MODELS TO ESTIMATE THE NUTRITIVE VALUE AND INTAKE OF CONSERVED FORAGES

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Introduction

Ruminant animals have a unique ability to produce high quality human food from resources (pastures, forages, by-products) that otherwise cannot be utilised for food production. This is based on the symbiosis between rumen microbes and host animal: the host provides ideal conditions for anaerobic fermentation in the rumen and the microbes produce volatile fatty acids (VFA) and microbial cells that are the main energy and protein sources for the host animal. Rumen microbes can ferment both fibre and non-fibre carbohydrates (NFC), whereas mammalian enzymes can digest only NFC. However, microbial fermentation in the rumen does not take place without losses, since part of the digestible energy is lost as methane and fermentation heat, and microbial cells are not completely digestible in the animal.

Variation in nutritive value of forages is much greater than that of concentrate feeds. Therefore, an accurate and precise determination of the nutritive value of forages is required for optimal utilization of farm-produced forages in ruminant livestock. Determination of forage feeding value is also important in attempts to minimize the impact of ruminant livestock production systems on the environment. Most emphasis in research has focused on the determination of forage digestibility/energy concentration and protein value. However, the intake potential has a much greater influence on nutrient intake than forage nutrient concentrations. Intake potential can be considered as an intrinsic characteristic of forages at the time of harvest, but the changes in forage composition during conservation can markedly influence the extent to which this potential is realized. The intake potential of forage is usually closely associated with its nutritive value emphasizing the importance of accurate estimation of forage digestibility.

The methods available for the evaluation of the nutritive value include chemical analysis, *in vitro* digestibility with rumen bacterial inoculum or enzymes, *in situ* incubations in nylon bags

in the rumen and near infrared reflectance spectroscopy (NIRS). With ensiled forages both the extent and type of in-silo fermentation affect intake potential stressing the importance of the analysis of silage fermentation characteristics. In addition to the analysis of feed parameters, appropriate models are required to predict nutritive value and intake potential from these parameters.

The objectives of this paper are to discuss the models and methods used in the evaluation of the nutritive value and intake of conserved forages. A special emphasis is focused on the digestibility determination due to its fundamental role in determining both energy and protein value of forages as well as intake potential.

Digestibility

Faecal energy is a greater and more variable loss of feed gross energy than methane and urinary energy, and therefore accurate determination of digestibility is a prerequisite in the determination of feed metabolizable energy (ME) concentration and of forage/diet intake potential. Much effort has been directed toward developing empirical regression equations that relate various chemical components to digestibility. These attempts have not been very successful because of the lack of real causal relationships, and large interspecies and environmental effects on the relationship between composition and digestibility (Van Soest 1994). Within year and forage species the relationship between chemical parameters and digestibility are usually reasonably good, but in global regression analysis over years and forage types the relationships were poor and residual standard deviation (RSD) was not markedly less than the standard deviation (SD) of digestibility in the data (Huhtanen et al., 2006a). Because forage indigestible NDF (iNDF) fraction in cell wall is attributable to cross-linking between lignin and hemicellulose when plants mature (Van Soest, 1994), attempts have been made to predict digestibility and iNDF from lignin concentration in DM or NDF (see Traxler et al., 1998). Despite this biological relevance, it has not been successful due to relatively high proportional errors in lignin analyses, as well as differences between forage types in lignin to iNDF ratio (Huhtanen et al., 2006). Within forage type permanganate lignin was clearly better predictor of digestibility than fibre fractions and CP, but RSD was greater than with biological methods.

Definitions

NDF (g/kg DM) = neutral detergent fibre. NDF represents the cell wall fraction of feed that be digested only by microbial enzymes. Pectins and β-glucans are also plant cell wall, but they are completely digested in ruminants. Digestibility of NDF is highly variable.

