



Intake, apparent digestibility, rumen fermentation and nitrogen efficiency in sheep fed a tropical legume silage with or without concentrate

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ABSTRACT

Legume silage can increase the forage quality of the diets as well as supply it with nitrogen, calcium and phosphorus. The objective was to evaluate the intake, apparent digestibility, rumen fermentation and nitrogen efficiency in sheep fed a tropical legume silage with or without concentrate. Twelve crossbred sheep with an average initial body weight of 32.2 ± 1.26 kg, with six animals cannulated in the rumen were distributed into four 3×3 Latin squares. The treatments were 1) *Stylosanthes* silage without concentrate (StS), 2) *Stylosanthes* silage with concentrate (StS+C), and 3) corn silage with concentrate (CS+C). StS diet showed lowest intake, except for neutral detergent fiber (NDF). The diets StS+C and CS+C showed similar intake of dry matter (DM) and crude protein. The intake of total digestible nutrients was higher for CS+C diet than diets StS+C and StS. Animals fed CS+C diet had lowest ruminal pH. The nitrogen use efficiency was similar for the diets with concentrate. In conclusion, StS+C diet replacing CS+C diet decreases the intake of total digestible nutrients.

Key words: alternative foods, corn silage, crude protein, neutral detergent fiber, *Stylosanthes*.

INTRODUCTION

Legume silage for livestock systems it is interesting because is an excellent source of protein and the crop requires less use of nitrogen fertilizers than grasses (Heinritz et al. 2012). In addition, sources of protein used in feedlot diets have high cost, like soybean meal (Millen et al. 2009). Thus, legume

silage can reduce the cost of diet as well as supply it with nitrogen, calcium and phosphorus (Baxter et al. 1984).

Stylosanthes cv. Campo Grande (stylo; *Stylosanthes macrocephala* and *S. capitata*) is a legume developed in Brazil. This cultivar showed good adaptation to infertile soils, mainly sandy soils, and with an annual production of 8-15 ton of DM ha⁻¹ (Fernandes et al. 2005, Moreira et al. 2015). In addition, recent studies with *Stylosanthes*

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cv. Campo Grande have shown that it is possible to obtain well-fermented silage when harvested at the time of flowering. This legume silage stabilizes with pH 4, and lactic acid and acetic acid concentrations of 5 and 3.6%, respectively, and concentration of ammonia around 10% of total nitrogen, being able to replace corn silage in diets of feedlot beef cattle without affecting feed intake or performance (Souza et al. 2014, Silva et al. 2016, Silva et al. 2017).

Therefore, it was hypothesized that stylo silage (StS) could replace corn silage (CS) in diets for sheep. The intake and digestibility of nutrients, ruminal ammonia-nitrogen (NH₃-N), ruminal pH, and nitrogen balance were evaluated in sheep fed diets containing StS with and without concentrate and corn silage with concentrate.

MATERIALS AND METHODS

The experiment was conducted at the Animal Science Department of the Federal University of Viçosa (UFV) following the procedures for humanitarian animal care and management guidelines from the ethics committee at the UFV.

EXPERIMENTAL PROCEDURE

Stylo and corn plants were harvested at the pre-blooming stage and one-third milk-line, respectively. Crops were chopped using a stationary chopper (2 mm theoretical chop length) and packed in laboratory-scale silos with capacity of 550 kg, with a packing density of 550 kg/m³.

Treatments consisted of diets containing stylo silage without (StS) or with concentrate (StS+C) and corn silage with concentrate (CS+C). The forage:concentrate ratio was 50:50 on a DM basis. Concentrate was made with ground corn and soybean meal.

Diets were formulated according to the amount of crude protein (CP) of StS, 117 g/kg of DM. The mixture of urea and ammonium sulfate (9:1) was used to adjust the CP content of corn silage

(Table I). The mineral mix was offered *ad libitum* in feeders adapted in the cages.

Twelve crossbred sheep (predominantly Santa Inês) with average initial body weight of 32.2 ± 1.26 kg, six of which were rumen-cannulated. The animals were housed in a covered barn in individual cages equipped with feeders and drinking water systems. The animals were fed twice daily at 0800 h and 1600 h for *ad libitum* intake, allowing for a maximum of 15% orts.

Each experimental period lasted 16 days, with 11 days for adaptation and five days for samples and data collections. The animals were weighed at the beginning and at the end of each experimental period. Total collections of orts, feces and urine were performed during the first four days of each sampling period. Leather bags were used for the

TABLE I
Proportion of ingredients and chemical composition of the diets (g/kg of dry matter).

