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VOLUNTARY INTAKE AND "in vivo" DIGESTIBILITY IN SHEEP FED WITH "Guash" *Leucaenaleucocephala* IN CHIAPAS, MEXICO

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ABSTRACT

Foliage from fodder trees is a nutritional option that can improve small ruminant systems. A trial was conducted to evaluate the effect of *Leucaena leucocephala* foliage on voluntary intake and the apparent digestibility in sheep fed with *Panicum maximum L*. cv. Tanzania. Five "Pelibuey" male sheep (30 ± 1.0 kg), enclosed in metabolic cages, were fed on five diets based on *P. maximum* mixed with different inclusion levels of *L. leucocephala* respectively (T_1= 100-0, T_2 = 80-20, T_3 = 60-40, T_4 = 40-60 y T_5 = 20-80 %). Treatment diets were arranged in a Latin Square 5 x 5 and randomly allocated to each animal. Each experimental period lasted 12 days, 7 for adaptation to diet and 5 for measurements. The diets had a similar concentration of metabolizable energy ME (MJ / kg^{-1} DM). The results obtained for the voluntary intake of DM ($kg^{0.75}$), NFD, AFD, hemicellulose, cellulose and lignin were significantly different (P <0.05). The largest voluntary DM intake was obtained with the T3 and T_4diets (79.72 and 75.01 g / kg $W^{0.75}$). Similar behaviour was observed for NFD intake (T_3 = 550.90 and T_4=506.26 g/$sheep^{-1}$). The coefficient of apparent digestibility of DM, did not present any statistical variation (P <0.05). With regard to the ME concentration treatments, there were no significant differences (P <0.05); however T_2resulted in the highest concentration of ME (9.27 MJ / kg DM^{-1}). The trial

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showed that the foliage of *L. leucocephala* is an important local source of nitrogen which improves the nutritional value of a diet of *P. maximum* in sheep.

Keywords: Fodder tree, agroforestry, silvopastoral systems, supplementation, sheep.

INTRODUCTION

Livestock are components of several farming systems in the world and are increasingly viewed as important pathways for rural households to escape poverty (Pica-Ciammarra et al 2015;Smith et al 2004). In Mexico, scarce economic resources, poor training, low quality and quantity of feeds are major constraints, limiting sheep systems productivity among smallholder farmers (Echavarría-Chairez, et al 2010). In southeastern Mexico, within a tropical area that borders Central America, the raising sheep of for meat is mainly based on extensive management. It faces many technical and environmental constraints, particularly the annual drought between January and may that causes shortages in the availability and quality of pasture, thus affecting animal productivity (Espinoza-García et al 2015; Nuncio et al 2001).

Mexican farmers have used tree foliage in their animal production systems for centuries, especially for the raising of sheep and goats in the more arid central and northern regions of Mexico, using local browse or trees that grow naturally on their fallow and pasture land (Stienen, 1990;). In southeastern Mexico, silvopastoral systems have emerged over the last decade, using a wide variety of species of trees and shrubs with fodder potential (Jimenez et al 2007; Jiménez et al 2008; Olivares et al 2014). In this tropical region, most fodder trees are multi-purpose, providing products such as wood, firewood, human food, and environmental services such as soil erosion control, carbon sequestration and high biodiversity (Marinidou et al 2013).

There is a wide diversity of native fodder trees throughout Latin America (Murgeitio et al., 2011) and the genus *Leucaena* (Guash: local name in Chiapas, México) has a distribution range that extends from Mexico to South America. The species *Leucaena*is a multiple use tree that provides a cheap source of protein for animal feed (Norton et al 1994). Its fodder potential is based on high nitrogen content and biomass production. The leaves of *Leucaenaleucocephala* possess high nutritious value (20-30% of dry matter), are high in crude protein (20-34%), and present a good range of *in-vitro* digestibility (50-60%), (Jimenez et al, 2008).