NDS (g/kg DM) = neutral detergent solubles. NDS can be digested by both mammalian and microbial enzymes. Originally NDS was defined as DM – NDF, but because ash does not provide any energy to the animals, organic matter (OM) – NDF could nutritionally be a more appropriate definition for NDS. NDS is considered as a uniform nutritional entity, i.e. it has a constant (0.98) true digestibility (Van Soest, 1994). There is indication that some differences exist in the true digestibility of NDS between forage types (Huhtanen et al., 2006).

iNDF (g/kg DM) = indigestible NDF. Digestibility of iNDF is by definition constant (zero) and therefore iNDF is a uniform nutritional entity. It is an intrinsic feed character and it is usually determined a long-term *in situ* incubation in the rumen (Huhtanen et al., 1994) or *in vitro* (Van Soest et al., 2004). Nylon bags of small pore size (6 – 17 µm) is used to avoid particle losses and long incubation time to ensure that maximum extent of digestion is obtained.

pdNDF (g/kg DM) = potentially digestible NDF = NDF – iNDF. Digestibility of pdNDF is variable, but variation is much less variable than total NDF digestibility. Typically the digestibility of pdNDF is approximately 0.85 in sheep fed forages at maintenance level (Huhtanen et al., 2006a) and 0.75 in dairy cows fed mixed grass silage based diets (Nousiainen et al., 2009).

uNDF (g/kg DM) = undigested NDF = faecal NDF. uNDF is a fraction of DM that is excreted in faeces. Because pdNDF is not completely digested in the animal within the residence time in the digestive tract, uNDF is always greater than iNDF. In the literature terminology can be confusing, since iNDF is sometimes used to denote uNDF.

updNDF (g/kg DM) = undigested pdNDF = uNDF – iNDF. This fraction represents the variable loss of potentially digestible OM in faeces, since faecal NDS is mainly of metabolic and endogenous origin and represents obligatory loss of potentially digestible OM.

NDFD (fraction of unity) = NDF digestibility = (NDF – uNDF) / NDF.

pNDFD (fraction of unity) = potential extent of NDFD = pdNDF / NDF = (NDF – iNDF) / NDF

pdNDFD (fraction of unity) = pdNDF digestibility = (pdNDF – uNDF) / pdNDF = [(NDF – iNDF) – uNDF) / (NDF – iNDF). Digestibility of pdNDF is always higher than that of NDF. Note that pdNDFD is not the same as pNDFD.

dNDF (g/kg DM) = digested NDF = NDFD × NDF = pdNDFD × pdNDF = NDF - uNDF. Note that dNDF is smaller than pdNDF, since pdNDF is not completely digested.

Digestible NDS (digestible NDS) = a + b × NDS (b =true digestibility ~ 0.98, a = metabolic faecal OM ~ 100 g/kg DMI). Metabolic faecal OM consists of undigested microbial cells synthesized in the rumen, microbial cells produced in the hind-gut, sloughed epithelial cells and digestive enzymes that are not reabsorbed.

D-value (g/kg DM) = concentration of digestible OM = dNDF + dNDS;

Biological methods

Owing to the constraints in routine measurements of *in vivo* digestibility, several *in vitro* laboratory methods have been used for estimating forage digestibility. The two-stage rumen fluid *in vitro* technique developed by Tilley and Terry (1963) and Goering and Van Soest (1970) are the most widely used methods. Both techniques are based on the incubation of forage samples in rumen fluid for 48 h followed by incubation in pepsin-HCl (Tilley and Terry, 1963) or extraction with neutral detergent (Goering and Van Soest, 1970). Tilley and Terry (1963) demonstrated a close correlation between DM digestibility (DMD) determined *in vivo* and *in vitro* and reported that the values determined based on *in vitro* incubations were almost the same as DMD determined in sheep.

