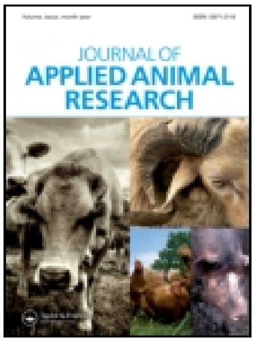

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Milk production and reproduction of dual-purpose cows with a restricted concentrate allowance and access to an association of *Leucaena leucocephala* and *Cynodon nlemfuensis*

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The aim of the study was to evaluate the productive and reproductive performance of dual-purpose (DP) cows with a restricted concentrate allowance and access to a forage association of Leucaena (*Leucaena leucocephala*) and Stargrass (*Cynodon nlemfuensis*). Twenty-four multiparous Holstein × Zebu cows were used during the first 98 days postpartum. Treatments were control (CT): nightly grazing of Stargrass + commercial concentrate (3.97 kg dry matter [DM]/d), and Leucaena (LT): nightly grazing of Stargrass + commercial concentrate (1.97 kg DM/d) + 4 h grazing on an association of Leucaena/Stargrass. The actual time spent browsing Leucaena represented 38% of the total time of consumption. The bite rate of Leucaena was 22 5 bites/min with a bite size of 0.541 ± 0.08 g DM. The estimated intake of Leucaena per cow was 503 ± 228 g DM/d. Milk yield per cow was similar ($P > 0.05$) between treatments (CT = 12.28 ± 0.6 kg/d, LT = 11.97 ± 0.6, TL = 11.9 0.6) with a similar composition ($P > 0.05$). There were no differences ($P > 0.05$) between treatments for changes in live weight (CT −29.6 kg, LT −33.3 kg) and body condition (CT −1.3, LT −1.4, in a 1–9 scale). Population of ovarian follicles per cow was similar between treatments throughout the experiment. Pregnancy rate was 33% for CT and 25% for LT, with no significant differences ($P > 0.05$). Grazing an association of Leucaena and Stargrass can replace part of the concentrate without detrimental effects on production and reproduction in DP cows.

Keywords: milk production; reproduction; Leucaena; dual-purpose

1. Introduction

Dual-purpose (DP) systems are those in which income is divided between milk and beef. DP cattle systems contribute most of the milk and meat produced in the tropics (Holmann et al. 1990), and they are characterized by using crossbred (*Bos taurus* × *Bos indicus*) cows. The low quality and availability of pasture during the dry season are the main constraints to ruminant production under tropical conditions (García 1991), and feed supplementation has become a common and necessary strategy to DP systems.

Milk production and reproduction in DP cows have been successfully improved by the use of feed supplements based on cereals (Aguilar-Pérez et al. 2009a); however, the high cost of grains and their low availability are the main limitations to this approach, which has prompted the search for local food alternatives to reduce the use of cereal-based concentrates. Leucaena (*Leucaena leucocephala*) is a tropical legume native to Mexico and Central America that has been widely used in the tropical regions of the world (Dalzell et al. 2006). Foliage of Leucaena is well accepted by cattle and, due to its high percentage of crude protein and digestibility (Jones 1994; Ayala et al. 2006), it has also been proposed to help to reduce dependence on grains in supplemental feeding. Leucaena can be successfully associated with grass to provide high-quality livestock forage (Rivera et al. 2009; Murgueitio et al. 2011).

The aim of this study was to evaluate milk production and reproductive performances of DP cows with restricted concentrate feed and access to an association of Leucaena/Stargrass (*Cynodon nlemfuensis*). It was hypothesized that such management could replace part of the concentrate provided without adverse effects on productive and reproductive performances of DP cows.

