## *IN VITRO* DEGRADATION KINETICS OF PROTEIN AND CARBOHYDRATE FRACTIONS OF SELECTED TROPICAL FORAGES

# CINÉTICA DE DEGRADAÇÃO IN VITRO DAS FRAÇÕES DE PROTEÍNA E CARBOIDRATO EM FORRAGEIRAS TROPICAIS

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ABSTRACT: Whereas obtain detailed information about nutrient composition and degradation rates of carbohydrate and protein fractions of tropical forages is essential to determine how much of each nutrient can be used by the animal and the main limiting causes to the level of production. A descriptive study was conducted to evaluate the degradation rate of protein and carbohydrate fractions and understand degradation synchronism of carbohydrates and protein fraction in the rumen of goats fed Tifton 85, mulberry and leucaena forages. Contents of crude protein (CP), nonprotein nitrogen (NPN), neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were measured to obtain the protein fractions A, B<sub>1</sub>, B<sub>2</sub> and C. Degradation profiles of nitrogen fractions were obtained in vitro incubating the forages samples with proteases from Streptomyces griseus. Contents of sugars, starch and soluble fiber (neutral detergent NDSF) were analyzed to determine the fractions A and  $B_1$  from total carbohydrates (TC), whereas for fraction B<sub>2</sub>, C and degradation rate of fraction B<sub>2</sub>, the gravimetric technique of *in vitro* degradation of the fiber was used through kinetic interpretation of degradation profiles. It was adopted descriptive statistics to summarize the dataset, to describe the data, tables were compiled and used sample average as position measurement. Regarding Tifton 85, the sum of fractions A and  $B_1$  was 51.61 g/100g CP and the fraction  $B_2$  was 38.74 g/100g CP. Thus, the portion of slowly degradable protein from this forage is higher and tends to escape from rumen contributing with amino acids in the small intestine. Differently, most of the protein from mulberry and leucaena are present as highly degradable protein in rumen (B<sub>1</sub>), requiring supplementation with readily fermentable carbohydrate for a better utilization of nitrogen compounds. Considering the partitions of protein in different compartments of the plant, possibly the mixture between forages promotes a better balance for the use of this nutrient by animals. Fractions representing about 80g/100g of the protein from Tifton 85 presents problems to be used by animals. This means that, despite the high levels of protein in Tifton 85, metabolizable protein deficit may occur at any time after the intake of forages by the animals. Mulberry and leucaena are extremely degradable feed, both for cellular content and cell wall, with high possibility of presenting good synchronization between degradation of carbohydrate and protein.

KEYWORDS: Cncps. Rumen. Starch. Sugars.

### INTRODUCTION

Determination of nutritional composition using protein and carbohydrates fractions, as well as parameters related to the degradation process in the ruminoreticulum, are important for prediction of the nutritive value of feeds and the estimation of animal performance in ruminant production systems. The model developed at Cornell University, known as CNCPS - Cornell Net Carbohydrate and Protein System (SNIFFEN et al., 1992; RUSSELL et al., 1992; FOX et al., 1992) uses passage and degradation rates for each fraction of protein and carbohydrate. The interaction of the different feed components was considered in this system aiming to maximize microbial production, reduce nitrogen losses by the animal and estimate rumen escape of nutrients from microbial and dietary origin.

The Tifton 85 is indicated for goat production systems in pastures, because has high nutritive value and productive characteristics, such as high leaf stem ratio and high density of forage, which facilitates the apprehension of the forage by the animal and indeed increases the intake of digestible energy (MOTT, 1981). However, little is known regarding the degradation of protein and carbohydrate fractions in the rumen of animals fed Tifton-85 managed under light interception as criteria, where the plant has a higher proportion of leaves and lower stems and dead tissues, which leading higher contents of protein and lower indigestible fiber (CARNEVALLI et al., 2001). However, there are no studies that quantify the fractions of carbohydrates and protein in the Tifton-85 managed by 95% light interception as criteria.

The leucaena and mulberry are forages with potential use for goats production systems, and can be used as protein bank (BASAGLIA, 1993, GARCIA et al., 1996). The protein bank is an integrated system, where a part of the pasture area is reserved for planting forage of high nutritional value, with the function of supplementing the diet of animals in grazing. The leucaena and mulberry are appropriate for the formation of protein bank because these forages have high protein contents. Furthermore is very important understand how is the availability this protein in the rumen.

