https://doi.org/10.22319/rmcp.v11i1.4565

Article

Forage yield and nutritional quality in *Leucaena leucocephala* and *Megathyrsus maximus cv.* Tanzania in an intensive silvopastoral system

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Abstract:

The biomass and nutritional value of *Leucaena leucocephala cv*. Cunningham (5,000 trees ha^{-1}) and *Megathyrsus maximus cv*. Tanzania was assessed in a silvopastoral system in a tropical hot sub-humid climate in the rainy and dry seasons. Samplings were done from August-October 2014 (rainy) and March-April 2015 (dry). The treatments were harvests at 20-, 30-, 40- and 50-d intervals in both seasons, randomly assigned to twelve (24 m^2) paddocks. The grass accounted for most of the total available forage in the silvopastoral system (80 *vs* 20 %). In both seasons, the association yielded more forage at the 50-d interval (5,300 kg DM ha^{-1} in the rainy season and 1,620 kg DM ha^{-1} in the dry season) (P<0.05). During the rainy season, crude protein (CP) in the tree forage was higher at the 50-d interval (22 %; *P*<0.05), but did not change over the intervals during the dry season (28 %; *P*>0.05); neutral detergent fiber (NDF) did not change over time (44 %; *P*>0.05) and acid detergent fiber (ADF) increased in the 50-d interval (25 %, *P*<0.05) but *in vitro* dry matter digestibility (IVDMD) decreased (49 %; *P*<0.05), regardless of season. Crude protein (10%; *P*<0.001) and IVDMD (58 %; *P*<0.03) of *M. maximus* remained high from the 20- to 40-d intervals, although in both seasons NDF and ADF fractions significantly increased after the 40-d interval. The evaluated silvopastoral system attains highest yield between the 40- and 50-d intervals. The highest nutritional quality of *M. maximus* was at 40 ds, after which the nutritional quality of *L. leucocephala* may compensate for the lost nutritional quality of the grass, regardless of season.

Key words: Tree-grass association, Silvopastoral system, Nutritional quality, Season.

Received: 30/07/2017

Accepted:07/01/2019

Introduction

Climatic conditions in the tropical regions of Mexico favor livestock forage production. However, the favorable temperatures and rainfall of the summer/fall rainy season can become limiting during the winter/spring dry season[1]. The low temperatures and high cloudiness common during the transition from rainy to dry seasons can decrease growth, and the absence of precipitation in the spring can slow growth in grasses, resulting in poor forage quality and shortages in quantity[2,3].

Associating grasses and forage trees in silvopastoral systems can extend forage availability year-round while improving forage chemical-nutritional quality[4,5]. Different associations of grasses and forage trees can be more productive than grass-only pastures[6,7], and lengthen forage availability, even in conditions of seasonal precipitation[8]. Moreover, forage nutritional quality (tree foliage plus grasses) is superior to grass monocultures[5,9]. Grass/forage tree associations can therefore increase total forage production and improve the quality of livestock diets[10,11].

Obtaining optimum forage yield and quality requires evaluation of possible forage tree/grass combinations to ensure they are viable, convenient and manageable. *Megathyrsus maximus* cv. Tanzania is widely accepted by producers because of its high production capacity, 10 to 14 % protein content, good digestibility (up to 60-70 %), acceptance by livestock, and adaptability to diverse edaphic and climatic conditions[12,13]. In the state of Veracruz, Mexico, this cultivar exhibits high forage production (up to 8,317 kg DM ha^{-1} at a 42-d interval in the rainy season; up to 1,027 kg DM ha^{-1} at a 35-d interval in the dry season)[14]. The productive potential of this forage grass can be complemented by forage trees grown in rows and high densities[15]. The legume tree *Leucaena leucocephala* is the most widely used tree species in silvopastoral systems because it contributes to improving cattle diet quality[16] and increasing the amount of forage available in silvopastoral systems. When managed properly it conserves its green leaves in the dry season (March-June) and becomes the most important forage source. Moreover, it provides better nutritional quality than grasses during this season, when both forage availability and crude protein content are lower[17,18]. It also positively affects biomass production capacity and the chemical composition of the grasses with which it is associated[9,16].

