Kinetics of transit and degradation of the fiber from guinea grass silages enriched with waste from soybean pre-cleaning

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ABSTRACT - The objective was to study the kinetics of transit and degradation of the fiber from guinea grass and the waste from soybean pre-cleaning (WSPC), ensiled with different proportions of mass (0, 100, 150, and 200 g WSPC/kg total mass). Four crossbred (Gyr × Holstein), fistulated cattle with an average body mass of 400±50 kg were organized in a 4 × 4 Latin square experimental design. The fiber utilized in the study of the transit kinetics was stained with chromium mordant, whereas the *in situ* technique was adopted for the degradation kinetics. The level of inclusion of WSPC only affected the true digestibility and the mean retention time. The addition of waste from soybean pre-cleaning to the silage of guinea grass is beneficial, in terms of kinetics of digestion and passage, at up to levels close to 100 g/kg, because after this quantity the fiber digestion and passage in and through the reticulo-rumen are impaired and there may be alterations in the ruminal environment that will affect the use of silage by animals.

Key Words: by-product, mathematical models, mean retention time, true digestibility

Introduction

The need to reduce costs and the impact on the production of animal-derived products leads to a greater focus on the rational use of all feed resources available. Thus, methods have been sought for the proper use of agroindustrial waste or by-products in animal feeding. The knowledge of the right use of these residues can certainly contribute to reducing the costs with animal feeding and the environmental impact (Carvalho Junior et al., 2010).

Several products originated from the industrialization or harvest, such as peelings and bagasse, have been produced on a large scale in Brazil. Yet, the largest part of this material has been underused in animal feeding due to its low nutrient concentration. Thus, research investigating the possibility of using these products, especially regarding their nutritional quality, has shown to be increasingly promising.

The investigation of the possibility and forms of utilization of a waste or any ingredient as feed for ruminant animals necessarily involves a study of its chemical composition. However, this chemical evaluation alone is not sufficient to decide on which is the best way to optimize the input of nutrients from this feed or from the diet to a given animal category. The detailed study of the kinetics of degradation and transit of particles is an important tool to aid this decision making.

The kinetics of the fiber degradation, used as an evaluation trait for several feeds, when estimated using the technique of nylon bags incubated in rumen-fistulated cattle (Orskov et al., 1980), provides a fast and simple estimate of the degradation of nutrients and the evaluation of many feeds at the same time, having a low cost and rapidity as compared with the *in vivo* method. The *in situ* technique enables the contact of the evaluated feed with the ruminal environment, which makes it the best way to simulate this medium, although the feed is not subjected to all digestive events such as chewing, rumination, and the passage (Van Soest, 1994).

The passage rate of feed particles through the reticulo-rumen depends on properties of the feed itself as well as those of the animal in question. This passage kinetics has a direct influence on the degradation of the feed, increasing or decreasing it (AFRC, 1993). Therefore, the study of the kinetics of transit of particles of this feed through the gastrointestinal tract of ruminants provides useful tools to estimate the digestibility and also the input of nutrients to the animal.

Thus, the objective was to study the kinetics of transit and degradation of the fiber from guinea grass and the waste from soybean pre-cleaning, ensiled at different mass proportions.

Material and Methods

For the experiment, the guinea grass (*Panicum maximum* cv. Mombaça), was harvested at 60 days of growth (regrowth), and at the moment of ensiling, the waste from soybean pre-cleaning (WSPC) was added at increasing levels to the total mass. The studied treatments were formed based on these levels of addition of WSPC. Silages with different proportions of guinea grass mass-to-WSPC were formed, as follows: guinea grass silage without addition of WSPC (control); silage containing 900 g/kg guinea grass + 100 g/kg WSPC; silage containing 850 g/kg guinea grass + 150 g/kg WSPC; and silage containing 800 g/kg guinea grass and 200 g/kg WSPC.

The WSPC has great potential for ruminants (Table 1), and was obtained from the pre-cleaning of soybean in a grain processing and storage plant, and contains the coarsest fraction of the material harvested in the field (pods with unripe beans, pods with defective beans, leaves and pieces of soy-plant stems). This by-product is generated in soybean-producing regions at proportions of up to 1%.

The ensiling process started with a mechanical cut (tractor-ensiling machine set) of the guinea grass, and the WSPC was subsequently added at the mass proportions described above, and the total material was inserted in mini-silos (PVC containers with capacity of 12 kg of fresh matter, at a storage density of 500 kg/m³; Bunsen valves were adapted on the lids to enable gas flow). After filling, these containers were sealed hermetically with adhesive tapes to prevent air exchange with the environment.

