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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*

MARIA B. BOTTINI-LUZARDO[1], CARLOS F. AGUILAR-PÉREZ[2], FERNANDO G. CENTURIÓN-CASTRO[2], FRANCISCO J. SOLORIO-SÁNCHEZ[2] AND JUAN C. KU-VERA[2]

[1]*Unidad Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Guerrero, Cuajinicuilapa, Guerrero, Mexico.* www.uagro.mx
[2]*Facultad de Medicina Veterinaria y Zootecnia, Universidad de Yucatán, Mérida, Yucatán, Mexico.* www.veterinaria.uady.mx

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

Correspondence: M.B. Bottini-Luzardo, U.A. Medicina Veterinaria y Zootecnia 2, Universidad Autónoma de Guerrero, Federal Acapulco – Pinotepa Nacional, km 198, CP 41940, Cuajinicuilapa, Guerrero, Mexico.
Email: mariabluzardo@yahoo.es

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.

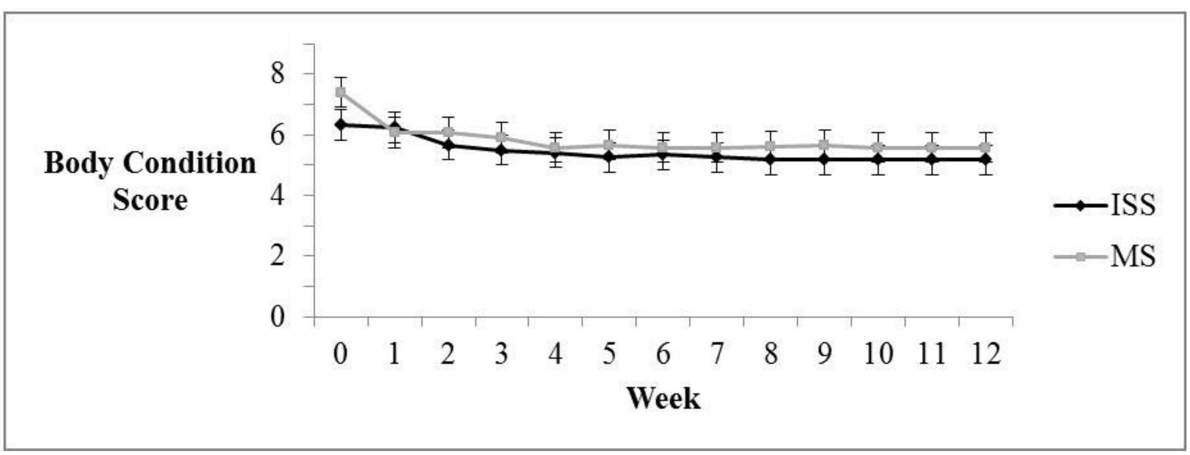

Figure 1. Body condition scores in crossbred cows grazing an intensive silvopastoral system (ISS) of leucaena-grass and a grass monoculture system (MS). Vertical bars indicate SD.

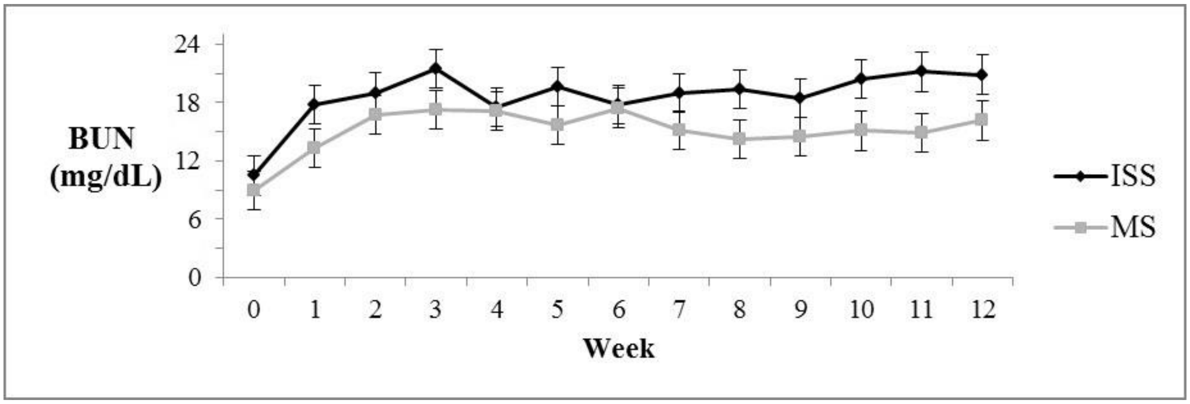

Figure 2. Blood urea nitrogen in crossbred cows grazing an intensive silvopastoral system (ISS) of leucaena-grass and a grass monoculture system (MS). Vertical bars indicate SD.

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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