In this way, obtain detailed information about nutrient composition and degradation rates of carbohydrate and protein fractions of these forages is essential to determine how much of each nutrient can be used by the animal and the main limiting causes to the level of production. Therefore, our objectives were to evaluate the degradation rate of protein and carbohydrate fractions and understand degradation synchronism of carbohydrates and protein fraction in the rumen of goats fed Tifton 85, mulberry and leucaena forages.

#### MATERIAL AND METHODS

Studied forages were Tifton 85 (*Cynodon* spp.), mulberry tree variety mucheie II (*Morus alba*, L.), and the legume leucaena cv. Peruvian (*Leucaena leucocephala* (Lam.) de Wit). Plants were grown in the Zona da Mata region of Minas Gerais, in a soil area classified as Yellow-Red Latosol clay type.

Tifton 85 was harvested when plants intercepted 95% of sunlight, presenting average height of 20cm and 16 days old. Mulberry and leucaena leaves were collected when new shoots were 50 days old, and plants presented average height of 1.09 and 1.04 m respectively. The forages sample collections were based on the selection made by lips prehension and material collected was composed only of leaves.

During harvest, ten samples of each forage were taken and dried in a laboratory oven at 55 °C during 72 hours, then ground to pass through a 1 mm sieve and analyzed for dry matter (DM), total nitrogen (TN), fat and ash using techniques described by AOAC (1990); neutral detergent fiber corrected for ashes and protein (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991). Determination of neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were performed according to the technique described by Licitra et al. (1996), and lignin in sulfuric acid

(LDA), as described by Goering and Van Soest (1970).

Estimated values of total carbohydrates (TC) and non-fibrous carbohydrates (NFC) were obtained from equations proposed by Sniffen et al. (1992) and Van Soest et al. (1991), respectively:

Protein was fractionated into 4 subfractions (A,  $B_1$ ,  $B_2$ , and C) (FAVORETO et al., 2008), instead of five subfractions (A,  $B_1$ ,  $B_2$ ,  $B_3$  and C) as originally proposed in CNCPS (SNIFFEN et al., 1992).

Rumen degradation rates of protein fractions  $B_1$  and  $B_2$  were obtained via *in vitro* incubation of samples with commercial bacterial proteases of *Streptomyces griseus*, following procedures described by Krishnamoorthy et al. (1983).

Once degradation profiles of the crude protein and protein fractions were obtained, the degradation rates of the protein fractions were estimated by curve peeling (MERTENS, 2005), according to the following model:

$$R(t) = \sum_{i=1}^{2} B_i \exp(-kd_i t) + C$$
(1)

Where  $B_i$  and  $kd_i$  whichever i = 1 and 2 corresponds the potentially degradable protein fractions and their respective degradation rates; C is the unavailable protein fraction.

Contents of starch and neutral detergent soluble fiber (NDSF) were obtained using the methodology of Hall et al. (1999). Water-soluble carbohydrates contents were determined by spectrophotometry observing the formation of bluegreen color when these compounds were heated in antrona solution in strongly acid medium (DERIAZ, 1961).

To obtain NDFap *in vitro* degradation forages samples were incubated in penicillin flasks (100 ml) with ruminal fluid and reducing solution according to Goering and Van Soest (1970).

The ruminal fluid was originated from a cow fistulated, which was fed diet (90:10 roughage concentrate). The animal had unrestricted availability with respect to water and complete mineral mixture (6% phosphorus).

The degradation profile of neutral detergent fiber (NDFap) was obtained by the procedure described above were interpreted kinetically, using the model proposed by Matis et al. (1989), Matis (1972) and Vieira et al. (2008) considering the time dependency order (N). Three replicates were used for each forage. In vitro degradation ...

$$\mathbf{R}(t) = \left[\mathbf{R}_0 - \mathbf{U}\right] \left\{ \delta^N \exp(-k_d t) + \exp(-\lambda t) \sum_{i=0}^{N-1} \left(1 - \delta^{N-i}\right) (\lambda t)^i i! \right\} + U + e$$
<sup>(2)</sup>

Where: 
$$\delta = \lambda / (\lambda - k_d)$$
 (3)