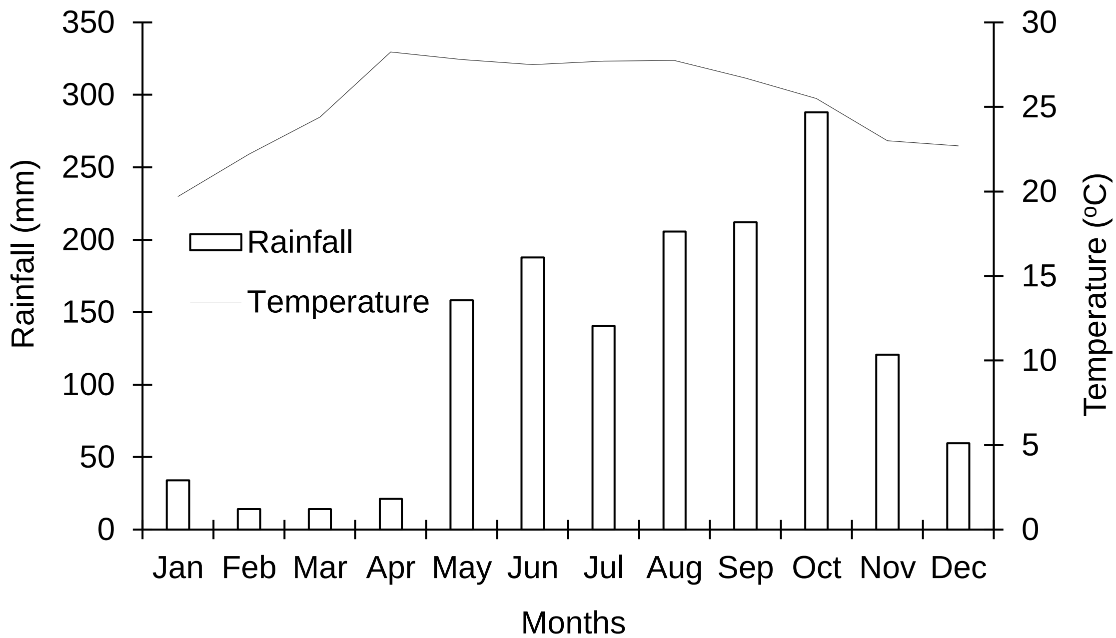
The grasses and trees in silvopastoral systems have different growth habits[19]. These determine their regrowth and forage production capacities over time[20] and need to be considered in management plans[21]. In *Leucaena* sp.-*M. maximus* (Tanzania) associations, rest intervals are recommended that allow the trees to recover without negatively effecting the grass. This means implementing intervals apt for both species. These cannot be overly long because the grass matures more rapidly and its nutritional quality changes apace[13], but *Leucaena* sp. requires more time to recover than grasses[22]. Season can also affect the recovery rate of each component. The present study objective was to determine the rest interval resulting in the highest forage production and best forage nutritional quality in a silvopastoral system containing *L. leucocephala* and *M. maximus*, in the rainy and dry seasons, in a warm weather climate under a seasonal rainfall regime.

Material and methods

Experimental site location and characteristics

The experiment was done in Juan Rodríguez Clara Municipality, in the state of Veracruz, Mexico (18°00′11″ - 17°59′5″ N; 95°16′29″ - 95°16′30″ W). Located at 107 m asl, regional climate is warm sub-humid with summer rains (AW_2), with the highest mean temperature (28 °C) in April and the lowest (20 °C) in January[23]. During the study period rainfall was highest (235 mm/month) from August to October and lowest (28 mm/month) from December to April[24] (Figure 1).

