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REGULAR ARTICLES

Dry matter intake and digestibility of rations replacing concentrates with graded levels of *Enterolobium cyclocarpum* in Pelibuey lambs

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Abstract The aim of the study was to evaluate the effect of graded levels of *Enterolobium cyclocarpum* pods in the ration on feed intake and digestibility by Pelibuey lambs. Five dietary treatments were imposed where ground pods replaced concentrate diet at 0, 20, 30, 40 and 50 % of dry matter (DM), respectively. The concentrate portion was composed of ground sorghum, soybean meal, cane molasses and minerals. Five entire Pelibuey lambs with initial body-weight 34±2 kg were allocated in the treatments in a 5×5 Latin square design. Values of dry matter intake (DMI) and dry matter (DMD) and organic matter (OMD) digestibility were measured and metabolisable energy intake (MEI) estimated. Rumen degradation constants for *E. cyclocarpum* were also measured. There were no differences ($P>0.05$) in average DMI (86.6 g/kg$^{0.75}$) and OMI (81.2 g/kg$^{0.75}$) among treatments. As the level of incorporation of *E. cyclocarpum* pods increased, voluntary DMI and OMI increased, whereas apparent DMD and OMD decreased linearly. Average digestible DM (65 g/kg$^{0.75}$) and OM (61 g/kg$^{0.75}$) intakes were similar ($P>0.05$) among treatments. Similarly, MEI (0.976 MJ ME kg$^{0.75}$/day) was not different ($P>0.05$) among treatments. The potential rumen degradation (A+B) of ground pods of *E. cyclocarpum* was 866.4 g/kg DM. Ground pods of *E. cyclocarpum* can be employed for lamb feeding up to 50 % of the ration, without affecting DMI, DM apparent digestibility and MEI.

Keywords Feed intake · Tropical legume pods · Rumen degradation

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Introduction

In tropical Mexico, intensive sheep farming systems have been expanding during the last few years to meet the raising demand of mutton by the increasing human population. However, such systems are highly dependent on imported grain for feeding of the flock. The price of grains has been increasing recently, reducing profitability of sheep production. There are foliages and pods of tropical trees and shrubs with good chemical composition (crude protein, neutral detergent fibre) and digestibility which hold potential for sheep feeding in Mexico (Garcia-Winder et al. 2009; Chay-Canul et al. 2009). *Enterolobium cyclocarpum* Jacq. Griseb. (1860) is one of such plants. It is a leguminous tree which shed its pods during the dry season, and these in turn can be easily collected manually for animal feeding purposes (Moscoso et al. 1995). The yield of pods of *E. cyclocarpum* is about 86 kg per tree, containing 15.6 % crude protein (Andrade et al. 2008). Pods also contain 63 % nitrogen-free extractives suggesting the presence of substantial amounts of soluble carbohydrates (Serratos et al. 2008). Moscoso et al. (1995) incorporated 36 % of ration dry matter as ground

pods of *E. cyclocarpum* in Katahdin × Blackbelly lambs, reporting average daily weight gains (ADG) of 223 g. The foliage of *E. cyclocarpum* is also employed by farmers in several regions of Mexico as animal feed (Castro et al. 2006). In addition, the foliage of *E. cyclocarpum* may have environmental benefits due to its content of saponins [19 mg/g dry matter (DM)] (Hess et al. 2003) reported to reduce ciliate protozoa in the rumen (Koenig et al. 2007) and thus methanogenesis (Morgavi et al. 2010). In addition, reduction of ciliate protozoa in the rumen could increase N availability and efficiency of N utilization, by decreasing N excretion in the faeces of ruminants (Calsamiglia et al. 2010).

The foliage and pods of *E. cyclocarpum*, therefore, have potential to be incorporated in the ration of growing lambs to increase profitability of smallholder sheep farmers. However, when pods of *E. cyclocarpum* are fed in large amounts (75 % of ration DM) to cattle, it resulted in toxicity (Negrón et al. 1993) giving rise to skin lesions in the way of a severe serous dermatitis as well as lymphocytosis. The aim of this trial was to determine the optimum level of inclusion of ground pods of *E. cyclocarpum* fed to Pelibuey sheep.

