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Review article

Recent developments in forage evaluation with special reference to practical applications

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The present re-evaluation of a dataset of systematically collected laboratory analyses and *in vivo* digestibility information for several types of silages gives convincing evidence of the biological weaknesses of feed characterisation based on the proximate feed analysis. The problems include intrinsic failures of the analysis in describing cause-response relationships between forage composition and digestibility, and heavy dependency of the equations on forage specific and environmental factors. It is concluded that proximate analysis is not suitable for characterisation of neither forages nor concentrate feedstuffs. *In vitro* pepsin-cellulase solubility of organic matter (OMS) and concentration of indigestible neutral detergent fibre (iNDF) predicted forage organic matter digestibility (OMD) with an acceptable accuracy for practical feed evaluation purposes provided that forage type dependent correction equations were employed.

The revised detergent system dividing forage dry matter (DM) into almost completely available neutral detergent solubles (NDS), and insoluble residue (neutral detergent fibre, NDF) shows potential for future development. The combined use of long-term *in situ* ruminal incubation and NDF fractionation can be used to divide forage DM into three biologically meaningful fractions: NDS, iNDF and potentially digestible NDF (pdNDF). The summative models can then be used to predict forage D-value, i.e. apparently digestible organic matter in forage (g kg^{-1} DM). The models sum digestible NDS, which can be determined by Lucas equation, and digestible NDF (dNDF), which is the amount of pdNDF that is actually digested during any specific fermentation or retention time. Forage type specific summative models were as good as regression equations based on OMS or iNDF in predicting forage D-value and general summative models gave better results than general equations based on iNDF and especially OMS.

If the goal is to reduce prediction error of D-value below 15 g kg^{-1} DM, forage type specific prediction equations should be used regardless of whether they are based on OMS, iNDF or summative models. Another option in the future may be dynamic models, which can incorporate simultaneously the two important dynamic processes constraining feed digestion in ruminants: the rates of NDF passage and degradation (k_d).

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However, a vital prerequisite to employ dynamic models in practical feed evaluation is that iNDF and k_d can be easily and reliably determined from on-farm forages. Although a NIRS prediction equation for iNDF will be adopted in practical use in the near future in Finland, the methodology for estimating k_d warrants further research.

Key words: silage, prediction, cell wall quality, digestibility, near infrared reflectance spectroscopy

Introduction

The main objective of feed evaluation techniques is to predict the availability of nutrients and feeding value of feeds for animal production systems. The methods available include chemical analysis, *in vitro* digestibility with rumen bacteria or enzymes, *in situ* incubation in nylon bags and near infrared reflectance spectroscopy (NIRS). Feed evaluation of forages is more important than that of concentrate feedstuffs due to the large variation in the nutritive value of forages and the large contribution of forage to total diet dry matter (DM) compared to individual concentrate ingredients. In addition to direct influence of forage quality on nutrient digestibility in ruminant diets, it also indirectly affects total nutrient supply, because of the large impact of both digestibility and silage fermentation characteristics on silage DM intake (Rinne 2000, Huhtanen et al. 2002).

Accurate estimation of forage digestibility is a prerequisite for diet formulation, economic evaluation of forages and prediction of animal responses. Determination of *in vivo* digestibility is time consuming and expensive for routine and even research use; therefore, different biological and chemical laboratory methods have been developed to estimate digestibility of forages. Advantages and disadvantages of different methods have been discussed in detail in many recent reviews (Steg et al. 1990, Weiss 1994, Coleman et al. 1999, Beever and Mould 2000, Cherney and Cherney 2003). From these reviews, it can be concluded that biological laboratory methods seldom estimate digestibility values directly. This does not mean that these methods are not useful, because they often have close empirical relationships to *in vivo* digestibility, but it does imply that empirical correction equations are required for estimating *in vivo* digestibility from *in vitro* and *in situ* measurements. These correction equations are often specific for different forages, environments and even laboratories (Weiss 1994, Van Soest 1994, Nousiainen 2004).

The relationships between chemical and even biological measurements to digestibility can be markedly different for the main grass species used for silage in Finland, i.e. timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis)*, to those estimated elsewhere. Temperature and light intensity influence lignification of the cell wall (Deinum et al. 1968, Van Soest 1994), which affects the relationship between fibre and digestibility. Grasses grown in northern latitudes had higher digestibility at the same stage of maturity than those grown at latitudes closer to the equator (Deinum et al. 1968).

In Finland, a data set has been compiled of systematically collected information on grass and leguminous silages to evaluate laboratory methods for predicting *in vivo* digestibility of forages with the final aim to develop a rapid, accurate and precise method based on NIRS for analysing farm samples. A series of papers has been published from this work (Nousiainen et al. 2003a, b, 2004, Nousiainen 2004, Huhtanen et al. 2005, Rinne et al. 2006). The objectives of this paper are to (1) re-evaluate and discuss the methods routinely used for forage analysis, (2) describe alternative methods of predicting digestibility using regression equations and summative models, (3) present the sources of variation in estimating digestibility and (4) make implications of the data for developing practical analysis for farm samples.

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Description of data

The dataset used in this analysis consisted of information for Finnish silages with *in vivo* digestibility determined in sheep fed at approximately maintenance level of feeding using total faecal collection method. The silages were harvested over 9 years in 1994–2003 from mixed timothy (*Phleum Pratense*) meadow fescue (*Festuca pratensis*) leys in primary growth (PG, n = 33) and in regrowth (RG, n = 27) and ensiled with formic acid based additives in pilot-scale tower silos or farm-scale bunker silos. The digestibility of both PG and RG silages was varied by systematically changing harvesting date. The silages are described in Nousiainen et al. (2003a, b) with a few added observations. The data set of pure legume silages including red clover (*Trifolium pratense*, n = 15) and galega (*Galega orientalis*, n = 4) is described by Rinne et al. (2006) with the exception that only feeds of Finnish origin are used in this analysis. Further, data from whole-crop silages prepared from barley (*Hordeum vulgare*, n = 5) and wheat (*Triticum aestivum*, n = 2) were included in the data set. Characteristics of the silages used are presented in Table 1.

Chemical methods in forage characterisation

Proximate analysis

The proximate feed analysis has been in use for more than 100 years. The following components of DM are analysed: ash, crude protein [CP = ni-

Table 1. Description of ash, crude protein (CP), neutral detergent solubles (NDS), neutral detergent fibre (NDF), lignin and indigestible NDF (iNDF) concentrations, organic matter pepsin-cellulase solubility (OMS) and *in vivo* digestibility of organic matter (OMD) and NDF (NDFD) of different forage types.

	In dry matter, g kg^{-1}								
	Ash	CP	NDS	NDF	iNDF	Lignin[a]	OMS	OMD	NDFD
Primary growth grass (n = 33)									
Mean	72	148	357	568	79	32	757	0.733	0.739
Standard deviation	8.2	35.0	67.1	70.1	39.1	10.5	70.5	0.0606	0.0701
Regrowth grass (n = 27)									
Mean	94	144	376	533	106	28	757	0.694	0.701
Standard deviation	8.5	25.4	27.8	34.0	28.2	4.8	27.8	0.0339	0.0493
Legume (n = 19)									
Mean	99	211	532	369	109	43	754	0.707	0.627
Standard deviation	14.3	38.1	70.3	75.6	52.5	18.6	65.5	0.0623	0.0815
Whole crop (n = 7)									
Mean	74	114	495	432	119	27	758	0.686	0.515
Standard deviation	10.7	7.2	71.3	63.2	30.6	8.1	69.3	0.0471	0.0300
All (n = 86)									
Mean	85	158	413	502	97	33	757	0.711	0.684
Standard deviation	15.6	43.0	92.9	100.3	41.2	12.8	58.0	0.0552	0.0919
Minimum	49	79	265	274	17	17	628	0.581	0.477
Maximum	122	301	627	669	211	79	878	0.840	0.869

[a] Analysed as permanganate lignin (Robertson and Van Soest 1981); n = 31 and n = 84 for primary growth grass and all silages, respectively.

trogen (N) × 6.25], ether extract (EE) and crude fibre (CF). The nitrogen free extract (NFE) is calculated as:

NFE = organic matter (OM) – (CP + EE + CF) [1]

It is typically assumed that CF is the least digestible fraction of feed fibre and that NFE represents the highly digestible carbohydrates. However, this assumption is often not correct, especially for forages because a large proportion of indigestible lignin and hemicellulose is solubilised during CF extraction (see Van Soest 1994). Consequently, forage NFE is comprised of components that vary from completely unavailable lignin to completely available fractions such as soluble carbohydrates and organic acids.

Apparent digestibility of NFE was less than that of CF in a large number of cases (Van Soest 1975). In the data set of 52 grass silages (Nousiainen et al. 2004), the digestibility of CF was higher than that of NFE in 31 cases. The difference between CF and NFE digestibility decreased ($P < 0.01$) with advancing maturity of grass ensiled, i.e. the earlier the grass was harvested, the greater the difference in the digestibility of CF and NFE.

Heterogeneous availability of grass NFE fraction can be demonstrated by the Lucas test (see Van Soest 1994). The purpose of the Lucas test is to identify ideal nutritional entities that have uniform digestibility over a wide range of feedstuffs by plotting the digestible nutrient concentration in DM against the nutrient concentration in DM. The slope of regression estimates the true digestibility and the intercept is an estimate of the metabolic and endogenous faecal matter (M) for the nutrient, which consists of unabsorbed digestive juices, microbial debris from the rumen and microbial cells from the hindgut fermentation. The true digestibility of silage NFE estimated by the Lucas test had a high standard error (±0.15 units of digestibility) and positive intercept. A positive intercept is not biologically possible, because at zero concentration of a nutrient there cannot be a positive amount digested.

The variable true digestibility of NFE indicates that it is not an ideal nutritional entity, which is not surprising considering that forage NFE fraction contains a range of chemical components differing in their availability. Consistent with this, silage NFE concentration had no correlation to OM digestibility (OMD). Faecal NFE output as grams per kg DM intake was closely related to lignin concentration (faecal NFE = 46.8±9.8 + 2.24±0.31 × Lignin, residual mean squared error (RMSE) = 18.8, $R^2 = 0.51$). The regression coefficient of lignin suggests that one gram lignin protected 2.24 g of carbohydrates recovered as NFE fraction in faeces, most likely hemicellulose. The intercept of regression may be interpreted as the metabolic and endogenous faecal component resulting from the error of using factor 6.25 for N to calculate faecal CP (more detailed discussion later).

Crude fibre also cannot be regarded as an ideal nutritional entity, because the Lucas test showed a significant ($P < 0.01$) positive intercept (112±19.8), and a variable and low slope (0.36±0.065) as an estimate of true digestibility. Although CF and NFE had significant positive correlations in the Lucas test, they are not ideal nutritional entities because their slopes were variable and intercepts were positive. Of the proximate analyses, only CP and EE behave as ideal nutritional entities and they typically comprise <0.20 of OM in forages. Attempts to identify a larger ideal nutritional fraction using the proximate analysis, e.g. CF-free OM (OM – CF), were unsuccessful because the combined fraction of CP, EE and NFE was non-ideal due to the impact of variability in NFE among forages (Fig. 1).

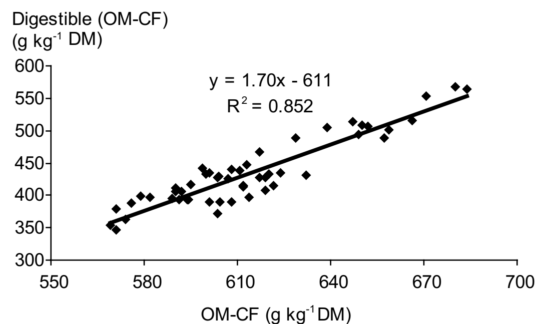

Fig. 1. The uniformity of organic matter (OM) minus crude fibre (CF) concentration determined with the Lucas test; data comprising of silages made from primary growth and regrowth grass.