R(t) corresponds to the residue of incubation after a certain time t(h); R(0) is equivalent to potentially degradable fraction (%); U corresponds to the undegradable fraction (%);  $k_d$  is the degradation rate of potentially degradable fraction of NDFap (h<sup>-1</sup>) (neutral detergent fiber corrected for ashes and protein). N represents the time dependency order related to the preparation of substrate for digestion;  $\lambda$  is the preparation rate of the substrate for degradation; *i* denotes the time dependency variation order; t is the independent variable time (h); and *e* corresponds to the random error, supposing Niid (0,  $\sigma^2$ ) where the random error is assumed to be independent and identically normally distributed with mean zero and variance  $\sigma^2$ . The equation was adjusted for the degradation profiles using the Marquardt algorithm of PROC NLIN procedure from SAS. The increase of time dependency order was applied to improve the adjustment of the model to the data. The criterion of choice for the best model was the likelihood probability calculated from Akaike criterion, simplified as follows:

$$\Delta_{AIC} = n_t \log_e (SQres_2 / SQres_1)$$
(4)  

$$P_r = \exp(-\Delta_{AIC} / 2) / [1 + \exp(-\Delta_{AIC} / 2)]$$
(5)

where, *nt* refers to the number of data in the degradation profile of fiber; *SQres* corresponds to the residual sum of squares; *Pr* refers to the likelihood probability (BURNHAM and ANDERSON, 2004; VIEIRA et al., 2008).

NDF corrected for protein content and ashes (NDF ap,VAN SOEST et al., 1991) was obtained by boiling the samples in neutral detergent solution, and then the crucible with the NDF residue was taken to a muffle furnace at 600°C during 3 hours in order to obtain the insoluble ash residue neutral detergent.

Correction for protein was performed using the result from neutral detergent insoluble protein (NDIP). Fractions  $B_2$  and C were obtained as follows:

$$B_2(\% TC) = (1 - U) XNDF_{ap}$$
(6)  
$$C(\% TC) = UXNDF_{ap}$$
(7)

where, U is equivalent to undegradable fraction (%); *NDFap* represents the neutral detergent fiber corrected for ashes and protein.

Cumulative gas production from fermentation was obtained after *in vitro* anaerobic incubations in acclimatized room at 39°C based on methodologies described by Malafaia et al. (1999). Degradation rates of fractions A and  $B_1$  of carbohydrates were estimated by the combination of the gravimetric and gas production *in vitro* techniques. Once the gas production profile of dry matter was obtained, the final volume of the gas was estimated from the adjustment to the model:

$$\mathbf{V}_{t} = \mathbf{V}_{f} \left\{ 1 - \left\{ \delta^{N} e^{-kt} + e^{-\lambda t} \sum_{i=0}^{N-1} (1 - \delta^{n-i}) (\lambda t)^{i} / i! \right\} \right\} + e \qquad (8)$$
  
$$\delta = \lambda I (\lambda - \mathrm{kd}) \qquad (9)$$

This model is an adaptation of the model from Vieira et al. (2008) to the gas production profile, in which *Vt* corresponds to the accumulated volume of gas at time t, expressed in mL/100 mg of incubated DM; *Vf* refers to maximum volume produced;  $\lambda$  corresponds to substrate preparation for digestion; *k* refers to the degradation rate expressed in h<sup>-1</sup>; and the other parameters are the same as the previous model.

When fractions A' and B' were detected in non fiber carbohydrate (NFC) profile, the final volume of gases was estimated by the adjustment to the following model:

$$V_{t} = V_{f1} \left\{ 1 - \left\{ \delta_{1}^{N} e^{-k_{1}t} + e^{-\lambda t} \sum_{i=0}^{N-1} (1 - \delta_{1}^{N-i}) (\lambda t)^{i} / i! \right\} \right\} + V_{f2} \left\{ 1 - \left\{ \delta_{2}^{N} e^{-K_{2}t} + e^{-\lambda t} \sum_{i=0}^{N-1} (1 - \delta_{2}^{N-i}) (\lambda t)^{i} / i! \right\} \right\} + e$$
Where:  $\delta_{1} = \lambda / (\lambda - k_{1}); \delta_{2} = \lambda / (\lambda - k_{2})$ 

$$10$$

This model was also adapted from heterogeneous GnG1 by Vieira et al. (2008), where, V(t) is the accumulated volume of gas, expressed in mL/100 mg incubated DM;  $Vf_1$  represents the maximum volume produced;  $\lambda$  is the preparation rate of substrate for digestion;  $k_1$  is the degradation rate of the carbohydrate fraction, expressed as h<sup>-1</sup>;  $k_2$ is the degradation rate of fraction B<sub>1</sub> of carbohydrates, expressed in h<sup>-1</sup>; the other parameters are the same as the previous models.

