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RUMEN CHARACTERISTICS OF YOUNG BULLS FED DIETS BASED ON GRASS AND COMMERCIAL CONCENTRATE SUPPLEMENTED WITH *Hibiscus rosa-sinensis* AND *Saccharin*

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ABSTRACT

Aiming at evaluating hibiscus (*Hibiscus rosa-sinensis*) and saccharin as supplements for young bulls, four crossbred animals were displayed in a Latin square experimental design. The following treatments were performed: 1 – 75% pasture + 25% concentrate; 2 – 50% pasture + 25% concentrate + 25% hibiscus; 3 – 50% pasture + 25% concentrate + 25% saccharin; and 4 – 25% pasture + 25% concentrate + 25% hibiscus + 25% saccharin. Diet composition, rumen fermentation, DM and CP degradation rates were evaluated. Rumen pH values varied from 5.82 to 6.98, for diets supplemented with hibiscus, at 9h post-feeding, and with saccharin, at the feeding moment. Ammonia N contents did not present great changes when only hibiscus or saccharin were added to the diet, but there was a significant effect when both were added (P<0.001). Rumen SCFA concentrations were lower due to saccharin presence in the diet (P<0.01). Saccharin diet presented higher values for readily soluble fraction for DM and CP (P<0.01). Saccharin promoted a higher effect on DM degradation of total diets (P<0.05). Hibiscus and saccharin inclusion increased the degradation of diets for grazing young bulls (P<0.05). Including these feed to the concentrate, rumen environment and functionality were improved. Thus, the partial replacement of concentrate by the tested feed is viable.

KEYWORDS: Rumen fermentation; degradation rate; degradability.

COMPORTAMENTO RUMINAL DE TOURINHOS ALIMENTADOS COM DIETAS DE GRAMÍNEA E CONCENTRADO COMERCIAL SUPLEMENTADAS COM HIBISCO E *Sacharina*

RESUMO

Com o objetivo de avaliar hibisco e sacharina como suplementos para tourinhos, quatro animais cruzados foram distribuídos em um delineamento experimental de quadrado latino. Os tratamentos foram: 1 – 75% pasto + 25% concentrado; 2 – 50% pasto + 25% concentrado + 25% hibisco; 3 – 50% pasto + 25% concentrado + 25% sacarina; e 4 – 25% pasto + 25% concentrado + 25% hibisco + 25% sacarina. Foram avaliadas a composição das dietas, a fermentação ruminal e as taxas de degradação da MS e da PB. Os valores de pH ruminal variaram de 5,82 a 6,98 – para as dietas suplementadas com hibisco, 9 horas após a alimentação e, com sacarina, no momento da alimentação. Os teores de N amoniacal não tiveram grandes alterações quando apenas hibisco ou sacarina foram acrescidos à dieta, mas houve efeito significativo quando ambos foram acrescentados (P>0,001). A concentração de ácidos graxos de cadeia curta no líquido ruminal diminuiu em virtude da presença de sacarina na dieta (P<0,01). A dieta com sacarina apresentou maior fração prontamente solúvel da MS e da PB (P<0,01). A sacarina promoveu maior efeito na degradação da MS das dietas totais (P<0,05). A inclusão de hibisco e de sacarina aumentou a degradação das dietas de tourinhos alimentados a pasto (P<0,05). Ao incluir esses alimentos, o ambiente e a funcionalidade ruminal foram melhorados. Considera-se viável, na suplementação, a substituição parcial do concentrado pelos alimentos testados.

PALAVRAS-CHAVE: Fermentação ruminal; taxa de degradação; degradabilidade.

INTRODUCTION

Feed for ruminants in Mexican tropical regions is based on low quality pastures, which are rich in fibers and poor in protein and energy; besides, when pastures are used alone, during part of the year, they can only provide low production levels. Furthermore, livestock in such areas present serious problems due to lack of forage caused by dry weather or floods. The lack of rain impair the quality of the pasture due to the reduction of leaves and increase of fiber levels. Consequently, animal weight loss occurs and in severe conditions some of the animals may die, especially the younger ones (ALAYÓN *et al*., 1998).