Due to difficulties in obtaining rumen fluid in commercial laboratories, standardization of the *in vitro* incubation system, variation in the activity of rumen fluid and increasing concern over the use of surgically modified animals, enzymatic *in vitro* procedures for the determination of forage digestibility have been developed and evaluated (Jones and Theodorou, 2000). The cellulase method differs from measurements of *in vivo* digestion in at least two key aspects: no endogenous matter is produced such that solubility reflects true rather than apparent digestibility,

and the capacity of commercial enzymes to degrade cell wall carbohydrates is lower than that of rumen microbes (McQueen and Van Soest, 1975; Nousiainen, 2004). Therefore regression equations are required to predict organic matter digestibility (OMD) from cellulase solubility. In a recent evaluation based on 86 forage samples of known *in vivo* digestibility measured in sheep the coefficient of determination (R^2) was 0.80 and RSD 0.025 units (Huhtanen *et al.*, 2006a). Because the relationship between pepsin-cellulase solubility (OMS) and *in vivo* OMD was highly dependent on forage type, using a forage specific correction equation was found to increase the R^2 of this relationship to 0.93 associated with a decrease in RSD to 0.0152. Recent study (Jančík et al., 2011) indicates that the relationship between OMS and OMD may also vary between the species of primary growth grass.

Determination of iNDF concentration either by extended *in vitro* or *in situ* incubation divides forage OM into three components: NDS, iNDF and pdNDF. Two of these fractions (NDS, iNDF) are nutritionally uniform entities that have a constant digestibility; NDS is (almost) completely digestible and iNDF by definition is indigestible. The close relationship between iNDF determined by 12-d *in situ* incubations in nylon bags of small pore size and *in vivo* OMD was first demonstrated by Nousiainen et al. (2003) and later confirmed by a larger number of samples of different forage types (Huhtanen et al. 2006a). In the later study the relationship between iNDF and *in vivo* OMD was more uniform for iNDF compared with the OMS (0.019 vs. 0.025). For the prediction of D-value the following simple regression model was derived from this data:

> D-value (g/kg DM) = 891±18.5 - 1.19±0.043 × iNDF - 0.09±0.021× pdNDF – 1.02±0.14 × Ash (n=86, R^2 = 0.91, RSD =15.7)

All parameters were statistically significant ($P < 0.001$) and biologically meaningful. The deviation of the intercept from 1000 (by 109 g/kg) is biologically equivalent to the metabolic faecal OM output. The coefficient -0.09 for pdNDF indicates that maximum digestibility of forage pdNDF was 0.91, whereas the coefficient < -1.0 for iNDF suggests a decrease in pdNDF digestibility with increased iNDF concentration. An advantage of iNDF is that it can be successfully calibrated for NIRS (Nousiainen et al., 2004)

Combining the data from a recent study by Krizsan et al. (2011) and Huhtanen et al. (2006a) resulted in total 128 forages. Also in the combined dataset iNDF predicted *in vivo* OMD more precisely than OMS (R^2 = 0.87 vs. 0.78; RSD = 0.0216 vs. 0. 0278). Greater RSD in the

combined dataset may be due to more heterogeneous set of samples or greater RSD of *in vivo* OMD. In the study of Krizsan et al. (2011) iNDF tended to under-predict OMD of lucerne samples and over-predict OMD of straw samples, especially treated straw.

Sources of RSD

It is often believed that prediction errors of OMD by the *in vitro* methods are entirely due to the inaccuracy of the *in vitro* methods. However, there is also random variation in determination of *in vivo* OMD. Van Soest (1994) stated that SD between the animals in carefully conducted digestibility experiments is 0.02 units, i.e. standard error (SE) of OMD estimates in typical digestibility trials with 4 sheep per feed would be 0.01. The mean SE in digestibility trials conducted according to Latin square was smaller (0.007; Nousiainen, 2004), probably because animal variation is excluded from the residual. Unless the number of sheep in digestibility trials is increased above four, 0.007 – 0.010 units could be considered as the minimum RSD of OMD prediction by the laboratory methods. This excludes sampling errors, random variation in the laboratory methods and true variation in the relationship between laboratory and *in vivo* estimates of OMD. In our datasets SD of replicate samples was 5 g/kg for iNDF and OMS methods, and 9.5 – 11.0 g/kg for the *in vitro* methods using 48 or 96 h incubation in rumen fluid. Sampling error (4 samples per feed) for OMS was 8 g/kg. Smaller SD for the iNDF than rumen fluid *in vitro* methods rumen is probably due to the longer incubation period.

