastoreo de vacunos en los diferentes departamentos de Paraguay. En total fueron colectados 13 diferentes géneros y especies de pastos y se identificaron 20 especies de insectos, de los órdenes Hymenoptera (familia Formicidae, 5 especies); Isoptera (Termitidae, 3 especies); Hemiptera (Lygaeidae y Cercopidae, 1 y 6 especies, respectivamente); Lepidoptera (Noctuidae, 2 especies); y Orthoptera (Acrididae, 3 especies). Los daños más comunes observados en las pasturas fueron daños por consumo de follaje (25%), corte de láminas foliares (25%) y amarillamiento y secado de hojas (35%).

Palabras clave: Familias, identificación, muestreo.

Introduction

Paraguayan cattle ranching has experienced a significant improvement in number and quality in the last 20 years, and Paraguay is the seventh largest beef exporter in the world (ARP 2017). The country currently has 15 million hectares being used for livestock, and sown (5.6 million ha) and natural pastures (10 million ha) are the primary feed source for cattle, since it is the most economic and practical approach to meat production (Glatzle and Stosiek 2001; ARP 2017). This has created an ideal environment for the proliferation of different genera of insects, which can be harmful to forage crops (Fowler 1979; Glatzle 1999; Benítez 2002; Sarubbi 2016). Many

Correspondence: H.J. Sarubbi, Facultad de Ciencias Agrarias, Universidad Nacional de Asunción, Campus Universitario, San Lorenzo, Paraguay. Email: humberto.sarubbi@agr.una.py

of these insects are widely distributed over the American continent and cause a range of symptoms from defoliation to death of plants (Gallo et al. 2002; Brandão et al. 2011). Knowing the distribution and potential hosts of insects and damage caused is an important step in the development of adequate management strategies (Picanço et al. 1999; Nakano 2011).

The objective of this work was to identify harmful insects and determine their distribution throughout Paraguay, plant hosts infested and description of damage caused.

Materials and Methods

First, a review of the insect collection of the Plant Protection Area of the Faculty of Agricultural Sciences, National University of Asunción was carried out, in order to produce a list of registered pests causing damage to pastures. Subsequently, insect collections were carried out at random, during the years 2014–2017, in the 4 seasons of the year (1 collection per season, 16 in total), on different species of Poaceae showing insect damage. The work was carried out in open areas of paddocks destined for cattle grazing in the following Regions: Western Region or Chaco: Alto Paraguay (APY), Boquerón (BOQ), Villa Hayes (VHA); and Eastern Region: Amambay (AMA), Concepción (CON), San Pedro (SPE), Canindeyú (CAN), Caaguazú (CAG)), Alto Paraná (APA), Central (CEN), Cordillera (COR), Paraguarí (PAR), Guairá (GUA), Caazapá (CAZ), Itapúa (ITA), Misiones (MIS) and Ñeembucú (ÑEE). Pasture samples were collected from the following species: *Cenchrus ciliaris* (CC), *Cenchrus purpureus* (CP), *Chloris gayana* (CG), *Cynodon nlemfuensis* (CN), *Digitaria eriantha* (DE), *Megathyrsus maximus* (MM, a range of cultivars), *Paspalum notatum* (PN), *Urochloa brizantha* (UB, a range of cultivars), *Urochloa decumbens* (UD), *Urochloa mosambicensis* (UM), *Urochloa ruziziensis* (UR) and *Urochloa arrecta* × *Urochloa mutica* (UA × UM; tangola grass).

Insect pests were collected, recording date, location, host and type and extent of damage, were photographed and immediately deposited in plastic containers for identification. The collection was manual using an entomological sweep net (50 cm ring diameter and 1 m bag length). The samples were transported to the Entomology Laboratory and examined with a stereoscope for identification. Insects were identified using the following reference sources: Fowler (1979), Kidono (1982), Glatzle (1999), Valério et al. (1999), Gallo et al. (2002), Sarubbi (2016) and Tolotti et al. (2018).

Results and Discussion

Twenty (20) species of insects in the orders Hymenoptera, Isoptera, Hemiptera, Lepidoptera and Orthoptera were found on 12 pasture hosts (Table 1). In Hymenoptera, 6 were in Formicidae family; in Isoptera, 3 were in Termitidae; in Hemiptera, 1 was in Lygaeidae and 6 in Cercopidae; in Lepidoptera, 2 were in Noctuidae; and in Orthoptera, 3 were in Acrididae.