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Detergent analysis

The fundamental problems associated with NFE and CF fractions in the proximate feed analysis were realised by Paloheimo (1953), who initiated research to develop improved analytical methods for plant cell wall. In the pioneering work, Paloheimo and co-workers (Paloheimo and Paloheimo 1949, Paloheimo and Vainio 1965) used weak hydrochloric acid and a two-stage ethanol extraction to remove cellular contents and to describe vegetable fibre. Despite the appropriate criticism against fractionating feed carbohydrates into CF and NFE, these methods were too laborious, not applicable to faecal samples and the fibre residue was contaminated with protein. Based on these ideas, Van Soest (Van Soest 1967, Van Soest and Wine 1967) developed the neutral detergent fractionation, which used detergents to remove protein and isolate dietary fibre easily in feeds and faeces.

Neutral detergent fibre (i.e. NDF) is widely accepted as an estimate of forage cell wall content, with the major exception that cell wall pectin is extracted. This means that the neutral detergent solubles (NDS) defined as:

$$NDS = DM - NDF \quad [2]$$

contains ash, sugars, starch, organic acids, soluble proteins and lipids and also soluble cell wall carbohydrates like β-glucans and pectin, but they are readily degraded in the rumen (Van Soest 1994). Because ash contributes no energy to the animal, ash can further be subtracted from NDS resulting in neutral detergent soluble OM. Later in this paper, NDS refers to ash free neutral detergent solubles.

The original method (Van Soest and Wine 1967) was modified by Robertson and Van Soest (1981) and Van Soest et al. (1991) by including the use of a heat-stable amylase to remove starch, but they removed sodium sulphite to minimize the losses of phenolic compounds, which isolated a fraction they called neutral detergent residue. The official method approved by AOAC (Mertens 2002a) uses both heat-stable amylase and sodium sulphite. Results can be calculated in four different ways in the official NDF method (with ash, with ash and blank corrected, ash-free, and ash-free and blank corrected). The effects of blank correction are minimal (Mertens 2002a), but especially for forage samples, ash-free values are lower. To avoid confusion, it is important to describe in detail how the NDF analysis was conducted. In the present work, NDF was analysed without the use of amylase except for the seven whole-crop silages, but with sodium sulfite and measured ash-free without blank correction.

Neutral detergent divides the feeds into a soluble fraction that is rapidly and almost completely available and a fibre fraction that is slowly and incompletely degraded by microbial enzymes. The neutral detergent soluble fraction has a high and relatively constant true digestibility across most feeds, which indicates that it is an ideal nutritional entity (Van Soest 1994). When a wide range of feeds (n = 504) was evaluated, Weisbjerg et al. (2004) reported a complete true digestibility for NDS fraction and an endogenous faecal output of 90.2 g kg^{-1} DM intake. In the present data, the true digestibility (0.963) of NDS for all silages was significantly ($P = 0.04$) different from unity. There were some differences between the forage types both in the estimated endogenous faecal output and the true digestibility of NDS (Table 2). The RMSE of the Lucas test was higher for all data compared with that of each forage type. The intercept was lower ($P < 0.01$) for whole-crop silages than the overall intercept, whereas the slopes for PG and whole-crop silages were higher ($P < 0.01$) and that of RG tended ($P < 0.06$) to be lower than the overall slope. The true digestibility of NDS was higher for PG silages compared with RG silages (1.015 vs. 0.925; $P < 0.01$). This difference may be explained by higher content of substances, such as waxes and cutins, in the NDS of RG compared to PG grass that have low availability *in vivo* (see Van Soest 1994). The true NDS digestibility above unity for whole-crop silages may partly be attributed to a small number of samples, but it may also be associated with reduced endogenous output with increased NDS concentration.

Nitrogen content in faecal NDS was estimated by regression to be 72 g kg^{-1} (Fig. 2), a value simi-

Table 2. Faecal metabolic output (intercept, g kg^{-1} DM) and true digestibility (slope) of the neutral detergent solubles fraction (NDS) of different forage types by regressing intake of NDS against apparently digestible NDS.

Forage	n	Intercept	s.e.[a]	Slope	s.e.	RMSE[b]	Adj. R^2
All	86	–92	7.4	0.963	0.018	15.1	0.972
Primary growth grass	33	–101	6.0	1.015	0.017	6.3	0.991
Regrowth grass	27	–90	10.2	0.925	0.027	4.1	0.978
Legume	19	–101	18.0	0.962	0.034	10.0	0.979
Whole-crop	7	–136	4.0	1.111	0.008	1.4	1.000

[a] Standard error
[b] Residual mean squared error

lar to that reported by Van Soest (1994). Although there were statistically significant ($P < 0.01$) differences between the forage types in the faecal N to NDS ratio, the numerical differences were relatively small (66.2, 63.4, 74.7 and 78.7 g N kg^{-1} NDS for the PG and RG grass, legume and whole-crop silages, respectively). The differences in this ratio may be associated with the hind-gut fermentation (higher N concentration in intact microbial cells than in partially digested microbial cell walls) and variation in faecal N output of feed origin (legumes). The high true digestibility of NDS and close relationship between faecal N and NDS suggests that most of faecal NDS and N are of microbial and endogenous origin. Markedly lower N concentration in faecal endogenous OM than in feed protein (70 vs. 160 g kg^{-1} DM) results in a large error in calculating faecal NFE concentration.

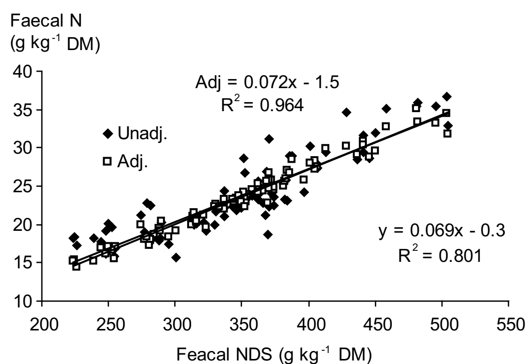

Fig. 2. The relationship between faecal neutral detergent solubles (NDS) and faecal nitrogen (N) concentrations estimated by single regression analysis and by a mixed model regression with random study effect of all forage types.

The low N concentration in metabolic and endogenous faecal OM creates errors for the calculation of endogenous losses for CP, which should be 14 × N instead of 6.25 × N for any fraction calculated using CP. For example faecal NFE concentration is calculated as:

$$\text{Faecal NFE} = \text{OM} - \text{CF} - \text{CP}\ (6.25 \times \text{N}) - \text{EE} \quad [3]$$

Because the faecal NFE uses factor 6.25 to calculate faecal CP instead of the factor 14 based on the true N content of faecal endogenous OM, proximate analysis system results in an erroneously high concentration of NFE in faeces, which is supposedly of non-structural carbohydrate origin. However, faecal NFE contains significant undigested plant cell wall components, primarily hemicellulose and lignin, as indicated by the relationship between faecal NFE and lignin. The proportion of faecal NFE that is undigested cell wall can be calculated as:

$$\text{Faecal NFE (Cell wall)} = 349 \pm 19.1 - 39 \pm 26.6 \times \text{OMD} \quad [4]$$

On average, 0.63 of the faecal NFE was endogenous matter, which is overestimated because the traditional coefficient of 6.25 × N was used, and 0.37 undigested NDF. In the data of Van Soest (1994), the proportion of cell wall fraction in faecal NFE was more than half, which may be related

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to lower digestibility of the grasses in that data set. In the present data, the concentration of the cell wall fraction in faecal NFE (g kg^{-1} DM intake) was strongly correlated (RMSE = 10.7; R^2 = 0.808) to the apparent OMD of the silage.

Analogous to NFE in the proximate analysis system a fraction can be calculated by difference in the detergent analysis that represents soluble or non-fibre carbohydrates. Because non-structural carbohydrates are typically determined analytically as starch plus sugars, this fraction is commonly called non-fibre carbohydrate (NFC) or neutral detergent soluble carbohydrate to indicate that it is calculated from fibre analysis:

$$NFC = OM - NDF - CP\ (6.25 \times N) - EE \quad [5]$$

The true digestibility of NFC from grass silages (0.96±0.026) was not significantly different from unity (P = 0.11). When the PG and RG silages were analysed separately, the true digestibility was 1.03 for both silages, but the intercept was more negative for the regrowth silages (–54 vs. –42 g kg^{-1} DM). These estimates of endogenous loss of NFC are erroneously high because the N correction factor for faecal NFC should be 14 instead of 6.25. Theoretically, the endogenous losses of NFC should be zero because there is little carbohydrate in endogenous animal secretions and microbial debris. Faecal NFC (OM – NDF – CP (6.25 × N) – EE) averaged 139 g kg^{-1} faecal DM (or 43 g kg^{-1} DM intake) for 52 grass silages. The value of 43 g kg^{-1} DM intake for the apparent faecal output of NFC is close to the intercept of the regression between lignin concentration and faecal NFE output, i.e. faecal NFE that is not related to dietary cell wall fraction.

The detergent system provides conceptually sound basis for understanding the physical and biochemical factors that influence the digestibility of feed fractions and causal relationships behind digestibility. If the feed fraction has a true digestibility close to unity and it behaves uniformly among feed types, the faecal output per kg DM intake should not be related to dietary concentration of the fraction or OMD. With proximate analysis, only relatively small proportion of forage OM (CP and EE) behaves uniformly compared with the NDS fraction in the detergent system. For example, in the primary growth silages (n = 27), both the dietary concentration (362 vs. 196 g kg^{-1} DM) and faecal output (94.8 vs. 56.5 g kg^{-1} DM intake) of uniformly behaving entities were markedly greater when based on the detergent system. The faecal output of NFE, CF and NDF decreased with increasing digestibility (Fig. 3), whereas faecal output of CP and NDS were not related to diet digestibility or dietary concentrations. The relationship was stronger for NDF (R^2 = 0.993) than for

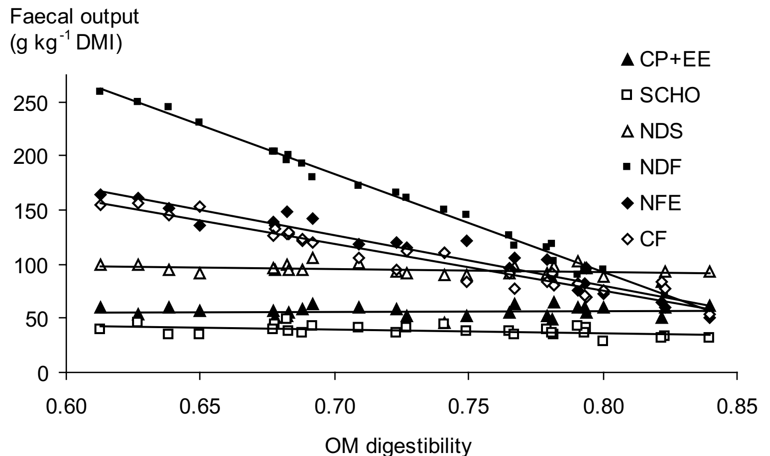

Fig. 3. The relationships between organic matter (OM) digestibility and faecal output of feed components per kg DM intake. Data from primary growth silages (n = 27). CP = crude protein, EE = ether extract, SCHO = soluble carbohydrates, NDS = neutral detergent solubles, NDF = neutral detergent fibre, NFE = nitrogen free extract, CF = crude fibre.

NFE and CF (R^2 = 0.926 and 0.923, respectively).

The advantages of the detergent system compared with the proximate analysis are: larger fraction which behaves uniformly (1), one instead of two fraction of which faecal output varies with digestibility (2) and a closer relationship of faecal fibre (CF vs. NDF) output to OMD (3). These analyses confirm the statement of Paloheimo et al. (1968), that dividing feed carbohydrate fraction into NFE and CF has no scientific justification and limited biological utility; therefore the use of NFE to evaluate forages should be ended. In spite of the limitations of CF analysis to describe the plant cell wall fraction and calculating the more easily available carbohydrate fraction as a difference, proximate analysis is still the basis of calculating feed energy values in most current feed evaluation systems in Europe.

Methods to estimate forage digestibility

Empirical relationships

Chemical composition

Much effort has been directed toward developing regression equations that relate various chemical components to digestibility, although these attempts have not been very successful because of large interspecies and environmental variation (Van Soest 1994). In the present work, the relationships between selected chemical parameters [CP, NDF, acid detergent fibre (ADF) and lignin] and OMD by regression analysis were evaluated as a reference for comparison to more biologically based models. Statistical significance of the prediction errors between the forage types was tested by one-way analysis of variance using GLM procedure of SAS (SAS 1999).

