Artificial rumen and NIR techniques to assess degradability kinetics of the major components of maize

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Introduction

Determination of forage components' degradability kinetics requires not only the development of standardised methods for the incubation phase and thus for the study of the disappearance of dry matter, but also the use of rapid analytical methods for the determination of the intrinsic composition of the large number of samples resulting from kinetics assessment.

This study aims to develop a simple and reproducible method to determine the degradability kinetics of the dry and organic matter (starch, fibres) of the whole maize plant. To achieve this objective an artificial rumen for the incubation of samples in standardised conditions and near infrared spectrometry for the determination of the biochemical composition of residues are used.

Material and methods

Samples

Maize whole plant samples (silage cut) are dried in a ventilated oven at 60°C for 48 hours and then ground (grid 4 mm). The ground material is weighed (twice per incubation period) in tarred nylon bags, 50 µm porosity (ANKOM Technology Corporation USA) with a padding level of 45 mg cm².

The sample weight is 3 g for the first incubation period (from nought to six hours) and 5 g for the other periods. The bags are sealed by heating and weighed again after sealing.

Artificial rumen

A large capacity fermenter (artificial rumen) was developed for this study. It consists of three incubators (mini washing machines) located in an airtight room saturated with CO_2 and kept at 39°C. This room is designed to allow the control of temperature and pH during fermentation. To simulate ruminal movements, the incubation tank rotates for one minute every five minutes. Each incubator can accommodate 800 g to 850 g of ground material.

Rumen juice is collected from adult Holstein oxen fitted with rumen fistula and fed two equal meals per day with a standardised ration consisting of 50% alfalfa hay (rumiluz), 40% spelt (*Triticum spelta*) and 10% wheat straw.

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Each individual fermenter contains 5.6 l of filtered rumen juice mixed with 15.2 l of Tilley and Terry buffer and artificial saliva. Kinetics are based on matter disappearance measurements at 0, 0.5, 1, 3, 6, 12, 24 and 48 hours after introduction of the bags into the fermenter, and at the same time a sample of rumen juice solution is taken to measure the pH. After removal from the fermenter, the bags are dropped into cold water, washed three times for one minute, spin-dried, oven-dried at 60°C for 48 hours, and then weighed to determine the loss in dry matter. The t_0 bags are just dropped in cold water and then dried following the procedure describe before.

Each incubator contains standard samples of alfalfa hay (rumiluz) to correct bias due to inter-batch variations.

Near infrared spectroscopy

All spectra of incubation residues are individually collected with a Foss-NIRSystems 6500 scanning monochromator. To develop predictive models for starch, ash and NDF (Neutral Detergent Fibre), some residues (grinding 4 mm) are grouped together to have enough matter to perform the wet chemistry (reference methods). In order to include a wide range of spectral data and as large a range of biochemical values as possible, residues are grouped by two different selection techniques: some according to spectral data and others according to incubation time.

Spectral data of grouped residues are collected before performing reference methods. After the completion of wet chemistry, reference values and spectral data are combined to develop predictive models. These models are then used to predict the composition of all individual residues.

Data processing

Degradation values of observed data are computed in order to determine the Orskov and Mac Donald model parameters (Equation 1) by iterative processing with the SAS software (Proc NLIN, Marquardt method).

Orskov–Mac Donald model :

$$y = B0 + B1 * [1 - Exp(-B2 * T)] \quad (1)$$

where: y = Component disappearance at time t in %; B0 = Proportion of component immediately soluble in %; B1 = Proportion of component potentially degradable in %; B2 = Rate of disappearance of B1 in %/hour; T = Time.

Then, the effective degradability (Equation 2) based on the Orskov and Mac Donald parameters is estimated for a theoretical outflow rate of 4.5 per hour.

Effective degradability (Ed in %):

$$\mathbf{ED} = B0 + \frac{(B1 * B2)}{(B2 + K)} \quad (2)$$

where: K = Rumen outflow rate (K = 4.5% h^{-1} in the case of this study).

Finally, in order to avoid bias due to fermentation pattern between artificial rumen, a corrected effective degradability (EDC 4.5 %) is calculated as follows (Equation 3):

$$EDC\ (4.5\%) = ED - 0.6 \times (\text{observed degradation of alfalfa hay after 12 hours} - 45) \quad (3)$$

In the same way, and to integrate a response delay, predicted NIR composition data are computed to determine the Orskov and Mac Donald modified model parameters by iterative processing for the following equations (4 and 5):

$$Y \text{ (delay corrected)} = B0+B1*\{1-\exp[-B2(T-L)]\} \quad (4)$$

$$ED \text{ (delay corrected)} = B0+B1 \quad *[(B2/(B2+K)]*(\exp(-K*L)) \quad (5)$$

Results and discussion

Artificial rumen

To assess the protocol standardisation of kinetics determination, in addition to control of the oxen supply (stable rumen juice), the buffer solution, the temperature and the incubation period were tested to estimate the repeatability.