The *Urochloa* and *Megathyrsus* genera represent the most important pasture grasses in the country, and consequently the greatest variety of insects was found on them. A unique case was observed in *Urochloa arrecta* × *U. mutica* (tangola grass), which was the exclusive host of *Blissus antillus* (grass bug). In relation to the occurrence of insects across Regions, some species, such as defoliating ants and cicadas, cover the whole country, while other species are confined to certain regions or places, such as the locust (*Staurorhectus longicornis*) and the cutter ant (*Atta vollenweideri*) in Chaco. The types of damage to pastures most commonly observed were: leaf consumption 25% [caterpillars (*Mocis latipes*, *Spodoptera frugiperda*) and locusts], cutting 25% (cutter ants) and leaf yellowing and drying 35% [grass bug and spittlebug (*Notozulia entreriana* and *Mahanarva fimbriolata*)] (Table 1).

Insects with the highest number of species (12) and distribution were cutter ants and spittlebug as mentioned by Fowler (1979), Kidono (1982), Glatzle (1999), Benítez (2002) and Sarubbi (2016). Valério (2006) and Tolloti et al. (2018) consider that spittlebugs are among the most important harmful insects of tropical pastures, attacking several genera, species and varieties, as observed in this research, as they were present in the whole territory of Paraguay and with a wide host range.

Incidence of *Blissus antillus* in tangola pasture agrees with reports of Valério et al. (1999) and Fazolin et al. (2012), who found that tangola grass (a natural *Urochloa* hybrid) and *Urochloa arrecta* were the only hosts of *Blissus antillus* in Brazil.

The damage caused by termites is considered indirect since this species develops mounds that are obstacles for agricultural machinery, causing loss of useful area in the paddocks.

Occurrence of most of these insects is seasonal and some appear in large numbers at specific times. Some which can cause serious damage are: caterpillars (*Mocis latipes*), bug (*Blissus antillus*) and cicadas (*Notozulia entreriana* and *Mahanarva fimbriolata*), as was mentioned by Gallo et al. (2002) and Tolotti et al. (2018).

Table 1. Insect pests (common name in parenthesis) identified in different pasture species of Paraguay.