Because most prediction errors between forage types were significant, the next step was to estimate prediction accuracy of regression equations based on forage specific relationships between chemical parameters and digestibility. Finally, relationships within study and forage type were estimated using the MIXED procedure of SAS with trial (forage) as a random factor (random intercept). This model excludes variation resulting from differences such as animals in digestibility trials, animals in iNDF determination, enzyme activity in OMS determination, and the year effect between forage chemical composition and digestibility, i.e. the analysis describes the relationships between the independent and dependent variables within a study (Tables 5 and 6). The regression equations were considered acceptable predictors, when the prediction error was less than one-third of the standard deviation of the reference population, when the regression was biologically sound and they fit several forage types irrespective of environmental factors (see Nousiainen 2004 and Fig. 4).

The concentrations of feed chemical fractions using general equations were poorly related to *in vivo* OMD of silages (Table 3, Fig. 4). Although the relationships were statistically significant, prediction error using CP, NDF and ADF as independent variables was not markedly less than the stand-

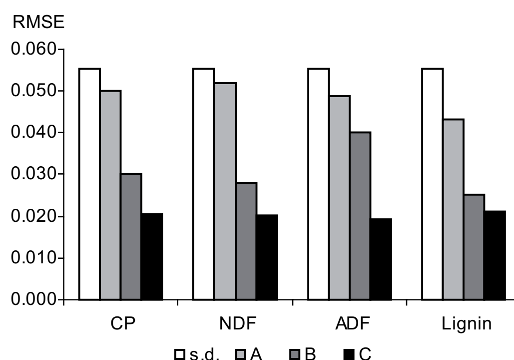

Fig. 4. Residual mean squared errors (RMSE) of the regression equations between feed components and organic matter digestibility estimated with different models (s.d. = standard deviation of the data; A = general relationship; B = forage type specific equations; C = variation from a random study effect excluded). Data contains all forage types.

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ard deviation of OMD in the data (Fig. 4). Lignin was the best single predictor of OMD, but it explained proportionally only 0.43 of the variation and prediction error (42 g kg^{-1}) was too high for practical feed evaluation and ration formulation.

A large proportion of unexplained variation in global regression was related to forage type. This was demonstrated by significant differences in the prediction error among the forage types, when general relationships between a feed fraction and OMD were used (Table 4), and the use of forage specific equations decreased the prediction error markedly (Fig. 4). The decrease was greater for the cell wall components than for CP. The better relationship between NDF and ADF, and especially that of lignin is related to the fact that these components are causative factors and related to the biological availability, whereas CP has no direct effect on digestibility provided that minimum N requirements of rumen microbes are met. This reevaluation confirms previous findings and a more detailed discussion about the relationships between chemical feed components and OMD is given in the original papers (Nousiainen et al. 2003a, b, Rinne et al. 2006).

Van Soest (1994) stated that cell wall fractions predict the digestibility of regrowth silages poorly because the association between lignin and cellulose in them is weak. The present data support this view as indicated by poor general relationships between lignin and NDF. However, when the random study (= year) effect was included in the statistical model, lignin was strongly correlated to NDF. This suggests that environmental differences among years affect lignification of forage cell walls and lead to variable digestibilities at the same lignin concentration. Prediction errors were further reduced by excluding the random trial (forage) ef-

Table. 3. Predictions of *in vivo* organic matter digestibility of all forage types from feed chemical fractions (kg kg^{-1} dry matter) using a single regression equation (F) or a mixed model regression (M) with random trial(forage) effect.

Component	Model	Intercept	s.e.[a]	Slope	s.e.	P-value	RMSE[b]	Adj. R^2
Crude protein	F	0.623	0.021	0.560	0.126	<0.01	0.0501	0.179
	M	0.467	0.021	1.600	0.106	<0.01	0.0205	0.918
Neutral detergent fibre	F	0.811	0.029	–0.200	0.056	<0.01	0.0518	0.12
	M	1.096	0.029	–0.760	0.049	<0.01	0.0201	0.935
Acid detergent fibre	F	0.846	0.028	–0.460	0.094	<0.01	0.0489	0.215
	M	1.060	0.024	–1.200	0.072	<0.01	0.0192	0.925
Lignin	F	0.805	0.013	–2.860	0.358	<0.01	0.0418	0.431
	M	0.846	0.012	–4.190	0.274	<0.01	0.0208	0.869

[a] Standard error
[b] Residual mean squared error

Table 4. Residual mean squared errors of *in vivo* organic matter digestibility (OMD) predicted from chemical parameters assuming a general relationship between the chemical fraction and OMD. The values in bold are significantly (P < 0.05) different from zero.

	Primary growth grass	Regrowth grass	Legume	Whole-crop	RMSE[a]	P-value
Crude protein	**–0.025**	0.009	**0.031**	0.001	0.041	<0.01
Neutral detergent fibre	**–0.032**	0.010	**0.028**	**0.036**	0.040	<0.01
Acid detergent fibre	–0.032	0.009	**0.022**	**0.054**	0.036	<0.01
Lignin	**–0.017**	**0.027**	**–0.022**	**0.037**	0.032	<0.01

[a] Residual mean squared error

fects from the variation, i.e. within a trial and forage type, chemical composition was closely related to *in vivo* OMD (Fig. 4). It is concluded that feed fractions can not be used to predict OMD with acceptable precision, even when forage specific equations are used because the RSME of these equations are greater than 1/3 of the SD for the population of OMD.

Organic matter cellulase solubility

In vivo apparent digestibility (intake minus faecal output) determined with sheep by total faecal collection is the basis of most existing feed evaluation systems. For practical and often even for research purposes this method is too expensive, laborious and a large quantity of the feed is required. Therefore, laboratory *in vitro* methods have been developed and are widely used, based on ruminal fluid (introduced by Tilley and Terry 1963; extensively reviewed by Weiss 1994) or commercial fungal cellulases. Due to difficulties in obtaining rumen fluid in commercial laboratories and standardisation of the system, an enzymatic *in vitro* procedure in the determination of forage digestibility has been evaluated.

Enzymatic digestion procedures have been described and discussed in detail in a review by Jones and Theodorou (2000). Basically the method includes removing of cell solubles either by HCl-pepsin or neutral detergent followed by a 24 or 48 h incubation in buffered enzyme solution. The cellulase method differs from the *in vivo* digestion at least in two aspects: no endogenous matter is produced, i.e. solubility reflects true rather than apparent digestibility, and the capacity of commercial enzymes to degrade cell wall carbohydrates is less than that of rumen microbes (McQueen and Van Soest 1975, Nousiainen 2004). Nousiainen (2004) estimated that *in vitro* grass silage NDF solubility was 0.79 of the *in vivo* sheep NDF digestibility and only 0.67 of the potential NDF digestibility estimated by a 12 day ruminal *in situ* incubation. However, these differences do not preclude the use of enzymatic OM solubility (OMS) in predicting the *in vivo* digestibility provided that appropriate correction equations are used. The details of the OMS method used in Finland are described by Nousiainen et al. (2003a).

The present data indicates that the relationship between OMS and *in vivo* OMD is not uniform among the forage types (Table 5), because the prediction error within each forage type was markedly smaller than that estimated using the general correction equation. However, compared to chemical components the prediction error was much smaller for either the general or forage-specific equations suggesting that enzymatic hydrolysis reflects the mechanisms of digestibility better than concentra-

Table 5. Empirical relationships between pepsin-cellulase organic matter (OM) solubility (kg kg^{-1}) and in vivo OM digestibility determined with fixed (F) or mixed regression analysis with random study effect (M).

Forage	Model	Intercept	s.e.[a]	P-value	Slope	s.e.	RMSE[b]	Adj. R^2
Primary growth grass	F	0.103	0.0289	<0.01	0.83	0.038	0.0151	0.937
	M	0.077	0.0211	<0.01	0.86	0.027	0.0085	0.981
Regrowth grass	F	–0.070	0.1030	0.50	1.01	0.136	0.0193	0.676
	M	–0.154	0.0627	0.05	1.12	0.082	0.0091	0.921
Legume	F	0.002	0.0332	0.94	0.93	0.044	0.0122	0.962
	M	0.003	0.0332	0.93	0.93	0.044	0.0121	0.962
Whole-crop	F	0.182	0.0487	0.01	0.66	0.064	0.0109	0.947
	M	0.290	0.0996	0.21	0.52	0.129	0.0090	0.942
All	F	0.064	0.0348	0.07	0.86	0.046	0.0245	0.804
	M	0.040	0.0193	0.05	0.89	0.026	0.0099	0.964

[a] Standard error

[b] Residual mean squared error

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tions of components from proximal analysis or detergent fractionation. When the forage specific equation was used, prediction error for OMD in all data decreased to 15.3 g kg^{-1}. The prediction error (Observed-Predicted) of the general OMS equation was positively related to pdNDF concentration determined by 12 d ruminal *in situ* incubation ($P < 0.01$; $R^2 = 0.16$), suggesting that the relative efficiency of the enzyme system to solubilize forage OM decreased with increased concentration of NDF potentially digestible by rumen microbes.

Using a mixed model regression, which included a random year-of-study variable, decreased prediction error especially for all forages, but also for grass silages. The smaller prediction error with the mixed model analysis may be associated with the variation between the trials in activity of the enzyme and differences between the animals used for the *in vivo* determination. For RG grass, the association between OMS and OMD could also depend on climatic and environmental conditions as indicated by the poor overall and good within year-of-study relationship between lignin and NDF, and between iNDF and NDF. Consequently, at a certain OMD level, OMS for RG and legume silages is apparently higher than for PG silages (Table 1, Nousiainen et al. 2003b, Rinne et al. 2006).

In addition to forage-specific equations, the laboratory-specific equations may be needed. Despite serious attempts, the laboratories of Valio Ltd. and MTT were not able to standardise the OMS methods (Nousiainen 2004). There was a difference in the intercept, but the slope was 1.00 and R^2 high (0.97). The intercept difference suggests particle loss during the procedures (manual filtration vs. Tecator crucibles). Further evidence for the possible contribution of the particle losses during OMS procedures is provided by a comparison of the method described by Nousiainen et al. (2003a) and the Ankom filter bag system (Z.M. Kowalski et al., unpublished).

It is also noteworthy that in the study with legume silages (Rinne et al. 2006), *in vitro* OMD determined by Tilley and Terry (1963) method significantly underestimated *in vivo* OMD. In their original evaluation, Tilley and Terry (1963) speculated that despite a close relationship between *in vivo* and *in vitro* digestibility, these values are not identical and specific correction equations within laboratory and possibly within forage type may be needed. Weiss (1994) interpreted between-laboratory variations to suggest that ruminal *in vitro* systems need laboratory-specific correction equations.

Organic matter digestibility can be predicted from OMS of pre-ensiled herbage as precisely as from OMS of the resultant silages provided that silages are well preserved with low or moderate ensiling losses (Huhtanen et al. 2005). For practical ration formulation, sampling of herbage during silage harvesting allows more representative sampling and provides a better indication of the variation in silage digestibility than samples taken from the silos, especially those drilled from the top of large tower silos. Advance information of silage digestibility would also be useful in the planning of rations for the feeding period.

In conclusion, *in vitro* OMS provides more precise prediction of forage OMD than chemical feed analysis, when general equations are used (RMSE of 24 vs. 42 to 50 g kg^{-1} DM). However, to achieve accurate estimates (RMSE less than 20 g kg^{-1} DM), forage specific correction equations should be used. Solubility values may also be laboratory and methodology specific, which indicates that relationships between OMS and OMD must be developed for each laboratory setting.

