Research Note

EFFECT OF THE PHYSICAL FORM OF TROPICAL LEGUMES CRATYLIA ARGENTEA *(DESV.) KUNTZE,* CALLIANDRA CALOTHYRSUS *MEISN. AND* LEUCAENA LEUCOCEPHALA *(LAM. DE WIT) ON SELECTIVE CONSUMPTION BY LAMBS*[1]

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Tropical legumes can improve animal performance by increasing forage intake and digestibility and also indirectly, by fixing nitrogen in the soil, thus improving yield and crude protein content of grasses grown in association (Broderick, 1995). In the tropics, shrub legumes have a potential use in small ruminant feeding systems. Shrubs generally out-yield herbaceous legume species, are more tolerant to adverse management conditions, and produce good quality forage even during long dry seasons (Lascano and Ávila, 2002). However, their evaluation as forage for small ruminants has been limited. *Cratylia argentea* (Desv.) Kuntze (CA), *Calliandra calothyrsus* Meissner (CC) and *Leucaena leucocephala* (Lam. de Wit) (LL), are three potential tropical shrub legume species that need further evaluation as forage sources for sheep and goats. All three shrubs have previously been evaluated as supplements and in protein banks in ruminant grazing systems (Pamo et al., 2005; Espinoza et al., 1999; Sánchez and García, 1998). However, there is limited information on the effect of the physical form of these shrubs on palatability, preference, and ingestive behavior of small ruminants. The objective of this study was to evaluate in three different experiments the selective consumption of CA, CC, and LL by lambs when offered: under grazing conditions in pastures with grass-legume associations, as legume hay when combined with grass hay, or as fresh-cut legume herbage for animals in confinement.

Experiment 1 was conducted at the Agricultural Experiment Station of the University of Puerto Rico (UPR) located in the Municipality of Lajas. Shrub legumes CA, CC, and LL were planted initially in individual containers and later transplanted to a 53.5 x 48.3 m field with six 277.07 m^2 subdivisions (N = 2 per legume) in association with tropical grasses (TG; mainly guinea-grass, *Panicum maximum*). In each paddock, shrubs were planted in double rows with a distance of 1.5 m between plants and 4 m between rows. Sixty plants of each legume species were planted per paddock for a total of 120 plants per shrub to be evaluated. The seeds of these legumes were not inoculated with N-fixing bacteria before planting. At 134 d post-transplanting, a grazing selectivity trial of 21-d duration was conducted using six plots of the tropical legumes in double rows and in association with TG. Before the grazing trial, three plants of each legume species and two of grasses were randomly collected from each experimental plot and analyzed

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to determine contents of dry matter (DM), organic matter (OM), inorganic matter (IM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) using standard procedures (AOAC, 1990; Van Soest et al., 1991). To determine selective consumption of the shrubs, eighteen young lambs (μ = 22.8 kg body weight, BW) were randomly allotted to six groups of three animals each and the groups randomly assigned to graze the plots under a controlled schedule. During the 21-d trial, lambs were allowed to graze each paddock daily during six hours from 6:00 am to 12:00 pm. Shrub selectivity was determined daily by visual observations during a 60-minute period, in which the number of lambs consuming the shrub was recorded at 0, 15, 30, 45 and 60 minutes. The shrub acceptability was quantified using a scale with three levels of preference based on the number of lambs browsing or consuming the forage. The levels of preference were: no browsing (zero consumptive activity), moderate browsing (one to two lambs consuming), and high browsing (three or more lambs consuming the legume). These levels represent 0, 16 to 33, and 50% or more of the lambs browsing the shrubs, respectively.

Observation of lambs under grazing conditions indicated no browsing of the shrub legumes CA and CC during the 21-d trial (Table 1). Ingestive behavior of the animals under these treatments was limited to the consumption of TG (Table 1). *Leucaena leucocephala* was moderately or highly selected by lambs at the beginning (time zero) and four subsequent equally spaced intervals of the 60-minute observation period. The preference of the grazing lambs for LL was evident at the end of the grazing trial when a high grade of accumulative defoliation was observed, except in the highest parts of the plants out of reach of the animals.