2. Material and methods

2.1. Location

The experiment was carried out on the dairy research farm at the Faculty of Veterinary Medicine and Animal Science, University of Yucatan, Mexico. The Yucatan Peninsula is located in Southern Mexico between latitudes 16°06′ and 21°37′ North and longitudes 87° 32′ and 90°23′ West. The climate is warm and subhumid with most of the rain falling between June and October. The annual temperature is fairly constant throughout the year (24.5–27.5 °C). Relative humidity varies between

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65% and 100% and annual rainfall fluctuates between 415 and 1290 mm (García 1988).

2.2. Animals and treatments

Twenty-four multiparous Holstein × Zebu cows with an average calving weight of 497 ± 66 kg and a body condition of 5.1 ± 1.2 (where one was emaciated, nine were obese, Ayala et al. 1998) were used. Cows were allocated to treatments according to parity and to milk production of previous lactation so that each group was balanced. Each cow was incorporated into the treatment at calving, and measurements were recorded during 98 days postpartum. Treatments were the following:

(1) Control treatment (CT): commercial concentrate feed (3.97 kg dry matter [DM]/cow/d) + 16 h grazing in Stargrass;
(2) Leucaena treatment (LT): commercial concentrate feed (1.97 kg DM/cow/d) + 4 h grazing in an association of Leucaena/Stargrass + 12 h grazing in Stargrass.

There were 13 cows (CT = 7, LT = 6) which calved from June to October (rainy season) and 11 cows (CT = 5, LT = 6) which calved during April, May, November, and December (dry season). Feed concentrate was composed of ground sorghum grain (58%), soya bean meal (20%), wheat bran (20%), and minerals (2%). The chemical composition was 89.7% DM and 18.3% CP with an estimated concentration of 11.1 MJ ME/kg DM (MAFF 1975). The concentrate was divided into two equal portions and offered during each milking (06:00 and 15:00 h). Cows were milked mechanically using the calf to stimulate "milk let down." The calf was allowed to suck for 2 min and then was tied up near its dam until the end of milking. A restricted suckling scheme was followed, where the calf was kept with the cow in an adjacent pen after each milking. After the morning milking, cows from LT had access to paddocks of Leucaena/Stargrass from 09:00 to 13:00 h, while CT cows grazed only in Stargrass paddocks. After milking in the afternoon, LT and CT cows were managed as one herd and taken to the Stargrass paddocks, where they grazed from 17:00 to 5:00 h. The Stargrass paddocks covered an area of 14 ha, which was irrigated during the dry season.

The associated fodder (Leucaena/Stargrass) covered an area of 1.55 ha, with a density of 1930 plants of Leucaena/ha and an average height of 1.8 m. The area was divided into 13 paddocks with electric fence marking the boundaries and was irrigated during the dry season. Stocking rate was 3.9 animal units (AU)/ha in the dry season and 7.3 AU/ha in the rainy season (1 UA = 450 kg live weight). Each paddock was occupied for 5 and 7 days during the dry and rainy seasons, respectively. Rest periods for the paddocks were 36 and 44 days in dry and rainy seasons, respectively. Stocking rate in paddocks of Stargrass was kept at 3.2 AU/ha throughout the experiment, with occupation periods of one day and rest periods of 31 days.

2.3. Measurements

Milk yield was recorded and milk samples were taken every 14 days at both the morning and afternoon milkings without calf intervention and assisted by an intramuscular injection of 40 iu of oxytocin. The am samples were refrigerated (5 °C) and then mixed with the pm samples so that there were seven samples per cow throughout the experiment. Milk samples were kept frozen (−20 °C) until the end of the experiment after which they were thawed at room temperature to obtain two pooled samples, constituted by samples 1–3 and 4–7. This two pooled samples were sent to the laboratory for chemical analysis. Body condition and live weight (without fasting) were both recorded at calving and every 14 days after morning milking. Body condition was assessed visually by the same person, using a scale of 1–9, as reported by Ayala et al. (1998).

