**Ruminants** Full-length research article



Brazilian Journal of Animal Science e-ISSN 1806-9290 www.rbz.org.br

#### \*Corresponding author: braga.braga@discente.ufg.br

Received: July 8, 2022

Accepted: October 13, 2023

**How to cite:** Lino, R. A.; Braga, B. C.; Couto, C. J.; Villela, S. D. J.; Gomes, R. S.; Tamy, W. P.; Moreira, L. M. and Leonel, F. P. 2024. Kinetics of transit and rumen degradation of processed fiber from seedbed straw according to different non-protein nitrogen sources. Revista Brasileira de Zootecnia 53:e20220098.

https://doi.org/10.37496/rbz5320220098

#### Editors:

Marcio de Souza Duarte Cláudio Vaz Di Mambro Ribeiro Eduardo Marostegan de Paula

**Copyright:** This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

CC BY

# Kinetics of transit and rumen degradation of processed fiber from seedbed straw according to different non-protein nitrogen sources

Rayane Aparecida Lino<sup>1</sup> (D), Bruna Cardoso Braga<sup>2\*</sup> (D), Claudiney de Jesus Couto<sup>1</sup> (D), Severino Delmar Junqueira Villela<sup>1</sup> (D), Raphael dos Santos Gomes<sup>3</sup> (D), Wagner Pessanha Tamy<sup>4</sup> (D), Leonardo Marmo Moreira<sup>5</sup> (D), Fernando de Paula Leonel<sup>5</sup> (D)

- <sup>1</sup> Universidade Federal dos Vales do Jequitinhonha e Mucuri, Departamento de Zootecnia, Diamantina, MG, Brasil.
- <sup>2</sup> Universidade Federal de Goiás, Departamento de Zootecnia, Goiânia, GO, Brasil.
- <sup>3</sup> Instituto Federal de Educação, Ciência e Tecnologia de Rondônia, Colorado do Oeste, RO, Brasil.
- <sup>4</sup> Universidade Federal Fluminense, Departamento de Zootecnia e Desenvolvimento Agrossocioambiental Sustentável, Niterói, RJ, Brasil.
- <sup>5</sup> Universidade Federal de São João del-Rei, Departamento de Zootecnia, São João del-Rei, MG, Brasil.

**ABSTRACT** - The present study presents a comparative evaluation of the transit kinetics of straw briquette in response to the dietary addition of non-protein nitrogen sources in the form of a mineral supplement. Four rumen-cannulated, castrated Holstein-Gir crossbred cattle, weighing an average of  $380\pm22.64$  kg, were distributed into a  $4 \times 4$  Latin square design (four supplements with non-protein nitrogen sources  $\times$  four experimental periods). The following non-protein nitrogen sources were studied: conventional urea, slow-release urea, extruded urea, and monoammonium phosphate. During the experiment, the animals were housed in individual stalls with concrete floors where they received a basal diet consisting of straw briquette, potato starch, and the mineral supplement, the latter whose variation was only in the non-protein nitrogen sources did not affect the parameters of transit or degradation kinetics of straw fiber briquette. These results can be associated with the low nitrogen content limited by the types of supplements and the particle size of straw briquette, which is smaller due to processing.

Keywords: Brachiaria straw, briquetting, extruded urea, passage rate, residue, slowrelease urea

## **1. Introduction**

Brazil is the world's largest producer of forage seeds and, as such, generates a large volume of residue that causes problems for the subsequent occupation of the area. The permanence of straw in seed production fields forms windrows that inhibit regrowth and make harvesting under the windrows difficult. Tropical-forage-grass seed production fields occupy an area equivalent to 140,000 ha per year, generating an average of 20 t of straw per hectare. It is thus estimated that 2.8 million tons of this lignocellulosic material are discarded each year in Brazil (Catuchi et al., 2017).

Among the alternatives to optimize production, the use of alternative foods from agricultural residues can be considered. This is possible due to the ability of ruminants to transform plant residues into

nutrients (Van Soest, 1994). As it comes from a mature forage, the residue from the production of tropical seeds is characterized by having low nutritional value, with high levels of fibrous carbohydrates and low levels of crude protein (CP), non-fiber carbohydrates, and minerals. An alternative to solve this problem is the compaction of straw through a process called briquetting (Souza and Cardoso, 2003). Processing takes place by means of temperature and pressure, which can result in some destabilization of the lignocellulosic matrix of the original material, causing an increase in the rate of ruminal degradation of the fiber.

Diets with a high concentration of cell wall restrict the access of microorganisms to the food particle, with a consequent decrease in digestibility (Akin, 1979). This limitation is mainly due to the lower rate of degradation and passage of the fibrous fraction through the rumen. For this type of feed, besides its composition, information on its transit and degradation kinetics is essential, so it can be strategically used in ruminant diets.

The total CP present in low quality fibrous foods is little used by rumen microorganisms and does not meet the requirements to maintain adequate fermentation (Oliveira, 2007). The low protein content of these straws can be overcome with the use of protein supplements. Ammonia is essential for microbial protein synthesis in the rumen and can be supplied through protein and non-protein nitrogen (NPN) sources such as urea (Berger et al., 1994).

Nonetheless, conventional urea has a fast degradation in the rumen, which can oftentimes reduce the efficiency of its utilization due to the low synchronization with fermentable carbohydrates in tropical diets. This has fostered the development of new sources of NPN, which provide greater synchronization and rumen efficiency. Slow-release urea degrade less rapidly in the rumen, with potential claims of improved synchronization of ruminal ammonia with energy digestion (Salami et al., 2020, 2021).

Therefore, the hypothesis is that slow-release urea and extruded urea provide greater synchronization with the fermentable carbohydrates and, therefore, improve the degradation dynamics and the transit kinetics of the fiber from the residue of the processed forage harvest. The present study was thus carried out to investigate the dynamics of degradation and transit kinetics of fiber from processed forage-seed harvest residue in response to the use of alternatives sources of NPN to urea in supplements formulated for a low intake.

## 2. Material and Methods

Research on animals was conducted according to the institutional committee on animal use (CEUA EPAMIG01/2019). The experiment was carried out in São João del-Rei, Minas Gerais, Brazil (Latitude: 21°8'11" South; Longitude: 44°15'43" West), from June to August 2020.