Items	Treatments ¹		
	StS	StS+C	CS+C
Proportion of ingredients			
<i>Stylosanthes</i> silage	1000	500	-
Corn silage	-	-	500
Ground corn	-	458	454
Soybean meal	-	36.6	36.2
Urea/ammonium sulfate (9:1)	-	-	5.00
Sodium bicarbonate	-	5.00	5.00
Chemical composition			
Dry matter, g/kg	291	582	589
Organic matter	914	946	963
Ether extract	28.6	32.0	28.9
Crude protein	117	118	114
Neutral detergent fiber	643	394	331
Acid detergent fiber	504	275	208
Indigestible neutral detergent fiber	351	181	98.6
Non-fiber carbohydrates	125	401	503
Lignin	140	79.2	30.6

¹StS = *Stylosanthes* silage without concentrate, StS+C = *Stylosanthes* silage with concentrate, CS+C = corn silage with concentrate.

fecal collection and at the end of each experimental period a fresh sample of approximately 350 g per animal was stored. Urine collection was performed using collector funnels that were attached to the cages and drained into a bucket on the ground containing 100 mL of 20% (v/v) sulfuric acid. After 24 h, the weight and the total volume of urine were recorded, and an aliquot of 5% of the daily volume was stored in a freezer. A composite sample was made for each animal after four days of collection.

To determine ruminal pH and $\text{NH}_3\text{-N}$ concentration, samples were taken on the fifth day of each sampling period, prior to feeding and 2, 4 and 6 h after feeding. Approximately 50 mL of rumen fluid was collected through of rumen cannula and the pH was immediately determined using a digital pH meter. After measuring pH, 1 mL of a 50% sulfuric acid (v/v) solution was added to each sample and were stored at -15°C for subsequent analysis of $\text{NH}_3\text{-N}$ concentration.

LABORATORY ANALYSIS

Samples of feed, orts, and feces were dried at 55°C for 72 h in a forced air oven and ground in a Wiley mill (Wiley mill, Arthur H. Thomas, PA, USA) with a 1-mm screen. The contents of DM, organic matter (OM), crude protein (CP), ether extract (EE), and acid detergent fiber (ADF) were determined according to AOAC (1990); neutral detergent fiber (NDF; Mertens 2002), sulfuric acid lignin (Robertson and Van Soest 1981) and indigestible neutral detergent fiber (Huhtanen et al. 1994) were also measured. Ruminal $\text{NH}_3\text{-N}$ was determined using a colorimetric method according to Chaney and Marbach (1962). The concentrations of non-fibrous carbohydrates (NFC; Detmann and Valadares Filho 2010), total digestible nutrients (TDN; Weiss 1999) and metabolizable energy (ME; NRC 2001) were calculated.

Urine samples were analyzed for creatinine using the Picrate Alkaline method (Labtest

Diagnóstica, MG, Brazil) and urea by the enzymatic-colorimetric method (Urea CE; Labtest Diagnóstica).

STATISTICAL ANALYSIS

Data were analyzed using the MIXED procedure of SAS (Statistical Analysis Software, Inc., Cary, NC), based on four 3×3 Latin square design and balanced for the residual effects of treatments (Lucas 1957). Two Latin squares were with rumen-cannulated animals and the two others with animals without cannula. The effects of the model were the experimental diets as fixed effects and Latin square, animal and experimental periods as random effects. Ruminal pH and concentration of $\text{NH}_3\text{-N}$ were analyzed through PROC REG; the diet (D), the sampling time (T) and the interaction between them ($D \times T$) were considered to be as fixed effects. The scheme of repeated measures was used, where sampling times (0, 2, 4, and 6 hours after feeding) were repeated for each experimental unit (Kaps and Lamberson 2004). Data were submitted to the analysis of variance and Tukey-test was used for comparisons of the means. All statistical procedures were performed using 0.05 as the critical probability level for type I error.

RESULTS

Nutrients intake, except NDF, were lower for StS diet. The intake of DM, OM, EE, and CP were higher for diets containing concentrate. The CS+C diet showed lower NDF intake than the diets StS+C and StS. The TDN intake was higher for the CS+C diet than the diets StS+C and StS (Table II).

Apparent digestibility of NDF was not affected by the diets. Digestibility of DM, OM, CP and dietary contents of TDN and ME were higher for the diet CS+C than the diets StS+C and StS. The StS diet showed the lowest apparent digestibility of DM, OM, CP and lowest dietary contents of TDN and ME (Table II).