Forage availability from the Stargrass paddocks was measured every 14 days, using a 0.5 × 0.5 m (0.25 m^2) metal square by a modification of the technique reported by Cox (1980). The metal square was dropped 10 times in each paddock, attempting to cover all the area in a zigzag pattern. The grass inside the square was cut at 5 cm height from the ground and then weighed. A representative sample was taken and kept frozen until chemical analysis. Samples of Leucaena were also taken for chemical analysis, every 14 days, trying to resemble the behavior of grazing cows. The intake of concentrate was recorded daily during milking by weighing the food offered and rejected. Monthly samples of concentrate were taken for chemical analysis.

Milk samples were analyzed to determine the concentration of fat by the Gerber technique (ILCA 1988), lactose by colorimetry (Saidén et al. 1999), and total nitrogen by combustion, using a LECO CN-2000 (series 3740, LECO Corporation). Crude protein was calculated as N × 6.25. The DM of feeds was determined by drying the forage in an oven to constant weight at 60 °C for 48 h. Acid detergent fiber was determined in Stargrass and Leucaena samples (Van Soest et al. 1991) in order to calculate metabolisable energy (MAFF 1975).

Feeding behavior in the Leucaena/Stargrass association was evaluated in 10 cows, through direct observation, at 21, 46, 70, and 95 days postpartum. The procedure was to observe each cow for 30 seconds, every 5 min for a total time of 194 ± 28 min. Variables recorded were the following: time spent consuming Leucaena, time spent consuming Stargrass, time spent ruminating, and time spent in other activities. Effective time of consumption of Leucaena was derived from the total observed time. The consumption of Leucaena was estimated in seven cows, during the rainy season only, through the bite simulation technique of hand plucking (Prates 1974) and using the data from the feeding behavior. The number of uninterrupted bites and the time spent on it were measured 11 times for each cow. With this information, the bite rate was calculated, i.e., the average number of bites per minute. At the end of the observation, the cows' bite was simulated 10 times, hand plucking the amount of Leucaena that resembled the bite observed in each animal.

Samples of urine were collected weekly from the two groups of cows, from the second week postpartum, to determine the concentration of urea by SERA-PAK Plus kit (Bayer®) and allantoin, according to the method described by Chen and Gomes (1995).

The ovaries of each cow were examined by transrectal ultrasonography (Pie Medical Ecograph with a 6–8 MHz linear rectal transducer, Falco Vet-100, Holland) every week, beginning on day 7 postpartum. The number and size (diameter) of follicles were recorded, and they were classified as small follicles (3–5 mm), medium follicles (6–9 mm), or large follicles (>9 mm). Cows were checked for pregnancy during the ovarian ultrasonography, and pregnancy rates were reported at the end of the experiment.

2.4. Statistical analysis

Milk yield was analyzed using the MIXED procedure for repeated measures of SAS (2002). Live weight at calving was included as a covariate. Milk composition, live weight, and body condition score were analyzed using the general linear model (GLM) procedure of SAS (2002). The initial models included effects of treatment, season of calving, and its interactions. Season of calving and interactions with diet were not significant, and only treatment effect was considered in the final models. Data are presented as means ± standard error. Data on feeding behavior and concentrations of urea and allantoin were analyzed as repeated measures, considering the dry (April, May, November, and December) and rainy (June–October) seasons and days in milk (21, 46, 70, and 95) as fixed effects (for feeding behavior), and the effect of diet (for concentrations of urea and allantoin).