After the period for stabilization of the material (30 days), the experimental silos were open to determine the chemical composition and to mark the fiber of the silages under study.

For the estimate of the fiber transit kinetics, approximately 3 kg of silage from each treatment were placed in a container to boil for one hour with a commercial neutral detergent at the rate of 100 g of dry sample for 100 mL of detergent per liter of water, as described by Udén et al. (1980). After this procedure, the material was filtered in a cotton-fabric bag and washed with running water from tap

until the water was clear to remove the soluble contents, and then dried in an oven at 55±5 ºC for 72 h.

Next, a potassium dichromate solution (K_2Cr2O_7. $2 H_2O$) was made at the proportion of 13% chromium in relation to the weight of the fiber to be stained. This solution was diluted in water in a glass container with previous immersion of the fiber in this solution. The container was covered with foil and kept in an oven at 105 ºC for 24 h. After this procedure the material was conditioned in a cotton-fabric bag and washed in running water to remove the excess potassium dichromate. Afterwards, the material was immersed in a commercial ascorbic acid solution at the proportion of half the weight of the fiber, and left to rest for one hour until reaching an intense green color. Soon after this, the material was once again conditioned in a cotton-fabric bag and washed several times until complete clearing of the water, and then dried in a forced-ventilation oven at 55±5 ºC for 72 h, thus generating the chromium-mordant NDF.

Four Holstein × Gyr crossbred castrated cattle with an approximate weight of 400 kg, cannulated in the rumen according to the technique described by Leão and Coelho da Silva (1980) and Leão et al. (1978), were distributed in a Latin square design with four treatments, four animals, and four experimental periods. The animals were kept in a confined regime, in individual pens, and fed a diet consisting of guinea grass supplied *ad libitum* plus 2 kg of a concentrate based on corn, soybean meal (25% CP), and a mineral mix during the trial. The feed was supplied twice daily, at around 07.00 h and 15.00 h.

Stained samples were placed directly in the rumen via ruminal cannulae at the amount of 200 g per animal. Feces were collected at times zero (immediately after administering the chromium-complexed fiber), 1, 2, 4, 6, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 56, 64, 72, 80, 88, 96, 120, 132, 144, and 196 h, to estimate the passage-kinetic parameters of the particles. After these collections at the indicated times, the samples were dried in a forced-ventilation oven at 55±5 °C for 72 h and then processed in a mill with 1-mm-mesh sieve. Approximately 200 mg of feces respective to each incubation time, animal, and period were diluted in a nitric-perchloric acid solution to remove the organic matter and for subsequent reading of the chromium concentration by atomic absorption spectrometry, following the methodology described by Kimura & Miller (1957).

The profiles of concentration of the marker in the feces were described by the generalized two-compartment model suggested by Matis et al. (1989). The parameters generated in this model provide estimates that explain the dynamics of the rate of passage or transit of fibrous particles through the gastrointestinal tract of ruminants in general.

Table 1 - Chemical values of waste from soybean pre-cleaning (WSPC) and guinea grass

Variable	WSPC	Guinea grass
Dry matter (g/kg)	737.3	262.7
Crude protein (g/kg)	192.3	71.4
Neutral detergent fiber (g/kg)	513.7	737.1
Acid detergent fiber (g/kg)	313.3	377.7

Concurrently with the study of transit kinetics, the ruminal degradability of the fiber from the silages was also analyzed. To this end, the *in situ* technique was used, which consists of incubating the materials under study for pre-set times in the rumen of the animals. Nylon bags (13 × 7 cm; 50-µm pore) containing approximately 1 g of sample each were used, maintaining a bag surface ratio of 25 mg DM/cm^2, in accordance with recommendations of Kirkpatrick and Kennelly (1987).

The incubation times adopted were 0, 6, 18, 48, and 96 h, according to Sampaio (1994), such that at each time all treatments were incubated in each animal. The bags were tied in sequential order on the links of a chain, which contained a weight on its extremity. This weight worked as an anchor, causing the bags to be immersed in the ruminal content, which promoted an effective activity of the ruminal microorganisms on the samples.

Bags were incubated in the rumen in a reversed chronological order (infused at the times set and (all) removed at the end of the countdown).

After they were removed, bags were washed in running water until complete clearing and then conditioned in a forced-ventilation oven at 55±5 ºC for 72 h. The bags corresponding to time zero were not incubated in the rumen but underwent the same cleaning process, simultaneously with the others.