Indigestible neutral detergent fibre

A part of the forage cell wall is unavailable to microbial digestion in ruminants, even if total tract residence time of fibre could be extended to infinite time (Allen and Mertens 1988, Van Soest 1994). This forage DM fraction can be called indigestible fibre, here referred to as indigestible NDF (iNDF). In addition to NDS, iNDF represents by definition a uniform feed fraction with zero true digestibility. Potentially digestible fibre (pdNDF) may then be calculated as:

$$pdNDF = NDF - iNDF \qquad [6]$$

Several methods may be used to divide forage NDF to potentially digestible and indigestible fractions, e.g. end-point measurement with long-term

(up to 144 h) *in vitro* batch rumen fluid incubation (Traxler et al. 1998) or fitting time-dependent (0–96 h) *in vitro* or *in situ* NDF nylon bag degradation data to single digestion pool rumen model (Wilman et al. 1996a). The ultimate extent of NDF digestion may not be reached with *in vitro* batch system and the *in situ* estimates may be biased due to crucial drawbacks of the traditional nylon bag procedure as discussed earlier (Nousiainen 2004, Nousiainen et al. 2004). The slow rate of NDF digestion within the nylon bags with small pore sizes leads to prolonged NDF digestion (Huhtanen et al. 2006), and the difference between the extent of digestion reached at 96 and 288 h incubations increased as the digestibility of silage decreased (Rinne et al. 2002). Because forage iNDF fraction is attributable to cross-linking between cell wall lignins and hemicellulose when plants mature (Van Soest 1994), several attempts to predict iNDF from lignin concentration in DM or NDF have been made (see Traxler et al. 1998). Despite this biological conjecture, it has not been successful due to relatively high proportional errors in lignin and iNDF analyses, as well as differences between forage types in lignin to iNDF ratio, which may also be prone to climatic factors.

The Cornell Net Carbohydrate and Protein system uses a factor 2.4 × lignin concentration in NDF in describing iNDF of forages (Van Soest et al. 2005). This factor is presumed to be universal across forage species and growth environments. Validation of this concept with data containing several forage species (corn, alfalfa, grasses, wheat straw) resulted in satisfactory regression (R^2 = 0.94) between observed and predicted (2.4 × lignin) iNDF (Van Soest et al. 2005). However, the present data does not support a generally applicable relationship between permanganate lignin and iNDF measured by 12 d *in situ* fermentation, although the overall slope was 2.4 (Fig. 5). The slopes for individual forages species varied between 2.8 and 5.5, and a general regression equation predicted iNDF with an unsatisfactory accuracy (R^2 = 0.56; RMSE = 27.4 g kg^{-1} DM). If forage-specific relationships were used, the RMSE for predicted iNDF decreased to 14.9 g kg^{-1} DM. This confirms the previous findings (Nousiainen et al. 2004) and suggests that a universal lignin equation describing the iNDF fraction did not exist as we measured them. The forage type specific lignin equations may be used to predict iNDF if *in situ* estimates are not available.

To determine forage iNDF concentration, a long-term (12 d) *in situ* incubation has been used at MTT to ensure complete digestion of potentially digestible NDF. The small pore size (6 or 17 μm) combined with a relatively large open surface area of the nylon bag cloth used allows moderate mi-

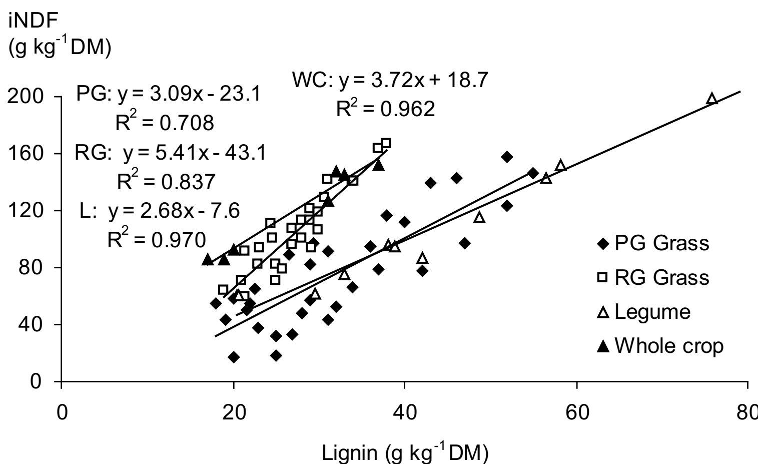

Fig. 5. The relationship between lignin and iNDF concentrations estimated by fixed regression analysis of silages made from primary (PG) or regrowth (RG) grass and leguminous (L) or whole-crop (WC) forages; overall regression y = 2.4x + 18 (R^2 = 0.555, residual squared mean error = 27.4 g kg^{-1} DM).

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crobial activity within the bags (Huhtanen et al. 1998) and prevents particle in- and out-flow from the bags. Indigestible NDF determined by long *in situ* incubation has been used to describe ruminal cell wall kinetics (Tamminga et al. 1989), and as digestibility marker for estimating total and ruminal digestibility (Huhtanen et al. 1994, Ahvenjärvi et al. 2000). The details of the procedures are described by Huhtanen et al. (1994) and Ahvenjärvi et al. (2000). After *in situ* incubation, the residues are washed with water and treated with neutral detergent solution to remove microbial matter.

An inter-laboratory ring test in Nordic countries showed large differences in the iNDF estimates (Lund et al. 2004) leading to standardization of the method, which was succesful in removing the between-laboratory differences (J. Nousiainen et al. unpublished). Currently the method includes the use of polyester bags with 10–17 µm pore size and 10–20 mg cm^{-2} sample to surface ratio. Ruminal incubations (288 h) should be conducted with two cows fed with forage based diets (forage to concentrate ratio at least 60:40). The use of NIRS in predicting grass silage iNDF has also been evaluated with promising results (Nousiainen et al. 2004), but the standardization of the reference method is a vital prerequisite in developing robust calibrations.

Previous results for grass silages (Nousiainen et al. 2003b, Nousiainen 2004) suggested that iNDF can be used in a general linear regression equation to predict forage OMD relatively universally over a range of species and harvesting conditions. The intercept of this equation represents a theoretical maximum of forage OMD provided that all NDF is potentially digestible and that the rate of pdNDF digestion (k_d) is the only factor limiting digestibility when the forage is fed to sheep at maintenance level of feed intake. The slope of the regression describes the decline in OMD with increasing iNDF concentration. However, when forage-specific equations for PG, RG, legume and whole-crop silages are compared, the relationship between iNDF and OMD was not uniform (Table 6). This can be judged both by the variable intercepts and slopes between the different forage types.

For PG grass and whole-crop silages the slope of the iNDF equation seems to be equal (about −1.5) irrespective of the model used (fixed vs. mixed) and suggests that one gram iNDF protects 1.5 gram NDF (or OM) from digestion in sheep

Table 6. Empirical relationships between forage indigestible neutral detergent fibre concentration (kg kg^{-1}) and *in vivo* organic matter digestibility determined with fixed (F) or mixed regression analysis and corrected for the random study effect (M).

Forage	Model	Intercept	s.e.[a]	P-value	Slope	s.e.	RMSE[b]	Adj. R2
Primary growth grass	F	0.852	0.0064	<0.01	–1.52	0.07	0.0159	0.932
	M	0.851	0.0062	<0.01	–1.51	0.05	0.0086	0.979
Regrowth grass	F	0.802	0.0137	<0.01	–1.03	0.13	0.0180	0.718
	M	0.829	0.0108	<0.01	–1.30	0.08	0.0072	0.963
Legume	F	0.831	0.0095	<0.01	–1.14	0.08	0.0175	0.921
	M	0.832	0.0097	<0.01	–1.15	0.07	0.0129	0.956
Whole-crop	F	0.867	0.0134	<0.01	–1.52	0.11	0.0082	0.970
	M	0.867	0.0134	<0.01	–1.52	0.11	0.0082	0.970
All	F	0.834	0.0053	<0.01	–1.26	0.05	0.0190	0.883
	M	0.839	0.0051	<0.01	–1.32	0.04	0.0106	0.964

[a] Standard error
[b] Residual mean squared error

fed at maintenance. The respective slope, however, is lower for RG grass (−1.3) and especially for legume (−1.15) silages. This may be explained by the variable relationship between iNDF concentration and rate of pdNDF digestion (k_d) among the forage types resulting in different apparent OMD at the same iNDF concentration (see Rinne et al. 2006) and also suggests that pdNDF is not a uniform nutritional entity. Especially legumes at high iNDF concentration are still relatively highly digestible as compared to grasses (Wilman et al. 1996b, Van Soest 1994). Nevertheless, despite the lack of uniform behaviour, the iNDF regression equation may be very useful in predicting forage OMD, especially if forage specific OMS equation cannot be used (see Tables 5 and 6).

Summative models

Background and methods

Most of the existing feed evaluation systems use the total amount of digestible nutrients expressed as grams in feed DM to determine feed metabolisable energy value (MAFF 1975, MTT 2006). However, the analytical procedures and equations to estimate digestible nutrients vary between systems. Van Soest (1967) developed a comprehensive system of feed analysis and its application to forages. He divided the feed into NDS fraction which is essentially completely available but its digestibility is apparently incomplete, because of faecal endogenous and microbial material. The second fraction corresponds to fibre (NDF) and its availability is controlled by structural features that link cellulose, hemicellulose and lignin. The fibre fraction is not uniform between forages. Goering and Van Soest (1970) presented a summative model to describe availability of forage DM:

$$dDM = NDFD \times NDF + 0.98 \times NDS - M \quad [7]$$

where dDM = digestible DM, NDFD = coefficient of NDF digestibility, and M = microbial and endogenous faecal DM losses. Theoretically this model is sound, but generally NDFD is not known. Conrad et al. (1984) modified this model by dividing feeds into NDS and potentially digestible NDF. They applied surface area law (mass raised to power 0.67) to calculate NDF that is covered by lignin, and this proportion was multiplied by lignin-free NDF to estimate available NDF. Their available, lignin-free NDF component, i.e., $(NDF–L) \times (1– L^{2/3} / NDF^{2/3})$ was an attempt to estimate pdNDF in the current terminology. They assumed that the digestibility of available lignin free NDF was 0.75 to calculate TDN at maintenance level of intake. Weiss et al. (1992) revised the Conrad et al. (1984) model and it was adopted by NRC (2001) to estimate total digestible nutrients (TDN). Huhtanen (2003) evaluated the NRC (2001) model using *in vivo* sheep digestibility data. The predicted and observed digestible OM concentrations (D-value, g kg^{-1} DM) were relatively well correlated, but there was a considerable slope bias. The NRC (2001) system clearly underestimated the D-value of high quality grass silages. This suggests that this system is not uniform for forages grown in different environmental conditions. The major problem was that the potential maximum of 0.75 for the digestibility of lignin free NDF is clearly too low for high quality grasses grown in northern latitudes.

Because the *in vivo* pdNDF digestibility is markedly less variable than the total NDF digestibility, and because the fraction subjected to this variation (pdNDF vs. NDF) is smaller, the accuracy of the summative systems based on three fractions (NDS, pdNDF and iNDF) could be improved compared to systems dividing feeds only to total NDF and ND solubles. In the present data, the coefficient of variations of pdNDF and NDF digestibility were 0.064 and 0.135, and respective concentrations 403 and 500 g kg^{-1} DM. Exclusion of the whole-crop silages from the data decreased the coefficient of variation in pdNDF digestibility to 0.041. In the Lucas test for the pdNDF fraction, the overall coefficient of determination was high (R^2 = 0.95) and the intercept was close to zero (Fig. 6), but obviously the high R^2 reflected partly a large range in the pdNDF concentration. The intercept was significantly positive for PG ($P = 0.01$) and legume ($P = 0.08$) silages and negative ($P = 0.001$) for the whole-crop silages. Both the negative and positive intercepts are biologically impossible, because the amount absorbed can not be positive at

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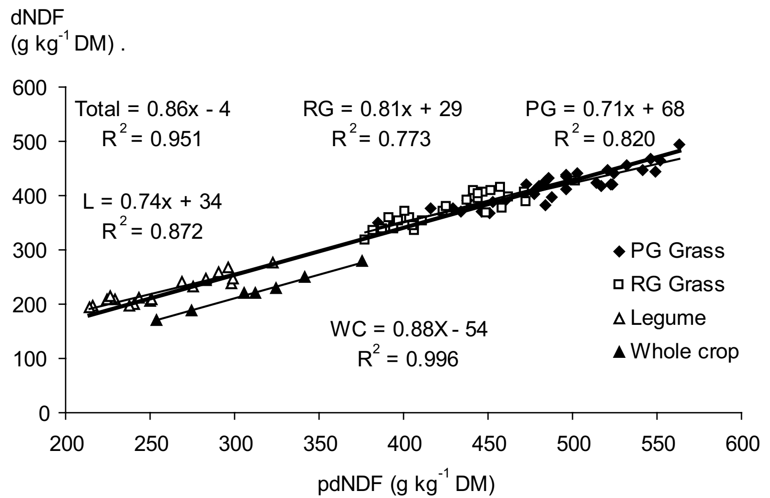

Fig. 6. Lucas test for digestible neutral detergent fibre (dNDF) of silages made from primary (PG) or regrowth (RG) grass and leguminous (L) or whole-crop (WC) forages.

zero intake and there is no faecal endogenous and microbial excretion of digestible fibre.