Experiment 2 evaluated the selective consumption of the three shrub legumes conserved as hay and fed in association with grass hay. The feeding trial was conducted at the small ruminant project, Alzamora Farm of the UPR, Mayagüez Campus. The shrubs CA and CC were mechanically harvested and sun-dried during 96 h at the UPR Agricultural Experiment Substation in Corozal, while LL plants were manually harvested at the Substation in Lajas and dried artificially in large paper bags at 65° C/96 h. By this procedure the leaflets of LL that tend to fall off upon drying were fully retained along

TABLE 1.—*Selective ingestion of shrub legumes by lambs under grazing conditions (Experiment 1).*

Time (min)	Legume[1]	Selectivity (browsing) by sheep[2]
	CA	None
0	CC	None
	LL	Moderate
	CA	None
15	CC	None
	LL	High
	CA	None
30	CC	None
	LL	High
	CA	None
45	CC	None
	LL	Moderate
	CA	None
60	CC	None
	LL	High

[1]CA= *Cratylia argentea*; CC= *Calliandra calothyrsus*; LL= *Leucaena leucocephala;*

[2]Based on the number of lambs browsing the shrub: None = zero lambs browsing; Moderate = one to two lambs browsing; High = three or more lambs browsing.

with still adhering foliage and stems. Eighteen lambs (BW, μ = 27.3 kg) were divided into six groups of three animals each. The groups were randomly assigned to one of the three experimental treatments and housed in 3.66 x 2.44 m feeding pens provided with automatic waterers and a double compartment feeder. The diets had in common 50% commercial tropical grass hay (TGH) composed mainly of guinea-grass (*Panicum maximum* Jacq.) in combination with 50% dried CC, CA or LL. Separately, individual forage portions were offered in quantities equivalent to 3% of animal BW on a dry matter basis (DMB), provided daily at 0900 h; orts were weighed separately and sampled to determine chemical composition (AOAC, 1990, Van Soest et al., 1991) and DM intake of each forage. The experiment was of 15 days duration with four days of adaptation to the facilities, six days of diet adaptation and five days of comparative data collection. Forage intake data (legumes and grasses) were analyzed according to a completely randomized design with two replicates per treatment using the General Lineal Model procedure of SAS (SAS Inst., 1990).

Chemical composition of the legume shrubs and grasses conserved as hay showed the expected high protein contents in the former; *L. leucocephala* (21.4%) having the highest (P<0.05), followed by CA (20.2%) and CC (17.7%) (Table 2). By contrast, the CP content of TGH (4.9%) did not reach even a third of that of the CC hay or a fourth of those of CA and LL hay. Among the legumes, cell-wall (NDF) content was highest (P<0.05) in CA hay (68.1%) and progressively lower in hay of CC and LL (61.2 and 58.6%). The NDF content of CA hay was even higher than that found in TGH (67.2%). In related research, CP and NDF values of 19% and 67%, and 21.5% and 70.1% were reported in CA hay and leaves, respectively (Wilson and Lascano, 1997; Lascano and Ávila, 2002). The NDF content of the TGH used in this experiment can be considered as typical, but the CP content (4.9%) was rather low (Toranzos et al., 2000; Randel and Méndez, 1989). In the trial to determine DM, CP and NDF consumption of the shrub legumes conserved as hay, significant differences (P<0.05) in the amount of forage offered, refused and consumed were observed (Table 3). Daily DM consumption and orts of CA and LL were similar (1,210 and 1,214 g, and 18 and 28 g, respectively); however, in CC, intake was lower (802 g) and orts were higher (426 g). Consumption of hays of CA and LL were 98.5 and 98.9% of the total offering, but this figure was only 65% in the case of CC hay. These observations indicate a greater acceptability and intake of CA and LL than of CC hay by sheep. Crude protein consumption was similar for CA and LL, but inferior in CC, while NDF intake by lambs fed CA was higher (P<0.05) than in those under the CC and LL treatments (Table 3). There were differences (P<0.05) in the TGH offering in association with the three shrub legumes, being higher (P<0.05)

