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## Short Communication

# Particle passage kinetics and neutral detergent fiber degradability of silage of pineapple waste (aerial parts) under different packing densities

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**ABSTRACT** - The objective of this study was to determine the kinetics of *in situ* degradability parameters of the dry matter (DM) and neutral detergent fiber (NDF) and the passage of materials originating from the ensilage of the waste from pineapple cultivation (aerial parts). The four treatments utilized were silage of pineapple waste compacted at 600, 700, 900 and 1000 kg/m$^3$. After ensiling the material from the pineapple cultivation, the particle-transit and rumen-degradation kinetics were analyzed. For the analysis of particle transit, chromium was utilized as a marker to mark the fiber. Passage rates were determined by retrieving the markers in the feces of the animals. In the degradation assay, samples were incubated in nylon bags for 0, 6, 18, 48 and 96 hours. The behavior observed in the regression curves of the variables analyzed describes high correlation between them, i.e., the time during which the silage is retained in the rumen influences its digestibility and its degradation rate. Although the silage compacted at 900 kg/m$^3$ shows a larger potentially digestible fraction, it is recommended that it be ensiled at a compaction density of approximately 750 kg/m$^3$ due to the lower cost and shorter mean retention time in the rumen-reticulum and rumen fill, thereby increasing the ruminal degradation and passage dynamics.

Key Words: compaction, mean retention time in the rumen, pineapple silage, rumen fill

## Introduction

The digestive transformations are determined by factors intrinsic of the feedstuff and by their interactions with the kinetic processes. Thus, the quantitative expression of the kinetic processes of digestion and passage is necessary to more precisely estimate the quantity and composition of the digested nutrients and their utilization efficiency by the animal (Ellis et al., 1994).

The particle passage kinetics in ruminants has been estimated with the use of markers complexed with the fibrous fraction of the feed in a single-dose procedure, with subsequent fecal samplings at pre-determined time intervals, aiming to characterize the excretion curve of this marker.

Among the various markers utilized to estimate the passage rate, chromium (Cr)-complexed fibers, called chromium mordants, have the advantage of more simple procedures during the laboratory analyses (Lira et al., 2000).

Another important parameter to be studied when considering the quality of the silage is dry matter degradability. In addition to its quick and easy performance, the *in situ* technique requires a small sample of the feed and allows close contact with the rumen environment, simulating a certain feeding regime in the rumen (temperature, pH, buffering, substrate, enzymes), because the material to be analyzed is exposed to rumen conditions commonly found (Orskov and Mcdonald, 1979; Orskov et al., 1980), although the feed is not always subjected to all the digestive events such as chewing, rumination and passage (Vieira, 1997). With this procedure, it is possible to find the effective degradability and the degradation rate of the forage utilized, which enables the calculation of a more adequate diet, and consequently more efficient production.

Given the importance of evaluating feeds to elaborate high-quality diets, which minimizes costs and promotes better animal productive performance, and given the diversities in the degradation rate of roughage feeds, the present study was conducted to evaluate the particle passage kinetics and the degradability of the neutral detergent fiber from the silage of the pineapple cultivation waste (aerial parts) compacted at different packing densities. The objective of the study was to verify if the differences in the compression levels affect the parameters related to passage rate and degradability of silages.

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## Material and Methods

This study was conducted on Experimental Farm Risoleta Neves, from the UFSJ/EPAMIG partnership, in the period from February to May 2011.

The pineapple cultivation waste (aerial parts after harvesting the fruit) was acquired in the municipality of Frutal/MG, Brazil and transported to Universidade Federal de São João Del Rei, in São João Del Rei, Minas Gerais, Brazil. The material was ensiled in experimental silos made from buckets of known dimensions, closed with lids provided with Bunsen valves, fitted and sealed with lids covered with plastic and adhesive tape.

The material was chopped with a stationary shredder powered by an electric motor. Sixteen experimental silages were utilized, in four treatments with four replications: silage of pineapple waste compacted at 600, 700, 900 and 1000 kg/m$^3$.

Four rumen-fistulated crossbred cattle of approximately 25 months of age and average live weight of 300 kg were utilized in the experiment. The animals remained stabled in individual stalls of concrete floor, partially covered with tile. The animals were randomly distributed into the four blocks of the production assay, at one animal per block, with four replications per treatment.

The following supplementation was provided: 25 kg/day of sorghum silage, mineral salt, and concentrate (70% corn and 30% soybean) at 1 kg/day, and all the animals had free access to water and mineral mixture.

