Precision and accuracy of the NDF rumen degradability of hays measured by the Daisy fermenter.

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ABSTRACT: An inventory of 162 hay samples from Austrian permanent grasslands was used to obtain information about the precision of the *in vitro* NDF degradability (NDFd) measured by the Daisy fermenter and its accuracy to predict in situ NDFd. The within forage standard error of the *in vitro* NDFd triplicate, obtained in five consecutive incubations, was equal to ± 2.8 %, while the effect of the four jar positions in the fermenter were not significant. The cutting frequency had a great impact on the in situ NDFd of hays, which ranged ($P<0.01$) from values of 32.9, 43.1 and 48.3% in hays obtained from 2, 3 and 4 cuts/season, respectively.The regression analysis between the *in vitro* and *in situ* NDFd values (measured at 48h and effective, k=3%/h) allowed to obtain a satisfactory degree of correlation ($R^2 = 0.83 – 0.86$) and a moderate level of accuracy (RSE = ± 3.48 %).

Key words: Rumen Degradability, NDF, Hay

Introduction – The NDF rumen degradability (NDFd) greatly influences the net energy content and the voluntary intake of fibrous feeds (NRC, 2001; Oba and Allen, 1999). A simple, cheap and fast *in vitro* rumen fermentation technique is now available in several labs (Robinson et al., 1999; Adesogan, 2005). In addition, the *in vitro* NDFd is used to calculate the NE contents of feeds (NRC, 2001) and also in models (Milk 2006) able to predict the milk yield from dairy cows (Shaver, 2006). However, the precision of the *in vitro* measures and the accuracy of their *in vivo* predictions have yet not well explored.
Present work has used an inventory of hay samples from Austrian permanent grasslands to obtain information about the precision of *in vitro* NDFd measured by the Daisy fermenter and its accuracy to predict *in situ* NDF degradability.

Material and methods – Hay samples were obtained from an experiment held at the Federal Agricultural Research and Education Centre Raumberg-Gumpenstein (Styria, Austria) to evaluate the effect of 3 cutting frequencies (2, 3 and 4 cuts/season) and 3 level of N fertilisation (60, 160 and 240 kg N/ha) on the nutritional value of permanent grassland hays. The experiment, replicated in 3 locations and in 6 years (1998 – 2003), is described by Gruber et al. (2006).
Each sample was analysed in triplicate for the *in vitro* NDFd according to Robinson et al. (1999). In brief, 250 mg of milled sample were introduced in filter bags (55 × 50 mm), which were placed in digestion jars filled with pre-warmed (39°C) buffer solutions and rumen inoculum collected from rumen-fistulated steers fed at maintenance. Four jars (24 bags/jar) were then inserted into a Daisy incubator (Ankom, Tech. Co., Fairport, NY, USA) for 48 h. A total of 162 hays were tested during 5 subsequent incubations.
The *in situ* NDFd was measured on a reduced inventory of 81 hays, which was obtained by selecting systematically the samples within the experimental factors (cutting frequency, fertilization, location and year). Nylon bags (pore size 53 um, 20 × 10 cm) were filled with 6 g of air dried material and inserted in the rumen of 4 cannulated steers (1100 kg LW) for 0, 3, 6, 10, 14, 24, 34, 72, 96, 120 h (see Gruber et al., 2006). The NDF in the residues was analyzed by using the NIRS procedure (Infralyzer 500, Bran & Lübbe, 1100 – 2200 nm, 10 nm intervals, software.