In Mexican tropic, the finisihing of weaned animals may be carried out with the use of trees and forrage bushes or sugarcane (ARANDA-IBÁÑEZ & OSORIO, 2003). Integrating trees and bushes to the feed improves the animals weight gain because the leaves of such plants present high protein levels. In Mexico, there is a great variety of trees and bushes species with potential to be used in ruminant production systems in the tropics. *Hibiscus rosa-sinensis* is one of them. It is known in Mexico as *tulipán*, and it has been tested in some regions as ruminat feed due to its good nutritional quality.

Sugarcane is considered a strategic forrage resource for dry periods because, in tropical regions, there are favorable conditions for a high dry matter (DM) yield, comparing with other forrages. This plant has a great quantity of soluble carbohydrates, presented preponderantly as sacharosis, but with low rates of crude protein (CP), about 2.5%. However, enriched sugarcane, or saccharin, can be obtained by aerobic fermentation in solid state of clean and ground sugarcane (without the leaves and the tips) combined with urea and mineral salt. It contains protein and non-protein nitrogen compounds obtained by microbial mass increment. Its true protein rate ranges from 11% to 16% DM (ELÍAS *et al*., 1990).

Therefore, the objective of this study was to determine the feed degradation rate and ruminal fermentation of young bulls fed different combinations of pasture, *Hibiscus rosa-sinensis*, saccharin and concentrate.

MATERIAL AND METHODS

Animals and diets

Four (F1: Brown Swiss x Brahman) 2-year-old young bulls were used. The animals were rumen-fistulated with permanent flexible cannula for four periods of 21 days each, being 14 days for acclimatization and seven for measurements and sample collection. The animals were housed in individual stalls and fed two different treatments, with water and mineral salt at will. The feed, supplied once a day, was composed of African star grass (*Cynodon plectostachyus*) and concentrate with or wothout the addition of hibiscus (*Hibiscus rosa-sinensis*) and saccharin. The grass and the *Hibiscus rosa-sinensis* were collected in paddocks and offered fresh to the animals (29.56% and 23.66% DM for grass and hibiscus, respectively).

Treatments

Four different diets were evaluated:

* Diet 1 – 75% pasture + 25% concentrate;
* Diet 2 – 50% pasture + 25% concentrate + 25% hibiscus;
* Diet 3 – 50% pasture + 25% concentrate + 25% saccharin; and
* Diet 4 – 25% pasture + 25% concentrate + 25% hibiscus + 25% saccharin.

Evaluated variables

Rate and extent of DM and CP degradation in the rumen were evaluated by *in situ* technique (ØRSKOV *et al*., 1980), using young bulls fed experimental diets. Nylon bags with samples of African star grass, hibiscus, saccharin, concentrate and the experimental total diets were incubated in duplicate for 3, 6, 12, 24, 48, 72, 96 and 120 hours. After incubation, the bags were repeatedly washed in a domestic whashing machine, until the water used for washing was clean. Afterwards, the bags were dried in a forced-air circulation hothouse, at 60°C, for 48 hours.

The disappearance of DM and CP from the feed and the total diets was calculated as the difference between the original and the residual material. Data of rumen disappearance were adjusted by the exponential model proposed by ØRSKOV & McDONALD (1979) and modified by McDONALD (1981), being p = A, for t < t0; and p = a+b(1–exp–ct), for t > t0, in which p is degradability in time t; A is the initial solution or readily soluble fraction; t0 is colonization time (lag time); a and b are mathematical constatns of the model; and c is degradation rate. From this model, it can be derived that B = (a+b)–A, being B the insoluble degradable fraction and the potential degradability determined by the addition of fractions A and B. Crude protein rate was determined by micro-Kjeldahl method.

For rumen pH, rumen fluid was colected from ventral sac T 0, 3, 6, and 9 hours after the diet supply. In these samples, rumen pH was measured by a potentiometer (10 Fisher Scientific model).

After rumen pH measurement, 4 mL of rumen fluid were sampled, acidified with 1 mL of metaphosphoric acid concentrated at 25% (weight:volume) and stored under freezing for further concentration analyses of ammoniacal N and short-chain fatty acids (SCFA): acetate, propionate and butyrate. Ammoniacal N concentrations were determined by indophenol method (McCULLOUGH, 1967), by the use of a Perkin Elmer Lambda 25 spectophotometer, and the SCFA concentrations by gas chromatography (ERWIN *et al*., 1961), using a Varian Star chromatography system.

