Supplementation with foliage of *L. leucocephala*. Its effect on the apparent digestibility of nutrients and methane production in sheep

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Four growing-fattening sheep of the Pelibuey breed, with liveweight of 25 kg ± 3.5, canulated in the rumen were used in a change over design to determine the effect of supplementing *L. leucocephala* foliage on the intake, *in vivo* methane production and apparent digestibility (AD) of DM, OM and NDF. The diets consisted of 63% of fresh forage of *Pennisetum purpureum* cv. Cuba CT-169 and 37 % of concentrate (A) or 63 % de fresh forage of Pennisetum, 27 % of Leucaena and 10 % of concentrate (B). The experimental period was of 18 d: twelve of adaptation and six of sampling. The feed offer and rejection were weighed to determine the total intake. The chamber method (tunnels) with open circuit was used to collect the gas expired by the animals. The methane of the collected gas was determined by gas chromatography. The supplementation with leucaena foliage increased (P <0.01) the DM intake (% LW) and OM (kg) in 19 %, compared with the control treatment. It did not affect either the DM AD or the NDF AD. The total volume of produced methane in $L.d^{-1}$ did not differ between treatments (5.63 and 6.65, respectively). However, when these values were expressed in function of the DM intake, a methane diminish of 16 % was observed in the treatment with leucaena. Under these experimental conditions, the inclusion of 27 % of *L. leucocephala* in a basal diet of *P. purpureum* increased the DMand OM intake, and reduced, in 15.6 %, the methane production in L/kg consumed DM, without affecting the apparent digestibility of nutrients in sheep.

Key words: *digestion, methanogenesis, rumen, intake, leucaena*

Methane production (CH_4) by ruminants is an unavoidable consequence of the carbohydrates fermentation in the rumen. It is not only an ecological problem as the gas is considered the second of highest repercussion in the green house effect, but also represents a loss (7-12 %) of the feed crude energy that, as consequence, diminishes productivity (Soliva *et al*. 2003).

In the last years, the interest for using the plants to manipulate the ruminal fermentation and reduce methanogenesis is growing (Navas-Camacho *et al*. 1994, Hess *et al*. 2003, Galindo *et al*. 2006 and Delgado *et al*. 2007 and 2012). The secondary compounds in the plants, mainly tannins and saponnins, seem to be responsible for these effects. Studies conducted in Cuba indicated that the foliages of *Gliricidia sepium*, *Sapindo saponaria*, *Arachis pintoi*, *Stysolobium aterrimum* and *Leucaena leucocephala*,and many others, have defaunating effect (Galindo *et al*. 2006 and 2009). The methanogenic bacteria live in a symbiotic relation with the protozoa and defaunation may reduce the emissions between 20 and 50 %.

Leucaena is possibly one of the most used plants as animal feeding in Cuba. It is used as protein bank, in silvopastoral and agroforestal systems. At present, there are technologies that prove its good results (Álvarez *et al*. 2006). The leucaena's foliage has high levels of polyphenols, tannins and saponnins. Besides, its potentiality to reduce methanogenesis under *in vitro* conditions has been proved (Galindo *et al*. 2009). However, proving whether it keeps this potentiality under *in vivo* conditions is of practical interest.

The objective of this experiment was to determine the effect of supplementing with foliage of *L. leucocephala* on the intake, nutrients AD and methane production in growing sheep.

Materials and Mehtods

Animals and treatament. Four growing-fattening male sheep of the Pelibuey breed, eight months of age and liveweight of 25 kg ± 3.5, canulated in the rumen were used in a change over design with two treatments (A and B) and two replicates (animals) per treatment. The diets consisted of 63 % of fresh forage of *Pennisetum purpureum* cv. Cuba CT-169 and 37 % of concentrate (control diet, A) or 63 % of fresh forage of *Pennisetum purpureum* cv. Cuba CT-169, 27 % of *Leucaena leucocephala* and 10 % of concentrate (experimental diet, B). The concentrate was composed of 51.5 % of soybean meal, 40.5 of ground maize, 4.0 % of urea and 4.0 % of calcium phosphate.

Experimental procedure. The experiment was conducted in the Institute of Animal Science. The Pennisetum was harvested in the dry season (November-December), during the flowering season, with four months of establishment. The leaves were cut daily manually, up to a length of 5-10 cm. The leucaena foliage (leaves with petioles) was harvested daily in grazing areas. The chemical composition of the diets is presented in table 1.

