Optimising calibration to measure degradability parameters of alfalfa hays and dehydrated forages

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Introduction

Hay-making has been the traditional conservation method for lucerne cultivated in Spain although, in the last few years, the quantity of dehydrated forage has been higher than hay-making.[1] Among the advantages attributed to dehydration, we can mention the following: culture intensification, diversification of the forage use and independence from the weather conditions.[2] The dry matter and nitrogen ruminal degradability of these forages differ widely according to the dried process characteristics. For this reason, it is necessary to know the degradability characteristics to obtain a good and accurate application of actual feeding systems. However, the availability of *in vivo* and *in situ* degradability values for this kind of forage is limited because these methods require working with fistulated animals which is rather complicated and requires intensive and expensive labour. Even though we use the easier *in situ* methodology, the dynamics and logistics result in considerable work, due to limitations on the number of samples and number of bags that can be placed in an animal and the different time intervals required to perform kinetic studies. Therefore, a simple method is necessary to estimate the feed degradation characteristics. In this way, near infrared (NIR) reflectance spectroscopy has been widely used to predict degradation characteristics of forage.[3,4]

The aim of this study was to confirm the potential of NIR to optimise work conditions to avoid duplicated efforts in collaborative trials on animal feed evaluation between research institutions.

Materials and methods

In situ degradation

Two sets, with a total of 50 samples of lucerne preserved as hay or dehydrated forage, were evaluated using the *in situ* method: 40 from the SERIDA Research Centre located in the northwest of Spain and ten from SIA in the North of Spain. The technique of nylon bags was used to determine the kinetics of nitrogen (N) degradation. The fractions, soluble (a), insoluble potentially degradable (b), the fractional degradation rate of the slowly degradable fraction (c) and the effective degradability (ED) were estimated according to the equation obtained by Ørskov and McDonald.[5]

The trials were carried out with three four-year-old castrated rams (Fleichchaff x Rasa Aragonesa in the SIA trials and Manchega in the SERIDA trials) provided with a rumen cannula, 60–65 kg aver-

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age live weight and adapted to a ration at maintenance level with alfalfa hay and concentrate [60 : 40 dry matter (DM) basis]. The forages were milled through a 2 mm screen and weighed in dacron bags with a 50 ± 10 micron pore size (Ankom Technology Corporation) at 12.5 mg cm^{-2}. The incubation times were 0, 2, 4, 8, 12, 16, 24, 48 and 72 h.

Parameters (a), (b) and (c) were estimated individually for each animal by a nonlineal regression. The adjustment of the kinetic values of rumen degradation to that equation was made by the procedure NLIN from the SAS statistical package.[6] The effective degradability (ED) of the DM and N were calculated for a rumen outflow rate (k) of 2% h^{-1},[7] by the equation $ED = a + [(b * c) * (c + k)^{-1}]$.

NIR scanning and calibration procedures

The lucerne samples were ground at 1 mm and scanned using a Foss NIRSystem 6500 monochromator with transport sampler, over a wavelength range from 400 to 2500 nm in steps of 2 nm.

Three sets of NIR spectra were created. The first set consisted of the lucerne spectra from SERIDA (40 samples) in their original form, the second included the spectra from SERIDA and five samples from SIA and the third set consisted of spectra from SERIDA and ten samples from SIA. The spectra from SIA were standardised using a single sample standardisation with one sample spectrally close to the centre of the population.[8] Every sample was measured in two replicates and the average of the replicate spectra obtained as log $1/R$ (R = Reflectance) was used in the calibration. Calibration equations were obtained with WINISI II v 1.5 sofware (Infrasoft International, Port Matilda, PA, USA), using a full wavelength range and modified partial least square as the regression method. The standard normal variate method (SNV)[9,10] was used for scatter correction and the second derivative as the mathematical treatment.

Results and discussion

The sample sets were characterised by the statistic listed in Tables 1–4: range and standard deviation (SD) for (a), (b) and ED of the dry matter and ED for nitrogen.

Generally, no significant differences were found between field-dried and dehydrated forages in terms of ED of dry matter, although the conservation in dehydrated form presented higher values than hay with respect to (b) fraction and lower values concerning the (a) fraction. This phenomenon can be explained by the larger exchange of nutrients, which can take place between fractions (b) and (a) in hays in relation to dehydration.

In terms of nitrogen ED, the lucerne preserved as dehydrated forage showed lower values of ED than hay forage (Table 4).