TABLE 2.—*Chemical composition of shrub legumes and tropical grasses conserved as hay (Experiment 2).*

Component (%)[1]	CA[2]	CC[2]	LL[2]	TGH[2]	SEM[3]
DM	90.0 a[4]	92.1 a	89.3 a	90.1 a	0.2
OM	93.2 a	92.6 a	93.1 a	88.3 b	1.0
IM	6.8 b	7.4 b	6.9 b	11.7 a	1.3
CP	20.2 a	17.7 b	21.4 a	4.9 c	48
NDF	68.1 a	61.2 b	58.6 b	67.2 a	34

[1]Dry Matter Basis

[2]CA= *Cratylia argentea*; CC= *Calliandra calothyrsus*; LL= *Leucaena leucocephala*; TGH = Tropical grass hay

[3]Standard error of the mean

[4] Means with unlike superscripts in the same row differ (P<0.05)

TABLE 3.—*Voluntary intake of hay of three shrub legumes by confined sheep (Experiment 2).*

	Legume			
Legume (g/d)[1]	CA[2]	CC[2]	LL[2]	SEM[3]
Offered				
DM	1,228.0 b[4]	1,228.0 b	1,242.0 a	0.0
CP	247.8 a	217.9 b	267.0 a	0.0
NDF	836.3 a	751.7 b	727.8 c	0.0
Orts				
DM	18.0 b	426.0 a	28.0 b	15.3
CP	3.6 b	75.7 a	15.0 b	5.1
NDF	12.3 b	260.7 a	16.4 b	9.4
Intake				
DM	1,210.0 a	802.0 b	1,214.0 a	15.3
CP	244.4 a	142.3 b	252.0 a	5.1
NDF	824.0 a	490.8 c	711.4 b	9.4

[1]Dry Matter Basis
[2]CA = *Cratylia argentea*; CC = *Calliandra calothyrsus*; LL = *Leucaena leucocephala*
[3]Standard error of the mean
[4]Means with unlike superscripts in the same row differ (P<0.05)

with LL than CC and CA. However, TGH consumption was higher when combined with CC than with CA and LL hay (data not shown). Total DM consumption values of the three lambs per treatment were 2,374; 1,998; and 2,386 g/d in diets containing CA, CC and LL hays in combination with TGH, representing respectively 96%, 81% and 96% of the total forage offered, and 2.90%, 2.43% and 2.88% animal BW (DMB). Expressed in this way daily forage intake of diets containing CC in combination with TGH was 0.57 percentage units lower that the expected value of 3% (DMB), whereas, in diets containing forages of the other two shrubs intake reached the expected level.

Experiment 3, conducted at the Lajas Substation, evaluated the selectivity and consumption of fresh-cut CC and LL provided at 50% of total forage offered and employed procedures similar to those previously described in *Experiment 2*, except that only two shrub legumes were evaluated. Twelve crossbred lambs (μ = 23.1 kg) were randomly assigned to four pens, each pen representing a replicate per treatment. Pens were equipped with a double wooden feeder and buckets to provide water ad libitum. Two diets containing 50% grass hay (*Brachiaria brizantha* cv. Mulato) and 50% fresh cut shrub (CC or LL) offered daily at 3% of lamb initial BW (DMB) were evaluated. Legumes were offered individually in the double wooden feeder. Animals were adapted to the experimental diets during six days followed by a five-day data collection period. Forage samples from each of the three trials were analyzed at the UPR Department of Animal Science, Animal Nutrition Laboratory, to determine DM (65° C/48 h), IM (550° C/12 h), OM (100 - IM), CP, NDF, ADF, and hemicellulose (NDF - ADF) using previously cited procedures. Forage consumption data were analyzed according to a completely randomized design with two replicates per treatment using the General Lineal Model procedure of SAS (SAS Inst., 1990). Forage chemical composition data were analyzed as a complete randomized block design with two replicates per treatment (plot). In both statistical analyses, given a significant variable response at P<0.05, the Tukey t- test was used for mean separation.