Experimental design

Data were analyzed as a 4 x 4 latin square, with four animals and four periods (STEEL & TORRIE, 1980), by the GLM procedure of the statistical package SAS (2001). Variables which compose rumen fermentation products were analyzed as measures repeated in time.

RESULTS AND DISCUSSION

The chemical composition of the feeds used in the experiments is presented in Table 1. African star grass showed lower levels of crude protein (CP), true protein (TP) and dry matter (DM) soluble fraction, and higher levels of neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose. The concentrate had the highest levels of CP and TP. The high pretein levels in saccharin were due to aerobic fermentation of the yeast naturally found in sugar cane and to the addition of urea, ammonium sulfate, soybean meal and sorghum.

Table 1. Chemical composition of the diet ingredients

	Fraction[(1)]							
	DM	CP	TP	NDF	ADC	Hem	OM	A_{MS}
Ingredients								
African star grass (*Cynodon plectostachyus*)	29.56	10.73	9.44	75.24	44.65	30.59	92.78	17.14
Hibiscus	23.66	13.51	11.89	35.10	21.26	13.85	93.00	33.83
Saccharin	39.86	17.27	15.20	37.25	20.02	17.23	95.75	30.19
Concentrate	90.14	22.85	20.11	34.02	7.51	26.52	94.68	22.38
Diets[(2)]								
Diet 1	44.71	13.76	12.11	64.93	35.36	29.57	93.26	18.45
Diet 2	43.23	14.46	12.72	54.90	29.52	25.39	93.31	22.62
Diet 3	47.28	15.40	13.55	55.44	29.21	26.23	94.00	21.71
Diet 4	45.81	16.09	14.16	45.40	23.36	22.05	94.05	25.89

(1) DM: dry matter (% of original matter); CP: crude protein (% DM); TP: true protein (% DM); NDF: neutral detergent fiber (% DM); ADF: acid detergent fiber (% DM); Hem: hemicellulose (% DM); OM: organic matter (% DM); A_{DM}: readily soluble fraction of DM

(2) Diet 1: 75% pasture + 25% concentrate; Diet 2: 50% pasture + 25% concentrate + 25% hibiscus; Diet 3: 50% pasture + 25% concentrate + 25% saccharin; and Diet 4: 25% pasture + 25% concentrate + 25% hibiscus + 25% saccharin.

Saccharin CP and TP values stand out. They can be explained by the way in which food is processed. In Mexico, the country where the experiment was conducted, saccharin is produced by mixing 68.7% cane sugar (ground leafless stalks), 1.5% urea, 0.5% mineral mixture, 0.3% ammonium sulfate, 5% Vitafert ® (cultivation of live lactobacillus), 4% soybean meal and 20% sorghum meal. This ingredients mixture, submitted to open-air fermentation, for about 24 hours, increases the protein content and quality.

The organic matter was similar for the four feeds. Hibiscus and saccharin had the lowest levels of hemicellulose and the highest of DM soluble fraction. These results were reflected in the diets, as hibiscus and saccharin increased levels of CP and TP in readily soluble fraction of DM (A_{DM}), and decreased levels of the different fibrous fractions.

Rumen pH at different times after feeding is presented in Table 2. Rumen pH decreases over time after feeding, but the pH at 6 and 9 hours is higher for saccharin supplementation than for diet 1. These values are related to the concentration of SCFA and ammoniacal nitrogen, which were lower for saccharin diet at those times. This effect of SCFA concentration in the decrease of pH was reported by Russel (1998).

The highest pH values were observed right before feeding (time 0) and decreased with time, until they reached the lowest values at nine hours after feeding.

Just as the inclusion of saccharin, hibiscus has also increased the concentration of ammoniacal N (Table 2). The effect of the interaction between saccharin and hibiscus was also observed. There was no change in the concentration of ammoniacal nitrogen by adding saccharin or hibiscus. However, the concentration decreased when both were added to the diet. This effect may be related to rates of degradation of the soluble part of saccharin and hibiscus protein (Table 7). All diets reached maximum concentration of ammoniacal nitrogen at 3 hours after feeding, decreasing thereafter.