Four rumen-cannulated, castrated Holstein-Gir crossbred cattle, weighing an average of 380±22.64 kg, were distributed into a 4 × 4 Latin square design. Throughout the experiment, the animals remained stabled in individual stalls with concrete floors and partially covered with clay tiles. Treatments were as follows: supplement containing conventional urea (CUS), supplement containing slow-release urea (SRUS), supplement containing extruded urea (EUS), and supplement containing monoammonium phosphate (MAPS).

A basal diet was used to meet the nutritional requirements of cattle with a body weight of 400 kg according to the NASEM (2016) (Tables 1 and 2). Diets were supplied twice daily to the animals, which had *ad libitum* access to water. The four diets varied only in the source of NPN in the low-intake supplement, which characterized each treatment. Orts were not sampled since the objective was to study fiber transit and degradation kinetics.

Straw briquette, used to compose the diet, is a product obtained from the industrial processing of residues from the production of tropical forage seeds (*Urochloa brizantha*, cv. MG4), composed of stems, leaves, and remaining inflorescences. This processing consists of grinding and subsequently compacting the material and cutting the briquettes formed. The potato starch, originated from the processing for the production of straw potatoes (Croques company, São João del-Rei, MG, Brazil), was

chosen to be used in the diet because of their low protein content. Straw briquette was hydrated at the ratio of 2:1 (2 L of water to 1 kg of straw briquette) and supplied to the animals together with starch and mineral supplement. To allow an equal concentration of NPN between the sources, its content was fixed by its concentration in monoammonium phosphate. Mineral supplements containing the test sources (Table 3) were inserted directly into the rumen once a day via cannula, throughout the trial period. This procedure was adopted to ensure the complete supply of NPN for each treatment.

In the basal diet, the protein and fiber contents were analyzed. Total nitrogen was determined by method 981.10 of AOAC (2012). The CP content was calculated by multiplying the percentage of N by 6.25. Neutral detergent fiber (aNDF) was determined according to Mertens (2002), without the addition of sodium sulfide and with the addition of thermostable alpha-amylase. The aNDF content was corrected for protein and ash for all samples (Licitra et al., 1996).

The experiment lasted 92 days, which were distributed into four experimental periods of 23 days each. Of these, the first 14 were used for the animals to adapt to the diets, and the subsequent nine for sample collection.

#### Table 1 - Centesimal composition of the standard diet used in the study

| Ingredient   | %    |  |
|--|------|--|
| Straw briquette - straw from forage-seed harvest, processed <sup>1</sup> | 85   |  |
| Potato starch  | 13.5 |  |
| Mineral supplements containing different sources of NPN                  | 1.5  |  |
| Total  | 100  |  |

NPN - non-protein nitrogen.

<sup>1</sup> Processing: grinding and compression-pelleting.

#### Table 2 - Chemical composition of ingredients (g/kg of DM)

|                    | 1     | 0     | 0  |       |       |        |      |        |
|--------------------|-------|-------|----|-------|-------|--------|------|--------|
| Feedstuff          | DM    | СР    | CF | aNDF  | ADF   | Lignin | MM   | Starch |
| Straw briquette    | 918.2 | 51.2  | ND | 736.6 | 439.4 | 35.2   | 54.5 | ND     |
| Potato starch      | 957.5 | 3.3   | ND | 40.8  | 29.5  | ND     | 4.3  | 801.4  |
| Mineral supplement | 925.0 | 312.5 | ND | ND    | ND    | ND     | 1000 | ND     |

DM - dry matter; CP - crude protein; CF - crude fat; aNDF - neutral detergent fiber; ADF - acid detergent fiber; MM - mineral matter; ND - not detectable.

#### **Table 3 -** Chemical composition of low-intake supplement supplied to animals

| Warranty level                |        |
|-------------------------------|--------|
| Calcium (g/kg)                | 160    |
| Phosphorus (mg/kg)            | 90     |
| Sodium (mg/kg)                | 65.35  |
| Sulfur (mg/kg)                | 5      |
| Magnesium (g/kg)              | 5      |
| Cobalt (mg/kg)                | 24     |
| Copper (g/kg)                 | 1600   |
| Iron (mg/kg)                  | 600    |
| Iodine (mg/kg)                | 60     |
| Manganese (g/kg)              | 3600   |
| Selenium (mg/kg)              | 12     |
| Zinc (mg/kg)                  | 4800   |
| CP (g/kg)                     | 312.46 |
| NPN Eq CP <sup>1</sup> (g/kg) | 312.46 |

<sup>1</sup> Crude protein equivalent from non-protein nitrogen refers to the sources of non-protein nitrogen in the low-intake supplement, which characterized each treatment.

#### 2.1. Particle transit kinetics

To evaluate the parameters of particle transit kinetics, the chromium marker (Cr mordant) was fixed to the roughage fiber, in an adapted version of the procedure described by Udén et al. (1980). The straw briquette samples were dried in a forced-air oven at 55±5 °C for 72 h. Subsequently, this material was boiled with water and neutral detergent for 1 h. The proportion of ingredients used was 100 g of dry sample to 100 mL of detergent and 1 L of water. Then, the material was filtered through a cotton fabric bag and washed in running water until the water was clear to remove the soluble components. After the filtering process, the fiber was returned to the forced-air oven at 55±5 °C, where it remained for 72 h. At the end of the washing and drying process, the fiber was placed in a suitable container and immersed in a solution of potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>2</sub>, 2H<sub>2</sub>O) at the ratio of 13% of chromium relative to the fiber weight. The container with the fiber was completely covered with aluminum foil and oven-dried at 105 °C for 24 h. Afterwards, a second wash was performed inside a cotton fabric bag to remove excess potassium dichromate. After this second wash, the material was immersed in a commercial ascorbic acid solution at the proportion of half the fiber weight. The immersion remained at rest for 1 h, until it reached an intense green color. Then, a third wash was performed, again in a cotton fabric bag, and the procedure was repeated until the water was completely clear. After washing, the fiber was dried in a forced-air oven at 55±5 °C for 72 h.