Materials and methods

Description of the experimental site

The experiment was carried out at the University of Yucatan in Merida, Yucatan located at 20°52′04″ N, 83°32′2″ W, South Mexico. The climate in the region is hot, subhumid, with a mean annual rainfall of 984.4 mm and an average temperature of 26.8 °C (García 1973).

Experimental design and treatments

Five entire male Pelibuey sheep with a body weight (BW) of 34±2 kg (mean±standard deviation) were used. Five treatments were formulated to incorporate 0, 20, 30, 40 and 50 % of complete pods of *E. cyclocarpum* in the ration. The experimental design was a 5×5 Latin square (Cochran and Cox 1991).

Experimental animals and management

Sheep were housed individually in metabolic crates, each provided with a feeder and a water bucket. The metabolic crates were housed in an open building with a metal ceiling and concrete floor. Before the start of the trial, all sheep were dewormed with Ivermectin® (1 % solution; 1 ml for each 50 kg live weight), and an intramuscular injection of vitamins ADE (1 ml for each 10 kg live weight) was also applied.

Source of feeds and feeding

Pods of *E. cyclocarpum* were collected manually under the trees located north of the city of Merida, Mexico in April and May. Eight collections were made and stored in plastic bags. The pods were then mixed, and samples of 100 g were taken from all the bags to separate its components (seeds, husks and complete pods), which were ground separately through a 1-mm sieve. Samples were thoroughly mixed, and subsamples were taken for separate chemical analysis of seeds, husks and complete pods.

Complete pods of *E. cyclocarpum* were ground through a 3-mm sieve with a hammer mill (Azteca, Monterrey, Nuevo León, México). Five dietary treatments were formulated to incorporate complete pods of *E. cyclocarpum* in the ration. The concentrate diet consisted of mixtures of ground sorghum, soybean meal, cane molasses and minerals. The meal of complete pods of *E. cyclocarpum* replaced the concentrate diet by 0, 200, 300, 400 and 500 g/kg DM (Table 1).

The mixtures represented 90 % of the DM offered while the other 10 % was fresh chopped Taiwan grass (*Pennisetum purpureum*). Fresh grass and experimental rations were given simultaneously in the feeder. The experiment lasted 60 days divided in five periods of 12 days, each experimental period had 7 days of adaptation and 5 days for measurements.

Rations were formulated according to AFRC (1993) for male sheep of 34 kg BW to cover maintenance requirements and a weight gain of 250 g animal^{-1} day^{-1} with rations containing metabolisable energy (ME) of 13.5 MJday^{-1}, metabolisable protein (MP) of 102 gday^{-1}, and DM intake of 1.1 kgday^{-1}. Sheep were fed twice daily in equal halves at 0900 h and 1700 hours. Water was freely available during the day.

Measurements of voluntary feed intake

Sheep were fed ad libitum allowing a refusal of at least 10 g kg^{-1} of the feed offered the previous day. Feed refusals were

Table 1 Composition (in grams per kilogram DM) of experimental rations

Ingredients	Level of incorporation of pods				
	0	20	30	40	50
E. cyclocarpum	0	200.0	300.0	400.0	500.0
Ground sorghum	666.02	492.0	408.0	322.3	235.7
Soybean meal	136.61	110.7	94.6	80.4	67.0
Cane molasses	79.45	79.5	79.5	79.5	79.5
Minerals	17.86	17.9	17.9	17.9	17.9
Taiwan grass	100.00	100.0	100.0	100.0	100.0

weighed at 0900 hours the following day. Voluntary DM intake was calculated as the difference between feed offered and the amount rejected the following day.

Estimation of apparent digestibility and metabolisable energy

Apparent digestibility of DM and organic matter (OM) was carried out by total collection of faeces (Schneider and Flatt 1975), taking a 10 % sample of faeces produced daily during the last 5 days of each experimental period. At the end of each period, daily faecal samples for the 5 days were thoroughly mixed and aliquots were taken and frozen at −4 °C until analyzed. ME of ration (in megajoules per kilogram DM) was estimated according to the procedures described by McDonald et al. (2002), based on digestible organic matter in dry matter.