The study was divided in four experimental periods, with a duration of 15 days, two of which were for animals to adapt to the diets, and eight days were used to evaluate the passage rate of the solid phase.

The experimental silos were opened at the beginning of the experiment, in February 2011. The chemical analysis (Table 1) was determined according to AOAC (1990).

From the 7th to the 15th day of each period, feces were collected to estimate the curve of excretion of the marker and, consequently, from the data generated, the particle passage kinetics was determined, utilizing chromium (Cr mordant) as marker, attached to the cell wall of the forage of the respective treatment (compaction level), by adapting the procedures described by Udén et al. (1980).

Table 1 - Chemical composition of silages under different compaction pressures

| Parameters | Compaction pressures | | | |
|---|---|---|---|---|
| | 600 kg/m$^3$ | 700 kg/m$^3$ | 900 kg/m$^3$ | 1000 kg/m$^3$ |
| Dry matter (%) | 39.06 | 38.40 | 33.92 | 33.43 |
| Crude protein (%) | 6.62 | 6.87 | 5.88 | 6.59 |
| NDF (%) | 54.10 | 53.95 | 54.61 | 55.37 |
| ADF (%) | 44.35 | 36.95 | 35.70 | 35.77 |
| Ether extract (%) | 4.90 | 3.96 | 4.75 | 3.72 |
| Lignin (%) | 7.48 | 8.75 | 8.49 | 8.21 |

NDF - neutral detergent fiber; ADF - acid detergent fiber.

First, samples of the forages were dried in forced-ventilated oven at 60±5 ºC for 72 hours. Next, this material was boiled with neutral detergent for one hour, at the ratio of 100 g dry of dry sample per 100 mL detergent and 1 L water. After this procedure, the material was filtered in cotton bag, washed with running tap water until the water was clear, to remove the soluble contents, then dried at 60±5 ºC, for 72 hours. A potassium dichromate ($K_2Cr_2O_7.2H_2O$) solution was added to this fiber, at the ratio of 13% chromium in relation to the weight of the fiber to be marked. After, the potassium dichromate solution was diluted in a glass container, with subsequent immersion of the fiber. This container was covered with aluminum foil and dried in an oven at 105 ºC for 24 hours. After this procedure, the material was conditioned in cotton bag and washed in running water to remove the excess dichromate. Afterwards, the material was immersed in a commercial solution of ascorbic acid at the proportion of half the weight of the fiber, and left to stand for one hour, until reaching an intense green color. Immediately after, the material was once again conditioned in cotton bag and washed, which was repeated until the water was completely clear, and dried in a forced-ventilation oven at 60±5ºC for 72 hours. Two hundred grams of marked fiber were supplied through a rumen cannula at the beginning of each experimental period.

After this, individual collections of feces were performed, beginning at zero time, along with administration of the mordanted fiber, resuming at pre-determined times until 192 h (0; 1; 2; 4; 6; 8; 10; 12; 14; 16; 20; 24; 28; 32; 36; 40; 44; 48; 56; 64; 72; 80; 88; 96; 108; 120; 132; 144; and 192 h). Feces were collected directly from the rectum of animals, with the aid of plastic gloves.

Samples of feces were pre-dried in a forced-ventilation oven at 65 ºC for 72 hours, ground in cyclone-type mill (2 mm mesh sieve) and analyzed for the content of chromium (Cr) by atomic absorption spectrophotometry after nitro-perchloric digestion, according to the methodology described by Kimura and Miller (1957).

To determine the rumen kinetics of the dry matter (DM) and neutral detergent fiber (NDF) from the silages of the materials from experimental silos, the *in situ* technique was adopted, as described by Mehrez and Orskov (1977) and Nocek (1985), according to recommendations of Kirkpatrick and Kennelly (1987).

Incubation times were of 0, 6, 18, 48 and 96 hours. After being removed, the bags were washed in running water until it showed clear; after, they were dried at 60±5 ºC in a forced-ventilation oven for 48 hours and taken to a dissector. Subsequently, their respective weights were determined. Bags corresponding to time zero were not incubated in the rumen, but they were washed along with the others.

The bags were attached to the links of a chain in a sequence (row) and immersed in the rumen content, with the chain anchored to the weight. Bags were incubated in the rumen in reverse chronological order (bags infused at the determined times and all removed at the end of the time count). Subsequently, they were washed together in a container with running water until the water did not present signs of residues from rumen content. Next, they were taken to forced-ventilation oven at 65 ºC for 48 hours, and their dry weight was determined on an analytical balance, following methodology described by AOAC (1990).