Final volume of Vf was estimated and the levels of NDFap = fiber carbohydrate (FC) and NFC = total carbohydrates (TC) - FC were considered to predict the contributions of each of these components (FC and NFC) to Vf, based on the presumption that the volume of gas produced by each unit of monomer from carbohydrate that was assimilated and fermented by microbial mass is the same for fibrous and non-fibrous carbohydrates (BEUVINK et al., 1992; SCHOFIELD and PELL, 1995; HALL et al., 1998).

It was adopted descriptive statistics to summarize the dataset, so that the conclusions generated in this study can not be applied to a larger population, in other words, only apply to group of samples from this study. To describe the data, tables were compiled and used sample average as position measurement.

#### **RESULTS AND DISCUSSION**

We adopted 4 protein subfractions (A,  $B_1$ , B<sub>2</sub>, and C) (FAVORETO et al., 2008), instead of five subfractions (A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and C) as originally proposed in CNCPS (SNIFFEN et al., 1992). The subfractions  $B_1$  and  $B_2$  of original model were determined to characterize proteins of different However, chemical nature. there are counterarguments to this fractionation (VAN SOEST, 1994; BRODERICK, 1995), because the kinetic behavior of protein degradation process in cellular content is very similar, indicating a nutritionally uniform behavior to the sum of CNCPS

**Table 1.** Chemical composition of tropical forages

fractions  $B_1$  and  $B_2$ . Thus, the use of fraction  $B_1$  of Favoreto et al. (2008) as equivalent to  $B_1+B_2$  from CNCPS can be considered as adequate. Strong favorable argument for the use of only four fractions of nitrogen compounds is related to laboratory techniques that are very accessible due to the procedures of routine analysis and the low cost.

High contents of protein and nonprotein nitrogen (NPN) (Tables 1 and 2) found in this work can be related to the age of Tifton 85 (16 days), which was collected when the shoots intercepted 95% of sunlight. Within this condition, plants measured approximately 20 cm of height, featuring early stage of development. As plants advanced to maturity, nitrogen present in cellular content, readily available to degradation, is used for sustaining tissue synthesis or stems, with less availability to the animal. Accordingly, young plants present higher contents of NPN and fraction  $B_1$ .

Item		Forage	
	Tifton-85	Mulberry	Leucaena
$DM^{a}$ (g.kg-1)	187.4	224.2	255.4
$OM^{b}$ (g.kg-1)	868.2	876.8	925.5
$\text{EE}^{c}(g.\text{kg}^{-1})$	23.9	42.5	36.3
$CP^{d}$ (g.kg <sup>-1</sup> )	229.0	261.1	283.9
$CHOT^{e}$ (g.kg <sup>-1</sup> )	615.4	573.2	605.4
$NFC^{f}(g.kg^{-1})$	127.4	408.9	389.3
$NDF^{g}(g.kg^{-1})$	650.5	270.4	353.0
$NDFa^{h}(g.kg^{-1})$	612.2	231.8	306.1
$NDFap^{i}(g.kg^{-1})$	488.3	164.2	216.1
$ADF^{j}(g.kg^{-1})$	281.0	125.3	155.9
$\text{NDIP}^{k}$ (g.kg-1)	112.3	69.0	93.7
$ADIP^{1}(g.kg^{-1})$	22.9	21.1	41.9
$LDA^{m}(g.kg^{-1})$	74.8	50.6	88.7
LDA:NFDap (%)	15.3	30.8	41.04
Sugars (g.kg <sup>-1</sup> )	17.2	29.5	23.4
Starch $(g.kg^{-1})$	1.9	1.9	00.7
$NDSF^{n}(g.kg^{-1})$	117.2	344.2	329.7

<sup>a</sup>dry matter, <sup>b</sup>organic matter, <sup>c</sup>ethereal extract; <sup>d</sup>crude protein, <sup>c</sup>total carbohydrate, <sup>f</sup>non-fibrous carbohydrates, <sup>g</sup>neutral detergent fiber; <sup>h</sup>NDF corrected for ash, <sup>i</sup>NDF corrected for ash and protein, <sup>j</sup>acid detergent fiber; <sup>k</sup>neutral detergent insoluble protein, <sup>l</sup>acid detergent insoluble fiber, <sup>m</sup>acid detergent lignin, <sup>n</sup>neutral detergent soluble fiber.