The concentration of short-chain fatty acids in rumen fluid decreased due to the presence of dietary saccharin. There was also an interaction effect between saccharin and hibiscus and, in diets without saccharin, hibiscus increased the concentration of SCFA in rumen fluid. However, in diets with saccharin this concentration decreased with the addition of hibiscus. Moreover, values varied according to time after feeding (Table 2): the lowest concentrations were observed for postprandial times 0 and 3 hours, increasing gradually until 9 hours

Table 2. Variables of rumen fermentation of rumen-fistulated young bulls fed African star grass (*Cynodon plectostachyus*) and concentrate and supplemented with hibiscus or saccharin

Diet[(1)]	Time	Variables						
		pH	$N\text{-}NH_3$ mg/dl	SCFA mmol/l	Acetate %	Propionate %	Butyrate %	Acet: Prop Ratio
Diet 1	0h	6.68	23.04	71.26	68.30	18.32	12.71	3.65
	3h	6.41	34.04	76.84	67.89	18.04	13.14	3.66
	6h	5.84	28.36	73.94	66.52	18.44	14.05	3.47
	9h	5.85	20.31	78.32	67.31	18.38	13.88	3.67
	SEM[(2)]	0.17	1.33	2.09	0.51	0.72	0.79	0.13
Diet 2	0h	6.63	27.87	72.40	66.84	18.86	14.26	3.64
	3h	6.31	31.22	84.06	63.83	20.59	14.59	3.02
	6h	5.87	29.19	90.49	65.75	20.07	13.62	3.23
	9h	5.82	25.33	87.22	66.59	20.16	13.45	3.36
	SEM[(2)]	0.16	1.55	5.25	1.42	0.90	0.61	0.21
Diet 3	0h	6.98	24.11	63.99	68.40	18.80	12.80	3.65
	3h	6.55	34.33	62.27	69.32	18.24	12.44	3.82
	6h	6.49	29.55	71.78	68.01	18.69	13.31	3.66
	9h	6.39	27.73	72.67	67.85	18.57	13.58	3.69
	SEM[(2)]	0.16	2.40	5.15	1.37	0.98	0.64	0.23
Diet 4	0h	6.75	15.85	62.29	68.48	19.48	12.04	3.52
	3h	6.44	19.28	53.94	68.41	18.95	12.65	3.65
	6h	6.20	18.02	55.62	67.89	18.85	13.26	3.65
	9h	6.04	16.62	63.52	67.34	19.60	13.07	3.47
	SEM[(2)]	0.05	2.60	5.55	0.90	0.78	0.46	0.21
Effects								
Saccharin		*	**	**	ns	ns	ns	ns
Hibiscus		ns	**	ns	ns	ns	ns	ns
Time		***	**	*	ns	ns	ns	ns
Saccharin x Hibiscus		ns	***	*	ns	ns	ns	ns
Saccharin x Time		ns	ns	ns	ns	ns	ns	ns
Hibiscus x Time		ns	ns	ns	ns	ns	*	ns
Saccharin x Hibiscus x Time		ns	ns	ns	ns	ns	*	ns

(1) Diet 1: 75% pasture + 25% concentrate; Diet 2: 50% pasture + 25% concentrate + 25% hibiscus; Diet 3: 50% pasture + 25% concentrate + 25% saccharin; and Diet 4: 25% pasture + 25% concentrate + 25% hibiscus + 25% saccharin.

(2) SEM = standard error of the mean; ns = not significant (P>0.05); * = P≤0.05; ** = P≤0.01; *** = P≤0.001

Changes in the concentration of SCFA are related to the kind of diet and the feed degradation rates. Saccharin showed lower degradation rate than hibiscus (Table 4).

There was no effect of saccharin, hibiscus or time in diets (Table 2) on acetic acid proportion in rumen. Values ranged from 63.8% to 69.3%. The same was observed for propionic acid proportion, which ranged from 18.0% to 20.5%.