Insect order, family and species	Host[1]	Occurrence (Region and collection site)[2]	
Hemiptera: Cercopidae			
Deois flavopicta (Salivazo)	CN, MM, UB	AMA 22°08'03.7" S 56°28'41.4" W CAG 24°57'06.9" S 56°21'46.8" W ITA 27°04'07.2" S 56°36'21.7" W	SPE 23°44'11.4" S 56°29'38.4" W APA 25°27'36.2" S 55°02'34.4" W
Deois mourei (Salivazo)	CN, MM, UB, UD	CEN 25°19'41.1" S 57°31'11.0" W CAG 25°28'10.8" S 56°32'18.3" W MIS 27°07'19.8" S 56°46'40.2" W	SPE 24°05'05.6" S 57°06'17.7" W APA 25°26'06.7" S 54°46'37.3" W
Deois rubropicta (Salivazo)	CN	AMA 22°40'15.7" S 56°02'41.8" W	
Deois schach (Salivazo)	PN, MM, UB	CAG 25°24'37.4" S 55°34'16.6" W	
Mahanarva fimbriolata (Salivazo)	CP, UB	AMA 22°07'39.0" S 56°27'47.4" W SPE 24°02'53.0" S 56°27'33.4" W COR 25°14'51.4" S 57°08'32.7" W CAG 24°57'55.3" S 56°20'58.7" W APA 25°25'09.7" S 55°23'28.5" W MIS 26°59'38.1" S 56°47'25.5" W	CON 23°27'44.1" S 57°24'28.7" W CEN 25°19'40.8" S 57°31'09.8" W PAR 25°30'18.1" S 57°10'07.0" W CAZ 26°09'30.2" S 56°21'49.5" W GUA 25°43'09.9" S 56°11'01.5" W ITA 26°23'41.0" S 55°30'54.4" W
Notozulia entreriana (Salivazo)	CC, CN, CG, DE, MM, UB, UD, UR	BOQ 23°26'20.4" S 60°75'12.5" W VHA 24°55'58.2" S 57°33'50.1" W PAR 26°08'35.0" S 56°42'49.9" W CAG 24°57'08.2" S 56°21'40.9" W ITA 27°12'03.1" S 56°06'51.8" W	APY 20°11'03.6" S 59°32'23.8" W SPE 23°45'02.0" S 56°29'34.2" W CEN 25°19'28.8" S 57°31'16.4" W APA 25°25'48.6" S 55°22'52.6" W
Hemiptera: Lygaeidae			
Blissus antillus (Chinche de las gramíneas)	UA×UM (tangola)	VHA 23°31'54.0" S 58°36'44.2" W	
Hymenoptera: Formicidae			
Acromyrmex heyeri (Akekẽ)	PN	MIS 27°07'35.6" S 56°41'57.3" W	
Acromyrmex landolti fracticornis (Akekẽ kapi´i)	CC, CN, CG, DE, MM, PN, UB, UD, UM, UR	APY 20°10'59.8" S 59°32'18.0" W BOQ 21°58'13.9" S 59°59'59.7" W SPE 23°43'54.8" S 56°29'31.4" W COR 25°14'56.0" S 57°08'51.6" W GUA 25°43'08.3" S 56°11'01.9" W	VHA 23°31'53.7" S 58°36'06.7" W CON 23°24'23.5" S 57°19'29.0" W CEN 25°12'24.8" S 57°24'31.7" W PAR 26°08'38.7" S 56°42'52.1" W MIS 27°07'15.0" S 56°46'44.7" W
Atta capiguara (Ysaú kapi´i)	CN, MM, PN, UB, UD, UR	SPE 23°44'20.7" S 56°29'30.3" W APA 25°10'24.3" S 54°42'01.2" W	CAG 25°24'14.1" S 55°31'56.1" W
Atta laevigata (Ysaú akã vidrio)	CN, MM, PN, UB, UD, UR	CON 22°24'58.6" S 56°41'37.6" W SPE 24°43'13.8" S 56°30'11.3" W	AMA 22°40'21.7" S 55°55'25.5" W
Atta vollenweideri (Ysaú chaco)	CC, CN, CG, DE, MM, UM	APY 20°15'55.1" S 59°32'42.7"W VHA 23°31'60.0" S 58°36'58.2" W	BOQ 22°00'31.8" S 60°00'15.3" W
Isoptera: Termitidae			
Cornitermes bequaerti (Kupi´i takuru chimenea)	CN, MM, PN, UB, UR	CON 23°01'33.2" S 56°35'46.4" W SPE 24°20'37.5" S 56°25'13.2" W	CAN 24°21'23.3" S 55°04'11.6" W CAG 25°24'06.7" S 55°48'49.8" W
Cornitermes cumulans (Kupi´i takuru)	CN, MM, PN, UB, UD, UR	CON 23°24'26.9" S 57°19'36.7" W SPE 24°22'43.4" S 56°25'07.8" W COR 25°04'56.6" S 57°23'22.4" W CAZ 26°09'42.5" S 56°21'44.6" W APA 25°29'15.5" S 54°49'21.4" W	CAN 24°21'34.3" S 55°04'28.6" W CEN 25°19'46.5" S 57°31'23.8" W PAR 25°23'26.2" S 57°03'34.1" W CAG 24°58'48.2" S 56°20'58.9" W MIS 26°40'39.7" S 57°06'04.4" W
Procornitermes striatus (Yvy kupi´i)	CN, MM, PN, UB	CEN 25°19'43.5" S 57°31'10.0" W	
Lepitoptera: Noctuidae			
Mocis latipes (Falsa medidora)	CC, CN, DE, MM, UB	APY 22°02'00.1" S 59°53'14.3" W MIS 26°34'33.7" S 56°54'46.0" W	BOQ 21°58'49.4" S 60°00'24.1" W CAG 24°58'03.7" S 56°21'02.3" W
Spodoptera frugiperda (Cogollero del maíz)	CC, CN, DE, MM, UB, UD, UM, UR	BOQ 23°15'53.6" S 60°43'53.8" W PAR 25°27'44.9" S 57°15'57.8" W APA 25°27'32.4" S 55°02'57.5" W	CAG 24°58'12.1" S 56°21'00.3" W ÑEE 25°57'07.5" S 57°46'37.8" W ITA 27°01'10.2" S 55°55'12.6" W
Orthoptera: Acrididae			
Rammatocerus pictus (Langosta)	CC, CN, MM	APY 20°11'31.4" S 59°31'48.1" W	BOQ 21°06'46.5" S 60°31'23.6" W
Schistocerca cancellata (Langosta migratoria)	CC, CN, CG, MM	APY 20°16'36.3" S 59°07'22.0" W	BOQ 21°19'15.7" S 60°27'10.6" W
Staurorhectus longicornis (Langosta de pastura)	CC, CN, CG, MM	APY 20°11'55.7" S 59°32'03.2" W	BOQ 23°14'35.4" S 60°45'49.6" W

[1]Hosts: CC (*Cenchrus ciliaris*), CP (*Cenchrus purpureus*), CG (*Chloris gayana*), CN (*Cynodon nlemfuensis*), DE (*Digitaria eriantha*), MM (*Megathyrsus maximus*), PN (*Paspalum notatum*), UB (*Urochloa brizantha*), UD (*Urochloa decumbens*), UA×UM (*Urochloa arrecta* × *Urochloa mutica*), UM (*Urochloa mosambicensis*) and UR (*Urochloa ruziziensis*).

[2]Occurrence according to the records of the entomological collection of the Plant Protection Area of the Facultad de Ciencias Agrarias, Universidad Nacional de Asunción and collections made by the authors.

Regions: AMA = Amambay; APA = Alto Paraná; APY = Alto Paraguay; BOQ = Boquerón; CAG = Caaguazú; CAN = Canindeyú; CAZ = Caazapá; CEN = Central; CON = Concepción; COR = Cordillera; GUA = Guairá; ITA = Itapuá; MIS = Misiones; ÑEE = Ñeembucú, PAR = Paraguarí, SPE = San Pedro; and VHA = Villa Hayes.

