Prediction of NDF degradation characteristics of grass and grass/clover forages based on laboratory methods[*]

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ABSTRACT

The degradation profiles of neutral detergent fibre (NDF) for typical Danish forages (25 grass and grass/clover samples) were evaluated using rumen nylon bag incubations.

In vitro digestibility of organic matter (OM) and NDF was performed by two different standard laboratory methods, one based on Tilley and Terry rumen fluid (T and T) and one on enzymes (ENZ). Concentrations of NDF, acid detergent fibre (ADF) and acid detergent lignin (ADL), ash and crude protein (CP) in feeds were determined. The ability to predict *in situ* potential NDF degradability and *in situ* fractional rate of NDF degradation based on *in vitro* digestibilities and on chemical analyses was tested using multiple regression analysis.

A considerable variation was found in NDF degradation between samples. The indigestible NDF (INDF) proportion was determined after 504 h *in situ* incubation and ranged from 0.047 to 0.246 of NDF. Fractional rate of NDF degradation (c) varied from 0.022 to 0.150 per hour. The potential degradable NDF fraction (b) showed high correlation with ADL content and ADL/NDF ratio. Fractional rate of degradation (c) showed the highest correlation with *in vitro* enzymatic NDF digestibility (ENZ$_{NDF}$) and NDF and ADL content. This shows that potential degradability of NDF is mostly correlated to lignification of fibre, whereas fractional rate of degradation is mostly correlated to digestibility.

It was possible to describe 0.87 of the variation in the potentially degradable NDF fraction (b) and 0.83 of the variation in fractional rate of degradation (c) using all available information on the feeds. Equations based on *in vitro* OM digestibility and chemical analyses described 0.83 and 0.85 of the

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variation in b and c, respectively, and equations based only on NDF content and calculated *in vivo* OM digestibility (sheep) were able to describe approximately 0.80 of the variation in b and c.

INTRODUCTION

The nutritive value of forages for ruminants highly depends on the ratio between cell content and cell walls and on the ability of the rumen microorganisms to degrade the plant cell walls (Waldo, 1986) as cell content has a high digestibility. The primary function of the NDF fraction in ruminant diets is to provide energy for microbial synthesis and to the animal *via* short chain fatty acids produced during fermentation, but also to secure rumen function and animal health by adding structural value to the diet (Mertens, 1994). The utilization of the fibre fraction in ruminant diets varies highly within and between forage types and is highly influenced by associative effects (Stensig and Robinson, 1997).

Analysis of the content of fibre (cell wall constituents) in ruminant feeds is based on the NDF analysis (Van Soest et al., 1991). The detergent system is a rapid procedure for determining the insoluble cell wall matrix and estimating its major subcomponents, as hemicellulose, cellulose and lignin can be determined from NDF, ADF and ADL analysis (Van Soest, 1994).

Based on either long time *in vitro* or *in situ* incubations, the NDF fraction can be divided into potentially digestible NDF (DNDF) and indigestible NDF (INDF) (Allen and Mertens, 1988).

Degradation rate of INDF is zero and is therefore defined as an "ideal nutritional entity" (Ellis et al., 1999). Lignin is often regarded as the primary factor limiting digestibility of forages (Besle et al., 1994; Van Soest, 1994) and thereby the magnitude of INDF. Lignin is indigestible and from its interaction with other cell wall constituents it reduces the proportion of potentially digestible fibre (Traxler et al., 1998). The estimation of INDF is critical for an accurate description of digestion kinetics, although the INDF fraction is not related to the animals capability of digestion, but is an intrinsic characteristic of the feed (Mertens, 1993).

Potential degradability and rate of degradation of NDF are key values in new feed evaluation systems. Therefore laboratory methods (chemical analysis and *in vitro* digestibilities) need to be developed for prediction of NDF degradation characteristics, as *in situ* techniques are resource demanding and require access to cannulated animals (Madsen and Hvelplund, 1994) and therefore not acceptable as routine methods in feed evaluation. Potential degradability can also be determined based on long term *in vitro* incubations. Mertens (1993) recommends *in vitro* compared to *in situ* methods, because fermentation conditions can be optimized,

contamination due to influx of fine particles from the rumen content is eliminated and particle loss from bags avoided. However, the *in situ* method is preferred by most researches, as it is believed to be closer to actual *in vivo* conditions, especially when an effective degradability is calculated from the degradation profile weighted with a fractional passage rate. However, for routine analysis in practical agriculture the *in situ* method is too laborious.

The objective of the present study was to develop equations to predict NDF degradation characteristics based on laboratory methods (Tilley and Terry *in vitro* digestibility, enzymatic *in vitro* digestibility and concentration of NDF, ADF and ADL in the feed).

MATERIAL AND METHODS

Twenty-five samples of grass and grass/clover forages, of which 13 were silages, were selected from a bank of samples previously examined at our laboratory for protein and dry matter (DM) nylon bag degradability. Grass was mainly ryegrass (*Lolium perenne*) and clover was mainly white clover (*Trifolium repens*). Samples were selected to cover as large a variation as possible in DM degradability after 96 h rumen incubation (from 0.794 to 0.980), assuming that this selection would give a similar variation in NDF degradability. Samples had been freeze dried and milled through a 1.5 mm screen before the previous examinations, and since then stored in a deep freezer.