A Monte Carlo simulation study was conducted to evaluate feasible levels of RDS in predicting OMD by the laboratory methods. The dataset (n = 86 forages) of Huhtanen et al. (2006a) was used to develop synthetic data by using the relationship between OMS and OMD estimated by a simple regression analysis (RSD = 0). The following sources of errors (SE; g/kg) were assumed for OMS: sampling error 4 ($8 / \sqrt{4}$) and error of duplicate OMS analysis 3.5 ($4 / \sqrt{2}$) ; and for OMD: random error of Latin Square (4×4) digestibility trial 0.007 and random error of group of four sheep (SD = 0.020 → SE = 0.010). Mean RSD of OMD prediction in 1 000 simulations was 0.0126 units that is smaller than observed RSD within forage type (0.0152). Excluding the effect of sheep group RSD reduced to 0.0082 that is smaller than observed RSD adjusted for the random study effect (0.0106). Greater observed values could result from the true variation in the relationship between OMS and OMD. However, it should be noted that both OMS and OMD were analyzed in the same laboratory. When the datasets are

compiled from different laboratories, RSD is likely to increase because greater between than within laboratory variation in determination *in vitro* digestibility and also because of between laboratory differences in the *in vivo* digestibility (Cochran and Galyean, 1994).

Mechanistic models

Models, which are based on fractionating feed components into entities that behave uniformly, can be considered mechanistic. Van Soest (1967) developed a comprehensive system of feed analysis and its application to forages. He divided the feed into NDS fraction that is essentially completely available, but its digestibility is apparently incomplete and variable because of faecal endogenous and microbial non-cell wall material. The second fraction corresponds to the plant cell wall and its availability is controlled by structural features that link cellulose, hemicellulose and lignin together. Cell wall fraction is not uniform between forages. Goering and Van Soest (1970) presented a summative model to describe availability of forage DM:

dDM = 0.98 × NDS + NDFD × NDF – M, where dDM = digestible DM, NDFD = coefficient of NDF digestibility, and M = microbial and endogenous faecal output. Theoretically this model is sound, but generally NDFD is not known. Conrad et al. (1984) developed this model further by dividing feeds into NDS and pdNDF. 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 obtain an estimate of the pdNDF. Weiss et al. (1992) revised the Conrad et al. (1984) model and it was adopted by NRC (2001) to estimate total digestible nutrients (TDN). In the revised model NDS fraction was divided into CP, fatty acids (FA) and NFC. The NFC fraction is calculated as difference (OM – CP – Fat – NDF). Neutral detergent fibre in the equation is corrected for NDF-bound CP (NDICP) to avoid double subtraction; NDICP is also included in total CP concentration.

The model was evaluated with data from digestibility trials with sheep fed at the maintenance level conducted in Finland (Huhtanen et al., 2006a). The data included 77 silages (primary and regrowth timothy-meadow fescue grass, red clover, and whole crop barley or wheat). Lignin was analysed as permanganate lignin (NRC model use ADL = acid detergent lignin). Concentration of NDICP was assumed to be 0.15 of total CP (Rinne et al., 1997). During in-silo fermentation a large proportion of NDICP is degraded (Jones et al., 1992; Rinne et al., 1997) and the proportion of NDICP is less than in dried forages. Truly digestible NDF is

$$tdNDF = 0.75 \times (NDF - NDICP - Lignin) \times [1 - (Lignin/(NDF - NDICP))^{0.667}].$$

Truly digestible CP (tdCP) is calculated as tdCP = CP × exp (-1.2 × ADICP/CP), where ADICP is acid detergent insoluble protein. An empirical equation was derived by regressing iNDF concentration against proportion of ADICP of CP:

ADICP (g/kg CP) = 0.256 × iNDF (g/kg DM) + 6.7 (N = 36; R^2 = 0.76, RMSE = 5.0). The samples were from the dataset used in the model evaluation.

