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Research Papers

Short Communications

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Research paper

Water use, root activity and deep drainage within a perennial legume-grass pasture: A case study in southern inland Queensland, Australia

Uso de agua, actividad radicular y drenaje profundo en una pastura leguminosa-gramínea perenne: Un estudio de caso en el sur de Queensland, Australia

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Abstract

Water use and depth of water extraction of leucaena (*Leucaena leucocephala*) and Rhodes grass (*Chloris gayana*) pasture, irrigated with desalinated coal seam water (a by-product of the coal seam gas industry), were monitored to provide background information on root activity, spatial and temporal water use and deep drainage over a 757-day period from August 2011 to August 2013. Methodology comprised measurement of soil water from surface to 4 m depth using 8 EnviroSCAN probes connected to dataloggers positioned within leucaena twin rows and within the Rhodes grass inter-row. Just over 581,000 individual moisture measurements were collated and are reported here. Water extraction (and by inference root activity) of leucaena and Rhodes grass showed marked seasonal fluctuation with deepest and highest water extraction occurring during the first growing season; water extraction was greatly diminished during the following drier and cooler seasons due to the negative influences of lower soil moisture contents, lower temperatures and increased defoliation on pasture growth. The highest values of deep drainage below 4 m depth occurred when high rainfall events corresponded with high soil water storage in the entire profile (0–4 m depth). Given that water usage by both leucaena and Rhodes grass was greatest in the upper layers of soil (<1.5 m), future research should focus on how the level of competitive interaction might be managed by choice of row spacing and frequency of irrigation. Further studies are needed, including: (a) physical sampling to determine the depth of active roots; (b) how defoliation affects rooting behaviors and water use of leucaena; and (c) modelling of the water and salt balances of leucaena and grass inter-row systems using data from this study, with various levels of irrigation, to investigate the risks of deep drainage over an extended climate sequence.

Keywords: Active rooting depth, agroforestry, *Chloris gayana*, *Leucaena leucocephala*, water extraction.

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Resumen

En el presente estudio se caracterizó el uso del agua y la profundidad de extracción de agua en una pastura compuesta por leucaena (*Leucaena leucocephala*) y pasto Rhodes (*Chloris gayana)*, irrigada con agua desalinizada proveniente de vetas de carbón [un subproducto de la industria del gas de vetas de carbón (*coal seam gas, CSG*)], para generar información sobre la actividad radicular, el uso de agua en el espacio y tiempo, y el drenaje profundo durante un período de 757 días (agosto de 2011 hasta agosto de 2013). La metodología consistió en mediciones de la humedad de suelo desde la superficie hasta 4 m de profundidad utilizando 8 sondas EnviroSCAN conectadas a dataloggers situados dentro de las hileras dobles de leucaena y las franjas del pasto entre las hileras de leucaena. Se presenta la compilación de algo más de 581.000 mediciones individuales de humedad que fueron ejecutadas. La extracción de agua (y, por inferencia, la actividad radicular) de leucaena y el pasto Rhodes mostraron una marcada fluctuación estacional, con mayor y más profunda extracción de agua durante el primer ciclo de crecimiento. La extracción de agua se redujo en gran medida durante las subsiguientes temporadas más frías y más secas, debido a los efectos negativos de la humedad de suelo más baja, las temperaturas más bajas y el incremento de la defoliación sobre el crecimiento del pasto. Los valores más altos de drenaje a una profundidad mayor de 4 m se registraron cuando eventos de alta precipitación correspondían con un alto almacenamiento de agua a lo largo de todo el perfil (0–4 m de profundidad). Se necesitan estudios adicionales, incluyendo: (a) muestreos para determinar las profundidades hasta las cuales se encuentran raíces activas; (b) cómo la defoliación afecta el sistema radicular y el uso de agua de leucaena; y (c), mediante el uso de los datos de este estudio, modelando los balances de agua y de sales en sistemas silvopastoriles con hileras de leucaena y franjas de pasto, con varios niveles de riego, para investigar los riesgos de drenaje profundo durante una secuencia climática extendida.

Palabras clave: Agroforestería, *Chloris gayana,* extracción de agua, *Leucaena leucocephala,* profundidad radicular.

Introduction

Intensive production systems such as *Leucaena leucocephala* (leucaena)-grass pastures are the key to enhancing profitable cattle production in northern Australia. With an area greater than 200,000 ha in Queensland, leucaena-grass pastures have been shown to be productive, profitable and sustainable (Shelton and Dalzell 2007). Furthermore, irrigation of leucaena can increase beef production by 3–6 times compared with dryland plantings (Shelton and Dalzell 2007).

Over the past decade, coal seam gas (CSG) exploration in southern Queensland has expanded rapidly, generating a large amount of water as a by-product of the gas extraction process, which must be put to beneficial use. Irrigated systems, capable of using large volumes of water with minimal risk impact on natural aquifers, are needed.

The decision by CSG companies to irrigate leucaena combined with Rhodes grass (*Chloris gayana*) was based on the hypothesis that the roots of trees and grass occupy different soil strata when growing in association (Schroth 1999) and are capable of maximizing water use in the profile and minimizing deep drainage. In the case of leucaena-grass pasture systems, there is limited information concerning root distribution and water uptake. According to Poole (2003) and Radrizzani (2009), approximately 60% of root biomass of a leucaena-grass pasture was concentrated in the top 0.4 m of the soil profile, with root abundance decreasing rapidly at greater depths, although some roots reached a depth of 6 m under 5–10-year-old leucaena. However, other studies have reported maximum root depth at only 2.8 m in 28-month-old leucaena (Dhyani et al. 1990) and at 2.6 m in 38-year-old leucaena in alley cropping with pasture (Radrizzani 2009) in soils with physical restrictions. Both of these studies reported a restrictive rock layer at these depths, which prevented leucaena from exploring deeper into the regolith.

Technologies for soil water monitoring have advanced over the past decade. EnviroSCAN (Sentek Pty. Ltd., Stepney, South Australia) capacitance systems are used in Australia and other countries to accurately monitor soil water content for irrigation management by measuring the electrical constant of the soil (Jabro et al. 2005). Precise measurements of soil water are critical for a better understanding of water use by crops and pastures and for irrigation scheduling. For instance, water management can be used to prevent or promote flushing of excess soil salt via drainage below the rooting zone.

Accordingly, as a prelude to a formal program of research, this study was designed to monitor soil water extraction under a leucaena-Rhodes grass pasture using EnviroSCAN to provide background information on: (a) the maximum depth of water extraction (and by inference root activity); (b) the amount and pattern of water extraction; and (c) the likelihood of deep drainage below 4 m depth.

Materials and Methods

Site details

Moisture usage was monitored at Santos' Fairview gas field north-east of Injune, Queensland (25°44'40" S, 149°3'19" E), where 234 ha of *Leucaena leucocephala* ssp. *glabrat*a and *Chloris gayana* was being irrigated using desalinated CSG water under 4 centre-pivot irrigation systems. The leucaena (cvv. Wondergraze and Tarramba) was sown in November 2009 in twin rows (1 m apart) with 8 m spacing between the centers of the paired hedgerows. Oats, ryegrass and Rhodes grass (cv. Finecut) were sown between the leucaena twin rows in March-April of 2010 but from 2011 onwards, the alley-ways between the leucaena twin rows were dominated by Rhodes grass. The soil types were Black and Red Vertosols (Isbell 1996), and at all locations the soil profile was >2 m depth to the C horizon and 3-4 m to regolith (substrate).

The subtropical climate has an annual rainfall of 628 mm and average maximum and minimum temperatures of 33.6 and 19.6 °C, respectively, in the hottest month (January) and 20.1 and 3.2 °C in the coolest month (July) (Bureau of Meteorology 2014). An automatic weather station recorded daily rainfall, maximum and minimum temperatures, wind speed, total radiation and potential evapotranspiration (PET) using the Penman-Monteith equation (Allen et al. 1996).

Soil water measurements

Volumetric soil water content was monitored at 4 sites using 8 EnviroSCAN probes connected to dataloggers (RT6 logger, Sentek Pty. Ltd.) with a sampling interval of 15 minutes. Each EnviroSCAN probe had 7 capacitance sensors located at 0.1, 0.3, 0.6, 1.2, 2, 3 and 4 m below ground level and data were collected over 757 days from August 2011 to August 2013. Four probes (1–4) were positioned within the leucaena twin rows and 4 probes (5–8) within the Rhodes grass inter-row sward at 2 sites, 2 and 4 m from the center of the leucaena twin rows. Field capacity point (FC) and wilting point (PWP) were estimated using IrriMAX 9.1.1 software tools (Version 9.1.1, Sentek Pty. Ltd.). Total plant-available water (PAW) was calculated from the difference between FC and PWP (Figure 1).

The sensors were installed following the recommendation of Sentek Pty. Ltd., and an in-situ calibration equation was developed for each soil (SENTEK 2001).

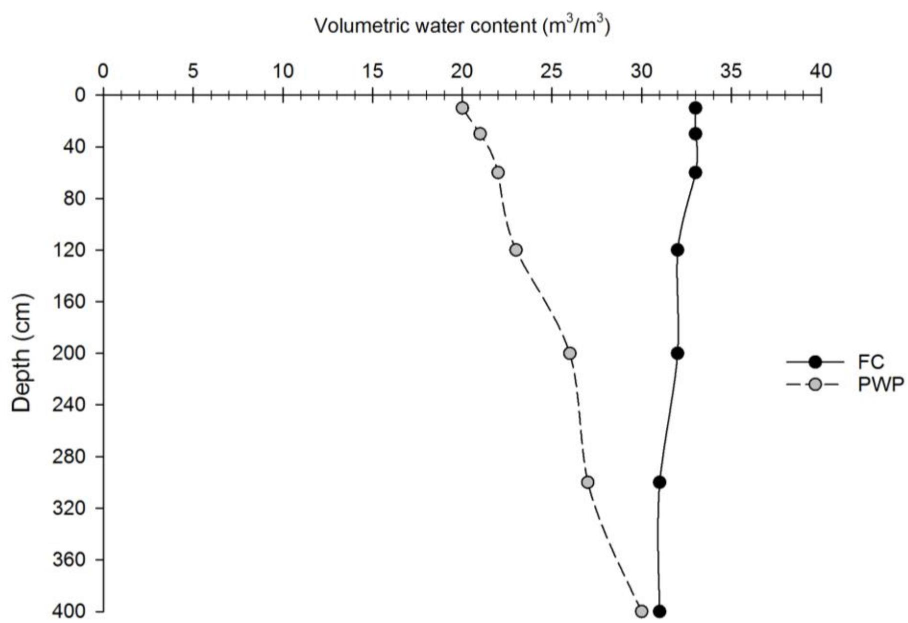

Figure1: Profile of soil water content used for the study. FC = field capacity; PWP = permanent wilting point.

Depth of water extraction

Depth of water extraction, assumed to be indicative of the maximum depth at which roots were actively taking up water, was estimated using the IrriMAX 9.1.1 software tools by measuring the depletion of water in the soil profile during days when no precipitation was recorded. Using the graphing tools of IrriMAX 9.1.1, it was possible to observe the activity of roots as defined by daily extraction patterns of >0.1 mm per day. Using this method, it was possible to generate a large database reflecting the extent and depth of water extraction (root activity) per month at each probe.

Water uptake and deep drainage

Decreases in soil water content could be due to evapotranspiration, plant water uptake (WU), runoff (R) or drainage (D). The EnviroSCAN data were used to calculate WU and D for the top 4 m of soil profile from 1 August 2011 to 27 August 2013 at 15-minute intervals. Any change in soil water content between 18:00 and 06:00 h was assumed to be drainage, as evaporation and plant uptake were assumed to be negligible during the night (Ward et al. 2014). Runoff was minimized by the high ground cover of the pasture but could not be estimated by the EnviroSCAN probes.

Daily water use (mm/d) at different depths (0.1, 0.3, 0.6, 1.2, 2, 3 and 4 m) was calculated using IrriMAX 9.1.1 software. Daily WU for the whole profile was obtained by interpolation between sensors.

Deep drainage (mm) below 4 m depth was estimated for all probes.

Statistical analyses

A total of 72,635 data points was logged for each probe, totalling 581,080 data points during the 757 days of study. Basic statistics were used to compare depth of water extraction, soil water extraction and deep drainage below 4 m depth data and averages and standard errors were calculated for these parameters plus potential evapotranspiration. Within leucaena twin rows, the averages for probes 1–4 (n=4) were used; within the grass inter-row, the data for probes located 2 and 4 m from leucaena twin rows were pooled (n=4). Data were pooled for the soil types as there were no differences in water use.

Results

Site information

A total of 552 mm rain was recorded during the first growing season (October 2011–May 2012), and only 338 mm during the second growing season (October 2012–May 2013) (Figure 2a). Rainfalls during the cool dry seasons (June–September) were 55, 149 and 7 mm for 2011, 2012 and 2013, respectively. (Note: There was an unseasonably high rainfall event of 122 mm during the month of June 2012). The average monthly maximum and minimum temperatures for the growing seasons were 30.1 and 15.6 °C, respectively; values for the cool dry seasons were 21.3 and 5.4 °C. The average values for potential evapotranspiration (PET) were 4.5 and 5.3 mm/d for the first and second growing seasons, respectively. PET for the cool seasons was similar in 2011, 2012 and 2013 with an average of 2.9 mm/d.

Supplementary irrigation was applied from the beginning of the study period but ceased due to lack of available water in April 2012 for probes 1, 3, 4, 5 and 6 and in July 2012 for probes 2, 7 and 8, when 155 mm had been applied (Figure 2a). Grazing commenced in late 2010, 12 months after planting. Initially the pastures were rotationally grazed and cattle were moved to allow at least 50 days recovery. In February 2012, all leucaena was pruned to a height of 0.5 m above the ground to control excessive height and thereafter was continuously grazed.

Soil water content and plant available water

Over the 2 years of the study, the average stored soil water (0–4 m depth) within leucaena twin rows and within grass inter-rows varied from 1,244±7 to 940±41 mm. The average values for field capacity and wilting point were 1,168 and 937 mm, respectively. Thus, regardless of location, relative plant available water (PAW) varied from 100% in August 2011 to 1% in August 2013 (Figure 2b). The unusually high rainfall event in June 2012 refilled the soil profile; however, thereafter PAW decreased due to lack of rainfall and irrigation.

Depth of water extraction

Overall, depth of water extraction was deeper in the growing seasons than in cool dry seasons, regardless of probe locations (Figures 3a and 3b). In the first growing season, water extraction within leucaena twin rows (leucaena-dominant) extended to an average depth of 2.2±0.15 m (maximum depth of water extraction was 4 m) (Figure 3a). During the second growing season, depth of water extraction reached 1.9±0.20 m (maximum rooting depth was 4 m). Average depths of water extraction within the grass inter-row (Rhodes grass-dominant) during the first and second growing seasons were 1.8±0.15 and 1.2±0.9 m, respectively, while maximum depth of water extraction within the grass inter-row was 3.5 m (Figure 3b). Depth of water extraction was less than 0.9 m for both pasture types in the cool dry seasons (Figure 3b).

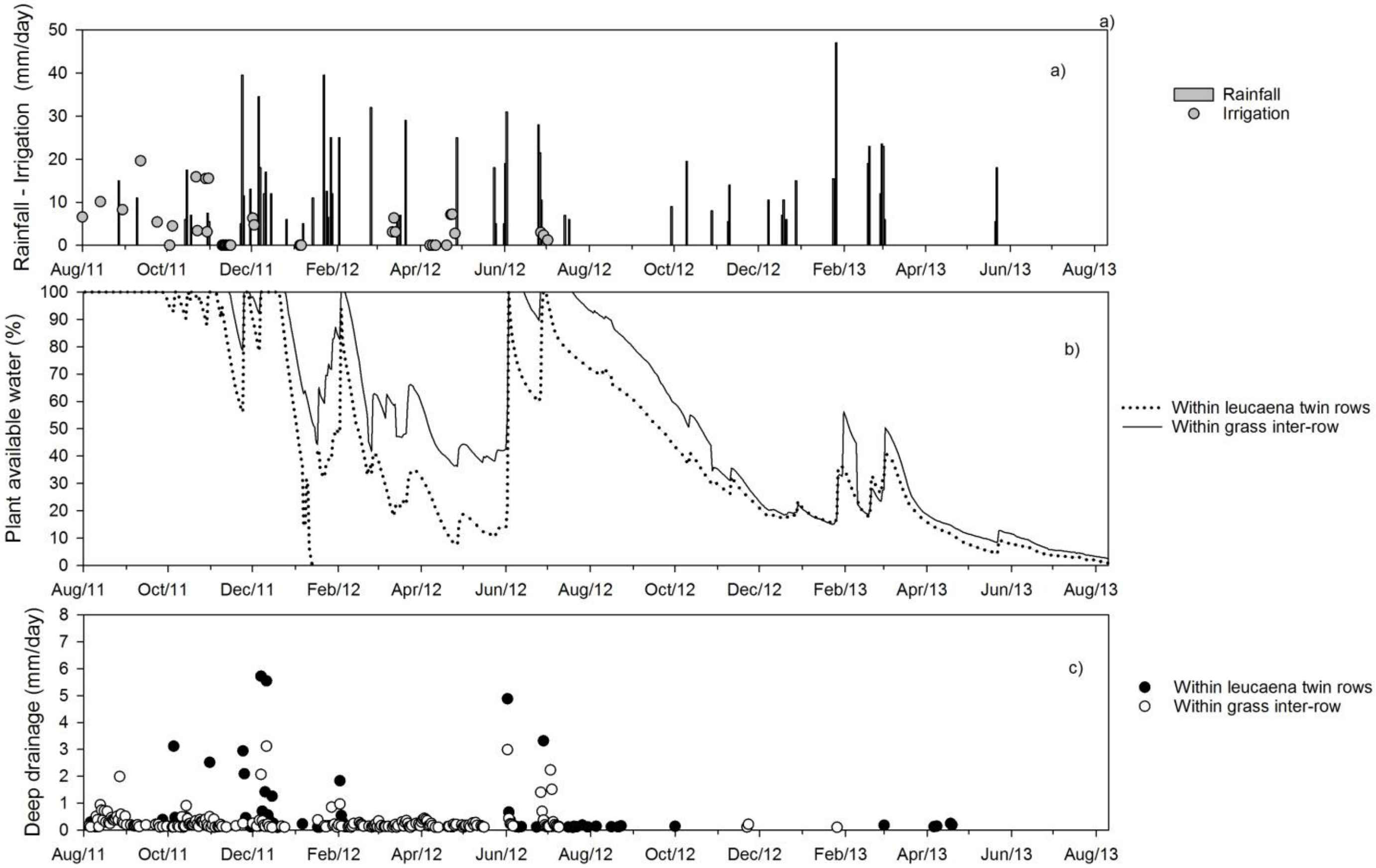

Figure 2. a) Rainfall and irrigation events; b) percentage of plant available water within leucaena twin rows and within the grass inter-row; and c) average daily deep drainage >0.1 mm/d within the leucaena twin rows and within grass inter-row during the period of study.

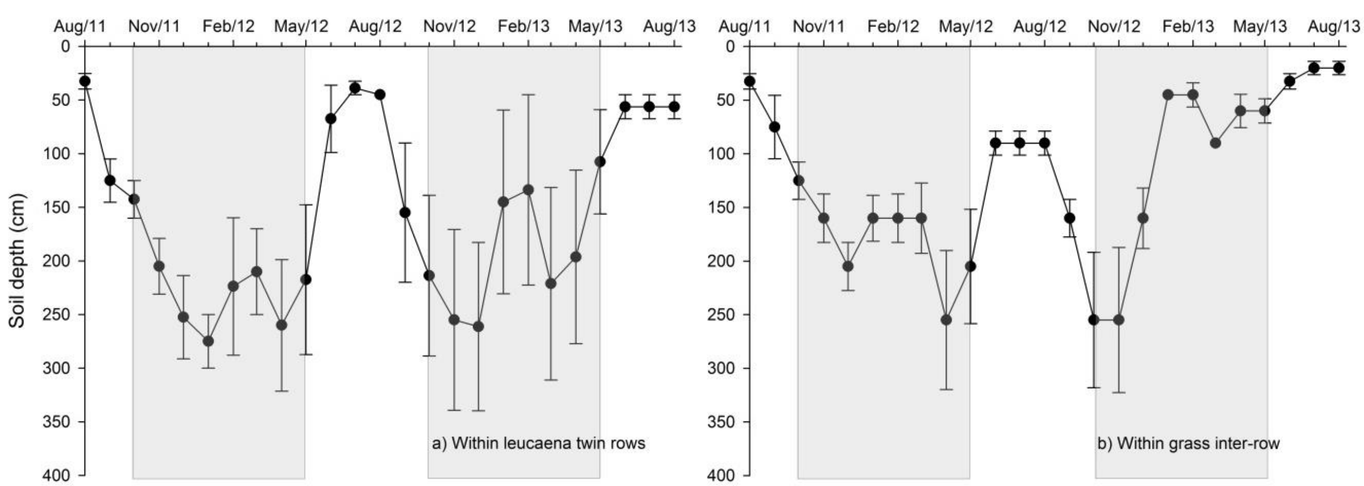

Figure 3. Monthly maximum depth of water extraction detected with IrriMax 9.1.1 software: a) within leucaena twin rows; and b) within the grass inter-row. Growing seasons are shown in light grey and standard error by bars (n=4).

Temporal and spatial patterns of water extraction

In general, greatest water extraction occurred in the first wet season. In all seasons, water extraction was highest in surface soil zones, and reduced with depth (Figure 4).

During the first growing season, total WU within leucaena twin rows (probes 1–4) was 675±181 mm; however, average WU was higher for probes 1 and 2 at 916±280 mm. An average (probes 1–4) of 77% of water was extracted from surface soil to 1.5 m depth, increasing to 99% for 1.5–3 m depth (Figure 4a; Table 1). During the second growing season, WU was lower at 303±61 mm, of which 75% was extracted from surface to 1.5 m depth, increasing to 94% for 1.5–3 m depth. During the cool dry seasons, the total WU within leucaena twin rows during 2012 was 81±16 mm, reducing to 40±8 mm in 2013, of which 100% was extracted from surface to 1.5 m depth (Table 1).