Nylon bag NDF degradability (pore size 37 µm) was measured after 0, 2, 4, 8, 16, 24, 48, 96, 168 and 504 h incubation according to Hvelplund and Weisbjerg (2000). Measurements were repeated in three dry Holstein cows fitted with a rumen cannula. The cows were fed twice daily, and had free access to drinking water. The feed ration was composed of grass hay (5.4 kg/day) and concentrate mixture (2.6 kg/day). Composition of concentrate mixture (g/kg of mixture) was soyabean meal (100), barley (420), oat (420), rapeseed meal (30) and sugar beet molasses (30). Furthermore, cows were fed granulated minerals (200 g/day) and a vitamin mixture (150 g per week).

To give proper amounts of residues for Ankom fibre filter bag analysis the amount of sample weighed into the nylon bags was: 0.50 g for 0, 2 and 4 h incubations; 0.75 g for 8 h incubation and 1.00 g for 16, 24, 48, 96, 168 and 504 h incubations, accepting that this will result in variable initial sample to bag surface ratio. After incubation, residual NDF in nylon bag residues was determined using an Ankom fibre analyser (Anonymous, 1998). Sodium sulphite and heat stable α-amylase (Anonymous, 1998) were used to dissolve protein and starch, and ash free NDF residues were subsequently determined by overnight drying at 100°C and subsequent combustion at 525°C.

Ash-free NDF, ADF and ADL were determined using a Fibertec analyser (Fibertec System M). NDF was determined according to Van Soest et al. (1991). An overnight pretreatment with α-amylase (A6380, Sigma) at 38°C according to Ferreira et al. (1983) was applied and subsequent followed by addition of sodium sulphite and a heat stable α-amylase (Termamyl, Novo Nordisk, Denmark) during NDF boiling. ADF and ADL were analysed according to Van Soest et al. (1991). CP was analysed according to the Kjeldahl method (AOAC, 1990). Ash was determined after combustion at 525°C (AOAC, 1990).

For *in vitro* OM digestibility two standard procedures were used. In the *in vitro* rumen fluid method (T and T) the feed samples were incubated for 48 h in buffered rumen fluid followed by 48 h in pepsin-HCl before determining the insoluble organic residue as difference in dry residue before and after combustion. The procedure was according to Tilley and Terry (1963) except for the determination of OM instead of DM residue. In the *in vitro* enzymatic method (ENZ), samples were first treated with pepsin-HCl in order to dissolve the protein. Then the solution was heated to 80°C. After washing, the samples were incubated with an enzyme-mix (Celluclast, NOVO; Novozym 188, NOVO; Gamanase, NOVO; Viscozym, NOVO; amyloglycosidase, Megazyme), which dissolve digestible cell wall carbohydrates and eventual starch. The non-solubilized residues were subsequently washed with hot water and then with acetone in order to extract any fat before determining the insoluble organic residue as difference in residue before and after combustion (Weisbjerg and Hvelplund, 1993). *In vitro* NDF digestibility (T and T_{NDF}; ENZ_{NDF}) was determinated using the above mentioned methods for *in vitro* OM digestibility (T and T and ENZ), but with modifications. For the T and T method (T and T_{NDF}) the rumen fluid incubation was followed by transfer of residues to fibretec crucibles using boiling water to arrest any microbial activity. NDF analyses were performed on Fibertec analyser (Fibertec System M) according to the standard procedure mentioned above, but without the pre-treatment with α-amylase. For the ENZ method (ENZ_{NDF}), the first washing with boiling water after incubation was followed by transfer of residues to fibretec crucibles for NDF determination as described above for T and T_{NDF}.

In vivo sheep OM digestibility based on ENZ analysis was calculated according to Søegaard et al. (2001): *in vivo* OM digestibility = 0.260 + 0.658 × ENZ. DNDF was estimated as the proportion of NDF degraded after 21 days (504 h) rumen incubation and was calculated as 1 - INDF. Modified *in vitro* OM digestibilities (ENZ_{mod} and T and T_{mod}) were calculated using the NDF residue as organic residue calculated as follows: (OM weighed out-NDF residue)/(OM weighed out).

For calculation of NDF nylon bag degradabilities, NDF residues at the respective incubation times were related to the NDF residue at zero h incubation, where bags were not rumen incubated, but only washed. Obtained

values are therefore corrected for eventually initial particle losses (Hvelplund and Weisbjerg, 2000). However, this is based on the assumption that NDF in small particles lost were representative for all NDF. NDF degradation profiles were calculated using equations both with lag time and without lag time. The soluble a fraction was omitted from the equations, as NDF per definition is insoluble, and as the calculation of degradability included correction of eventual initial particle losses. NDF degradation profiles were calculated using results from incubation times of 2, 4, 8, 24, 48, 96 and 168 h according to the following equations:

1. with lag time (McDonald, 1981)

$$\text{Deg (t)} = b \times (1 - e^{-c(t-lt)})$$

2. without lag time (Ørskov and McDonald, 1979)

$$\text{Deg (t)} = b \times (1 - e^{-ct})$$

Effective ruminal degradation of NDF was calculated using the models shown below and assuming a fractional passage rate of 0.02 per h. These models were used to get values for ranking of the samples, knowing that the simple assumptions on passage, which do not take into account selective retention of newly ingested NDF in the rumen, will underestimate *in vivo* NDF digestibility:

1. with lag time (McDonald, 1981)

$$ED = b \times (c/(c+k)) \times e^{(-k*lt)}$$

2. without lag time (Ørskov and McDonald, 1979)

$$ED = b \times (c/(c+k))$$

where:

Deg (t) = fraction of NDF degraded at time t of incubation
ED = effective ruminal degradation of NDF fraction
b = degradable NDF fraction
c = fractional rate of degradation of fraction b (h^{-1})
k = fractional passage rate from the rumen (h^{-1})
lt = lag time (h)

Estimates of b, c and lt were calculated using a Non Linear Model PROC NLIN (SAS Institute, 2000). Correlation coefficients between variables were computed using PROC CORR (SAS Institute, 2000). Multiple regression equations for prediction of degradation parameters were estimated using PROC STEPWISE (SAS Institute, 2000). Overparameterization was avoided by the restrictions that parameters should be significant at 0.15 levels and by using Mallows C_p for model selection (SAS Institute, 2000). The prediction ability of

the estimated models was tested in a cross-validation using the PRESS function in PROC REG (SAS Institute, 2000). PRESS is weighted with n-p as for RMSE (p is number of parameters in the equation, n number of observations), PRESS is the prediction error in a cross-validation where observations are left out one by one, and the model fitted without the observation is used to predict the observation.

RESULTS

The chemical composition and *in vitro* digestibility values of the experimental feeds are shown in Table 1. The CP content varied from 94 to 289 g/kg DM. With respect to feed type the CP varied from 145 to 289 g/kg DM for fresh forages and from 94 to 207 g/kg DM for ensiled forages. NDF content ranged from 229 to 577 g/kg DM. As expected, the NDF content was higher in late cut (564 g/kg DM, silage 25) than in silage from grass/clover cut 3 weeks before (434 g/kg DM, silage 24). ADF and ADL concentration in DM varied from 149 to 374 and from 8 to 44 g/kg DM, respectively.

In vitro T and T OM digestibility ranged from 0.478 to 0.840 and ENZ from 0.565 to 0.911. *In vitro* NDF digestibility ranged from 0.459 to 0.872 and from 0.389 to 0.883 using the T and T_{NDF} and ENZ_{NDF}, respectively. ENZ_{mod} and T and T_{mod} were highly correlated to OM digestibility determined using either ENZ ($r = 0.99$) or T and T ($r = 0.98$).

A wide variation in NDF degradability at different incubation times was found for the 25 forage samples (Figure 1). The NDF degradation after 8 h incubation varied from 0.121 to 0.723 and degradation after 96 h varied from 0.666 to 0.946. The proportion of NDF that is indigestible (INDF) ranged from 0.047 to 0.246 of NDF. To determine INDF long incubations seems necessary, as degradation continued after one week of incubation (168 h) and the increase from one week to three weeks (504 h) incubation was on an average 0.016 with a variation from 0 to 0.061 (results not shown).

Calculated degradation parameters for NDF are shown in Table 2 together with values for effective ruminal degradation (ED) of NDF calculated with a fractional passage rate of 0.02 per h. Data from 16 h incubations were excluded due to lower degradabilities for these incubations than expected from the profile for the rest of the incubations (Figure 1). 504 h incubation data were also excluded, because inclusion of these data resulted in larger residuals when fitting the degradation profiles. The lag time was generally short. On average lag time was 1 h and varied from zero to 4.5 h. As lag times were short, degradation parameters were also calculated with a simpler model without lag time. Due to the short lag times, inclusion of lag time in the model had only minor effect on

TABLE 1

Chemical composition (g/kg DM) and *in vitro* NDF and OM digestibility of fresh and ensiled grass and grass/clover

Feed	Chemical composition					In vitro digestibility					
	Ash	CP	NDF	ADF	ADL	ENZ_{NDF}	T and T_{NDF}	ENZ	T and T	ENZ_{mod}	T and T_{mod}
Fresh											
1	93	232	421	240	16	689	749	802	726	824	861
2	86	206	297	178	11	841	839	893	839	931	932
3	103	175	342	211	26	740	733	814	757	877	878
4	101	254	288	154	10	881	872	896	835	948	945
5	101	224	229	150	13	883	854	893	840	953	943
6	105	289	279	180	21	787	733	842	761	911	890
7	65	151	274	149	8	820	805	911	838	933	929
8	78	147	415	249	25	611	695	747	724	787	836
9	100	193	347	247	44	475	582	661	627	736	794
10	122	194	388	246	30	646	661	760	688	800	808
11	74	145	446	256	37	608	655	738	678	778	803
12	86	241	233	165	16	853	820	894	812	946	934
Silage											
13	129	94	303	192	26	445	525	740	697	772	808
14	107	118	512	321	38	507	583	635	626	673	726
15	104	128	378	240	13	646	745	781	767	812	867
16	90	195	373	231	16	646	725	795	772	818	861
17	116	175	364	265	23	632	683	798	732	821	847
18	133	207	383	250	15	710	811	811	778	847	901
19	107	143	366	253	24	611	728	781	755	807	867
20	82	184	414	273	33	515	629	714	682	737	801
21	89	169	543	366	34	530	488	646	514	699	676
22	107	206	485	321	25	527	577	694	603	723	753
23	113	154	577	374	32	539	500	646	478	688	658
24	94	162	434	280	17	601	720	766	730	798	856
25	73	119	564	348	39	389	459	565	528	593	644