A FA concentration of 20 g/kg DM was assumed. Variation in forage FA concentration is small and mainly related to the maturity at harvest (Advidsson, 2009). The effects of possible deviations from 20 on TDN would be minimal, since the model assumed complete digestibility for FA and 0.98 for NFC; a deviation of 10 g/kg DM only corresponds to 0.2 g/kg DM in digestible OM and higher TDN of FA would influence similarly on predicted TDN and *in vivo* TDN.

The model clearly underestimated tdNDF (Figure 1). The RSD was 57 g/kg and the mean bias (mean observed - mean predicted) was 44 (SE = 3.8; $P < 0.001$). The model under-predicted especially early harvested primary growth grass silages, whereas tdNDF of whole-crop silages was over-predicted. Under-prediction can partly be related to using permanganate lignin instead of ADL, since ADL concentrations are usually approximately 0.80 of that of permanganate lignin. However, using a relative value of 0.80 of determined lignin concentration decreased mean bias only from 44 to 32 g/kg DM and the range of residual did not markedly reduce (164 vs. 160 g/kg DM).

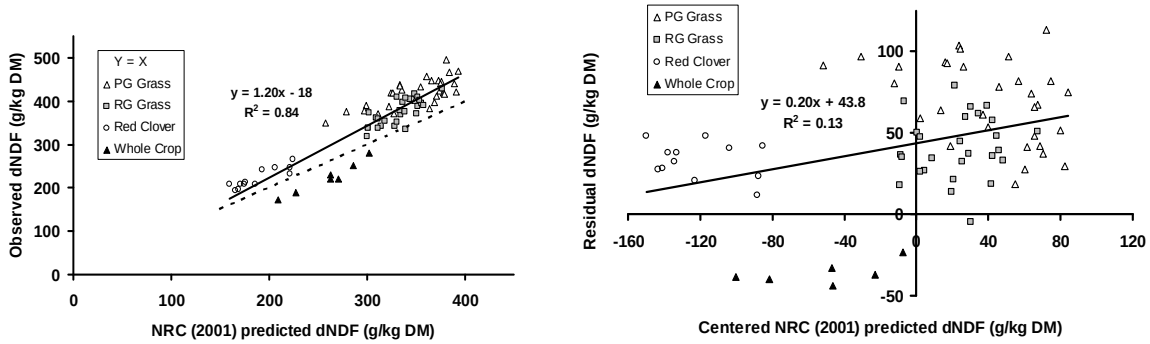

Figure 1. Observed against predicted (NRC, 2001) digestible NDF (dNDF) concentration (left) and the plot of residuals against centered predicted values of dNDF (right). Values were centered by subtracting mean dNDF from each data point.

The main reason for the poor performance of NRC (2001) model for predicting tdNDF is an assumption of a constant digestibility of 0.75 for pdNDF. This value is too low, since in the current dataset even the total NDFD exceeded 0.75 for 18 silages out of 77 silages. The observed mean value of pdNDFD *in vivo* (0.85; SD = 0.057) was clearly higher than the default value of the NRC (2001) model. Weiss et al. (1992) used a single compartment rumen model by Waldo et al. (1972) in deriving the value 0.75 for the pdNDFD. However, using the same mean rumen retention time (1 / (0.02/h) = 50 h) and digestion rate (0.076/h) in a two-compartment rumen model (Allen and Mertens, 1988) gives a similar value (0.87) to the current *in vivo* data. On average, the NRC (2001) model predicted pdNDF reasonably accurately (mean bias -17 g/kg; $P < 0.01$) and precisely ($R^2 = 0.90$) relative to the *in situ* pdNDF. However, the mean bias was highly variable between forage type ranging from 7 (primary growth grass) to -51 (whole crops). Interestingly, the surface area lignin model did not predict observed iNDF concentration more precisely than the linear model (R^2 0.50 vs. 0.50). Residual SD of tdNDF was acceptable (20.1) when analysed with the GLM procedure of SAS using forage type as a class variable, but the slope (0.82) was different from 1.0 ($P <0.05$) and the forage type effects were highly significant.