After this procedure, 200 g of marked fiber were placed directly into the rumen cannula at the beginning of each experimental period. Then, feces were collected individually, directly from the animals' rectum, at predetermined times up to 192 h (3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 60, 72, 96, 120, 144, and 192 h).

Feces samples were pre-dried in a forced-air oven at 55±5 °C for 72 h, ground in a Cyclone mill with a 1-mm mesh sieve, and analyzed to determine the chromium content by atomic absorption spectrophotometry, after nitric-perchloric digestion, following the methodology described by Kimura and Miller (1957).

#### 2.2. Parameters of rumen degradation kinetics

The technique proposed by Mehrez and Orskov (1977) and Nocek (1985) was adopted to determine the parameters of rumen degradation kinetics of dry matter (DM) and aNDF of straw briquette. Nylon bags (13 × 7 cm; pore diameter: 50  $\mu$ m) were used, maintaining a ratio of 25 mg DM/cm<sup>2</sup> of bag surface, as recommended by Kirkpatrick and Kennelly (1987).

The incubation procedure involved the use of a chain anchored to a weight. The nylon bags were tied individually to the links of the chain, sequentially to the incubation times, and immersed into the rumen content, allowing the action of rumen microorganisms on the samples.

The bags were incubated in the rumen of the animals in reverse chronological order (placing at the predetermined times and removal of all bags at the end of the time count). Incubation times were 0, 3, 6, 9, 12, 24, 36, 72, and 96 h. At the end of the incubation period, the bags were washed in running water until the water was completely clear and dried in a forced-air oven at 55±5 °C for 48 h. Subsequently, they were dried in a desiccator and the "oven-dried weight" was then determined. The incubation referring to zero time was not used, but the corresponding bags were washed simultaneously with the others. Subsequently, aNDF analyses were performed (AOAC method 2002.04) in all incubated samples.

#### 2.3. Estimation of particle transit kinetics parameters

The general form attributed to the markers' excretion profiles was the segmented one-compartment model (Pond et al., 1988):

$$C_t = d$$
, if  $t < tt$ , and

$$C_t = d + C_0 \times \frac{\lambda^N \times (t - tt)^{N-1} \times \exp[-\lambda \times (t - tt)]}{(N-1)!}, \text{ if } t \ge tt,$$
(1)

in which  $C_t$  is the concentration of the marker at time t, d is a biologically meaningless scale parameter required for the use of uncorrected reading data,  $C_0$  is the concentration of the marker in the rumenreticulum at t = 0,  $\lambda$  is the asymptotic rate of passage of the marker emerging from the reticulorumen, N represents a positive integer denoting the time-dependent order, and tt is the time the marker takes from the reticulo-omasal orifice to exit in the feces.

The conventional assumption of homoscedasticity was evaluated as follows (Pinheiro and Bates, 2000):

$$\sigma_{c_t}^2 = \sigma^2 \tag{2}$$

$$\sigma_{C_t}^2 = \sigma^2 \left(C_t\right)^{2\psi} \tag{3}$$

in which  $\sigma^2$  is the homogeneous residual variance ( $\sigma_{C_t}^2 = \sigma^2$ ) as shown by equation 2. Equation 3 represents the power-scaled ( $\psi$ ) residual variance ( $\sigma_{C_t}^2 = \sigma^2$ ) as a function of the expected mean,  $C_t$ . The correlation between the measures was also evaluated using the continuous autoregressive correlation (CAR1) of the nlme package of R (Pinheiro and Bates, 2000).

#### 2.4. Estimation of rumen degradation parameters

The following models were fitted to the degradation profiles:

$$R_t = A \times (\exp(-kt)) + U + e_t \tag{4}$$

$$R_t = A \times \left(1 - \left(\frac{t^c}{t^c + K^c}\right)\right) + U + e_t$$
(5)

$$R_{t} = A \times \left( \delta^{N} \exp(-kt) + \exp(-\lambda_{a}t) \sum_{i=1}^{N-1} \frac{(1 - \delta^{N-i})(\lambda_{a}t)^{i}}{i!} \right) + U + e_{t}$$
(6)

Equations 4, 5, and 6 represent the exponential, generalized Michaelis-Mentem (GMM), and generalized compartment (GNG1) models, respectively (López et al., 1999; Vieira et al., 2008), in which *A* is the potentially degradable fraction. In equations 4 and 6, *k* is the degradation rate of potentially degradable fractions; in equation 5, *c* is a scale parameter and *K* (h) represents the time taken until half of the substrate is consumed (half-life). In equation 6, *N* is a positive integer representing the time-dependent order,  $\lambda_a$  (1/h) is the asymptote of the rate of preparation for digestion,  $\delta = \lambda_a/(\lambda_a - k)$  is a constant, *U* is the indigestible fraction, and  $e_t$  is the random error. The models were fitted to the degradation profiles using the nlme package procedure of R (Pinheiro and Bates, 2000). The best fit of the model to the profile was evaluated by computing the corrected Akaike information criteria and its derived measures (Akaike, 1974).

The conventional assumption of homoscedasticity was tested using the nlme package of R (Pinheiro and Bates, 2000).

Variance was modeled as shown below (Pinheiro and Bates, 2000):

$$\sigma_{R_{\star}}^2 = \sigma^2 \tag{7}$$

$$\sigma_{R_{\star}}^2 = \sigma^2 = \sigma^2 \left(R_{\star}\right)^{2\psi} \tag{8}$$

in which  $\sigma^2$  is the residual homogeneous variance as shown by equation 7. Equation 8 represents the power-scaled ( $\psi$ ) residual variance as a function of the expected mean,  $R_t$ . The correlation between measures was also evaluated using the CAR1 of the nlme package of R (Pinheiro and Bates, 2000).

#### 2.5. Fitting of models

The models were fitted to the profiles of *in situ* fiber degradation and marker excretion using the nlme package of R software (Pinheiro and Bates, 2000). The best fit of the model to the profile was

evaluated by computing the information criteria derived from the corrected Akaike criterion (Akaike, 1974; Sugiura, 1978; Burnham and Anderson, 2004). Additionally, the decision on the best model to describe the profile was made based on the recommendations of Vieira et al. (2012).