After the drying process, the bags were open and their contents were analyzed for the levels of dry matter (DM; method 967.03, AOAC, 1998) and neutral detergent fiber (NDF; method 2002.04, Mertens, 2002)

The model utilized to estimate the parameters of degradation kinetics of the fiber consists of a simple first-order equation, which was proposed by Smith et al. (1971). A parameter representing the discrete lag time was added to this model; this inclusion was suggested by Mertens and Loften (1980) and reaffirmed by Vieira et al. (2008a), who justified its use by the substantial improvement of its predictive ability.

Variables A and U, both expressed in $g\,kg^{-1}$, derived from the degradation kinetics study, were normalized as proposed by Waldo et al. (1972) so that they could demonstrate a suitable proportion between each other, becoming An and Un. The variables tested in the degradability study were: potentially digestible fraction of the normalized fiber (An), indigestible fraction of the normalized fiber (Un), discrete lag (L), digestion rate (c), mean retention time in the rumen-reticulum (MRT), true digestibility (TD) and fiber rumen-fill effect (RF). Variable L, expressed in hours, represents the time for preparation and colonization of the substrate in the rumen until the effective start of digestion. Variable c,

in turn, quantifies the proportion of fiber digested in the rumen per time unit, and is expressed in h^{-1}.

The turnover, or mean retention time in the reticulo-rumen (MRT), was estimated based on biological interpretations, in which both ascending and descending phases of the profile of excretion of the markers in the feces exerted an influence on the retention of the particles in the reticulo-rumen (Vieira et al., 2008b). This parameter in the model is expressed in hours, and was estimated according to the equation of Matis et al. (1989).

The fiber true digestibility coefficient (TD) is dimensionless and was estimated using the model deduced by Vieira et al. (2008b), as well as the rumen fill (RF), expressed in days, which was obtained by using the model to estimate the ruminal content, from the same author, but the multiplication by the fiber intake was discarded, as can be seen below:

$$RF = A\left\{v\sum_{i=1}^{N}\left[\lambda^{i-1}/(\lambda+c)^i\right] + \lambda^N/\left[(\lambda+c)^N(k+c)\right]\right\} + U[N/\lambda + 1/k].$$

The parameters of the adjusted model for the kinetics of passage and degradability profiles were estimated through the NLIN procedure of SAS (Statistical Analysis System, version 9.0) software. Both the Newton and Marquardt algorithms were used. Initially, the preferred form or algorithm was that of Newton, due to its good performance in terms of convergence; however, whenever the correlations among the estimates of the parameters were high, we opted for Marquardt's algorithm.

The choice of the best version for the time-dependency order (N) and consequently of the best model to explain the passage rate was evaluated by the calculation of Akaike's information criterion ($AICc_h$) (Akaike, 1974; Burnham and Anderson, 2004). The $AICc_h$ was calculated as the sum of the squares of the error (SSE_h), number of estimated parameters including the residual variance (Θ_h), and the size of the sample (n_h) for all the different versions of N, $\forall h = 1,2,…,6$. The differences between the $AICc_h$ (Δ_h) values, the probability of likelihood (w_h), and the evidence ratio (ER_h) were obtained according to the models proposed by Vieira et al. (2012).

The estimates of the parameters of the profiles of passage rate were adjusted according to robust-regression procedures (Beaton and Tukey, 1974), to reduce the effect of discrepant observations (outliers) and eliminate subjectivity during the appreciation of these points.

The adopted statistical model was as follows:
$$Y_{ij} = \mu + \alpha_i + \beta_j + e_{ij},$$
in which the fixed effect is represented by the effect of the mean (μ) and by the effect of treatment (α_i), whereas the

random effect is attributed to the effect of the animal (β_j) and to the effect of the random error (e_{ij}).

Homogeneous and heterogeneous variance and covariance matrix structures were tested (Vieira et al., 2012), and then Akaike's criterion was used to choose the best structure. The equations proposed by Neter and Wasserman (1974) were utilized to calculate the points of maximum for x and y on variable *TD* and their confidence intervals.

Results

Changes in the chemical variables of the silages according to the level of enrichment of guinea grass with WSPC occurred (Table 2), but were not the focus of this study.

Table 2 - Concentrations of protein (CP) and fiber (NDF) in the dry matter of the silage, as a function of the enrichment with the waste from soybean pre-cleaning (WSPC)

Variable	Level of WSPC (g kg⁻¹)				
	0	50	100	150	200
CP (g kg⁻¹)	6.3d	7.8c	9.3b	12.2a	12.9a
NDF (g kg⁻¹)	74.9a	69.62b	68.33b	63.83c	63.45c

Means followed by different letters in the same row differ statistically from each other.
CP - crude protein; NDF - neutral detergent fiber.