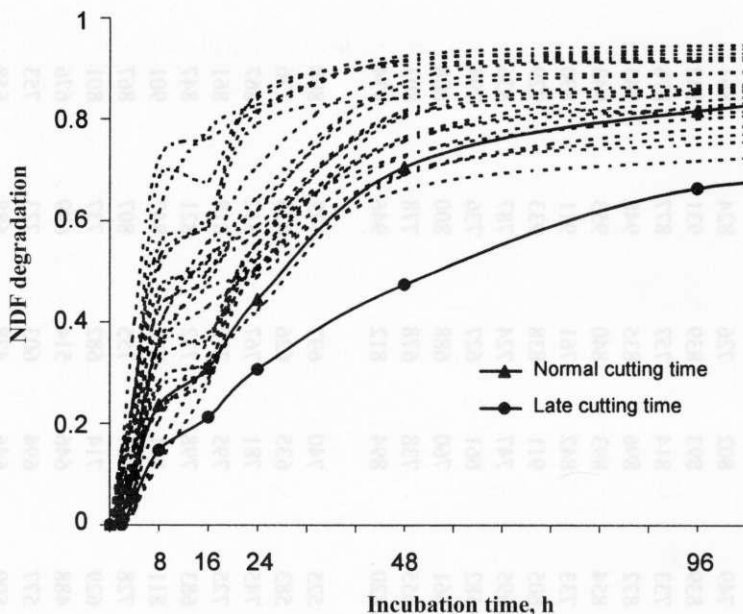for abbreviations see list of abbreviations

Figure 1. NDF degradation profiles based on *in situ* incubation data. The two full line curves show NDF degradability of silage samples from the same sward varying 3 weeks in cutting time

ED. Therefore, in further calculations, only results from the models without lag time were used.

The potentially degradable NDF fraction (b) varied from 0.725 to 0.939. The b value estimated from the degradation profile up to 168 h incubation was then on average 0.022 (variation from 0.005 to 0.069) lower than the DNDF found after 504 h incubation. The fractional rate of degradation (c) varied from 0.022 to 0.150 per h. Effective degradability calculated with a fractional passage rate of 0.02 per h varied from 0.390 to 0.805.

The degradation parameters b and c, and NDF degradability found after 504 h incubation were correlated to most of the chemical fractions and *in vitro* digestibilities given in Table 1. The correlations are shown in Table 3. DNDF and b were highly correlated, and they were both highly correlated to ADL content and ADL/NDF ratio, and less to *in vitro* digestibilities (see also Figures 2 and 3). Fractional rate of NDF degradation (c) was mostly correlated to *in vitro* digestibilities (Figure 4), and less to ADL content. c seemed to be nonlinearly related to NDF concentration (Figure 5).

The stepwise procedure was used to find the combinations of chemical and *in vitro* digestibility characteristics for prediction of b, c and DNDF in multiple regressions (Table 4). ADF and ADL together with ENZ_{NDF} could explain 0.87 of the variation in b. ADL/NDF ratio and ENZ_{NDF} could explain 0.83 of the variation

NDF degradability characteristics of fresh and ensiled grass and grass/clover

Feed	Without lag time				With lag time[4]			
	b	c (h^{-1})	ED^1	DNDF^2	lt^3 (h)	b	C (h^{-1})	ED^1
Fresh								
1	0.889	0.065	0.680	0.919				
2	0.935	0.083	0.753	0.941	1.8	0.919	0.122	0.762
3	0.885	0.075	0.697	0.907				
4	0.932	0.106	0.785	0.953	0.9	0.924	0.131	0.788
5	0.939	0.118	0.803	0.951	1.1	0.929	0.161	0.807
6	0.869	0.103	0.728	0.893	1.3	0.857	0.145	0.733
7	0.934	0.087	0.759	0.942	0.9	0.926	0.104	0.763
8	0.862	0.060	0.648	0.893				
9	0.725	0.056	0.535	0.754				
10	0.774	0.053	0.561	0.809				
11	0.823	0.045	0.572	0.850	0.8	0.817	0.050	0.573
12	0.912	0.150	0.805	0.934	1.2	0.901	0.224	0.807
Silage								
13	0.775	0.041	0.520	0.811				
14	0.828	0.053	0.602	0.867				
15	0.932	0.049	0.660	0.937	1.5	0.918	0.059	0.665
16	0.883	0.048	0.625	0.900				
17	0.876	0.047	0.612	0.889	1.3	0.865	0.054	0.616
18	0.932	0.062	0.704	0.950	1.2	0.919	0.075	0.709
19	0.851	0.041	0.571	0.866	0.7	0.845	0.044	0.572
20	0.807	0.040	0.538	0.834	1.3	0.797	0.046	0.540
21	0.882	0.031	0.538	0.896	4.5	0.852	0.045	0.539
22	0.893	0.034	0.560	0.910	3.5	0.868	0.046	0.564
23	0.889	0.038	0.582	0.905	2.0	0.874	0.046	0.583
24	0.870	0.033	0.539	0.895	0.3	0.867	0.033	0.539
25	0.738	0.022	0.390	0.776	0.7	0.733	0.023	0.390

[1] effective ruminal degradation of NDF fraction assuming the fractional rate of passage to be 0.02 h^{-1}

[2] NDF degradability after 504 h *in situ* incubation

[3] lag time

[4] results not shown for samples when estimated lag time was zero

in c. ADF, ADL, ENZ$_{mod}$ and NDF × NDF content could explain 0.88 of the variation in DNDF.