Additional estimates were obtained by the NLMIXED package of SAS (Statistical Analysis System, University Edition), using the estimates from the nlme package of R as input.

As aNDF has no soluble component in the rumen fluid phase, the estimated parameter coefficients were normalized by assuming the correction proposed by Waldo et al. (1972), as follows:

$$An = \frac{A}{(A+U)} \tag{9}$$

$$Un = \frac{U}{(A+U)} \tag{10}$$

The effective degradability coefficient (ED), mean residence time in the rumen-reticulum (MRTR), and mean total residence time (MTRT) were calculated following the equations:

$$ED = \frac{k}{(k+\lambda)} \tag{11}$$

$$MRTR = \frac{N}{\lambda}$$
(12)

$$MTRT = \frac{N}{\lambda} + tt$$
(13)

The estimated parameters and additional estimates were compared by contrasts against the control treatment, which is conventional urea (CUS). Contrasts were considered significant when P<0.05.

#### **3. Results**

#### 3.1. Fiber degradation kinetics

The generalized compartment model combined with scaled residual variance and random effect on parameter A showed the lowest Akaike information criterion (Table 4). The following parameters were evaluated: potentially degradable fraction, degradation rate, indigestible fraction, and parameter  $\lambda_a$ . The source of NPN had an effect on the parameters of fiber degradation kinetics. For the parameters potentially degradable fraction, parameter  $\lambda_a$  and indigestible fraction differences were detected in the contrast EUS vs. CUS. In degradation rate, in all tested contrasts, SRUS vs. CUS, US vs. CUS, and MAPS vs. CUS, differences were detected (Table 5).

| Model                    | Random effect <sup>1</sup> | AICc <sub>r</sub> | $\Delta_{r}$ | W <sub>r</sub> | $ER_r$ | $\Theta_{r}$ |
|--------------------------|----------------------------|-------------------|--------------|----------------|--------|--------------|
| Eq. (8) and (10), N = 5  | А                          | -652.0            | 0            | 0.390          | 1      | 23           |
| Eq. (8) and (10), N = 5* | А                          | -650.7            | 1.3          | 0.204          | 1.9    | 24           |
| Eq. (8) and (10), N = 5  | U                          | -650.5            | 1.5          | 0.184          | 2.1    | 23           |
| Eq. (8) and (10), N = 5* | U                          | -649.3            | 2.7          | 0.101          | 3.9    | 24           |
| Eq. (8) and (10), N = 6  | U                          | -647.5            | 4.5          | 0.041          | 9.5    | 23           |

Table 4 - Best-fit models for degradation kinetics

AICc<sub>r</sub> - Akaike information criterion corrected for model r;  $\Delta_r$  - difference in AICc between the models;  $w_r$  - likelihood probability of model r;  $ER_r$  - evidence ratio of model r;  $\Theta_r$  - number of parameters of model r.

<sup>1</sup> Random effect of animal × period interaction.

\* Continuous autoregressive correlation.

| Devenueter  |                     | Contrast (P-value) |                     |                     |              |             |              |
|-------------|---------------------|--------------------|---------------------|---------------------|--------------|-------------|--------------|
| Parameter   | CUS                 | SRUS               | EUS                 | MAPS                | SRUS vs. CUS | EUS vs. CUS | MAPS vs. CUS |
| An          | 0.2144±0.0075       | 0.2027±0.0045      | 0.3013±0.0048       | 0.2075±0.0043       | 0.2143       | < 0.0001    | 0.4694       |
| $\lambda_a$ | 0.1159±0.0187       | 0.1052±0.0090      | 0.3033±0.0343       | 0.1099±0.0107       | 0.6028       | < 0.0001    | 0.7771       |
| k           | 0.0097±0.0024       | 0.0427±0.0058      | $0.0198 \pm 0.0020$ | 0.0361±0.0048       | < 0.0001     | 0.001       | < 0.0001     |
| Un          | $0.7856 \pm 0.0075$ | 0.7973±0.0045      | 0.6987±0.0048       | $0.7925 \pm 0.0043$ | 0.2143       | < 0.0001    | 0.4694       |

| Table 5 - Parameters of fiber degradation kinetics, follow | ved by their respective standard errors |
|--|---|
|--|---|

An - potentially degradable fraction;  $\lambda_{v}$  - (1/h) asymptote of the rate of preparation for digestion; k - degradation rate of the potentially degradable fraction; Un - indigestible fraction.

<sup>1</sup> CUS - conventional-urea supplement; SRUS - slow-release urea supplement; EUS - extruded urea supplement; MAPS - monoammonium phosphate supplement.

#### 3.2. Particle transit kinetics

The segmented one-compartment passage rate model combined with scaled residual variance and random effect on parameter *d* (biologically insignificant scale parameter) was the one that showed the lowest Akaike information criterion (Table 6). The following parameters were evaluated: asymptotic rate of passage from the reticulorumen, mean residence time in the reticulorumen compartment, transit time from the reticulo-omasal orifice to the feces, mean total residence time, and effective degradability. The source of NPN had an effect on the parameters of particle transit kinetics. Of all the parameters tested, only for effective degradability was a difference detected between the SRUS vs. CUS, US vs. CUS, and MAPS vs. CUS (Table 7).

| Table 6 - Best-fit models for t | transit kinetics |
|---------------------------------|------------------|
|---------------------------------|------------------|

| Model                  | Random effect <sup>1</sup> | AICc <sub>r</sub> | $\Delta_{r}$ | W <sub>r</sub>    | ER <sub>r</sub>   | $\Theta_{\rm r}$ |
|------------------------|----------------------------|-------------------|--------------|-------------------|-------------------|------------------|
| Eq. (1) and (3), N = 3 | d                          | 5021.9            | 0            | 1                 | 1                 | 23               |
| Eq. (1) and (3), N = 2 | No                         | 5148.1            | 126.2        | 10 <sup>-28</sup> | 10+27             | 22               |
| Eq. (1) and (2), N = 2 | No                         | 5148.3            | 126.4        | 10 <sup>-28</sup> | 10 <sup>∓27</sup> | 21               |
| Eq. (1) and (2), N = 3 | C <sub>0</sub> ; tt        | 5153.7            | 131.8        | 10-29             | $10^{\mp_{28}}$   | 23               |
| Eq. (1) and (2), N = 3 | C <sub>0</sub> ; λ         | 5155.2            | 133.3        | 10-29             | 10+28             | 23               |

AICc<sub>r</sub> - Akaike information criterion corrected for model r;  $\Delta_r$  - difference in AICc between the models;  $w_r$  - likelihood probability of model r;  $ER_r$  - evidence ratio of model r;  $\Theta_r$  - number of parameters of model r; t - transit time from the reticulo-omasal orifice to the feces; *d* is a biologically meaningless scale parameter required for the use of uncorrected reading data;  $C_0$  is the concentration of the marker in the rumen-reticulum at t = 0;  $\lambda$  is the asymptotic rate of passage from the reticulorumen.