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

The model utilized to estimate the kinetic parameters of fiber digestion are based on a simple first-order equation (Smith et al., 1971).

The variables tested in the present study were: standardized potentially digestible fraction (Bn); standardized fiber indigestible fraction (Un), digestion rate (c), mean retention time in the rumen-reticulum (MRT), true digestibility (TD) and rumen fill (RF) of the fiber. Fractions B and U were standardized to demonstrate adequate proportion between each other (Waldo et al., 1972). The variable c represents the fractional rate of fiber digestion per unit of time.

The turnover, or mean retention time in the rumen-reticulum (MRT), was estimated based on biological interpretations, in which both ascending and descending phases of the profile of excretion of markers in the feces have an influence on the retention of particles in the rumen-reticulum (Vieira et al., 2008). The MRT of particles in the rumen-reticulum was estimated according to the equation suggested by Matis et al. (1989). The dimensionless true digestibility coefficient and the rumen fill effect (expressed in days) of the fiber (TD) were estimated through the models deduced by Vieira et al. (2008).

The model fitting parameters for the passage and degradability kinetics profiles were estimated through command NLIN of software SAS (Statistical Analysis System, version 9.0). Both algorithms of Newton and Marquardt were utilized. Initially, the preferred form or algorithm was that of Newton, due to its good performance in terms of convergence; however, whenever the correlations between the estimates of the parameters were high, the Marquardt algorithm was chosen.

The selection of the best version for the order of time dependence $N$ and, consequently, of the best model to explain the passage rate, was evaluated by calculating Akaike's information criterion ($ALCc_h$) (Akaike, 1974; Burnham and Anderson, 2004), which was calculated by the sum of the square errors ($SSE_h$), number of parameters estimated, including the residual variance ($\Theta_h$) and the size of sample ($n_h$) for all the different versions of $N$ ($\forall h$=1,2,...,6.). The differences between the values of $N$ ($\forall h$=1,2,...,6.), the probability of likelihood ($w_h$) and the evidence rate ($ER_h$) were also calculated with the equations described by Vieira et al. (2012).

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

Statistical analyses of the estimates of the digestion and transit kinetics parameters through the rumen were conducted. The statistical model adopted was the following:

$$Y_{ij} = \mu + a_i + a_j + e_{ij}$$

The fixed effects are the mean ($\mu$) and the treatments ($a$); the random effects are attributed to the animal ($a$) and to the error ($e$). The statistical model was fitted utilizing procedure PROC MIXED of SAS (version 9.0). The significance level adopted was 0.05, unless otherwise stated. Different models were tested for the structure of the variance and covariance matrices. The likelihood of these models was verified from Akaike's criterion and from the likelihood criteria derived from it, i.e., $\Delta_h$, $w_h$, and $ER_h$.

The following structures were tested: variance component, compound symmetry with constant correlation and homogeneous variance, compound symmetry with constant correlation and heterogeneous variance, heterogeneous variances allocated in the main diagonal with null covariances and unrestricted structure of variance and covariances (Vieira et al., 2012). The equations proposed by Neter (1974) for the calculation of maximum or minimum points and the respective confidence interval were used for parameters MRT, TD, RF and c.

## Results and Discussion

The calculation of Akaike's criterion allows for comparing multiple hypotheses and, with the results obtained, select the model that best predicts the reality according to the group of data in question. The variance components structure was that which produced the most likely results among all variance and covariance structures tested based on Akaike's criterion.

Among all the parameters tested, only variables Bn and Un did not express significance for the regression analyses (Table 2).

As for fractions Bn and Un, the treatment with compaction at 900 kg/m$^3$ showed to be more efficient, providing a larger potentially digestible fraction and consequently smaller indigestible fraction in relation to the other treatments.

Parameters c, MRT, TD and RF presented significant values for the regression analysis of the different treatments, both with similar behavior in a quadratic function (Figure 1).