Regression analyses were also performed where all measures except *in vitro* NDF digestibilites were included. ADF, ADL and ENZ × ENZ could explain 0.83 of the variation in parameter b. ADL/NDF ratio, ENZ, ENZ × ENZ and content of CP could explain 0.85 of variations in c. ADL, ENZ and NDF × NDF could explain 0.83 of the variation in DNDF.

TABLE 3

Correlation between NDF degradation characteristics (b[1], c[2] and DNDF[3]) and chemical fractions and *in vitro* digestibilities

Parameter	b	c	DNDF
b		.	
c	0.49*		
DNDF	0.99*	0.52*	
NDF[4]	-0.34	-0.78*	-0.33
ADF[4]	-0.37	-0.78*	-0.37
ADL[4]	-0.82*	-0.55*	-0.82*
ADL/NDF[5]	-0.80*	-0.13	-0.80*
CP[4]	0.39	0.66*	0.41*
T and T_{NDF}[6]	0.69*	0.73*	0.70*
ENZ_{NDF}[6]	0.75*	0.86*	0.76*
T and T[6]	0.55*	0.68*	0.55*
ENZ[6]	0.69*	0.77*	0.68*
T and $T_{mod.}$[6]	0.58*	0.72*	0.58*
$ENZ_{mod.}$[6]	0.66*	0.83*	0.66*

[1] potentially degradable fraction
[2] fractional rate of degradation (h[-1])
[3] NDF degradability after 504 h *in situ* incubation
[4] g/kg of DM
[5] ADL/NDF ratio
[6] *in vitro* OM and NDF digestibilities, see list of abbreviations
* significance was declared when P<0.05

TABLE 4

Multiple regression equations for prediction of parameters of NDF degradation (b[1], c[2] and DNDF[3]). Units for chemical analysis are kg/kg DM and for digestibilities kg/kg

Y variable	X variables and regression parameter estimates	R^2	RMSE[4]	PRESS[5]
Based on all analyses				
b	$0.607 + 0.632\,ADF - 4.29\,ADL + 0.318\,ENZ_{NDF}$	0.87	0.024	0.029
c	$-0.116 + 0.512\,ADL/NDF + 0.227\,ENZ_{NDF}$	0.83	0.013	0.016
DNDF	$0.253 + 0.40\,2\,ADF - 3.19\,ADL + 0.672\,ENZ_{mod.} + 0.434\,(NDF \times NDF)$	0.88	0.021	0.025
Based on *in vitro* OM digestibility and chemical analyses				
b	$0.404 + 0.943\,ADF - 3.06\,ADL + 0.551\,(ENZ \times ENZ)$	0.83	0.027	0.032
c	$0.458 + 0.430\,ADL/NDF - 1.49\,ENZ + 1.16\,(ENZ \times ENZ) + 0.167\,CP$	0.85	0.013	0.017
DNDF	$0.364 - 2.49\,ADL + 0.630\,ENZ + 0.622\,(NDF \times NDF)$	0.83	0.024	0.029
Based on calculated *in vivo* OM digestibility and NDF content only				
b	$-0.542 + 0.860\,(NDF \times NDF) + 1.66\,Sheep$[6]	0.78	0.031	0.035
c	$0.135 - 1.10\,NDF + 1.20\,(NDF \times NDF) + 0.210\,Sheep$[6]	0.81	0.014	0.018
DNDF	$-0.380 + 0.784\,(NDF \times NDF) \times 1.49\,Sheep$[6]	0.79	0.026	0.030

[1] potentially degradable part
[2] fractional rate of degradation (h[-1])
[3] NDF degradability after 504 h *in situ* incubation
[4] root mean square error
[5] PRESS is the prediction error in a cross-validation
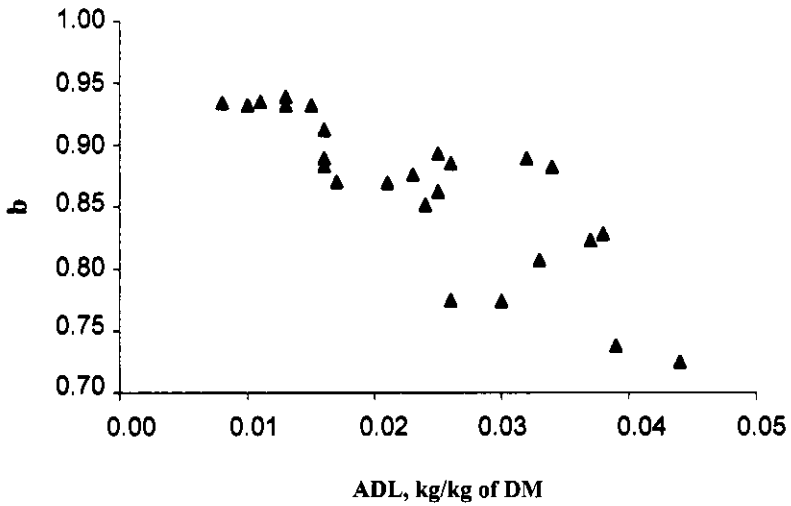[6] *In vivo* sheep digestibility of OM (predicted from *in vitro*)