Rumen degradation of feedstuffs

Additionally, measurements of rumen degradation of DM of pods of *E. cyclocarpum* were carried out in four ruminally cannulated crossbred cows (*Bos taurus* × *Bos indicus*). The cannulas were of 10 cm internal diameter (Bar Diamond Inc., Parma, ID, USA). Cows weighed 350±7 kg (mean± standard deviation) and were fed ad libitum for 2 weeks with a ration based on chopped *Pennisetum purpureum* grass and supplemented with 1 kg commercial concentrate.

Samples of pods, husks and seeds were ground to pass a 3-mm sieve. The in situ technique of Ørskov et al. (2000) was employed using nylon bags of dimensions 7×14 cm (width, length), with a pore size of 53 μ (Lockertex, Warrington, England). Samples of 5 g were introduced in each bag. Rumen incubation times were: 0, 6, 12, 24, 36, 48, 72 and 96 h. Bags were introduced in duplicate to each cow at each incubation time. For this, lingerie bags (20×40 cm) provided with a zipper were used. Time 0 (T0) was measured by taking duplicate nylon bags containing the appropriate samples and washed using a domestic washing machine without being incubated in the rumen. Incubation (starting at 0800 hours) was carried out in reverse order to the incubation times, introducing incubation time 96 h first. Thus, all bags were withdrawn at the same time, introduced to buckets with water at room temperature to stop microbial activity and washed with tap water to eliminate excess digesta adhered to the bags. Then the bags were washed in an automatic washing machine for 6 cycles until the draining water was clear. In order to quantify the cold water-soluble fraction (T0), additional nylon bags containing the appropriate feed samples were washed in a washing machine (Whirlpool Mod ELA8624FQ0) using tap water at room temperature. Thereafter, the bags were introduced into a forced-air oven at 60 °C for 48 h to estimate the soluble fractions (T0) by the difference in weight before and after washing (T0) and/or incubation.

Percent dry matter degradation in the rumen was determined as the difference between dry matter incubated in the rumen and that remaining after incubation in percent (Ørskov and McDonald 1979). In order to estimate rumen degradation constants for DM, percent loss at different incubation times was introduced to the software NOWAY (Rowett Research Institute, Aberdeen, Scotland) and data were adjusted to the nonlinear equation proposed by Ørskov and McDonald (1979). Rumen degradation data of *E. cyclocarpum* were used to estimate metabolisable energy and protein supplies in the ration by using the appropriate equations described in AFRC (1993).

Chemical analysis

DM of pods, feeds and faeces was determined by introducing samples to a forced-air oven at 60 °C for 48 h. N content of samples was assayed by combustion using a LECCO CN-2000 series 3740 (LECCO Corporation) analyzer. OM was determined by combustion of samples in a muffle furnace at 600 °C for 6 h. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined as suggested by Van Soest et al. (1991). Crude fibre (CF) and ether extract (EE) were analyzed by the procedures described by AOAC (1980).

Statistical analysis

Data of feed intake and apparent digestibility were subjected to analysis of variance for a 5×5 Latin square model (Cochran and Cox 1991). In situ rumen degradability data were analysed as a complete randomized model. Treatment means were assayed with the Tukey's test. Additionally, surface response analysis was performed to assess the linear or quadratic terms of the responses (SAS 2004).

Results

Chemical composition

The chemical composition of rations was similar among treatments, although the ADF content increased gradually as the level of incorporation of ground pods of *E. cyclocarpum* was augmented (Table 2). Table 3 shows the chemical composition of the seeds, husks and complete pods of *E. cyclocarpum*. The seeds had numerically highest concentration of crude protein (CP), EE and NDF, but had the lowest concentrations of CF, ADF and lignin, followed by complete pods. Husks are the components with the greatest concentration of fibrous fractions and lowest crude protein.

