Scientific Paper

Effect of different proportions of *Moringa oleifera:Cenchrus purpureus* on voluntary intake and nitrogen balance

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Abstract

Four male goats of the Alpine breed, two years old and 37,94 ± 4,87 kg of LW, were used, confined in individual metabolism cages during 60 days and distributed in a Latin square design (4 x 4), in order to evaluate the effect of different proportions of *Moringa oleifera*:*Cenchrus purpureus* cv. Cuba OM-22 on the voluntary intake and nitrogen balance. The treatments consisted in the inclusion of increasing levels of *M. oleifera* (0, 20, 40, 80 %) in substitution of *C. purpureus* cv. Cuba OM-22 (100, 80, 60, 20 %), and a control to which concentrate feed (6 g kg LW^{-1}) and forage from cv. Cuba OM-22 were offered *ad libitum*. The results of dry matter intake relative to live weight (3,5 % LW, $p < 0{,}0001$), metabolic weight (88,99 g kg $LW^{0{,}75}$, $p < 0{,}0004$) and protein intake (220 g d^{-1}, $p < 0{,}0001$) benefitted the ration 80:20 % *M. oleifera:C. purpureus*. Similar statistical performance was shown by the ingested nitrogen (35,20 g day^{-1}, $p < 0{,}0001$), retained nitrogen (19,78 g day^{-1}, $p < 0{,}0001$) and apparent nitrogen digestibility (92,31 %, $p < 0{,}0001$). It is concluded that the utilization of rations with inclusion of *M. oleifera:C. purpureus* cv. Cuba OM-22 in goat feeding improves voluntary intake and nitrogen utilization, although the best performance was achieved with 80 % of *M. oleifera*:20 % of *C. purpureus*; which increased digestibility and retention, and it also decreased the nitrogen excretion to the environment.

Keywords: goats, digestibility, nitrogen retention

Introduction

Goats in Latin America and the Caribbean are managed in grass grazing with insufficient biomass production to cover the feed and nutrient demand, mainly in the season with lower forage availability, situation that could be attenuated by using forage trees and shrubs which can satisfy their requirements (Sánchez, 2001).

Among these tree species, *Moringa oleifera* has been included in research protocols in Cuba aimed at studying its chemical composition and digestibility (Rodríguez *et al*., 2017), the utilization of biomass in the elaboration of mixed silages with grasses (Gutiérrez *et al*., 2013), the characterization of ruminal fermentation and improvement in the voluntary intake (Gutiérrez *et al*., 2015), as well as the productive performance of ruminants (García-López *et al*., 2017).

The utilization of this forage plant (fresh as well as processed) as complement in the conventional diets used in ruminant feeding offers a feeding alternative (Quintanilla-Medina *et al*., 2018).

In spite of all the results obtained, at present there is the need of developing feeding strategies adjusted to the requirements of ruminants, which help to prevent the excessive excretion of fecal and urine nitrogen to the environment. Based on the above-stated facts, the objective of this study was to evaluate the effect of different proportions of *M. oleifera* and *Cenchrus purpureus* cv. Cuba OM-22, on the voluntary intake and nitrogen balance in the goat species.

Materials and Methods

Location. The trial was developed during May-June (2014) in the department of Ruminant Management and Feeding, belonging to the Institute of Animal Science (ICA, for its initials in Spanish); which is located at 22º 53' North latitude, 82º 02' West longitude and at 92 m.a.s.l., in the San José de las Lajas municipality –Mayabeque province, Cuba.

Experimental procedure

Work was done with the forage plants *M. oleifera* cv. Supergenius and *C. purpureus* cv. Cuba OM-22, with approximately 50 and 27 days of age, respectively; they were cultivated on a typical Ferralitic Red soil, of fast desiccation and uniform profile (Hernández-Jiménez *et al*., 2015), with neither fertilization nor irrigation.

The forages were manually harvested during the first morning hours (8:00 am), were ground in a mechanical grinder until reaching an approximate particle size of 3,0-5,0 cm; and were mixed afterwards, according to the proportion, into the ration.

Four goat males of the Alpine breed, two years old and 37,94 ± 4,87 kg LW^{-1} (1,21 ± 1,48 kg $LW^{0,75}$), were used; they were confined in individual metabolism cages and distributed in a Latin square design (4 x 4), with four experimental periods of 15 days each (10 of adaptation and five of data collection), which corresponded to 16 total experimental units. Before the beginning of the experiment (30 days), deworming of the animals was carried out with Labiomec® (dose of 0,2 mg kg LW^{-1} 1 % ivermectin, from the entrepreneurial group Laboratorio Farmacéutico, LABIOFAM) and Praziquantel (dose of 10 mg kg LW^{-1}, produced by the pharmaceutical laboratories Bela Farma). Likewise, a unique dose was applied (2 mL) of the highly concentrated vitamin compound AD_3E FORTE (from the Medicine Research and Development Center of Cuba); and at the end of each period, at 48-hour intervals, double application was made (2 mL $animal^{-1}$) of B complex vitamins (produced by LABIOFAM).