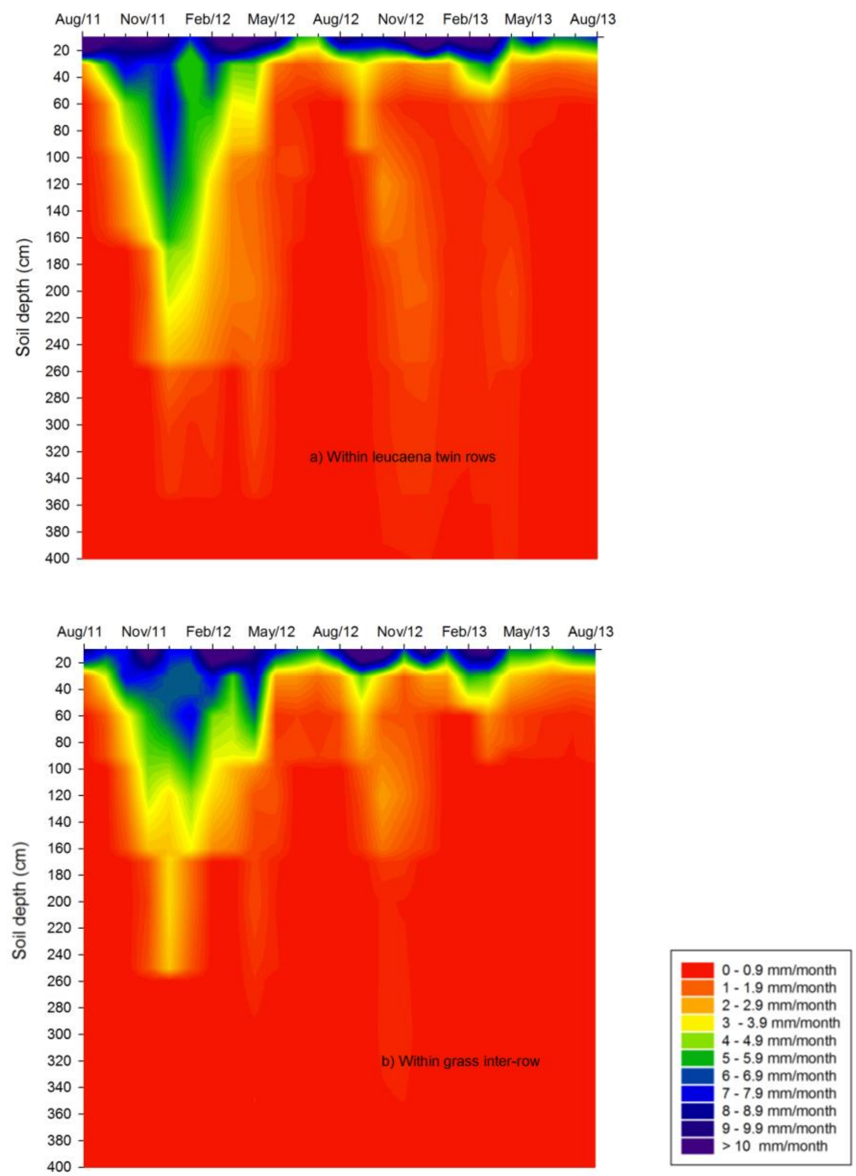

Figure 4. Patterns of average water extraction: a) within leucaena twin rows; and b) within the grass inter-row per 0.1 m soil layer from August 2011 to August 2013. The monthly amount of water extracted per layer is expressed by different colors (mm/month).

Table 1. Accumulated total water extraction per layer and total deep drainage below 4 m depth within leucaena twin rows and within the grass inter-row during the growing and cool dry seasons of 2012 and 2013. Standard errors are presented in italics.

Depth (m)	Average total water extraction per season (mm)											
	Within leucaena twin rows (probes 1–4) (n=4)				Within leucaena twin rows (probes 1–2) (n=2)				Between leucaena twin rows (probes 5–8) (n=4)			
	1st GS (304 days)	1st CDS (122 days)	2nd GS (243 days)	2nd CDS (88 days)	1st GS (304 days)	1st CDS (122 days)	2nd GS (243 days)	2nd CDS (88 days)	1st GS (304 days)	1st CDS (122 days)	2nd GS (243 days)	2nd CDS (88 days)
0–0.5	256 ±*58*	62 ±*16*	181 ±*25*	38 ±*8*	339 ±*80*	87 ±*19*	223 ±*14*	42 ±*18*	322 ±*66*	113 ±*18*	196 ±*8*	65 ±*19*
0.5–1	152 ±*34*	12 ±*3*	21 ±*7*	2 ±*2*	191 ±*62*	10 ±*6*	21 ±*2*	0	163 ±*37*	28 ±*4*	35 ±*35*	0
1–1.5	111 ±*29*	4 ±*3*	25 ±*9*	0	152 ±*25*	1 ±*1*	31 ±*6*	0	131 ±*18*	7 ±*3*	23 ±*23*	0
1.5–2	75 ±*26*	1 ±*1*	23 ±*10*	0	111 ±*37*	0	25 ±*11*	0	40 ±*8*	1 ±*1*	5 ±*2*	0
2–2.5	56 ±*22*	0	21 ±*10*	0	83 ±*37*	0	20 ±*11*	0	16 ±*9*	0	1 ±*1*	0
2.5–3	15 ±*12*	0	13 ±*9*	0	25 ±*15*	0	8 ±*4*	0	1 ±*1*	0	1 ±*1*	0
3–3.5	10 ±*7*	0	11 ±*8*	0	15 ±*7*	0	6 ±*3*	0	0	0	1 ±*1*	0
3.5–4	0	0	8 ±*7*	0	0	0	0	0	0	0	0	0
Total	675 ±*181*	81 ±*16*	303 ±*61*	40 ±*8*	916 ±*280*	97 ±*30*	334 ±*99*	42 ±*14*	673 ±*107*	149 ±*21*	262 ±*23*	65 ±*19*
DD (mm)	32 ±*9.4*	11 ±*4*	5 ±*1.4*	2 ±*1*	43 ±*7.6*	17 ±*5.0*	8 ±*2.3*	2 ±*0.3*	39 ±*9.4*	16 ±*2.5*	7 ±*1.6*	2 ±*0.4*
R (mm)	552	149	338	7	552	149	338	7	552	149	338	7
IR (mm)	126	26	0	0	103	26	0	0	103	26	0	0
Δ SWC (mm)	248	-62	74	15	278	-109	130	22	248	-45	112	25

GS: growing season; CDS: cool dry season; DD: depth drainage; R: rainfall; IR: irrigation; and Δ SWC: change in soil water content.

During the first growing season, WU within the grass inter-row of probes 5–8 averaged 673±107 mm (Figure 4b; Table 1). However, the spatial patterns of water uptake were different from those within leucaena rows, with 92% of water extracted from surface to 1.5 m depth. During the second growing season, total water extracted was greatly reduced to 262±23 mm, with 89±5% extracted to 1 m depth. During the first cool dry season, average total water uptake was 149±21 mm (Table 1), with 97% extracted from surface to 1.5 m depth. During the second cool dry season, total water uptake was lower at 65±19 mm, with 100% of water being extracted from surface to 0.5 m depth (Table 1).

Deep drainage below 4 m depth

Deep drainage below 4 m for the study period was 50±12.5 and 64±15.4 mm for the leucaena and grass inter-row, respectively. This is 4.1 and 5.4% of total rainfall plus irrigation.

It was greatest when significant rainfall events occurred when moisture content of soil profile was near FC (Figures 2a and 2b; Table 1). Thus highest deep drainage occurred when rainfall events refilled the soil profile to more than 1,200 mm, i.e. ≥100% PAW (Figures 2a and 2c). Deep drainage within leucaena twin rows was 31.5±9.4 mm during the first growing season, but lower at 4.5±1.4 mm during the second growing season. In the first cool dry season of 2012, deep drainage was 11.1±4 and 1.8±1 mm during the cool dry season of 2013.

Within the grass inter-row during the first and second growing seasons, deep drainage volumes were 38.7±9.4 and 6.6±1.6 mm, respectively. These volumes were similar to the 43±7.6 and 8.3±2.3 mm of deep drainage registered for probes 1 and 2 located within leucaena twin rows. By comparison deep drainage volumes within the grass inter-rows during the cool dry seasons were 16±2.5 and 2.4±0.4 mm for 2012 and 2013, respectively.

Discussion

The motivation for this study was based on the requirement that ground water extractions, as part of the CSG process, must be used for beneficial purposes, e.g. irrigation of agricultural crops and pastures. As CSG water varies in availability from limited to excess volumes, the potential outcomes of such variable irrigation scheduling need to be better understood.

The objective of this study was to monitor and describe the water extraction (and by inference apparent root activity) and deep drainage of an irrigated leucaena-grass pasture grown on Vertosols. The methodology comprised 2 years of detailed monitoring of spatial and temporal patterns of water extraction, and hence root activity, and deep drainage below 4 m depth. Data showed that all parameters varied depending on rainfall events, season and management of the leucaena-grass pastures.

Root activity and water extraction

Depths of water extraction and water uptake patterns, shown so dramatically in Figure 4, are of particular interest in agroforestry systems as trees and grasses are considered to occupy different soil strata when grown in association (Schroth 1999). In this survey, water extraction was used as a proxy for depth of rooting activity. Maximum depth of water extraction and water use (WU) were modestly greater within leucaena twin rows (leucaena-dominant) than within the grass inter-row (Rhodes grass-dominant). When growing at maximum capacity in the first growing season, water extraction within leucaena twin rows extended to an average depth of 2.2±0.15 m with a maximum depth of 4 m. By contrast, mean depth of water extraction within the grass inter-row was 1.8±0.15 m with a maximum depth detected of 3.5 m. It is unlikely that roots of grass reached 3.5 m depth, and it is possible that lateral roots of leucaena were exploiting soil moisture under the grass inter-row. Further studies are needed, including physical sampling of plant roots, to determine the origin of active roots.

The percentage of total WU within leucaena twin rows below 1.5 m depth was 25% (leucaena-dominant) compared with just 10% between rows (Rhodes grass-dominant). This suggested that there was only a small degree of complementarity in water use between the trees and grass, with leucaena accessing water deeper in the soil profile. Various authors mention that, in successful agroforestry systems, trees can access water resources that the crop or grass would not otherwise access (Cannell et al.1996; Schroth 1999; Fernandez et al. 2008). This assertion was not strongly supported in this study.

These results confirm those reported by Poole (2003), who found that maximum rooting depth for another tropical grass (buffel grass, *Cenchrus ciliaris*) was 1.7 m in Grey Vertosols in central Queensland, Australia. However, the depth of water extraction and by inference active rooting depth of leucaena observed in this study was much shallower than that reported by Poole (2003), who found physical evidence of roots of 5–10-year-old *L. leucocephala* to 5.9 m depth. Rooting depths similar to ours have been reported at 2.8 m in 28-month-old leucaena (Dhyani et al. 1990), at 2.6 m in 38-year-old leucaena in alley cropping with pasture (Radrizzani 2009) and at 2 m in an alley cropping system with maize (Rao et al. 1993).

Active water extraction by leucaena was shallower during the second growing season due to the combined effects of lower rainfall, absence of irrigation and severe defoliation by pruning and grazing. This was unexpected as leucaena has a reputation for continuing to grow during prolonged dry periods, when upper layers of the soil profile are dry (i.e. soil water content <PWP); this attribute is often cited as one of its major production advantages (Shelton and Dalzell 2007). We postulate that the more severe defoliation experienced in the second growing season may have contributed to the lower WU of leucaena during this time. The effects of continuous heavy grazing were also severe on Rhodes grass, as depth of water extraction reduced from 1.5 m to 0.5 m. During the cool dry seasons, the shallow depths of water extraction by both species (0.66±0.18 m) could be attributed to lower temperatures, which would have limited plant growth (Cooksley et al. 1988; Moore et al. 2006).

Water uptake patterns

Water uptake was greatest in the upper soil profile and decreased with depth. This pattern reinforces the findings of Callow (2011), who reported that the capacity of warm season forages to extract soil water generally decreased with depth.

Season had a strong influence on total water extraction, which was highest in the first growing season due to high evapotranspiration demands associated with rapid growth of the pasture and adequate soil water content leading to deeper root exploration by both leucaena and Rhodes grass.

The amount of water extracted during the cool dry seasons was much lower than during the growing seasons as low soil water levels coupled with lower temperatures, as well as defoliation, would have limited plant growth. The influence of defoliation on WU requires further study. Overall, the amounts of water extracted were lower than those reported by Narain et al. (1998) at a location receiving an average of 1,523 mm of rainfall. In a 4-year study of water use under different land uses, which included a leucaena monoculture and a leucaena-grass system, they reported average WUs of 1,528 and 1,397 mm/yr, respectively. They found similar seasonal differences in water extraction between growing and cool dry seasons, with water extraction limited by low available soil moisture and reduced plant growth during winter.

Water use of leucaena versus grass

There was some evidence that leucaena extracted more water than grass alone as its greater depth of rooting made a modest difference in water uptake. Water extracted within the grass inter-row (Rhodes grass-dominant) was 25% lower than that extracted within leucaena twin rows. According to Schroth (1999), while depth of root exploration is important, it is necessary also to consider root distribution and root activity within the soil profile.

Deep drainage below 4 m depth

Although the potential advantages of leucaena-grass systems in controlling deep drainage is hypothesized (Shelton and Dalzell 2007), there are few data on the amount of deep drainage that occurs in leucaena-grass pastures. However, there are considerable data on deep drainage in pasture and native vegetation (Owens et al. 2004; Silburn et al. 2009; Tolmie et al. 2011). In this study, daily deep drainage below 4 m differed between growing seasons and cool dry seasons. Deep drainage was greatest when significant rainfall events or frequent irrigation occurred at times when the soil moisture profile was near field capacity. Thus higher daily deep drainage occurred during the first growing season and the cool dry season of 2012 following an unseasonal rainfall event. During the late phase of the study, when rainfall and corresponding soil moisture values were much lower, average drainage was low. There was no major difference between deep drainage within leucaena twin rows and within the grass inter-row.

Poole (2003) modelled the probability of deep drainage under leucaena-buffel grass pastures, buffel grass only and annual summer grain (sorghum) cropping over a 100-year period and also found that higher rates of deep drainage were related to higher rainfall events. The model predicted that there would be less deep drainage under leucaena-grass pastures than under buffel grass pastures and grain sorghum annual cropping. In soils without limitation, the probability of annual deep drainage of 50 mm (over a 100-year period) was 85% for annual sorghum cropping, 60% for buffel grass pastures and 20% for leucaena-grass pastures. Robinson et al. (2010), using simulation modelling for Goondoola Basin in a semi-arid region of Queensland, found that deep drainage was strongly related to soil type and vegetation; clearing native vegetation and introducing crops and pastures increased deep drainage. Pastures with deeper roots (2.4 m depth), such as leucaena-grass pasture, growing on 6 different soil types had 25 mm less of deep drainage than wheat cropping.

The study period had below average to average rainfall and greater deep drainage would be expected in wetter years and with greater irrigation, although growth and water use may also be greater. Modelling of the water and salt balances of leucaena and grass inter-row systems using data from this study, with various levels of irrigation, is recommended to investigate the risks of deep drainage over an extended climate sequence.

Conclusions

EnviroScan sensors were a useful tool for characterizing spatial and temporal patterns of water extraction, and by inference root activity of leucaena-Rhodes pasture. A marked seasonal water extraction was observed which was greater during growing seasons and lower in cool dry seasons. Both leucaena and Rhodes grass extracted a greater amount of water in the upper layers, suggesting high levels of competition for water resources between species. Low rainfall, defoliation and low temperatures negatively affected depth of water extraction and therefore reduced total water extraction. There was some evidence that leucaena roots were active slightly deeper in the soil profile than roots of Rhodes grass.

The highest values of deep drainage below 4 m occurred when rainfall events coincided with soil moisture near to 100% PAW. Therefore, irrigation should be avoided at this time. Deep drainage below 4 m within leucaena twin rows differed little from that within the grass inter-rows.

Given that water usage by both leucaena and Rhodes grass was greatest in the upper layers of soil (<1.5 m), future research should focus on how the level of competitive interaction might be managed by choice of row spacing and frequency of irrigation. Also, additional studies are needed, including: (a) physical sampling to determine the depth and distribution of active roots; and (b) how defoliation affects rooting behavior and water use of leucaena. Modelling of the water and salt balances of leucaena and grass inter-row systems using data from this study, with various levels of irrigation, is recommended to investigate the risks and advantages of deep drainage to manage soil salt profiles.

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Research paper

Ecuaciones de calibración en espectroscopía de reflectancia en el infrarrojo cercano (NIRS) para predicción de parámetros nutritivos en forrajes tropicales

Near infrared spectroscopy (NIRS) calibration equations to predict nutritional quality parameters of tropical forages

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Resumen

El estudio tuvo como objetivo desarrollar y evaluar ecuaciones de calibración para predecir los parámetros de calidad nutritiva de especies forrajeras tropicales utilizando espectroscopía de reflectancia en el infrarrojo cercano (NIRS, por su sigla en inglés). En total fueron analizadas 1,991 muestras de tejido de gramíneas y leguminosas heterogéneas por su edad de rebrote, estado fenológico y partes de las plantas recolectadas, época de muestreo y sitio o lugar de procedencia. En grupos aleatorios de muestras se realizaron análisis químicos de referencia para proteína cruda (n=315), fibra detergente neutro (n=243), fibra detergente ácido (FDA) (n=156), digestibilidad in vitro de la materia seca (n=449) y digestibilidad de fibra detergente neutro (DFDN) (n=238). Las curvas de calibración se calcularon mediante el uso de errores estándar de calibración, validación cruzada (SEVC) y predicción. La precisión de cada ecuación se calculó por el coeficiente de determinación (R^2) y el índice RPD (relación entre la desviación estándar y el SEVC). La validación se realizó con muestras externas resultando coeficientes de correlación (r) >0.90 para los parámetros, con excepción de FDA (r=0.72). Los resultados indican que la predicción de los parámetros de calidad nutritiva con el uso de NIRS en forrajes tropicales es confiable y rápida; no obstante la determinación de la fracción de FDA por este método analítico requiere un mayor ajuste para incrementar su confiabilidad.

Palabras clave: Calidad nutritiva, gramíneas, leguminosas, métodos analíticos, nutrición animal.

Abstract

The objective of this study was to develop NIRS calibration curves to predict nutritional quality parameters of tropical forage species. For this a total of 1,991 samples of tropical forages (grasses and legumes) were employed. These samples showed a high heterogeneity in regrowth age, vegetative state and parts of the plants collected, sampling period and sample origin. Chemical analysis was performed to determine crude protein, neutral detergent fiber, acid detergent fiber (ADF), in vitro digestibility of dry matter and digestibility of neutral detergent fiber. A group of samples was used for each chemical parameter to develop the calibration curves. The curves were chosen taking into account the standard errors of: calibration; cross validation (SECV); and prediction. To evaluate the accuracy of each equation the coefficient of determination (R^2) and the RPD (ratio performance deviation) index (relation between standard deviation and SECV), which assesses the predictive power of the equations, were calculated. Validation was performed with external samples and results showed correlation coefficients (r) of >0.90 for all parameters except for ADF (r=0.72), demonstrating the precision of the predictive equations. However, the determination of ADF by this analytical method requires further work to increase its reliability.

Keywords: Analytical methods, animal nutrition, forage quality, grasses, legumes.

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Introducción

Los forrajes son la principal fuente de alimento para bovinos; por tanto, el conocimiento de su valor nutritivo en forma oportuna y confiable es de vital importancia para el éxito del negocio ganadero, ya que condiciona directamente el desempeño productivo y reproductivo de los animales. Los análisis químicos tradicionales utilizados para determinar la composición de los forrajes representan altos costos en el tiempo, requerimiento de mano de obra calificada, y utilización de reactivos químicos que en algunos casos pueden ser contaminantes peligrosos. La espectroscopía de infrarrojo cercano (NIRS, sigla en inglés) es una técnica considerada como una herramienta rápida y confiable para la determinación de parámetros de calidad de forrajes, principalmente gramíneas y leguminosas en pasturas, ensilaje y henos, entre otros (Castro et al. 2005; Ibáñez y Alomar 2008).

El presente trabajo tuvo como objetivo específico desarrollar curvas de calibración NIRS para una rápida y confiable determinación de proteína cruda (PC), fibra detergente neutro (FDN), fibra detergente ácido (FDA), digestibilidad in vitro de la materia seca (DIVMS) y digestibilidad de fibra detergente neutro (DFDN) en muestras de plantas forrajeras tropicales (gramíneas y leguminosas), con el fin de apoyar programas de selección y mejoramiento de forrajes tropicales.

Materiales y Métodos

El estudio se realizó en el Laboratorio de Calidad de Forrajes del Centro Internacional de Agricultura Tropical (CIAT), localizado en el municipio de Palmira (Valle del Cauca, Colombia). El equipo NIRS se mantiene en ambiente estable (21.3 °C y humedad relativa de 53%).

Muestras

En total fueron analizadas 1,991 muestras de forrajes tropicales, de las cuales previamente existían análisis químicos de diferentes parámetros, representando 62 especies de leguminosas herbáceas y arbustivas y 26 especies de gramíneas. Entre las leguminosas se incluyeron *Canavalia brasiliensis*, *Canavalia* sp., *Centrosema brasilianum*, *Clitoria ternatea*, *Cratylia argentea*, *Cratylia mollis*, *Cratylia* sp., *Desmodium velutinum*, *Dioclea guianensis*, *Dioclea virgata*, *Flemingia macrophylla*, *Leucaena diversifolia*, *Leucaena* sp., *Leucaena trichandra*, *Stylosanthes guianensis*, *Tadehagi triquetrum* y *Vigna unguiculata*. Entre las gramíneas predominaban hibridos de *Brachiaria* y *Brachiaria humidicola*; además en el parámetro DFDN se utilizaron *B. brizantha*, *B. mutica*, *Axonopus* sp. y *Alopecurus pratensis* entre otras. Las muestras provinieron de diferentes localidades en Colombia: Popayán (Cauca), Palmira (Valle del Cauca), Pasto (Nariño) y Santander de Quilichao (Cauca), y distintas edades de rebrote (4, 5, 6, 7, 8, 10, 12, 16 y 20 semanas) que crecieron en épocas secas o lluviosas. Para los análisis se utilizaron hojas o plantas enteras.

Obtención de los espectros

Las muestras fueron escaneadas en un equipo monocromador FOSS-NIRSystem II modelo 6500 (Foss NIRSystem, Silver Spring, Washington, USA), con un rango de longitudes de onda de 400 a 2,500 nm de reflectancia. Las muestras fueron colocadas en celdas "simple cup" (Black), circulares metálicas de 3.5 cm de diámetro interno y 1 cm de espesor, provistas de una ventana de cuarzo para muestras en harina [US-ISIH-0307 (FOSS and Infrasoft International, USA, 2005)]. Cada muestra se escaneó por duplicado, utilizando el software ISIScan (IS-2250) versión 2.71 (FOSS and Infrasoft International, USA, 2005). Una vez recolectados los espectros se generó un archivo .nir.

Determinación de composición química de referencia (LAB)

Las muestras de tejido frescas fueron secadas en un horno a 60 °C (gramíneas) o liofilizadas (leguminosas) durante 96 h y pasadas por un molino Thomas Wiley Laboratory Mill Model 4 con mallas de 1 mm. En un número de muestras seleccionadas aleatoriamente se determinaron PC, FDN, FDA, DIVMS y DFDN para análisis de referencia, siguiendo los protocolos propuestos por los autores de las referencias que aparecen en el Cuadro 1. Para la determinación de FDN y FDA se utilizaron submuestras independientes; el procedimiento no fue secuencial. En el proceso de análisis de FDN no se aplicó enzima amilolítica. Se adicionó sulfito de sodio solo en las leguminosas arbustivas que presumiblemente contenían taninos.

Una vez obtenidos los datos de referencia se generó un archivo .txt.