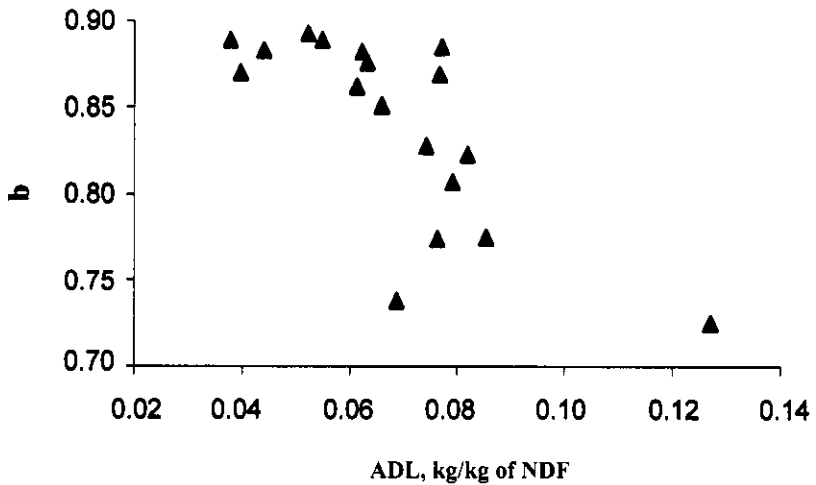Figure 2. Potential degradability (b) versus ADL concentration in DM

Figure 3. Potential degradability (b) versus ADL proportion of NDF

Regression analyses were also performed based on only NDF content and sheep OM digestibility predicted from ENZ digestibility as NDF content and OM digestibility values are present in most feed tables. Equations based on these two measures were able to describe 0.78, 0.81 and 0.79 of the variation in b, c and DNDF, respectively.
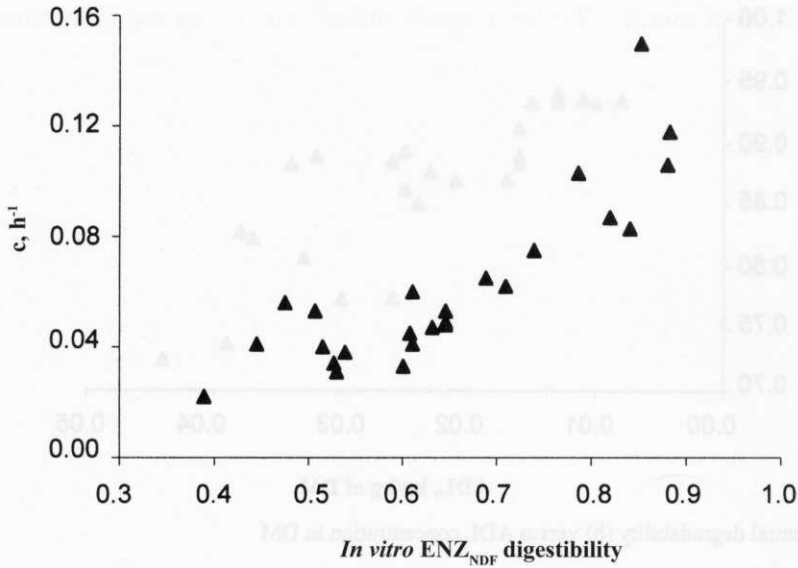
Figure 4. Fractional rate of degradation (c) versus *in vitro* ENZ_{NDF} digestibility
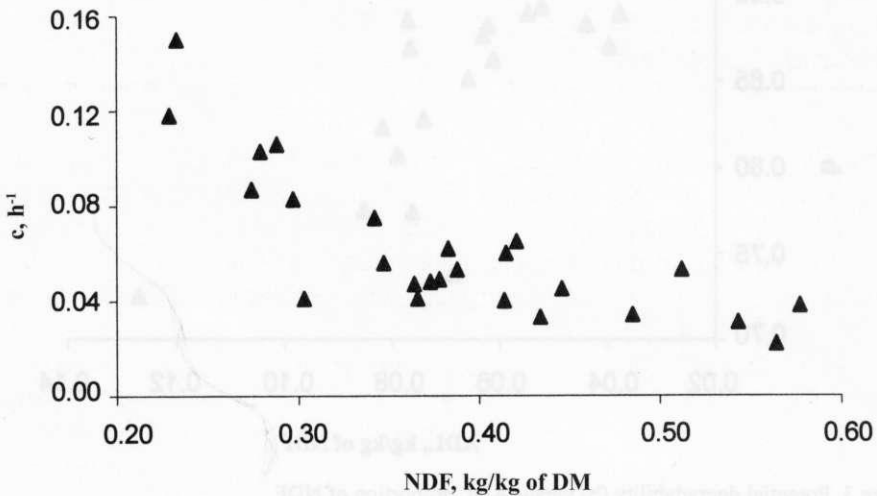

Figure 5. Fractional rate of degradation (c) versus NDF concentration in DM

DISCUSSION

The present experiment has clearly demonstrated a large variation between grass and grass/clover samples in chemical composition and *in vitro* digestibility of OM and NDF. The chemical composition (CP, NDF, ADF and ADL content)

of the experimental forages (Table 1) was in the same range as found in other studies.