Table 2. Protein fractions present in tropical forage

	· · ·	Forage	
Fractions (g/100g CP)	Tifton-85	Mulberry	Leucaena
A	31.12	13.89	24.89
$B_1$	20.50	59.66	42.07
$B_2$	38.47	18.34	18.26
С	9.90	8.10	14.78

Furthermore, the nitrogen fertilization prior to sampling may have increased the nitrogen availability in soil, leading to a luxury consumption of nitrogen, as already reported by Balsalobre (2002), and contributing to increase the protein contents, especially of fraction A. Thus, tropical pastures from intensive production systems for milk or meat, where high contents of N are applied aiming high stocking rates, will present higher crude protein contents, once collected at the right moment (ROMERO et al., 2008). However, these high protein contents in plants can generate excessive ammonia inside rumen and be excreted as urea.

The Tifton 85 presented 51.61g/100g CP as a sum of fractions A and B1 (NPN, peptides and amino acids readily available in rumen) that are nitrogen source readily degradable (Table 2). High proportions of these fractions can result in loss of N in rumen, as ammonia, because large amounts of soluble fraction require higher supply of readily degradable carbohydrates for synchronization of fermentation between carbohydrates and proteins (NOCEK and RUSSELL, 1988). Thus, if the contents of readily degradable carbohydrate is low and there is high proportion of soluble protein (fraction  $B_1$ ) in rumen, the consequence would be high losses of nitrogen via urea. Some can be recycled to the rumen via saliva or rumen wall, however much of the nitrogen must be metabolized and removed from the body through urea cycle (OWENS and ZINN, 1988), in a process that spends energy.

The fraction  $B_2$  was 38.74 g/100g CP in Tifton 85 (Table 2), representing the higher portion of protein in tropical forages and corresponds to the protein adhered to cell wall, with potential to be degraded but, however, with low rate of degradation. Although Tifton 85 was at early stage of development, more than 45% of fibrous protein is linked to the fibrous fraction of plants, which reduces the possibility of utilization by the animals.

Therefore, the fractions that represent about 80% of protein from Tifton 85 (A,  $B_2$  and  $C_2$ ) present problems of utilization by the animal. As for fraction A (NPN), if the supply of rapidly

fermentable carbohydrates is not adequate, the loss of NPN as ammonia can be high. Fraction  $B_2$  has slow degradation rate, but can be used in the small intestine, whereas fraction C, which represented 9.90% crude protein, is not usable by animal. This means that, despite the high levels of protein in Tifton 85, a deficit of metabolizable protein may occur at any time after the intake (BALSALOBRE, 2002).

The sum of fractions A and  $B_1$  from mulberry and leucaena were 73.55 g/100g CP and 66.96g/100g CP respectively; whereas fraction  $B_2$ was 18.34 g/100g CP and 18.26 g/100g CP respectively, evidencing a higher amount of protein linked to non-fibrous fraction from those plants.

Regarding Tifton 85, the sum of fractions A and  $B_1$  was 51.61 g/100g CP and the fraction  $B_2$  was 38.74 g/100g CP. Thus, the portion of slowly degradable protein from this forage is higher and tends to escape from rumen contributing with amino acids in the small intestine. In contrast, most of the protein from mulberry and leucaena are present as highly degradable protein in rumen ( $B_1$ ), requiring readily fermentable carbohydrate supplementation to improve nitrogen utilization (RUSSELL et al., 1992). Considering the partitions of protein in different compartments of the plant, possibly the mixture between forages promotes a better balance for the use of this nutrient by animals.

Digestion rates of nitrogen fractions (Table 3) influence the escape of protein to intestines and are important to meet the requirements of rumen microorganisms for adequate microbial growth. Among studied forages, leucaena presented a lower degradation rate of fraction B<sub>2</sub> (0.0631  $h^{-1}\pm 0.024$ ). This fraction of protein presents low degradation rate for being linked to fibrous compounds that have high degradation rate (0.1808  $h^{-1}\pm 0.009$ ), indicating a complete dissociation between the degradation processes of nutrients. The fraction B<sub>2</sub> contributes with most part of the protein that reaches the small intestine without being degraded in rumen. Thus, the indigestible residue of this fraction together with fraction C represents a large portion of the ingested protein that is excreted in the feces.

Table 3. Contents, means and standard errors (SE) of the estimated degradation rates of nitrogen fractions.