Cuadro 1. Protocolos desarrollados para análisis de referencia (LAB).

Parámetro	Análisis de referencia
PC	Micro Kjeldahl, Temminghoff (2010)
DIVMS	Tilley and Terry modificado por Moore (1970)
FDN	Van Soest et al. (1991)
FDA	Van Soest et al. (1991)
DFDN	Hoffman et al. (2006), Hall and Mertens (2008)

Calibración

Una vez obtenidos los análisis de referencia (LAB) para PC, FDN, FDA, DIVMS y DFDN, la información se guardó en un archivo Excel con extensión .txt para formar un colectivo para cada parámetro. A partir de los archivos creados (.txt y .nir) se generó un archivo .cal utilizando el software WinISI III (IS-1485) version 1.6 (FOSS and Infrasoft International, USA, 2005). Este archivo se sometió a un análisis estadístico RMS (Root Mean Square) para evaluar la repetitividad espectral de los datos (Urbano-Cuadrado 2004). Dependiendo de la complejidad del parámetro se estableció un límite de selección. En los casos de PC, FDA y DIVMS se utilizó un RMS de 6,000, mientras que para FDN y DFDN se trabajó con un RMS de 3,000.

Finalmente, se realizó un primer análisis de componentes principales (PCA) para reducir la variación de referencia, mejorar las características espectrales y excluir muestras denominadas como anómalas u "outliers", las cuales arrojaran un GH (distancia de Mahalanobis) >3.0. Una vez depurado el colectivo, se sometió nuevamente a PCA para seleccionar para la validación del 10–20% del número de muestras que conforman el colectivo de calibración.

Para la calibración se implementaron los 3 modelos de regresión disponibles en el programa WinISI: regresión de componentes principales (PCR), cuadrados mínimos parciales (CMP), y cuadrados mínimos parciales modificados (CMPM). Se realizaron diferentes combinaciones de tratamientos matemáticos, donde el primer término indicaba el orden de la derivación o diferenciación (primera o segunda derivada), el segundo término la amplitud o distancia entre segmento a sustraer, el tercer término la longitud del segmento que debería ser suavizado, y el cuarto termino el número de veces que se promedió cada segmento. El modelo de corrección implementado fue la combinación de SNV (variable estándar normal) y DETREND, donde SNV corrige los problemas ópticos mientras que DETREND corrige la tendencia de los datos. Las diferentes combinaciones se realizaron con las longitudes de onda completa (400–2,500 nm) e infrarrojo (1,100–2,500 nm).

Selección de las ecuaciones

La selección de las mejores ecuaciones de calibración para cada parámetro está dada por la relación entre el coeficiente de determinación (RSQ) más alto, error estándar de calibración (SEC), error estándar de validación cruzada (SEVC) y error estándar de predicción (SEPc) más bajo y homogéneo entre sí (Solis et al. 2001; Alomar et al. 2003; Vásquez et al. 2004). La evaluación de las ecuaciones en relación con su grado de precisión se hizo a través del índice RPD (ratio performance deviation), el cual es una herramienta estadística que evalúa la relación entre la desviación estándar del análisis químico y el error estándar de validación cruzada (SD/SEVC), siendo considerada como una ecuación con alto poder de predicción si la relación es >3 (Cozzolino y Moron 2004; Arana et al. 2005; Shenderey et al. 2010).

Validación

Una vez seleccionadas las ecuaciones, se realizó la validación cruzada utilizando las muestras de los colectivos y la validación externa mediante la determinación del coeficiente de correlación (r) entre datos de referencia y los datos de predicción.

Análisis estadístico

Los datos fueron analizados y verificados con el paquete estadístico SAS 9.2 para LINUX (SAS Institute Inc., Cary, NC, USA, 2008). Los colectivos definidos para la calibración se sometieron a un análisis de estadísticas descriptivas [media, desviación estándar (DE), valor máximo (max), valor mínimo (min) y coeficiente de variación (CV)]. Se calculó el coeficiente de correlación de Pearson, la correlación (r) entre los resultados de referencia (LAB) y los datos de predicción por NIRS; estos análisis fueron realizados tanto con los datos de calibración como de validación. Se usó el test de Kolmogorov-Smirnov (Zar 1984), que consiste en una prueba no paramétrica para determinar la bondad del ajuste entre sí de 2 distribuciones de probabilidad, ya que compara las funciones de distribución empírica de la muestra vs. la que se desea contrastar. Si la probabilidad del estadístico de la prueba es P>0.05, no hay suficiente evidencia para rechazar la hipótesis nula, indicando que la distribución de los datos obtenidos a partir del modelo NIRS (predicción) es igual a la distribución de los datos de referencia.

Resultados

En el Cuadro 2 se presenta un resumen de la caracterización química de las muestras utilizadas en la calibración, indicando el número de muestras (N) que conforman cada colectivo con representación de leguminosas y gramíneas forrajeras. Para el caso de digestibilidad in vitro de la materia seca (DIVMS) se obtuvieron 2 ecuaciones que compartieron las mismas muestras de leguminosas, pero las muestras de gramíneas eran de edades de corte diferentes (5 y 20 semanas). Los resultados mostraron que el coeficiente de variación (% CV) fue amplio y representó la heterogeneidad del colectivo debido a la variabilidad en familia, género, edad de corte, origen de las muestras y partes de la planta cosechada. Estos factores se tomaron en cuenta al momento de generar el colectivo de calibración.

Los resultados de los parámetros estadísticos muestran que la eficiencia predictiva de las ecuaciones expresadas como errores estándar (SEC, SEVC y SEP_C) fue baja (Cuadro 3). El coeficiente de determinación que indica el ajuste del modelo fue de R^2 >0.90. El índice RPD (relación DE/SEVC) fue >3 lo cual indica que el poder de predicción de las ecuaciones seleccionadas es alto.

Una vez seleccionadas las ecuaciones se realizaron las validaciones respectivas., utilizando las mismas muestras de los colectivos de calibración (Cuadro 3). Los coeficientes de correlación (r) calculados, que relacionan los valores de predicción obtenidos por la técnica NIRS con los de referencia, presentaron valores superiores a 0.90 (P<0.001), lo cual fue confirmado con el test de Kolmogorow-Smirnov (Cuadro 3), con una probabilidad P>0.05.

En el Cuadro 4 se incluyen los parámetros estadísticos de la validación con muestras externas tomando como base al error estándar de predicción (SEPv), el coeficiente de determinación para validación (R^2_V), los valores GH (distancia de Mahalanobis al centro de la población) y NH (distancia de Mahalanobis al vecino más próximo).

Fue evidente una alta correlación entre la predicción por NIRS y datos de referencia (LAB), con excepción de FDA que presentó una correlación r = 0.72.

Cuadro 2. Caracterización química (% en base seca) de las muestras utilizadas en los colectivos de calibración.

Colectivo	N	Media (%)	DE	Min (%)	Max (%)	CV (%)
PC	315	16.4	6.7	4.4	30.6	41.2
FDN	243	55.2	17.3	20.0	79.7	31.4
FDA	156	24.8	7.2	14.5	48.0	29.2
DIVMS[1] (5 sem)	204	63.3	8.3	32.1	75.0	13.2
DIVMS[1] (20 sem)	245	56.8	8.7	24.0	72.2	15.4
DFDN	238	37.9	13.9	5.2	58.7	36.6

[1]DIVMS incluyendo gramíneas de 5 resp. 20 semanas de rebrote.
N: Número de muestras; DE: Desviación estándar; Min: Valor mínimo; Max: Valor máximo; CV: Coeficiente de variación = (DE/Media) x 100.

Cuadro 3. Parámetros estadísticos y eficiencia predictiva de las ecuaciones de calibración.

Parámetro	N_C	SEC	SEVC	SEP_C	R^2	RPD	r	P
PC	310	0.8	0.9	0.9	0.99	7.3	0.99	0.95
FDN	228	1.5	3.5	3.2	0.99	4.9	0.98	0.98
FDA	155	1.7	2.1	1.6	0.95	3.5	0.97	0.90
DIVMS[1] (5 sem)	195	1.7	2.0	1.8	0.95	4.4	0.97	0.52
DIVMS[1] (20 sem)	243	1.8	2.0	1.6	0.96	4.7	0.98	0.92
DFDN	230	2.1	3.2	3.6	0.95	4.4	0.96	0.86

[1]DIVMS incluyendo gramíneas de 5 resp. 20 semanas de rebrote.
N_C: Número de muestras para calibración; SEC: Error estándar de calibración; SEVC: Error estándar de validación cruzada (error del NIRS); SEP_C: Error típico de predicción (calibración); R^2: Coeficiente de determinación; RPD: Relación DE/SEVC; r: Coeficiente de correlación; P: Probabilidad según test de Kolmogorow-Smirnov.

Cuadro 4. Parámetros estadísticos de validación externa.

Parámetro	N_V	SEP_V	R^2_V	GH	NH	r
PC	32	1.8	0.83	2.6	1.3	0.94
FDN	30	4.0	0.74	0.8	0.1	0.98
FDA	20	4.2	0.52	0.9	0.4	0.72
DIVMS[1] (5 sem)	21	6.7	0.81	0.0	0.0	0.93
DIVMS[1] (20 sem)	26	6.3	0.85	4.2	1.9	0.89
DFDN	40	4.3	0.93	0.8	0.1	0.96

[1]DIVMS incluyendo gramíneas de 5 resp. 20 semanas de rebrote.

N_V: Número de muestras para validación; SEP_V: Error típico de predicción (validación); R^2_V: Coeficiente de determinación (validación); GH: Distancia de Mahalanobis al centro poblacional; NH: Distancia de Mahalanobis al vecino más próximo; r: Coeficiente de correlación.

Discusión

Los resultados de este estudio mostraron una marcada variación en las concentraciones de diferentes parámetros de calidad de los forrajes tropicales evaluados, lo cual sugiere que se usaron muestras muy representativas de la calidad. Según Cozzolino et al. (2003) y Valenciaga y Saliba (2006) no existe un número mínimo definido de muestras para una calibración satisfactoria; no obstante es necesario evaluar productos heterogéneos con un mínimo de 100 muestras. Los colectivos de calibración desarrollados en este trabajo contenían un número superior a 150 muestras para cada parámetro con sus respectivos datos de laboratorio, lo cual sugiere una alta representatividad de las muestras usadas.

Por diseño, los coeficientes de variación (CV) para los diferentes parámetros de calidad medidos en este estudio fueron altos (13.2–41.2%) debido a la heterogeneidad de las muestras en relación con familia, género, especie, partes de la planta cosechada, origen de las muestras y edad de rebrote (Cuadro 2). En DIVMS se ha encontrado un CV del 20.1% para *Panicum maximum* (Vásquez et al. 2004).

Según Shenk y Westerhaus (1996) y Williams (2003) la precisión para la calibración con base en los valores de R^2 se puede definir en los niveles siguientes: valores de R^2 entre 0.50 y 0.65 indican que se puede discriminar entre valores altos y bajos (por ej., para selección en mejoramiento genético); valores de R^2 entre 0.66 y 0.81 para hacer predicciones aproximadas; valores de R^2 entre 0.82 y 0.90 indican una precisión de las predicciones muy alta; y ecuaciones con valores de R^2 mayores que 0.91 permiten predicciones excelentes. Con las ecuaciones generadas en este trabajo se obtuvieron R^2 >0.95 (Cuadro 3) que indican una muy alta confiabilidad de estas ecuaciones para predecir calidad nutritiva de gramíneas y leguminosas forrajeras tropicales.

Como se mencionó anteriormente, la selección de la mejor ecuación de calibración está dada por la relación entre el coeficiente de determinación (R^2) más alto y los errores más bajos. Para PC, FDA, DIVMS (5 semanas) y DIVMS (20 sem), los SEC, SEVC y SEP_C registrados presentan una diferencia muy baja. Para FDN y DFDN se encontraron valores con una marcada diferencia en los SEVC y SEP al ser comparados con el SEC (Cuadro 3). Estas diferencias son posiblemente el resultado de la complejidad bioquímica de la pared celular en el grupo de las especies tropicales analizadas.

Cada una de las ecuaciones obtenidas en este trabajo presenta valores de RPD superiores a 3 (Cuadro 3), lo cual representa el valor límite para aceptar una ecuación en función de su capacidad de predicción. El valor de RPD más alto reportado se encontró en PC (7.3) y el más bajo en FDA (3.5). Esto se reflejó en las altas correlaciones (r) obtenidas al comparar los datos de referencia (LAB) con la predicción por NIRS para las mismas muestras de los colectivos de calibración (Cuadro 3), lo que demuestra el alto poder de predicción de las ecuaciones obtenidas en este estudio. Lo anterior fue confirmado al comparar la distribución empírica (test de Kolmogorow-Smirnov) donde se obtuvo en todas las ecuaciones de P>0.05. Esto demostró, además, que existe una alta relación entre la distribución de los datos obtenidos a partir del modelo NIRS y la distribución de los datos de referencia (LAB), mostrando así la alta relación existente entre lo esperado a nivel biológico con lo obtenido en la predicción (Cuadro 3).

Las ecuaciones seleccionadas se validaron con el colectivo de muestras externas (Cuadro 4) obteniendo un R^2 >0.80, obteniéndose buenas predicciones. Para FDN y FDA, teniendo en cuenta los diferentes niveles de precisión presentados por Williams (2003) para estos parámetros, se sugiere utilizar estas ecuaciones únicamente para discriminar valores altos y bajos, por ejemplo, en trabajos de selección y mejoramiento genético. Para FDA se sugiere mejorar el colectivo con muestras con valores altos de FDA y de esta forma incrementar la confiabilidad de este método de determinación.

En este estudio se observaron correlaciones de 0.89 (P<0.001) para DIVMS o por encima de 0.90 para otros parámetros. Sin embargo, para FDA se presentó una correlación de 0.72 (P<0.001) entre valores estimados con la ecuación y valores medidos por medio de análisis químico.

En comparación con resultados reportados en trabajos anteriores, las ecuaciones obtenidas en esta investigación presentan una eficiencia de predicción alta, siendo respaldada esta afirmación por estadísticas comparativas donde se obtuvieron correlaciones superiores a 0.90, mostrando así que las ecuaciones generadas pueden ser utilizadas con confianza en análisis de uso rutinario.

Conclusión

Los resultados de esta investigación permitieron comprobar la viabilidad de la técnica NIRS para la predicción de parámetros de calidad en forrajes tropicales, mostrando dicha predicción una alta correlación con los datos de laboratorio y por ende con la información a nivel biológico.

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Research paper

Feeding and fertilization practices and greenhouse gas emissions in specialized dairy farms of Dos Pinos in Costa Rica

Prácticas de alimentación animal y de fertilización, y emisión de gases de efecto invernadero en granjas lecheras de Dos Pinos, Costa Rica

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Abstract

Emissions of methane (CH_4) and nitrous oxide (N_2O) based on the feeding systems of 104 dairy farms in Costa Rica were estimated using IPCC procedures. This study indicated that farmers' decisions, which determine the feeding strategies for lactating cows, have a substantial impact on CH_4 emissions per kg of milk. Lower CH_4 emissions per kg milk were estimated on farms with high-producing cows consuming rations with lower neutral detergent fiber concentrations and higher amounts of concentrates. Hours spent in pasture did not influence estimated grass intake or CH_4 emissions. However, higher feed efficiency appeared to be a key factor in reducing CH_4 emissions per kg of milk. The study also showed that higher N_2O emissions were associated with higher amounts of commercial nitrogen fertilizer application; however, the main source of N_2O emissions was from the manure deposited during the grazing period. Future approaches to reduce farm gate emissions of CH_4 per kg of milk in specialized dairy farms could include incorporating dietary fats in rations, feeding adequate amounts of concentrates and feeding forage at a more digestible stage. These findings are strongly influenced by the assumptions made in calculating CH_4 and N_2O emissions but do highlight the critical areas which affect greenhouse gas emissions.

Keywords: Feed efficiency, fertilization, forage, manure, methane, nitrous oxide.

Resumen

Se estimaron las emisiones de metano (CH_4) y óxido nitroso (N_2O) en 104 granjas lecheras en Costa Rica, utilizando los procedimientos del IPCC. El estudio indica que las decisiones de los productores respecto a las estrategias de alimentación de sus vacas en ordeño tienen un impacto sustancial en las emisiones de CH_4 por kg de leche. Se estimaron emisiones de CH_4 bajas por kg de leche en aquellas granjas donde las vacas de alta producción consumían raciones con concentraciones menores de fibra detergente neutro y cantidades mayores de concentrados. Las horas dedicadas al pastoreo no influyeron en las estimaciones del consumo de pasto ni en las emisiones de CH_4. Sin embargo, una mayor eficiencia alimenticia parecía ser un factor clave en la reducción de las emisiones de CH_4 por kg de leche. El estudio también mostró que emisiones de N_2O más altas estaban asociadas con la aplicación de mayores cantidades de fertilizantes comerciales de nitrógeno. Sin embargo, la principal fuente de emisiones de N_2O fueron las excretas de las vacas durante el pastoreo. Futuras estrategias para reducir, a nivel de granja, las emisiones de CH_4 por kg de leche en las

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explotaciones lecheras especializadas, podrían incluir la incorporación de grasas alimenticias en las raciones, alimentación con cantidades adecuadas de concentrados y alimentación con forrajes más digeribles. Aunque estos resultados estén fuertemente influenciados por los supuestos que se emplean en los cálculos de las emisiones de CH_4 y N_2O, sí realzan las áreas críticas que determinan las emisiones de gases de efecto invernadero a nivel de granja lechera.

Palabras clave: Eficiencia alimenticia, excreta, fertilización, forraje, metano, óxido nitroso.

Introduction

The specialized dairy industry of Costa Rica can play an important role in helping the country reduce its national inventory of the 3 main gases that trap heat in the atmosphere: carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). For ease of comparison and interpretation, greenhouse gas emissions are typically expressed as carbon dioxide equivalent (CO_2-eq) to account for the differing amounts of each gas released and its effectiveness in trapping heat. Chacón et al. (2009) calculated that agriculture contributed 37% of all greenhouse gas emissions of Costa Rica in 2005, with the livestock sector responsible for the majority of these emissions.

Dairy farmers' decisions on how to feed their cows have a substantial impact on emissions of CH_4 produced during fermentation of feed, primarily in the rumen and secondly in the caecum of the large intestine. These emissions are referred to as enteric emissions, or emissions from the digestive system. In addition, farmers' decisions on how to fertilize pastures have a substantial impact on the emissions of N_2O from the soil. Furthermore, decisions on how to manage manure (feces + urine) collected from the barn may also influence substantially CH_4 and N_2O emissions.

The objective of this article is to share the results of a study we conducted to estimate the impact of farmers' management decisions on the emissions of CH_4 from lactating cows and N_2O from pastures on specialized dairy farms associated with Costa Rica's largest dairy cooperative, Dos Pinos. The study focused on the important sources of emissions within the farm, often referred to as "farm gate" emissions, but did not provide a full account of the carbon footprint of milk production, which would require estimating the emissions associated with the production of all inputs used on farms and the emissions associated with transport, milk processing, packaging and storing until consumption. Specifically, we studied the following relationships:

- Enteric CH_4 emissions associated with the farmers' decisions on how to feed lactating cows;
- Nitrous oxide emissions from soils associated with the farmers' decisions about:
  - Nitrogen (N) fertilizing of grazed pastures with commercial fertilizers;
  - N fertilizing of cut-and-carry pastures with commercial fertilizers; and
  - Organic N fertilizing of grazed pastures through manure (feces + urine) deposited by the cows during the grazing period.

Materials and Methods

Source of the data

Most of the data for this study were obtained in a survey conducted in December 2013 and January 2014 among producers of the Cooperativa Dos Pinos (see Figure 1 for locations). Since the amount of feed consumed and the chemical composition of the diet are critical in estimating CH_4 emissions, farmers were asked to list all feeds and amounts offered to their lactating cows. We relied also on the equations of the National Research Council (NRC 2001) to determine how much feed cows consumed per day, and subtracted the amount of feed offered in the dairy from total feed consumption to determine intake of grass from pasture. Similarly, as N applied per hectare and per year is critical in estimating N_2O emissions, farmers were asked to list all fertilizers and amounts applied during each pasture rotation cycle or each cut-and-carry cycle, as well as the number of hours that cows spent in the pasture each day (to calculate the proportion of manure N deposited in the pasture).

Pasture distribution frequency

Data from the 104 farms allowed the identification and evaluation of the most dominant pasture and forage species. Georeferenced farms, including their grass species and forage inventory, were categorized using the ecological life zones of Costa Rica (Bolaños et al. 1999) established after the Holdridge life zones (Holdridge 1967) and related with climate data from WorldClim database (Hijmans et al. 2005) in order to calculate

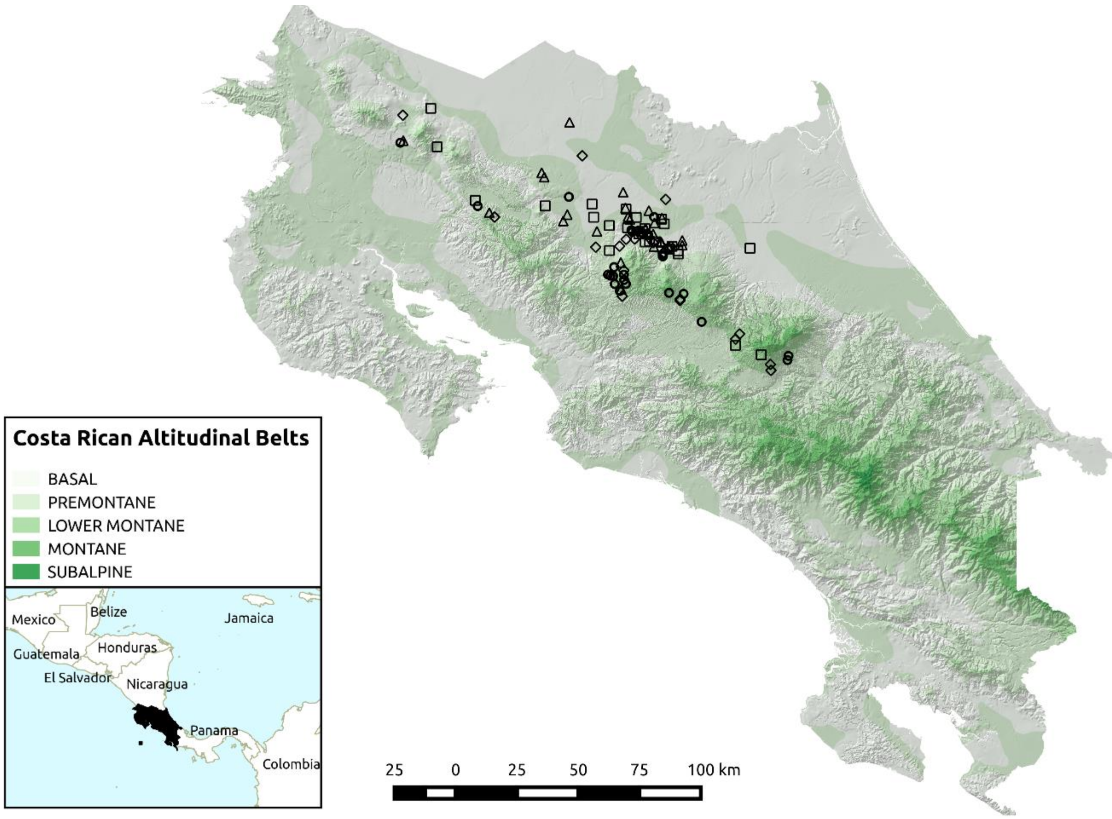

Figure 1. Distribution of locations of the 104 specialized Cooperativa Dos Pinos dairy farms in Costa Rica included in the study labeled in quartiles of partial carbon footprint (kg CO_2-eq per kg of fat-and-protein corrected milk) (Triangle = high emitters: 0.67 to 1.17; diamond = medium-high emitters: 0.59 to 0.67; squares = medium-low emitters: 0.51 to 0.59; circle = low emitters: 0.38 to 0.51); map colors represent 5 elevation zones based on Holdridge's ecological life zones (Bolaños et al. 1999).

average elevation, rainfall and temperature for each grass species. Grass species frequency distribution within ecological life zones was summarized using the grass species reported in each farm and information obtained from the "Digital Atlas of Costa Rica" (Ortiz-Malavassi 2009).