As for butyric acid, either saccharin or hibiscus did not produce any effect, but there was an interaction between time and hibiscus, and diets with this ingredient showed higher proportion at times 0 and 3 hours, and similar ones to the others at other times. There was also a triple interaction among saccharin, hibiscus and time: the addition of hibiscus increased butyric acid concentration in diets without saccharin, at 0 and 3 hours; however, there was no difference in the diets with saccharin. At 6 and 9 hours, no difference was found for either saccharin or hibiscus.

The acetate: propionate ratio was not affected by saccharin, hibiscus or time after feeding.

The final products of rumen fermentation depend on the kind of diet, and the acetate: propionate ratio is usually lower for concentrated food (grains) than for forages (BLAXTER, 1962). The reduction of this ratio decreases methane (CH_4) production and increases energy retention by the animal (WOLIN, 1960). Some rumen bacteria that digest starch produce significant amounts of propionate, but many fibrolytic bacteria produce large amounts of succinate, an intermediate which is eventually converted to propionate (HUNGATE, 1966). Cereal grains fermentation often reduces rumen pH, and this fact is more significant in cases of high food intake (SLYTER, 1966). Lactate may be an intermediate in the conversion of starch to propionate (HUNGATE, 1966). When the pH is lower than 5.3, acetate: propionate ratio is significantly increased as well as H_2 amount. These results indicate that propionate-producing bacteria can be much more sensitive to pH than acetate- and H_2-producing bacteria (RUSSELL, 1998).

TAMMINGA (1992) estimated that 50% of dietary N is lost by ruminants (in case of dairy cows) by urinary excretion. Of these 50%, approximately 30% is lost due to inefficient rumen metabolism. In addition, the efficient use of ammonia in the rumen is a central factor that determines the economic costs and environmental impact of ruminant production (HRISTOV *et al.*, 2003). If it is not used for the production of microbial mass, rumen ammonia detoxifies in the liver (LOBLEY *et al.*, 1995) and a significant portion of it is lost through urea urinary excretion.

Some studies (CECAVA et al., 1990) showed that protein sources susceptible to degradation in the rumen have increased the efficiency of microbial protein synthesis and / or the flow of microbial N in the small intestine, compared with proteins which are more resistant to degradation in the rumen. These conditions may be due to both an increased concentration of ammoniacal nitrogen and products from the protein breakdown, necessary for microbial growth (HESPELL, 1979). In most cases, the effects of the concentration of proteolysis growth factors on protein synthesis are confused with the ammoniacal N concentrations in the rumen. The yield efficiency and the microbial growth are also affected by the amount of organic matter fermented in the rumen (ROHR et al., 1986). Theoretically, the appropriate concentrations of ammoniacal nitrogen can maximize microbial growth if concentrations of other nutrients are insufficient (CECAVA et al., 1991).

Corn-based concentrate can increase the energy availability in all parts of the gastrointestinal tract, because it is more likely to increase the non-structural carbohydrates (starch) supply (CECAVA et al., 1991). Ammonia is the preferred N source for fibrolytic bacteria (HUNGATE, 1966) and is also required by starch-fermenting bacteria for microbial protein synthesis (COTTA & RUSSEL, 1982). SCHAEFER et al. (1980) reported that no more than 5 mg / dL of ammoniacal N is required to achieve the maximum microbial growth. However, WALLACE (1979) observed that the increased rates of *in situ* degradation of DM and CP of barley grains were accompanied by bacterial growth, which increased when the concentration of ammoniacal N changed from 9.7 to 21.4 mg / dl rumen fluid. Similarly, MEHREZ et al. (1977) reported that *in situ* degradation rates were maximized in ammoniacal N concentrations above 20 mg / dl of rumen fluid.

Constants of DM rumen degradation found in African star grass pasture, used as the basis of animal diet, are presented in Table 3. There was no effect of saccharin or hibiscus in none of the constants of DM rumen degradation.

Dry matter degradation rates of saccharin, hibiscus and concentrate are shown in Table 4.

Saccharin presented the highest values for readily soluble fraction (A) and hibiscus for fermentable insoluble fraction (B) and degradation rate (c). The concentrate presented the highest potential for degradation (A + B). The readily soluble fraction (A) and potentially degradable fractions (A + B) as well as the effective degradability (ED) of the diets increased with the inclusion of saccharin (Table 5). Hibiscus only affected effective degradability (ED).

