Forage organic matter digestibility: NIRS predictions based on *in vivo* values and standardisation of *in vitro* determinations

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Abstract

The determination of forage organic matter digestibility (OMD) is of great importance to ruminant production but can be expensive and time consuming. We evaluated an *in vitro* OMD determination and near infrared spectroscopy (NIRS) OMD prediction using 199 forage samples from 24 regions around Switzerland, which were selected according to the diversity of their fibre and protein content, botanical composition and geographical origin. The *in vitro* OMD was determined with a Daisy II incubator (Ankom Technology), by 48 h digestion of 250 mg sample (in a filter bag) with rumen inoculum (from three cows) in a buffer solution (pH=6.8) at 39.5 °C and anaerobic conditions. Five samples with known *in vivo* OMD values were repeated in each batch to standardise rumen inoculum activity. The standardisation procedure was evaluated with three validation samples. Near infrared spectrometry (NIRS) was performed with a FT-NIR (NIRFlex N-500, Büchi, Flawil, Switzerland) with 3 replicates per sample, 32 scans/replicate from 4,000 to 10,000 cm^{-1} and 8 cm^{-1} of resolution. The NIRS OMD model was based on *in vivo* values. The *in vitro* and NIRS OMD determinations showed moderate to good correlations with ADF, NDF and lignin content, R^2 ranging from 0.24 to 0.85.

Keywords: OMD; *in vitro* digestibility; digestibility with NIRS; permanent and intensive meadows

Introduction

With increasing pressure on animal production and environmental concerns, tools are needed to perform frequent determinations of forage organic matter digestibility (OMD), thereby enabling the optimisation of grassland management. Although tables with OMD values exist, they are based on average values, often not reflecting grasslands specificities, e.g. year, botanical and chemical composition etc., which ultimately influence OMD. This trial aimed to evaluate fast and economical techniques (*in vitro* and near infrared spectrometry (NIRS)) for determining OMD of forages. We focused on the standardisation of rumen inoculum activity for *in vitro* OMD determination and the predictability of NIRS models.

Materials and methods

Standardisation of rumen inoculum

Different rumen inoculums were used per batch, i.e. a mix of the ruminal fluid of one fistulated cow and of two additional cows (from a pool of four cows) vacuum probed through the oesophagus. In each *in vitro* batch, 5 standard samples were included for the standardisation of rumen inoculum activity, as well as 3 validation samples for the evaluation of the standardisation procedure, all 8 (standard and validation samples) with known *in vivo* OMD values. A Daisy II incubator (Ankom technology, Macedon, NY, USA) was used for the *in vitro* OMD determination. The incubator is designed to perform a 48 h digestion by slowly rotating four 2-litre bottles at 39 °C with a capacity of 25 samples each. To prevent oxidation the buffers and the ruminal fluid were maintained at 39 °C under anaerobic conditions at all times. The *in vitro* OMD determination was performed with 0.2500±0.0001 g of milled and dried sample, sealed inside a filter bag. The incubation bottles were each filled with 1,330 ml of buffer [266 ml (10 g l^{-1} KH_2PO_4, 0.5 g l^{-1} $MgSO_47H_2O$, 0.5 g l^{-1} NaCl, 0.1 g l^{-1} $CaCl_22H_2O$, 0.5 g l^{-1} urea) + 1,330

ml (15 g l^{-1} Na_2CO_3, 1 g l^{-1} Na_2S9H_2O)] and were maintained at pH 6.8 and 39 °C. To each incubation bottle 25 filter bags were added that contained either forage (standard and validation samples) or were empty (at least 3 empty bags: blanks). After about 30 min of mixing at 39 °C, 400 ml of ruminal inoculum was added to each bottle, fluxed with CO_2 and incubated for 48 h. The result was calculated as the mass lost during incubation, corrected for ash content. Three determinations per sample were performed, each in a different *in vitro* batch (nine batches in total).

Predictability of NIRS model

An FT-NIR (NIRFlex N-500, Büchi, Flawil, Switzerland) was used with 3 replicates per sample, 32 scans/replicate from 4,000 to 10,000 cm^{-1} and a resolution of 8 cm^{-1}. The NIRS OMD model was built in 2018 based on approximately 100 samples whose *in vivo* OMD values span back to 1990. The study was based on circa 800 forage samples from 32 meadows (23 permanent and 9 intensive) distributed within 24 regions across Switzerland, ranging from 400 to 1000 m in altitude. First, second and third cuts (year 2018) were sampled, with nine samples per meadow × cut. The evaluation of the botanical composition showed values ranging between 60 to 98% grass, 0 to 10% legumes and 1 to 35% forbs for the first cut and between 35 to 98% grass, 1 to 20% legumes and 1 to 74% forbs for the subsequent cuts. The variation of the botanical composition, within the same meadow and cut, could reach 17% for legumes and 25% for forbs. The samples were oven dried at 60 °C for 24 h and milled to pass through a 1 mm sieve. The nutrient and chemical composition of all samples was determined by NIRS (Ampuero Kragten and Wyss, 2014). From this parent database, 199 samples were chosen for *in vitro* (Daisy II incubator) and NIRS determination of OMD while maximising the diversity in fibre, protein and botanical content as well as the geographical origin of the sample subset. Based on the mass availability of forage, 3 samples were chosen for *in vivo* determinations (Pacheco *et al.*, 2018) and further evaluation of *in vitro* and NIRS procedures with recent values of OMD *in vivo*.