Estimating methane and nitrous oxide emissions

Calculations of CH_4 and N_2O emissions are complex and include a large degree of uncertainty. However, we used equations recommended by the international scientific organization responsible for studying greenhouse gas emissions and climate change, known by its English acronym as IPCC (Intergovernmental Panel on Climate Change; IPCC 2016). Estimated daily CH_4 emissions were converted to annual emissions based on the number of lactating cows on the farm, which in turn were converted to amounts of CO_2-eq to account for the fact that CH_4 is 21 times more potent than CO_2 (the main greenhouse gas) at trapping heat in the atmosphere and changing the climate (Dong et al. 2006). Finally, we calculated the emissions of CO_2-eq from CH_4 per kg of milk produced by the herd after standardizing milk production to a common fat and protein content, referred to as fat-and-protein-corrected milk (FPC-milk) as recommended by the International Dairy Federation (IDF 2010).

The N_2O emissions per hectare from commercial fertilizer were estimated as 1% of the N applied on grazed pasture and cut-and-carry areas (de Klein et al. 2006), and subsequently converted to annual N_2O emissions based on respective areas within the farm. To estimate the N_2O emissions from manure, i.e. the N voided by the cows in feces and urine during the daily grazing period, we used a N balance approach, assuming that, on average, the N consumed daily that is not excreted in the milk on that day is voided in feces and urine (Dong et al. 2006; Olmos Colmenero and Broderick 2006). The N_2O emitted from pastures for lactating cows was then calculated as the sum of the N_2O emitted from commercial fertilizers and that arising from manure. This amount was then converted to CO_2-eq to account for the fact that N_2O is 310 times more potent than CO_2 at trapping heat in the atmosphere and changing the climate (de Klein et al. 2006). Finally, we calculated the emissions of CO_2-eq from N_2O per kg of FPC-milk produced by the herd.

Partial carbon footprint of milk

The emissions from the farm were calculated as the sum of the annual emissions of CH_4 from lactating cows and the annual emissions of N_2O from the land of the farm (from the 3 sources discussed above). Then, the partial carbon footprint (CO_2-eq/kg FPC-milk) was calculated as total farm emissions divided by the FPC-milk produced annually by the lactating cows.

Determining farms with high and low emissions

Our goal was to determine farm characteristics that influenced estimated emissions of CH_4, N_2O and the partial carbon footprint. Thus for each of these emissions, we listed the 104 farms in the study from the lowest to the highest emitter and then divided the farms into 4 groups of 26 farms each, including the lowest emitters (first quartile: bottom 25% of the farms), the medium-low emitters (second quartile: 26th to 50th percentiles of the farms), the medium-high emitters (third quartile: 51st to 75th percentiles of the farms) and the highest emitters (fourth quartile: top 25% of the farms). Then, for each group, we calculated and tabulated the average of selected variables, describing farm characteristics and management decisions of the producers.

Results

Pasture distribution frequency

A wide variety of pasture and forage species was found on the farms (Tables 1 and 2). Even though the distribution frequency of species varied among the different climatic zones depending on elevation and rainfall, several pasture species were found in a number of climatic zones (Table 1). Most abundant species in the basal climatic zone were tanner [*Brachiaria* (now *Urochloa*) *arrecta*], brizantha [*Brachiaria* (now *Urochloa*) *brizantha*] and ratana (*Ischaemum ciliare*). In the premontane climatic zone the most dominant species were found to be estrella (African star grass, *Cynodon nlemfuensis*) and brizantha, while kikuyu (*Pennisetum clandestinum*) and estrella were predominant in the lower montane zone.

The use of cut-and-carry forage species was widespread among the surveyed dairy farms (Table 2). Most frequently used species were king grass (*Pennisetum* hybrid), kikuyu (*P. clandestinum*) and cameroon (*P. purpureum*). Most forage species were used in more than one climatic zone except kikuyu and rye grass (*Lolium perenne*), which were grown in the lower montane climatic zone only.

Table 1. Most important grazed pasture species with average elevation (Elv) of their respective farms, average rainfall (R), average temperature (T) and use frequency ranking in climatic zones.

Species	Elv	R	T	Use frequency ranking in climatic zones[1] (n)		
	(masl)	(mm/yr)	(ºC)	1st	2nd	3rd
Pennisetum clandestinum (n=15)	1,847	2,800	16	LM (15)		
Brachiaria arrecta x mutica (n=6)	600	3,631	23	B (3)	P (2)	M (1)
Ischaemum ciliare (n=19)	268	3,644	25	B (11)	P (8)	
Panicum maximum (n=3)	300	3,903	25	P (2)	B (1)	
Brachiaria brizantha CIAT-26110 (cv. Toledo) (n=3)	400	4,178	25	B (2)	P (1)	
Brachiaria decumbens (n=6)	500	3,552	24	B (4)	P (2)	
Brachiaria arrecta (n=23)	535	4,311	24	B (14)	P (9)	
Brachiaria brizantha CIAT-26124 (n=23)	287	3,627	25	B (12)	P (11)	
Cynodon nlemfuensis (n=41)	690	3,303	23	P (22)	B (10)	LM (9)
Brachiaria hybrid CIAT line FM 9201/1873 (cv. Mulato) (n=3)	133	3,618	26	P (2)	B (1)	
Brachiaria brizantha (n=8)	450	3,689	24	P (5)	B (3)	
Lolium perenne (n=1)	1,700	2,924	17	LM (1)		

[1]Climatic zones categorized according to Holdridge (1967) as LM = lower montane; B = basal; P = premontane; M = montane. 1st = most frequent; 2nd = second most frequent; 3rd = third most frequent. Numbers in parentheses indicate the number of farms (n) in which species were observed.

Table 2. Most important cut-and-carry forage species with average elevation (Elv) of their respective farms, average rainfall (R), average temperature (T) and use frequency ranking in climatic zones.

Species	Elv (masl)	R (mm/yr)	T (ºC)	Use frequency ranking in climatic zones[1] (n) 1st	2nd	3rd
Pennisetum clandestinum (kikuyu) (n=9)	1,755	2,808	17	LM (9)		
Pennisetum sp. (maralfalfa) (n=11)	636	3,854	23	B (5)	P (4)	LM (2)
Pennisetum sp. (king grass) (n=12)	608	4,196	24	B (6)	P (6)	
Digitaria swazilandensis (suazi) (n=4)	250	3,676	25	B (2)	P (2)	
Panicum maximum cv. Mombaza (n=6)	467	4,275	24	B (4)	P (2)	
Pennisetum purpureum (cameroon) (n=7)	900	3,393	22	B (3)	LM (3)	P (1)
Lolium perenne (rye grass) (n=4)	1,775	2,666	17	LM (4)		

[1]Climatic zones categorized according to Holdridge (1967) as LM = lower montane; B = basal; P = premontane; M = montane. 1st = most frequent; 2nd = second most frequent; 3rd = third most frequent. Numbers in parentheses indicate the number of farms (n) in which species were observed.

Methane emissions

How much methane is produced? Estimated CH_4 emissions averaged 266 g/cow/d, but varied considerably (standard deviation = 55 g/cow/d). To give ourselves confidence in the prediction of CH_4 emissions, we compared our results with the predictions obtained from the equation proposed by Moraes et al. (2014), which was based on ration and animal characteristics, and the equation proposed by Ramin and Huhtanen (2013), which was based solely on feed consumption (dry matter intake). These equations yielded averages of 231 and 326 g/cow/d, respectively. Although the difference between these estimates was substantial, our estimation of CH_4 emissions was within the range of these literature values. Our estimate of annual CH_4 emissions was 97 kg/cow (266 g/cow/d x 365 d), which was higher than the IPCC (tier 1) value of 63 kg/cow (Dong et al. 2006). The IPCC value, however, assumed a considerably lower level of milk production (800 kg/cow/yr) than those produced on the farms in this study (range 4,000–7,000 kg/cow/yr). Estimated CH_4 emissions expressed as CO_2-eq/kg FPC-milk averaged 419 g, but ranged from 316 to 636 g for different farms. As indicated in Table 3, lactating cows in farms of the first, second, third and fourth quartiles had average emissions of 342, 386, 428 and 519 g CO_2-eq/kg FPC-milk, respectively.

What factors are associated with high and low methane emissions? Cow characteristics, concentrate feeding and time in pasture had a marked influence on our estimated CH_4 emissions per kg of FPC-milk (Table 3). Our interpretation of these effects is as follows:

1. Farms that emitted the lowest amount of CH_4 per kg of FPC-milk were those where CH_4 emissions per cow were highest. Methane production per cow was a reflection of the amount and the composition of the feed consumed by the cow, regardless of milk production. Thus, in general, cows that consumed more feed produced more CH_4 but also produced (proportionally) more milk. Results indicated that less CH_4 was produced per kg of FPC-milk when cows consumed more feed and produced more CH_4 per day but also produced more milk.
2. Estimated emissions of CH_4 per kg of FPC-milk were lowest in herds that had the highest feed efficiency. Feed efficiency is calculated as milk production (kg/d) per unit of dry matter intake (feed consumption, kg/d), and is a partial reflection of the farmer's ability to feed and manage cows to produce the highest possible amount of milk for each kg of feed consumed.
3. Estimated emissions of CH_4 per kg of FPC-milk were lowest when cows were fed more concentrates. As concentrate feeding (DM basis) increased from 3.3 to 6.1 kg/cow/d, estimated CH_4 emissions decreased from 0.52 to 0.34 kg CO_2-eq/kg FPC-milk. These results are consistent with those reported by Aguerre et al. (2011), indicating that increasing the proportion of concentrates and reducing the proportion of forage in the diet decreases CH_4 emissions per kg of milk produced.

Does diet composition make a difference? To investigate further the effects of diet composition on estimated CH_4 emissions, we grouped the 104 farms into 4 quartiles based on amounts of concentrate offered to the cows. Average amount of concentrate (DM basis) offered ranged from 2.1 kg/cow/d (low concentrate users) to 7.2 kg/cow/d (high concentrate users). Results summarized in Table 4 indicate that producers who fed more concentrates to their cows did not offer greatly different amounts of by-product feeds or forage dry matter in the

Table 3. Estimated enteric methane (CH_4) emissions and feeding practices for specialized dairy farms (n=104) ranked in quartiles according to estimated level of enteric CH_4 emissions[1].

Parameter	CH_4 emission quartile[1] (g CO_2-eq/kg FPC-milk)			
	4th	3rd	2nd	1st
	519	428	386	342
CH_4 (kg/cow/d)	0.23	0.25	0.27	0.32
CH_4 (kg/cow/yr)	83	92	99	115
Cow characteristics:				
Cow body weight (kg)	408	410	426	438
FPC-milk (kg/cow/d)	9.7	13.0	16.0	20.0
DMI[2] (kg/cow/d)	13.8	15.2	16.6	18.3
Feed efficiency (kg FPC-milk/kg DMI)	0.70	0.85	0.96	1.08
Feeding and management strategies:				
Concentrate (kg DM/cow/d)	3.3	4.2	5.5	6.1
Time in pasture (h/cow/d)	18	17	17	16

[1]1st, 2nd, 3rd and 4th quartile = farms with low, medium-low, medium-high and high enteric CH_4 emissions expressed as CO_2-eq per kg of fat-and-protein-corrected milk (FPC-milk), respectively.
[2]DMI = Dry matter intake estimate based on NRC (2001) equation.

form of purchased hay or silage or grass from cut-and-carry pastures. Interestingly grass dry matter intake, which declined slightly when more concentrate was offered to the cows, was not affected by time in the pasture, which was increased by farmers who offered more concentrates to their cows. Overall, as concentrate feeding increased, total dry matter intake also increased as did milk production and feed efficiency (Table 4). Dietary neutral detergent fiber concentrations observed in this study were high for lactating cow diets (NRC 2001) and were likely to limit the total amount of feed that cows could consume and process per day (Mertens 1997). Feeding additional concentrates with low neutral detergent fiber would result in higher total feed consumption, higher milk production and thus higher feed conversion efficiency (Table 4). As a result of these compounded effects, increasing the amount of concentrates fed to the cows was estimated to increase daily CH_4 emissions by the cows, but reduced the CH_4 emissions expressed as kg CO_2-eq/kg of FPC-milk produced on the farm (Table 4). The low levels of crude fat reported in Table 4 suggested that inclusion of supplemental dietary fats may be an avenue to reduce CH_4 emissions from dairy cows in Costa Rica. Although fats are normally minor constituents of dairy cow rations, a slight increase in fat concentration may reduce CH_4 emissions/kg FPC-milk (Martin et al. 2010).

Does time in pasture make a difference? The answer is "probably not". As shown above in Table 3, the average time spent in pasture daily declined only slightly from the highest CH_4-emitting herds to the lowest CH_4-emitting herds. Similarly, the estimated amount of grass dry matter consumed from the pasture was almost identical among the 4 groups of farms. This result suggested that consumption of grass was not limited by time in pasture.

Nitrous oxide emissions

How much nitrous oxide is produced? Most common N fertilizers for cut-and-carry forage production were: a chemical NPK fertilizer (10-30-10) applied on 13% of the farms; urea applied at least once per year on 11% of the farms; and ammonium nitrate on 11% of the farms (Nutran, 33.5% N). Common N fertilizers applied on pastures were: ammonium nitrate (Nutran, 33.5% N) used on 37% of the farms; urea on 32% of the farms; magnesium ammonium nitrate (21% N, 11% Ca, 7.5% Mg) applied on 24% of the farms; and a chemical NPK fertilizer 10-30-10 applied on 21% of farms.

Estimated N_2O emissions expressed as kg N_2O/ha/yr are presented in Table 5. Estimated emissions from the application of commercial N fertilizer averaged 2.76 kg N_2O/ha/yr but the standard deviation was high (2.57 kg N_2O/ha/yr) indicating high variation among farms. Nevertheless, the average value was comparable with emission values of 1.23 kg N_2O/ha/yr without fertilizer application and 2.44 kg N_2O/ha/yr after applying 200 kg N/ha/yr to a kikuyu pasture reported by Montenegro and Herrera (2013). When expressed as kg CO_2-eq/kg FPC-milk, N_2O emissions averaged 198 g CO_2-eq/kg milk, but ranged from 56 to 536 g CO_2-eq/kg milk. As indicated in Table 5, average N_2O emissions on farms in the first, second, third and fourth quartiles were 108, 157, 200 and 328 g CO_2-eq/kg FPC-milk, respectively.

Table 4. Estimated enteric methane (CH_4) emissions and feeding practices for specialized dairy farms (n=104) ranked in quartiles according to the amounts of concentrates consumed by the lactating cows[1].

Parameter	Concentrate consumption quartile[1] (kg DM/cow/d)			
	4th	3rd	2nd	1st
	7.2	5.2	4.2	2.1
Enteric CH_4 emission				
g CH_4/cow/d	304	265	243	251
g CO_2-eq/kg PFC-milk	371	394	429	481
Dietary ingredients				
Concentrates (kg DM/cow/d)	7.2	5.2	4.2	2.1
By-products (kg DM/cow/d)	1.7	1.8	1.0	1.3
Forage[2] (kg DM/cow/d)	1.1	1.2	2.1	1.2
Grazed pasture[3] (kg DM/cow/d)	7.7	7.7	7.2	9.5
DMI (kg/cow/d)	17.8	15.9	14.6	14.1
Time on pasture (h/cow/d)	18	18	16	17
Estimated dietary composition				
Crude protein (% DM)	14	13	12	12
Neutral detergent fiber (% DM)	39	45	49	54
Crude fat[4] (% DM)	2.9	2.7	2.5	2.4
Milk production and efficiency				
FPC-milk[5] (kg/cow/d)	17.7	14.3	12.2	11.7
Feed efficiency (kg FPC-milk/kg DMI)	0.99	0.90	0.84	0.81

[1]1st, 2nd, 3rd and 4th quartile = farms with low, medium-low, medium-high and high concentrate consumption by lactating cows, expressed as kg per cow per day, respectively.
[2]Forage dry matter offered in the barn included purchased hay, purchased silage in plastic bales and grass from cut-and-carry pastures.
[3]Grazed pasture intake calculated by difference between dry matter intake (DMI) estimated from NRC (2001) equation and the sum of all other dietary ingredients.
[4]Crude fat = total fat measured by ether extract procedure.
[5]FPC-milk = fat-and-protein-corrected milk production (IDF 2010).

Table 5. Estimated nitrous oxide (N_2O) emissions and fertilizer practices for specialized dairy farms (n=104) ranked in quartiles according to estimated level of N_2O emissions[1].

Parameter	N_2O emission quartile[1](g CO_2-eq/kg FPC-milk)			
	4th	3rd	2nd	1st
	328	200	157	108
N_2O emissions (kg/ha/yr) from:				
Commercial fertilizer on grazed pasture	4.2	3.6	2.2	1.1
Commercial fertilizer on cut-and-carry grass	2.2	1.5	1.1	0.7
Manure (feces + urine) on grazed pasture	6.3	7.8	8.3	6.0
N fertilizer (kg N/ha/yr):				
Commercial fertilizer on grazed pasture	267	229	138	68
Commercial fertilizer on cut-and-carry grass	141	96	70	42
Manure (feces + urine) on grazed pasture	201	249	265	192
Time on pasture (h/d)	18	17	17	15
N balance of the cow (g/cow/d):				
Nitrogen intake	303	337	343	315
Milk nitrogen	57	77	80	83
Manure nitrogen	245	260	263	232
N use efficiency[2] (%)	19	23	23	26

[1]1st, 2nd, 3rd and 4th quartile = farms with low, medium-low, medium-high and high N_2O emissions expressed as CO_2-eq/kg fat-and-protein-corrected milk produced (FPC-milk), respectively.
[2]Nitrogen use efficiency (%) = 100 x milk N (g/d) / N intake (g/d).

What factors are associated with high and low nitrous oxide emissions? Table 5 shows estimated N_2O emissions in relation to N fertilizer application from commercial fertilizers and manure N deposited by the cows during grazing. Main factors influencing N_2O emissions per kg FPC-milk and interpretation of these effects are as follows:

1. On all farms, the main source of N_2O emissions was manure deposited by the cows during grazing rather than commercial fertilizers. Among quartiles, estimated N_2O emissions from manure ranged from 6.0 to 8.3 kg N_2O/ha/yr, whereas estimated N_2O emissions from commercial fertilizer applied to pasture and to cut-and-carry grass ranged from 1.1 to 4.2 and from 0.7 to 2.2 kg N_2O/ha/yr, respectively. The amounts of commercial fertilizer N applied to pasture and to cut-and-carry grass annually were 176 and 87 kg N/ha/yr, but the average amount of manure N deposited by lactating cows during grazing was estimated as 227 kg N/ha/yr. As suggested by the data presented in Table 5, on at least 75% of the farms (those in the 1st, 2nd and 3rd quartiles), the amount of N deposited in feces and urine by cows during grazing was higher than the amount of N applied as commercial fertilizer.
2. Extremes in estimated N_2O emissions per kg FPC-milk were associated with extremes in amount of commercial N application. In this study 13 farmers (13% of the farmers) applied no commercial fertilizer to pasture grazed by lactating cows, while 15 farmers (14% of the farmers) applied more than 300 kg N/ha/yr. These extremes in N application explained in large part the variation in N_2O emissions observed in this study.
3. Estimated N_2O emissions per kg FPC-milk from manure deposited by the cows during grazing depended on a combination of factors, but remained fairly consistent among all farms in the study. The amount of N deposited on the pasture by cows depended upon the length of time spent on pasture per day and the amount of manure excreted per day. The latter increased with level of dry matter intake (and milk production) and with N concentration in the manure, which in turn depended partly on crude protein concentration in the diet. Crude protein concentration was low in the pasture for lactating cows (NRC 2001), but increased with the amount of concentrate fed to the cows (Table 4).
4. Estimated N_2O emissions per kg FPC-milk were lowest on farms in which N use efficiency for milk production was highest. Nitrogen use efficiency, or the percentage of the N consumed by the cows which was converted to milk N, was low, averaging 19%, among the high N_2O-emitting farms, but was substantially better, averaging 26%, among the low-emitting farms.

Does the amount of commercial fertilizer applied make a difference? To investigate further the effects of the application of commercial fertilizer on N_2O emissions, we grouped the 104 farms of this study into 4 quartiles based on amount of N fertilizer applied per hectare of pasture. Average N application ranged from a low 20 kg N/ha among the low N fertilizer users to a high 383 kg N/ha among the high N fertilizer users. Data in Table 6 indicated that estimated dry matter intake, milk production and feed efficiency were not affected by level of commercial N application to grazing pasture. As a result, increases in N applied as commercial fertilizer resulted in increases in N_2O emissions per hectare of pasture and N_2O emissions expressed as g CO_2-eq/kg PFC-milk. Technical support personnel in Dos Pinos indicated that current recommendations for commercial N application are approximately 250 kg N/ha/yr. These recommendations are made regardless of the amount of manure N deposited by cows during grazing, which, as indicated in Table 5, ranged from 192 to 265 kg N/ha/yr. Our data do not support recommendations for high commercial N application on grazing pasture under the assumption that more fertilizer means more grass (i.e. more feed) for the cows and thus more milk per cow. The feeding practices and estimated dietary composition findings described above support this conclusion because grass intake was relatively constant on all farms regardless of feeding strategies. This conclusion is consistent with recent findings at the University of Costa Rica, indicating that cows consume only 30–45% of the total amount of grass biomass available in pasture (Villalobos and Sánchez 2010; Villalobos et al. 2013).

Table 6. Estimated nitrous oxide (N_2O) emissions and cow performance for specialized dairy farms (n=104) ranked in quartiles according to level of commercial N fertilizer application[1].