The experimental treatments consisted in a ration constituted by increasing quantities of *M. oleifera* (20, 40 and 80 %), as replacement of *C. purpureus* (80, 60, 20 %), and a control with forage of *C. purpureus* cv. Cuba OM-22 *ad libitum* and concentrate feed (6 g kg LW^{-1}) on only one occasion (8:30 am). The ration was supplied at a rate of 3,5 % of the live weight on dry basis, minimum consumption quantity for goats in maintenance status according to Morand-Fehr (2005), more than 25 % to try to decrease selectivity and refuse level the next day. All the animals had access to water and mineral salts *ad libitum*.

The distribution frequency of the ration was in equal parts at two times during the day (10:30 am and 4:30 pm); the material in the feeding trough was removed in intermediate daily hours between the offers.

The concentrate feed was supplied on only one occasion (8:30 am). During five consecutive days per period, through the traditional system (offer-refuse), voluntary intake was calculated; the ration and the animals were individually weighed to adjust the feed intake.

Chemical indicators. Proximal chemical analysis of all the feedstuffs was carried out, according to the AOAC (2005); the neutral detergent fiber (NDF), by the method suggested by Goering and Van Soest (1970); and *in vitro* dry matter digestibility, through the KOH indirect method. The metabolizable energy of the grass forage was estimated according to Martín (1979), and the metablizable energy of the tree and the concentrate feed, according to Baxter (1964). The analyses (table 1) were conducted in the laboratories of analytical services of the Institute of Animal Science (LASEICA, for its initials in Spanish).

In order to study the nitrogen balance in the first five sampling days per period, daily samples were taken of the offered and refused feed. The total fresh feces collected from each animal were immediately refrigerated at 20 ºC and stored, to integrate four of them later into a composite sample. In the case of urine collection, under the cage a mesh was placed to hold the particles of feces and feedstuffs; the urine was kept in plastic containers with 10 mL of hydrochloric acid and it was frozen at a temperature of -4 ºC, to prevent its volatilization, until its later analysis. The total nitrogen content was analyzed by the micro-Kjeldahl method. The following variables were determined: feces fecal nitrogen (Nf), urine nitrogen (NU), ingested nitrogen (NI), retained nitrogen (NR); NR = (NI – Nf – NU) and apparent nitrogen digestibility (AND) = (NI – NF)/ NI x 100.

Statistical analysis. Variance analysis was carried out, after proving that the assumptions of variance homogeneity and normality were fulfilled. When necessary, Duncan's test was applied to express differences among means. Likewise, simple correlations and linear and multiple regressions

Table 1. Chemical composition of the feedstuffs used in the ration.

Feedstuff	DM (%)	CP (%)	ME (MJ/kg DM)	NDF (%)	IVDDM (%)
C. purpureus	26,40	4,0	7,91	81,84	52,49
M. oleifera	21,28	19,0	9,79	66,34	64,93
Goat supplement	88,10	16,4	10,25	8,50	-

DM: dry matter, CP: crude protein, ME: metabolizable energy, NDF: neutral detergent fiber.

among variables were made, and the statistical criteria of equation adjustment were stated. For processing the information the statistical package Infostat (Di Rienzo *et al*., 2012) was used.

Results and Discussion

Voluntary intake

Table 2 shows that the absolute dry matter intake (kg day^{-1}) differed among treatments (p = 0,001); similar statistical performance was shown by the dry matter intake relative to live weight (% LW, g kg LW^{0,75}). The highest value was reached with 80 % of inclusion of *M. oleifera* in the ration, similar to the control, although the last one included supplementation (6 g kg LW^{-1}) with a fast fermentation energy and nitrogen substrate which favors the enzymatic activity of microorganisms and the diet digestion (Blas-Beorlegui *et al*., 1995).

Likewise, the results of the multiple regression (Y_{DMI} = 0,0076 + 0,1322 MTEI – 0,0015 CPI; R^2 = 99,23; R^2 aj. = 98,89, ± SE = 0,01, p = 0,0001) indicated that the dry matter intake in the experimental rations was determined by increases in the protein and energy ingestion. Similar statistical performance was shown by the relation between protein and energy intake, which linearly increased ($Y_{CP:\ ME}$ = 6,552 + 0,1281 % *M. oleifera*; R^2 = 98,65, R^2 aj. = 97,77, ± SE = 0,44, p = 0,001) with the doses of *M. oleifera* in the ration.