<sup>1</sup> Random effect of animal × period interaction.

## Table 7 - Parameters of fiber transit kinetics in the animals' gastrointestinal tract, followed by their respective standard errors

| Devenueter | Treatment <sup>1</sup> |                     |                |                | Contrast (P-value) |             |              |
|------------|------------------------|---------------------|----------------|----------------|--------------------|-------------|--------------|
| Parameter  | CUS                    | SRUS                | EUS            | MAPS           | SRUS vs. CUS       | EUS vs. CUS | MAPS vs. CUS |
| λ          | 0.055±0.0025           | 0.056±0.0024        | 0.058±0.0032   | 0.055±0.0028   | 0.7775             | 0.469       | 1            |
| MRTR       | 54.5455±2.5199         | 53.5714±2.2508      | 51.7241±2.8308 | 54.5455±2.7846 | 0.7779             | 0.4651      | 1            |
| tt         | 10.07±0.7940           | 10.84±0.7085        | 12.31±1.0032   | 11.46±0.8043   | 0.4781             | 0.0929      | 0.104        |
| MTRT       | 64.6155±2.0564         | 64.4114±1.9213      | 64.0341±2.1399 | 62.695±2.4286  | 0.9433             | 0.847       | 0.5527       |
| ED         | $0.1503 \pm 0.0059$    | $0.4327 \pm 0.0103$ | 0.2103±0.0071  | 0.3964±0.0122  | < 0.0001           | < 0.0001    | < 0.0001     |

 $\lambda$  - asymptotic rate of passage from the reticulorumen; MRTR - mean residence time in the reticulorumen compartment; tt - transit time from the reticulo-omasal orifice to the feces; MTRT - mean total residence time; ED - effective degradability.

<sup>1</sup> CUS - conventional-urea supplement; SRUS - slow-release urea supplement; EUS - extruded urea supplement; MAPS - monoammonium phosphate supplement.

## 4. Discussion

Because it is a mature material, straw from seed production has low nutritional value (i.e., high fiber content, low protein content). This translates into poor degradation of this fiber, as evidenced by the indigestible fraction (Un) values (Table 5). The high levels of lignin and the incrustation of this lignin onto the other components of the cell wall (cellulose and hemicellulose) found in plants at an advanced stage of maturity make it difficult for microorganisms to access the feed particle, reducing its digestibility (Akin, 1979). In *Cynodon nlemfuensis* pastures, Oliveira et al. (2013) found that the potential degradability of aNDF was inversely proportional to the age of the plants (83.2, 80.3, 76.8, and 75.3%, respectively for 28, 48, 63, and 79 days of regrowth). Moreover, rumen retention time increases and voluntary intake decreases, ultimately compromising animal performance (Lazzarini et al., 2009).

The size of the potentially degradable and indigestible fractions is characteristic of the substrate, and changes in the ruminal environment cause variations in the rate of degradation by microorganisms (Ørskov, 2000). These effects have a greater impact in tropical conditions relative to aNDF, given its role as the main energy substrate for growth and as a determining factor in the rumen-fill process, which increases as forage quality decreases (Vieira et al., 1997).

The incorporation of NPN sources into strategic low-intake supplementation could improve the degradation and transit parameters of fiber, even if it has a relatively low quality (Galo et al., 2003; Pires et al., 2004; Santos and Pedroso, 2011). The increase in the aNDF degradation rate through the addition of nitrogenous compounds reiterates their priority nature in the supplementation of animals kept on pastures during the dry season, a situation in which energy extraction from fibrous carbohydrates becomes limited due to deficiency of nitrogenous compounds for the synthesis of enzymatic systems of ruminal microorganisms (Costa et al., 2008; Detmann et al., 2009; Lazzarini et al., 2009; Sampaio et al., 2009).

Positive alterations in the escape of particles from the ruminal environment are associated with the use of nitrogen supplements in low-quality forages and are strongly related to an increase in total intake by the animal (McCollum and Galyean, 1985). Experiments carried out in several places in the United States showed an average increase of 22% in the intake of ammoniated forages (Berger et al., 1994). In nine experiments with growing cattle, the animals that received forage treated with ammonia gained 163 g/day more than those that received untreated straw. In eight tests with pregnant cows, ammonia-treated hay and straw provided a 313-g higher daily gain compared with that of animals that received untreated forages (Kunkle, 1998).

Although urea positively affects ruminal fermentation parameters and microbial growth in ruminants, it is rapidly degraded, making it difficult for microorganisms to readily utilize it and leading to waste of nitrogen sources. Greater synchrony of urea hydrolysis with carbohydrate degradation could improve the efficiency of NPN incorporation into microbial protein (Firkins, 1996). It is believed that the main effect of slow-release urea compared to conventional urea is to balance ammonia concentrations throughout the day.

In the present study, conventional urea showed a lower rate of degradation of the potentially degradable fraction of the fiber and effective degradability than the other sources in the NPN test, slow-release urea, extruded urea, and monoammonium phosphate. Considering that conventional urea is the most readily soluble source of the four NPN sources under study, this may have caused an excess of ammoniacal nitrogen in the rumen, which is not used efficiently by ruminal microorganisms for microbial protein synthesis, and therefore, is absorbed by the rumen wall and metabolized in the liver. Its excess in the liver increases the energy costs associated with nitrogen metabolism, the "urea cycle", to finally be able to excrete urea via urine. With this, the animal starts directing the ingested energy to eliminate urea (Verbic, 2002).