Forage		Contents (g	$g.kg^{-1}$ DM)		Degradation rate (h <sup>-1</sup> )		
$A$ $B_1$ $B_2$	С	<b>B</b> <sub>1</sub>	<b>B</b> <sub>2</sub>				
Tifton-85	71.29	46.95	88.13	22.68	0.2570±0.005	0.0798±0.022	
Mulberry	36.28	155.75	47.87	21.16	$0.3036 \pm 0.003$	0.1076±0.034	
Leucaena	70.69	119.48	51.85	41.97	$0.1005 \pm 0.002$	0.0631±0.024	

In vitro degradation...

Mulberry presented high proportion of soluble N (A+B<sub>1</sub>), about 73% of total CP, and high degradation rate of fraction B<sub>1</sub> (0.3036 h<sup>-1</sup>±0.003), inferring that its protein tends to be highly degraded in rumen, which can result in significant losses of N, if synchronization is not adequate with the fermentation of carbohydrates in rumen.

Carbohydrate fractions of Tifton 85 were 54.08 g/100g TC as fraction  $B_2$  and sum of

Table 4. Carbohydrate fractions present in forage plants

fractions  $A+B_1$  were 20.67 g/100g TC (Table 4). The higher proportions of fibrous carbohydrates reaffirm the high importance of these compounds, particularly to the potentially degradable fraction, as a source of energy for the animals fed pasture, and also demonstrate that the non-fibrous carbohydrates present in tropical grasses such as Tifton 85, rarely exceed 20g/100g of total carbohydrate.

		Forage		
Fractions (g/100g TC)	Tifton-85	Mulberry	Leucaena	
A	2.80	5.17	3.90	
$B_1$	17.87	66.12	60.40	
$B_2$	54.08	20.87	25.69	
С	25.25	7.84	10.03	

Mulberry and leucaena presented 71.29g/100g TC and 64.30 g/100g TC represented by fractions  $A+B_1$ , suggesting the high quality of these feed, due to the high proportion of highly fermentable carbohydrates. Fraction  $B_2$  presented only 20.87 and 25.69 g/100g TC, in mulberry and leucaena, respectively and differently from Tifton-85, these forages presented higher percentages of non-fiber carbohydrates.

Fraction of total carbohydrates А corresponds to soluble sugars, such as glucose and disaccharides that function as reserve in plants, and are quickly fermented in rumen (SNIFFEN et al., 1992). Fraction  $B_1$  have starch and pectin with an intermediate degradation rate, ranging from 0,3 to 0,7 h<sup>-1</sup> because pectin is rapidly digested, but some types of starch are slowly degraded. Tifton 85, in this study, presented sugar content of 1.72g/100g DM and starch of 0.19g/100g DM, much lower values than those obtained by Ribeiro et al. (2001), who found for the same grass at 28 days, protein content of 17.58g/100g CP, CNF of 5.29g/100g, NDFap of 67.29g/100g, 3.95g/100g of sugars and 1.72 g/100g of starch in DM. Possibly the lower concentration of sugars and starch in Tifton 85 of this study happened due to the early stage of development (16 days), with low carbohydrate as reserve.

Among fractions A+B1, mulberry and leucaena presented, respectively, 2.95 and 2.34 g/100g DM of sugars; 0.19 and 0.07 g/100g DM of starch; and 34.42 and 32.97g/100g DM of neutral detergent soluble fiber (NDSF). Therefore, the main composition of CNF is NDSF and not starch (reserve carbohydrate).

Forages studied had low contents of starch (Table 1), then laboratory analysis of this component was not needed because this technique is extremely difficult and have high cost. Indeed, starch contents can be obtained by the difference: Starch = CNF – neutral detergent soluble fiber (NDSF) – Sugars.

Digestion rates found for fraction B<sub>2</sub> of tifton-85 (Table 5) was higher than those obtained by Malafaia et al. (1999), Cabral et al. (2000) and Ribeiro et al. (2001), possibly because of the early stage of development at the moment of sampling that made this forage present highly degradable fiber. In fact, when Sniffen et al. (1992) worked with alfalfa hay, they obtained degradation rates of fiber of 0.080 h<sup>-1</sup>, that was very close to that obtained in this study for Tifton 85, which leads us to classify this forage as good quality. Degradation rates of fractions A and  $B_1$  in tifton 85 (Table 5) were similar to the results found by Sniffen et al. (1992), in alfalfa hay, with degradation rate of fraction  $B_1$  between 0.35 and 0.40h<sup>-1</sup>. The high degradation rate of fraction B<sub>1</sub> is originated mainly from NDSF that presents higher proportion than starch.