Mean bias in TDN was smaller than in tdNDF (18 vs. 44 g/kg DM), but random variation was large as indicated by relatively low R^2 (Figure 2). The residuals varied markedly between the forage types. *In vivo* TDN was calculated by adding 1.25 × FA (20 g/kg DM) to correspond the NRC (2001) TDN equation. The mean bias was significant ($P < 0.001$). Metabolic TDN (70 g/kg DM) in NRC (2001) was determined by Weiss et al. (1992). This value is clearly lower than the observed faecal output of NDS (105 g/kg DM), especially considering that metabolic fat component was multiplied by 2.25. On OM basis NRC (2001) faecal metabolic output would be 62-64 g/kg DM. Indigestible components of CP and NFC accounted for 10.5 g leaving about 30 g/kg DM difference in faecal metabolic output. Part of this can be attributed to indigestible lipid components, but also to the metabolic output of CP and NFC which were greater (39 and 38 g/kg DM) than the values (27 and 31 g/kg DM) determined by Weiss et al. (1992).

Although the errors in tdNDF and tdNDS partly counterbalanced, RSD for the NRC (2001) model remained rather high (41 g/kg DM), and even root mean squared error (RMSE) that describe random variation across the regression line was relatively high (37 g/kg DM). A simple bivariate empirical regression model (TDN = iNDF + Ash) was much more precise (RMSE = 17.8) than the NRC (2011) model in predicting TDN. The residual of the NRC (2001)

model was strongly (R^2 = 0.61) and negatively related to iNDF/NDF ratio, whereas the correlation to the corresponding Lignin/NDF ratio was poor (Figure 3). This indicates that the Lignin/NDF ratio does not describe accurately the digestibility of pdNDF fraction. This is not very surprising, since both permanganate lignin (Buxton and Russell, 1988) and ADL (Jung et al., 1997) had a stronger influence on the NDFD of grass than on the NDFD of legumes.

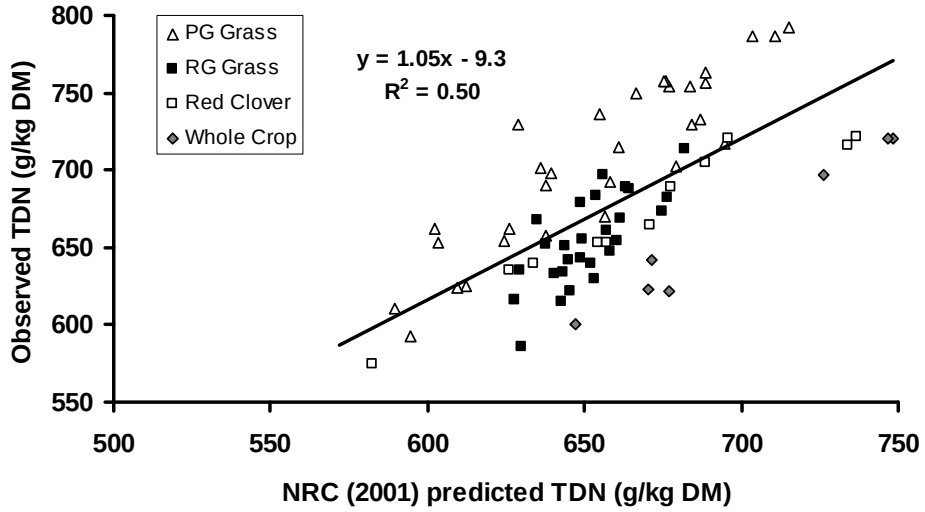

Figure 2. Observed against predicted (NRC, 2001) TDN concentration.