- Period of incubation

The parameters of the Orskov and Mac Donald model and the ED were compared for several samples on the basis of two incubation periods: 48 and 72 hours. The results obtained are described in Table 1 and Figure 1.

Table 1. Dry matter degradability of maize whole plant sample : comparison of Orskov's parameters and the effective degradability for two incubation lengths

Parameters	*48 h data*	*72 h data*
B0	*11.5863*	*11.4886*
B1	*47.6720*	*46.8679*
B0+B1	*59.2583*	*58.3565*
B2	*0.0999*	*0.1040*
ED 4.5%	*44.4510*	*44.2001*

- Repeatability of dry matter degradability between two artificial rumens

Twenty (20) samples (160 observations) were incubated in the same conditions in two different incubators in order to compare the results obtained for the kinetics. The results are explained in Figure 2.

The results allow the application of the protocol to a large number of samples in order to compare them for their dry matter degradability kinetics and to try to develop NIR models to predict residue composition and thus degradability kinetics of some major components.

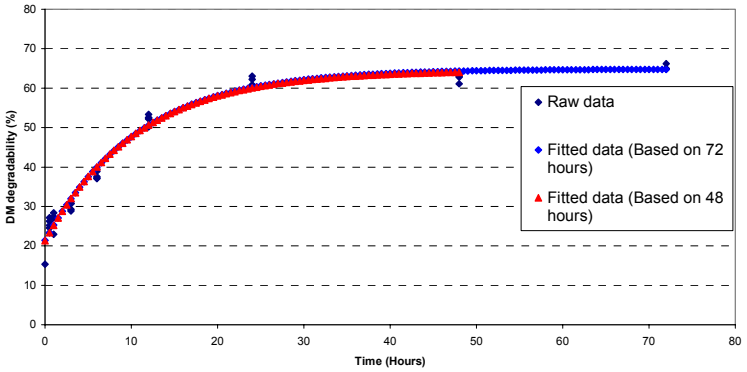

Figure 1. Comparison between fitted data on basis of two incubation times

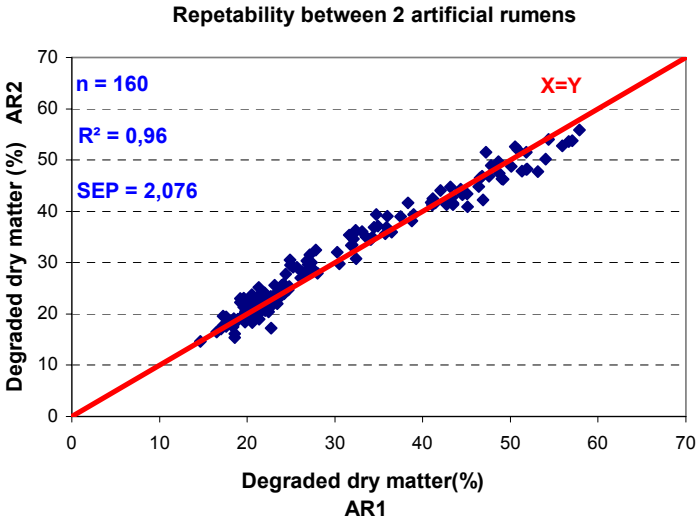

Figure 2. Comparison between observed degradation data for the same sample in two artificial rumens (incubators) in the same conditions

Near infrared spectroscopy model development

NIR analyses were performed using a Foss-NIRSystems 6500 apparatus between 400 and 2500 nm on all the residues. The first step was to detect possible outliers in the spectral database obtained for the grouped residues. The spectra of those samples are explained in first derivative 1,1,1 in Figure 3. No outlier is detected; normal noise appears in visible region below 800 nm.

The study of Mahalanobis distances gives the same results: no outlier and a typical distribution of the GH values (Math used: SNVD-D1,4,4-800-2498/6nm).

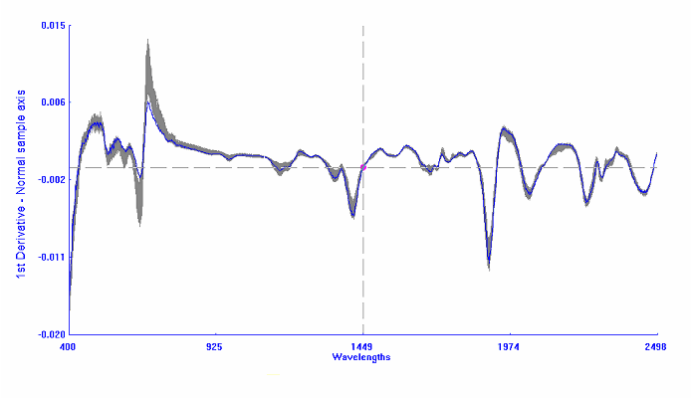

Figure 3. Spectral data of grouped residues in first derivative 1,1,1

The NIR calibration models obtained for the first batch of samples (n=228) are explained in Table 2 and the results obtained for starch in Figure 4. At this stage, the models to analyse the residues of nylon bag seem good. Very low SECV for ash, high R² for starch and NDF are observed. The accuracies for these two parameters are at the same level as the levels observed for maize forage samples.

