https://doi.org/10.22319/rmcp.v10i2.4455

Artícle

Replacement of alfalfa with *Tithonia diversifolia* in lambs fed sugar cane silage-based diets and rice polishing

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Abstract:

The aim was to evaluate the effect of replacing alfalfa (AA) with *Tithonia diversifolia* (TD) in sugar cane silage (SCS) based lamb diets, both with or without supplementation with rice polishings (RP), on *in vitro* digestibility, nitrogen retention and productive performance. The experimental diets (D) contained the following: D1) 68.6 % of SCS and 29.4 % of TD; D2) 63.7 % of SCS and 34.3 % of AA; D3) 46 % of SCS, 22.6 % of TD and 29.4 % of RP; and, D4) 44.1 % of SCS, 24.5 % of AA and 29.4 % of RP. Diets were isoproteinic and isocaloric. The *in vitro* digestibility of dry matter and organic matter were greater ($P<0.05$) with D2 compared to D1 and increased ($P<0.05$) with RP supplementation, with no difference between D3 and D4. Similarly, productive performance was not different (P>0.05) between diets containing TD or AA. Nevertheless, RP improved (P<0.01) dry matter intake, average daily gain and total body gain with both forages. In conclusion, in sugar cane silage based diets, the replacement of alfalfa with *Tithonia diversifolia* has no effect on DM digestibility, N retention or productive performance. Complementation with rice polishings improves DM digestibility, N retention and productive performance in animals fed forage rich diets such as those utilized in this study.

Key words: *Tithonia diversifolia*, Digestibility, Productive performance, Nitrogen retention.

Received: 31/03/2017

Accepted: 20/02/2018

Introduction

In tropical regions, sugar cane forage is frequently utilized by farmers in ruminant feed(1). Due to its low cost of dry matter produced and harvest concur with the poor availability of forage, although daily cut is a common practice may not be economical because of the labor implied(2). The ensiling of sugar cane is a forage conservation method that can increase NDF digestibility(3) and soluble carbohydrates(2,4,5). Some positive effects on digestion and productive performance encourage its research, for example, crude protein of ensiled sugar(6) and feed efficiency was higher in dairy cows fed with inoculated sugar cane silage respect fresh sugar cane(7). However, its TDN value is low and the N content of sugar cane silage is insufficient to maintain N equilibrium due to its poor N content(8).

The shrub commonly called “gold button” or “wild sunflower” (*Tithonia diversifolia*) contains between 14 and 28 % of crude protein, and has high ruminal degradability of dry matter and low phenol and tannin content(9). Crude protein of *Tithonia diversifolia* is similar to alfalfa but the former is higher in rusticity. Moreover, the price of alfalfa is high because of its low availability in these regions. As a cheap source of protein, *Tithonia diversifolia* may be utilized up to a 30 % level of inclusion without negative effect(10,11) on productive performance and digestibility.

Energy and N supplements increase the efficiency of ruminal microorganisms in forage fed ruminants(12). Nevertheless, nitrogen digestibility may be altered by the energy source because of the different site of the digestion(13). Rice polishing is a byproduct with similar metabolizable energy and greater protein values respect high used grains such as sorghum and corn[14] because of its elevated protein and lipid content[15] . Positive N-balance has been observed with diets that include 25 to 50 % of rice polishings in the concentrate mixture of lactating goats[16] and cows[17]. Also, rice polishing inclusion in diets based on sugarcane has improved the productive performance of cattle[18,19], but there is no evidence about the influence of rice polishing supplementation in diets based on sugar cane silage. The objective of this study was to evaluate the effect of the replacement of alfalfa with *Tithonia diversifolia* in sugar cane silage based lamb diets with and without rice polishings complementation.

Material and methods

Animal management procedures were conducted within the guidelines of locally-approved techniques for animal use and care NOM-051-ZOO-1995; technical specifications for the care and use of laboratory animals.

This experiment was conducted at the Unidad Académica de Medicina Veterinaria y Zootecnia of the Universidad Autónoma de Nayarit located in Compostela, Nayarit, Mexico (21° 17´46´´N and 104° 54´ W).