The available nitrogen in foliage from several fodder trees (*L. leucocephala, Gliricidiasepium, Erythrinasp*) is very soluble, quickly degradable in the rumen and attaches to the cellular wall of fiber; therefore, the incorporation of this supplement is necessary in order to improve ruminal fermentation and animal response (Kass et al., 1990; Camero et al., 2001). Despite being a local

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resource *Leucaena* and easily accessible to producers, it is important to remember that excessive intake of this legume foliage, can cause a decline in animal intake due to the presence of anti-nutritive and toxics factors such as mimosine, condensed tannins, and flavanol glycosides (Jones, 1994; Ibrahim et al 2005).

The objective of this study was to evaluate the effect of the inclusion of increasing levels of *L. leucocephala* foliage in the diet of "Pelibuey" sheep fed with *P. maximum* cv. Tanzania, on the voluntary intake and apparent digestibility of DM.

MATERIALS AND METHODS

Study area

This study was conducted at a Silvopastoral Farm (Centro Agropecuario de Capacitación y Desarrollo Sustentable-(CACyDS) in Chiapa de Corzo, Chiapas, Mexico (16° 42' N y 93° 00' W), a dry tropical area located at 400-450 m above sea level in the Central Valley of Chiapas State. This area receives 900 mm rainfall per annum. Minimum and maximum temperatures vary from 17.5 to 26.0°C, respectively. The vegetation includes short grasses and trees and shrubs, especially *Leucaenaleucocephala, Gliricidiasepium*, *Guazumaulmifolia, Erythrinasp and Ceibapentandra.*

Animals, feeds and treatments diets

Five male Pelibueysheep (LW 30 ± 1 kg) were maintained in metabolic cages in the CACyDS, They were provided with feeders, waterers and individual feces and urine collectors (Lascano et al., 1990). Treatments consisted of diets with varying proportions of grass *P. maximum* cv. Tanzania and foliage (leaves and petioles) of *Leucaenaleucocephala*: 100-0 (T_1), 80-20 (T_2), 60-40 (T_3), 40-60 (T_4) and 20-80 (T_5) % respectively.

Different mixtures of chopped green forage *P. maximum* and L. *leucocephala L.* foliage were offered daily. These were harvested in the silvopastoral area where sheep grazed on pasture. *L. leucocephala* foliage was harvested from mature trees within the silvopastoral farm. The daily amount of biomass offered was adjusted according to the initial weight and the consumption requirements of DM, considering the nutritional requirements of the adult sheep (NRC, 2007). The animals were treated against external and internal parasites and were in a healthy condition before and during the experiment. Water was offered ad libitum and mineral salt was included daily with 10 g $sheep^{-1}$.

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Experimental design

Treatment diets were arranged in a Latin Square 5 x5 with five sheep. The five treatments were then randomly allocated to each animal. Each experimental period lasted 12 days, 7 for adaptation to the diet and 5 for recording measurements. (Table 1). The variables were: a) Chemical composition of the diet (b) Voluntary intake of DM g sheep^{-1} (c), apparent digestibility of DM , d) Concentration of metabolizable energy (MJ / kg-^{1} DM)

Table 1. Experimental design

Periods	Sheep				
	1	2	3	4	5
I	T2	T4	T5	T1	T3
II	T1	T3	T4	T5	T2
III	T4	T1	T2	T3	T5
IV	T3	T5	T1	T2	T4
V	T5	T2	T3	T4	T1

Treatments: T1= 100% *P. maximum cv*. Tanzania ;T2= 80% *P. maximum cv*. Tanzania and 20% *L. Leucocephala;* T3= 60% *P. maximum cv*. Tanzania and 40% *L. Leucocephala;* T4= 40% *P. maximum cv*. Tanzania and 60% *L.Leucocephala;* T5= 20% *P. maximum cv*. Tanzania and 80% *L. Leucocephala*

Metabolism trial (feed intake and determination of apparent digestibility)

During the experiment, a quantitative collection of feeds, refusals and feces was conducted to determine the apparent digestibility of the diets. Animals were acclimatized to the metabolism cages for 3 days after the 7 day adaptation period and prior to the 5 day collection period. Representative samples of foliage, concentrate, refusals and feces were collected daily and dried at 105 ◦C to determine the daily intake of DM for each animal. Other representative samples of each material, by animal for refusals and feces, were collected daily over the 10 day collection period, bulked, mixed, sub-sampled and ground to pass a 1 mm sieve for subsequent laboratory analysis.