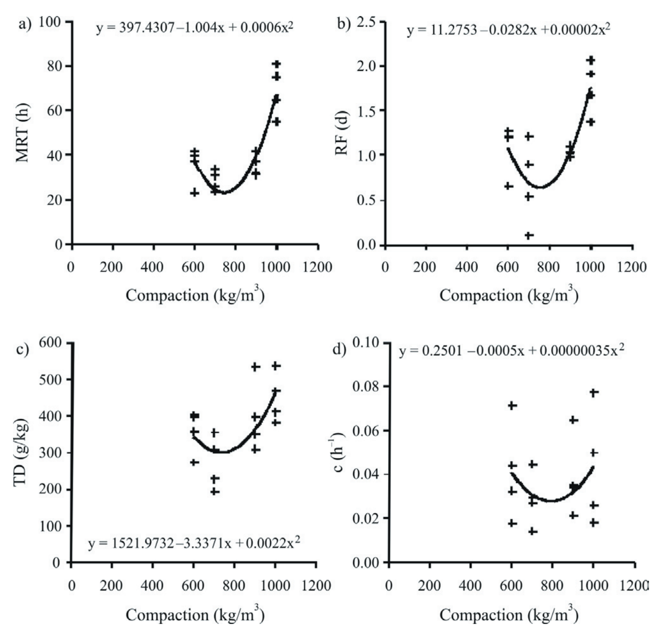
The behavior presented in the regression curves of the analyzed variables describes a high correlation between them in the different treatments. With increase in the compaction pressure in the treatments, the MRT and TD of silages increased as well; the time of permanence of the silage in the rumen has an influence on its digestibility and on its degradation rate. In fact, the longer the feed remains in the rumen, the more digestive processes it will go through, so it will be more degraded (Figures 1a and 1c).

This increase in compaction pressure in the treatments also had an effect on RF, which led to a dramatic increase in this parameter (Figure 1b). If a feed causes rumen fill in an animal, this animal will have its voluntary intake affected, given that intake is also regulated by mechanisms of rumen fill, and the animal may undergo nutritional restrictions.

According to the results obtained for the different treatments in the regression analyses, it can be observed, through the minimum points found in the graphs (Table 3), that there is a mechanism that decelerates the rumen dynamics of degradation and passage with very accented increase in the silage packing density.

These mechanisms which decelerate the rumen dynamics of fiber indicate that packing densities above 800 kg/m$^3$ are not justifiable, because, in addition to the higher energy and financial costs to obtain high compaction densities, the final result has a negative influence on the ruminal degradation and passage dynamics. Further studies should be conducted to verify the cause and effect of the results obtained in the present study.

The silage compacted at around 750 kg/m$^3$ provided greater positive effect from the rumen physiology standpoint, especially due to its shorter retention time in the organ and lower rumen fill.




Crosses correspond to the mean values for each treatment.
(a) Mean retention time in the rumen (MRT).
(b) Rumen fill (RF).
(c) True digestibility (TD).
(d) Digestion rate (c).

Figure 1 - Regression curves and their respective equations according to the different compactions for the variables

Table 2 - Means of least squares and respective errors for the confidence interval at 95% for the standardized potentially digestible (Bn) and indigestible (Un) fiber fractions

| Compaction (kg/m$^3$) | 95% Confidence intervals | |
|---|---|---|
| | Bn | Un |
| 600 | 0.559±0.053 | 0.441±0.053 |
| 700 | 0.546±0.053 | 0.454±0.053 |
| 900 | 0.627±0.053 | 0.372±0.053 |
| 1000 | 0.579±0.053 | 0.421±0.053 |

Table 3 - Minimum point for X and Y and confidence interval for X related to variables

| Parameters | Minimum X | Y at the minimum X | Confidence interval for X | |
|---|---|---|---|---|
| | | | Min. | Max. |
| MRT | 745 (kg/m$^3$) | 23.39 (h) | 737 (kg/m$^3$) | 754 (kg/m$^3$) |
| RF | 754 (kg/m$^3$) | 0.64 (d) | 744 (kg/m$^3$) | 765 (kg/m$^3$) |
| TD | 732 (kg/m$^3$) | 300.5 (g/kg) | 705 (kg/m$^3$) | 759 (kg/m$^3$) |
| c | 789 (kg/m$^3$) | 0.0277 (h$^{-1}$) | 762 (kg/m$^3$) | 816 (kg/m$^3$) |

MRT - mean retention time in the rumen; RF - rumen fill; TD - true digestibility; c - digestion rate.

## Conclusions

It is recommended to ensile pineapple waste at a compaction density of 750 kg/m$^3$ of natural matter, because this density provides the best digestion and passage characteristics to the ensiled forage.

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## ERRATUM

The Short Communication "**Particle passage kinetics and neutral detergent fiber degradability of silage of pineapple waste (aerial parts) under different packing densities**", was published in Revista Brasileira de Zootecnia, v.43, n.1, p.49-53, 2014, without the Acknowledgments section included.

Where it should read:

### Acknowledgments

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