Conclusions

This study has provided an overview of the range of insects which occur in pastures in Paraguay. Whether or not active measures to control them should be undertaken would depend on the extent of damage they cause and the impact on both pasture production and resultant animal performance. Observations on degree of damage to pastures under a range of conditions should supply some information on which to base decisions.

References

(Note of the editors: All hyperlinks were verified 5 May 2020.)

ARP (Asociación Rural del Paraguay). 2017. Introducción a Paraguay y su sector cárnico. ARP, Asunción, Paraguay. bit.ly/35wlZ9h

Benítez E. 2002. Listado de nombres científicos y vulgares de plagas agrícolas y forestales del Paraguay. Universidad Nacional de Asunción, San Lorenzo, Paraguay.

Brandão CRF; Maye-Nunes A; Sanhudo CED. 2011. Taxonomia e filogenia das formigas-cortadeiras. In: Della Lucia TMC, ed. Formigas cortadeiras: Da biología ao manejo. Editora UFV, Viçosa, MG, Brazil. p. 27–48.

Fazolin M; Tomazini MJ; Estrela JLV. 2012. Pragas das culturas de importância econômica para o Estado do Acre. Documentos 127. Embrapa Acre, Rio Branco, AC, Brazil. bit.ly/3fp26Fp

Fowler H. 1979. Las hormigas cortadoras del Paraguay de los géneros *Atta* Fabricius y *Acromyrmex* Mayr: Bionómico, distribución y sistemática. Informes científicos ICB-UNA 2:30–57.

Gallo D; Nakano O; Silveira Neto S; Carvalho RPL; Baptista GC; Berti Filho E; Parra JRP; Zucchi RA; Alves SB; Vendramim JD; Marchini LC; Lopes JRS; Omoto C. 2002. Entomologia agrícola. p. 484–493. FEALQ, Piracicaba, SP, Brazil.

Glatzle A. 1999. Compendio para el manejo de pasturas en el Chaco. Editorial El Lector, Asunción, Paraguay.

Glatzle A; Stosiek L. 2001. Perfiles por país del recurso pastura/forraje Paraguay. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. bit.ly/2xG6zTo

Kidono H. 1982. Algunas observaciones sobre las cigarritas de las pasturas, salivazo en el Paraguay. Universidad Nacional de Asunción, San Lorenzo, Paraguay.

Nakano O. 2011. Entomologia econômica. p. 280–293. ESALQ, Piracicaba, SP, Brazil.

Picanço M; Leite GLD; Mendes MC; Borges VE. 1999. Attack of *Atarsocoris brachiariae* Becker, a new pest of pasture-lands in Mato Grosso, Brazil. Pesquisa Agropecuária Brasileira 34:885–890. (In Portuguese). doi: 10.1590/S0100-204X1999000500022

Sarubbi H. 2016. Plagas de pasturas en Paraguay. Universidad Nacional de Asunción, San Lorenzo, Paraguay.

Tolotti A; Azevedo WS; Valiati VH; Carvalho GS. 2018. Cigarrinhas das pastagens en gramineas forrageiras no Brasil. Editora Evangraff, Porto Alegre, RS, Brazil.

Valério JR. 2006. Considerações sobre a morte de pastagens de *Brachiaria brizantha* cv. Marandu em alguns estados do Centro e Norte do Brasil: Enfoque entomológico. Comunicado Técnico 98. Embrapa Gado de Corte, Campo Grande, MS, Brazil. bit.ly/3fmm2sx

Valério JR; Vieira JM; Valle LCS. 1999. The occurrence of *Blissus antillus* Leonard (Hemiptera: Lygaeidae: Blissinae) in Tangola pasture in Mato Grosso do Sul State, Brazil. Anais da Sociedade Entomológica do Brasil 28:527–529. (In Portuguese). doi: 10.1590/S0301-80591999000300020

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Short Communication

Pasto Certo® version 2.0 - An application about Brazilian tropical forage cultivars for mobile and desktop devices

Pasto Certo® version 2.0 – Una aplicación sobre cultivares brasileños de especies forrajeras tropicales para dispositivos móviles y de escritorio

SANZIO CARVALHO LIMA BARRIOS[1], CAMILO CARROMEU[1], MÁRCIO APARECIDO INÁCIO DA SILVA[2], EDSON TAKASHI MATSUBARA[2], CACILDA BORGES DO VALLE[1], LIANA JANK[1], MATEUS FIGUEIREDO SANTOS[1], GISELLE MARIANO LESSA DE ASSIS[3], LEONARDO LAZARINO CRIVELLARO[2], THALLYSON DANCHEN TEIXEIRA GONÇALVES[4], JOSÉ MARCOS QUEIROZ JÚNIOR[5], ANDERSON RAMIRES CANDIDO[6], WYVERSON KIM ROCHA MACHADO[6], BEATRIZ TOMÉ GOUVEIA[7], ALANA APARECIDA AMARILHA NOBRE[2] AND AYHAN LIELL ZANELLA[5]