Forages mulberry and leucaena have fibrous components of high degradation (Table 5). Fractions A and  $B_1$  were joined for calculation of the degradation rate of NFC in leucaena because the separation of what would come from each fraction in gas production curves was not possible.

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-	Contents (g.kg <sup>-1</sup> DM)				Degradation rate (h <sup>-1</sup> )		
Forage	А	$\mathbf{B}_1$	$B_2$	С	А	$\mathbf{B}_1$	$B_2$
Tifton-85	17.25	109.92	332.73	155.36	0.78±0.036	0.41±0.023	$0.084 \pm 0.016$
Mulberry	29.55	379.38	119.39	44.85	0.35±0.023	$0.07 \pm 0.032$	$0.275 \pm 0.005$
Leucaena	23.61	365.66	155.53	60.72	60.72 0.1965±0.015*		$0.1808 \pm 0.009$

Table 5. Contents, means and standard errors (SE) of the estimated degradation rates of carbohydrate fractions.

\* Degradation rate A+B1;

**Table 6.** Observed frequencies of the minimum sum of squares of errors (SSE) and cumulative frequency distribution of the nu for number of runs of sign of the errors as partial measurements of the quality of fit (Likelihood probability based on the Akaike's Information Criterion) for the generalized compartmental model of digestion (GCMD) for NDFap degradation of Tifton-85.

Characteristic			N <sub>a</sub> in GCMI	)	
	2	3	4	5	6
Minimum SSE	0.0177	0.0161	0.0154	0.01514	0.0150
No. runs of sign <sup>b</sup>					
2	0.9896	0.9980	0.9991	0.9993	0.9994
3		0.8425	0.9177	0.9402	0.9486
4			0.6758	0.7460	0.7752
5				0.5850	0.6233
6					0.5400

<sup>a</sup> Associated to 35 degradation profiles; <sup>b</sup>Computed from profiles that yielded significant parameters after fitting each model.

**Table 7.** Observed frequencies of the minimum sum of squares of errors (SSE) and cumulative frequency distribution of the nu for number of runs of sign of the errors as partial measurements of the quality of fit (Likelihood probability based on the Akaike's Information Criterion) for the generalized compartmental model of digestion (GCMD) for NDFap degradation of Mulberry.

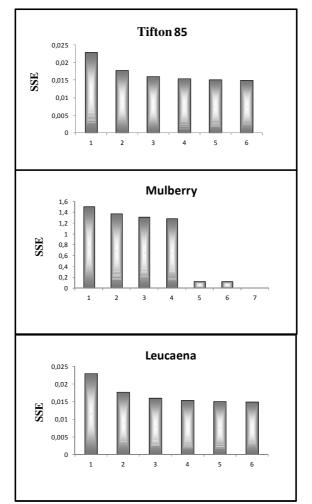
Characteristic			N <sub>a</sub> in GCME	)	
	2	3	4	5	6
Minimum SSE	1.37479	1.31498	1.28078	0.12409	0.12596
No. runs of sign <sup>b</sup>					
2	0.8090	0.8982	0.9317	1.0000	1.0000
3		0.6757	0.7629	1.0000	1.0000
4			0.6070	1.0000	1.0000
5				1.0000	1.0000
6					0.4386

<sup>a</sup> Associated to 33 degradation profiles; <sup>b</sup>Computed from profiles that yielded significant parameters after fitting each model.

**Table 8.** Observed frequencies of the minimum sum of squares of errors (SSE) and cumulative frequency distribution of the nu for number of runs of sign of the errors as partial measurements of the quality of fit (Likelihood probability based on the Akaike's Information Criterion ) for the generalized compartmental model of digestion (GCMD) for NDFap degradation of Leucaena.

Characteristic			N <sub>a</sub> in GCME	)	
	2	3	4	5	6
Minimum SSE	0.0177	0.0161	0.0154	0.0151	0.0150
No. runs of sign <sup>b</sup>					
2	0.9896	0.9980	0.9991	0.9993	0.9994
3		0.8425	0.9177	0.9402	0.9486
4			0.6758	0.7460	0.7752
5				0.5850	0.6233
6					0.5400

<sup>a</sup> Associated to 35 degradation profiles; <sup>b</sup>Computed from profiles that yielded significant parameters after fitting each model.