In general, the response found could be ascribed to the fact that the use of trees increases the microbial activity and stimulates the efficiency of utilization of metabolizable energy (Jeovanny, 2008).

Another element of the diet that could show the energy quantity available in the rumen that is used for microbial growth is fermented organic matter (FOM), substrate used for the proliferation of bacteria, of which microorganisms synthesize as average per day 145 g of protein per kilogram of FOM (Stern *et al*., 1994). Thus, when considering the average protein intake per treatment, it can be inferred that the experimental rations achieved higher truly fermentable substrate at rumen level (0,99 ± 0,45 kg FOM^{-1}) than the control (0,52 ± 0,08 kg FOM^{-1}), although it was higher (1,52 ± 0,37 kg FOM^{-1}) with 80 % of *M. oleifera* in the ration.

On the other hand, when considering that to reach the efficiency in microbial synthesis, according to Stern and Hoover (1979), 30 g of N per kilogram of FOM are needed as average, only in the experimental groups the average the average value reached the limit (29,70 ± 13,58 g N kg FOM^{-1}), but surpassed by 80 % of *M. oleifera* (45,52 ± 10,97 g N kg FOM^{-1}), which should have generated higher quantity of energy and nitrogen to favor the growth of microorganisms and increase the microbial protein synthesis (Nocek and Russell, 1988), unlike the control in which the lowest value was obtained (15,64 ± 2,25 g N kg FOM^{-1}). Likewise, the regression results (Y g N kg FOM^{-1} = 12,602 + 0,3881 % *M. oleifera*, R^2 = 76,66, R^2 aj. = 75,84, ± SE= 6,5, p = 0,0001) between the grams of N per kilogram of FOM and the *M. oleifera* doses in the ration expressed improvements in the ruminal fermentation.

Table 2. Mean values of the nutrient intake for goats fed with different rations.

Indicator	*M. oleifera*:*C.purpureus*				SE ±	*p*
	0:100	20:80	40:60	80:20		
DMI, kg d^{-1}	1,30^{a}	1,24^{a}	1,24^{a}	1,37^{b}	0,01	0,0010
DMI, % LW	3,58^{b}	3,26^{a}	3,20^{a}	3,58^{b}	0,06	0,0001
DMI, g kg LW^{0,75}	87,71^{b}	80,88^{a}	79,65^{a}	88,99^{b}	1,73	0,0004
CPI, g d^{-1}	75,58^{a}	87,11^{a}	123,59^{b}	219,99^{c}	7,30	0,0001
MEI, MJ d^{-1}	10,70^{a}	10,25^{a}	10,71^{a}	12,93^{b}	0,50	0,0023
g CP: MJ ME	7,08^{a}	8,50^{b}	11,55^{c}	17,02^{d}	0,02	0,0001
NDFI, g d^{-1}	0,920	0,970	0,930	0,950	0,05	0,8187
NDFI, % PV	2,54	2,57	2,42	2,49	0,04	0,0649

DMI: dry matter intake, CPI: protein intake, MEI: metabolizable energy intake,
NDFI: neutral detergent fiber intake.
a, b, c, d: different letters on the superscripts in the same row indicate significant differences for p < 0,05.

The results of dry matter intake relative to live weight (% LW) in this study were higher than the ones reported by Gutiérrez *et al*. (2012) with basically fibrous diets composed by *Brachiaria brizantha* hay and supplementation at a rate of 6 g kg LW^{-1}, along with an activator of rumen fermentation for goats in maintenance. However, they were similar to the results found by Bacallao (2013) in lactating creole goats (3,4 % LW^{-1}), fed with a fresh integral ration composed by *Saccharum officicinarum* (sugarcane), *C. purpureus*, plus chicken dung (8 g kg LW^{-1}) as source of NPN.

Based on the similarity in the absolute dry matter intake (kg day^{-1}) and on the fact that the average energy intake values (11,14 ± 2,28 MJ kg DM^{-1}) exceeded the minimum requirements (10 MJ kg DM^{-1}) established by the NRC (2001) for maintenance goats, then it could be inferred that the dry matter intake could have also been associated to metabolic regulations, as a result of the increase in the energy intake (Krehbiel *et al*., 2006).

Concerning the NDF intake, all the treatments exceeded the mean value (1,53 % LW) reported by Carvalho (2002) for the goat species, who evaluated different NDF levels from a basic diet with Tifton-85 (*Cynodon* sp.) hay.