TABLE II
Nutrient intake, total apparent digestibility, and nitrogen efficiency of sheep fed *Stylosanthes* silage without concentrate (StS), *Stylosanthes* silage with concentrate (StS+C), and corn silage with concentrate (CS+C).

Items	Treatments			SEM ¹
	StS	StS+C	CS+C	
Intake, g/day				
Dry matter	528b	907a	988a	72.8
Organic matter	480b	860a	962a	69.3
Ether extract	16.8b	25.5a	25.2a	3.50
Crude protein	64.7b	110a	112a	8.54
Neutral detergent fiber	344a	346a	304b	31.7
Total digestible nutrients	238c	586b	749a	48.5
Intake, g/kg of body weight				
Dry matter	16.5b	27.4a	29.0a	1.56
Neutral detergent fiber	10.7a	10.4a	8.91b	0.77
Digestibility, g/kg of dry matter				
Dry matter	385c	620b	711a	30.2
Organic matter	453c	663b	744a	25.2
Crude protein	519c	612b	662a	17.4
Neutral detergent fiber	465	472	519	29.6
Total digestible nutrients	449c	653b	762a	21.6
Energy content, Mcal/kg of dry matter				
Metabolizable	1.58c	2.12b	2.53a	0.08
Nitrogen use efficiency, g/day				
Nitrogen intake	10.2b	17.6a	18.5a	1.28
Nitrogen feces	4.84b	6.98a	6.59a	0.58
Nitrogen urine	6.39	7.18	7.45	0.79
Nitrogen balance	-1.04b	3.48a	4.46a	0.82
Urinary urea nitrogen	3.78	3.62	4.55	0.51
Creatinine, mg/kg of body weight	28.6	26.2	29.6	2.98

Means followed by the same letter in the rows are not significantly different based on a Tukey-test ($P < 0.05$). ¹SEM: standard error of the mean.

Animal fed StS diet showed lowest N-intake, N-feces, and N-balance than the diets with concentrate. Urinary excretion of nitrogen, urea nitrogen and creatinine were not affected by the treatments. The diets StS+C and CS+C had similar N-intake, N-feces, and N-balance (Table II).

Ruminal pH and $\text{NH}_3\text{-N}$ were affected by the interaction between diets and sampling time. The pH decreased faster for the CS+C diet than the diets StS+C and StS. The effect of diets was significantly for ruminal pH, with averages 6.22, 6.42 and 6.85 for CS+C, StS+C and StS, respectively (Figure 1).

Animal fed StS diet showed higher ruminal $\text{NH}_3\text{-N}$ four and six hours after feeding (Figure 2).

DISCUSSION

Low DM intake for StS diet agrees with estimated intake by NRC (2007) for maintenance of sheep. Probably the NDF intake was a limiting factor and resulted in lower TDN intake for StS diet. Since the NDF intake was similar between StS and StS+C and the last had similar DM intake than the CS+C diet. High lignin content and consequently iNDF could

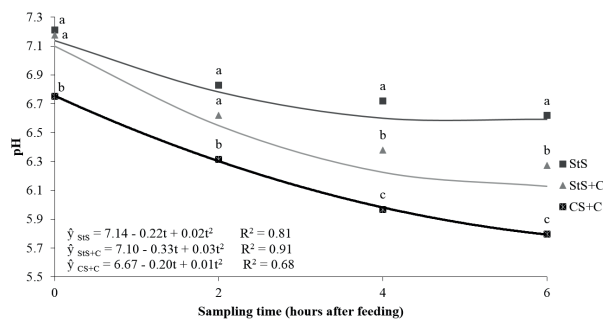


Figure 1 - Ruminal pH as a function of sampling time of sheep fed *Stylosanthes* silage without concentrate (StS), *Stylosanthes* silage with concentrate (StS+C), and corn silage with concentrate (CS+C). Different letter in the points are significantly based on a Tukey-test ($P < 0.05$). Effects of diets ($P < 0.01$), time ($P < 0.01$) and interaction ($P = 0.04$); standard error of mean (0.05).