Parameter	N fertilizer application quartile[1] (kg N/ha/yr)			
	4th	3rd	2nd	1st
	383	197	102	20
N_2O release (kg/ha/yr)	6.02	3.10	1.60	0.32
N_2O release (kg CO_2-eq/kg FPC-milk)	0.25	0.22	0.17	0.13
Cow performance:				
FPC-milk[2] (kg/cow/d)	14.9	14.4	15.2	14.4
DMI[3] (kg/cow/d)	16.0	15.7	16.5	15.8
Feed efficiency (kg FPC-milk/kg DMI)	0.91	0.89	0.90	0.89

[1]1st, 2nd, 3rd and 4th quartile = farms with low, medium-low, medium-high and high levels of N fertilizer applied per hectare of pasture grazed by lactating cows, respectively.
[2]FPC-milk = fat-and-protein-corrected milk production (IDF 2010).
[3]DMI = Dry matter intake estimated from NRC (2001) equation.

Table 7. Estimated greenhouse gas [methane (CH_4) + nitrous oxide (N_2O)] emissions and fertilizer practices for specialized dairy farms (n=104) ranked in quartiles according to partial carbon footprint[1].

Parameter	Partial carbon footprint ($CH_4 + N_2O$) quartile[1] (kg CO_2-eq/kg FPC-milk)			
	4th	3rd	2nd	1st
	0.82	0.62	0.56	0.47
Source of emissions (%)				
CH_4 from enteric fermentation	63	68	71	75
N_2O from fertilizer[2] on grazed pasture	15	10	7	5
N_2O from fertilizer[2] on cut-and-carry grass	1	0	1	0
N_2O from manure[3] on grazed pasture	21	21	21	20
Farm characteristics:				
Lactating cows (head)	47.7	54.9	51.3	69.7
Grazing + cut-and-carry pastures (ha)	24.9	19.8	18.8	21.8
Stocking rate[4] (cows/ha)	2.4	3.1	3.3	3.5
Elevation (masl)	392	481	854	1123
Precipitation (mm/yr)	3,500	3,572	3,632	3,277
Temperature (°C)	24.5	24.1	22.1	20.6

[1]1st, 2nd, 3rd and 4th quartile = farms with low, medium-low, medium-high and high levels of $CH_4 + N_2O$ emissions expressed as CO_2-eq/kg fat-and-protein-corrected milk produced (FPC-milk), respectively.
[2]Emissions associated with commercial N fertilizer application.
[3]Emissions associated with manure (feces + urine) from cows during grazing.
[4]Average stocking rates (average lactating cow numbers divided by average area of grazing + cut-and-carry pastures) differed substantially because of large standard deviations for some quartiles for lactating cow numbers or area of grazing + cut-and-carry pastures.

Partial carbon footprint

The sum of estimated CH_4 and N_2O emissions for each farm was determined giving an average of 617 g CO_2-eq/cow/d, but ranged from 383 to 1,021 g CO_2-eq/kg of FPC-milk. When farms were stratified into groups according to total emissions, average emissions were 467 g in the low emitting group, 556 g in the medium-low emitting group, 624 g in the medium-high emitting group and 821 g CO_2-eq/kg FPC-milk in the high emitting group. These emissions should not be interpreted as a complete carbon footprint of milk production by specialized dairy farms of Dos Pinos, but rather a first step towards a partial farm gate carbon footprint. In 2010, the Food and Agriculture Organization of the United Nations reported average emissions of CO_2-eq/kg FPC-milk at farm gate, ranging from 1,300 to 7,500 g from various regions of the world (FAO 2010). Although our study included some of the most important sources of greenhouse gas emissions from within the farm (enteric CH_4 plus N_2O from fertilizer and manure deposition from cows in the pasture), there were not enough reliable data to estimate other sources of emissions, which were not accounted for in this study. Some of these sources include:

- Emissions associated with the production and transport of feed ingredients such as concentrates, by-product feeds and purchased forages (hay and bagged silage).
- Emissions associated with collection, storage and application of manure deposited by cows in the barn, which are recognized as an important source of greenhouse gases (Dong et al. 2006).
- Emissions of CO_2 associated with the use of fuel and electricity on the farm.

What farm-related factors are associated with high and low overall GHG emissions? All management factors discussed above for estimated CH_4 and N_2O emissions also influence overall GHG emissions. Table 7 shows the percentage of estimated emissions associated with each of the 4 sources of GHG included in this study. Overall, CH_4 emissions accounted for 69%, N_2O emissions from commercial fertilizer applied to grazed pasture accounted for 9%, N_2O emissions from commercial fertilizer applied to cut-and-carry pasture accounted for 1%, and N_2O emissions from manure deposited by the cows during grazing accounted for 21% of the estimated emissions in this study. Partial carbon footprint was reduced on farms that had a lower proportion of N_2O emissions from commercial fertilizer applied on grazed pasture, but a greater proportion of enteric emissions, reflecting the "dilution effect" of greater milk production (Table 7). In contrast, contribution of N_2O emissions from manure deposited by the cows during grazing did not vary whether the farm was a high emitter or a low emitter. Although there were large variations in stocking rates within each quartile, data in Table 7 indicated that the partial carbon footprint was lower on farms with higher stocking rates (lactating cows/ha of grazed and cut-and-carry pastures), which most likely reflected more intensive feeding management practices (milk production, feed consumption, feed conversion efficiency and N use efficiency).

In addition, data in Table 7 indicated a strong relationship between the partial carbon footprint and farm characteristics that are fixed (conditions that may not be changed such as elevation) or unlikely to change in the near future unless there is a main restructuring of the farm (buying/selling of land or building new facilities to accommodate a larger herd size). Partial carbon footprint was reduced with higher elevation and lower average temperature (Table 7). This relationship is likely to reflect changes in feeding and fertilizer practices in distinct ecosystem zones of the country (see Figure 1), but the data from this study were insufficient to explore whether these fixed characteristics may have a direct effect on partial carbon footprint. Current knowledge, however, suggests that both CH_4 emissions from dairy cows and N_2O emissions from pasture may be influenced in part by bio-physical (soil type) and environmental (temperature and humidity) conditions. For example, heat stress in dairy cattle, which depends upon a combination of temperature and relative humidity, reduces feed consumption and milk production. Thus, higher temperature and humidity in the lowland humid tropical regions may have a substantial effect on CH_4 emissions from cows. In regard to N_2O emissions, recent research by Montenegro (2013) suggested that the redistribution of water and nitrates (a precursor of N_2O) due to the topography (slope of the terrain) had a substantial impact on emissions from highly fertilized pasture.

Discussion and Conclusions

The estimates of CH_4 and N_2O emissions we have derived are dependent on the assumptions in the particular equations we used. Thus none of the values presented here should be considered as definitive for the actual amounts of these gases released from dairy farms in Costa Rica, i.e. the partial carbon footprint. However, the principles, which have been demonstrated, indicate where the areas of greater release exist and where effort should focus to reduce emissions.

While this study estimated emissions of CH_4 and N_2O from specific sources within the farm, additional data are needed for a complete assessment of the carbon footprint of milk production in specialized dairy farms of Costa Rica, or for a complete life cycle assessment. Implications and recommendations made here relate only to reducing emissions from the specific sources within the farm (farm gate boundaries). We found that decisions made by dairy producers, which determine the strategies of feeding lactating cows, have a substantial impact on CH_4 emissions per kg FPC-milk produced on the farm. The fact that lower CH_4 emissions/kg milk were observed on farms with high-producing cows consuming rations with lower neutral detergent fiber concentrations and higher amounts of concentrates highlights the importance of focusing on high production per cow. The key factor influencing CH_4 emissions was the amount of milk produced per cow, which was strongly controlled by the amount of concentrate fed. Higher feed conversion efficiency (more kg milk produced/kg of feed consumed) was a key factor in reducing CH_4 emissions per kg FPC-milk produced on the farm. We could not detect any effect of hours spent in pasture on grass intake or CH_4 emissions.

Future approaches to reducing farm gate emissions of CH_4 in Costa Rican specialized dairy farms may include the following:

- Inclusion of dietary fats in rations. Dietary fats are known to reduce CH_4 emissions from dairy cows (Knapp et al. 2014). As long as concentration of dietary fats does not exceed approximately 6.5% of dry matter intake, no negative impacts are expected but additional benefits may result through increased energy intake and alleviation of heat stress (because the processing of fat by the cow produces less heat than processing of fiber).
- Inclusion of adequate amounts of concentrates in the diet. High quality concentrate feeds also increase the energy (and protein) supply. Compared with an all-grass diet, the inclusion of an adequate amount of concentrate should increase feed conversion efficiency and reduce CH_4 emissions per kg FPC-milk produced on the farm.
- Increased forage digestibility would be an alternative approach to providing cows with a higher quality (i.e. energy) diet without reducing the proportion of forage in the diet (and thus avoiding increasing dependence on imported grains). Forage digestibility varies with plant maturity at the time of harvest (for preserved forages) or at the time of grazing (for pasture-based systems).
- Focusing on genetic improvement of dairy herds by recording individual milk yields of cows and using sires from high-producing dams to place selection pressure on high yield potential would produce replacement animals with the potential to produce higher yields if fed correctly.

This study demonstrated also that decisions made by the farmer, e.g. relating to fertilizing of grazed pastures and cut-and-carry pastures, have substantial impacts on estimated N_2O emissions per kg FPC-milk produced on the farm. While estimated higher N_2O emissions were associated with higher amounts of commercial fertilizer applied, the main source of N_2O estimated in this study was manure (feces + urine) deposited by the cows during the grazing period. These emissions were influenced by a number of factors including: hours of grazing, feed intake (which influenced the amount of manure produced per day), crude protein content of the ration and the level of milk production of the cows. Most of these factors are determinants of N use efficiency (conversion of dietary N into milk N). Since any N consumed by a cow but not used for milk production is excreted as manure (urine plus feces), this study has shown that N_2O emissions were reduced substantially on farms that achieved higher N use efficiency.

As opposed to increasing concentrate feeding, which increased CH_4 emissions per cow, but decreased CH_4 emissions per kg of milk produced on the farm, increasing N fertilizer levels had detrimental effects on emissions of both N_2O/ha pasture and N_2O/kg milk produced on the farm. Thus to avoid unnecessary N_2O emissions, researchers and technical support groups in Costa Rica should:

- Develop standards for applying commercial N fertilizer to pasture designed not to maximize grass production, but rather to produce an economical and high-quality feed for lactating dairy cows, while not leading to excessive N_2O release; and
- Develop practices to quantify and account for organic N deposited by the cows during the grazing periods.

A combination of these 2 factors would allow the reduction of commercial fertilizer usage and would reduce the greenhouse gas emissions associated with the synthesis, transport and application of commercial fertilizer on pasture.

Recommendations for future studies

Future studies should focus on data collection for evaluation of sources of emissions not included in this study, particularly emissions from manure deposited by cows in the barn (collection, storage and land application) and emissions associated with the production and transport of feed ingredients such as concentrates, by-product feedstuffs and purchased forages (hay and bagged silage). Additional areas would be factors influencing feed efficiency and N use efficiency on Costa Rican dairy farms as a means to improve productivity, reduce emissions and possibly increase profitability. Given the relationships observed in this study between estimated greenhouse gas emissions and the biophysical locale of the farm (elevation, rainfall and temperature), future research should focus on identifying “unavoidable” and “acceptable” levels of emissions as well as emissions that can be reduced with proper management techniques. Finally, data should be collected to determine the actual and potential carbon sequestration (e.g. in tree plantations) or carbon offsets (e.g. bio-digestion) on Costa Rican dairy farms. The road toward carbon neutrality should include measures and practices to reduce emissions of greenhouse gases, promote carbon sequestration and offset (avoid) emissions within and outside the farm gates.

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Research paper

Milk yield and blood urea nitrogen in crossbred cows grazing *Leucaena leucocephala* in a silvopastoral system in the Mexican tropics

Rendimiento de leche y nitrógeno ureico en sangre de vacas cruzadas pastando Leucaena leucocephala *en un sistema silvopastoril en el trópico mexicano*

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Abstract

The aim of the study was to assess milk yields, estimate the intake of crude protein (CP) and determine the concentrations of blood urea nitrogen (BUN) in early post-partum crossbred cows grazing irrigated *Leucaena leucocephala* (leucaena) in a silvopastoral system relative to those in an irrigated grass monoculture. Twenty-four multiparous cows were randomly allotted at calving on the basis of previous milk yields to 2 grazing treatments: grass monoculture system (MS) of *Cynodon nlemfuensis* (n=12); and an intensive silvopastoral system (ISS) composed of leucaena and *C. nlemfuensis* (n=12). Cows were supplemented with sorghum grain (ISS) or a conventional concentrate (MS) during milking to ensure availability of metabolizable energy (ME) and CP required for milk production. Mean estimated intake of leucaena was 5.1±1.3 kg DM/d and estimated CP intakes were 1,479±3.3 and 1,258±3.3 g/d for ISS and MS, respectively (P>0.05), while estimated intakes of ME were 161±1.3 and 131±1.4 MJ/d for ISS and MS, respectively (P<0.05). Milk yields were 13.5 and 14.5 kg/cow/d for cows on ISS and MS, respectively (P>0.05). Concentrations of BUN were 19.1 mg/dL for cows in ISS and 15.3 mg/dL for cows in MS (P<0.05). We conclude that intake of leucaena and sorghum grain in an irrigated silvopastoral system was sufficient to substitute for expensive concentrate in the diets of lactating cows grazing irrigated grass monoculture. However, the higher levels of BUN found in ISS suggest a lower efficiency of N utilization in this treatment. Restricting consumption of leucaena might be a means of improving efficiency of its use and this warrants investigation.

Keywords: Cattle, crude protein, *Cynodon nlemfuensis*, leucaena, tropical pastures.

Resumen

El objetivo del estudio fue evaluar la producción de leche, estimar el consumo de proteína cruda (PC) y determinar la concentración de nitrógeno ureico en sangre (NUS) durante el posparto temprano de vacas cruzadas, pastando *Leucaena leucocephala* (leucaena) en un sistema silvopastoril. Veinticuatro vacas multíparas fueron asignadas aleatoriamente el día del parto a 2 tratamientos, ambos bajo irrigación: Un sistema de monocultivo (SM) de *Cynodon nlemfuensis* (n=12) y un sistema silvopastoril intensivo (SSPi) de leucaena con *C. nlemfuensis* (n=12). En ambos tratamientos se suplementaron las vacas durante el ordeño para cubrir sus requerimientos de energía

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metabolizable (EM) y PC, usando sorgo (SSPi) o concentrado convencional (SM). El consumo de leucaena fue de 5.1±1.3 kg materia seca/d. El consumo de PC fue de 1,479±3.3 g/d y 1,258±3.3 g/d en SSPi y SM, respectivamente ($P>0.05$) y el consumo de EM fue de 161±1.3 y 131±1.4 MJ/d en SSPi y SM, respectivamente ($P<0.05$). No se encontraron diferencias ($P>0.05$) en la producción de leche entre sistemas (13.5 y 14.5 kg/vaca/d en SSPi y SM, respectivamente). La concentración de NUS fue de 19.1 mg/dL para vacas en SSPi y 15.3 mg/dL en SM ($P<0.05$). Se concluye que el consumo de leucaena y sorgo en un sistema silvopastoril bajo irrigación fue suficiente para sustituir el concentrado en la dieta. Sin embargo, las concentraciones de NUS en el SSPi sugieren una menor eficiencia de utilización de N. Restringir el consumo de leucaena podría ser una medida para mejorar la eficiencia de su uso y esto debe ser investigado.

Palabras clave: *Cynodon nlemfuensis*, ganado bovino, leucaena, pastos tropicales, proteína cruda.

Introduction

Milk yield in dual-purpose systems in the tropics depends, to a large extent, on the use of expensive imported concentrates. Providing high quality forages is a possible alternative approach, which could be more economical. *Leucaena leucocephala* (leucaena), a leguminous tree with high crude protein (CP) concentration in its foliage (14–34%, García et al. 2008), has been widely used for improving the diets of ruminants in the tropics. It was shown that using leucaena in silvopastoral systems enabled a reduction in level of concentrates fed to dairy cows (Peniche-González et al. 2014), and provided environmental services such as increased carbon sequestration (Ferguson 2013) and nitrogen fixation in the soil (Orwa et al. 2009). Silvopastoral systems involving leucaena are in widespread use in Australia, Africa, Cuba, Mexico and South America (Broom et al. 2013).

However, while leucaena has a high concentration of rumen-degradable protein (80%, Eb-Pareja 2015), it has a low concentration of metabolizable energy (Tinoco-Magaña et al. 2012), which could affect the efficiency of utilization of nitrogen by rumen micro-organisms (Poppi and McLennan 1995). Nocek and Russell (1988) pointed out that degradability of CP in the rumen in excess of 60% may lead to nitrogen losses, even with high availability of fermentable carbohydrates. Ruiz (2013) found that both blood urea nitrogen and urinary nitrogen excretion increased linearly in cows fed increasing levels of leucaena, suggesting inefficient use of nitrogen in the gastrointestinal tract. A better understanding could be obtained by determining how much leucaena is consumed by grazing animals. However, it is difficult to measure dry matter intake by grazing cattle (Coleman et al. 2014) and it becomes even more difficult when an attempt is made to measure the intake of different plants/species in a silvopastoral system (i.e. grasses, legumes) such as the so-called intensive silvopastoral system (ISS). An ISS is defined as a type of agroforestry for direct grazing where more than 8,000 forage shrubs/ha are combined with improved grasses and tree species (Calle et al. 2012). Being able to assess the intake of CP from leucaena by cattle grazing such silvopastoral systems would assist in the development of strategies to increase the efficiency of nitrogen utilization.

Blood urea nitrogen (BUN) is a reflection of the efficiency of utilization of dietary protein in the rumen (Fadel et al. 2014), with values ranging between 6.0 and 19.0 mg/dL, with the desirable level being 15.0 mg/dL (Rhoads et al. 2006). It is well known that BUN in ruminants is positively correlated with the concentration of CP in the diet, especially if the animal has insufficient fermentable energy in the rumen for the efficient utilization of dietary nitrogen (Poppi and McLennan 1995).

The aims of the present study were to measure milk yields, estimate the intake of CP and determine the concentrations of BUN in early post-partum crossbred cows grazing *L. leucocephala* in an intensive silvopastoral system in comparison with a grass monoculture.

Materials and Methods

Location

The experiment was carried out at the Faculty of Veterinary Medicine and Animal Science, University of Yucatan, South Mexico, located at the east of Yucatan Peninsula (16º06'–21º37' N, 87º32'–90º23' E). Climate in the region is warm and subhumid with rains between May and October. Average temperature fluctuates between 24.5 and 27.5 ºC (INEGI 2014). The experiment was carried out from March 2013 to March 2014.

Animals and treatments

Twenty-four multiparous (≥3 calvings) crossbred cows (Holstein and Brown Swiss x Zebu), with proportions of European genes ranging from 50 to 75%, were used. Mean body weight and body condition (scale 1–9, with 1 being the lowest score) were 509±74.0 kg and 6±0.3, respectively. Parturitions occurred throughout the year of the study, being uniform between seasons. Each cow remained in the experiment only during the first 12 weeks post-partum. The cows were divided into 2 homogeneous groups based on previous milk yield records, and assigned at calving to the following grazing treatments: MS, a monoculture of African stargrass (*Cynodon nlemfuensis*); and ISS, an intensive silvopastoral system including leucaena (*Leucaena leucocephala*) and stargrass. Leucaena was distributed in rows at 2 m spacing with 0.15 m between plants within rows, giving a density of 36,000 plants/ha. The grass was established between leucaena rows.

Cows in MS (n=12) rotationally grazed 3 ha of stargrass at a stocking rate of about 2.0 AU/ha [1 animal unit (AU) = 450 kg live weight]. Occupation time in each paddock was 3 d, followed by a rest period of 27 d. Cows in ISS rotationally grazed 4.3 ha of stargrass-leucaena at a stocking rate of about 2.4 AU/ha, with 3 d of occupation, followed by 56 d of rest. Stocking rates were defined based on previous results according to forage availability (Aguilar-Pérez et al. 2001; Peniche-González et al. 2014) and were achieved and kept constant during the experiment through the "put-and-take" technique (Crowder and Chheda 1982), using additional cows as necessary. Both groups were on the pastures from 08:00 to 15:00 h and from 17:00 to 5:00 h. Paddocks in both treatments were irrigated to maintain forage yield throughout the experiment.

Milk yield. Cows were machine-milked twice a day (06:00 and 16:00 h), allowing the calves access for the initial 5 minutes to stimulate "milk let down". After that, the calves were withdrawn and tied up near their dams. Calves suckled the cows for a short time after milking. Milk yield was measured using Waikato® milk recorders every 14 d without the presence of the calf. On these occasions, an intramuscular injection of oxytocin (20 IU per cow) was used in order to empty the udder.

Body condition. Body condition score was recorded at calving and then every week using a 1–9 scale, with 1 being the lowest score (Ayala et al. 1992).

Supplement allocation

Cows in both treatments were supplemented according to their milk yields (AFRC 1995), with adjustments as necessary every 14 d following recording of milk yields and the field estimation of forage consumption, fully described below. Because of the different forages and their composition (Table 1), sorghum grain was used to provide energy to ISS cows, while a commercial concentrate, composed of 60% corn grain, 12% soybean meal and 28% soy hulls, was used to provide protein and energy to MS cows.

Half of the daily supplement allowance was offered at each milking and all was consumed. A field estimation of forage intake was performed monthly in both treatments from forage availability in the paddocks before and after grazing and taking into account the number of grazing cows. The allocation of supplements for each cow was based on its milk yield, the estimated deficit of ME and CP from forage and forage intake. Based on the above, concentrate allocation was calculated to be 0.38 kg/kg milk produced.

Table 1. Chemical composition of the diets in an intensive silvopastoral system (ISS) of leucaena-grass and a grass monoculture system (MS).

Variable[1]	ISS			MS	
	Leucaena	Stargrass	Sorghum	Stargrass	Concentrate
DM (g/kg FM)	321	274	950	274	890
CP (g/kg DM)	153	80	86	62	123
ADF (g/kg DM)	247	361	-	361	-
Ash (g/kg DM)	78	99	13	99	38
ME (MJ/kg DM)	9.3	9.2	12.4	8.9	11.2

[1]DM: dry matter; FM: fresh matter; CP: crude protein; ADF: acid detergent fiber; ME: metabolizable energy.

Forage availability in MS was recorded using a 0.5 x 0.5 m (0.25 m^2) metal quadrate, by a modification of the technique reported by Cox (1980). Ten samples were taken from each paddock on each occasion, attempting to cover all the area in a zig-zag pattern. The grass inside the square was cut at 5 cm height from the ground and then weighed. Forage availability in ISS was recorded as described by Bacab-Pérez and Solorio-Sánchez (2011): using a 4 m^2 quadrate, the edible forage of leucaena (leaves and young stems) was harvested. In addition, grass inside the quadrate was cut at 5 cm from the ground. Ten samples were taken from each paddock on each occasion. Subsamples of grass and leucaena were selected and oven-dried at 60 ºC to constant weight (AOAC 1980). Forage samples were analyzed for CP by the Dumas method (Jung et al. 2003) and for acid detergent fiber (ADF) by the filter bag technique (Contreras et al. 1999). Metabolizable energy in grass and leucaena samples was estimated according to the following equations (MAFF 1978):

Grass ME (MJ/kg DM) = 15.9 - 0.019 ADF

Leucaena ME (MJ/kg DM) = 12 - 0.019 ADF

where:

ME is metabolizable energy; and ADF is acid detergent fiber.