Chemical analyses

Ground samples of feeds, refusals and feces were analyzed for dry matter (DM) by drying samples at 105 ◦C for 24 h in a forced air oven. Chemical analyses were conducted in duplicate. Ash content was determined by ignition of the feeds in a muffle furnace at 550 ◦C for 3 h (AOAC, 1990). The CP was determined by the Kjeldahl method (AOAC, 1990; ID 954.01). Ash-free neutral detergent fibre (NDF), ash-free acid detergent fibre (ADF) and acid detergent

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lignin (ADL) were determined using methods described by Van Soest et al. (1991). Metabolizable energy (ME) contents of the treatment diets were estimated according to the formulae (MAFF, 1975):

$ME(MJ\ kg^{-1}) = 0.15$ or $0.16DOMD\%$,
$DOMD\% = 0.98DMD\% - 4.8$

where DOMD% is digestible organic matter in DMand DMD ($g\ kg^{-1}$ DM) is the 48 h DM degradability (adopted due to its high correlation to the in vivo digestibility coefficient) (Chenost et al., 1970)

Voluntary Intake and Apparent digestibility

To estimate voluntary intake (sheep g MS^{-1}), the food offered and refused after 24 h was weighed (Minson, 1982). Apparent digestibility was estimated by the total collection of feces method (Schneider and Flatt ,1975).

Statistical analyses

The data were analyzed using the GLM procedure in SAS Software (SAS, 2015). The mathematics model used for intake and apparent digestibility of DM and chemical composition data was the following: $Yijkl=\mu+Si+PJ+Tk+Eijkl$, where μ was the overall mean, *Si* the fixed effect of square, *PJ* the fixed effect of period, *Tk* the fixed effect of treatments, and the *Eijkl* the random residual error. Differences were declared significant if $P<0.05$.

RESULTS AND DISCUSSION

Chemical composition of feeds

The chemical compositions of the feeds used in the treatments are presented in Table 2. As expected, *Leucaena* fodder presented higher crude protein (CP) content and lower neutral detergent fiber (NDF) concentrations when compared to the tropical grasses. *P. maximum* presented good protein, and a high content of NDF and acid detergent fiber (ADF). It can be observed that the values of PC for *P. maximum* were slightly higher than expected, possibly due to the effects of association with *Leucana* in the silvopastoral system. In this regard, Ruiz et al (2015) indicates the importance of legumes, such as *Leucaena,* fixing atmospheric N through a symbiotic relationship with bacteria belonging to the *Rhizobium* genus, improving productivity and quality of the biomass of grasses associated with silvopastoral systems. The DM content of *L. leucocephala* (89.6%) was similar to that reported by Nouel et al. (2006) who in evaluating voluntary intake and the apparent digestibility of diets based on a tropical legume tree, found values of 92.5% MS. Ku. Vera et al. (2010) in studies of fodder tree species in the states of Yucatan and Chiapas (Mexico), reported values for *L. leucocephala*, for OM, CP, NDF and FDA of 91.9, 18.6, 34.6 and 18.2%, respectively similar to those obtained in the present study.

Table 2. The chemical composition (dry base %) of *P. maximum* L. cv Tanzania and *Leucaenaleucocephala.*

Constituents	*P. maximum* L.	*L. leucocephala*
Dry matter	90.2	89.6
Ash	15.2	9.9
Crude protein	11.7	25.3
Crudefibre	31.2	17.3
Etherextract	2.1	3.5
Carbohydrates	39.8	44.1
Neutral detergent fibre	65.6	31.6
Acid detergent fibre	44.2	27.2
Hemicellulose	20.6	3.6
Organic Matter	88.7	91.3
Number of samples	6	6

CP values obtained in this study, for different levels of *L. leucocephala* inclusion, were reported as above the critical value (7%) by Minson (1982). Furthermore, the contents of OM and NDF (91.3 and 31.6%, respectively) suggest that the use of leaves of this legume permitted a favorable increase in voluntary intake of DM. as well as digestibility of the basal diet of animals (Norton et al., 1994).

