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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 was not significant. The cutting frequency had a great impact on the *in situ* effective 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 medium degrees of correlation ($r^2 = 0.69 – 0.71$; $P<0.01$) and low levels of accuracy (RSE = 4.0 -4.6 %).

Key words: Rumen degradability, NDF, Hay, *In vitro* NDFd.

INTRODUCTION – The NDF rumen degradability (NDFd) is measured by a simple, cheap and fast *in vitro* rumen fermentation technique (DaisyII incubator, Ankom, Tech. Co., Fairport, NY, USA), which is now available in several labs (Robinson *et al.*, 1999; Adesogan, 2005). The Daisy NDFd is directly used to calculate the NE contents of feeds (NRC, 2001) and the voluntary intake of cows (Oba and Allen, 1999), which are included in Milk 2006 model 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 not yet well explored.
The present work has used an inventory of hay samples from Austrian permanent grasslands to obtain information about the precision of the *in vitro* NDFd 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 (1999 – 2004), 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 systematically selecting the samples within the experimental factors (cutting frequency, fertilization, location and year). Nylon bags (pore size 53 µm, 20 x 10 cm) were filled with 6 g of air dried material and inserted in the rumen of 4 cannulated steers (1100 kg LW) for 3, 6, 10, 14, 24, 34, 72, 96, 120 h (see Gruber *et al.*, 2006). The NDF in the residues was analysed 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, 1999, Marquardt method) 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 was 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:
$y = \mu + \alpha_i + \varepsilon_i$ (model 1); $y = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$ (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. Adjusted values for the year effect were used to generate two dimensional graphs (SAS, 1999)

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 different jar positions in the fermenter as the average values obtained during the five incubations for the different jars were numerically similar and not statistically different (from 51.6 to 53.0%, data not shown). Other factors are probably important sources of variability, such as the bag characteristics and preparation (porosity, dimensions, amount of substrate, etc.; Adesogan, 2005).
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), which also did not generate significant interactions with the cutting frequency.

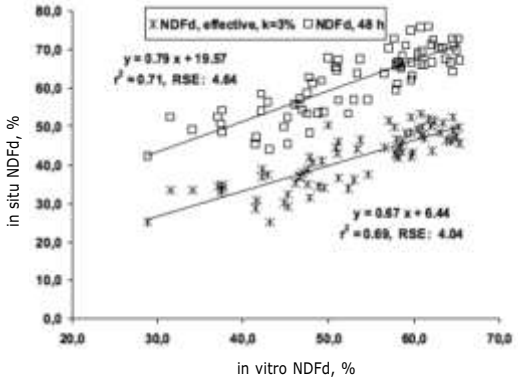
Table 1. Chemical composition and in situ and in vitro NDF degradability (NDFd) of hays.

		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}	133 ^{B}	161 ^{A}	*	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	%	0.7 ^{B}	2.8 ^{A}	1.6 ^{B}	ns	ns	**	ns	3.6
- b	%	62.1 ^{C}	65.9 ^{B}	73.8 ^{A}	ns	ns	ns	ns	4.5
- c	%/h	3.7 ^{C}	5.2 ^{B}	5.9 ^{A}	ns	ns	ns	*	1.0
- lag	h	1.9	1.1	1.1	ns	ns	ns	ns	1.3
NDFd, in situ, 48 h	%	51.0 ^{C}	62.6 ^{B}	69.8 ^{A}	ns	ns	ns	**	3.5
NDFd, in situ, effective	"	32.9 ^{C}	43.1 ^{B}	48.3 ^{A}	ns	ns	*	**	3.3
NDFd, in vitro, 48 h	"	42.4 ^{C}	55.0 ^{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.*

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 *in situ* rumen NDF degradation: the potentially degradable NDF fraction and its rate of degra-

dation significantly increased (from 62.1 to 73.8%, and from 3.7 to 5.9%/h, respectively, P<0.01) when hays were cut from 2 to 4 times for 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 (P <0.01) from 42.4 to 54.0 and to 60.6 for forages obtained from 2, 3 and 4 cuts for season respectively. *In vitro* NDFd values were 25-30% higher than effective *in situ* values.



The regression analysis between the *in vitro* and *in situ* NDFd values (adjusted for the year effect, figure 1) have a medium degree of correlation (r^2 = 0.69 – 0.71; P<0.01) and a low level of accuracy (RSE = 4.0 - 4.6 %). A partial explanation for the high standard error of the prediction could be due to the long experimental work requested for the *in situ* measures (1999-2004), which could have contributed to the overall *in situ* variability. On the contrary the *in vitro* measures were all produced in a restricted experimental period (within 4 weeks in 2006).
The precision and accuracy of the *in vitro* technique need to be improved before the NDFd application in prediction animal models. Additional research focused on methodology and *in situ*/*in vitro* relationship are requested.

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