To determine ME concentration for the concentrate and sorghum given to the cows in the MS and ISS, respectively, 3 samples were taken as the experiment progressed. Proximate analyses (AOAC 1980) were performed for sorghum and concentrate. Metabolizable energy concentration was estimated according to the following equation (MAFF 1978):

ME (MJ/kg DM) = 0.012 CP + 0.031 EE + 0.005 CF + 0.014 NFE

where:

ME is metabolizable energy; CP is crude protein; EE is ether extract; CF is crude fiber; and NFE is nitrogen-free extract.

Estimation of forage intake

Dry matter intakes of grass and leucaena were estimated once per cow on day 45 post-partum, using the n-alkane technique (Dove and Mayes 1991). The marker alkane C32 (dotriacontane) was dosed (500 mg/cow/d) in 100 g of labelled wheat bran. Each cow was dosed twice a day (250 mg of dotriacontane in 50 g of wheat bran) during each milking, for 12 consecutive days. Grab samples of feces were taken from the rectum after each milking on the last 5 days of the dosing period, and grass and leucaena samples were hand-plucked from the area where the cows were grazing. The morning and afternoon feces from each cow were pooled daily, as were forage samples, oven-dried (70 ºC for 72 h for feces and 60 ºC for 48 h for forage) and ground.

The alkane was extracted from feces and forages as indicated by Aguilar-Pérez et al. (2009) and the alkane concentrations were determined by gas chromatography using an Agilent Technology 7820AGC chromatograph, fitted with a flame ionization detector (FID) and a column Agilent J&W GC, 19091N-133, of 30 m x 0.320 mm x 0.25 µm. Two microliters of alkane extract were injected into the gas chromatograph at temperatures of 280 and 340 °C, to the injector and detector, respectively, using H_2 as carrier gas at a flow rate of 40 mL/min (Aguilar-Pérez et al. 2009).

The n-alkanes C33 (tritriacontane) (Aguilar-Pérez et al. 2009) and C29 (nonacosane) (Sánchez et al. 2009) were used as internal markers for grass and leucaena, respectively. Dry matter intakes of forages were calculated by the method of Dove and Mayes (1991) applying the following equation:

$$\text{Intake (kg DM/d)} = \left(\frac{F_i}{F_j} \times D_j\right) / \left(H_i - \frac{F_i}{F_j} \times H_j\right)$$

where:

F_i is concentration of odd alkane in feces;

H_i is concentration of odd alkane in plant;

F_j is concentration of even alkane in feces;

H_j is concentration of even alkane in plant; and

D_j is the internal marker dosed.

Blood urea nitrogen (BUN)

Blood samples were taken by coccygeal venipuncture in vacutainer heparinized tubes, once a week, 5 minutes after the morning milking. Samples were centrifuged at 3,500 rpm for 15 min, and plasma was separated and stored at -20 °C until analysis. Blood urea was determined using the UV kinetic urea test (Wiener Lab., Rosario, Argentina); urea values were then multiplied by 0.467 to convert them to BUN.

Experimental design and statistical analysis

A completely randomized design with 2 treatments (ISS and MS) was used. Intakes of CP and ME were analyzed by the general linear model (GLM) procedure. Milk yield and BUN values were analyzed as repeated measures by the MIXED procedure with an autoregressive structure of covariance. Systems were the fixed effects and the cows the random effects. Body condition score was analyzed using the non-parametric test of Mann-Whitney. There were no effects of month of calving or the interaction month x treatment on response variables; therefore, only treatment effects are reported. Blood urea nitrogen data were transformed to $\log_{10}$ and results were reported as antilogarithm. Means were compared using the Tukey test at P<0.05 probability level. Results were expressed as mean and standard error (s.e.) and means and standard deviation (SD). Analysis of all data was performed using the statistical package SAS (SAS 2009).

Results

Estimated composition of the diets of individual cows for both treatments varied greatly (Table 2); leucaena represented 27.0–39.0% of the diet in ISS, with a mean of 34.2% of total DM consumed.

Concentration of C33 alkane in *C. nlemfuensis* was 117–270 mg/g and concentrations in feces were 100–250 mg/g for cows in MS, and 110–200 mg/g for cows in ISS. Concentration of C29 was 50–150 mg/g in leucaena and 27–120 mg/g in feces.

Estimated CP and ME intakes of individual cows showed large variation on both systems (Table 3), but only ME intake was different (P<0.05) between systems.

Body condition scores on both systems were similar (P>0.05) throughout the experiment (Figure 1), with SD of 0.5 points for cows in ISS and 1 point for cows in MS.

Table 2. Estimated dry matter intakes (kg DM/cow/d) of components of the diet of crossbred cows grazing an intensive silvopastoral system (ISS) of leucaena-grass and a grass monoculture system (MS).

Diet component	ISS			MS		
	Minimum	Maximum	Mean±s.e.	Minimum	Maximum	Mean±s.e.
Leucaena	2.6	6.9	5.1±1.8	-	-	-
Stargrass	2.7	8.5	4.9±2.4	4.6	10.4	7.4±1.4
Sorghum	2.6	9.5	4.8±2.2	-	-	-
Concentrate	-	-	-	2.2	10.7	4.6±3.1
Total			14.8±2.1			11.9±2.2

Table 3. Estimated intakes of crude protein (CP) and metabolizable energy (ME) and milk yields in crossbred cows grazing an intensive silvopastoral system (ISS) of leucaena-grass and a grass monoculture system (MS).

Variable	ISS			MS		
	Minimum	Maximum	Mean±s.e.	Minimum	Maximum	Mean±s.e.
CP (g/cow/d)	1,234	1,981	1,479±1.0a[1]	999	1,917	1,258±1.0a
ME (MJ/cow/d)	129	227	161±1.0a	101	194	131±1.0b
Milk (kg/cow/d)	10.0	25.0	13.5±1.1a	10.0	25.0	14.5±1.1a

[1]Means within rows followed by different letters are significantly different at P<0.05.

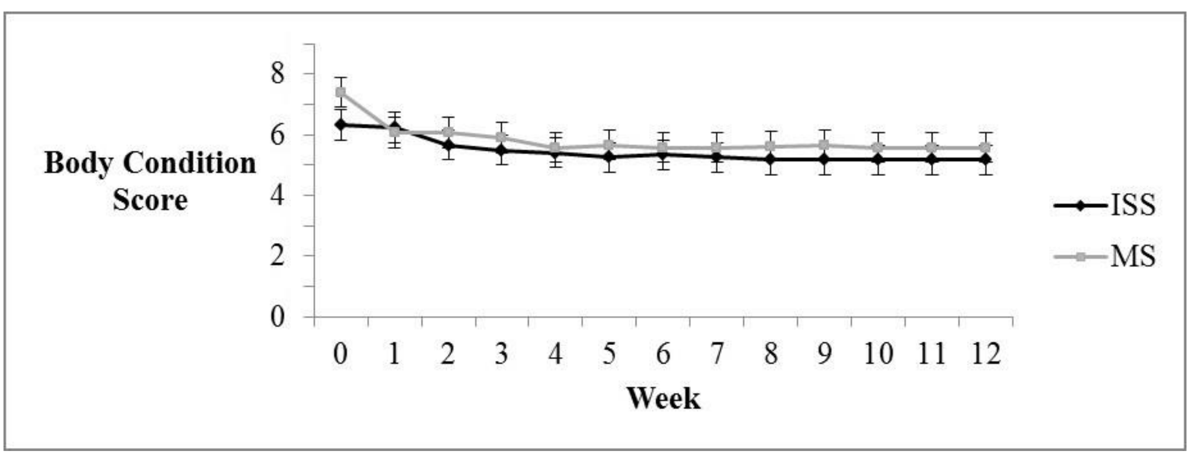

Figure 1. Body condition scores in crossbred cows grazing an intensive silvopastoral system (ISS) of leucaena-grass and a grass monoculture system (MS). Vertical bars indicate SD.

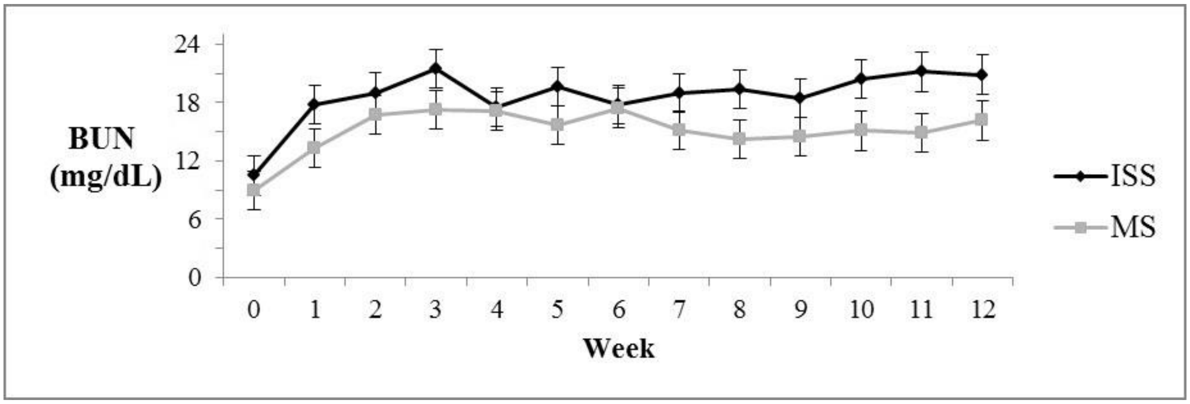

Figure 2. Blood urea nitrogen in crossbred cows grazing an intensive silvopastoral system (ISS) of leucaena-grass and a grass monoculture system (MS). Vertical bars indicate SD.

Figure 2 shows that, in general, blood urea nitrogen concentration was higher ($P<0.05$) in the ISS (mean 19.3±2 mg/dL) than in the MS (15.3±2 mg/dL) system.

Discussion

In this study, our data suggest that leucaena comprised 34% of the average DM intake by grazing cows in the ISS, which was similar to the 30% reported by Sierra-Montoya (2014) in cows grazing a silvopastoral system with leucaena in Colombia. These intakes are within the recommended percentages of inclusion of forage from tropical trees in the diets of ruminants (Norton 1998). The results highlighted the variation in acceptance of leucaena by different animals. All cows did not consume similar proportions of grass and leucaena, but the range in leucaena proportion (27–39%) as estimated by the alkane method was much narrower than the range of 8–83% recorded by Buck et al. (2011) for steers grazing leucaena-grass pastures in Australia. This variability can be attributed to individual preferences by animals for different forages reported by Cárdenas et al. (2011). Another possible explanation could be the effects of the environment on the cows within a group as has been suggested by Friggens et al. (1998) or differences in individual nutrient requirements mentioned in some studies (Kyriazakis 2003; Hristov and Giallongo 2014).

Estimated CP intakes using the alkane method in both systems were similar but ME intakes were higher in the silvopastoral system. Based on forage composition and the estimated forage intakes, leucaena contributed almost 50% of the CP consumed by cows in ISS. This could provide an economic advantage over cows in MS, where the main CP source was an expensive concentrate. These results also indicate that CP in leucaena was able to support a milk yield similar to that provided by the protein in the concentrate. This outcome is in agreement with findings of other authors who have partially replaced concentrate supplements with leucaena in diets of lactating buffaloes (Garg and Kumar Sanijiv 1994) and cows (Peniche-González et al. 2014). It also agrees with the findings of Flores et al. (1979), who pointed out that leucaena was superior to formaldehyde-treated casein as a protein source for lactating cows. Since body condition scores did not differ between treatments, it seems that milk yields truly reflected nutrients supplied by the diets.

An important finding in our research is the CP concentration of *C. nlemfuensis* in the ISS, which was higher than that found in the monoculture. This difference may result from nitrogen fixation by the legume, which allowed the star grass in the ISS to maintain an adequate level of protein despite being grazed at a later age than in the MS. Casanova-Lugo et al. (2014) found a higher CP concentration in pastures associated with legumes. The use of leguminous plants for nitrogen fixation from the atmosphere would allow an environmentally friendly and economically sustainable production system due to reduced use of nitrogen fertilizers (Peoples et al. 1995).

The higher BUN concentrations for cows in ISS than for those in MS throughout the study period suggest a lower efficiency of utilization of nitrogen in the rumens of cows on the ISS. Other authors, e.g. Ruiz (2013) and Arjona (2015), have observed increasing values of BUN when leucaena intake was equal to or higher than 30% of ration DM. Since leucaena is such a valuable forage, it seems desirable to restrict consumption to levels below those consumed in this study.

Since estimated ME intakes in the ISS were greater than in the MS, one might expect either increased weight gains by cows in ISS or increased milk yields. It is possible that the rate of ruminal degradation of CP in leucaena (Miranda et al. 2012), as well as the asynchrony between the rates of fermentation of CP (during grazing) and the energy supplement (during milking), caused peaks of concentration of NH_3 and energy in the rumen at different times, resulting in absorption of surplus ammonia into the blood stream, resulting in elevated BUN in ISS cows. Lazarin et al. (2012) found a linear increase in the concentration of BUN in cows consuming CP and energy at different times of the day or diets with high contents of rapidly degradable protein.

Conclusion

This study has shown that a silvopastoral system based on African stargrass and leucaena with sorghum supplement can support milk yields equal to those of stargrass plus conventional concentrates. Replacement of the expensive concentrate supplements should result in a more economic production system on top of the environmental benefits of the legume. Economic assessments are needed to determine the profitability of such a strategy and the appropriate proportions of stargrass and leucaena in such systems. Strategies for capturing the excess amounts of N released in the rumen to improve the efficiency of N utilization should be pursued.

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Research paper

Growth and nutritional evaluation of napier grass hybrids as forage for ruminants*

Producción y evaluación nutritiva de híbridos del pasto elefante como forraje para rumiantes

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Abstract

Napier grass is a perennial, tropical C-4 grass that can produce large amounts of forage. However, low temperatures and drought stress limit its productivity and nutritive value as a forage. To overcome these limitations, pearl millet × napier grass hybrids (PMN) were developed. It was hypothesized that PMN hybrids were more drought-tolerant, produced higher yields, and had higher nutritive value than napier grass varieties. The yield and nutritive value of 4 napier grass varieties (Bana grass, Mott, MB4 and N51) and 4 PMN hybrids (PMN2, PMN3, 5344 and 4604) were determined with or without irrigation in a strip plot design in Hawaii. Hybrid PMN3 outperformed napier grass varieties and the other hybrids for yield, while 5344 showed higher nutritional content and digestibility than most other grasses. Dry matter yields during the 110-day study period ranged from 10.3 to 32.1 t/ha without irrigation and 19.6 to 55.8 t/ha with irrigation, indicating that moisture stress was limiting performance in raingrown pastures. Only hybrids PMN3 and PMN2 and variety MB4 showed significant growth responses to irrigation. Further work is needed to evaluate the hybrids in a range of environments over much longer periods to determine if these preliminary results can be reproduced over the long term. Similarly, feeding studies with animals are needed to determine if the in vitro data for digestibility are reflected in superior performance for the promising hybrids.

Keywords: Biomass, cattle, in vitro digestion, nutrient content, *Pennisetum*, tropical grasses.

Resumen

Pasto elefante (*Pennisetum purpureum*) es una gramínea tropical C-4 que puede producir grandes cantidades de forraje. Sin embargo, temperaturas bajas y sequía limitan su productividad y valor nutritivo. Para superar estas limitaciones, se desarrollaron híbridos *P. glaucum* (sin. *P. americanum)* × *P. purpureum*, bajo la hipótesis que, en comparación con las variedades del pasto elefante, los híbridos son más tolerantes a la sequía y más productivos, y tienen mayor valor nutritivo. En este estudio se determinaron la producción de materia seca (MS) y el valor nutritivo de 4 variedades de pasto elefante (los cultivares Bana, Mott, MB4 y N51) y de 4 híbridos (PMN2, PMN3, 5344 y 4604), con o sin riego, en un diseño de parcelas divididas con tratamiento en franjas (*strip plot*) en Hawaii, USA. El híbrido PMN3 superó las variedades de pasto elefante y los otros híbridos respecto al rendimiento de MS, mientras que el híbrido 5344 mostró una mayor concentración de nutrientes y una digestibilidad más alta que la mayoría de las otras gramíneas.

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Las producciones de MS durante los 110 días del estudio oscilaron entre 10.3 y 32.1 t/ha sin riego y entre 19.6 y 55.8 t/ha con riego, lo que indica que la falta de humedad estaba limitando el rendimiento. Solo los híbridos PMN3 y PMN2 y la variedad MB4 respondieron al riego con producciones significativamente más altas. Se necesitan más estudios para evaluar los híbridos en una diversidad de ambientes y durante periodos mucho más largos para determinar si estos resultados preliminares pueden ser reproducidos a largo plazo. Del mismo modo, se necesitan estudios de alimentación con diferentes clases de animales para determinar si las digestibilidades in vitro más altas de los híbridos promisorios se reflejan en superiores producciones animal.

Keywords: Biomasa, calidad nutritiva, contenido de nutrientes, digestibilidad in vitro, gramíneas tropicales, *Pennisetum*.

Introduction

Forages are a necessary component of diets for ruminants, as they provide the fiber needed to optimize rumen function. Napier grass, *Pennisetum purpureum* Schumach. (2n=28), is a robust perennial grass that has been widely used as a tropical forage, producing greater dry matter (DM) yield than other tropical grasses (Boonman 1997; Hanna et al. 2004). Although it has low protein concentration, it can provide a satisfactory forage source for dairy cows, if supplemented with legumes and protein concentrates (Nyambati et al. 2003). Napier grass is an outcrossing species with a low self-fertilization rate. It is replicated vegetatively due to its low seed set and low seed viability. Pearl millet, *Pennisetum glaucum* (L.) R.Br. (2n=14), a major warm-season cereal and an annual bunchgrass grown in arid and semi-arid regions of the world, is a crop with an outcrossing rate of more than 85%. Pearl millet crosses easily with napier grass to produce sterile interspecific hybrids (3n=21), which are more vigorous than the parent species (Burton 1944) and have high biomass potential (Hanna et al. 2004).

In this age of Global Warming one of the major concerns for cattle industries is how the changing climate will affect forage quality and availability. The Intergovernmental Panel on Climate Change (IPCC 2013) concluded that elevated greenhouse gas concentrations are likely to lead to general drying of the subtropics by the end of this century, creating widespread stress on agricultural crops and pastures. The Panel suggested that every degree Celsius increase in seasonal temperature would lead to 2.5–16.0% direct yield loss of the major grain crops (IPCC 2013). Battisti and Naylor (2009) calculated that, by the end of this century, there is a 90% possibility that average summer temperatures will exceed the current hottest recorded temperature throughout the world. Higher temperatures will also lower soil organic matter content by oxidation and further result in reduction of crop yields and land quality. These climatic changes can have serious effects for meeting food, forage, water and energy needs of human and livestock populations. Hence, it is necessary to develop/identify crop varieties that are tolerant of heat and heat-induced water stress.

Several researchers (Gupta and Bhardwaj 1975; Ogwang and Mugerwa 1976; Gupta and Mhere 1997) have indicated the vast potential for improvement in the yield and quality of pearl millet × napier grass (PMN) hybrids over napier grass. The interspecific hybrids produce more tillers and leaves and grow faster than their parents (Gupta and Mhere 1997). Pearl millet is drought-tolerant and is also resistant to most pests and diseases. Hence, PMN hybrids have been developed, which have high seed set and viable seeds. Additionally, with over 21,000 pearl millet accessions worldwide, there is high potential to transfer desirable traits into napier grass. The PMN hybrids can play a major role in producing high quality forages with lower water demands for the cattle industry in tropical regions of the world.

Biomass yield and chemical composition of napier grass vary significantly depending on variety, age, season, location and management practices (Ogoshi et al. 2010; Rengsirikul et al. 2011; Xie et al. 2011). Field trials in Hawaii have shown the ratoon crop yield was 13% higher than the plant crop for Bana grass, a napier grass variety (Osgood et al. 1996). In addition, the nutritive value of forage affects the utilization by animals, which in turn affects the production of animals as well as emissions of methane, a major greenhouse gas (Mirzaei-Aghsaghali and Maheri-Sis 2011). There are limited data on the nutritional content and digestibility of PMNs, a situation that must be corrected before recommendations for animal feeding are made. It was hypothesized that PMN hybrids would be more drought-tolerant and nutritious than napier grass varieties. The objective of this study was to evaluate the growth and nutritional value of some PMN hybrids and napier grass varieties under both rain-grown and irrigated conditions to identify the most productive of these forages for use in the cattle industry.

Materials and Methods

Germplasm

In the study, 4 PMN hybrids were used: PMN2, PMN3, 5344 and 4604; and 4 napier grass varieties (cultivars): Bana grass, Mott, MB4 and N51. PMN2 and PMN3 are pearl millet × napier grass interspecific hybrids resulting from a field-pollinated polycross utilizing 6 napier grass varieties (OB06, 514B, N51, OB07, Green and Bana grass) and cytoplasmic male-sterile (CMS) pearl millet varieties (ICMA 07333 and ICMA 00999). They were selected from a screening of over 800 progeny from these crosses on the basis of rapid growth, plant morphology (e.g. smooth and soft leaves, high leaf:stem ratio, leaf area, stem thickness) and disease resistance. Hybrid 5344 is a PMN hybrid from crossing of napier grass cv. Bana with the CMS pearl millet, Tift 23A1E1. Hybrid 4604 was derived from a polycross of napier grass cultivars N74, N23 and N14 with CMS pearl millet, Tift 23A1E1 (Osgood et al. 1997). MB4 is a wild napier grass accession collected on Maui Island (Hawaii, USA) in 2009. Bana grass is commonly used as a windbreak throughout Hawaii. Variety N51 (Hanna and Monson 1980) is another tall napier grass variety, and cv. Mott is a dwarf napier grass, N75 (Hanna and Monson 1988.

Field trials

The 4 napier grass varieties and 4 PMN hybrids were field tested both with and without irrigation treatment in a strip plot design, with each treatment consisting of 3 replicated plots at the College of Tropical Agriculture and Human Resources, Waimanalo Research Station, University of Hawaii, USA (21°20’18” N, 157°42’53” W; 18 masl), where annual rainfall averages 1,400 mm. Stem cuttings (~46 cm long) were planted in shallow furrows and urea and potassium chloride were applied at 33.6 kg/ha each. A similar second application of urea and potassium chloride was applied at 55 days after planting. All plots were drip irrigated at 100% pan evaporation for the first 30 days. Plots were 2 m × 6 m in size. Grasses were planted in 3 rows with 2 m between rows and overlapping stem cuttings placed in 2 m furrows. An area of 2 m^2 of the center row of the forages was harvested at about 20 cm above the soil on day 110 after planting, when samples were collected, chopped and dried in a forced-draft oven at 100 °C until constant weight. Dry matter yields were calculated. During the 110-day trial period, plots received 268 mm of rainfall and 18,915 liters of irrigation were applied (0.002 hectare-meter) (Figure 1). Plots used for the experiments contained silty clay soils in the Waialua series (isohyperthermic Pachic Haplustolls).