Excluding the whole-crop silages resulted in the following equation by the Lucas test:

$$\text{dNDF}(\text{g kg}^{-1}\text{ DM}) = 16.9\pm7.0 + 0.821\pm 0.017 \times \text{pdNDF (g kg}^{-1}\text{ DM) } (R^2 = 0.97) \quad [8]$$

where dNDF is digestible NDF and pdNDF potentially digestible NDF calculated as NDF − iNDF. This equation meets all other criteria of uniformity presented by Lucas (see Van Soest 1994) except that the intercept was slightly, although significantly ($P = 0.02$) positive. However, when legume silages were excluded from the data the intercept increased to 65 g kg^{-1} DM ($P < 0.01$) suggesting that pdNDF is not a uniform entity. Despite high R^2 values for the dNDF in the Lucas test, it can not be considered as fundamental biochemical cause-and-effect relationship, and therefore the summative approach in determining forage availability based on tdNDS and dNDF estimated by the Lucas concept must be essentially interpreted as an empirical approach.

Three different summative approaches were used to estimate the silage D-value. All methods had the same basic structure but the method used in estimating dNDF differed:

$$\text{D-value (g digestible OM kg}^{-1}\text{ DM)} = \text{tdNDS} + \text{dNDF} - \text{M} \quad [9]$$

where tdNDS (g kg^{-1} DM) is truly digestible NDS and M is faecal microbial and endogenous output of OM (g kg^{-1} DM). NRC (2001) estimated dNDF (g kg^{-1} DM) as follows:

$$\text{dNDF}_{\text{NRC}}\text{ (g kg}^{-1}\text{ DM)} = 0.75 \times (\text{NDF} - \text{Lignin}) \times [1 - (\text{Lignin/NDF})^{0.667}] \quad [10]$$

where NDF and lignin are expressed as g kg^{-1} DM. In this equation, the first part may be interpreted as potentially digestible NDF (i.e. pdNDF) and the latter part $[1 - (\text{Lignin/NDF})^{0.667}]$ digestibility of pdNDF. Both the original parameter values and those estimated form the present data were used. Mertens (2002b) derived a simple equation in which dNDF is a linear function of NDF and lignin:

$$\text{dNDF}_{\text{Mertens}} = a \times \text{NDF (g kg}^{-1}\text{ DM)} + b \times \text{Lignin (g kg}^{-1}\text{ DM)} \quad [11]$$

where *a* and *b* can be estimated by regression. Constant *a* is the digestibility coefficient of pdNDF and constant *b* is the product of the digestibility coefficient of pdNDF and the proportion of NDF

protected by lignin. This equation has no intercept, i.e. neither endogenous nor microbial excretion of NDF. The parameter values for both NRC and Mertens equations were estimated by the Solver tool (Fylstra et al. 1998) in Microsoft® Excel, which employs the Generalized Reduced Gradient (GRG2) non-linear optimization code (Lasdon et al. 1978).

The third summative equation in estimating D-value was based on applying the Lucas test both for NDS and pdNDF:

$$\text{D-value (g kg}^{-1}\text{ DM)} = \text{tdNDS (g kg}^{-1}\text{ DM)} + \text{dNDF (g kg}^{-1}\text{ DM)} \quad [12]$$

Because the effects of lignin and iNDF on digestibility and output of M were forage type specific, the summative models were tested both using all data and separately for each forages. The models of NRC (2001) and Mertens (2002b) were also tested by using iNDF instead of lignin. For all models, both general equations derived from all data and forage specific equations were used for dNDF and dNDS. The models were compared on the basis of residual mean squared errors were calculated as:

$$\text{RMSE} = \sqrt{\sum (\text{Observed} - \text{Predicted})^2 / n} \quad [13]$$

Mean squared prediction error (MSPE) was divided to components resulting from mean bias, slope bias and random variation around the regression line (Bibby and Toutenburg 1977).

Results and discussion

The NRC (2001) system clearly underestimated *in vivo* dNDF (Fig. 7), which agrees with Huhtanen (2003). The mean bias (observed – model predicted) was 48 g kg^{-1} DM, but it varied from 75 (PG silages) to –34 g kg^{-1} DM (whole-crop). The major problem was that the first part of the NRC equation [0.75 × (NDF – Lignin)] clearly underestimated the concentration of potentially digestible NDF (351 vs. 404 g kg^{-1} DM). However, the precision of the prediction was good (R^2 = 0.89), mainly because of the close relationship between forage NDF and $pdNDF_{NRC}$ concentrations (R^2 = 0.89). *In vivo* digestibility of NDF was higher than the maximum potential NDF digestibility of the NRC (2001) system (0.75) in 19 cases and that of lignin-free NDF in 39 cases out of 86.

In the present study, lignin was analysed as permanganate lignin, which results in higher values than ADL. The mean bias of the NRC (2001) system would probably have been smaller, if ADL had been used. Variation in $dNDF_{NRC}$ was more

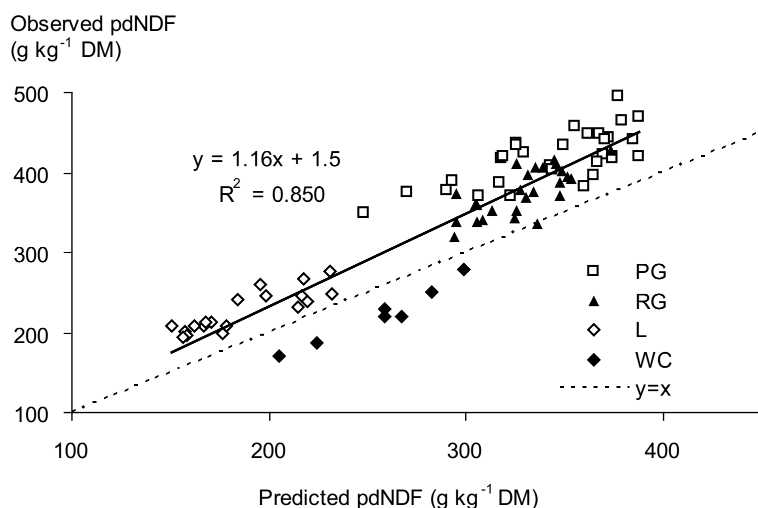

Fig. 7. The relationship between digestible neutral detergent fibre (dNDF) concentration predicted according to NRC (2001) and observed dNDF determined with sheep fed at maintenance level of feeding.

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closely associated with the NDF concentration than with the observed *in vivo* dNDF (R^2 0.91 vs. 0.75). This suggests that the components of the equation predicting pdNDF and its digestibility did not describe biological cause-and-effect relationships explaining the variation in these basal attributes of digestibility. This evaluation using the *in vivo* data for several forage species did not validate successfully the assumptions about the surface area law describing the effect of lignin on digestibility.

The results of the evaluation of the general summative models are shown in Table 7. Estimating the parameters from the present data decreased the prediction error of the NCR (2001) equation due to reduced mean bias. The parameter value describing the maximum digestibility of lignin free NDF increased from 0.75 to 0.966, which overestimated the concentration of pdNDF by 48 g kg^{-1} DM (452 vs. 404 g kg^{-1} DM). However, the precision of the prediction was acceptable ($R^2 = 0.89$). The power in the second component of function which assumes that lignin affects NDF digestibility according the surface area law, decreased from 0.67 to 0.515. As for the original NRC equation, simulated pdNDF digestibility by the model was not correlated with the *in vivo* data. The more complex function of lignin adopted in the NRC (2001) model did not improve the precision of the prediction compared to purely empirical prediction based on NDF concentration and linear relationship between NDF digestibility and lignin concentration (RMSE = 33.9). Mertens (2002b) equation resulted in a similar error to that of NRC and empirical approach. Interestingly, the prediction errors of NRC and Mertens equations were strongly correlated ($R^2 = 0.99$) indicating that the form of lignin function had no influence on D-value prediction.

When lignin was replaced with iNDF, the prediction error of pdNDF digestibility reduced markedly. This improvement can be attributed to the fact that direct determination of iNDF by 12 day *in situ* describes the fibre fraction that is completely unavailable for microbial digestion better than lignin concentration. The ratio between lignin and iNDF was not uniform between the forage types, a prerequisite for accurate and precise prediction of dNDF from simple or complex functions of lignin. The error in lignin analysis is absolute rather than proportional (Van Soest 1994), which can lead to

Table 7. Prediction of the digestible NDF (dNDF) and D-value using different summative equations for all forage types; general equations were used to predict both dNDF and digestible neutral detergent solubles.

Trait/method	Independent variable	Intercept	Slope	R^2	MSPE[a]	Distribution of MSPE		
						Bias	Slope	Random
dNDF (g kg^{-1} DM)								
NRC (2001)	Lignin	11	0.971	0.853	33.6	0.000	0.005	0.994
NRC (2001)	iNDF	9	0.977	0.954	18.7	0.001	0.012	0.988
Mertens (2002b)	Lignin	9	0.975	0.849	34.0	0.000	0.004	0.996
Mertens (2002b)	iNDF	8	0.978	0.957	18.5	0.001	0.011	0.988
Lucas test	dNDF	0	1.001	0.952	19.1	0.000	0.000	1.000
D-value (g kg^{-1} DM)								
NRC (2001)	Lignin	–104	1.162	0.551	35.3	0.000	0.023	0.976
NRC (2001)	iNDF	–80	1.124	0.904	17.0	0.001	0.103	0.896
Mertens (2002b)	Lignin	–121	1.188	0.542	35.7	0.000	0.029	0.971
Mertens (2002b)	iNDF	–78	1.121	0.911	16.4	0.001	0.106	0.893
Lucas test	dNDF	–152	1.235	0.908	18.3	0.000	0.264	0.736

[a] Mean squared prediction error

high proportional errors in lignin analysis of forages of low lignin concentration. Predicting dNDF assuming pdNDF as a uniform nutritional entity described pdNDF almost as precisely as the models of NRC (2001) and Mertens (2002b).

The prediction error of D-value was not higher than that of dNDF in spite of relatively large (16 g kg^{-1} DM) error in predicting dNDS from Lucas equation. The non-additivity of the errors was mainly due to the negative correlation between the errors in dNDF and dNDS, i.e. the errors were partly counterbalanced. For example, all models underestimated dNDF for whole-crop silages, but because faecal endogenous output was underestimated, the overall D-value was predicted fairly accurately for the whole-crop silages. The slope bias in D-value predictions also suggests interactions between dNDF and dNDS components.

When the forage specific equations were applied to predict both dNDF and tdNDS (Table 8), prediction errors were markedly reduced compared with the general equations. Prediction errors for dNDF were only 11–12 g kg^{-1} DM for the three models based on iNDF, and the models describing the mechanisms of digestion were slightly better. The more complex NRC (2001) model was not better than the simpler Mertens (2002b) model. This provides further evidence that the theoretical surface law of lignin protection does not predict digestibility of pdNDF more accurately than the empirical relationships between iNDF and pdNDF.