Results and discussion

Figure 1A shows the dispersion of the 9 *in vitro* batches by plotting the *in vitro* OMD of the 5 standard samples (single determination, but rumen inoculum from 3 cows) against their corresponding *in vivo* OMD values. Linear regressions per batch showed R^2 and slopes ranging from 0.961 to 0.998 and from 0.84 to 1.09 respectively (data not shown). Figures 1B and 1C show the corresponding values for the three validation samples per batch: B before and C after the correction by a batch-regression equation built with the standard samples. The potential of this form of standardisation should be more evident when using more diverse rumen inoculums. Indeed, a limited number of cows was used in this trial with limited variation in breed, feeding management, health status, etc. The precision was evidently improved by averaging the *in vitro* OMD values over 3 batches (Figure 1D). In Figures 1A and 1D, *in vitro* total average OMD and NIRS OMD values are respectively denoted by X and — signs.

As expected, high correlation coefficients were obtained for first-cut forages between ADF, NDF and lignin respectively and both: *in vitro* OMD values (0.81, 0.67 and 0.63), and NIRS OMD values (0.85, 0.72 and 0.84). However, when using all samples, R^2 decreased, e.g. NIRS: 0.36, 0.24 and 0.71. This probably reflects fibre quality and amount changes between primary growth and regrowth of forage, showing the necessity of including more *in vivo* OMD values of regrowth samples (higher cuts) in the NIRS prediction model.

The diversity of the chemical composition of the 199 samples used for testing the predictability of NIRS model is described in Table 1.

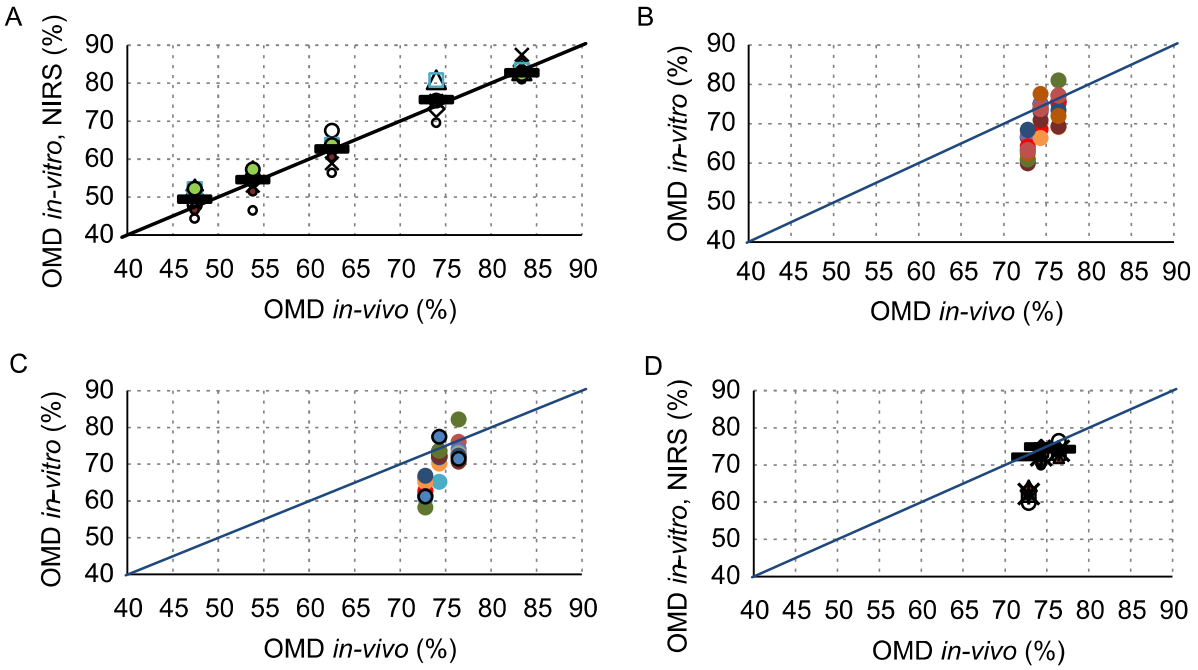

Figure 1. *In vitro* (and NIRS) organic matter digestibility (OMD) vs *in vivo* OMD. (A) Standard samples, per batch values (single determination, different rumen inoculum); (B) validation samples, per batch values; (C) validation samples, per batch values corrected by the corresponding linear regression equation of standard samples; (D) validation samples averaged over three batches. X: *in vitro* OMD total average; NIRS OMD values.

Table 1. Chemical composition (% in dry matter) of the 199 forage samples determined with NIRS.[1]

	DM	CP	CF	ADF	NDF	Lignin	CA	WSC
Average	93.8	15.9	23.2	27.1	45.7	2.9	10.5	9.9
SD	1.3	5.1	5.9	5.8	10.7	1.1	1.9	3.0
Min	91.3	6.8	8.7	11.7	18	0.5	6.5	0.3
Max	97.4	33.9	36.7	39.7	66.9	6.5	16.4	20.0
RMSEP	0.33	0.55	0.80	1.03	1.52	0.40	0.63	0.68

[1] DM = dry matter in % of fresh matter; CP = crude protein; CF = crude fibre; ADF = acid detergent fibre; NDF = neutral detergent fibre; CA = crude ash; WSC = water soluble carbohydrates, SD = standard deviation; RMSEP = NIRS root mean square of prediction error.

Conclusions

The averaging of three batches as a standardisation technique of rumen inoculum activity shows an interesting potential for improving the accuracy of *in vitro* OMD determination. However, the NIRS technique seems more efficient, more economical and less time consuming for OMD evaluation, provided that the set of samples used for the modelling contains all the necessary sample diversity.

References

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