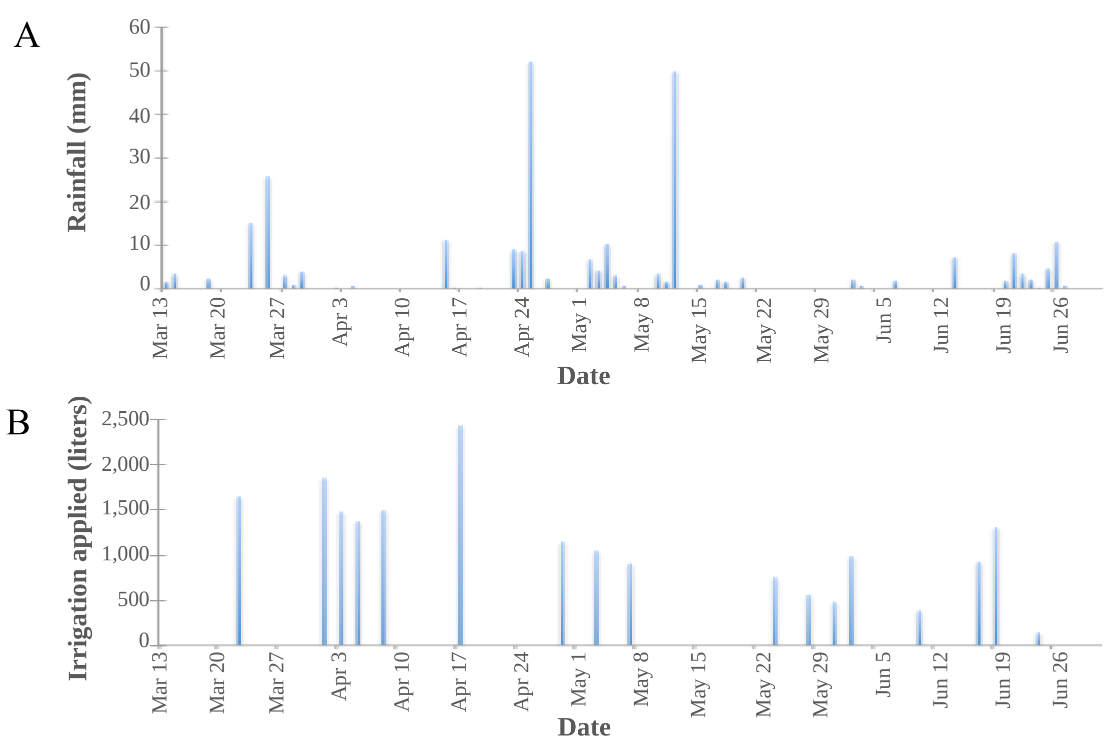

Figure 1. Rainfall and irrigation during trial period (13 March 2013 to 1st July 2013). A. Rainfall in mm per day. B. Irrigation applied in liters per application.

Sample analysis

The representative dried sample from each plot was ground to pass through a 1 mm screen using a Thomas Wiley laboratory mill. Subsamples were subjected to determination of basic proximate constituents, fiber and energy content, and in vitro digestibility parameters using near infrared spectroscopy, an approved method of AOAC (Association of Official Analytical Chemists, Gaithersburg, MD, USA) at a certified commercial lab (Dairy One Cooperative Inc., Forage Lab, Ithaca, NY, USA). The NIR analysis was conducted using Foss NIR systems model 6500 equipped with software win ISI ii v1.5. The laboratory uses broad-based calibrations for NIR by incorporating samples collected over several decades based on reference chemistry using traditional procedures. The analysis included dry matter, ash, crude protein, digestibility of protein, neutral detergent insoluble crude protein, starch, non-fiber carbohydrate, soluble carbohydrate, acid detergent fiber, neutral detergent fiber, lignin, total digestible nutrients, metabolizable energy, net energy for growth, net energy for lactation, net energy for metabolism, in vitro total digestibility, neutral detergent fiber digestibility and rate of digestion. It also included most of the minerals with importance to animal nutrition. For predicting TDN and net energy, the laboratory uses the summative energy equation of NRC (1988).

Statistical analysis

The dry matter yields, nutritional profiles and in vitro digestibility characteristics were compared using the MIXED procedure of SAS v. 9.2 (SAS Institute Inc., Cary, NC, USA) with a strip-plot arrangement of variety and treatments (with or without irrigation). Means were separated using the Tukey method, using pdmix macro of SAS. Differences were considered significant if $P<0.05$.

Results

Dry matter (DM) yields of the PMN hybrids and napier grass varieties showed a significant overall effect of variety *(*$P<0.0001$) and irrigation ($P=0.0002$), but variety × irrigation interactions were not significant ($P=0.2636$). Dry matter yields in the non-irrigated plots ranged from 10.3 t/ha (Mott) to 32.1 t/ha (PMN3), while corresponding yields in the irrigated plots ranged from 19.6 t/ha (Mott) to 55.8 t/ha (PMN3) (Figure 2). PMN3 had significantly higher DM yields under irrigation than the other varieties and hybrids, while Mott had significantly lower DM yields without irrigation than the other varieties and hybrids. While all varieties and hybrids produced higher yields when irrigated, the differences were significant only for MB4, PMN2 and PMN3 (Figure 2).

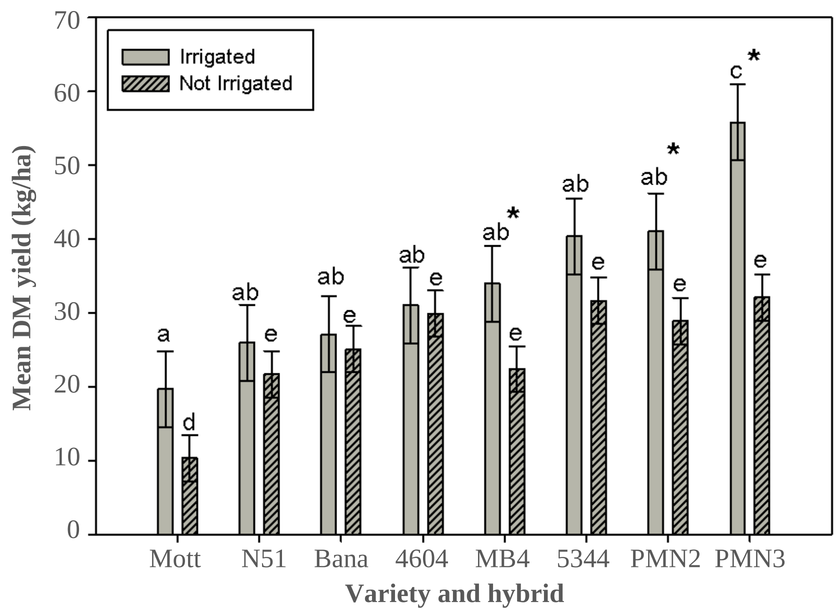

Figure 2. Mean dry matter (DM) yields for 110-day harvest of napier grass varieties (Mott, N51, Bana grass and MB4) and pearl millet × napier grass hybrids (PMN2, PMN3, 4604 and 5344) under irrigated ( ) and non-irrigated ( ) conditions. Letters indicate Tukey comparisons of mean DM yields within irrigated and non-irrigated treatments. Varieties and hybrids with the same letter are not significantly different. * denotes mixed model ANOVA significant difference between irrigated and non-irrigated DM yields for a variety/hybrid. Error bars are ±standard error of the mean.

In general, DM concentration of PMN hybrids was higher than that of napier grass varieties, with DM% of PMN2 (24.3%) and PMN3 (22.9%) being significantly higher (P<0.05) than those of 5344 (18.5%), Bana grass (18.1%) and N51 (17.9%) (Table 1).

Ash was lowest (P<0.05) in PMN2 (8.9%) and highest in 5344 (14.6%) (Table 1). No significant differences (P>0.05) in concentrations of acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), lignin and metabolizable energy (ME) were found among napier grass varieties and PMN hybrids (Table 1) or between irrigated and non-irrigated treatments (Table 2).

The in vitro dry matter digestibility (IVDMD) varied between varieties and hybrids, being significantly higher (P<0.05) in 5344 and Bana grass (70.0 and 68.0%, respectively) than in PMN2 (54.5%) (Table 3). Rate of digestion varied from 4.9%/h in 5344 to 2.7%/h in PMN2 (P<0.05).

Irrigation had no effect on mineral concentrations in forages but varieties and hybrids differed in concentration of minerals (Table 5), with the following ranges: calcium – 0.36% (5344) to 0.17% (PMN2) (P<0.05); potassium – 3.91% (N51) to 2.15% (PMN2) (P<0.05); phosphorus – 0.26% (Mott) to 0.19% (PMN2), 0.18% (N51) and 0.17% (MB4) (P<0.05); magnesium – 0.27% (4604) to 0.14% (Mott) (P<0.05); zinc – 24.8 ppm (PMN3) to 18.7 ppm (Bana) (P<0.05); copper – 10.7 ppm (MB4) to 7.3 ppm (PMN3, 5344) (P<0.05); selenium – 0.15 ppm (5344) to 0. 06 ppm (PMN2, N51) (P<0.05); and chloride – 1.27% (5344) to 0.82% (PMN2) (P<0.05).

The neutral detergent fiber (NDF) concentration of the grasses ranged between 68.4 and 73.3%, with no significant differences (P>0.05) (Table 1). Neutral detergent fiber digestibility (NDFD) of 5344 and Bana grass (56.7 and 53.2%, respectively) was significantly higher (P<0.05) than that of PMN2 (38.0%).

Table 1. Nutrient profile of forage from napier × pearl millet hybrids and napier grass varieties[1].

Variable	Hybrid				Variety				s.e.m.	P-value		
	PMN2	PMN3	5344	4604	Bana	Mott	MB4	N51		Var	Trt	Var × Trt
Dry matter (%)	24.2	22.9	18.5	21.6	18.0	20.6	20.4	17.9	0.88	<0.001	0.441	0.929
Ash (%)	8.9	11.4	14.6	11.2	14.1	12.7	13.2	11.3	0.90	0.002	0.706	0.718
Crude protein (%)	6.4	7.0	7.9	7.9	8.3	6.5	7.4	7.5	0.71	0.444	0.974	0.789
Digestibility of protein (%)	55.5	60.2	59.8	59.3	57.0	56.7	62.5	55.7	2.97	0.666	0.906	0.491
NDICP (%)	2.3	2.5	3.0	2.8	2.8	2.2	2.8	2.9	0.26	0.381	0.205	0.462
Starch (%)	1.6	1.4	1.7	0.7	1.7	1.3	1.8	1.2	0.24	0.079	0.157	0.011
NFC (%)	12.8a	10.6ab	10.2ab	10.8ab	10.9ab	9.6ab	8.8b	9.6ab	0.73	0.025	0.477	0.015
Soluble carbohydrate (%)	4.2	3.4	4.4	4.1	5.8	3.0	3.6	4.6	0.50	0.017	<0.001	0.072
ADF (%)	51.2	52.3	47.4	52.3	47.8	52.9	51.6	49.7	1.53	0.133	0.321	0.630
NDF (%)	73.3	72.5	68.9	71.7	68.4	72.2	72.2	73.0	1.41	0.143	0.631	0.344
Lignin (%)	6.6	6.5	5.2	8.2	5.5	6.2	6.3	6.6	0.64	0.084	0.288	0.817
TDN (%)	46.5	46.7	51.2	46.2	50.3	44.7	49.2	51.2	1.75	0.073	0.484	0.780
ME (Mcal/kg)	1.7	1.7	1.7	1.6	1.7	1.7	1.6	1.7	0.05	0.438	0.091	0.751
NEL (Mcal/kg)	0.71	0.73	0.90	0.73	0.86	0.70	0.78	0.80	0.056	0.146	0.405	0.490
NEM (Mcal/kg)	0.70	0.73	0.88	0.68	0.83	0.66	0.8	0.86	0.063	0.107	0.576	0.702
NEG (Mcal/kg)	0.16	0.16	0.35	0.18	0.33	0.10	0.25	0.31	0.060	0.043	0.332	0.704

[1]All data, except dry matter, are expressed on a dry matter basis.
ADF - acid detergent fiber; ME - metabolizable energy; NEG - net energy for growth; NEL - net energy for lactation; NEM - net energy for metabolism; NDF - neutral detergent fiber; NDICP - neutral detergent insoluble crude protein; NFC - non-fiber carbohydrate; TDN - total digestible nutrients; Trt - treatment; Var - variety.

Table 2. Nutrient profile of forage from napier × pearl millet hybrids and napier grass varieties grown under irrigated and non-irrigated conditions[1].

Variable	Hybrid								Variety								s.e.m.
	PMN2		PMN3		5344		4604		Bana		Mott		MB4		N51		
	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	
Dry matter (%)	23.6	24.9	22.7	23.0	18.5	18.5	22.1	21.0	16.9	19.1	20.1	21.2	20.5	20.4	17.9	17.9	0.9
Ash (%)	8.5	9.3	12.5	10.2	14.7	14.5	11.5	11.0	14.3	13.9	11.5	13.9	13.9	12.4	11.4	11.2	1.3
ADICP	0.9	0.9	0.7	1.0	0.8	0.7	1.0	0.8	0.8	0.8	0.8	0.9	1.1	1.0	0.9	0.9	0.1
AvCP	4.6	6.4	6.3	6.0	7.5	7.4	6.8	7.1	8.1	6.8	5.2	6.2	7.0	5.7	6.7	6.5	0.9
Digestibility of protein (%)	57.3	53.7	58.0	62.3	65.0	54.7	62.0	56.7	56.0	58.0	53.7	59.7	60.0	65.0	55.7	55.7	4.2
NDICP (%)	2.0	2.7	2.7	2.3	3.0	3.0	2.9	2.6	3.1	2.4	2.2	2.3	3.2	2.3	3.0	2.8	0.4
Crude fat (%)	0.8	1.2	1.1	1.0	0.9	1.4	1.2	1.1	1.1	1.2	1.1	1.4	1.2	1.2	1.4	1.5	0.2
Starch (%)	1.7	1.5	1.4	1.3	1.1	2.3	0.76	0.70	2.2	1.1	1.3	1.2	2.66	0.9	1.1	1.3	0.3
NFC (%)	12.8	12.8	10.2	11.1	8.3	12.0	9.6	12.1	10.8	11.0	10.7	8.6	10.8	6.7b	8.6	10.6	1.0
Soluble carbohydrate (%)	4.3	4.3	3.1	3.9	2.3c	6.6	3.3	5.0	4.5	7.2a	3.0	3.0	3.3	4.0	4.0	5.3	0.7
ADF (%)	53.2	49.2	51.1	51.2	48.6	46.1	51.7	52.9	47.7	47.9	55.2	50.5	50.1	53.1	50.7	48.6	2.2
NDF (%)	74.4	72.1	71.9	73.1	70.7	67.1	72.9	70.6	68.1	68.7	72.9	71.4	69.2	75.2	74.0	72.1	2.0
Lignin (%)	7.3	5.8	5.8	7.2	5.3	5.0	8.5	8.0	5.6	5.3	6.9	5.5	6.4	6.3	7.2	6.1	0.9
TDN (%)	44.0	49.0	47.7	45.7	49.7	52.7	46.0	46.3	50.3	50.3	44.0	45.3	50.7	47.7	50.0	52.3	2.5
ME (Mcal/kg)	1.7	1.8	1.7	1.7	1.6	1.8	1.6	1.6	1.7	1.7	1.6	1.7	1.6	1.6	1.6	1.7	0.1

[1]All data, except dry matter, are expressed on a dry matter basis.

ADF - acid detergent fiber; ADICP - acid detergent insoluble crude protein; AvCP - available crude protein; ME - metabolizable energy; NDF - neutral detergent fiber; NDICP - neutral detergent insoluble crude protein; NFC - non-fiber carbohydrate; TDN - total digestible nutrients.

Table 3. In vitro digestibility parameters of forage from napier × pearl millet hybrids and napier grass varieties.

Variable	Hybrid				Variety				s.e.m.	P-value		
	PMN2	PMN3	5344	4604	Bana	Mott	MB4	N51		Var	Trt	Var × Trt
IVTD (30 h, % of DM)	54.5	59.2	70.0	59.7	68.0	59.7	63.7	63.5	2.71	0.007	0.983	0.650
NDFD (30 h, % of NDF)	38.0	44.2	56.7	43.7	53.2	44.2	50.0	49.8	2.97	0.002	0.813	0.643
Rate of digestion (%/h)	2.7	3.3	4.9	3.7	4.4	3.3	4.0	4.1	0.35	0.002	0.305	0.797

IVTD - in vitro total digestibility; NDFD - neutral detergent fiber digestibility; Var - variety; Trt - treatment.

Table 4. In vitro digestibility parameters of forage from napier × pearl millet hybrids and napier grass varieties grown under irrigated and non-irrigated conditions[1].

Variable	Hybrid								Variety								s.e.m.
	PMN2		PMN3		5344		4604		Bana		Mott		MB4		N51		
	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	
IVTD (30 h, % DM)	50.3	58.7	62.3	56.0	69.7	70.3	59.7	59.7	69.7	66.3	58.3	61.0	65.7	61.7	62.3	64.7	3.8
NDFD (30 h, % NDF)	33.3	42.7	48.0	40.3	58.0	55.3	44.3	43.0	55.3	51.0	43.0	45.3	50.7	49.3	49.0	50.7	4.2
Rate of digestion (%/h)	2.3	3.0	3.6	3.0	5.1	4.6	3.8	3.6	4.9	3.9	3.2	3.3	4.2	3.8	4.1	4.1	0.5

IVTMD - in vitro total digestibility; NDFD - neutral detergent fiber digestibility.

Table 5. Mineral concentration in forage from napier × pearl millet hybrids and napier grass varieties[1].

Variable	Hybrid				Variety				s.e.m.	P-value		
	PMN2	PMN3	5344	4604	Bana	Mott	MB4	N51		Var	Trt	Var×Trt
Calcium (%)	0.17b	0.24ab	0.36a	0.27ab	0.31ab	0.29ab	0.20ab	0.21ab	0.035	0.0129	0.193	0.786
Potassium (%)	2.15e	2.50de	3.38abc	2.73cde	3.50ab	3.01bcd	3.23abcd	3.91a	0.016	0.0001	0.620	0.575
Phosphorus (%)	0.22ab	0.19b	0.20ab	0.21ab	0.19b	0.26a	0.17b	0.18b	0.262	0.0045	0.001	0.491
Magnesium (%)	0.18bc	0.21abc	0.23ab	0.27a	0.24ab	0.14c	0.22ab	0.20abc	0.018	0.0005	0.110	0.957
Sodium (%)	0.023	0.03	0.031	0.033	0.032	0.032	0.04	0.03	0.006	0.7372	0.573	0.774
Iron (ppm)	370	968	702	484	499	491	742	659	202.1	0.5213	0.554	0.793
Zinc (ppm)	23.7	24.8	22.8	19.5	18.7	22.7	22.2	21.0	1.742	0.229	0.386	0.795
Copper (ppm)	6.50c	7.33bc	7.33bc	8.5abc	9.83ab	10ab	10.67a	9.5ab	0.618	0.0002	0.850	0.914
Manganese (ppm)	35.7	61.5	49.3	40.0	41.5	35.0	45.0	44.3	8.338	0.4204	0.130	0.981
Molybdenum (ppm)	0.14	0.00	0.15	0.26	0.30	0.18	0.20	0.35	0.474	0.0568	0.476	0.693
Selenium (%)	0.06	0.08	0.15	0.11	0.11	0.07	0.14	0.06	0.020	0.174	0.320	0.664
Chloride ion (%)	0.82c	0.95abc	1.27a	1.16ab	1.05abc	0.93bc	1.14ab	1.18ab	0.069	0.0009	0.003	0.883

[1]All data are expressed on a dry matter basis.
Trt - treatment; Var - variety.

Table 6. Mineral concentration in forage from napier × pearl millet hybrids and napier grass varieties grown under irrigated and non-irrigated conditions[1].

Variable	Hybrid								Variety								s.e.m.
	PMN2		PMN3		5344		4604		Bana		Mott		MB4		N51		
	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	Irr.	Non-Irr.	
Calcium (%)	0.13	0.22	0.25	0.23	0.31	0.41	0.26	0.28	0.30	0.33	0.25	0.33	0.21	0.19	0.22	0.21	0.05
Potassium (%)	2.07	2.24	2.87	2.14	3.39	3.38	2.72	2.74	3.40	3.61	3.06	2.97	3.17	3.29	3.99	3.84	0.23
Phosphorus (%)	0.23	0.21	0.21	0.18	0.20	0.20	0.22	0.20	0.23	0.16	0.30	0.22	0.19	0.16	0.20	0.17	0.02
Magnesium (%)	0.16	0.20	0.21	0.21	0.22	0.24	0.26	0.30	0.22	0.27	0.13	0.15	0.22	0.23	0.20	0.20	0.03
Sodium (%)	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.04	0.02	0.03	0.04	0.03	0.03	0.03	0.01
Iron (ppm)	3967	344	1037	898	905	498	343	624	522	477	265	717	529	955	574	744	285.9
Zinc (ppm)	21.3	26.0	24.3	25.3	21.7	24.0	19.0	20.0	20.7	16.7	23.0	22.3	21.3	23.0	19.7	22.3	2.46
Copper (ppm)	6.00	7.00	7.67	7.00	7.67	7.00	8.33	8.67	10.33	9.33	9.67	10.33	11.00	10.33	9.33	9.67	0.87
Manganese (ppm)	30.3	41.0	57.7	65.3	50.0	48.7	33.7	46.3	42.0	41.0	24.0	46.0	38.7	51.3	39.3	49.3	11.79
Molybdenum (ppm)	0.15	0.13	0.10	0.00	0.10	0.20	0.27	0.25	0.33	0.27	0.17	0.20	0.20	0.20	0.27	0.43	0.07
Selenium (%)	0.05	0.07	0.10	0.06	0.18	0.12	0.14	0.09	0.09	0.13	0.07	0.07	0.14	0.13	0.07	0.06	0.03
Chloride ion (%)	0.76	0.89	0.96	1.01	1.17	1.38	1.11	1.22	0.88	1.23	0.85	1.02	1.08	1.21	1.12	1.25	0.10

[1]All data are expressed on a dry matter basis.

Discussion

The higher dry matter yields obtained from the irrigated plots indicated that moisture availability was a limiting factor for growth on the non-irrigated plots. The significantly higher DM yields for the newly developed hybrid, PMN3, under irrigated conditions in the study shows its potential for high levels of production when moisture levels are adequate. Although PMN3 had the highest DM yields under irrigated conditions, it was expected that the PMN hybrids would outperform napier grass under non-irrigated conditions due to the high drought tolerance of pearl millet varieties. It is possible that the 268 mm of rainfall during the 110-day trial prevented plant water stress from developing to a stage when significant yield differences would occur. The advantage of the new hybrids over napier varieties when water is limiting might not be as great as anticipated. Future trials will monitor plant water stress to address this issue.