Extruded urea showed improvement in all parameters of fiber degradation kinetics and effective degradability when compared with conventional urea. The solubility and concentration of ammoniacal nitrogen of extruded urea is lower than the value observed for conventional urea (Ítavo et al., 2016).

This fact indicates that by reducing solubility, urea extrusion allows a slower release of nitrogen in the ruminal environment when compared with conventional urea. Ítavo et al. (2016) worked with different combinations of NPN sources to obtain different NPN solubilization rates, urea + extruded urea + coated urea, urea + coated urea, urea + extruded urea, and extruded urea. Although the animals in the extruded urea treatment showed lower intake compared with the animals supplemented with the other sources, a higher weight gain was observed, which indicates that extruded urea, as it is a source of medium solubility nitrogen release, may have been used with greater efficiency by ruminal microorganisms and enabled adequate synchrony between the digestion of forage fibrous carbohydrate and the release of nitrogen from the source for microbial protein synthesis.

Ferreira et al. (2005) observed satisfactory results when comparing conventional urea with encapsulated urea, with the former showing more constant ammonia levels throughout the day, which resulted in greater fiber degradability. Dietary urea or slow-release urea supplementation improves the efficiency of microbial protein synthesis in Nellore steers (Corte et al., 2018). The synchronization between fermentable energy and degradable nitrogen in the rumen, promoted by using slow-release urea, maximizes microbial growth and promotes microbial protein synthesis. In the rumen, the microbial growth rate is slower than the urea degradation rate, and rapid ammonia release results in inefficient utilization of nitrogen and wastage of nitrogen sources (Xin et al., 2010). The coated urea supplementation improves milk production and fat content in dairy buffaloes through microbial protein turnover and increased fiber degradation (Aquino et al., 2014; Nadeem et al., 2014).

In general, the results regarding particle transit kinetics obtained in this study were similar to those described by Andrade (2018), who evaluated the parameters of corn silage and found an asymptotic rate of passage from the reticulorumen of 0.053, a mean total residence time of 62.1 min, and a mean residence time in the reticulorumen of 56.6 min. The similarity between the parameters, especially for the rate of passage, in the comparison of corn silage and straw briquette, can be said to be due to the particle size of straw briquette, which decreases with processing (grinding).

Disagreeing with these studies, Bourg et al. (2012) found no differences between a lipid-coated urea and urea on diet intake or digestibility in an N balance study using Holstein steers fed a diet based on steam flaked corn. In the study by Azevedo et al. (2008), neither protein supplementation nor urea encapsulation affected the aNDF degradation parameters. Likewise, in an experiment with grazing animals, Hess et al. (1994) did not observe differences in aNDF degradation rates after supplementing them with different protein sources (alfalfa hay, cottonseed meal, and corn gluten).

## **5.** Conclusions

When comparing parameters of fiber degradation and transit kinetics as a function of different sources of non-protein nitrogen, in low-intake supplements for cattle, it was found that, in general, the worst results are obtained with supplements that contained conventional urea.

## **Conflict of Interest**

The authors declare no conflict of interest.

## **Author Contributions**

**Conceptualization:** Lino, R. A.; Moreira, L. M. and Leonel, F. P. **Data curation:** Lino, R. A. and Braga, B. C. **Formal analysis:** Lino, R. A.; Braga, B. C.; Gomes, R. S.; Moreira, L. M. and Leonel, F. P. **Investigation**: Couto, C. J.; Villela, S. D. J.; Gomes, R. S.; Tamy, W. P. and Leonel, F. P. **Methodology:** Couto, C. J.; Gomes, R. S.; Moreira, L. M. and Leonel, F. P. **Project administration:** Lino, R. A. and Couto, C. J. **Resources:** Villela, S. D. J.; Tamy, W. P. and Leonel, F. P. **Software:** Villela, S. D. J. and Tamy, W. P. **Supervision:** Villela, S. D. J.; Gomes, R. S. and Leonel, F. P. **Validation:** Tamy, W. P. and Moreira, L. M. **Writing – original draft:** Lino, R. A. and Braga, B. C. **Writing – review & editing:** Braga, B. C.

### **References**

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716-723. https://doi.org/10.1109/TAC.1974.1100705

Akin, D. E. 1979. Microscopic evaluation of forage digestion by rumen microorganisms - a review. Journal of Animal Science 48:701-710. https://doi.org/10.2527/jas1979.483701x

Andrade, M. A. T. 2018. Uma abordagem cinética para avaliar o trânsito e a degradação de partículas sólidas de resíduos da agricultura no trato gastrintestinal de bovinos. Dissertação (M.Sc.). Universidade Federal dos Vales de Jequitinhonha e Mucuri, Diamantina.

AOAC. 2012. Official methods of analysis of AOAC International. 19th ed. AOAC International, Gaithersburg, MD.

Aquino, D. L.; Del Rosario, M. V. and Vergarra, K. F. 2014. Effect of augmented feeding with bypass amino acids and slow-release non-protein nitrogen supplements on milk peak, lactation persistency, milk quality and post-partum reproductive performance of Brazilian buffaloes. Philippine Journal of Veterinary and Animal Sciences 40:145-158.