Table 2 Chemical composition (in grams per kilogram DM) of experimental rations

Ingredients	Level of incorporation of pods				
	0	20	30	40	50
DM	860.2	869.5	868.9	873.8	869.9
CP	156.0	158.7	151.4	161.3	166.9
OM	941.7	938.0	936.0	934.6	932.3
ADF	38.2	81.1	99.8	126.1	140.6
NDF	461.9	351.8	303.9	294.9	291.8
EE	10.1	8.4	11.9	9.4	3.6
MP (g/kg DM)[a]	102.0	102.0	102.0	102.0	102.0
ME (MJ/kg DM)[a]	13.56	13.56	13.56	13.56	13.56

Mineral composition was taken from the label of a locally available commercial mixture

CP crude protein, *OM* organic matter, *EE* ether extract, *NDF* neutral detergent fibre, *ADF* acid detergent fibre, *MP* metabolisable protein, *ME* metabolisable energy

[a] Estimated as in AFRC (1993)

Intake and apparent digestibility of DM and OM

There were no differences ($P>0.05$) for dry matter and organic matter intakes (OMI) among treatments (Table 4). The intake of digestible DM (DDM) and OM (DOM) matter was similar among treatments ($P>0.05$), with a linear trend evident. Nevertheless, an increase in intake of DDM and DOM was observed as the level of *E. cyclocarpum* in the ration was increased.

There was no difference ($P>0.05$) in DM digestibility among treatments, nonetheless, DM and OM digestibility decreased linearly with increased levels of incorporation of *E. cyclocarpum* in the ration (Table 4). Apparent digestibility of DM was reduced by 8.97 % in the treatment with 50 % incorporation of pods of *E. cyclocarpum* relative to the control treatment (0 % *E. cyclocarpum*). Organic matter digestibility was significantly higher in the control (0 %) than in the other treatments. ME concentration was similar ($P>0.05$) for all treatments.

Table 3 Chemical composition (in grams per kilogram DM) of different fractions of pods of *E. cyclocarpum*

Chemical composition	Fraction of the pods of *E. cyclocarpum*		
	Husks	Seeds	Whole pods
CP	121.9	243.5	167.2
OM	960.1	961.6	957.3
CF	196.9	145.7	161.8
EE	15.0	22.1	15.9
NDF	325.1	399.3	353.6
ADF	255.6	214.8	231.7
Lignin	109.0	49.4	88.3

CP crude protein, *OM* organic matter, *CF* crude fibre, *EE* ether extract, *NDF* neutral detergent fibre, *ADF* acid detergent fibre

Rumen degradation of DM

Table 5 gives the kinetics of DM degradation of seeds, husks and complete pods of *E. cyclocarpum* in the rumen. It can be observed that the soluble fraction (A) of complete pods and seeds were similar without significant differences ($P>0.05$) among components. However, when seeds and complete pods are compared with husks, the latter had a greater ($P<0.05$) readily degradable fraction (A). The potentially degradable fraction (B) was greater for the seeds, followed by complete pods and husks ($P<0.05$) among the three components. Potential degradation (A + B) of DM of complete pods of *E. cyclocarpum* was similar to that of husks ($P>0.05$), but lower to that of the seeds ($P<0.05$).

Discussion

The observed chemical composition of pods of *E. cyclocarpum* in the present trial is in agreement with data reported by other authors (Cecconello et al. 2003; Alvarez et al. 2003; Peralta et al. 2004). The observed mean dry matter intake (DMI) in Pelibuey sheep was lower than that reported by Moscoso et al. (1995), who registered DM intakes of 105, 105, 110 and 114 g/kg$^{0.75}$ at levels of incorporation of ground pods of *E. cyclocarpum* 0, 12, 24 and 36 % of ration DM. Peralta et al. (2004) found a reduction in DMI of hair sheep as the level of incorporation of pods of *E. cyclocarpum* was increased from 0 to 30 % of ration DM. The average DMI of 91 g/kg$^{0.75}$ observed with the ration containing 50 % incorporation of pods of *E. cyclocarpum* in the present study is higher than the 62.13 g/kg$^{0.75}$ found by Alvarez et al. (2003) in sheep fed a ration containing 30 % of pods of *E. cyclocarpum* in a mixture with corn grain and minerals. As regard to MEI, sheep in the present study consumed twice the amount of ME reported by Cantón et al. (1995) Those authors suggested that the amount of ME required by hair sheep for maintenance to be 0.490 MJkg$^{0.75}$, which may imply that the sheep in this work consumed nearly twice their maintenance energy requirement.