Nyambati et al. (2010) found that mean annual DM yields of Bana grass over 3 harvests were 10.3 t/ha and 22.1 t/ha in the following year over 6 harvests. In comparison, Bana grass yielded 27.1 t/ha under irrigation in this study over only 110 days. The difference is likely due to genotype by environment interaction. The DM yields of both PMN2 and PMN3 were well above the range (15–22 t/ha/yr) reported for napier grass in eastern Africa (Mugerwa and Ogwang 1976; Muia et al. 2001).

Nutritional quality of forage is at least as important as yield in the selection of the best grass variety for cattle. A dairy cow weighing 600 kg would require around 52.6 Mcal/kg of energy from its feed to produce 30 kg of milk with 4.04% of milk fat (Alderman and Cottrill 1993). To meet this requirement, she would need to eat about 17.5 kg DM/d of feed containing 3 Mcal ME/kg DM. Even when good quality forages are fed to cattle, the major limitation for milk production in any pasture-based diet is energy intake. All grasses in this study can provide only 1.7 Mcal ME/kg DM, and a cow would have to eat around 30.9 kg DM/d, equivalent to 128 kg fresh PMN2, 135 kg PMN3, 143 kg 4604 and 168 kg 5344, whereas 172 kg Bana grass and N51 would be required, because of differing DM concentrations. Hence, the ME density of these grasses would be the primary limitation to milk production, if they were the sole component of the diet. Harris et al. (1997) reported that even good quality ryegrass-white clover pasture did not contain sufficient energy for high-yielding dairy cows producing 30 kg milk/cow/d.

Maximum herbage dry matter intake that a grazing cow can consume from pasture is reported to be 2.5−4.2% of live weight (NRC 1988). This means, when the best quality pasture is fed, a cow weighing 600 kg could potentially consume 25 kg DM/d and would meet the ME requirement for more than 30 kg milk/d. With the limited ME concentration in the grasses in this study, a cow could not possibly consume sufficient pasture to give satisfactory milk yields.

Hemicellulose concentration in the grasses in this study was high and ranged from 19% (Mott) to 23% (N51), which is another factor which would limit intake and digestibility of the forage. In spite of higher NDF content, ME value of all the grasses was reasonably high (1.7 Mcal/kg DM); this higher ME is possibly associated with the presence of comparatively higher amounts of hemicellulose (30% of NDF) than in other grasses.

Though there are still unresolved mechanisms, lignin inhibits digestion of plant cell wall components (Morrison 1983). The higher the lignin concentration the lower the digestibility of forage, as lignification is negatively associated with NDF digestibility. Hybrid 4604 had the highest lignin concentration (8.2%), while 5344 had the lowest lignin concentration (5.2%). This might explain why 5344 had the highest in vitro dry matter digestibility of all grasses tested. However, Dehority and Johnson (1961) and Van Soest (1994) indicated that lignin concentration itself may not be the only factor influencing digestibility, as legumes tend to have twice the lignin concentration of grasses at the same digestibility.

Mineral deficiency is an important cause of reproductive failure and low production rates in ruminants. Forages neither contain all the minerals that animals require nor are they present in adequate quantity (Vargas and McDowell 1997). Mean calcium (Ca) concentration of the forage studied ranged from 0.17 to 0.36%, while the recommended Ca requirement for maintenance, growth and lactation in sheep ranges from 0.12 to 0.26% (Reuter and Robinson 1997). Hence, a complete diet of these forages should provide sufficient dietary Ca for sheep. Although ruminants can tolerate calcium:phosphorus ratios of more than 10:1 (Ternouth 1990), the recommended Ca:P ratio should be around 1:1–2:1 (Underwood 1981). All forages in this study had Ca:P ratios falling in this range. Recommended potassium

(K) concentration for maintenance of ruminants is 0.8% (ARC 1980), while the K concentration in the forages studied ranged from 2.15 to 3.91%. Therefore, ruminants grazing on the grasses studied would receive adequate levels of K. However, magnesium (Mg) absorption from the rumen is inhibited by high levels of dietary K (Judson and McFarlane 1998; McDowell 2003). Requirement of Mg decreases with the increase in level of Ca and soluble carbohydrates (Judson and McFarlane 1998). Metabolic requirements of Mg for adult sheep and goats are 1.5 and 1.6%, respectively (NRC 2007). None of the forages studied can meet the Mg requirement. While most grasses are deficient in sodium (Na), both the hybrids and napier grass varieties contain adequate amounts of Na for adult small ruminants. Non-fiber carbohydrate (NFC) concentration was significantly higher in PMN2 both under irrigated and non-irrigated conditions, which were 12.8 and 12.7%, respectively (Table 2). These carbohydrates are digested more rapidly than fiber, yielding weaker acids as by-products. Volatile fatty acids, primarily propionate, are produced from the fermentation of NFCs, which are absorbed from the rumen and are also used as a source of energy by ruminant animals. NFCs are also used by rumen microbes to make microbial protein, which in turn is utilized by animals as a protein source.

As the rate of digestion of PMN2 was slow, only 2.3% being digested per hour, feed intake would be limited due to rumen fill, whereas a faster rate of digestion in 5344 and Bana would result in higher intake and greater levels of production, if adequate feed is available to satisfy appetite.

The neutral detergent fiber digestibility (NDFD) was highest in 5344 and Bana grass, which again favors these grasses as fodder sources for livestock. The more digestible the NDF in pasture the more energy is available to the animal. The lower digestibility of NDF in PMN2 would be a contributing factor to the low rates of digestion in this hybrid.

Overall, the low crude protein, high fiber and low energy density of these grasses at this age would not sustain growing beef animals and would not meet the needs of cows in late stages of pregnancy and early lactation, or growing and lactating dairy animals, especially if fed in stalls, as the sole diet. However, under grazing conditions, animals are able to select a higher quality diet than the total on offer and would produce at a higher level.

Conclusion

This study suggests that the hybrids tested have some advantages over the napier grass varieties tested. While yield differences were small under rainfed conditions, only 2 hybrids (PMN2 and PMN3) and napier variety MB4 showed significant responses when irrigation was provided. This suggests that the main advantages of these hybrids might be seen in higher rainfall years or when irrigation is available. In terms of DM yield, PMN3 showed outstanding potential. While hybrid 5344 produced lower DM yields than PMN3, it had a much higher digestibility and displayed more rapid digestion than the other hybrids and varieties except Bana. Low IVDMD, low NDFD and slow rate of digestion are limiting factors in the use of PMN2.

PMN3 exhibited a positive combination of high biomass yield, favorable lignocellulosic content, good digestibility and high nutritional value. This hybrid appears to have definite potential for both cattle feeding (as forage) and as a source of bioenergy. For the growing conditions experienced in this study, hybrids PMN3 and 5344 seem preferable to napier grass varieties for forage production for ruminant feeding systems, even under rainfed farming conditions. Additional research is required to obtain detailed information on the long-term performance of PMN grasses, as well as their chemical composition and biomass yield at different cutting intervals and at different locations. Feeding studies would provide data on the palatability of these grasses and performance of stock consuming the forage.

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Short Communication

Neglected grass species of Southern Africa: Nutritive value of conserved *Hyperthelia dissoluta* harvested at different growth stages

Gramíneas descuidadas del sur de África: Valor nutritivo del forraje conservado de Hyperthelia dissoluta *cosechado en diferentes estados de crecimiento*

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Abstract

Native species like *Hyperthelia dissoluta* have great potential in livestock production but not much has been done to improve their contribution to that sector. This study examined 2 conservation methods (drying and ensiling) and 3 different growth stages, namely: elongation stage (January), early flowering (February) and late flowering stage (March) of *H. dissoluta* in terms of nutritional composition and digestibility. The method of conservation had a significant effect (P<0.05) on nutritive value, with silage having more P and CP than hay. Stage of growth had an effect (P<0.05) on all nutritional properties of both hay and silage: Phosphorus, Ca and CP concentrations and digestibility of hay and silage decreased with maturity, while NDF and ADF concentrations increased. Silage pH value was significantly higher at elongation (5.2) and late flowering growth stages (5.7) than at early flowering (4.4). Dry matter digestibility of the conserved material reached levels as high as 82% for silage made at the elongation stage with all values at least 60%. We conclude that *H. dissoluta* can be conserved as both silage and hay to produce a good quality feed. Harvesting at the early flowering stage would seem to provide a good compromise between quantity (not measured in this study) and quality of harvested forage. Further studies seem warranted to determine the acceptability and intake of the material by livestock, the advantages of adding fermentable carbohydrates during ensiling and DM yields in different areas and a range of seasonal conditions.

Keywords: Air drying, hay, perennial native grasses, plastic bag silo, quality silage.

Resumen

Especies nativas como *Hyperthelia dissoluta* tienen un gran potencial para la producción pecuaria, pero se ha trabajado poco para mejorar su contribución. En el presente estudio, conducido en Simbabwe, se evaluó el efecto de 2 métodos de conservación (henificación y ensilado) y 3 etapas de crecimiento: elongación (enero), floración inicial (febrero) y floración final (marzo), en la composición nutricional y la digestibilidad de *H. dissoluta.* El método de conservación tuvo un efecto (P<0.05) en el valor nutritivo, resultando en el ensilaje concentraciones de fósforo (P) y proteína cruda (PC) más altas que en el heno (0.63–1.69 y 4.5–8.2%, respectivamente, vs. 0.16–0.66 y 2.3–6.5%). La etapa de

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crecimiento tuvo un efecto (P<0.05) en todos los componentes nutritivos tanto del heno como del ensilaje: las concentraciones de P, calcio y PC y la digestibilidad disminuyeron con la madurez, mientras que las concentraciones de fibra (FDA y FDN) incrementaron. El pH del ensilaje fue significativamente más alto en la etapa de elongación y al final de la floración (5.2 y 5.7, respectivamente) que en la etapa inicial de la floración (4.4). La digestibilidad de la materia seca (MS) del forraje conservado presentó niveles elevados (60% y más), alcanzando 82% para ensilaje en la etapa de elongación. Concluimos que es posible producir de *H. dissoluta* tanto un heno como un ensilaje de buena calidad. Cosechando el pasto en la etapa de floración inicial parece ser un buen compromiso entre la cantidad del forraje (no determinada en este estudio) y su calidad. Se sugieren estudios para evaluar la aceptabilidad y el consumo del forraje conservado por el ganado, estudiar posibles ventajas de la adición de carbohidratos fermentables durante el ensilado y determinar los rendimientos de MS en diferentes áreas y condiciones estacionales.

Palabras clave: Calidad, ensilaje, gramíneas perennes nativas, heno, secado, silo de bolsas plásticas.

Introduction

Feed shortage, both in quantity and quality, is the principal constraint to livestock production in subtropical Africa (Smith 2002; Gusha et al. 2014). Here, almost every smallholder farmer faces this challenge during the annual dry period of 7–9 months (Mapiye et al. 2006; Ngongoni et al. 2006). The quality of forage generally declines as plants mature and become more fibrous and crude protein levels fall to as low as 2% dry matter (Smith 2002), resulting in accumulation of poor quality biomass, which is lowly digestible and low in nutrients (Ball et al. 2001). The biomass is either consumed by veld fire during the dry season or breaks down during the following rainy season. This results in low productivity, long calving intervals and high livestock mortality (Lukuyu et al. 2011). However, we consider that there are some native grass species, which have been neglected in terms of looking for solutions to these problems but which, because of their adaptation to local climatic conditions, may have the potential to modify the perennial feed deficit problems.

Hyperthelia dissoluta (Figure 1), also known as yellow thatching grass or yellow tambookie grass, is a perennial grass that can reach heights of 1–3 m (Burkill 1985) and can produce average yields of 26 t DM/ha (Skerman and Riveros 1990; Heuzé et al. 2012). It prefers sandy soils and is usually found in disturbed areas, savannas, field margins, fixed dunes, velds, roadsides and deciduous bushland (Heuzé et al. 2012). It is tolerant of fire and in trials in Zambia over 3 years, fire actually increased the population of *H. dissoluta* (Kativu 2011). It requires annual rainfall in excess of 425 mm and grows well from low lying areas to above 3,000 masl (Skerman and Riveros 1990; Heuzé et al. 2012). It is dispersed throughout tropical Africa, Southern Africa and Madagascar, and has spread to tropical America (Burkill 1985). Despite its positive attributes, *H. dissoluta* has been neglected and not fully utilized.

In order to improve livestock productivity we consider the use of native high-producing veld grasses is imperative. Harvesting should be done when pastures are at a vegetative stage and still nutritious and forage is conserved as hay or silage for use during times of deficit. Traditionally, exotic fodder species and cereals have been planted to produce hay and silage; some are in direct competition with humans for land to produce foodstuffs for human consumption (Gusha et al. 2014).

We decided to evaluate the potential of adapted native grass species, using *Hyperthelia dissoluta* as an example, as conserved forage for dry season feeding. The study aimed to identify the most suitable harvesting and conservation strategies to produce high quality herbage for livestock feeding.

Figure 1. Mature *Hyperthelia dissoluta*.

Materials and Methods

Study site

The study was conducted at Henderson Research Institute, 30 km north of Harare (17°35' S, 30°58' E; 1,300 masl). The area receives an average rainfall of 870 mm annually (www.drss.com), which falls mainly between November and late March. The vegetation consisted mainly of tree savanna or bush clump savanna with tall perennial grasses such as *Hyperthelia dissoluta* and *Hyparrhenia filipendula* on red clay soils. However, during the year of study, rainfall pattern and amount were below average (555 mm total, mostly late in the season; Table 1) and crop failures were experienced, so biomass produced was probably well below normal and is not considered in this study.

Silage and hay preparation

The experimental design was a 2 x 3 factorial. There were 2 methods of conservation of the grass (either ensiled or dried as hay) and 3 growth stages [elongation stage in January (early growth stage), early flowering stage in February (middle growth stage) and late reproductive stage in March (late growth stage)]. At each stage of harvest 60 kg per site of *H. dissoluta* was harvested by cutting with a sickle at 5 cm above the ground from 3 different sites within the farm. The harvested *H. dissoluta* forage was transported to the bio-assay laboratory at the University of Zimbabwe and chopped into approximately 3 cm pieces manually using machetes. Ten kilogram samples of the chopped herbage were placed in polythene bags with a thickness of 150 microns. The contents of the bags were compacted and the bags compressed to remove air, tied to prevent the entry of air and inserted into another polythene bag. Three samples were prepared at each site and each stage of growth. The bags were left to ferment for 3 weeks at room temperature of about 25 ºC. The other 30 kg herbage at each site was divided into three 10 kg heaps, which were spread and dried under shade for 7 days, when the dried material was stored in hessian bags at room temperature. A total of 9 bags of silage and 9 heaps of hay were made at each harvesting stage and were used as replicates in the study.

Nutrient chemical composition analysis

Representative samples were collected from each treatment bag at day 22 from the day of making and were oven-dried for 48 h at 60 ºC. The samples were then ground through a 1 mm sieve and analyzed for nitrogen (N), dry matter (DM), acid detergent insoluble nitrogen (ADIN) and ash according to AOAC (2000). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined using the method of Goering and Van Soest (1970). Phosphorus (P) and calcium (Ca) concentrations were determined by the spectrophotometer method (Danovaro 2009) and the EDTA method (Kaur 2007), respectively. Digestibility was determined according to Tilley and Terry (1963) and silage pH using a digital pH meter.

Table 1. Rainfall received during the period of study (2014/2015 season) and medium-term (2005–2015) rainfall at the study site.

Month	Rainfall (mm) during study period (2014/2015 season)	Rainfall (mm) during 2005–2015
October	0	87
November	0	168
December	138	187
January	108	109
February	109	88
March	88	170
April	112	67
May	0	0
June	0	0
July	0	0
August	0	0
September	0	0
Total	555	876

Statistical analysis

Analysis of variance was carried out using the Proc GLM procedure (SAS 2010). The following model was fitted:

$$y_{ij} = \mu + \alpha_j + \beta_k + (\alpha\beta_{jk}) + \varepsilon_{ij}$$

where:

y_{ij} = digestibility and nutritive content;
μ = the overall mean common to all observations;
α_j = effect of growth stage;
β_k = effect of conservation method;
$(\alpha\beta_{jk})$ = effect of the interaction of factors A and B; and
ε_{ij} = the residual error.

Results

Silage and hay harvested during the elongation stage of growth had the highest CP concentrations of 8.2 and 6.5% (DM basis), respectively (Table 1). Crude protein

Table 2. Nutrient composition (% DM) for hay and silage of *Hyperthelia dissoluta* conserved at 3 different harvest stages (early, middle and late, in summer 2014/2015).

Parameter	Conservation method						s.e.
	Hay			Silage			
	Early	Middle	Late	Early	Middle	Late	
DM	90.1a	90.2a	91.4a	25.9d	33.7c	38.0b	0.59
Ash	9.3a	6.4d	6.0d	8.4b	7.6c	6.2d	0.26
P	0.66b	0.19c	0.16c	1.69a	0.63b	0.64b	0.096
Ca	0.02b	0.02b	0.01c	0.03a	0.02b	0.01c	0.003
NDF	64.3e	68.5d	79.6a	54.9f	72.5c	75.9b	1.41
ADF	41.7d	49.5b	54.6a	42.2d	49.0bc	47.8c	0.71
ADIN	0.26a	0.26a	0.31a	0.18a	0.30a	0.28a	0.022
CP	6.5c	5.1d	2.3f	8.2a	7.6b	4.5e	0.19
Dig	73.1b	73.0b	59.5d	81.9a	75.3b	64.4c	2.59

Early = elongation stage; middle = early reproductive stage; late = late reproductive stage; DM = dry matter; P = phosphorus; Ca = calcium; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADIN = acid detergent insoluble nitrogen; CP = crude protein; and Dig = DM digestibility coefficient. Means within rows followed by different letters differ ($P<0.05$).

concentrations declined progressively with later harvesting to 4.5% for silage and 2.3% for hay at the late reproductive stage. Silage pH values (not reported in Table 2) were 5.2 at the elongation stage, 4.4 at the early reproductive stage and 5.9 at the late reproductive stage. Corresponding DM concentrations of the silages were 25.9, 33.7 and 38.0%, respectively. Phosphorus concentration in conserved forage declined significantly with age and at all stages was higher for silage than for hay (Table 1). Apparent digestibility of dry matter declined significantly as harvesting stage was delayed for both methods of conservation and was generally higher for silage than for hay.

Discussion

Though yield measurements were not taken because of unusual rainfall conditions and the native pastures, where *H. dissoluta* was sampled, presented a high variability of species mixtures, it was evident that biomass on offer increased, as could be expected, from the elongation growth stage to the early reproductive stage by an estimated 100% and from there to the late reproductive stage by an estimated 20%.

This study has produced some interesting information on the quality of hay and silage made from *H. dissoluta* at different stages of growth. The most striking finding was the high apparent in vitro DM digestibility of the conserved material. The highest digestibility coefficient obtained was 81.9% for silage made at the elongation stage of growth, while the lowest was 59.5% for hay made at late flowering. These values were much higher than the value of 56.5% reported by Heuzé et al. (2012). They are outstanding for a tropical grass and strongly suggest that this particular species warrants further study as a potential source of forage for livestock.

As would be expected, quality, in terms of CP and fiber concentrations and DM digestibility coefficients of the conserved material, declined as harvesting was delayed from the elongation stage to the late flowering stage. Moore et al. (1991) reported that at late maturity forages become lignified with reduced digestibility.

Neutral detergent fiber (NDF) represents the cell wall portion of a feed, comprising ADF and hemicellulose and affects feed intake, with intake declining as NDF concentration in feed increases (Bosworth and Hudson 2005). The desirable level of NDF in feedstuffs is <55% (Mertens 2009). In our study all treatments had values above this, which should depress intake. It would be expected, however, that intake of forage with DM digestibility in the range 60–82% would be quite acceptable, suggesting that feeding studies with animals to test acceptability of the material, possible intake levels and in vivo digestibility are needed.

Silage is preserved by lowering the pH through lactic acid formation. For grass silages the recommended pH for a well preserved silage is between 4.2 and 4.7 (Pyatt and Berger 2000; Bosworth 2005). The pH values for elongation and late flowering growth stages were above these recommended levels for grass silage. This could be related to low soluble sugars during the elongation stage of growth (Abia et al. 2006) combined with high moisture levels in the fast-growing crop, while low moisture levels at late flowering could have prevented adequate compaction to remove air effectively (Ball et al. 2001). Wilting of material with high moisture levels prior to ensiling can improve the quality of the resulting silage. The benefits from adding a source of readily available carbohydrate, e.g. molasses or maize meal, should be investigated. Pyatt and Berger (2000) reported that forages with a DM above 35% at ensiling, like the material ensiled at late flowering, have less efficient fermentation because they are difficult to compact.

While the decline in CP and P concentrations in conserved material with delay in harvesting time were to be expected, the consistently higher CP and P concentrations in silage compared with hay were surprising. MacDonald and Clark (1987) reported results from 8 separate studies, showing there is an average of 34% CP loss during the drying process. Thus in our study the lower CP levels in hay could be associated with leaf loss and weathering damage during drying.

Method of conservation had no significant effect at $P<0.05$ on the Ca levels but growth stage at ensiling had a significant effect in silage with a trend of increasing Ca concentration with maturity (Urrutia et al. 2011). The Ca concentration in both hay and silage was below the minimum requirement for all classes of livestock (Fox et al. 1988) and if hay made from *H. dissoluta* is to be fed as a complete ration, a Ca supplement like limestone flour should be fed (Fox et al. 1988). Silage contained adequate levels of P for livestock feeding but hay made at early or late flowering had insufficient levels of P, especially for lactating females (Coates and Ternouth 1992), and supplements may be necessary if this material constitutes a major part of the diet.

The CP concentrations in hay were generally lower than the levels found in previous studies of 6.4% in late summer (Heuzé et al. 2012). If these conserved fodders were to be fed as a major part of the ration for livestock, especially lactating females, a protein supplement would need to be added to the ration. Acid detergent insoluble nitrogen (ADIN) is an indicator of the quantity of N that is indigestible in the rumen and intestines, and reflects the quantity of heat-damaged silage or hay (Dilley et al. 2013). In this study ADIN concentration was similar for both methods of conservation and stages of harvest and the quantity of ADIN was less than 12% of the total N in the forages, indicating that little heat damage took place (Seglar 2003).

Conclusion

We conclude that *H. dissoluta* has potential to produce good quality forage, which could be conserved as silage or hay for dry season feeding of livestock. Timing of harvest would depend on whether quantity or quality of conserved material was more important. Whether hay or silage was made would depend to some extent on weather conditions during the optimal time of harvesting and the ability to reliably dry hay. More research is needed to determine if quality of *H. dissoluta* silage can be improved by incorporating additives containing fermentable carbohydrates, e.g. molasses or maize meal, at ensiling. Further studies seem warranted to confirm that the high in vitro digestibility levels recorded in this study can be repeated with animals and to determine acceptability and intake with and without N and P supplements. Dry matter yields of this forage during a range of seasons and on a range of soil types would provide evidence whether satisfactory DM yields can be obtained in a range of situations.

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