Figure 1: Study region mean monthly rainfall (mm) and temperature (°C) from 2014-2015



Experimental site and parcels

The experimental site was a 0.5 ha pasture planted in 2011 with a silvopastoral system constituting the grass *M. maximus* cv. Tanzania and the legume tree *L. leucocephala* cv. Cunningham. The grass was propagated via vegetative material and the trees by seed. The latter were planted at a 5,000 plants ha^{-1} density in rows 2.0 m apart with 1.0 m between plants. Prior to the experiment, the pasture had been grazed-browsed starting twelve months after planting using traditional management practices. It was grazed for 3 to 4 h a day after milking for approximately seven continuous days with irregular rest periods (>25 d) and an animal load of 20 to 27 AU. Within the site a 288 m^2 experimental area was marked off and divided into twelve 24 m^2 (6 x 4 m) plots, each of which was an experimental unit.

Soil physicochemical composition

Soil samples were collected at the experimental site using a zig-zag pattern. Eight samples were taken at a depth of 30 cm and these pooled to form a composite sample for physical and chemical composition analysis[25]. The analyses were performed in the Soil, Water and Plants Laboratory of the Postgraduate College (COLPOS), Veracruz Campus following established methods[25]. Soils in the pasture were a sandy loam containing 64 % sand, 17 % clay and 19 % silt (Bouyoucos method, AS-09). The pH was slightly acidic (6.6, electrometric method, AS-02), and organic matter content was low (0.15 %) (Walkley and Black method, AS-07). The chemical composition included 100 mg L^{-1} nitrates (cadmium method), 70 mg L^{-1} ammonium (Nessler method), 108 mg L^{-1} potassium (Turbidimeter method) and 27 mg L^{-1} phosphorus (Amino acid method). Soil electrical conductivity at the site was 45 dS m^{-1} as estimated with the saturation extract method[26].

Treatments and experimental design

The treatments consisted of four post-harvest rest intervals (20, 30, 40 and 50 d) randomly assigned to each of the twelve experimental plots, with three replicates per treatment. Two biomass evaluations were done, the first from August 22 to September 21, 2014 (rainy season) and the second from May 23 to April 22, 2015 (dry season).

Experimental procedure

The experiment was carried out from August 2014 to April 2015. In both periods an initial cut of the aerial biomass was made to standardize forage plant height: *M. maximus* was cut at 20 cm above ground level and *L. leucocephala* was pruned at 1.0 m height. Pruning consisted of cutting the main and/or most woody branches[27]. After the initial cut successive cuts were done according to the rest intervals defined for each treatment.

Variables

Total available biomass of the trees and grass, and forage nutritional quality at each interval were quantified. For each sampling period (rainy/dry season), four sampling points (2 x 1 m rectangles) were randomly assigned inside each of the three plots (replicates) of each treatment. Within each rectangle all new growth foliage (leaves and tender stems) on the trees was harvested as a means of simulating animal browsing, and simultaneously all grass green matter was harvested at 20 cm above ground level[12,28]. From each sampling point two sub-samples of green biomass were taken: one to quantify dry matter and the other for nutritional quality analyses. Both grass and tree foliage samples were dried in a forced air oven at 60 °C for 48 h.

Grass and tree foliage nutritional quality were assessed separately. Crude protein (CP) content was quantified with the Microkjeldahl method[29], neutral (NDF) and acid detergent fiber (ADF) with the filter bag technique (ANKOM2000; Ankom Technology, NY, USA) and *in vitro* dry matter digestibility (IVDMD) with the ANKOM Daisy incubator using Model F57 bags (ANKOM Technologies, Macedon, NY, USA)[30,31]. Analyses were performed at the Animal Nutrition Laboratory at the COLPOS Montecillo Campus.