In vitro OM digestibility measured by the ENZ method was as a mean 0.06 higher than the *in vitro* digestibility measured by the T and T rumen fluid method. Søegaard et al. (2001) found a similar difference between the two methods. The higher values obtained with the ENZ method is probably due to the more intensive washing of the residues with hot water and acetone compared to washing with cold water in the T and T method. Opposite to this, *in vitro* NDF digestibility was 0.07 higher when measured by the T and T method than by the ENZ method, indicating a higher cellulolytic activity of the rumen fluid used in the T and T method compared to the enzymes used in the ENZ method. When the *in vitro* OM digestibility was based on the NDF residue, the modified OM digestibility was as a mean 0.04 higher for the T and T_{mod} compared to the ENZ_{mod} method, in accordance with the higher *in vitro* NDF digestibility for the T and T method.

Compared to nylon bag NDF degradabilities after 48 h incubations, the values obtained by the *in vitro* NDF methods were lower for both T and T_{NDF} and ENZ_{NDF} method, indicating lower cellulolytic activity or a larger lag time *in vitro* than *in situ*. Further, particle losses from the nylon bag during rumen incubation above initial losses might explain the higher *in situ* degradabilities. Despite the difference in absolute values, high correlations were found between 48 h NDF degradability and both OM and NDF digestibilities: i.e. ENZ (r = 0.83), T and T (r = 0.74), ENZ_{NDF} (r = 0.86) and T and T_{NDF} (r = 0.82).

There was a significant negative correlation between NDF content and digestibility of OM and NDF (Table 3). This is in accordance with other *in situ* and *in vitro* NDF digestibility studies which showed that the concentration of NDF is the major factor determining forage quality (Valentin et al., 1999; Allen, 2000). Increasing *in vitro* NDF digestibility with decreasing NDF content was also found by Weiss et al. (1992).

Predicting forage DNDF from lignin concentration has been proposed in several studies (Conrad et al., 1984; Weiss et al., 1992). Many research groups have highlighted the relationship between lignification and NDF digestibility of forages (Jung and Vogel, 1986; Buxton and Russell, 1988). In this study NDF, ADF and ADL concentrations were positively correlated. ADL content was the only fibre measure showing significant correlation with DNDF (Table 3), which is in agreement with results obtained by Traxler et al. (1998). ADL concentration was positively correlated to the lignification of NDF (ADL/NDF ratio) and negatively to the NDF digestibility (results not shown), which has also been reported previously (Van Soest, 1967; Jung and Vogel, 1986).

DNDF varied from 0.75 to 0.95 in this study. Similar variations between forages (from 0.59 to 0.90) in DNDF using 21 days (504 h) rumen *in situ*

incubations were found by Lund (2002). With increasing maturity of ensiled or fresh grass NDF digestibility decreases. This is also obvious from this study, where potential degradability decreased from 0.895 (sample 24) to 0.776 (sample 25) for two silage samples from the same sward varying 3 weeks in cutting time.

The 16 h incubations gave lower degradabilities than expected from the degradation profile of the rest of the incubations (Figure 1). Therefore, these data were excluded from the calculations of degradation parameters. The reason for the lower 16 h values was probably that these samples were incubated at 16.00 h in the afternoon, whereas the rest of the bags were incubated at 8.00 h in the morning. Therefore, the results indicate that the afternoon incubation resulted in lower degradabilities than expected, probably due to lower microbial activity during the nighttime than during daytime. Incubation times up to 168 h resulted in underestimation of potential degradability compared to long-term incubations, as also found by Weisbjerg et al. (2001). Therefore, incubations up to 504 h seem necessary to determine the 'true' indigestible NDF fraction. This also means, that degradability values after 504 h incubation time were generally higher than expected from the other incubation times. The reason for this is probably, that fractional rate of degradation is not constant, but will decrease with increased incubation time (Van Soest, 2000). *In vivo* mean retention times are much lower than 504 h, therefore the very long incubation times are less interesting to describe the *in vivo* situation, and 504 h results were deleted before calculations of degradation parameters to get an optimal fit of the results from shorter incubation times. The higher degradability than expected for 504 h incubations when a constant rate of degradability is assumed might partly be due to a disappearance of small particles during degradation (Van Soest, 1994).

The potentially degradable NDF fraction b and the fractional rate of degradation c averaged 0.865 and 0.062 per hour, respectively. Effective degradability (without lag time) of NDF varied from 0.390 to 0.805 calculated using a value of 0.02 per hour for the fractional passage rate. Including lag time for ED calculations resulted only in small changes, and the parameters obtained from the model without lag time was therefore used for the regression analysis.

The potentially degradable fraction b was highly correlated to ADL content and ADL/NDF ratio whereas the fractional rate of degradation c mainly was correlated to *in vitro* digestibilities (Table 3), especially ENZ_{NDF} was highly correlated to c. This indicates, that a direct determination of NDF digestibility is necessary to get a high correlation with c. A possible option, if only OM digestibility is measured, is then to estimate *in vivo* NDF digestibility based on the Lucas principle as proposed by Weisbjerg et al. (2004).

Digestion in the rumen is a time dependent dynamic process and the result of a competition between the fractional rate of digestion in the rumen and the fractional rate at which feed components pass out of the rumen (Waldo et al., 1972). As the rumen degradation is a dynamic process, the effective rumen degradation is highly

affected by the fractional passage rate and will therefore depend on, for example, feeding level. Effective degradabilities presented in Table 2 are calculated at a fractional passage rate of 0.02 per hour using a simple one-pool rumen model. This model do not take into account the selective retention in the rumen of newly ingested NDF, and therefore the obtained values probably underestimate *in vivo* digestibility and should only be regarded as a ranking of the forages.