In this study, a more adequate adjustment in relation to the variance and covariance structures (Table 3) was observed in all variables when the homogenous variance and covariance structure was used, which is justified by the lower values found for $AICc_h$, Δ_h, and ER_h, and consequently the higher values observed for the probability of likelihood (w_h).

An effect of treatment (inclusion of WSPC) was found only on *TD* and *MRT*; the P-values obtained for these variables were 0.0524 and 0.0513, respectively.

The other parameters of the models (potentially digestible fraction, indigestible fraction, digestion rate, lag, and rumen fill) did not show to be significantly affected (Table 4).

The estimated behavior of the true-digestibility model showed a quadratic response (Figure 1), with P = 0.0374. The maximum point for the values of x in variable *TD* was 100.3 g kg⁻¹, with a confidence interval between 92.2 and 108.4 g kg⁻¹. The maximum point for Y, however, was 3.2, with a confidence interval between 2.6 and 3.7.

Unlike the trend observed for *TD*, *MRT* increased linearly, with P = 0.0499; according to the model estimate, there was an increase of 0.0514 h per unit of increment with the inclusion of WSPC (Figure 2).

Table 3 - Criterion of choice of the best adjustment of variance and covariance structure (VC), considering corrected Akaike's criterion ($AICc_h$), Δ_h, probability of likelihood (w_h) and evidence ratio (ER_h)

Parameter	VC	$AICc_h$	Δ_h	w_h	ER_h
An	Homogeneous	−2.6	0	0.984	1
	Heterogeneous	5.6	8.2	0.016	60.3
Un	Homogeneous	−2.6	0	0.984	1
	Heterogeneous	5.6	8.2	0.016	60.3
c	Homogeneous	−57.3	0	0.825	1
	Heterogeneous	−54.2	3.1	0.175	4.7
L	Homogeneous	91.4	0	0.995	1
	Heterogeneous	102	10.6	0.005	200.3
RF	Homogeneous	11.3	0	0.992	1
	Heterogeneous	21	9.7	0.008	127.7
TD	Homogeneous	−27.5	0	0.891	1
	Heterogeneous	−23.3	4.2	0.109	8.2
MRT	Homogeneous	88.7	0	0.981	1
	Heterogeneous	96.6	7.9	0.019	51.9

An - potentially digestible fraction (g kg⁻¹); *Un* - indigestible fraction (g kg⁻¹); *c* - digestion rate (h⁻¹); *L* - lag (h); *RF* - rumen fill (d); *TD* - true digestibility (dmls); *MRT* - mean retention time (h).

Table 4 - Inclusion levels of the waste from soybean pre-cleaning and their respective confidence intervals for the different parameters

Parameter	Inclusion level (g kg⁻¹) (95% confidence interval)			
	0	100	150	200
An (g kg⁻¹)	0.417 (0.247;0.587)	0.494 (0.324;0.664)	0.600 (0.430;0.770)	0.368 (0.198;0.538)
Un (g kg⁻¹)	0.583 (0.413;0.753)	0.506 (0.336;0.676)	0.400 (0.230;0.570)	0.632 (0.462;0.802)
c (h⁻¹)	0.023 (0.005;0.040)	0.039 (0.022;0.057)	0.012 (−0.005;0.030)	0.030 (0.013;0.048)
L (h)	17.139 (8.591;25.688)	11.186 (2.638;19.735)	8.490 (−0.059;17.038)	10.631 (2.083;19.180)
RF (d)	1.673 (1.370;1.9769)	1.937 (1.634;2.241)	1.942 (1.639;2.246)	2.020 (1.716;2.323)

An - potentially digestible fraction; *Un* - indigestible fraction; *c* - digestion rate; *L* - lag; *RF* - rumen fill.

Kinetics of transit and degradation of the fiber from guinea grass silages enriched with waste from soybean pre-cleaning
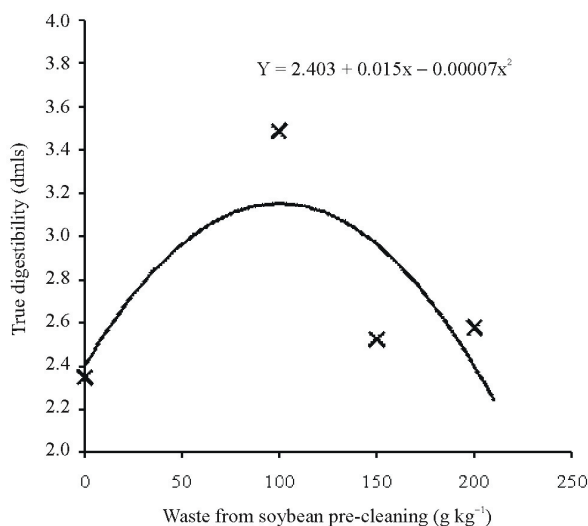
205