3. Results and discussion

Table 1 shows the chemical composition of concentrate and forages used in the study. The availability of Stargrass was similar ($P > 0.05$) for the dry (1692 ± 766 kg DM/ha) and the rainy (1797 ± 617 kg DM/ha) seasons, which was attributable to the irrigation of the paddocks. The chemical composition and availability of Stargrass were within the expected ranges for tropical pastures (Juarez-Lagunes et al. 1999). The crude protein of Stargrass and Leucaena were both above the minimum quantity (60 g CP/kg DM) necessary to meet the requirements of rumen bacteria (Minson 1990). The crude protein of Leucaena (25.9%) was higher than that reported for Leucaena forage (22.03%) but lower than that reported for Leucaena leaf meal (29.2%) (García et al. 1996). Such a difference could have been due to the Leucaena sampling technique, which was carried out by simulating the cows' bite. Cows spent 121 ± 33 min (mean ± SD) browsing and grazing in the Leucaena/Stargrass association. Effective time of consumption of Leucaena was 46 ± 17 min which represented 38% of the total time of consumption in the Leucaena/Stargrass association. The time devoted to the consumption of Leucaena was above the range (2–30%) reported in cattle browsing fodder trees (Ibrahim 1981; Bayer 1990). The biting rate was 22 ± 5 bites/min with an average size of 0.5 ± 0.1 g DM. This is in agreement with the study of Morales (2000) who reported 22 bites/min in a silvopastoral system during the rainy season in Yucatan, Mexico. The estimated average consumption of Leucaena was 503 ± 228 g DM/d, with a coefficient of variation of 45%. This consumption of Leucaena was low compared to previous research. Valdivia (2006) reported 2.6 and 2.3 kg DM animal/d in crossbred cows in a silvopastoral

Table 1. Chemical composition of feedstuffs (mean ± standard deviation).

	Concentrate	Grass	*L. leucocephala*
DM (g/kg)	897.2 ± 19.3	337.4 ± 70.0	303.3 ± 38.0
Crude protein (g/kg)	182.5 ± 7.8	79.4 ± 11.8	259.4 ± 25.0
ADF (g/kg)	–	391.72 ± 40.6	244.84 ± 39.7
Crude fiber (g/kg)	33.2 ± 3.2	–	–
Ether extract (g/kg)	27.0 ± 10.5	–	–
NFE (g/kg)	602.1 ± 13.6	–	–
Ash	52.2 ± 5.3	–	–
ME (MJ/kg DM)[a]	11.6	8.4 ± 0.7	9.4 ± 0.4

Note: ADF, acid detergent fiber; NFE, nitrogen-free extract; ME, metabolisable energy.
[a]Calculated from MAFF (1975).

system with and without energy supplementation, respectively. Bacab-Pérez and Solorio-Sánchez (2011) found higher consumption of *L. leucocephala* in cows that remained for 20 h in two silvopastoral systems but at higher densities of Leucaena and lower stocking rate compared with that of the present experiment. These authors reported consumption levels of 2.96 kg DM at 34,500 plants/ha and 3.0 AU/ha and 4.97 kg DM at 53,000 plants/ha and 2.5 AU/ha. It is likely that the technique employed for the estimation of Leucaena intake led to an underestimation of the actual amount consumed since cows spent 38% of their total grazing time in the association, actually browsing the legume. In general, the cows seemed to prefer Stargrass than Leucaena regardless of day postpartum (Table 2). Except for Leucaena on day 70 and Stargrass on day 95 postpartum, the time spent consuming both forages increased along the time (Table 2). It has been observed that cows in early lactation show a low voluntary intake which is gradually increased as lactation progresses (Garnsworthy & Topps 1982). It is possible that unexpected climatic events, which went unrecorded, may have resulted in the unusual patterns of grazing behavior registered on days 70 and 95 postpartum. The time spent ruminating decreased as the day postpartum advanced, although this result may be misleading, because the measurement was carried out only during the time the cows remained in the Stargrass/Leucaena paddocks. Table 3 shows that the cows spent more time consuming both forages during the dry season, which could be associated with the expected lower quality of the basal diet (Stargrass) during the dry season rather than to the availability of Stargrass, which was not different between seasons.