The Mertens (2002b) equation can be formulated in three different ways:

$$\text{dNDF} = a \times \text{NDF} + b \times \text{iNDF} \quad [14]$$

$$\text{dNDF} = a \times (\text{NDF} - \text{iNDF}) + b \times \text{iNDF} \quad [15]$$

$$\text{dNDF} = a \times (\text{NDF} - \text{iNDF}) \quad [16]$$

Equation [14] describes dNDF as a function of NDF and iNDF. Coefficient *a* can be interpreted as a maximum potential NDF digestibility and coefficient *b* representing a discount for dNDF related to iNDF. This equation also allows NDF and iNDF interact in such a way that possible effects of iNDF concentration on pdNDF digestibility can be accounted for. In equation [15], the fraction (NDF-iNDF) describes by definition potentially digesti-

Table 8. Prediction of digestible NDF (dNDF) and D-value using different summative equations from data comprising of silages made from primary or regrowth grass and leguminous or whole-crop forages; forage specific equations were used to predict both dNDF and digestible neutral detergent solubles.

	Independent variable	Intercept	Slope	R^2	MSPE[a]	Distribution of MSPE		
						Bias	Slope	Random
dNDF (g kg^{-1} DM)								
NRC(2001)	Lignin	15	0.960	0.944	20.9	0.000	0.005	0.994
NRC(2001)	iNDF	4	0.989	0.984	11.2	0.001	0.012	0.988
Mertens (2002b)	Lignin	13	0.964	0.946	20.5	0.000	0.004	0.996
Mertens (2002b)	iNDF	2	0.993	0.983	11.4	0.001	0.011	0.988
Lucas test	dNDF	0	1.001	0.980	12.4	0.000	0.000	1.000
D-value (g kg^{-1} DM)								
NRC (2001)	Lignin	–50	1.078	0.802	23.4	0.003	0.021	0.976
NRC (2001)	iNDF	–16	1.026	0.932	13.6	0.002	0.008	0.990
Mertens (2002b)	Lignin	–33	1.053	0.809	23.0	0.002	0.010	0.987
Mertens (2002b)	iNDF	–13	1.020	0.930	13.8	0.001	0.005	0.994
Lucas test	dNDF	–50	1.078	0.922	15.0	0.001	0.057	0.942

[a] Mean squared prediction error

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ble NDF, coefficient *a* is digestibility of pdNDF and *b* is a discount factor for iNDF allowing pdNDF digestibility to differ with iNDF concentration. The third equation [16] is a simplification from equation [15] and it assumes a constant digestibility for pdNDF (NDF – iNDF), i.e. the equation is Lucas model without M for pdNDF.

The three equations were compared and the results for PG grass and legume silages are shown in Table 9. Coefficient *a* and RMSE of the models were similar for equations [14] and [15], and represent the digestibility of pdNDF. In equation [14] the coefficient *b* is associated both with iNDF and the effect of iNDF on the digestibility of pdNDF, whereas in equation [15] coefficient *b* describes the additional effect of iNDF on pdNDF digestibility. Indigestible NDF had a strong negative effect on pdNDF digestibility of PG grasses (–0.317). In contrast, iNDF had only a minor effect on pdNDF digestibility of legume silages and consequently, the simple equation [16] did not increase markedly the prediction error. However, for the PG silages, equation [15] resulted in a smaller prediction error due to the strong impact of iNDF on pdNDF digestibility.

The results suggest that equation [15] includes the basic nutritional concepts of fibre digestion: it separates NDF into potentially digestible and indigestible fractions (1), and that the equation is flexible allowing interactions between pdNDF and iNDF to influence the digestibility of pdNDF (2). Compared with equation [16] or the Lucas equation allowing an intercept, equation [15] markedly reduced the prediction error. This effect may be associated to the curvilinear relationship between maturity and pdNDF concentration, whereas iNDF increases linearly with advancing maturity. Although equation [14] predicts pdNDF equally well to equation [15], interpretation of the coefficients is biologically more difficult.

The summative approach based on uniform nutritional entities and biochemical cause-and-effect relationships for non-uniform entities, predicted silage D-value at least as accurately as the best empirical equation using either OMS or iNDF as independent variables. When the general relationships were used, the summative approach was markedly better than OMS (Table 10). This can mainly be attributed to forage specific relationships between OMS and OMD. Results in Table 10 suggest that only minor reductions in RMSE are gained by the use of forage-specific equations compared to general equations for summative models and those using iNDF. These equations would reduce by a factor of four the number of parameters that must be estimated and are consistent with the uniform nutritional availability of the Lucas test.

Re-evaluation of the different approaches reveals that for accurate and precise prediction of D-

Table 9. Comparison of three versions of Mertens (2002b) equation (for description of equations, see text) in predicting forage D-value using data of silages made from primary growth grass or legumes.

Forage	Equation	Coefficient		RMSE[a]	Regression for potentially digestible NDF		
		a	b		Intercept	Slope	R^2
Primary growth grass	[14]	0.901	–1.218	10.14	30	0.928	0.912
	[15]	0.901	–0.317	10.14	30	0.928	0.912
	[16]	0.849	0.000	15.23	68	0.838	0.820
Legume	[14]	0.886	–0.927	9.72	30	0.867	0.871
	[15]	0.886	–0.042	9.72	30	0.867	0.871
	[16]	0.868	0.000	9.90	34	0.852	0.872

[a] Residual mean squared error

value forage specific equations are needed irrespective the method used (empirical vs. summative, see Table 10). Basically, this is because among the forages faecal output of NDS is not constant (1), and because the relationship between iNDF concentration and the rate of pdNDF digestion is variable (2). An advantage of the summative systems is that they are based on physical and biochemical factors that influence the availability of various feed fractions. It is possible that sometimes the errors counterbalance each other, the case being especially likely for the methods that use iNDF as independent factor. The strong empirical relationship between iNDF concentration and OMD reported by Nousiainen (2004) was also confirmed by the results obtained from this larger dataset.

Dynamic models

Several reviews have discussed the mathematic modelling of ruminal cell wall digestion and strengths and weaknesses of the experimental methods used to determine the parameter values required in the models (Mertens 1993, Illius and Allen 1994, Ellis et al. 1999, Huhtanen et al. 2006). The recent knowledge of digestion and passage kinetics has been incorporated into the Nordic dairy cow model Karoline (Danfær et al. 2005). This model predicted accurately and precisely the amount of NDF digested. The sensitivity analysis demonstrated that iNDF is a key parameter in estimating nutrient supply from the digestive tract (Huhtanen et al. 2006), which is consistent with the close relationship between forage iNDF concentration and D-value. Digestion in the ruminant digestive tract is the competition between the rates of digestion (k_d) and passage (k_p). When the rate of digestion in relation to passage increases, digestibility of pdNDF increases. The variation in pdNDF digestibility must therefore be associated with differences in the rates of digestion and passage.

Previous discussion of the methods to describe feed availability clearly demonstrated that the scope to decrease the prediction error of D-value is rather limited with traditional regression equation and summative approaches. An additional source of variation is the variable faecal NDS secretion, and a more thorough understanding of the underlying biological mechanisms causing this variability (in this data from 81 (whole-crop) to 121 (legumes) g kg^{-1} DM intake) is needed to improve the models in predicting forage D-value.

Both the empirical and summative approaches were limited in their ability to explain the variation in pdNDF digestibility related to rates of passage and digestion, which had a range of 0.11 to 0.15 units for grass and legume silages. A large proportion of this variation is related to differences in the rate of digestion attributable to intrinsic feed fac-

Table 10. Prediction of D-value (g kg^{-1} DM) from organic matter pepsin cellulase solubility (OMS) and indigestible neutral detergent fibre concentration (iNDF) using empirical relationships or the summative approach according to Mertens (2002b).

Method	Equation[a]	Intercept	Slope	R^2	MSPE[b]	Distribution of MSPE		
						Bias	Slope	Random
OMS	G	–29.2	1.05	0.816	22.3	0.000	0.008	0.992
	S	–5.1	1.01	0.929	13.8	0.000	0.001	0.999
iNDF	G	–1.3	1.00	0.893	16.9	0.000	0.000	1.000
	S	–49.7	1.08	0.802	14.3	0.000	0.000	1.000
Mertens (2002b)	G	–78.3	1.12	0.912	16.3	0.001	0.106	0.893
	S	–13.6	1.02	0.930	13.7	0.001	0.005	0.994

[a] G = General equation for all forages; S = Forage type specific equations.
[b] Mean squared prediction error

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tors, since at maintenance level the differences in compartmental residence time are unlikely to be large enough to explain the observed differences in pdNDF digestibility. For example, if k_d is 0.05 per h, mean compartmental residence time should increase from 50 to 90 h to increase pdNDF digestibility from 0.80 to 0.90. Similarly, with 50 h compartmental residence time k_d should increase from 0.05 to 0.09 per h to increase pdNDF digestibility from 0.80 to 0.90, respectively. Attempts to establish relationships between feed chemical fractions and k_d of fibre have had little success (for review see Huhtanen et al. 2006). The relationships may be reasonable within a forage type, but overall relationships are poor. There are two prerequisites for the dynamic models to improve predictions of D-value: the method must be accurate in predicting the true pdNDF digestion rate (1) and it must be more precise than the current empirical approaches (2). Until now the progress in this area has been limited by the lack of *in vivo* validation data. Most of the k_d studies have compared different laboratory and *in vitro* methods and the data has been mainly qualitative ranking of feedstuffs.

The studies conducted at MTT have suggested that *in vitro* gas production technique (for review see Schofield 2000) is a promising tool for estimating k_d of NDF. When the parameter values derived from gas production kinetics of isolated NDF were used in dynamic rumen models, *in vivo* NDF digestibility was predicted both accurately and precisely (Huhtanen et al. 2001, Rinne et al. 2006). The data from *in vivo* digestion trials can be used to estimate digestion rate by solving the equation of Allen and Mertens (1988) for k_d by assuming a fixed compartmental residence time (Huhtanen et al. 2006). Digestion rates estimated from isolated silage NDF with *in vitro* gas production technique and those calculated from the *in vivo* data were strongly correlated ($R^2 = 0.90$) without mean bias. In contrast, ruminal *in situ* incubation markedly underestimated the *in vivo* digestion rate (Huhtanen et al. 2006).

The current empirical and summative models are probably accurate and precise enough to predict the D-value at maintenance level and hence are suitable for calibration of NIRS equipment for practical feed evaluation of farms samples. However, the future feed and ration evaluation models such as Karoline (Danfær et al. 2005) need accurate and precise estimates of the kinetic parameters of NDF digestion. The existing energy values predicted from feed digestibility at the maintenance level still form the sound basis for feed evaluation systems, but dynamic models are needed to cope with the interactions between dietary components at different feeding levels.

Near infrared reflectance spectroscopy

Implementation of computerised chemometrics based on near infrared reflectance spectroscopy (NIRS) of homogenised feed samples was a major innovation that made the recent developments of forage evaluation research available for practical farmers. Since Norris et al. (1976) first introduced the NIRS equations for predicting forage quality, much success has been achieved in developing NIRS for the forage analysis (for a review, see Deaville and Flinn 2000). The parallel development of computers, optical devices and calibration software have stimulated the progress of NIRS applications in feed analysis. The purpose of this chapter, however, is not to review the theory behind NIRS, instrumentation, sample treatment and presentation, mathematical treatments of spectral data and calibration methods; instead, the reader is referred to the numerous textbooks and reviews (see e.g. Williams and Norris 1987, Windham et al. 1989, Reeves 2000). Herein the developments in NIRS equations for predicting D-value of forages that are typically produced in Finland are discussed.

Near infrared reflectance spectrum (usually from 1100 to 2500 nm) of forages contains specific absorbance regions e.g. for water and protein (see Deaville and Flinn 2000), which both can be predicted relatively accurately by NIRS. In contrast, the predictions of forage fibre characteristics and OMD in particular are more challenging because these traits are not definite chemical entities and do not have specific absorbance bands in the NIR spectrum. Therefore, NIRS has been criti-

cized as a "black box" method for predicting feed characteristics of value for animal nutrition. However, as indicated by Deaville and Flinn (2000), interpretation of published NIRS equations reveal that OMD of forages is often associated to spectral regions near to 1650–1670 and 2260–2280 nm (see Fig. 8). Nousiainen et al. (2004) demonstrated that these regions were negatively correlated to grass silage iNDF as determined with long (288 h) ruminal *in situ* incubation and that the standard normal variate and de-trended (see Barnes et al. 1989) correlation spectrum for lignin and iNDF showed much resemblance. Previous findings by Russell et al. (1989) also relate these spectral regions to lignin bonding, thus providing scientifically valid background for determining forage fibre characteristics by NIRS.