The linear increase in DMI observed in the current study may suggest that the amount of DM of pods of *E. cyclocarpum* readily degradable (fraction a=567.3 g/kg DM) reduces physical fill of the rumen. As the level of incorporation of pods increased, the content of the readily degradable fraction consumed was also augmented, resulting in a quick fermentation of the material consumed and reducing retention time of digesta in the gastrointestinal tract (Chilibroste et al. 1997; Kyriazakis 2003). However, the

Table 4 Voluntary feed intake, apparent digestibility of DM, and intake of digestible DM and digestible OM by Pelibuey lambs fed graded levels of *E. cyclocarpum* in the ration

	Treatments[a]						
	0	20	30	40	50	SEM	Linear effect
Intake (g/kg$^{0.75}$)							
DM	73	87	88	94	91	6.0	–a[b]
OM	69	81	83	88	85	5.7	–a[b]
ME	0.9	1.0	1.0	1.0	0.9	0.07	–b[b]
Apparent digestibility (g/kg DM)							
DM	780	772	758	722	710	21.9	–a[b]
OM	795c[c]	776cd[c]	760cd[c]	723cd[c]	709d[c]	19.9	–a[b]
Intake (g/kg$^{0.75}$)							
DDM	58.88	67.29	66.85	67.59	64.65	5.02	–b[b]
DOM	55.82	63.45	62.70	63.21	60.17	4.74	–b[b]

DM dry matter, *OM* organic matter, *ME* metabolisable energy, *DDM* digestible dry matter, *OMD* digestible organic matter

[a] Levels of incorporation of pods of *E. cyclocarpum*

[b] Rows with the same letter indicate linear effect ($P>0.05$)

[c] Means in the same row with different letters indicate statistical difference Tukey ($P>0.05$)

values of DMI and OMI in the current trial were lower than those reported by Moscoso et al. (1995) in hair sheep which may be influenced by the high concentration of ADF in the ration, a result of the increased levels of *E. cyclocarpum* in the ration. The increase in the level of intake has a positive effect on rate of passage from the rumen provoking rapid outflow from this organ which in turn results in a reduction in apparent digestibility. This was observed in the present study, since the linear increase in DMI and OMI was reflected in the linear reduction in digestibility as the level of incorporation of complete pods of *E. cyclocarpum* in the ration was increased. The mean intakes of DDM and DOM were similar among treatments, suggesting a balance among the linear reduction in digestibility and the linear increase in intake (Shem et al. 1995; McDonald et al. 2002). In fact, in another trial carried out in this laboratory, Esquivel-Mimenza et al. (2010) recorded ADG of 239 g in sheep fed a ration containing 50 % of ration DM as ground pods of *E. cyclocarpum*. There were no behavioural changes observed in sheep due to saponins known to be present in the pods as reported in cattle by Negrón et al. (1993). Recent data from this laboratory (Ku-Vera et al. 2012) confirm the fact that it is possible to sustain daily weight gains of up to 240 g in hair sheep fed with 45 % of ration dry matter as ground pods of *E. cyclocarpum*.

Table 5 Kinetics of rumen DM degradation of different components of pods of *E. cyclocarpum*

Constant	Component of pods of *E. cyclocarpum* (g/kg DM)				*P* value
g/kg DM	Husks	Seeds	Pods	SEM	
T0	628.0a	443.0c	529.0b	7.04	<0.05
A	701.3a	510.4b	567.3b	19.21	<0.05
B	128.2c	424.2a	299.1b	13.04	<0.05
A+B	829.5b	937.3a	866.4b	9.88	<0.05
C	0.038	0.028	0.039	0.06	>0.05

Means in the same row with different letters indicate statistical difference Tukey ($P>0.05$). Kinetics of rumen DM degradation adjusted to the exponential equation: $P=A+B\ (1-\exp^{-C\times t})$ (Ørskov et al. 2000) where A is the intercept of exponential equation of fitted values on "*y*", B potentially degradable fraction, A+B degradation potential, C rate of degradation of "B" (per hour) and T0 DM loss of feed soluble in water without incubation in rumen

The observed negative linear reduction in apparent digestibility of DM may have been influenced ($R^2=0.25$) by the increase in the concentration of ADF (38.2, 81.1, 99.8, 126.1, 140.6 g/kg DM) as the level of *E. cyclocarpum* was augmented (0, 20, 30, 40 and 50 %). This may be due to the fact that the fibrous fraction of feedstuffs has considerable influence upon dry matter digestibility, being important both the composition as the amount of fibre. This association shows a negative correlation among ADF content and digestibility (McDonald et al. 2002; Kyriazakis 2003).