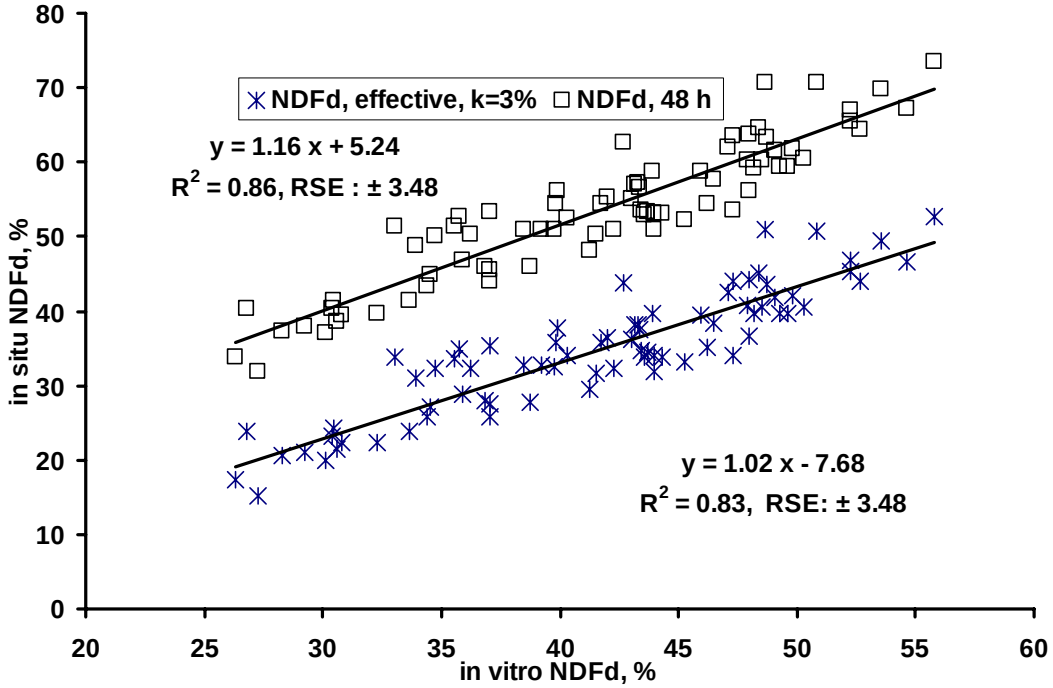
Unscrambler, vers. 9.1) The NIRS calibration was carried out using known NDF contents of 209 residues (r = 0.930, the RMSEC and RMSEP being 20.1 and 22.4 g NDF/kg DM, respectively).
Degradability data were interpolated (PROC NLIN SAS, 1989) with the following model :
$= a + b \times (1 - \exp(-c \times (t - L)))$, where a = immediately soluble fraction, b = potentially degradable fraction, c = degradation rate, t = incubation time (h), L = lag time (h). The effective degradability has been calculated at a rumen passage rate of 0.03/h (k), as follows:
$= a + [(b \times c) / (c + k)] \times \exp(-k \times L)$.
In vitro triplicate measures of NDFd were analysed with the following two models:
$y = \mu + \alpha_i + \varepsilon_{ijl}$ (model 1); $y = \mu + \alpha_i + \beta_j + \varepsilon_{ijkl}$ (model 2)
where μ = overall mean, α = fixed effect of forage (i = 1,162; model 1) or incubation (i = 1,5; model 2), β = fixed effect of jar j = 1,4. Chemical composition and NDF degradability (in situ and in vitro) were analysed with the following model: $y = \mu + \alpha_i + \beta_j + \delta_k + \gamma_l + (\alpha\beta)_{ij} + \varepsilon_{ijkl}$,where μ = overall mean, α = fixed effect of cutting frequency (i = 1,3), β = fixed effect of N fertilisation (j = 1,3), δ = fixed effect of year (k = 1,6), γ = fixed effect of location (l = 1,3). The NDFd measured *in situ* (Y_{ij}) was regressed on *in vitro* NDFd (X_{ij}) according to the following linear mixed model: $Y_{ij} = B_0 + B_1X_{ij} + s_i + e_{ij}$, where s_i = random effect of year. Parameters were estimated using the MIXED procedure of SAS (1999) and adjusted values for the year effect were used to generate a two dimensional graphs.