The *T. diversifolia* was harvested at the university 60 d after the last cut. The forage was then chopped to obtain a particle size of between 2 and 3 cm and sun dried for 72 h and turned every 24 h. The sugar cane silage was prepared utilizing whole sugar cane plants harvested (24 mo after sowing) at a farm with clay soil near the experiment location. Sugar cane (SC) plants were chopped to obtain a particle size of between 2 and 6 cm. Three (3) percent of homemade inoculum (10 % of sugar cane molasses, 0.5 % of urea, 5 % of poultry waste, 1 % of yogurt and 83.5 % of water) [20,21], 1 % of urea, 0.1 % of ammonium sulfate and 0.25 % of diammonium phosphate (on wet basis) were added to SC by spraying it on each layer of sugar cane during ensiling. Sugar cane forage was compacted with a tractor at each 30 cm layer, with the compacted SC forage then covered with polyethylene and 15 cm of soil during 45 d.

Chemical analyses

The experimental diets (Table 1) and their ingredients were analyzed for dry matter (DM), crude protein (CP), ash and ether extracts (EE) determination(22) Also, the neutral detergent fiber (NDF) and the acid detergent fiber (ADF) were determined(23). Gross energy was determined by calorimetric bomb(24). The digestibility of the dry and organic matter were determined *in vitro*(25).

Table 1: Ingredients, chemical composition and gross energy of experimental diets

Item	AA	TD	AA+RP	TD+RP
Ingredient (%):				
Sugar cane silage	63.73	68.63	44.12	46.08
T. diversifolia	-	29.41	-	22.55
Alfalfa	34.31	-	24.51	-
Rice polishing	-	-	29.41	29.41
Mineral premix	0.98	0.98	0.98	0.98
Salt	0.98	0.98	0.98	0.98
Composition (%):				
DM	91.33	93.12	91.14	92.71
CP	17.44	17.5	17.1	17.47
Ash	9.14	9.8	8.59	10.3
EE	1.01	1.05	3.92	3.47
NDF	50.55	52.85	49.84	44.75
ADF	32.16	34.65	27.16	26.19
Lignin	16.54	14.71	18.65	11.4
GE,(Mcal/kg	3.76	3.76	3.97	3.86

TD= sugar cane silage plus *Tithonia diversifolia*, AA= sugar cane silage plus alfalfa, TD+RP= sugar cane silage, *Tithonia diversifolia*, and rice polishing, AA+RP= sugar cane silage plus alfalfa, and rice polishing, DM= dry matter, CP= crude protein, EE= ether extract, NDF= neutral fiber detergent, ADF= acid detergent fiber, GE= gross energy.

Mineral premix contains P (30%), Co (30 ppm), I (13 ppm), 26 ppm of Se (organic plus organic), Cu (230 ppm), 1170 ppm of Zn (organic plus organic), Mn (150 ppm), Cu (150 ppm), Cr (110 ppm), Fe 80 (ppm), Mg (0.9 ppm), buffer (60%), NaCl (1.35%), Vitamin (A 200,000 UI), Vitamin D (100,000,000 UI), Vitamin E (100,000 UI), Ionophore (1200 ppm) and Ca (3%).

Animals and diets

Four (4) Blackbelly uncastrated male sheep (35 ±1.2 kg of body weight) with cannulas in the rumen were assigned to a 4 x 4 latin square design to determine N retention. These animals were utilized as donors of rumen fluid to determine *in vitro* digestibility of dry matter and organic matter. Housed in individual metabolic cages (60 x 180 cm) with steel mesh flooring. The experiment consisted of four experimental periods of 21 d, 14 for diet adaptation and 7 for urine and feces collection. On adaptation period, feed was offered at 110 % of that consumed the previous day so sheep had *ad libitum* access. During the samples collection period, sheep received the 90 % of the feed offered *ad libitum*. Each animal received a different experimental diet (Table 1) in each period. Two experimental diets included alfalfa because of its similar protein content range respect to *T. diversifolia* and the proved high nutritive value of alfalfa. The urine from each animal was collected in a bucket placed at the bottom of the metabolism cage while the feaces were collected in a bag collocated in the animal. A sample of 10 % of the urine and faeces collected were taken and frozen at -4 °C.