In ruminants, as the level of intake is increased, a faster rate of passage ensues, which decreases rumen degradation of feedstuffs since rumen microorganisms have less time to enzymatically attack feed components. Thus, the linear increase in voluntary intake in this trial may help to explain the linear decrease in ration digestibility, as a result of the smaller retention time of digesta in the gastrointestinal tract which in turn is a result of the lower time available for degradation of

fibrous components in the rumen (Djouvinov et al. 1994; Shem et al. 1995; Illius et al. 2002). OM digestibility for the treatment with 40 % incorporation of *E. cyclocarpum* was similar to the *in vitro* digestibility of DM reported by Moscoso et al. (1995) with 36 % incorporation of pods of *E. cyclocarpum* (723 g/kg OM vs 733 g/kg DM, respectively). OM digestibility showed the same trend towards a linear reduction as the level of pods of *E. cyclocarpum* was increased as observed for DMD, although only differences were observed among treatments 0 and 50 % incorporation of pods of *E. cyclocarpum* in the ration. This agrees with a 22 % reduction in OM digestibility when foliage (187 g/kg DM) of *E. cyclocarpum* was incorporated in the ration for sheep relative to a control ration without *E. cyclocarpum* (Koenig et al. 2007). Although in this trial saponins were not analyzed nor their effect on protozoa population can be ascertained, it may be assumed that they may have played some effect on ration digestibility as proposed by Mora et al. (1991) who suggested that protozoa were responsible for 34 % of fibre digestion which may help to explain the linear and significant reduction in digestibility of OM of experimental rations (0 vs 50 %). These results suggest that pods of *E. cyclocarpum* may be incorporated up to 40 % of ration DM without affecting utilization of other nutritional components of the ration.

The smaller potential degradation of complete pods and husks of *E. cyclocarpum* may be influenced by its content of ADF and lignin compared with that in the seeds which contain a high amount of readily degradable monosaccharides (38.9 %) and lower percentage of fibrous fractions (Serratos 1989). Under farm conditions, the seeds of *E. cyclocarpum* tend to pass intact through the gastrointestinal tract, being easily observed in faeces of ruminant animals, something that was not observed in the present trial since the seeds (and husks) were ground to pass a 3-mm sieve. Potential degradation of DM of complete pods of *E. cyclocarpum* (866.4 g/kg DM) observed in this trial was similar to that found (887.0 g/kg^{-1} DM) by other authors (Cecconello et al. 2003; Pinto et al. 2004). The rumen degradation data, however, cannot be directly compared since the regions in which both studies were carried out are rather different. It was observed that the husks of *E. cyclocarpum* had a higher potential degradation (829.5 vs 639.0 g/kg DM) compared with the values found by Cecconello et al. (2003). Even when complete pods of *E. cyclocarpum* contain a high concentration of ADF and lignin, the fractions quickly and potentially degradable as well as the potential degradation are high, since Ayala et al. (1997) showed that as the level of the fibrous fraction is increased, rumen degradation of DM is reduced (Table 5). However, potential rumen degradation of complete pods of *E. cyclocarpum* is superior to that determined for other tropical fruits with good potential for ruminant feeding such as those of *Phitecellobium saman* and *Caesalpinia coriaria* (707 and 683 g/kg DM, respectively).

Conclusion

Incorporation of increasing levels of ground pods of *E. cyclocarpum* in the ration of Pelibuey sheep had no effect on intake and apparent digestibility of DM and OM. At 50 % incorporation of pods, OM digestibility was reduced. Ground pods of *E. cyclocarpum* showed a high potential DM degradation in the rumen which explains the good intake of the ration and the relative high DM digestibility recorded in this trial. It is concluded that ground pods of *E. cyclocarpum* can be incorporated in the ration of Pelibuey sheep up to 50 % of dry matter.

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