The animals were placed in metabolism cages, with free access to water and feeds. The feed was offered during the morning hours. The experimental period was of 18 d, twelve of adaptation and six of samplings. The offered and rejected feed was weighed to determine the total intake. Representative samples of the feeds were

Table 1. Chemical composition of the diets

Indicators	Chemical composition, % DB				
	Ingredients			Diets	
	Forage *P. purpureum* CT -169	Leucaena	Concentrated	Diet A, without leucaena	Diet B, with leucaena
DM	24.46	26.28	-	-	-
Residual DM	89.40	89.56	90.88	90.22	89.94
CP	14.09	24.42	19.80	16.20	16.90
NDF	51.97	48.51	-	32.74	45.80
OM	89.01	92.20	95.13	91.27	88.57

taken during the experimental period and determined DM and once they were dried, they were mixed and placed into clean flasks for their further chemical analysis. Total collection of feces was carried out for five days to estimate the total apparent digestibility of the nutrients. The feces samples were weighed and mixed daily and 10 % of the total weight was frozen for its further chemical analysis. The DMAD was calculated from the formula DMAD=[(DM consumed – DM excreted)/DM consumed]*100

Determination of methane. The open circuit tunnel method was used for determining the methane. Metabolism cages (200 x 82 x 147 cm) adapted for this purpose were used to obtain individual chambers (tunnels). Each chamber was covered with polyethylene and opened a whole in the rear part (5 cm diameter), to which a plastic tube of 5 cm diameter was inserted with a vacuum pump connected to extract the gas let out by each animal. Another whole opened in the tunnel side, near the exit, made possible taking samples and measuring the air rate at the tunnel exit. A domestic fan was installed inside each chamber for the air-recirculation. The air rate inside the tunnel was measured with a manual anemometer. During the adaptation period, the tunnels were uncovered to keep the air flow. During the three days of consecutive sampling, each chamber was sealed with the animals inside, once the clean up was carried out and the feed was offered.

The sampling of the gas let out by the animals was conducted every one hour inside the tunnel and at the exit of the extractor. For this, a 50 cc syringe was used. The gas samples were placed in vacuum tubes of 10 mL. For determining the methane, 1 µL of the gas collected was taken and injected in the gas chromatographer Philips PU-4400 (fire ionization detector (FID) of helium as gas carrier (1mL.min^{-1}) at oven temperature of 60 ºC (attenuation at 200 ºC and temperature detector). The pure CH_4 (99.5 % pureness) was used as pattern. The methane concentration was determined by the formulas:

CH_4, liters =air volume at the tunnel exit* concentration of CH_4 in the tunnel

Air volume at the tunnel exit= flow rate (m/h) (measured with the anemometer) x exit area (m^2) x time (24h). (Correct all the values to the standard conditions of pressure and temperature)

Chemical composition. The DM, ash, OM and CP were determined according to AOAC (1995). The NDF was measured by the methodology of Goering and van Soest (1970).

Statistical analysis. The general linear model, SSPS system, was used to control the effects of the animal, treatment and period. When necessary, the differences between means were analyzed according to Duncan (1955).

Results and Discussion

Table 2 presents the results in respect to intake and apparent digestibility of the nutrients.

The supplementation of *P. purpureum* with leucaena foliage increased (P <0.01) the DM intakes (% liveweight of the animals) in 19 % compared with the control treatment. The OM ingestion was higher (P <0.01) in the ration with the legume foliage.

Similar results in respect to the higher DM intake when including leucaena in the rations of tropical grasses were previously reported by Delgado *et al*. (1996), Vitti *et al*. (2005), Babayeni and Bamikole (2006) and Longo *et al*. (2008). This plant is a legume with excellent chemical composition and acceptability, complementing the nutrients supply to the animals when offered in the proper proportions. This justifies its positive effects on the intake.

There were no differences between treatments for the apparent digestibility of the DM, OM and NDF (table 2). The DMAD ranged between 45 and 47 %, while that of the NDF was of 39 and 40 % for the treatments A and B, respectively.

The results of the literature are contradictory compared with the effect of the inclusion of tree and shrub forages on the apparent digestibility of the nutrients. The presence of tannins may affect the ruminal digestibility and apparent digestibility of DM, NDF and protein.

Studies conducted with tannin forages showed intake increase, without changes on the apparent digestibility of nutrients (Kaitho *et al*. 1998 and Abdulrazak *et al*. 2006).