The aim of the present study was to present prediction equations for the NDF degradation parameters. Three sets of equations are presented (Table 4). For all three sets the stepwise procedure in SAS (SAS Institute, 2000) was used to find the best equations. The first set was based on all available measures in this study. However, as all these analyses will seldom be available, a second set of equations was based on *in vitro* OM digestibility and chemical analysis. The third set of equations was based on NDF concentration and predicted *in vivo* sheep OM digestibility which is information available in most feedstuff tables.

With the increasing restriction on prediction parameters used, the explanation ability of the equations (R^2) decreased with approximately 0.10. Even with the third set of equations the R^2 for the prediction equations for the different degradation parameters were approximately 0.80. This shows that it is possible to describe most of the variation in NDF degradation parameters using common feed analyses. Compared to this study with grass and grass/clover, similar studies on whole crop cereals showed poorer correlations between NDF degradation parameters and chemical composition and *in vitro* digestibilities (Weisbjerg et al., 2003).

Multiple regression analysis can be questioned, as they can result in parameters without biological sense. An example on this is the positive parameter values for ADF concentration and lignification (ADL in proportion of NDF) found in some of the equations in Table 4. These unbiological parameter values are probably due to the strong correlation between *in vitro* digestibility and lignification. Therefore, the two first sets of equations should rather be regarded as the potential for explanation than a proposal for equations for practical use. The third set of equations is based on only NDF concentration and OM digestibility, analysis that are commonly performed as standard on feed samples from practice. These equations can therefore be used in practice on forage samples believed to be covered by the sample population examined in this study. However, extrapolation of the equations to be used on samples from other populations (forage types, climatic regions) needs further examinations. The single analysis that showed the highest correlation with fractional rate of degradation (c) was ENZ_{NDF}, and for potential NDF degradability (b and DNDF) ADL concentration and ADL in proportion of NDF showed a high correlation (Table 3). Therefore predictions using simple linear regressions could also be an option.

The degradation parameters, which we try to estimate from laboratory methods, are not directly measured values, but are estimated from the degradation

profile using the PROC NLIN (SAS, 2000). The PROC NLIN simultaneously estimate fractional rate of degradation (c) and potential degradability (b), and these estimates are normally highly correlated. Therefore, each of the parameters can have a high error on the prediction, although they as a set of parameters are well estimated. Due to the possible high error on the original prediction of the individual parameters (c and b), the prediction of the parameter estimates using alternative methods/measures like in this study, cannot be expected to give very low prediction errors, and therefore the explanation obtained in this study is satisfactory. This also underlines, that degradation parameters should always be regarded as a set of parameters, not as individual parameters.

CONCLUSIONS

The present study showed that common laboratory analyses could be used for prediction of both potential degradability and rate of degradation of NDF for grass/clover forages.

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STRESZCZENIE

Ocena rozkładu NDF traw i mieszanki trawy/koniczyna na podstawie metod laboratoryjnych

Oznaczono rozkład włókna NDF typowych duńskich zielonek (25 prób traw i mieszanki trawy/koniczyna) stosując inkubację woreczków nylonowych w żwaczu.

Strawność substancji organicznej (SO) *in vitro* oznaczono dwiema różnymi standardowymi metodami: jedną w oparciu o metodę Tilley'a i Terry stosując płyn żwaczowy (T+T), drugą - enzymatyczną (ENZ). W paszach oznaczono zawartość NDF, ADF, ADL, popiołu i białka ogólnego (CP). Możliwość określenia *in situ* potencjalnej rozkładalności NDF oraz *in situ* tempa rozkładalności NDF, w oparciu o strawności oznaczone *in vitro* oraz analizy chemiczne, badano stosując wielokrotną analizę regresji.

Stwierdzono znaczną zmienność w rozkładzie NDF pomiędzy próbami. Udział niestrawnego NDF (INDF) oznaczony po 504 godz. inkubacji *in situ* wynosił od 0,047 do 0,246 NDF. Tempo rozkładu NDF (c) wahało się od 0,022 do 0,150 na godz. Stwierdzono wysoką korelację pomiędzy frakcją potencjalnie rozkładalnego NDF (c) a zawartością ADL oraz stosunkiem ADL/NDF, najwyższą korelację pomiędzy tempem rozkładalności (c) a enzymatyczną *in vitro* strawnością NDF (ENZ_{NDF}), a także zawartością NDF i ADL. Wyniki te wskazują, że potencjalny rozkład NDF jest głównie skorelowany z lignifikacją włókna, podczas gdy tempo rozkładu - ze strawnością.

Możliwe jest, że zmienność w potencjalnie rozkładalnej frakcji NDF (b) wynosi 0,87, a w tempie rozkładu (c) 0,83, jeżeli zastosuje się wszystkie dostępne dane dotyczące pasz. Na podstawie równań, opierających się na oznaczeniu *in vitro* strawności OM oraz wynikach analiz chemicznych, zmienność frakcji b i c wynosi odpowiednio 0,83 i 0,85, a opartych tylko na zawartości NDF i obliczonej *in vivo* (na owcach) strawności OM, zmienność ta wynosi odpowiednio około 0,80 dla b i c.