Twenty (20) Blackbelly x Pelibuey male lambs (2 mo old) with a mean body weight of 11.5 ± 2.99 kg were used in the feeding trial. Animals were allotted in individual pens (0.95 x 1.1 m) with a rice straw bed that was turned every other day and replaced every 2 wk to keep them dry. Each lamb was provided individual feed and waterers. The experimental diets were formulated to meet nutrient requirements of hair breed lambs(26) recommendations (Table 1). These were assigned randomly to each lamb were both isocaloric and isoproteinic. Each diet was provided to 5 lambs. Animals were fed *ad libitum* once daily at 0800 h. The first 7 d were for adaptation to treatment followed by 119 trial days.

Initial and final body weights were obtained after the morning meal using an electronic scale. Body weight gains were calculated by subtracting the previous weight from the current weight. The average daily body weight gains were calculated by dividing body weight gain by the number of days between two weighing days.

Statistical analyses

The data obtained from the trial to nitrogen balance was analyzed as a 4 x 4 Latin square, while the data from the feeding experiment was analyzed as a completely randomized design using the GLM procedure of SAS[27]. Comparisons between treatment means were analyzed with Tukey test (P<0.05) when significant treatment differences were detected.

Results

Chemical composition of the feeds

The chemical composition and gross energy of the ingredients comprising the experimental diets are presented in Table 2. The crude protein, NDF, and ADF of *Tithonia diversifolia* were 30.7, 21 and 30 % higher than alfalfa, respectively. However, the lignin content calculated for alfalfa is 15.9 % higher compared to *T. diversifolia*. The silage pH enabled optimal conservation and was reflected in terms of odor and color.

Table 2: Chemical composition (%) of diet ingredients on dry matter basis

	T. diversifolia	**Alfalfa**	**SCS**	**RP**
DM	28.31	93.71	29.44	89.99
CP	23.54	18.01	13.36	14.37
Ash	16.82	10.94	8.72	7.08
EE	1.32	1.63	1.09	4.99
NDF	56.20	46.41	49.71	13.66
ADF	37.14	28.70	31.30	6.55
Lignin	19.10	22.13	13.42	3.21
GE, Mcal/kg	3.65	3.73	3.89	4.2
pH			3.57	

SCS= sugar cane silage; RP= rice polishing; DM= dry matter; CP= crude protein; EE= ether extract; NDF= neutral detergent fiber; ADF= acid detergent fiber; GE= gross energy.

The chemical composition and gross energy of the experimental diets are presented in Table 1. Fractions (NDF and ADF) of the diets decreased while ether extracts increased with the addition of the rice polishings.

In vitro digestibility of dry matter

The *in vitro* digestibility of dry matter (DDM) and organic matter (OMD) are shown in Table 3. The inclusion of the rice polishings increased (P<0.05) DDM and OMD levels in the TD diet only. The lowest digestibility level was observed with the TD diet, despite the similar NDF and ADF content compared to AA diet.

Table 3: *In vitro* dry (DDM) and organic matter (OMD) digestibility of the experimental diets (%)

Item	AA	TD	AA+RP	TD+RP	SEM	*P*-value
DDM	63.91 b	59.82 c	65.13 b	68.93 a	0.3	<0.001
OMD	67.74 b	62.29 c	68.09 b	73.27 a	0.19	<0.001

TD= sugar cane silage plus *Tithonia diversifolia;* AA= sugar cane silage plus alf alfa, TD+RP; sugar cane silage, *Tithonia diversifolia*, and rice polishing; AA+RP= sugar cane silage plus alfalfa, and rice polishing

SEM= standard error of the mean.

ab Means in the same row that do not have a common letter differ (P<0.05).

Nitrogen balance

Nitrogen (N) intake, N absorption and N retention (% and g of intake) increased (P<0.05) with RP complementation, while urinary levels of N increased with no RP (Table 4). N retention as a percentage of the N absorbed was 30% greater with *T. diversifolia* compared to alfalfa when no RP was added to diets. Nitrogen retention as a percentage of the N absorbed increased with RP supplementation in both alfalfa and TD diets.