[1]*Embrapa Gado de Corte, Campo Grande, MS, Brazil.* embrapa.br/gado-de-corte
[2]*Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.* ufms.br
[3]*Embrapa Acre, Rio Branco, AC, Brazil.* embrapa.br/acre
[4]*Universidade para o Desenvolvimento do Estado e da Região do Pantanal – Anhanguera/Uniderp, Campo Grande, MS, Brazil.* uniderp.com.br
[5]*Universidade Católica Dom Bosco, Campo Grande, MS, Brazil.* site.ucdb.br
[6]*Universidade Estadual de Mato Grosso do Sul (UEMS), Aquidauana, MS, Brazil.* uems.br
[7]*Universidade Federal de Lavras, Lavras, MG, Brazil.* ufla.br

Abstract

A brief outline of the second version of Pasto Certo®, released by Embrapa and partners in February 2019, is presented. It is an improved and updated version of Pasto Certo® 1.0, an application that describes Brazilian commercial tropical forage cultivars. The application helps the user to identify and differentiate cultivars, provides recommendations and information on use restrictions of each cultivar, and compares different cultivars in terms of a number of characteristics. In comparison with the first version (published in 2017), new features of Pasto Certo® 2.0 are: (1) 7 cultivars of forage legumes (genera *Arachis*, *Cajanus* and *Stylosanthes*) were added to the original 16 grass cultivars (*Urochloa* spp. and *Megathyrsus maximus*); (2) the user can choose between Portuguese, Spanish and English languages; (3) information on commercial seed sources in Brazil is included; (4) a guide to selecting the most suitable cultivar for specific conditions is provided; and (5) the application is available for different platforms (Android, iOS and WEB - www.pastocerto.com).

Keywords: Grasses, legumes, *Megathyrsus maximus*, pastures, software, *Urochloa*.

Resumen

Como una ayuda para la selección y manejo de cultivares brasileños de forrajeras, dirigida a productores ganaderos, técnicos, agrónomos, zootecnistas y el comercio de semilla en zonas tropicales, en Febrero 2019 la Empresa Brasileira de Pesquisa Agropecuária (Embrapa) con la colaboración de entidades asociadas, puso a disposición la aplicación Pasto Certo® 2.0, una versión mejorada y actualizada de Pasto Certo® 1.0. La aplicación asiste en la selección de cultivares comerciales, proporciona recomendaciones para cada uno de ellos y suministra información sobre posibles restricciones de uso, teniendo en cuenta las características de los diferentes cultivares. En comparación con la primera versión,

Correspondence: Sanzio Carvalho Lima Barrios, Embrapa Gado de Corte, Av. Rádio Maia, 830, Zona Rural, Campo Grande, CEP 79.106-550, MS, Brazil. Email: sanzio.barrios@embrapa.br

publicada en 2017, nuevas funcionalidades de Pasto Certo® 2.0 son: (1) la inclusión de siete cultivares de leguminosas forrajeras de los géneros *Arachis*, *Cajanus* y *Stylosanthes*, al total de 16 cultivares de gramíneas (*Urochloa* spp. y *Megathyrsus maximus*); (2) el usuario puede seleccionar para consulta entre los idiomas portugués, español e inglés; (3) se presenta información sobre fuentes comerciales de semilla en Brasil; (4) se incorporó una herramienta de selección de cultivares para diferentes condiciones específicas; y (5) la aplicación está disponible para varias plataformas (Android, iOS y WEB - www.pastocerto.com).

Palabras clave: Gramíneas, leguminosas, *Megathyrsus maximus*, pasturas, software, *Urochloa*.

Background and Development

Brazil is the world's leader in tropical forage seed production and exports, with a local market of about US$ 600 million/year. Cultivars of *Urochloa* and *Megathyrsus maximus* represent more than 90% of this market and consequently the cultivated pasture area in Brazil, which covers roughly 100 million hectares (José 2012). Despite this unquestionable significance there is no easily accessible platform for farmers, technicians, agronomists, veterinarians and seed dealers, plus other users, which describes the main characteristics of *Urochloa* and *Megathyrsus maximus* cultivars, either released by Embrapa or in the public domain. While this information exists, it is scattered in various types of publications, such as Embrapa series (Valle et al. 2004, 2017; Jank et al. 2017), folders (various Embrapa cultivar information folders) and several scientific articles, but generally relates to a single cultivar. There are other excellent forage platforms, such as Tropical Forages (tropicalforages.info/) and Feedipedia (feedipedia.org/), which describe various species and provide considerable related information. However Pasto Certo® offers an electronic software platform composed of a web tool and a mobile application for users, which allows quick and integrated access to the characteristics of the main tropical forage cultivars released by Embrapa and others in the public domain.