Urea concentration in urine was statistically higher ($P < 0.05$) for the cows that had access to the association Leucaena/Stargrass (13.0 vs 10.8 mg/dl). The concentration of allantoin in urine was not different ($P > 0.05$) between CT (2367.8 mg/l) and LT (2087.7 mg/l). Even though the estimated consumption of Leucaena was low in the present study, the higher excretion of urea in the LT group would be a reflection of the high content of nitrogen of Leucaena foliage (Muinga et al. 1995). Those results and the similar excretion of allantoin in the CT and LT would imply that microbial protein supply to the duodenum was similar in both treatments and that the efficiency of N utilization by the rumen bacteria was low (Chen & Gomes 1995) probably as a consequence of the lower consumption of energy from the concentrate in the LT. Thus, energy supplementation in cows grazing legume fodder should be an important practice to optimize N utilization in the rumen. Recently, Arjona-Alcocer et al. (2012) were able to reduce urinary urea excretion in sheep fed 50% (DM) of Leucaena forage in their rations, when they were supplemented with readily fermented energy in the way of cane molasses.

Milk yield and milk composition were not different ($P > 0.05$) between treatments (Table 4) and both groups of cows showed similar live weight and body condition score losses (Table 5). This implies that cows in both treatments had to use their body reserves to meet their energy requirements for lactation. It has been shown that such losses in early lactation are common even for DP cows in the tropics, whose nutritional requirements are not as high as those for high-merit dairy cows (Aguilar-Pérez et al. 2009a; Tinoco-Magaña et al. 2012). Milk yield in LT (11.97 kg/cow/d) was higher compared with that reported in previous studies with DP cows and

Table 2. Feeding behavior (time spent) of DP cows in an association of *L. leucocephala*/*C. nlemfuensis*, measured at different time after calving.

Time (minutes)	Days postpartum				
	21	46	70	95	SEM
Consuming Leucaena	30.00[a]	59.52[b]	33.50[a]	61.07[b]	8.51
Consuming Stargrass	42.50[a]	81.30[b]	93.50[c]	83.45[d]	11.90
Rumination	56.25[a]	40.47[a]	21.00[b]	17.26[b]	9.33
Other activity	39.37[a]	48.69[b]	27.50[c]	42.02[d]	9.17

Notes: Means with different superscripts within the same row are different ($P < 0.05$).
SEM = standard error of means.

Table 3. Feeding behavior (time spent) of DP cows in an association of *L. leucocephala*/*C. nlemfuensis*, measured in the dry and in the rainy seasons.

Time (minutes)	Dry season	Rainy season	SEM
Consuming Leucaena	54.9[a]	37.1[b]	8.51
Consuming Stargrass	99.8[a]	50.6[b]	11.90
Rumination	19.6[a]	47.9[b]	9.33
Other activity	15.1[a]	63.7[b]	9.17

Notes: Means with different superscripts within the same row are different ($P < 0.05$).
SEM = standard error of means.

Table 4. Milk yield and milk composition of DP cows during the first 98 days postpartum with (LT) or without access (CT) to an association of *L. leucocephala*/*C. nlemfuensis* (mean ± standard error).

	Treatment		
	LT	CT	*P*
Milk yield (kg/d)	11.97 ± 0.60	12.28 ± 0.60	0.71
Milk composition (g/kg)			
Fat	34.41 ± 0.15	34.87 ± 0.15	0.83
Protein	29.68 ± 0.83	30.57 ± 0.83	0.46
Lactose	54.62 ± 0.24	51.86 ± 0.24	0.43

Table 5. Live weight and body condition of DP cows with (LT) or without (CT) access to an association of *L. leucocephala* and *C. nlemfuensis* (mean ± standard error).

	Treatment		
	LT	CT	*P*
Live weight (kg)			
Initial	490.4 ± 19.3	502.8 ± 19.3	0.65
Final	457.1 ± 17.8	473.2 ± 17.8	0.51
Change	−33.3 ± 7.6	−29.6 ± 7.6	0.72
Body condition (score)			
Initial	5.29 ± 0.36	4.87 ± 0.36	0.42
Final	3.83 ± 0.23	3.54 ± 0.23	0.38
Change	−1.45 ± 0.36	−1.33 ± 0.36	0.80