Statistical analysis

Variables for biomass (total, grass and tree) and nutritional quality (CP, NDF, ADF and IVDMD), were analyzed with a completely randomized design using a 4 x 2 factorial arrangement: four pasture rest periods and two seasons. The model included the effects of treatment (intervals 20, 30, 40 and 50 d), season (rainy and dry) and the season/interval interaction. The analyses were run with the GLM (Generalized Linear Model) procedure of the SAS statistical package[32]. When statistical differences (P<0.05) were identified between treatments the LSMeans (Least Square Means) method was used to compare average biomass and nutritional quality for the grass and tree foliage.

Results

Forage biomass

Total forage biomass (*M. maximus* + *L. leucocephala*) differed (P<0.001) in response to the season/interval interaction (Table 1). In the rainy season production was highest at 50 d (P<0.05), lowest at 20 d (P<0.05) and did not differ between 30 and 40 ds. In the dry season the highest yields were at 40 and 50 d, which did not differ (P>0.05), production was moderate at 30 d, which did differ from 40 d (P<0.05), and was lowest at 20 d, although this did not differ from 30 d (P>0.05).

Table 1: Forage biomass for *Megathyrsus maximus cv.* Tanzania, *Leucaena leucocephala cv.* Cunningham and total biomass (*M. maximus* + *L. leucocephala*; kg DM ha^{-1}) in a silvopastoral system at 20-, 30-, 40- and 50-d rest intervals in the rainy and dry seasons

	Rainy			Dry		
Rest Intervals	Mm	Ll	Total	Mm	Ll	Total
20	1140 ± 240^{c}	60 ± 20^{c}	1200 ± 24^{c}	330 ± 250^{c}	40 ± 20^{a}	370 ± 240^{c}
30	2270 ± 240^{b}	100 ± 20^{c}	2370 ± 24^{b}	680 ± 240^{bc}	20 ± 20^{a}	700 ± 240b^{c}
40	2330 ± 260^{b}	300 ± 20^{a}	2630 ± 26^{b}	1090 ± 240^{ab}	30 ± 20^{a}	1120 ± 230^{ab}
50	5110 ± 240^{a}	190 ± 20^{b}	5300 ± 24^{a}	1580 ± 240^{a}	30 ± 20^{a}	1610 ± 230^{a}

Mm = *M. maximus*; Ll = *L. leucocephala*.

[abc] Different letter superscripts in the same column indicate significant difference (*P*< 0.05).

The individual forage contributions of *M. maximus* and *L. leucocephala* also differed in response to the season/interval interaction (*P*<0.001). In the rainy season the amount of forage produced by both species increased as the interval lengthened. Between the 20- and 50-d intervals biomass for *M. maximus* increased from 1,140 to 5,110 kg DM ha^{-1} and that of *L. leucocephala* from 60 to 190 kg DM ha^{-1}. Between the same intervals in the dry season *M. maximus* availability increased from 330 to 1,580 kg DM ha^{-1}, whereas *L. leucocephala* biomass remained low (around 30 kg DM ha^{-1} at all intervals) (*P*>0.05) (Table 1). The grass (*M. maximus*) contributed more biomass than the legume tree (*L. leucocephala*) in both seasons: 96.3 vs. 88.6% in the rainy season; 97.7 vs. 88.0% in the dry season.

Biomass chemical-nutritional quality

Tree foliage CP contents differed in response to the season/interval interaction (*P*<0.05). In the rainy season CP content ranged from 22 to 29 % with the highest content at the 20-d interval (*P*<0.05) (Table 2). In contrast, during the dry season CP did not vary between intervals (*P*>0.05), but was higher overall than in the rainy season. This interaction had no effect on any other tree nutritional quality variable (*P*>0.05).

Table 2: Crude protein content (%) of *Leucaena leucocephala cv.* Cunningham foliage in a silvopastoral system in association with *M. maximus* cv. Tanzania at 20-, 30-, 40- and 50-d intervals in the rainy and dry seasons

Intervals	Rainy Season	Dry Season
20	29 ± 1.2^{a}	29 ± 1.2^{a}
30	23 ± 1.2^{b}	26 ± 1.2^{a}
40	23 ± 1.2^{b}	30 ± 1.2^{a}
50	22 ± 1.2^{b}	28 ± 1.2^{a}

[ab] Different letter superscripts in the same column indicate significant difference (*P*<0.05).