Table 4: Nitrogen balance of lambs fed sugar cane silage based diets supplemented either *Tithonia diversifolia* or alfalfa and rice polishing

Item	AA	TD	AA+RP	TD+RP	SEM	*P*-value
N intake, g	14.35 b	13.16 b	19.64 a	17.38 a	1.06	<0.01
Fecal N, g	1.77	1.72	1.66	1.65	0.16	>0.05
N absorbed, %	87.71 b	86.96 b	91.50 a	90.48 a	0.77	<0.01
Urinary N, g d^{-1}	9.94 a	7.98 b	8.11 b	7.86 b	0.4	<0.01
Retention, g d^{-1}	3.12 b	3.46 b	7.74 a	7.87 a	1.24	<0.05
N Retention, %	21.54 b	26.28 b	38.86 a	45.24 a	4.91	<0.05

AA= sugar cane silage plus alfalfa; TD= sugar cane silage plus *Tithonia diversifolia;* TD+RP= sugar cane silage, *T. diversifolia*, and rice polishing; AA+RP= sugar cane silage plus alfalfa, and rice polishing

SEM= standard error of the mean.

ab Means within each row followed by different letter differ significantly (P<0.05).

Productive performance

The productive performance of lambs fed different experimental diets is shown in Table 5. Feed intake, average daily weight gain, and total weight gain increased (P<0.05), while feed conversion decreased (P=0.02), with the inclusion of rice polishings in diets with either TD or alfalfa. No difference was observed between alfalfa and TD, either with or without RP complementation.

Table 5: Productive performance of lambs fed sugar cane silage plus either alfalfa or *Tithonia diversifolia* with or without rice polishing

Item	AA	TD	AA+RP	TD+RP	SEM	*P*-value
Initial BW, kg	10.5 a	11.9 a	10.9 a	12.4 a	2.76	>0.05
Final BW, kg	13.3 b	14.35 ab	19.5 a	18.5 ab	3.37	>0.05
DMI, g d^{-1}	336.5 b	341.5 b	512.1 a	443.7 a	45.98	<0.001
ADG, g d^{-1}	23.7 b	20.8 b	72.5 a	51.7 a	15.07	<0.05
Total gain, kg	2.82 b	2.47 b	8.62 a	6.15 a	1.79	<0.001
F:G	16.5 ab	19.04 a	7.5 c	8.71 bc	5.19	<0.05

AA= sugar cane silage plus alfalfa; TD= sugar cane silage plus *Tithonia diversifolia*; TD+RP= sugar cane silage, *Tithonia diversifolia*, and rice polishing; AA+RP= sugar cane silage plus alfalfa, and rice polishing

BW = body weight, DMI= dry matter intake, ADG= average daily gain in grams per day; F:G= feed conversion.

abc Means in the same row followed by different letter differ significantly (P<0.05).

Discussion

The ADF content of *T. diversifolia* in this study is greater than the values reported by different authors(10), who utilized *T. diversifolia* leaves only, while the CP content concurred with others(10,28). The nutrient content of *T. diversifolia* depends on age and plant part(29) while the higher ash content of *T. diversifolia* compared to alfalfa may be explained by its elevated concentration of minerals(30) such as Ca, P, and Mg(31). Higher NDF and ADF content in the non-RP diets reflect the higher fiber fraction concentration in *T. diversifolia* and alfalfa compared to RP, while EE content increased with the addition of RP. Concurs with the higher ether extracts in rice polish based concentrate compared to concentrate with wheat bran observed(17). The crude protein content of sugar cane silage (13.36 %) was higher than that without the addition of inoculums(8). Silage pH value reflected good fermentation(8) and was around to those reported in pure sugar cane and sugar cane plus 0.5 and 1 % of formic acid(32).

The protein solubility (40 %) of *T. diversifolia* fodder(33) is one limitation for the use of high levels of this forage in lamb diets(28), a limitation that may be avoided by including rapidly fermentable carbohydrates(34). There is a synergy between protein and sugar supplementation because the microbial population is improved to a greater extent when protein is supplied in addition to sugar rather than when they are supplied separately(35).

Rice polishings facilitated the digestion of the TD diet due to the increased energy caused by the increment in ether extract and highly fermentable carbohydrates in the rice polishings. Moreover, the lower NDF content in the diets containing RP led to greater digestibility due to the negative relationship between NDF and digestibility. While complementation have increased the total diet digestibility of organic matter, the fiber-based energy have presented greater digestibility levels than a grain based supplement(36). In order to maintain an acceptable intake and digestion of low quality forages requires the synchronization of the degradable protein and carbohydrate supply from the energy supplement(12).