The software platform was constructed by students of the Computer Science College (FACOM) of the Federal University of Mato Grosso do Sul under the auspices of the Association for the Promotion of Research in Forage Breeding (UNIPASTO). It followed the steps of computational requirements, inclusion of forage technical information, software architecture design, construction of the software itself and finally validation by Embrapa employees. At the completion of these steps, the platform was created, validated and the mobile application (version 1.0, initially available only for Android operating systems) was released to users in March 2017 (Barrios et al. 2017).

Since its release, the application gained great acceptance by users and several suggestions for improvements were received, which motivated the development team to release version 2.0. Both the brand and the software Pasto Certo® - versions 1 and 2 are the property of Embrapa at the 'Instituto Nacional da Propriedade Industrial' (INPI) in Brazil. Representatives of the co-operating institutions agreed that Pasto Certo® would be an open-source software with BSD (Berkeley Software Distribution) license. The application can be downloaded at Google Play and Apple Store or accessed directly at www.pastocerto.com.

Description

Version 2.0 of Pasto Certo® is comprised of 23 forage cultivars (10 of *Urochloa*, 6 of *Megathyrsus maximus*, 3 of *Arachis*, 2 of *Cajanus* and 2 of *Stylosanthes*) and more than 160 variables, which describe these cultivars, grouped into 6 categories: identity, morphology, agronomy, performance under grazing, use in integrated systems and images of the plant from germination to adult stages. The cultivars are arranged in rectangular cards, represented by a photograph and the respective common and scientific names. To access data on a cultivar, either tap on the photograph or type the cultivar´s common name (or part thereof) (Figure 1A).

Three useful interactive features are available on Pasto Certo® 2.0: Firstly, a comparison between cultivars for different variables, where the user can select up to 4 cultivars to compare simultaneously for as many variables as are of interest (Figure 1B); Secondly, the feature 'choice of forage cultivars for pasture establishment', where the user answers 8 technical questions and is presented with cultivar suggestions for his/her specific needs (available only for *Urochloa* and *Megathyrsus maximus* cultivars) (Figure 2); and Thirdly, a contact list of more than 30 partner companies in Brazil, indicating where to obtain seeds of these tropical forage cultivars. Several of these companies have subsidiaries in Latin American countries.

The application is user-friendly, has Portuguese, Spanish and English versions and is automatically updated where an internet connection is available once the application is opened and usage starts. However, Pasto Certo® 2.0 is designed to work both online and offline, i.e. does not require internet connection.