Results and conclusions – The within forage standard error of the *in vitro* NDFd was equal to ± 2.8 %, which indicates a limited repeatability of the measure. This could not be attributed to difference in jar position in the fermenter as the average values obtained during the five incubations for the different jars were numerically similar and not statistically significant (from 51.6 to 53.0%, data not shown).
As can be seen from Table 1, the cutting frequency (CF) had a great impact on the chemical contents of hays and scarce effects were found for the N fertilisation (only on CP and b degradability fraction), which also did not generate significant interactions with the cutting frequency.
Increasing the number of cuts for season allowed grasses to be harvested at an earlier growth stage (high leaf/stem-ratios) and this increased the CP, EE and ash contents and lowered their fiber contents. Moreover, there was a great impact of CF on the rumen NDF degradation: the potentially degradable NDF fraction and its rate of degradation significantly increased (from 64.2 to 80.9%, and from 3.7 to 6.3%/h, respectively, $P < 0.01$) when hays were cut from 2 to 4 times per season. The effective NDFd, calculated at rumen turn-over rate of 3 %/h, ranged ($P<0.01$) from values of 32.9, 43.1 and 48.3% in hays obtained from 2, 3 and 4 cuts/season, respectively.
These *in situ* variations were associated to similar deviations in *in vitro* values of NDFd, which changed from 42.4 to 54.0 and to 60.6 for forages obtained from 2, 3 and 4 cuts per season respectively.
The regression analysis between the *in vitro* and *in situ* NDFd values (adjusted for the year effect) allowed to obtain the graph showed in figure 1, which have a satisfactory degree of correlation (R^2 = 0.83 – 0.86) and a moderate level of accuracy (RSE = ± 3.48 %).
In conclusion, the precision of the *in vitro* technique requires to be improved and, as the jar position is not relevant, probably others factors are important sources of variability, such as the bag characteristics and preparation (porosity, dimensions, amount of substrate, etc.; Adesogan, 2005). NDFd measured *in vitro* in this study is well related to in situ values, but the standard error of the prediction is moderately high. This could be due to the effects of several factors present in the model (CF, fertilization, year, location) and also to an ample variability in in situ related to the requested long experimental work for the *in situ* measures. However, the satisfactory relation obtained between *in vitro* and *in situ* data stimulates additional research to better define the accuracy in the prediction by dedicated/specific experiments including other categories of fibrous feeds.

Table 1: Chemical composition and in situ and in vitro NDF degradability (NDFd) of hays.

		Factors and interactions in the model							
		Cutting frequency			F	FxC	Y	L	
		2	3	4					SE
Chemical composition:									
-Crude protein	g/kg DM	104 ^{C}	132 ^{B}	16 ^{A}	ns	ns	**	ns	13
-Ether extract	“	18 ^{C}	22 ^{B}	25 ^{A}	ns	ns	**	**	2
-Ash	“	103 ^{B}	111 ^{B}	125 ^{A}	ns	ns	**	ns	19
-NDF	“	643 ^{A}	577 ^{B}	529 ^{C}	ns	ns	**	ns	3
NDFd, *in situ*									
- a	%	-1.3 ^{B}	-1.7 ^{B}	-5.7 ^{A}	ns	ns	**	ns	5.0
- b	%	64.2 ^{C}	70.5 ^{B}	80.9 ^{A}	ns	ns	**	**	4.0
- c	%/h	3.7 ^{C}	5.5 ^{B}	6.3 ^{A}	ns	ns	**	**	0.9
- lag	h	1.0 ^{A}	0.3 ^{B}	0.2 ^{B}	ns	ns	**	ns	0.9
NDFd, *in situ*, 48 h	%	51.3 ^{C}	63.2 ^{B}	70.5 ^{A}	ns	ns	**	**	3.5
NDFd, *in situ*, effective	“	32.9 ^{C}	43.1 ^{B}	48.3 ^{A}	ns	ns	**	**	3.4
NDFd, *in vitro*, 48 h	“	42.4 ^{C}	54.0 ^{B}	60.6 ^{A}	ns	ns	**	ns	4.8

^{A,B,C} = P < 0.01; ** = P < 0.01; F : N fertilisation; Y : year; L : location.



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