None of the studied variables for crude protein degradation of the grass was affected by hibiscus. There was an effect of saccharin on the increase of the potentially degradable fraction (A + B), as shown in Table 6.

TABLE 3. Constants of DM rumen degradation of African star grass (*Cynodon plectostchyus*) by animals fed four different experimental diets[(1)]

Animal Feed[(1)]	Variables[(2)]					
	A	B	A+B	c	t_0	ED
Diet 1	18.67	45.73	64.39	0.0347	- 1.48	35.28
Diet 2	19.88	49.08	68.96	0.0266	- 2.98	34.54
Deit 3	22.49	47.35	69.84	0.0334	- 1.80	38.99
Diet 4	22.88	46.03	68.89	0.0259	- 3.58	36.35
SEM[(3)]	1.52	1.47	1.80	0.0067	1.20	1.47
Effects						
Saccharin	ns	ns	ns	ns	ns	ns
Hibiscus	ns	ns	ns	ns	ns	ns
Saccharin x Hibiscus	ns	ns	ns	ns	ns	ns

[(1)] Diet 1: 75% pasture + 25% concentrate; Diet 2: 50% pasture + 25% concentrate + 25% hibiscus; Diet 3: 50% pasture + 25% concentrate + 25% saccharin; and Diet 4: 25% pasture + 25% concentrate + 25% hibiscus + 25% saccharin.
[(2)] A: readily soluble fraction (%); B: fermentable soluble fraction (%); A+B: potentially degradable fraction (%); c: degradation rate; t_0: tempo de colonização (h); ED: effective degradability (%)
[(3)] SEM = standard error of the mean; ns = not significant (P>0.05)

TABLE 4. Constants of DM rumen degradation of saccharin, hibiscus and concentrate in African star grass-based diets[(1)]

Feed	Diet[(1)]	Variables[(2)]					
		A	B	A+B	c	t_0	ED
Saccharin	Diet 3	50.79	40.24	91.02	0.0221	-15.10	61.48
	Diet 4	47.64	38.23	85.87	0.0373	-11.93	62.63
Hibiscus	Diet 2	22.90	62.29	85.19	0.0627	2.73	54.45
	Diet 4	23.79	58.93	82.73	0.0803	1.83	56.90
Concentrate	Diet 1	41.15	56.89	98.04	0.0387	-10.50	62.11
	Diet 2	42.22	54.07	96.29	0.0373	-10.28	63.05
	Diet 3	40.15	57.73	97.88	0.0312	-8.18	60.59
	Diet 4	41.46	55.94	97.40	0.0277	-10.40	59.26

(1) Diet 1: 75% pasture + 25% concentrate; Diet 2: 50% pasture + 25% concentrate + 25% hibiscus; Diet 3: 50% pasture + 25% concentrate + 25% saccharin; and Diet 4: 25% pasture + 25% concentrate + 25% hibiscus + 25% saccharin.
(2) A: readily soluble fraction (%); B: fermentable soluble fraction (%); A+B: potentially degradable fraction (%); c: degradation rate; t_0: tempo de colonização (h); ED: effective degradability (%)

TABLE 5.Constants of DM rumen degradation of diets based on African star grass (*Cynodon plectostchyus*) supplemented with hibiscus or saccharin

Dieta[(1)]	Variables[(2)]					
	A	B	A+B	c	t_0	ED
Diet 1	24.40	47.67	72.07	0.0357	- 4.23	42.00
Diet 2	26.89	51.24	78.13	0.0376	- 3.08	46.62
Diet 3	34.74	47.35	82.07	0.0294	- 6.50	50.03
Diet 4	35.08	47.12	82.22	0.0398	- 5.05	53.72
SEM[(3)]	1.72	1.07	1.30	0.0047	0.81	1.19
Effects						
Saccharin	**	ns	**	ns	*	***
Hibiscus	ns	ns	ns	ns	ns	*
Saccharin x Hibiscus	ns	ns	ns	ns	ns	ns