The essential advantage of NIRS is the speed and economy of forage evaluation. The accuracy of NIRS results is related to the scope of the data set used to calibrate for forage digestibility predictions. A wide range of reference values are needed for NIRS calibration data set to predict forage digestibility, even when intended to be applied to a specific forage type. For forage D-value prediction, *in vivo* digestibility would be the most logical reference method, as was the first application in Finland (Hellämäki 1992). Though the reference data set included a reasonable number of samples (n = 90) and the performance of calibration was satisfactory [standard error of calibration 15.3 g kg^{-1}], it resulted in biased predictions when applied to unknown samples. The evident reason for this was too narrow a range of D-values in the reference data (SD 36 g kg^{-1}).

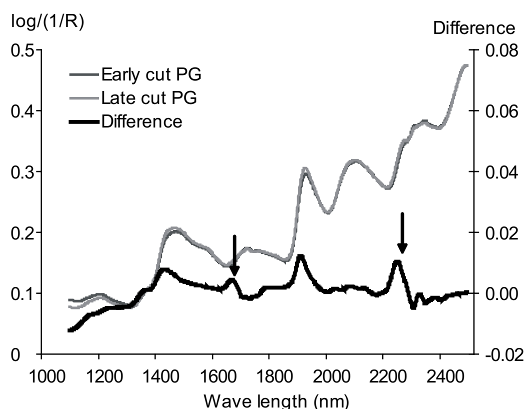

Fig. 8. Near infrared reflectance spectrum of very early (D-value 764 g kg^{-1} DM) and late (D-value 586 g kg^{-1} DM) cut silages made from primary growth (PG) grass in 1996; the arrows in the difference spectrum indicate the important wave length areas that are associated with digestibility.

Further, much spectral variation is caused by the unhomogeneous nature of forages attributable to species and variety differences, ensiling methods, harvest (primary vs. regrowth) and possibly climatic factors. As a consequence, several hundred reference samples are required for a multi-species forage population (Deaville and Flinn 2000), which makes the use of *in vivo* digestibility data as a reference method essentially unpractical and expensive. Hence, a biologically valid *in vitro* reference method is needed, i.e. validation against *in vivo* data. Due to obvious advantages for a commercial forage laboratory, the pepsin-cellulase method as described by Friedel (1990) was chosen for the reference method (Klemetti et al. 1995), and in addition to *in vivo* samples, the calibration data set was extended with data from on-farm silages to increase the D-value range and spectral variation. The resulting calibration performed acceptably (validation R^2 = 0.752 and SEP/SD = 2.1), but produced unrealistically high D-value predictions for silages made from regrowth grass. Later it appeared that the single correction equation for OMS introduced by Friedel (1990) does not generally apply to different forage types (discussed earlier in this paper).

In conclusion, the total prediction error of a NIRS D-value calibration is strongly dependent on the biological validity of the *in vitro* reference method. Nousiainen (2004) compared different reference methods for grass silage D-value and demonstrated that when the proportion of reference error increases, the total NIRS prediction error (observed$_{in\ vivo}$ vs. predicted$_{NIRS}$) increased significantly. Thus a good NIRS calibration and validation statistics does not automatically guarantee acceptable total prediction performance, and if not

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recognised, this may lead to serious misuse of NIRS in nutritional applications.

The calibration and validation statistics of NIRS D-value equations for forages used between the years 2003–2005 in Finland is shown in Table 11. The calibrations were produced either using general or forage type specific (2003 vs. 2004 and 2005) OMS prediction equations. The number of samples also differed between the calibrations as well as math treatment of the spectral data (first vs. second order derivatization in calibration 2003 and 2004 vs. 2005, respectively). The calibrations were applied to experimental silages, and the total prediction errors ($Observed_{in\ vivo}$ vs. $Predicted_{NIRS}$) were calculated (Table 12). Despite the best cross-validation results for the 2003 calibration (i.e. best precision), it produced the lowest accuracy of the D-value estimates compared to *in vivo*, mainly owing to over- and under-prediction of silages made from regrowth and primary growth grass with general OMS equation, respectively. This problem was only partly solved in the 2005 calibration as

Table 11. Near infrared reflectance spectroscopy calibration and validation statistics for silage D-value (J. Nousiainen et al. unpubl.).

Calibration	OMS equation[c]	N[b]	Mean	s.d.[d]	Calibration[a]			Cross validation		
					Math	SEC[e]	R^2	SECV[f]	R^2	SD/SECV
2003	General	750	672	34.7	1,4,4,0	10.8	0.903	11.7	0.887	2.97
2004	Specific	994	660	45.6	1,4,4,1	18.3	0.839	19.1	0.824	2.38
2005	Specific	1159	658	46.4	2,4,4,1	16.5	0.874	17.6	0.857	2.64

[a] For description of equipment, scanning, sample and spectral treatment and calibration methods, see Nousiainen et al. 2004
[b] Including on-farm produced grass, legume and whole crop silages
[c] Reference D-values were calculated as D-value = OM × OMD and pepsin-cellulase organic matter solubility was used to predict OMD either with general or species specific correction equation (see Table 4)
[d] Standard deviation of the reference population
[e] Standard error of calibration
[f] Standard error of cross validation (see Nousiainen et al. 2004)

Table 12. Comparison of total prediction performance of three near infrared reflectance spectroscopy D-value calibrations applied to primary growth (PG) grass, regrowth (RG) grass and legume silages and within forage species prediction performance with a calibration using forage-specific OMS equation as reference method.

	Intercept	Slope	R^2	MSPE[b]	Distribution of MSPE		
					Bias	Slope	Random
Calibration[a]							
2003	–19	1.02	0.623	31.9	0.04	0.00	0.96
2004	97	0.85	0.689	29.3	0.00	0.06	0.94
2005	9	0.99	0.783	23.7	0.00	0.00	1.00
Calibration 2005							
PG grass	30	0.97	0.902	19.9	0.28	0.01	0.71
RG grass	–68	1.08	0.688	22.8	0.50	0.01	0.49
Legume	100	0.85	0.661	29.6	0.03	0.06	0.92

[a] See Table 11
[b] Mean squared prediction error $= \sqrt{(\sum(Observed_{in\ vivo} - Predicted_{NIRS})^2/n)}$

indicated by decreases in under-prediction of PG silage from 13.6 to 10.7 and in over-prediction of RG silages from 34.5 to 16.3 g kg^{-1} DM. Consequently the proportion of bias for both PG (0.28) and RG (0.50) silage is still large (Table 12).

The harvesting year had a significant influence on NIRS prediction errors for D-value (Fig. 9). Apparently there are at least three sources or errors behind the year effects; animal differences in the *in vivo* experiments conducted in different years (1), variations in laboratory analyses between years (2) and variation in environmental conditions that may affect forage composition (3). Moreover, the effects of number of harvest and harvesting year seem to be additive for grass silages. With the last calibration (2005, Table 6) prediction error in D-value decreased to 14.8 g kg^{-1} DM when correcting results for year within forage effect. This figure is only slightly higher than the residual variation in digestibility trials of this data (13.8; Nousiainen 2004), but it is questionable whether the year effects within forage types can be totally excluded. However, it should be possible to reduce the prediction error to 16–17 g kg^{-1} DM with grass silages, i.e. to that attained without the mean bias error for the PG and RG silages, respectively.

It appears that despite attempts to use forage specific OMS equations, the bias between forage types still remained rather high. This may at least partly be associated with errors in coding the harvest (primary vs. regrowth) of on-farm samples used in the calibration data. Reference methods less dependent on forage type such as iNDF and summative models, or dynamic models in the future, may reduce this problem.

To evaluate the potential of different methods in calibrating NIRS for D-value prediction of on-farm forages, a comparison between *in vivo*, OMS, iNDF and summative model was made (Table 13). The reference values were based on either general or specific equations. All reference methods resulted in good calibration statistics (calibration $R^2 > 0.96$ and for cross-validation $R^2 > 0.91$; results not shown). This is in good agreement with the results presented previously by Nousiainen (2004), and describes the good precision of NIRS. The total prediction error (Observed$_{in\ vivo}$ minus Predicted$_{NIRS}$) was lowest for specific OMS and highest for general OMS. A noteworthy feature of the calibrations is that the difference in prediction error compared to OMS calibrations based on large data of on-farm samples (see Tables 12 and 13) is due to lower bias error. This describes the potential in improving the existing OMS calibration (2005, Table 12) either by correction the errors in coding the number of harvest or using iNDF or summative models as a reference.

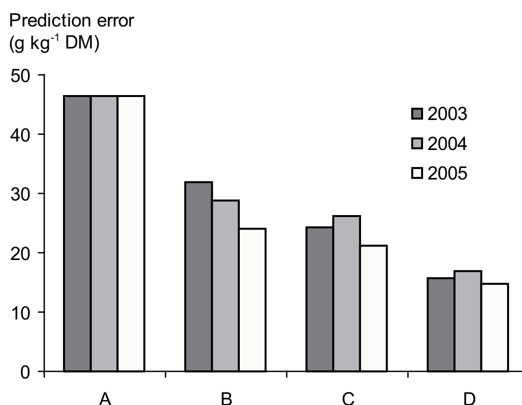

Fig. 9. Total D-value prediction errors (g kg^{-1} DM) of the three NIRS calibrations (see table 12): A = standard deviation of the reference population, B = NIRS calibration [$=\sqrt{(\sum(\text{Observed}_{in\ vivo}-\text{Predicted}_{NIRS})^2/n)}$], C = B and variation between forage types excluded, D = B and variation between year(forage) excluded.

Interpretation of sources of errors in determining D-value of forages

Provided that the true digestibility of NDS is unity and that the metabolic OM is constant, the variation in OMD is related only to pdNDF digestibil-

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Table 13. Comparison of near infrared reflectance spectroscopy calibration methods in predicting forage D-value based on experimental forages[a].

Reference for calibration[b]	Predicted$_{NIRS}$ vs. observed$_{in\ vivo}$				Mean bias, g kg^{-1} DM	MSPE distribution			Forage-specific MSPE g kg^{-1} DM			
	Slope	Intercept	R^2	MSPE[c], g kg^{-1} DM		Bias	Slope	Random	PG grass	RG grass	Legume	Whole crop
In vivo	0.97	17	0.946	12.0	–0.1	0.000	0.012	0.988	10.9	9.5	10.3	16.9
OMS S[d]	1.00	3	0.903	16.0	–0.4	0.001	0.000	0.999	15.9	16.1	16.0	17.2
OMS G[d]	1.01	–4	0.780	24.2	–1.9	0.006	0.000	0.994	26.9	23.8	25.6	16.1
iNDF S	1.01	–7	0.891	17.0	0.9	0.003	0.001	0.997	15.7	17.9	16.7	18.3
iNDF G	1.00	3	0.863	19.1	–0.2	0.000	0.000	1.000	17.1	21.1	19.1	19.6
Summative S	1.02	–14	0.894	16.8	0.9	0.003	0.003	0.994	15.3	18.5	16.8	16.9
Summative G	1.12	–75	0.886	18.1	–0.5	0.001	0.077	0.923	19.0	17.3	18.2	18.9

[a] Data comprising of silages made from primary (PG) or regrowth (RG) grass and leguminous or whole crop forages
[b] For description of equipment, scanning, sample and spectral treatment and calibration methods see Nousiainen et al. 2004
[c] Mean squared prediction error $= \sqrt{(\Sigma(\text{Observed}_{in\ vivo} - \text{Predicted}_{NIRS})^2/n)}$
[d] S = Forage type specific equations; G = General equation for all forages.

ity. If pdNDF concentration would be predicted accurately, prediction error of OMD in the present data set would be 0.0162. This error is partly related to the systematic and significant differences in faecal metabolic OM output between the forage types (Table 2), and partly to random variation in endogenous faecal output. Using forage specific equations decreased the prediction error of the faecal endogenous OM to 8.6 g kg^{-1}. This value may be considered as the potential minimum error of the laboratory methods in estimating forage OMD.