Nitrogen balance

The nitrogen balance (table 3) showed significant differences in the nitrogen ingestion (NI) and a trend to grow as *M. oleifera* increased in the ration (R^2 = 76,54, R^2 aj. = 75,80, ± SE = 5,05, p = 0,0001). These differences could have been influenced by variations in protein degradation, along with the dry matter (r = 0,58; p = 0,0001) and energy intake (r = 0,78, p = 0,0001).

The availability and synchronization of these nitrogen and energy substrates should have guaranteed the adequate fermentation and activity of rumen microorganisms (Clavero *et al*., 1997), especially when the animals consumed 80 % of *M. oleifera* in the ration; in this last one the intake was 25,64 ± 0,05 g NI kg DM^{-1}, and surpassed the 24 g NI kg DM^{-1} recommended by Tamminga and Verstegen (1992) as necessary minimum value to guarantee an adequate ruminal digestion, unlike the other experimental treatments 17,64 ± 6,06 g NI kg DM^{-1}) and the control (9,35 ± 0,20 g NI kg DM^{-1}) which were lower.

The results allow to assert that, although the energy availability should not have constituted a limiting factor –as stated above–, the differences in the nitrogen increase must have been associated with protein degradation. If it were increased, as should have happened in the treatment with the highest percentage of *M. oleifera* in the ration, accumulation of ammoniacal nitrogen at rumen level, its absorption and transformation in the liver would occur, and nitrogen would not be incorporated to the microbial mass, which would lead to an increase in the excretion of urine nitrogen (Salgado, 2006).

In the case of total nitrogen excretion, there were differences (p = 0,0001) and higher values when the animals consumed the rations with 40 and 80 % of *M. oleifera*, with regards to the treatment with 20 % of the tree in the mixture and the control, which were statistically similar. These differences were basically determined by urine excretion, not fecal excretion (p = 0,6167), although the latter was moderately related to the dry matter intake (r = 0,65).

Table 3. Nitrogen intake and balance of the ration.

Indicator	*M. oleifera:P. purpureum*				SE ±	*p*
	0:100	20:80	40:60	80:20		
Ingested nitrogen, g	$12,09^{a}$	$13,94^{a}$	$19,77^{b}$	$35,20^{c}$	1,16	0,0001
Excreted nitrogen, g	$7,11^{a}$	$10,06^{a}$	$13,88^{b}$	$15,42^{b}$	1,25	0,0001
Fecal nitrogen, g	2,97	2,31	2,69	2,83	0,37	0,6167
Urinary nitrogen, g	$4,14^{a}$	$7,75^{ab}$	$11,19^{bc}$	$12,59^{c}$	1,31	0,0002
Retained nitrogen, g	$4,99^{a}$	$3,88^{a}$	$5,90^{a}$	$19,78^{b}$	1,88	0,0001
Apparent nitrogen digestibility, %	$74,20^{a}$	$83,20^{b}$	$87,27^{b}$	$92,31^{c}$	1,68	0,0001
Ingested nitrogen kg MS^{-1}, g /kg	$9,35^{a}$	$11,27^{b}$	$16,01^{c}$	$25,64^{d}$	0,02	0,0001
Total excreted nitrogen/ingested nitrogen, %	$58,76^{ab}$	$74,84^{b}$	$71,31^{ab}$	$46,65^{a}$	8,71	0,0142

a, b, c, d: different letters on the superscripts in the same row indicate significant differences for $p < 0,05$.

Likewise, it was observed that the urine excretion was directly related (r = 0,56) with the percentage of *M. oleifera* in the ration; 80 % of *M. oleifera* exceeded the control and the treatment with 20 % of M. oleifera in 8,4 and 4,8 g of excreted urinary N, respectively. This effect could be corroborated with the increase in the apparent N digestibility, which significantly differed from that of the other treatments.

The results in nitrogen retention, in spite of differing and although the highest value was reached with 80 % of *M. oleifera* in the ration, showed a high degree of adjustment (r = 0,64) as *M. oleifera* in the ration increased, as well as adjustment with the dry matter intake (r = 0,62) and the protein intake (r = 0,90). Such performance does not seem to inhibit the utilization of available nitrogen by the rumen microorganisms when this tree forage is used in the ration (Valdes *et al*., 2015).

It is concluded that the utilization of rations with inclusion of *M. oleifera*:*C. purpureus* cv. Cuba OM-22 in goat feeding improves the voluntary intake and nitrogen utilization; the best performance was reached with 80 % *M. oleifera* and 20 % *C. purpureus*, which increased digestibility and retention, and decreased the nitrogen excretions to the environment.

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