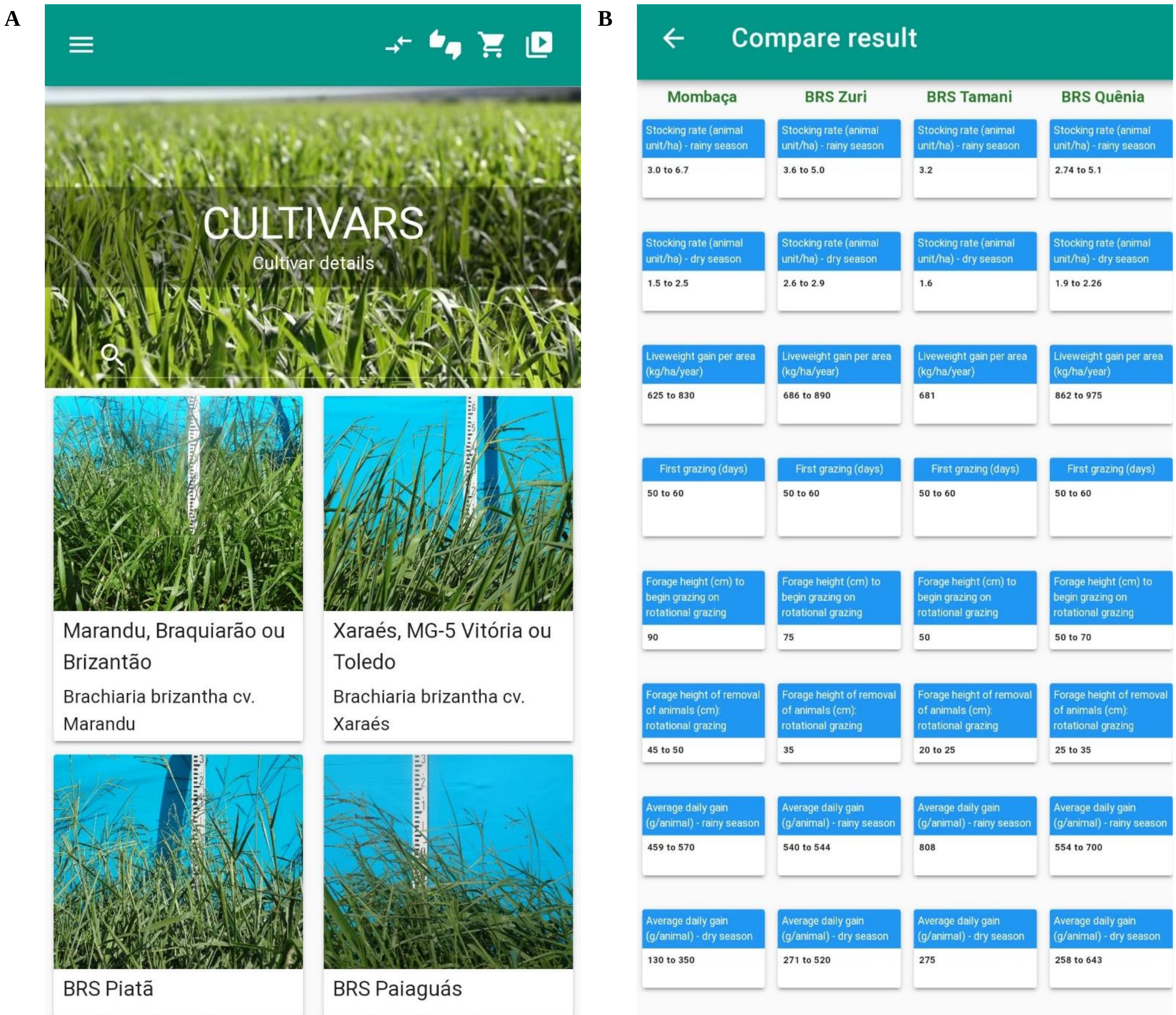

Figure 1. A) Main screen of Pasto Certo® with figure cards indicating the different cultivars in the app. At the left top is the MENU and at the right top are the interactive functions: compare cultivars; choice of forage cultivars for pasture establishment; where to buy tropical forage seeds in Brazil; and video gallery. **B)** Screen showing the comparison between *Megathyrsus maximus* cvv. Mombaça, BRS Zuri, BRS Tamani and BRS Quênia for several variables.

The application can be accessed free of charge and is available on 3 different platforms (Android, iOS and WEB - www.pastocerto.com). Pasto Certo® is thus an efficient tool to assist users in the comparison, choice, establishment and management of tropical pastures. Moreover, the application provides the capability for the user to send questions and suggestions to the administrative team responsible for Pasto Certo®, and thus to contribute to the continuous improvement of the package.