Intake and "in vivo" digestibility

Table 3 shows the results of voluntary intake of DM, OM and cell wall constituents. There were no significant differences (P> 0.05) among treatments; however, the consumption of 40 to 60% *L. leucocephala* resulted in a numerical increase in sheep intake. At this level of inclusion(40 - 60 % *L. Leucoephala*) , it was found that voluntary intake of DM per kilogram of metabolic

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weight ratio was higher. Soltan et al., 2013, found that grass-fed L. *Cynodondactylon* (Bermuda) supplemented with up to 50% of *L. leucocephala* in the ration, demonstrated significant differences in voluntary DM intake (1480 and 1249 g of DM/sheep^{-1}, respectively) when compared to a diet of grass alone. Ruiz et al. (2011**),** mention that in assessing voluntary intake, apparent digestibility and nitrogen excretion of urinary origin in sheep fed with 0, 15, 30, 45 and 60%, *L. leucocephala*, resulted in a voluntary DM intake of 950, 1010, 1200, 1115 and 1089 g sheep^{-1}, respectively, results similar to those reported in this essay. The effect observed in this study was most likely due to good ruminal fermentation of OM of the fodder tree species, permitting a greater voluntary intake of food (Ku et al., 2010).

Table 3. Voluntary intake (g/DM sheep^{-1}) and different levels of *L. leucocephala* foliage

Component	Treatments				
	T1	T2	T3	T4	T5
Dry matter	860.30^{a}	819.50^{a}	1062.98^{a}	1027.55^{a}	876.76^{a}
Dry matter (kg$^{0.75}$)	52.47^{b}	49.17^{b}	79.72^{a}	75.01^{a}	54.35^{b}
Organic matter	730.44^{a}	704.60^{a}	928.22^{a}	911.99^{a}	789.67^{a}
Neutral detergent fibre	502.66^{a}	496. 68^{a}	550.90^{a}	506.26^{a}	357.00^{b}
Acid detergent fibre	405.79^{ab}	395.16^{ab}	455.84^{a}	406.95^{ab}	325.32^{b}
Hemicellulose	135.71^{a}	101.516^{b}	95.10^{b}	100.46^{b}	31.68^{c}
Cellulose	279.11^{a}	245.31^{a}	280.40^{a}	224.59^{ab}	162.47^{b}
Lignin	95.80^{c}	116.35^{bc}	132.77^{abc}	169.91^{a}	158.62^{ab}

Means with different superscript within rows differ (P<0.05)

Treatments: T1= 100% *P. maximum cv*. Tanzania; T2= 80% *P. maximum cv*. Tanzania and 20% *L. Leucocephala;* T3= 60% *P. maximum cv*. Tanzania and 40%*L. Leucocephala;* T4= 40% *P. maximum cv*. Tanzania and 60% *L. Leucocephala;* T5= 20% *P. maximum cv*. Tanzania and 80% *L. Leucocephala*

As shown in Table 3, increasing the inclusion of *L. leucocephala* foliage to more than 60% of the DM, led to a slight depression in DM intake. This effect may be attributable to the scarce presence of the alkaloidmimosine in the foliage of *L. leucocephala*, considering that this metabolite can exert toxic effects on animals (McSweeney et al., 2003), causing a decline in animal intake, especially when levels of inclusion exceed 40% of the diet in animals (Barros-Rodriguez, et al., 2012). Sandoval et al. (2005),when studying cattle in southeast Mexico, identified a drop in voluntary DM intake when animals were offered *L. leucocephala* compared to *Brosimumalicastrum* (15.6 and 55.4 g MS / kg0.75).