Azevedo, E. B.; Patiño, H. O.; Silveira, A. L. F.; López, J.; Brüning, G. and Kozloski, G. V. 2008. Incorporação de uréia encapsulada em suplementos protéicos fornecidos para novilhos alimentados com feno de baixa qualidade. Ciência Rural 38:1381-1387. https://doi.org/10.1590/S0103-84782008000500029

Berger, L. L.; Fahey Jr., G. C.; Bourquin, L. D. and Titgemeyer, E. C. 1994. Modification of forage quality after harvest. p.922-966. In: Forage quality, evaluation, and utilization. Fahey Jr., G. C., ed. ASA, CSSA, SSSA, Madison. https://doi. org/10.2134/1994.foragequality.c23

Bourg, B. M.; Tedeschi, L. O.; Wickersham, T. A. and Tricarico, J. M. 2012. Effects of a slow-release urea product on performance, carcass characteristics, and nitrogen balance of steers fed steam-flaked corn. Journal of Animal Science 90:3914-3923. https://doi.org/10.2527/jas.2011-4832

Burnham, K. P. and Anderson, D. R. 2004. Multimodel inference: understanding AIC and BIC in model selection. Sociological Methods & Research 33:261-304. https://doi.org/10.1177/0049124104268644

Catuchi, T. A.; Soratto, R. P.; Francisquini Júnior, A.; Aranda, E. A.; Guidorizzi, F. V. C. and Tiritan, C. S. 2017. Nitrogen management, nitrogen use efficiency, and seed yield and quality of creeping signalgrass. Crop Science 57:2865-2874. https://doi.org/10.2135/cropsci2017.02.0096

Corte, R. R.; Brito, F. O.; Leme, P. R.; Pereira, A. S. C.; Freitas Jr, J. E.; Rennó, F. P.; Silva, S. L.; Tedeschi, L. O. and Nogueira Filho, J. C. M. 2018. The effects of partial substitution of soybean with urea or slow-release urea on finishing performance, meat quality, and digestion parameters of Nellore steers. Animal Production Science 58:2242-2248. https://doi.org/10.1071/AN16609

Costa, V. A. C.; Detmann, E.; Valadares Filho, S. C.; Paulino, M. F.; Henriques, L. T. and Mantovani, H. C. 2008. Degradação *in vitro* da fibra em detergente neutro de forragem tropical de baixa qualidade em função de suplementação com proteína e/ou carboidratos. Revista Brasileira de Zootecnia 37:494-503. https://doi.org/10.1590/S1516-35982008000300015

Detmann, E.; Paulino, M. F.; Mantovani, H. C.; Valadares Filho, S. C.; Sampaio, C. B.; Souza, M. A.; Lazzarini, I. and Detmann, K. S. C. 2009. Parameterization of ruminal fibre degradation in low-quality tropical forage using *Michaelis-Menten* kinetics. Livestock Science 126:136-146. https://doi.org/10.1016/j.livsci.2009.06.013

Firkins, J. L. 1996. Maximizing microbial protein synthesis in the rumen. Journal of Nutrition 126:1347S-1354S. https://doi.org/10.1093/jn/126.suppl\_4.1347S

Ferreira, R. N.; Oliveira, E. R.; Orsine, G. F.; Paula, A. A.; Oliveira, L. G.; Bittencourt, A. R. and Souza, S. N. 2005. Liberação de nitrogênio amoniacal no rumen com o uso de uréia encapsulada com polímero (Optigen 1200 Alltec). In: Anais da 42ª Reunião Anual da Sociedade Brasileira de Zootecnia. Goiânia.

Galo, E.; Emanuele, S. M.; Sniffen, C. J.; White, J. H. and Knapp, J. R. 2003. Effects of a polymer-coated urea product on nitrogen metabolism in lactating Holstein dairy cattle. Journal of Dairy Science 86:2154-2162. https://doi.org/10.3168/jds.s0022-0302(03)73805-3

Hess, B. W.; Park, K. K.; Krysl, L. J.; Judkins, M. B.; McCracken, B. A. and Hanks, D. R. 1994. Supplemental protein for beef cattle grazing dormant intermediate wheatgrass pasture: effects on nutrient quality, forage intake, digesta kinetics, grazing behavior, ruminal fermentation, and digestion. Journal of Animal Science 72:2113-2123. https://doi. org/10.2527/1994.7282113x

Ítavo, L. C. V.; Ítavo, C. C. B. F.; Dias, A. M.; Franco, G. L.; Pereira, L. C.; Leal, E. S.; Araújo, H. S and Souza, A. R. D. L. 2016. Combinações de fontes de nitrogênio não proteico em suplementos para novilhos Nelore em pastejo. Revista Brasileira de Saúde e Produção Animal 17:448-460. https://doi.org/10.1590/S1519-99402016000300011

Kimura, F. T. and Miller, V. L. 1957. Chromic oxide measurement, improved determination of chromic oxide in cow feed and feces. Journal of Agricultural and Food Chemistry 5:216-216. https://doi.org/10.1021/jf60073a008

Kirkpatrick, B. K. and Kennelly, J. J. 1987. In situ degradability of protein and dry matter from single protein sources and from a total diet. Journal of Animal Science 65:567-576. https://doi.org/10.2527/jas1987.652567x

Kunkle, W. E. 1998. Strategies for cost effective supplementation of beef cattle. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Ona. 7p.

Lazzarini, I.; Detmann, E.; Sampaio, C. B.; Paulino, M. F.; Valadares Filho, S. C.; Souza, M. A. and Oliveira, F. A. 2009. Dinâmicas de trânsito e degradação da fibra em detergente neutro em bovinos alimentados com forragem tropical de baixa qualidade e compostos nitrogenados. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 61:635-647. https://doi.org/10.1590/S0102-09352009000300017

Licitra, G.; Hernandez, T. M. and Van Soest, P. J. 1996. Standardization of procedures for nitrogen fractionation of ruminants feeds. Animal Feed Science and Technology 57:347-358. https://doi.org/10.1016/0377-8401(95)00837-3

López, S.; France, J.; Dhanoa, M. S.; Mould, F. and Dijkstra, J. 1999. Comparison of mathematical models to describe disappearance curves obtained using the polyester bag technique for incubating feeds in the rumen. Journal of Animal Science 77:1875-1888. https://doi.org/10.2527/1999.7771875x

McCollum, F. T. and Galyean, M. L. 1985. Influence of cottonseed meal supplementation on voluntary intake, rumen fermentation and rate of passage of prairie hay in beef steers. Journal of Animal Science 60:570-577. https://doi. org/10.2527/jas1985.602570x

Mehrez, A. Z.; Ørskov, E. R. and McDonald, I. 1977. Rates of rumen fermentation in relation to ammonia concentration. British Journal of Nutrition 38:437-443. https://doi.org/10.1079/bjn19770108

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beaker or crucibles: collaborative study. Journal of AOAC International 85:1217-1240.