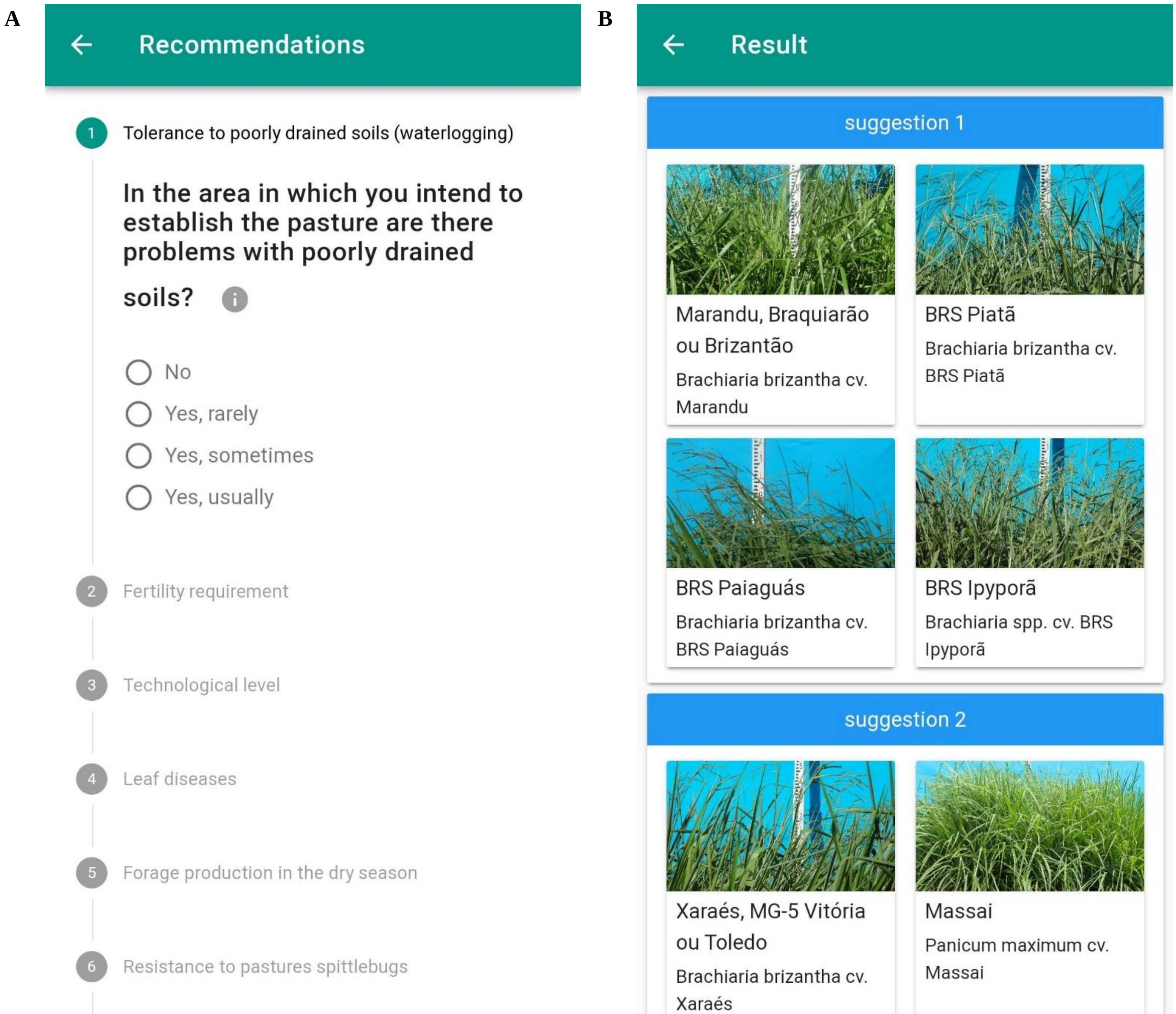

Figure 2. A) Main screen of "choice of forage cultivars for pasture establishment" function. Six of the 8 questions (tolerance of waterlogging, fertility requirement, technological level, leaf diseases, forage production in the dry season, resistance to pasture spittlebugs, frost tolerance and rainfall) are shown on the screen. This function is currently available only for *Urochloa* and *Megathyrsus maximus* cultivars. **B)** Screen showing an example of output from the "choice of forage cultivars for pasture establishment" function, based on the input (responses) of a user.

Perspectives

Pasto Certo® 2.0 has had a broad public acceptance, confirmed by the positive evaluation of the application and number of downloads surpassing 30 thousand. Moreover, improvements and adjustments have been requested by users, which will be incorporated into version 3.0. This new version is already under construction and the following items will be incorporated: inclusion of other forage genera (*Andropogon*, *Paspalum* and *Cenchrus*); and 2 new interactive functions.

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References

(Note of the editors: All hyperlinks were verified 19 May 2020.)

Barrios SCL; Carromeu C; Silva MAI da; Santos MF; Valle CB do; Jank L. 2017. Pasto Certo – versão 1.0® aplicativo para dispositivos móveis sobre forrageiras tropicais. Comunicado técnico No. 142. Embrapa Gado de Corte, Campo Grande, MS, Brazil. bit.ly/2ZnFShB

Jank L; Andrade CMS de; Barbosa RA; Macedo MCM; Valério JR; Verzignassi JR; Zimmer AH; Fernandes CD; Santos MF; Resende RMS. 2017. O capim-BRS Quênia (*Panicum maximum* Jacq.) na diversificação e intensificação das pastagens. Comunicado técnico No. 138. Embrapa Gado de Corte, Campo Grande, MS, Brazil. goo.gl/RHsH1v

José MR. 2012. Forrageiras: Uma grande parceira para o agronegócio. Anuário 2012. Associação Brasileira de Sementes e Mudas (Abrasem), Brasília, DF, Brazil. p. 22–23.

Valle CB do; Euclides VPB; Pereira JM; Valério JR; Pagliarini MS; Macedo MCM; Leite GG; Lourenço AJ; Fernandes CD; Dias Filho MB; Lempp B; Pott A; Souza MA de. 2004. O capim-xaraés (*Brachiaria brizantha* cv. Xaraés) na diversificação das pastagens de braquiária. Série Documentos No. 149. Embrapa Gado de Corte, Campo Grande, MS, Brazil. goo.gl/dQXGwU

Valle CB do; Euclides VBP; Montagner DB; Valério JR; Mendes-Bonato AB; Verzignassi JR; Torres FZV; Macedo MCM; Fernandes CD; Barrios SCL; Dias Filho MB; Machado LAZ; Zimmer AH. 2017. BRS Ipyporã ("belo começo" em guarani): Híbrido de *Brachiaria* da Embrapa. Comunicado técnico No. 137. Embrapa Gado de Corte, Campo Grande, MS, Brazil. bit.ly/2ZkfhlJ

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TGFT Editorial Team
A.A. 6713, Km 17 Recta Cali-Palmira, Cali, Valle del Cauca, Colombia.
Phone: +57 2 4450100 Ext. 3084
Email: CIAT-TGFT-Journal@cgiar.org