(1) Diet 1: 75% pasture + 25% concentrate; Diet 2: 50% pasture + 25% concentrate + 25% hibiscus; Diet 3: 50% pasture + 25% concentrate + 25% saccharin; and Diet 4: 25% pasture + 25% concentrate + 25% hibiscus + 25% saccharin.
(2) A: readily soluble fraction (%); B: fermentable soluble fraction (%); A+B: potentially degradable fraction (%); c: degradation rate; t_0: tempo de colonização (h); ED: effective degradability (%)
(3) SEM = standard error of the mean; ns = not significant (P>0.05); * = P≤0.05; ** = P≤0.01; *** = P≤0.001

TABLE 6.Constants of CP rúmen degradation of African star grass (*Cynodon plectostchyus*) by animals fed four different experimental diets[(1)]

Animal feed[(1)]	Variables[(2)]					
	A	B	A+B	c	t_0	ED
Diet 1	37.67	38.09	75.76	0.0381	1.83	52.24
Diet 2	39.43	39.90	79.33	0.0270	1.18	51.57
Diet 3	45.79	40.78	86.57	0.0387	- 2.28	61.48
Diet 4	46.17	38.41	84.58	0.0298	- 4.95	58.90
SEM[(3)]	5.10	3.69	1.73	0.0042	5.29	4.53
Effect						
Saccharin	ns	ns	**	ns	ns	ns
Hibiscus	ns	ns	ns	ns	ns	ns
Saccharin x Hibiscus	ns	ns	ns	ns	ns	ns

[(1)] Diet 1: 75% pasture + 25% concentrate; Diet 2: 50% pasture + 25% concentrate + 25% hibiscus; Diet 3: 50% pasture + 25% concentrate + 25% saccharin; and Diet 4: 25% pasture + 25% concentrate + 25% hibiscus + 25% saccharin.
[(2)] A: readily soluble fraction (%); B: fermentable soluble fraction (%); A+B: potentially degradable fraction (%); c: degradation rate; t_0: tempo de colonização (h); ED: effective degradability (%)
[(3)] SEM = standard error of the mean; ns = not significant (P>0.05); * = P≤0.05; ** = P≤0.01; *** = P≤0.001

TABLE 7. Constants of CP rumen degradation of saccharin, hibiscus and concentrate in African star grass-based diets

Feed	Diets[(1)]	Variable[(2)]					
		A	B	A+B	c	t_0	ED
Saccharin	Diet 3	62.41	33.08	95.49	0.0320	- 5.93	73.87
	Diet 4	60.80	33.07	93.87	0.0512	- 5.58	76.03
Hibiscus	Diet 2	26.09	69.17	95.26	0.0708	3.83	63.58
	Diet 4	36.52	57.75	94.26	0.1032	- 0.78	73.56
Concentrate	Diet 1	51.75	45.81	97.55	0.0254	- 15.22	65.55
	Diet 2	52.42	44.36	96.77	0.0283	- 12.33	66.85
	Diet 3	56.50	43.50	99.99	0.0159	- 17.43	65.49
	Diea 4	55.25	44.75	99.99	0.0146	- 19.33	64.07

[(1)] Diet 1: 75% pasture + 25% concentrate; Diet 2: 50% pasture + 25% concentrate + 25% hibiscus; Diet 3: 50% pasture + 25% concentrate + 25% saccharin; and Diet 4: 25% pasture + 25% concentrate + 25% hibiscus + 25% saccharin.
[(2)] A: readily soluble fraction (%); B: fermentable soluble fraction (%); A+B: potentially degradable fraction (%); c: degradation rate; t_0: tempo de colonização (h); ED: effective degradability (%)

Crude protein degradation rates of saccharin, hibiscus and concentrate are presented in Table 7. For protein degradability, saccharin showed the highest values of initial solubility (A) and effective degradability (ED). Hibiscus presented the highest values of degradable insoluble fraction (B), the highest rates of degradation (c), and also it showed a discrete colonization time (t0). The concentrate had the highest values of CP potential degradability (A + B).

The fraction A of the diet crude protein (Table 8) increased due to saccharin. Fraction B was also affected, either by inclusion of saccharin as hibiscus, and it showed an interaction effect: the addition of hibiscus increased fraction B, but the inclusion of saccharin, or saccharin and hibiscus, did not affect this fraction.