**Figure 1**. Patterns of the sum of squares of errors (SSE) distributed according to the order of time dependency (positive integer order of time dependency related to the preparation of NDF (Na) for digestion on the abscissa).

The degradation profile of neutral detergent fiber (NDFap) was obtained by the procedure

interpreted kinetically, using the model proposed by Matis et al. (1989), Matis (1972) and Vieira et al.

(2008) considering the time dependency order (N) (Figure 1). The criterion of choice for the best model was the likelihood probability calculated from Akaike criterion. In the case of Tifton the model 4, 5 and 6 is more likely than 3, we opted for the less complex model that was 3 (Table 6). For Mulberry the model chosen was the 5 once the comparison between models 5 and 6 not get gain in model fit (Table 7) and for Leucaena the model chosen was 4 once the comparison between 5 and 6 do not get gain in model fit (Table 8).

Total carbohydrate (TC) present in mulberry and leucaena was 71.29 and 64.30g/100g, respectively, in the form of non-fibrous carbohydrate (sugar, starch and NDSF), with high degradation rate, where 20.87 and 25.69 g/100g are in the form of potentially degradable fibrous carbohydrates, also with high degradation. Thus, these forages are highly degradable feed, both for the cellular content (in higher proportion) and for the cell wall (in a lesser extent), with high possibility of presenting good synchronization of degradation between carbohydrates and protein.

#### CONCLUSIONS

Fractions representing about 80g/100g of the protein from tifton 85 presented problems to be used by animals. This means that, despite the high contents of protein in Tifton 85, metabolizable protein deficit may occur at any time after the intake of forages by the animals.

Mulberry and leucaena are extremely degradable feed, both for cellular content and cell wall, with high possibility of presenting good synchronization between degradation of carbohydrate and protein.

**RESUMO**: A obtenção de informações detalhadas sobre a composição dos nutrientes e a taxa de degradação das frações de carboidratos e proteínas é essencial para determinar quanto de cada nutriente pode ser usado pelo animal e quais as principais limitações para o nível de produção. Um estudo descritivo foi conduzido para avaliar a taxa de degradação das frações de carboidratos e proteína e compreender o sincronismo de degradação dessas frações no rúmen de cabras alimentadas com as forrageiras Tifton-85, amoreira e leucena. Os conteúdos de proteína bruta (PB), nitrogênio não protéico (NNP), nitrogênio insolúvel em detergente neutro (NIDN) e nitrogênio insolúvel em detergente ácido (NIDA) foram mensurados para obter as frações de protéicas A, B<sub>1</sub>, B<sub>2</sub> e C. O perfil de degradação das frações de nitrogênio foram obtidas por incubação das forragens com proteases de Streptomyces griseus. Os conteúdos de açúcares, amido e fibra solúvel em detergente neutro (FSDN) foram analisados para determinar as frações A e B<sub>1</sub> dos carboidratos totais (CT), sendo que para obtenção da fração  $B_2$ , C e para taxa de degradação da fração  $B_2$ , a técnica gravimétrica de degradação da fibra in vitro foi utilizada através da interpretação do perfil cinético de degradação. Foi utilizada a estatística descritiva para analisar os dados e como medida de posição foi adotada a média. Em relação ao Tifton 85, a soma das frações A e  $B_1$ foram 51.61 g/100g PB e a fração B<sub>2</sub> foi 51.61 g/100g PB. Portanto, as frações de proteína de baixa degradação dessa forrageira são altas e tendem a escapar do rúmen e contribuir com aminoácidos no intestino delgado. Por outro lado, a maior parte da proteína da amoreira e leucena são altamente degradáveis no rúmen (B<sub>1</sub>), o que requer suplementação com carboidratos rapidamente fermentáveis para melhor utilização dos compostos nitrogenados. Considerando as partições da proteína nas diferentes partes da planta, é possível que a mistura entre essas forrageiras permita um melhor balanceamento para uso desse nutriente pelos animais. Cerca de 80g/100g de proteína do Tifton 85 apresentaram problemas de uso pelos animais. Isso significa que, apesar do alto nível de proteína nessa forrageira, deficiência de proteína metabolizável pode ocorrer em algum momento. Amoreira a leucena são alimentos com alta degradação, tanto do conteúdo celular como da parede celular, com alta possibilidade de apresentar boa sincronização entre a degradação de carboidratos e proteína.

PALAVRAS-CHAVE: CNCPS. Rúmen. Amido. Açúcares.

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