The CP content of LL was higher (P<0.05) than that of CC (23.7% vs. 16.7%) when offered as fresh-cut foliage (Table 4). Conversely, NDF content was numerically lower

TABLE 4.—*Chemical composition of fresh cut shrub legumes and tropical grass hay (Experiment 3).*

Component (%)[1]	CC[2]	LL[2]	TGH[2]	SEM[3]
DM	36.0 b[4]	28.8 b	89.1 a	3.6
OM	92.4 a	92.9 a	90.3 a	0.2
IM	7.6 a	7.1 a	8.7 a	0.3
CP	16.7 b	23.7 a	3.4 c	3.6
NDF	59.8 b	50.6 b	74.4 a	2.4

[1]Dry Matter Basis
[2]CC = *Calliandra calothyrsus*; LL = *Leucaena leucocephala*; TGH = Tropical grass hay
[3]Standard error of the mean
[4]Means with unlike superscripts in the same row differ (P<0.05)

in LL than in CC (50.6% vs. 59.8%). The TGH used in this trial had a low CP (3.4%) and high NDF content (74.4%). No significant differences were observed in DM offered, refused and consumed by the lambs between either fresh cut CC or LL combined with TGH. Daily DM consumption of CC and LL had the same mean: 1,032 g (Table 5). Consumption of fresh cut CC and LL diets represented 99.6% and 99.5% of the total forage offered daily, respectively, showing a high acceptability and essentially complete consumption of these legumes, when offered at 1.5% of the animal BW (DMB). Differences in CP and NDF intake were related to the chemical composition of the shrubs, CP consumption being higher (P<0.05) and NDF lower in lambs fed LL rather than CC (245 vs. 173 g/d, and 523 vs. 618 g/d, respectively. Quantities of TGH offered equivalent to 50% of the diet used with both shrub legumes, left uneaten and consumed were similar for both experimental treatments (data not shown). Total DM intake of the grass hay included in

TABLE 5.—*Daily intake of two fresh-cut shrub legumes mixed with tropical grass hay by groups of three sheep (Experiment 3).*

	Legume		
Legume:Grass mixture (g/d)[1]	CC	LL	SEM[2]
Offered			
DM	1,037 a	1,037 a	0.0
CP	174.2 b	245.7 a	0.0
NDF	620.5 a	525.1 b	0.0
Orts			
DM	4.30 a	4.60 a	1.75
CP	0.72 a	1.09 a	0.33
NDF	2.39 a	2.02 a	0.00
Intake			
DM	1,032 a	1,032 a	1.75
CP	173.4 b	244.6 a	0.33
NDF	617.9 a	522.8 b	0.99

[1]Dry Matter Basis
[2]CC = *Calliandra calothyrsus*; LL = *Leucaena leucocephala*
[3]Standard error of the mean
[4]Means with unlike superscripts in the same row differ (P<0.05).

the fresh-cut legume diets was 99.2% and 99.1% of the offering, which represented 2.97 and 2.96% of the lamb BW daily for treatments based on CC and LL, respectively.