TABLE 8. Constants of CP rumen degradation of diets based on African star grass (*Cynodon plectostchyus*) supplemented with hibiscus or saccharin

Diets[1]	Variables[2]					
	A	B	A+B	c	t_0	ED
Diet 1	41.10	37.12	80.22	0.0361	- 0.40	55.65
Diet 2	40.30	46.01	86.33	0.0392	0.18	58.53
Diet 3	53.88	38.45	92.28	0.0279	- 6.45	65.81
Diet 4	52.84	37.97	90.81	0.0402	- 6.25	68.03
SEM[3]	2.93	1.21	1.73	0.0042	2.29	2.72
Effect						
Saccharin	**	*	**	ns	*	*
Hibiscus	ns	*	ns	ns	ns	ns
Saccharin x Hibiscus	ns	**	ns	ns	ns	ns

[1] Diet 1: 75% pasture + 25% concentrate; Diet 2: 50% pasture + 25% concentrate + 25% hibiscus; Diet 3: 50% pasture + 25% concentrate + 25% saccharin; and Diet 4: 25% pasture + 25% concentrate + 25% hibiscus + 25% saccharin.
[2] A: readily soluble fraction (%); B: fermentable soluble fraction (%); A+B: potentially degradable fraction (%); c: degradation rate; t_0: tempo de colonização (h); ED: effective degradability (%)
[3] SEM = standard error of the mean; ns = not significant (P>0.05); * = P≤0.05; ** = P≤0.01; *** = P≤0.001

Degradation rate and extension may be related to microbial growth. On one hand, RUSSEL et al. (1980) showed that urea infusion increased proportionally the amount of associated bacteria and total SCFA concentration; however it seemed to have little or even no impact at all on rumen degradation. This fact suggests that ammoniacal N concentration for the maximun microbial growth differs from the maximum feed degradation. On the other hand, WALLACE (1979) observed that the increase of DM and CP degradation occureed along with bacaterial growth, when ammoniacal N concentration increased from 9.7 to 21.4 mg/dl.

Energy availability has an important modifying influence on rumen degradation of the feed. Thus, as the amount of bacteria can increase in response to increased ammonium concentrations, the degradation of the fiber in the nylon bags was not affected, because the entry of cellulolytic bacteria was limited due to the attack of bacteria to the fibrous material in the rumen. The production of SCFA in the rumen is somehow related to rumen pH, which is an important regulator of the efficiency of microbial synthesis and amino acids absorption (Russell & Dombrowski, 1980).

Increasing CP content of the diet may have increased not only animal production (WU & SETTER, 2000), but also the concentrations of rumen ammoniacal N and blood urea N, and therefore it led to higher N losses through urine (CASTILLO *et al*., 2001). Nitrogen loss in the rumen was reported by TAMMINGA (1992) as a major contributor to N losses from the animal as a whole. If the rumen degradable protein is not used for microbial protein synthesis, it can be converted into ammoniacal N. Ammoniacal N can be absorbed by the rumen wall, detoxify urea in the liver (LOBLEY et al., 1995) and gradually be excreted via urine. A certain amount of rumen degradable protein can pass to the rumen and contribute to the duodenal flow of amino acids and peptides (CHOI et al., 2002). Ammoniacal nitrogen excess of the rumen degradable protein increases microbial protein synthesis of ruminants (HRISTOV et al., 2004). This excess of degradable protein in the diet of ruminants may not be used efficiently for microbial protein synthesis and it is lost in large quantities through urinary N excretion. Mixed rumen bacteria produce less methane and ammonia when the pH is lower than 6.0 (ERFLE et al., 1982).

CONCLUSION

The inclusion of hibiscus and saccharin increased the degradation of pasture-based diets offered to young bulls. The inclusion of such forrages along with the concentrate improved rumen behavior of the animals. Because of that, it is possible to carry out the partial replacement of the concentrate used in feeding supplementation of ruminants when these feeds are available, representing an economic gain to the producers. Therefore, this is a viable alternative to improve animal production in tropical regions.

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