Figure 1 - Quadratic regression model adjusted for the true digestibility according to the applied treatments and its estimated equation.
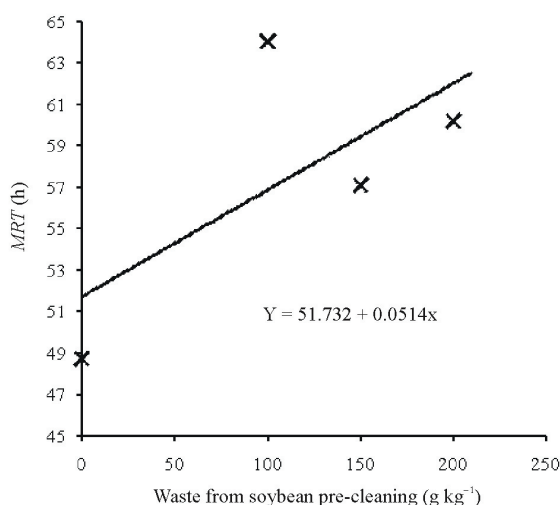

Figure 2 - Model of increasing linear regression adjusted for the mean retention time (MRT) according to the applied treatments and its estimated equation.

Discussion

The calculation of Akaike's criterion (Akaike, 1974) allows us to compare different hypotheses, and based on the obtained results, select the one that best predicts reality according to the evaluated dataset. Although the homogeneous variance and covariance structure has a lower number of parameters, it may, in some cases, result in in worse adjustment, with higher values on both Akaike's corrected criterion and delta and evidence ratio, and lower estimates for the probability of likelihood. However, this behavior was not observed in the present study.

Mathematical models applied to animal nutrition have been used to quantify the nutritional value of diets and estimate the animal performance. These models are based on the evaluated substrate and on the ruminal digestion kinetics to estimate the availability of nutrients (carbohydrates and proteins) for the microbial growth (Abreu et al., 2014). The optimization of diets for ruminants has been widely investigated, especially concerning their ability to ingest and consume fiber (Vieira et al., 2008a; Jardim et al., 2013).

Theoretically, it is expected that with the increase in the *MRT* values, the *TD* estimate will also increase, given that the longer a feed remains in the rumen, the more it will undergo the effects of fermentation and degradation. However, in the present study it was found that the digestibility of the fiber from the silage had a quadratic response, whereas the time of retention of the fibrous particles inside the reticulo-rumen responded linearly. This type of behavior is not uncommon, and has already been reported in the literature (Vieira et al., 1997). The occurrence of this phenomenon may be related to the characterization of the ruminal microorganisms, which may vary according to the type of feeding (Van Soest, 1994). With the marked increase in the source of rapidly fermentable feed, the pattern of ruminal microorganisms might have been modified, which means that very high levels of inclusion of the WSPC in the silage might have affected negatively the amount of fiber-degrading bacteria, and this fact might have resulted in the decrease in *TD* of the fiber from the silages. Still, mathematically speaking, the nature of the equations utilized for the estimate of the parameters of *TD* and *MRT* differs. The digestion and time of retention of the particles in the rumen result from the degradation and passage; in the model used to calculate *TD* these rates are on the numerator and denominator, and compete against each other, which may lead to the observed quadratic effect. In the model used to estimate the *MRT*, however, the rates of degradation and escape are only on the denominator, and instead of competing they aggregate, generating an increasing linear effect.

In the animals fed forages, the flow of saliva and the rumination are stimulated, and the rate of dilution of the fluids is high (Russell, 2002). This fact indicates that the ruminal escape is facilitated by the salivation and dilution provided by forage feeds, and when roughage feeds are proportionally substituted for concentrate sources, which are more rapidly degraded in the rumen, the salivation and rate of dilution of the ruminal fluid decrease by around 15% (Russell, 2002), which may cause an elongation in the time of residence of this material inside the organ and a consequent increase in *MRT*.

Conclusions

Addition of the waste from soybean pre-cleaning to guinea grass silages benefits the kinetics of digestion and passage through the gastrointestinal tract of cattle, considering the inclusion levels of soybean hulls between 92 and 108 kg/1,000 kg of ensiled material. From this limit, the digestion and passage of the fiber in and through the reticulo-rumen are impaired, i.e., the clearance of the fiber by the reticulo-rumen is delayed, which may result in lower use of the silage by the animals.

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ERRATUM

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