Table 2. Effect of the supplementation with *L. leucocephala* on the intake and apparent digestibility of DM, OM, and NDF in sheep fed *P. purpureum* cv. CT-169

Indicators	Control	Control + Leucaena	Signification
DMI, kg	0. 86 ± 0.02	0.90 ± 0.02	NS
DMI % LW	2.79 ± 0.11	3.32 ± 0.11	**
OMI, kg	0.59 ± 0.02	0.70 ± 0.02	**
Apparent digestibility			
DM, %	45.01 ± 2.73	47.79 ± 2.37	NS
NDF, %	39.36 ± 2.98	40.67 ± 2.58	NS
OM, %	48.99 ± 2.87	52.39 ± 2.49	NS

** (P<0.01)

However, Longo *et al*. (2008) found in supplemented sheep, with leucaena levels between 20 and 60 % in the diet, significant reduction of the digestibility. This is attributed to diverse factors, mainly to the content of tannins and lignin of this legume. When foliages of trees and shrubs are used in the diet, the AD may vary depending on factors such as the supplementation level, species and animal category, type of foliage and basis pasture. The amount of condensed tannins and other secondary compounds present in the plants may also influence.

Table 3 presents the methane mean values produced in the rumen during the experimental periods.

Leucaena did not reduce the total volume of methane produced by the animals (5.93 and 6.55 L.day^{-1} for the treatments A and B, respectively). It did not modify either the results when expressed in relation with the metabolic weight (table 3). However, it is of interest that when the data were calculated in function of the consumed DM, the leucaena treatment diminished 15.6 % the CH_4 production in respect to the control treatment. This could have biological and environmental signification.

The methane emissions (g.kg DM^{-1} ingested) in animals fed legumes are usually lower (Whagorn *et al*. 2002) than those which diets are composed mainly of grasses, although this is not always valid (van Dorlang *et al*. 2007). The results in relation with the positive effect of these plants on reducing methane are explained by the presence of condensed tannins, lower fiber content, higher DM ingestions and faster passage from the rumen (Beauchemin *et al*. 2008, Jayanegara *et al*. 2012 and Patra 2012).

Different authors have demonstrated, under *in vitro* conditions, the capacity of leucaena and other shrubs to reduce the production of ruminal methane (González *et al*. 2007, Delgado *et al*. 2007, Soliva *et al*. 2008 and Galindo *et al*. 2010). However, the plants effect on the ruminal methanogenesis under *in vivo* conditions are not studied enough and the results vary in function of the experimental conditions. Hess*et al*. (2003) state that the tannins present in many legumes such as *Calliandra calothyrsus*, may be associated with the reduction of the methane production up to 50%, compared with the traditional diet of pasture alone. However, these authors also reported that species like *Cratylia argentea* and *Arachis pintoi* increased the methane levels, up to three or four times the determined amount is diets with pasture alone.

Woodward *et al*. (2001) found diminishing in the emission of CH_4 in sheep and dairy cows fed *Lotus corniculatus*, a legume with high levels of condensed tannins. The condensed tannins reduce the total population of protozoa in the rumen. Their presence modified the Entodiniomorphos, like a Holotrichia. This proves once again that the plant metabolites act as deafuning agents (Woodward *et al*. 2001). Recent studies carried out in grazing areas of *L. leucocephala* in association with mixture of natural pastures indicated that the ruminal protozoa reduced in the cows, when these trees were included in the system, apart from the inclusion level (30 or 100 % of the area) (Galindo *et al*. 2007). Posseti *et al*. (2008) proved that the inclusion of 20 and 50 % of leucaena hey in a ration of Bermuda grass (*Cynodon dactylon*) increased the levels of CH_4. However, there was interaction and the methane production diminished when adding yeasts.

In this study, the effect of leucaena foliage on the methane reduction was not statistically significant. It is possible that the percentage of foliage inclusion, the diet composition, the animal species, the experiment

Table 3. Effect of the supplementation with leucaena on the *in vivo* methane production in growing-fattening sheep.

Indicators	Control	Forage + Leucaena	SE (±)
CH_4 (L.d^{-1})	5.93	6.55	1.63
CH_4 (L.kg DM ingested^{-1})	9.04	7.63	2.06
CH_4 (L/LW$^{0.75}$)	0.53	0.59	0.14

duration, among other factors, would not allow reaching significant reductions of methane production. Studies to optimize the use of leucaena and other promising plants to achieve significant reductions of methane emissions to the environment under *in vivo* conditions without affecting the productive efficiency of animals may be addressed.

Under the established experimental conditions, the inclusion of 27 % of *L. leucocephala* in a basic ration of *P. purpureum* increased the DM and OM intake, and reduced in 15.6 % the methane production in L/kg DM consumed, without affecting the apparent digestibility of nutrients in sheep.

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