Tables 1–3 also summarise the calibration equation statistics that were obtained for kinetic parameters of DM. The values for ED of nitrogen are shown in Table 4. The ratio between the reference data

Table 1. Statistical results of calibrations and population characteristics for dry matter soluble fraction: a (%)

Population	*SEC*	R^2	*SECV*	r^2	*RER*	Range	*SD*
Set A (n = 40)	0.87	0.97	2.34	0.79	8.14	22.0-41.0	4.98
Set B (n = 40 + 5)	1.34	0.98	3.91	0.84	10.73	22.0-64.0	9.77
Set C (n = 40 + 10)	1.85	0.98	3.48	0.92	12.65	22.0-66.0	12.63

A: 40 alfalfa hays and dehydrated forages from SERIDA
B: 40 alfalfa hays and dehydrated forages from SERIDA + 5 alfalfa hays from SIA
C: 40 alfalfa hays and dehydrated forages from SERIDA + 0 alfalfa hays from SIA

Table 2. Statistical results of calibrations and population characteristics for dry matter degradable fraction: b (%).

Population	*SEC*	R^2	*SECV*	r^2	*RER*	Range	*SD*
Set A (n = 40)	1.12	0.95	3.28	0.52	7.02	36.0-59.0	4.77
Set B (n = 40 + 5)	1.39	0.97	4.04	0.70	9.15	22.0-59.0	7.43
Set C (n = 40 + 10)	1.76	0.95	3.60	0.81	10.55	21.0-59.0	8.22

A: 40 alfalfa hays and dehydrated forages from SERIDA
B: 40 alfalfa hays and dehydrated forages from SERIDA + 5 alfalfa hays from SIA
C: 40 alfalfa hays and dehydrated forages from SERIDA +10 alfalfa hays from SIA

Table 3. Statistical results of calibrations and population characteristics for dry matter effective degradability: ED (%).

Population	*SEC*	R^2	*SECV*	r^2	*RER*	Range	*SD*
Set A (n = 40)	0.92	0.97	2.15	0.84	10.69	50.0–73.0	5.37
Set B (n = 40 + 5)	0.90	0.98	2.34	0.88	12.82	50.0–80.0	6.82
Set C (n = 40 + 10)	1.18	0.98	2.36	0.91	13.12	50.0–81.0	7.95

A: 40 alfalfa hays and dehydrated forages from SERIDA
B: 40 alfalfa hays and dehydrated forages from SERIDA + 5 alfalfa hays from SIA
C: 40 alfalfa hays and dehydrated forages from SERIDA +10 alfalfa hays from SIA

Table 4. Statistical results of calibrations and population characteristics for nitrogen effective degradability: ED (%)

Population	*SEC*	R^2	*SECV*	r^2	*RER*	Range	*SD*
Set A (n =40)	2.76	0.67	3.90	0.34	4.87	65.0–84.0	4.81
Set B (n = 40 + 5)	2.44	0.79	3.66	0.51	5.19	65.0–84.0	5.27
Set C (n = 40 + 10)	2.50	0.80	3.86	0.51	5.44	63.0–84.0	5.53

A: 40 alfalfa hays and dehydrated forages from SERIDA
B: 40 alfalfa hays and dehydrated forages from SERIDA + 5 alfalfa hays from SIA
C: 40 alfalfa hays and dehydrated forages from SERIDA +10 alfalfa hays from SIA

range and standard error of cross-validation (*SECV*) was calculated (*RER*). Ideally, it should be at least ten.[11]

The relationship between the NIR data and the degradation characteristics have a tendency to improve by adding a small number of samples. The addition to samples recorded on the host instrument, which were standardised using a single sample standardisation procedure, maximise the range of composition on kinetic parameters—22.0–66.0 for (a); 21.0–59.0 for (b), 50.0–81.0 for ED of DM and from 63.0–84.0 for ED of N. The calibration statistics indicate a better correlation between NIR and reference data and good accuracy. The *RER* ratio also increased, for DM degradation parameters it was always above ten.

The relationship between the NIR data and the ED was weaker for N than DM (r^2 = 0.51 vs r^2 = 0.91) according to Atanassova *et al.*[3] and De la Roza *et al.*[4] ED of nitrogen values could be less accurate because no account was taken of possible microbial contamination of the bag residues. For this reason, the statistics obtained for (a) and (b) fractions of N also were of lower accuraty than statistics for (a) and (b) fractions of DM and are not included in this paper.

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