Neutral detergent fiber content (NDF) in the *L. leucocephala* foliage did not differ (*P*>0.91) between intervals (Table 3). In contrast, ADF was lowest at 20 ds (*P*<0.03), and higher (*P*<0.05) but not different among the remaining intervals (*P*>0.05). Foliage digestibility (IVDMD) was lowest at 50 d (*P*<0.05), while the remaining intervals were all higher by two to three percentage points (*P*<0.03).

Table 3: Neutral detergent fiber, acid detergent fiber and *in vitro* dry matter digestibility of *Leucaena leucocephala cv.* Cunningham foliage in a silvopastoral system in association with *M. maximus* cv. Tanzania at 20-, 30-, 40- and 50-d intervals

Intervals	NDF (%)	ADF (%)	IVDMD (%)
20	45 ± 1.0^{a}	21 ± 0.8^{b}	52 ± 0.7^{a}
30	44 ± 1.0^{a}	24 ± 0.8^{a}	52 ± 0.7^{a}
40	45 ± 1.0^{a}	23 ± 0.8^{ab}	51 ± 0.7^{ab}
50	45 ± 1.0^{a}	25 ± 0.8^{a}	49 ± 0.7^{b}

NDF= neutral detergent fiber; ADF= acid detergent fiber; IVDMD= *in vitro* dry matter digestibility.
[ab] Different letter superscripts in the same column indicate significant difference (*P*<0.05).

Overall CP and IVDMD values decreased in *M. maximus* with each subsequent harvest. Crude protein content (CP) varied between the intervals (*P*<0.01), with the highest value at 20 d (*P*<0.05), a slightly lower content at 30 and 40 d (*P*>0.05) and the lowest content at 50 d (*P*<0.05) (Table 4). Digestibility also varied between intervals (*P*<0.03) following a trend like that of CP, with the highest values at 20 d and the lowest at 50 d (*P*<0.05).

Table 4: Crude protein (%) and *in vitro* dry matter digestibility (%) of *Megathyrsus maximus cv.* Tanzania in a silvopastoral system in association with *Leucaena leucocephala cv.* Cunningham, at 20,- 30-, 40- and 50-d intervals in the rainy and dry seasons

Intervals	CP	IVDMD
20	12 ± 0.5[a]	60 ± 1.3 [a]
30	10 ± 0.5[b]	58 ± 1.3 [a]
40	10 ± 0.5[b]	58 ± 1.3 [a]
50	7 ± 0.5[c]	54 ± 1.3 [b]

CP= crude protein; IVDMD= *in vitro* dry matter digestibility.

[abc] Different letter superscripts in the same column indicate significant difference (P<0.05).

The season/interval interaction affected both NDF (P<0.002) and ADF (P<0.001) in *M. maximus*. During the rainy season NDF accumulation did not differ between the 20-, 30- and 40-d intervals (P>0.05), which were lower overall than at 50 d (P<0.05) (Table 5). In the dry season, NDF was highest at 40 and 50 d, followed by contents at 30 ds and 20 ds (P<0.05). The same trend held for ADF, with the highest contents (P>0.05) at 40 and 50 ds (no difference between them, P>0.05) and the lowest at 20 and 30 d (P<0.05).