According to Van Soest (1994), the minimum variability in carefully conducted digestibility trials is 0.020. Nousiainen (2004) reported a value of 0.0138 from studies included in the present data. As suggested by Van Soest (1994), a difference of 0.020 in digestibility can be taken as the lower limit of biological significance of digestibility of feeds. This difference corresponds to a difference of about 1 kg d^{-1} in milk yield or that almost 2 kg d^{-1} more concentrates should be fed to compensate for the lower silage digestibility (Rinne 2000).

Errors in the *in vivo* OMD predicted with different methods can result from systematic errors between the forage types, random errors between the trials and random errors in determination of *in vivo* digestibility within trials. Contribution of the forage type on the prediction errors of OMD were analysed by one-way ANOVA using the GLM procedure of SAS (1999). The significance of random study effect was tested using a mixed model analysis with a fixed effect of forage type and a random study effect. Possible contribution of the random variability of the *in vivo* trials was estimated as a relationship between the errors using a mixed model regression analysis with a random study effect. It may be assumed that a strong correlation of the errors in OMD predictions is at least partly related to random variability of the *in vivo* data. Data estimated using forage specific equations was used for this analysis.

When the general prediction equations were used for all forage types, the residual mean squared prediction errors were in many cases significantly different from zero, i.e. the prediction accuracy was dependent on the forage type (Table 14). The OMS method underestimated the *in vivo* D-value of PG silages and overestimated that of RG and whole-crop silages. Lignin either markedly overestimated (RG grass and whole-crop silages) or underestimated (PG grass and legume silages) D-

Table 14. Mean prediction errors[a] of D-value for primary growth and regrowth grass, legume and whole crop silages predicted using different laboratory techniques.

Method	Primary growth	Regrowth	Legume	Whole crop	RMSE	P-value
OMS[b]	**–19.9**[f]	**16.1**	1.8	**24.8**	14.9	<0.01
iNDF[c]	1.4	5.6	**–9.6**	–2.2	16.3	<0.05
Lignin	**20.8**	**–29.6**	**22.8**	**–39.2**	28.0	<0.01
NRC (2001)	**75.8**	**45.9**	**39.2**	**–34.0**	23.5	<0.01
Mertens (2002b)[d]	**28.0**	**–20.2**	3.0	**–48.1**	25.0	<0.01
Mertens (2002b)[e]	**6.1**	–1.9	–0.5	–10.5	16.0	<0.10

[a] (Observed – Predicted)
[b] Pepsin-cellulase solubility of organic matter
[c] Indigestible fibre (determined by 12 d *in situ* incubation)
[d] Estimated from lignin
[e] Estimated from iNDF
[f] Values printed in bold are statistically different from zero (P < 0.05)

value. The differences in the mean bias were large between the forage types irrespective of the prediction being based on empirical relationship or summative approaches. The greater effect of forage type with lignin may be related to differences in the ratio between lignin and iNDF among the forage types, as discussed by Nousiainen et al. (2004). The behaviour of iNDF and summative models was more uniform among forage types.

The random study effect was significant for OMS, iNDF and Mertens (2002b) summative equation based on iNDF, when the variation resulting from forage type was excluded. These effects may be attributed to differences between the animals used in the trials, differences in the activity of enzymes used in the determination of OMS and to differences in microbial activity in the rumen of the cows used for determination of the iNDF concentration of the forages. The study effect was not significant for lignin equations, which may be related to a greater variability of the errors being presumably attributable to high random variation in lignin analysis and/or climatic factors influencing cell wall lignification.

All prediction errors were significantly (P < 0.01) correlated with each other within a trial (Table 15). The relationship was strongest between the methods based on iNDF and summative system (Mertens 2002b; iNDF), probably because of the strong influence of iNDF on predicted OMD in the summative system. Significant relationships between the prediction errors within a trial even when the most contrasting systems (e.g. OMS vs. iNDF and OMS vs. summative system) were compared, suggests that random errors of the *in vivo* digestibility determinations had some contribution to the overall prediction error. If one method overestimated the *in vivo* OMD of a feed, the probability that another method also overestimated it, was high. Corresponding conclusions were made by Rinne et al. (2006) from similar analysis of legume silage data. Based on the mean standard error (0.0138) of OMD of the present data and 4 sheep per feed, confidence interval of P = 0.90 will be ±0.023.

Calculating a reference value as a weighted mean of *in vivo* OMD (0.50) and the mean of three other laboratory methods (0.50), i.e. excluding the method being evaluated, led to markedly reduced prediction errors of OMD. The mean squared prediction errors were 0.013, 0.011, 0.021 and 0.011 for OMS, iNDF, lignin and the summative system, respectively, when the values were based on forage specific equations. Except for lignin, these values are even slightly lower than the standard error of the *in vivo* data (Nousiainen 2004) and close to the theoretical minimum of about 0.008, when all the variation results from random variation in faecal

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Table 15. Relationships between the prediction errors of organic matter digestibility estimated by organic matter pepsin-cellulase solubility (OMS), indigestible neutral detergent fibre (iNDF) and lignin using the forage specific equations.

Y Variable	X Variable	A	s.e.[a]	B	s.e.	P-value	Adj. R2
OMS	iNDF	0.6	2.09	0.77	0.446	<0.01	0.391
OMS	Lignin	0.2	2.19	0.93	0.268	<0.01	0.228
OMS	Summative[b]	0.8	2.07	0.69	0.592	<0.01	0.578
iNDF	Lignin	–0.2	2.07	0.93	0.311	<0.01	0.459
iNDF	Summative[b]	0.5	0.63	0.39	0.969	<0.01	0.890
Lignin	Summative[b]	2.1	2.79	0.45	1.038	<0.01	0.514

[a] Standard error
[b] Mertens (2002b) equation with iNDF

endogenous OM. Correlation between the errors originating from different prediction methods (1), relatively large confidence interval of OMD even in carefully conducted digestibility trials (2) and markedly lower prediction errors when the reference value was based on a weighted mean of *in vivo* and other laboratory methods (3) all suggest that the true prediction error of the laboratory methods is likely to be smaller than the calculated errors suggest. Interestingly, the prediction errors of NIRS D-values were strongly correlated with the prediction errors of laboratory methods (Fig. 10). For OMS, this is partly attributed to using OMS predicted D-values as a reference method for NIRS calibrations, i.e. errors in reference values would automatically reflect errors in predicted values. However, the highly significant ($P < 0.01$) relationship between the errors within forage(year) strongly supports the earlier suggestion about random errors of the *in vivo* values. Consequently, the true errors of both the laboratory methods and NIRS are likely to be smaller than the estimated errors.

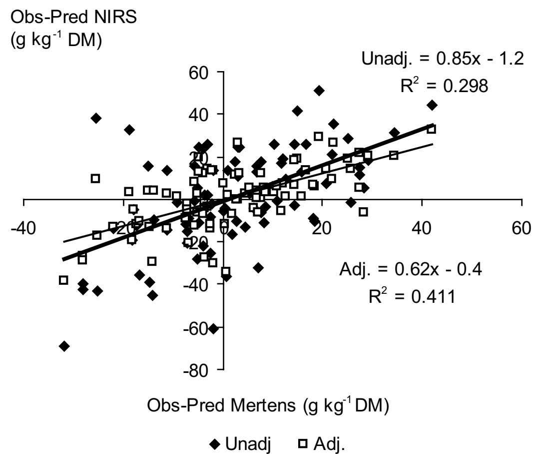

Fig. 10. Relationships between errors of D-value (Observed – Predicted) estimated by the summative model (Mertens 2002b) and by NIRS calibrated with forage-specific organic matter pepsin-cellulase solubility (calibration 2005).

Implications

The present re-evaluation based on a systematically collected dataset confirmed the weaknesses of the proximate feed analysis. The revised detergent system should be used instead. Predicting *in vivo* organic matter digestibility with the empirical equations using chemical parameters gave unsatisfactory results. Pepsin-cellulase solubility predicted forage OM digestibility with an acceptable accuracy but the drawback of the method is the forage type, environmental and laboratory dependency. To reduce the D-value prediction error further, regression equations based on indigestible NDF or

summative models using uniform feed fractions from the detergent analysis and long-term *in situ* ruminal incubation may be used. These methods are also an interesting alternative to pepsin-cellulase solubility as a reference for NIRS applications in practical feed evaluation. Another option in the future may be dynamic models. However, a vital prerequisite for using dynamic models in practical feed evaluation is that iNDF and k_d can be easily and reliably determined from on-farm forages. Although a NIRS prediction equation for iNDF will be adopted in practical use in the near future in Finland, the respective methodology for k_d warrants further research.

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SELOSTUS

Karkearehujen sulavuuden määrityksen viimeaikainen kehitys ja käytännön sovellukset

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Systemaattisesti kerätyn säilörehuaineiston perusteella tehty yhteenveto osoittaa selvästi ns. virallisen rehuanalyysin eli Weenden analyysin biologiset puutteet rehujen ravitsemuksellisen laadun kuvaajana. Analyysi ei kuvaa rehun kemiallisen koostumuksen ja sulavuuden välisiä syy-seuraussuhteita. Lisäksi tilastolliset yhteydet vaihtelevat huomattavasti eri kasvimateriaaleilla ja ympäristöolosuhteissa. Weenden analyysin käyttöä ei siis voi suositella karkea- eikä väkirehujen laadun kuvaamiseen. *In vitro* pepsiini-sellulaasiliukoisuus (OMS) ja sulamattoman kuidun (iNDF) pitoisuus sen sijaan ennustivat karkearehujen orgaanisen aineen sulavuuden riittävän tarkasti käytännön ruokinnansuunnittelua varten, edellyttäen että analyysitulokset muunnettiin sulavuudeksi rehutyyppikohtaisia korjausyhtälöitä käyttäen eli erikseen ensimmäisestä sadosta ja jälkikasvusta tehdyille nurmisäilörehuille, palkokasvisäilörehuille ja kokoviljasäilörehuille.

Detergenttikuituanalyysi, joka jakaa rehun kuiva-aineen liukoiseen ja lähes täysin käyttökelpoiseen solunsisällykseen (NDS) sekä liukenemattomaan kuituun (NDF), on Weenden analyysiä huomattavasti kehityskelpoisempi vaihtoehto. Kun kuituanalyysiin yhdistetään pitkä *in situ* pötsi-inkubaatio, rehun kuiva-aine saadaan jaettua kolmeen biologisesti mielekkääseen osaan: NDS, potentiaalisesti sulava kuitu (pdNDF) ja iNDF. Rehun D-arvo eli sulavan orgaanisen aineen pitoisuus kuiva-aineessa voidaan ennustaa ns. summatiivisella yhtälöllä. Yhtälössä lasketaan yhteen sulanut NDS, joka voidaan määrittää Lucasin yhtälöllä, ja sulanut kuitu (pdNDF-pitoisuus × pdNDF:n sulavuus tai vaihtoehtoisesti NDF-pitoisuus × NDF:n sulavuus). Rehutyyppikohtaiset summatiiviset yhtälöt ennustivat karkearehujen D-arvon lähes yhtä hyvin kuin OMS ja iNDF. Kun koko aineistoa tarkasteltiin yhdessä, summatiiviset yhtälöt olivat parempia kuin iNDF ja erityisesti OMS.

Jos D-arvon ennustevirhe halutaan saada pienemmäksi kuin 15 g/kg kuiva-ainetta, on käytettävä rehutyyppikohtaisia yhtälöitä riippumatta siitä, onko laskennan perusteena OMS, iNDF tai summatiivinen yhtälö. Toinen vaihtoehto tulevaisuudessa on dynaamisten mallien käyttö. Ne pystyvät samanaikaisesti huomioimaan kaksi tärkeää dynaamista prosessia, jotka rajoittavat rehun sulatusta pötsissä eli kuidun virtaus- ja sulatusnopeuden. Dynaamisten mallien käyttö edellyttää kuitenkin sitä, että rehuista voidaan helposti ja luotettavasti määrittää iNDF-pitoisuus ja kuidun sulatusnopeus. Maatilarehujen iNDF-määritys NIRS-menetelmällä toteutuu Suomessa lähiaikoina, mutta kuidun sulatusnopeuden määritys vaatii vielä lisätyötä.