Nadeem, M. S.; Pasha, T. N.; Jabbar, M. A.; Javed, K.; Khan, M. Z.; Naveed, S. and Ditta, Y. A. 2014. Effect of different nonprotein nitrogen (NPN) sources on performance of lactating Nili-Ravi buffaloes. Journal of Animal and Plant Sciences 24(Suppl. 1):1-4.

NASEM - National Academies of Sciences, Engineering, and Medicine. 2016. Nutrient requirements of beef cattle. 8th rev. ed. The National Academies Press, Washington, DC. https://doi.org/10.17226/19014

Nocek, J. E. 1985. Evaluation of specific variable affecting in situ estimates of ruminal dry matter and protein digestion. Journal of Animal Science 60:1347-1358. https://doi.org/10.2527/jas1985.6051347x

Oliveira, E. R.; Monção, F. P.; Góes, R. H. T. B.; Gabriel, A. M. A.; Moura, L. V.; Lempp, B.; Graciano, D. E. and Tochetto, A. T. C. 2013. Degradação ruminal da fibra em detergente neutro de gramíneas do gênero *Cynodon* spp em quatro idades de corte. Revista Agrarian 6:205-214.

Oliveira, J. S.; Zanine, A. M. and Santos, E. M. 2007. Processo fermentativo, digestivo e fatores antinutricionais de nutrientes para ruminantes. REDVET. Revista Electrónica de Veterinaria 8(2).

Ørskov, E. R. 2000. The *in situ* technique for the estimation of forage degradability in ruminants. p.175-188. In: Forage evaluation in ruminant nutrition. Givens, D. I.; Owen, E.; Axford, R. F. E. and Omed, H. M., eds. CAB International, London.

Pinheiro, J. C. and Bates, D. M. 2000. Mixed-effects models in S and S-PLUS. Springer, New York.

Pires, A. V.; Oliveira Júnior, R. C.; Fernandes, J. J. R.; Susin, I.; Santos, F. A. P.; Araújo, R. C. and Goulart, R. C. D. 2004. Substituição do farelo de soja por ureia ou amiréia na dieta de bovinos de corte confinados. Pesquisa Agropecuária Brasileira 39:937-942. https://doi.org/10.1590/S0100-204X2004000900014

Pond, K. R.; Ellis, W. C.; Matis, J. H.; Ferreiro, H. M. and Sutton, J. D. 1988. Compartment models for estimating attributes of digesta flow in cattle. British Journal of Nutrition 60:571-595. https://doi.org/10.1079/bjn19880129

Salami, S. A.; Devant, M.; Apajalahti, J.; Holder, V.; Salomaa, S.; Keegan, J. D. and Moran, C. A. 2021. Slow-release urea as a sustainable alternative to soybean meal in ruminant nutrition. Sustainability 13:2464. https://doi.org/10.3390/su13052464

Salami, S. A.; Moran, C. A.; Warren, H. E. and Taylor-Pickard, J. 2020. A meta-analysis of the effects of slow-release urea supplementation on the performance of beef cattle. Animals 10:657. https://doi.org/10.3390/ani10040657

Sampaio, C. B.; Detmann, E.; Lazzarini, I.; Souza, M. A.; Paulino, M. F. and Valadares Filho, S. C. 2009. Rumen dynamics of neutral detergent fiber in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. Revista Brasileira de Zootecnia 38:560-569. https://doi.org/10.1590/S1516-35982009000300023

Santos, F. A. P. and Pedroso, A. M. 2011. Metabolismo de proteínas. p.265-298. In: Nutrição de ruminantes. 2.ed. Berchielli, T. T.; Pires, A. V.; Oliveira, S. G., eds. Funep, Jaboticabal.

Souza, F. H. D. and Cardoso, E. G. 2003. Alternativa para o descarte de palhada resultante da produção de sementes de capim. Comunicado Técnico, 39. Embrapa Pecuária Sudeste, São Carlos.

Sugiura, N. 1978. Further analysis of the data by Akaike's information criterion and the finite corrections. Communications in Statistics 7:13-26. https://doi.org/10.1080/03610927808827599

Udén, P.; Colluci, P. E. and Van Soest, P. J. 1980. Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. Journal of the Science of Food and Agriculture 31:625-632. https://doi.org/10.1002/jsfa.2740310702

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. 2nd ed. Comstock Publishing Associates, Ithaca.

Verbic, J. 2002. Factors affecting microbial protein synthesis in the rumen with emphasis on diets containing forages. In: 29. Viehwirtschaftliche Fachtagung. BAL Gumpenstein, Irdning. Vieira, R. A. M.; Campos, P. R. S. S.; Silva, J. F. C.; Tedeschi, L. O. and Tamy, W. P. 2012. Heterogeneity of the digestible insoluble fiber of selected forages *in situ*. Animal Feed Science and Technology 171:154-166. https://doi.org/10.1016/j. anifeedsci.2011.11.001

Vieira, R. A. M.; Pereira, J. C.; Malafaia, P. A. M. and Queiroz, A. C. 1997. The influence of elephant-grass (*Pennisetum purpureum* Schum., Mineiro variety) growth on the nutrient kinetics in the rumen. Animal Feed Science and Technology 67:151-161. https://doi.org/10.1016/S0377-8401(96)01130-3

Vieira, R. A. M.; Tedeschi, L. O. and Cannas, A. 2008. A generalized compartmental model to estimate the fibre mass in the ruminoreticulum: 2. Integrating digestion and passage. Journal of Theoretical Biology 255:357-368. https://doi.org/10.1016/j.jtbi.2008.08.013

Waldo, D. R.; Smith, L. W. and Cox, E. L. 1972. Model of cellulose disappearance from the rumen. Journal of Dairy Science 55:125-129. https://doi.org/10.3168/jds.S0022-0302(72)85442-0

Xin, H. S.; Schaefer, D. M.; Liu, Q. P.; Axe, D. E. and Meng, Q. X. 2010. Effects of polyurethane coated urea supplement on *in vitro* ruminal fermentation, ammonia release dynamics and lactating performance of Holstein dairy cows fed a steam-flaked corn-based diet. Asian-Australasian Journal of Animal Sciences 23:491-500.