Table 2. NIR prediction models on grouped residues

Constituent	N	Mean	SEC	R²c	SECV	R²v
Starch	228	21.46	2.49	0.97	2.76	0.96
Ash	228	2.07	0.15	0.86	0.17	0.80
NDF	228	60.32	2.10	0.98	2.59	0.96

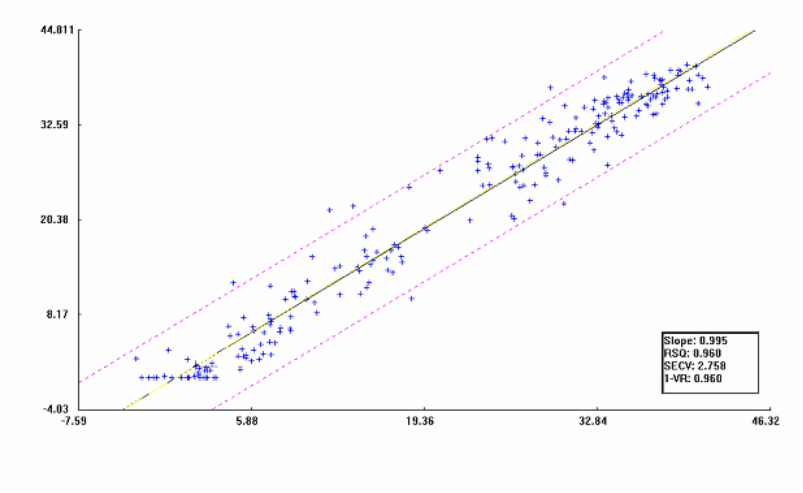

Figure 4. Starch calibration SNVD-D2,20,2. Cross-validation scatter plot

The addition of another sample set to the data confirms the performance of the models. Models used to predict each individual residue are described in Table 3. The problem would be to analyse individual bags because the calibration samples are averages of several bags and the individual sample could be outside the calibration boundaries. Thus, the Mahalanobis distances (GH and NH) must be verified carefully to avoid wrong prediction.

Table 3. Final prediction models for ash, starch and NDF on residues.

Constituent	n	Mean	SEC	R^2c	SECV	R^2v
Starch	302	22.06	2.49	0.97	2.63	0.96
Ash	304	2.05	0.13	0.85	0.16	0.78
NDF	300	59.79	2.37	0.97	2.46	0.97

Composition parameters degradability analysis

The performances observed for the incubation protocol (kinetics of dry matter disappearance) and for NIR prediction for ash, starch and NDF allow the application of the mathematical models described before to assess the parameters of kinetics for those composition components. Kinetic parameters obtained for three samples of maize whole plant are explained in Table 4 and the relation between the fitted data and the NIR predicted data in Figure 5.

The correlation between the NIR predicted and the fitted data is the same as that for observed and fitted dry matter degradation data.

Table 4. Example of starch kinetic parameters obtained for maize whole plant samples

Sample	1	2	3
B0	9,8022	15,0215	23,6637
B1	86,7292	77,9267	68,4519
B2	0,1705	0,1172	0,2321
Delay	2,2981	1,0696	5,6936
B0+B1	96,5314	92,9483	92,1156

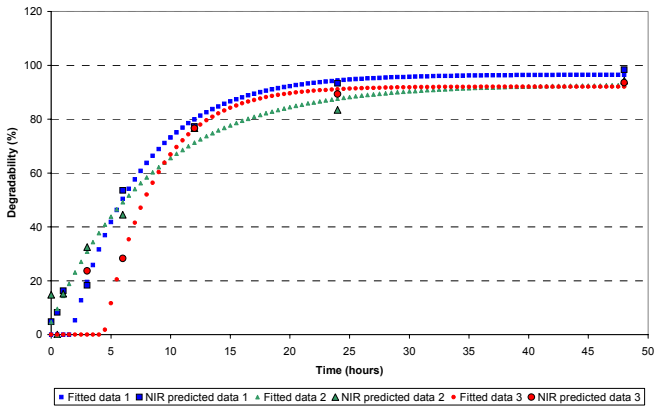

Figure 5. Fitted and NIR predicted data for starch degradation on the same three samples

Conclusions

This study did not aim to predict the real digestibility of forages but, through the application of the described protocols for in vitro incubation, NIR analysis and data treatments, to allow the comparison of samples not only for dry matter, but also for some of the major components of degradability kinetics in rumen juice.

The large capacity of the fermenter and the rapidity of the NIR technique allow analysis of a lot of samples from only a few fistulated animals.

To predict real forage-use efficiency, the technique will be correlated with in vivo measurements.

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