As to interpretation of results from the three experiments, the only one of the three shrub legumes evaluated that was browsed by lambs under the conditions of this research was LL. In the form of hay, CA and LL were highly acceptable to lambs, thus showing their potential for use in small ruminant feeding systems. When offered as fresh-cut foliage lambs consumed both CC and LL, but CC was not consumed under grazing conditions, and as hay this shrub was less acceptable than LL. It might best be used as fresh-cut foliage for feeding small ruminants. The factors that could influence ruminant voluntary consumption include those dependent on the plant (i.e., botanical fractions and chemical composition) and on the animal (i.e., ingestive behavior, age, size, sex, and physiological state). Results obtained in the grazing trial are in agreement with those of García and Medina (2006), who reported an active browsing behavior of lambs grazing different varieties of LL. Espinoza and co-workers (1999) also reported active browsing of LL by lambs in that section of the plants below 60 cm of height, similar to the browsing tendency observed in the present 21-day grazing trial. The grazing trial results show that LL is promising as a potential alternative for use as an integral part of small ruminant grazing systems. A recommended use might be to graze its lower sections and harvest for hay production the vegetative material above the 60-cm level. By contrast, CA and CC showed no promise as alternatives under grazing conditions in association with tropical grasses. In *Experiment 2*, we observed greater acceptability and intake of CA and LL hays than of CC; however, in contrast with the grazing experiment, appreciable intake of both CA and CC hays by lambs was observed. Ávila and Lascano (2002) reported that dairy cows consumed CA offered as hay, but rejected the same shrub offered as fresh foliage. Raaflaub and Lascano (1995) observed in sheep a lower intake of fresh cut CA than of the legume conserved as hay. Results from the present experiment suggest that supplementation of TG with CA hay could alleviate CP deficiencies in sheep diets. Masama and co-workers (1997) evaluated four levels of supplementation (0, 50, 100 and 150 g DM/d) using four legumes conserved as hay: *Acacia angustissima, Cajanus cajan, C. calothyrsus* and *L. leucocephala*, in sheep fed corn forage ad libitum, and found increased consumption of LL as the level of supplementation increased, but not a clear response to supplementation with the same levels of CC. Lascano and co-workers, (2003) studied the intake of CC from two different locations and registered respective consumption equal to 41% and 63% of the total legume offered. Norton and Waterfall (2000) reported that intake of DM and digestible DM from low-quality TG initially increased as the dietary percentage of supplemental CC increased, the relative increment being 18% of total DM intake and 19% of digestible DM intake at the lowest level of daily supplementation (0.8% LW/DMD). However, higher levels of supplementation decreased palatability and digestibility of the diet. Other studies reported that the acceptability of CC by ruminants depends on the level of inclusion in the diet and the stage of maturity of the plant (Salawu et al., 1997). The low selectivity and consumption of CC reported by different researchers is presumably associated with the high content of condensed tannins in the shrub (Lascano et al., 2003; Norton and Waterfall, 2000; Flores et al., 1998; Norton and Ahn, 1997; Salawu et al., 1997). The research literature indicates that high levels of condensed tannins depress intake and digestibility of forages such as CC; however, the physiological mechanism involved is still unclear.

In *Experiment 3*, lambs consumed LL and CC when offered as fresh-cut foliage. Pamo and co-workers (2005) also observed high levels of consumption by dairy goats of CC and LL offered as fresh-cut foliage, demonstrating excellent acceptability. These authors further found that an increase in milk production and decrease in incidence of abortion were associated with inclusion of the legume in the diet. Merkel et al. (1999) evaluated

the effect of supplementation with fresh-cut CC and two other legumes (*Gliricidia* and *Falcataria*), added in proportion to supply 25% and 50% of the CP in concentrate-based diets, obtaining in all cases similar legume consumption and gain in animal BW. In the present experiments the intake of grasses under grazing conditions or when offered as hay was not quantified. Previous research with heifers and dry cows has showed that grass consumption is not independent of legume intake. In a total of seven experiments conducted in tropical Puerto Rico with *Arachis glabrata* and LL, in all but one case legume supplementation decreased grass consumption, but increased total dietary DM intake (Randel, 2007; 2009); while in the one odd trial, supplementation with *Arachis glabrata* hay did not negatively affect grass intake by heifers. In theory, low levels of legume supplementation could positively affect intake of poor-quality grass by alleviating shortages of nutrients, such as available nitrogen, needed for the rumen fermentation. Other experiments have also shown associative effects on intake and vitro and in vivo digestibility of diets containing grass and legume mixtures (Rodríguez et al., 1998, 2009, 2010a, 2010b). Legume species, level of supplementation and stage of plant maturity have been identified as factors that influence the positive response to legumes associated with grasses in ruminant diets (Sandoval-Centeno, 2007).

In the present experiment, the selective consumption of CA, CC and LL by lambs was observed when offered under grazing conditions in pastures with grass-legume associations, as legume hay when combined with grass hay, or as fresh-cut legume herbage for animals in confinement. In summary, results of these experiments show that *Leucaena leucocephala* was the most versatile of the tropical shrub legumes evaluated under grazing conditions, as hay, and as fresh-cut foliage. However, all three of these shrubs, when offered in the appropriate physical form, might represent potential alternatives to serve as high protein supplements in small ruminant diets.

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