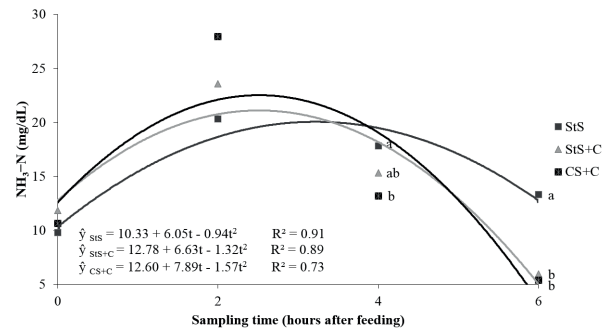


Figure 2 - Concentration of ruminal ammonia nitrogen ($\text{NH}_3\text{-N}$) as a function of sampling time of sheep fed *Stylosanthes* silage without concentrate (StS), *Stylosanthes* silage with concentrate (StS+C) and corn silage with concentrate (CS+C). Different letter in the points are significantly based on a Tukey-test ($P < 0.05$). Effects of diets ($P = 0.78$), time ($P < 0.01$) and its interaction ($P < 0.01$); standard error of mean (0.96).

explain the difference on TDN intake, as previously observed in other study with sheep (Silva et al. 2015). These fractions are related with decrease of intake by the ruminants. Intake restriction on diets containing high fiber content is mainly due the physical factors, which depends on the filling rumen capacity and on the fiber digestibility (Mertens 1994). Silage quality characteristics such as the pH, the concentrations of lactic acid, acetic acid, butyric acid and $\text{NH}_3\text{-N}$ are known to impair feed intake, however, in small ruminants, dietary NDF seems to be the main determinant for voluntary intake (Castro-Montoya and Dickhoefer 2018).

Although the StS+C diet showed higher digestibility than StS diet, the highest digestibility values were for CS+C diet. Probably due to the high lignin content of StS that acts as a barrier for the ruminal microorganisms during the fiber colonization and digestion. Because of that, diets StS and StS+C showed lower content of TDN than CS+C diet. The lack of effect on NDF digestibility among the diets could be due the diets with high NDF content and longer retention can decrease the intake and increase the digestibility, as previously observed in other studies with sheep (Mupangwa et al. 2000, Van Soest 1994).

Ruminal pH for the diets StS and StS+C decreased more slowly than CS+C diet, probably due lower digestibility and rumen production of organic acids, besides of higher buffering capacity of legume silages (Heinritz et al. 2012). Generally, ruminal pH affects directly the growth rate of ruminal microorganisms and diets with high proportion of roughage can increase the ruminal pH and improve the cellulolytic bacteria growth due the pattern of replacing the soluble carbohydrates by NDF (Church 1979, Van Soest 1994).

In this study, for StS diet, the pattern of ruminal $\text{NH}_3\text{-N}$ four and six hours after feed supply, show an imbalance for microbial growth between degradation rate of protein and energy, therefore, without energy in the rumen, the concentration of $\text{NH}_3\text{-N}$ can increase. In addition, the concentrate can improve nitrogen intake, digestion and retention due the accord with the dietary energy and nitrogen supply to the microbial growth (Van Soest 1994). Probably the imbalance between energy and protein resulted an excess of $\text{NH}_3\text{-N}$ in the rumen that was absorbed through the rumen wall. This $\text{NH}_3\text{-N}$ is converted into urea and can return to the rumen or may be eliminated in the urine (Poppi and McLennan 1995). Furthermore, urea excretion in urine is greater when in the

rumen the protein degradation rate exceeds the fermentation of carbohydrates, with consequent urea production and excretion, resulting in nitrogen and energy losses (Russel et al. 1992). However, the amount of recycled urea depends on the N-intake: when N ingestion is low then N cycling increases, because the urea pool in the metabolism is under physiological homeostatic control, which tends to remain constant (Van Soest 1994).

In the present work, decrease in the relationship between protein and intake of TDN was observed; even without affecting the N-balance, the lower content of metabolizable energy of diets containing StS and, consequently, lower intake of TDN, could result in impaired animal performance. Detmann et al. (2014) showed that the imbalance between energy and protein in diets for cattle affect the N-use efficiency and DM intake, and consequently, cattle performance. However, Castro-Montoya and Dickhoefer (2018) concluded that even though the legume silages in ruminant diets negatively affected DM intake and nutrient digestibility, no negative effects on performance were observed, particularly at legume inclusion levels below 400 g/kg DM. Thus, there is still a need for further studies with legume silage for sheep on performance and economic viability.

The constancy on the creatinine excretion may be related to the homogeneity of the sheep body weight. Creatinine is a product of muscle metabolism and their production and excretion is directly related to the metabolism of this tissue (Schutte et al. 1981). Liu and McMeniman (2006), in a study with sheep, reported relative constancy in creatinine excretion for any one diet irrespective of intake level, but affected by animals.

In conclusion, stylo silage had lower content of metabolizable energy in relation to corn silage, resulting in lower intake of TDN in diets for sheep. However, replacing diet StS+C by CS+C did not affect the nitrogen-use efficiency.

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