restricted access to paddocks of *L. leucocephala*. Razz et al. (2004) reported a milk yield of 9.59 kg/cow/d with 2 kg of concentrate plus 1 h in Leucaena paddocks. Faría et al. (2007) found 10.76 kg milk yield/cow/d with the same amount of concentrate but with 2 h grazing in Leucaena. In a previous study at the same experimental station and with the same cows that were used in the present experiment, Tinoco-Magaña et al. (2012) reported a milk yield of 10.55 kg/cow/d with 4-h access to the same Leucaena/Stargrass association but with only 1 kg of sorghum grain as an energy supplement. Therefore, it is clear that the level of concentrate and the genetic merit of cows are both crucial in determining the productive response in the experiments using Leucaena. The lack of differences in milk yield between the LT and CT is in accordance with Faría et al. (2007). Those authors reported a milk yield of 11.13 vs 10.76 kg/d in cows receiving 4 kg of concentrate without Leucaena and 2 kg of concentrate plus 2 h in Leucaena, respectively. They concluded that Leucaena was able to partially substitute the concentrate; however, they did not measure the consumption of Leucaena. In the present experiment, it is reasonable to assume that in LT, the cows could have compensated the low consumption of concentrate and Leucaena by a higher intake of grass. Garcia and Sanchez (2006) also reported a low consumption of *L. leucocephala* (0.105 kg DM/cow/day) and similar milk yield between cows with (9.06 kg/cow/d) and without (9.23 kg/cow/d) *L. leucocephala*, and they attributed that to the fact that the grass alone met the nutritional requirements of the cows.

The lack of difference in milk composition between treatments suggests a similar supply of milk precursors to the mammary gland in both groups of cows. The results are consistent with the findings of Bobadilla-Hernandez et al. (2007) and Faría et al. (2007) who did not find differences in milk composition of cows grazing grasses plus *L. leucocephala*. It has been reported that

Table 6. Population of ovarian follicles of different sizes and pregnancy rate of DP cows with (LT) or without (CT) access to an association of *L. leucocephala* and *C. nlemfuensis* (mean ± standard error).

	Treatment		
	LT	CT	*P*
Follicles, number			
3–5 mm	5.5 ± 1.3	6.4 ± 1.3	0.616
6–9 mm	1.8 ± 0.2	1.6 ± 0.2	0.502
> 9 mm	1.1 ± 0.2	0.9 ± 0.21	0.545
Pregnancy rate, number (%)	4/12 (33)	3/12 (25)	0.315

the composition of milk is not easily influenced by the effect of supplementation with legumes (Camero 1995; Hess et al. 1999; Aguilar-Pérez et al. 2001).

Population of small, medium, and large ovarian follicles were not different ($P > 0.05$) for cows in LT and CT. Pregnancy rate was also similar ($P > 0.05$) between treatments (Table 6). These results would reflect the similar changes in body weight and body condition showed by the groups. It is well known that body condition and body weight are functional indicators of energy status and rebreeding performance after calving (Randel 1990) and that the rate of body condition losses after calving may be more influential on reproductive performance than absolute body condition score at calving (Roche et al. 2000). Even though pregnancy rates found in the present experiment seemed to be low, they are consistent with pregnancy rates found at early lactation (90 days postpartum) in DP cows at the same experimental station (Aguilar-Pérez et al. 2009a, 2009b). In DP systems in Mexico, the interval between parturitions births exceeds 500 days, mainly caused by the extensive postpartum anoestrus period, originated by the negative effects exerted by suckling and the feeding of cows with low-quality pastures (Rojo-Rubio et al. 2009). Low pregnancy rates have been reported in cows grazing Leucaena (Jones et al. 1989; Hammond 1995) associated with the ingestion of the nonprotein amino acid mimosine and the effect of its metabolites 3,4-DHP and 2,3-DHP. However, these findings occurred in animals which were consuming higher amounts of Leucaena (>30% of total DM intake) than that estimated in the current experiment.

4. Conclusion

Grazing an association of *L. leucocephala*/*C. nlemfuensis* can replace part of the concentrate without detrimental effects on milk production and reproduction of DP cows.

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