Similarly, dry matter digestibility values between 60 and 66 %, and values of between 64 and 68 % for organic matter digestibility when *T. diversifolia* leaves were supplemented at levels of 10 and 40 % have been observed(37). In contrast, with the lower *in vivo* digestibility (41 to 53 %) in diets with 20, 35 and 50 of *T. diversifolia* combined with Taiwan grass and concentrate(28). The utilization of different diet composition and techniques for digestibility determination may explain the variable results observed in different trials. The Taiwan grass(28) contained a similar percentage of NDF to the levels for sugar cane silage recorded in this study. However, the protein in sugar cane silage is twice that of Taiwan grass.

There is a close relationship between *in vitro* digestibility and rumen undegradable protein digestibility (r^2=0.090) and a moderate relationship between rumen undegradable protein and CP (r^2= 0.48 and 0.42)(38).

Similar N retention as a percentage of N intake to diets with greater digestibility (80 %) caused by a greater amount of concentrate in the diet have been mentioned(39). In dairy cattle, NDF content is inversely related to N digestibility(40). Although the NDF content of *T. diversifolia* is greater than alfalfa, N retention as a percentage of absorbed N is 30 % greater in TD compared to AA diet, which can be attributed to the higher essential amino acid content of *T. diversifolia*(41), compared to alfalfa(42) which could be mobilized for tissue deposition or the synchrony between the degradation of energy and the protein contained in the ingredients of the diet. Similarly, greater concentration of serum protein have been found when animals, fed with threshed sorghum top, were supplemented with plant foliage(43).

The high amino acid content in *T. diversifolia* enhances its N availability(9), which concurs with the fact that protein supplements with sufficient essential amino acid content, such as fish meal, have recorded high digestion and absorption levels(44). Rumen undegradable protein digestibility may not be similar for all forages. Improved N retention is desirable since it has a favorable impact on both the environment and the production economy because of the reduction of N loss through urine and feces. Based on the present results, supplementation with forage with high quality protein may improve N retention with rice polishings complementation. On the other hand, greater N retention with lower levels of *T. diversifolia* in concentrate had been attributed to the anti-nutritive components of this forage(9). Nevertheless, no difference in N retention was observed when with alfalfa diets were compared to those with *T. diversifolia* diets in this study.

Dry matter intake was improved by RP supplementation in both alfalfa and TD diets. Similarly, higher total dry matter intakes when concentrate was added to the whole sugar cane plant diets of goats and lambs (11 kg live weight) compared to those without concentrate were observed; however, they did report live weight losses(45). Also, average daily gains (26.1 g/d) similar to those recorded in this study have been found in goats fed diets based on *Panicum maximum* plus 30 % of *T. diversifolia* and concentrate(11).

The DMI observed coincides with that of diets with dry matter digestibility between 64 and 68 %(9). Although the *in vitro* digestibility of the DM and OM in TD+RP was the highest, the ADG was similar (P>0.05) to that in AA+RP in response to the same dry matter intake. Intake is positively related to production(46) and depends on the ability of the feed to provide the nutrients needed(9).

The RP diets contained a lower NDF percentage due to the fact that the rice polishings contained around one third of the NDF of alfalfa or *T. diversifolia*. The NDF content of all the experimental diets was higher than the 25 % which promotes digestibility, intake and weight gain(47,48). The elevated levels of forage in the diet may limit energy intake and ADG(49). Nevertheless, the similar performance of animals fed AA and TD encourages the further study of the effect of *T. diversifolia* supplementation in diets with higher energy and rumen undegradable protein content and lower fiber content on the performance of young animals.

The superior N retention and DMI of lambs supplemented with RP was reflected in increased ADG. Rice polishings improved ADG, since its inclusion decreases fiber fraction percentage and increases the energy content of diets. The energy supplements influence animal performance and forage utilization, potentially increasing the opportunities for nutrient synchrony in the diet(12).

The substitution of alfalfa with *T. diversifolia* reduced the feed cost with and without rice polishings complementation, 133 % ($1.02 *vs* $2.38 MXN) and 49 % ($1.97 *vs* $2.93 MXN), respectively. The sugar cane silage-based diet supplemented with *T. diversifolia* and complemented with rice polishing showed the lowest cost per kg of weight gain.

Conclusions and implications

Alfalfa can be replaced by *Tithonia diversifolia* in fiber rich diets with no effect on dry matter intake, average daily gain and feed conversion. The inclusion of rice polishings in sugar cane silage based diets improves digestibility, N retention and productive performance because it increases energy and decreases the fiber content.

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