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It is worth noting that the L*eucaena* collected in this trial, may have had few toxic factors, allowing improved intake and scarce negative response of animals in T_4 and T_5. On a similar note, Borel (1990) mentions the existence of high variability in the characteristics of nutritional value and toxic factors in the foliage of fodder trees of the same species; this can be attributable to particular site conditions and provenance of the material used in the experiments.

In this study the NFD intake decreased as the amount of *leucaena* foliage in the sheep's diet of sheep of by up to 80% (357 g sheep^{-1}). This observation is most likely a result of the mature foliage offered and the concentration of structural carbohydrates in *Leucaenaleucocephala* (Table 3). With regard to the consumption of AFD and structural carbohydrates, there were fluctuations (P <0.05) in the results obtained in this trial.

Table 4 shows that there were no differences (P> 0.05) in the coefficient of apparent digestibility of DM. Good ruminal fermentation of OM was observed in T_2. However, results varied for other nutrients (P <0.05), particularly for NDF, ADF and cellulose, hemi cellulose and lignin. These values differ from obtained by Roa and Céspedes (2011) where a supplement of 5 kg of *Gliricidiasepium*was included to the pasture-based diet of sheep, reporting a digestibility of NDF and ADF of 62 and 59%, respectively.

Table 4. Apparent digestibility (%) of DM and different levels of *L. leucocephala* foliage.

Component	Treatments (%)				
	T1	T2	T3	T4	T5
Dry matter	57.51^a	59.46^a	56.71^a	54.23^a	58.74^a
Organic matter	60.41^{ab}	61.83^a	58.87^{ab}	56.29^b	60.57^{ab}
Neutral detergent fibre	54.61^a	56. 36^a	47.57^b	43.58^b	42.01^b
Acid detergent fibre	50.79^{ab}	54.96^a	45.30^b	33.90^c	37.09^c
Hemicellulose	66.65^c	61.81^{cd}	58.45^d	82.67^b	92.58^a
Cellulose	62.27^a	64.85^a	59.89^a	45.90^b	48.36^b
Lignin	43.39^a	46.42^a	19.60^b	20.26^b	27.86^b
ME (MJ/kg^{-1} DM)	9.06^a	9.27^a	8.83^a	8.44^a	9.09^a

Means with different superscript within rows differ (P<0.05). DM = Dry matter, ME = metabolizable Energy
Treatments: T1= 100% *P. maximum cv*. Tanzania; T2= 80% *P. maximum cv*. Tanzania and 20% *L. Leucocephala;* T3= 60% *P. maximum cv*. Tanzania and 40% *L. Leucocephala;* T4= 40% *P. maximum cv*. Tanzania and 60% *L. Leucocephala;* T5= 20% *P. maximum cv*. Tanzania and 80% *L. Leucocephala*

These results show that the level of inclusion of *L. leucocephala* foliage in the diet of sheep should be between 20 and 40%. Ku et al. (2006) note that the nutrient content and digestibility coefficient and MO of *L. leucocephala* show its great potential as forage for use in ruminants as a food strategy, especially during the dry season when food resources are scarce. ME content does not present any significant differences among treatments ($P <0.05$);however, T_2 presented the highest concentration.

CONCLUSIONS

In this study, sheep diet supplementation with *Panicum maximum* and *Leucaena leucocephala* leaves did not produce any significant differences ($P <0.05$) in the voluntary intake of DM. However, an increase in DM intake was observed when sheep consumed the T_3 = 60-40% and T_4 = 40-60%. The coefficient of apparent digestibility of DM remained unchanged at different levels including when *L. leucocephala* foliage was added to the sheep's diet. Inclusions of high levels of *Leucaena* (T_5) may not significantly depress DM intake; however it is essential that the toxic factors of the tree foliage used are assessed. The use of *L. leucocephala* foliage, a local resource with high nutritional value, as a supplement when feeding sheep with tropical grasses, can improve voluntary intake (g / DM $sheep^{-1}$).

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Conflict of interest. The authors declare that they have no conflict of interest.

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