Table 5: Neutral detergent fiber (%) and acid detergent fiber (%) of *Megathyrsus maximus cv.* Tanzania in a silvopastoral system in association with *Leucaena leucocephala cv.* Cunningham, at 20-, 30-, 40- and 50-d intervals in the rainy and dry seasons

	Rainy Season		Dry Season	
Intervals	NDF	ADF	NDF	ADF
20	69 ± 0.8[b]	37 ± 0.7[b]	62 ± 0.8[c]	28 ± 0.7[c]
30	68 ± 0.8[b]	38 ± 0.7[b]	66 ± 0.8[b]	32 ± 0.7[b]
40	69 ± 0.8[b]	39 ± 0.7[b]	70 ± 0.8[a]	36 ± 0.7[a]
50	74 ± 0.8[a]	44 ± 0.7[a]	71 ± 0.8[a]	36 ± 0.7[a]

NDF= neutral detergent fiber; ADF= acid detergent fiber.

[abc] Different letter superscripts in the same column indicate significant difference (P<0.05).

Discussion

Total biomass remained higher at the longest cutting interval (50 ds) in both seasons. This increase can be associated with the longer recovery time allowed both the tree and grass species, which allowed them to accumulate more root and stem reserves(33,34) and therefore exhibit more vigorous regrowth. However, the overall contribution of *L. leucocephala* biomass was lower than that of *M. maximus*, most probably due to the lower density of the legume tree (5,000 plants ha^{-1}) in the evaluated silvopastoral system(6). At densities of up to 35,000 trees ha^{-1} *L. leucocephala* is reported make a larger contribution to total forage biomass(7).

The season/interval interaction affected yield in both *L. leucocephala* and *M. maximus* because growth conditions for these species differ between seasons. This coincides with previous studies carried out in seasonal precipitation conditions(12), which show that the climatic conditions (temperature and precipitation) during the rainy season in the present study favored forage production in these species(35,36). For instance, in the rainy season the 50-d harvest was made at 25 °C and 287 mm accumulated rainfall, both of which favor growth in *L. leucocephala* and *M. maximus*. During the dry season, by contrast, low rainfall (21 mm) and higher temperatures (28 °C) prevented the plants from fully expressing their production potential. Moreover, when a silvopastoral system depends on moisture from rainfall, some rest intervals may coincide with periods of more homogeneous moisture availability than others; this was the case at the 40-d interval in which production values were similar to those at 30 d in both seasons(37,38).

Of note is that growth in *L. leucocephala* varied minimally during the dry season, exhibiting similar behavior among intervals. In part this is because moisture levels are more homogeneous over time in this season, changing little between intervals. In addition the response of *L. leucocephala* differs from that of grasses (the growth of which varies widely in the dry season), possibly because they have different growth habits and survival strategies(39,40). This allows legume trees like *L. leucocephala* to explore deeper soil layers in search of water and other resources.

Agroecological conditions (e.g. soil type and climate) can vary widely between regions and management strategies must respond in kind. This makes direct comparisons between the present results and those from other regions a challenging prospect(1,41). Total forage biomass in the present study at 50 ds in the rainy season (5,300 kg DM ha^{-1}) was comparable to the 4,350 kg at a 42-d interval reported for a *Cynodon nlemfuensis*-*L. leucocephala* association in the rainy season(42). However, it is lower than the 7,080 kg DM ha^{-1} at a 40-d interval in the rainy season reported for an association of *L. leucocephala* with *Brachiaria ruziziensis* (Dawar napier) and *Pennisetum* sp. (Taiwan A25) grasses(22). Variation is also apparent during the dry season. For instance, total biomass was 2,690 kg at a 45-d interval in the dry season for a *L. leucocephala*-*M. maximus* association under irrigation[7]. But it was 3,221 kg at a 42-d interval during the dry season in a *Cenchrus ciliaris*-*L. leucocephala* association without irrigation[43].

Forage biomass in the studied *L. leucocephala*-*M. maximus* silvopastoral system had the highest nutritional quality between the 40- and 50-d intervals. In *M. maximus* nutritional quality tended to decrease after 40 d as fiber fractions increased, and CP and IVDMD decreased. However, in *L. leucocephala* it varied little over time as the foliage retained relatively steady CP levels and IVDMD values while the fiber fractions (NDF and ADF) were unchanged. These results agree with previous reports that *L. leucocephala* can maintain protein content up to 70 d (24 %) during rainy seasons[44], and that grasses, regardless of species, decrease their nutrient concentration more rapidly than trees because their growth cycles are shorter and they reach maturity more quickly, consequently reducing their chemical-nutritional quality[20]. For example, nutritional quality is known to decline in the grass *C. nlemfuensis* in the rainy season beginning at a 42-d interval[45], as is also the case in the grasses *B. ruziziensis* (Dawar napier) and *Pennisetum* sp. (Taiwan A25) at a 40-d interval[22].

Season defined biomass nutritional quality. In *L. leucocephala* the foliage exhibited clearly higher protein levels (up to 8 percentage points) during the dry season. For *M. maximus* the dry season resulted in lower NDF and ADF contents. This was variable since in the rainy season the fiber fractions in this grass remained stable up to 40 ds and then increased significantly up to 50 d, while in the dry season fiber content gradually increased at longer intervals. The higher biomass nutritional quality in the dry season can be attributed to the fact that water deficit limits plant growth by retarding maturity. Less growth translates into less demand for cellular content metabolites to build structural tissue, maintaining NDF and ADF fractions more stable during the dry season[46]. For example, *M. maximus* had a lower proportion of stems in its biomass in the dry season than in the wet season. There are also reports of higher protein concentrations and lower fiber fractions in the foliage of *L. leucocephala*[44,47,48] and grasses[49,50] in the dry season.

Nutritional quality of *L. leucocephala* was not compared to *M. maximus* in the present study. However, the low digestibility of the *L. leucocephala* foliage coincides with previous reports[51,52] and has been attributed to greater lignification in trees. Although at the 50-d interval new *L. leucocephala* stems exhibited no signs of lignification, tree and bush branches require lignification to maintain their structure[45] and lignin content limits dry matter digestibility[53]. In *L. leucocephala* this may also be related to the presence of condensed tannins[54] which can reduce dry matter digestibility by binding to protein, making it unavailable in the small intestine[55]. Nonetheless, the growth habit and resource absorption strategies of trees allows them to maintain better nutritional quality for longer periods than in grasses[56]. In silvopastoral systems these components can therefore complement each other to consistently produce forage of greater overall nutritional quality.

Nutritional quality in the *L. leucocephala* studied here was similar to that reported for the same species (29 % CP, 49 % NDF, 23 % ADF and 59% IVDMD) at a 42-d interval[44], and at a 30-d interval in the dry season (30 % CP, 38 % NDF and 20% ADF)[48]. Nutritional quality of the *M. maximus* was comparable to that of *C. ciliaris* at a 42-d interval (11 % CP and 48 % IVDMD)[48,56], and *M. maximus* at 45-d intervals during the northwinds season (11 % CP, 62 % NDF and 59 % IVDMD)[16].

Conclusions and implications

Under the present study conditions the *L. leucocephala*-*M. maximus* association reached its highest production during the wet season at the 50-d interval. Nutritional quality decreased in *M. maximus* after 40 d while in *L. leucocephala* it remained largely unchanged up to 50 ds, regardless of season. Defining the optimal grazing point in a silvopastoral system such as this one requires quantification of the amount of available biomass and nutritional quality of both forage components. At 40 d *M. maximus* exhibited its highest nutritional quality, but total system forage biomass was 50 % lower than at 50 d. This means that, in terms of forage production, grazing is best done between 40 and 50 ds even though the *M. maximus* nutritional quality will be slightly lower within this interval. However, this nutrient shortfall is compensated for through the constant foliage quality of *L. leucocephala*. The forage biomass provided by the 5,000 ha^{-1} *L. leucocephala* density used in the present silvopastoral system is too low for practical purposes but could be changed by increasing sowing density.

Acknowledgements

Thanks are due to Mr. José Barrera Morfin for allowing to his silvopastoral system and for support throughout the field phase of this research.

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