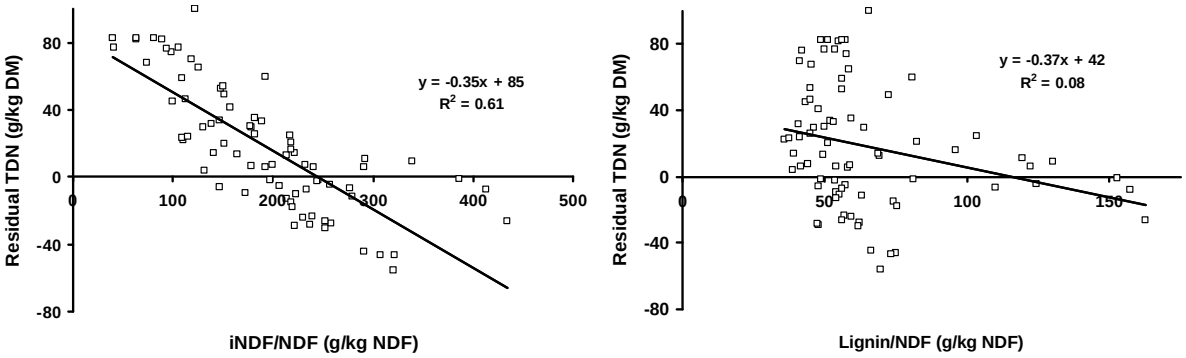

Figure 3. Relationships between iNDF/NDF and Lignin/NDF ratios to residual (observed – predicted) TDN.

Simplification of the NRC (2001) equation by using total NDF with NDICP correction and uniting NDS fraction (true digestibility = 0.98) only marginally increased RSD of tdNDF (44). It can be concluded that the major problems of the NRC (2001) TDN model are poor ability

of lignin to predict iNDF, constant and too low pdNDFD and underestimation of faecal metabolic TDN, even though the latter partly counterbalance the errors in tdNDF.

Mertens (2002) derived a simple equation in which dNDF is a linear function of NDF and lignin: dNDF = a × NDF (g kg^{-1} DM) + b × Lignin (g kg^{-1} DM),

where a and b can be estimated by regression. Constant a is the digestibility coefficient of pdNDF and constant b is the proportion of NDF protected by lignin. This equation has no intercept, i.e. neither endogenous nor microbial excretion of NDF. Huhtanen et al. (2006a) modified this model by replacing NDF with pdNDF and lignin with iNDF and estimated parameter values by Excel Solver. The simplified model of Mertens (2002) described dNDF as precisely as the surface area law, but the precision was markedly improved when pdNDF and iNDF were used in the model instead of NDF and lignin (RSD of D-value within a forage type 23.0 vs. 13.8 g/kg DM).

Mechanistic dynamic models

Waldo et al. (1972) developed a model that incorporates digestion and passage to estimate digestibility:

Digestibility (D) = $k_d / (k_d + k_p)$,

where k_d = digestion rate (1/h) and k_p = passage rate (1/h). This model is not adequate for NDF, since it does not take into account that only part of NDF is potentially digestible. For pdNDF the model can be described as:

D = pdNDF × $k_d / (k_d + k_p)$

The single compartment model indicates that digestibility is competition between digestion and passage; the faster the digestion rate in relation to passage rate the higher digestibility will be. Mertens (1993) concluded that assuming the rumen as a single compartment is not an adequate mathematical or biological representation of rumen functions. Mean rumen retention time (MRRT) in sheep fed at maintenance is approximately 50 h and its reciprocal passage rate 1/50 h = 0.02/h. Average *in vivo* pdNDFD of different forages (n = 86) was 0.85. To achieve this digestibility with a passage rate of 0.02, digestion rate should be 0.113 – an unrealistically high value. Digestion rate should be 0.18 to reach a pdNDFD of 0.90, which is not an unusual value for early harvested grass. Alternatively, using a realistic value of 0.06 for digestion rate, the passage rate should be only 0.0106 (MRRT = 94 h) to achieve pdNDFD of 0.85. With a

digestion rate of 0.08 MRRT should be 112 h to achieve a pdNDFD of 0.90 in a single compartment system. These examples clearly demonstrate that the single compartment rumen model is inadequate in predicting pdNDFD accurately with realistic parameter values. Despite this, in the literature this is the most commonly used model to describe NDF digestion or protein degradation (with protein an immediately degraded component is included in the model).

Allen and Mertens (1988) derived a model that includes selective retention of feed particles in the rumen:

$D = pdNDFD \times (k_d / (k_d + k_r) [1 + k_r / (k_d + k_p)]$, where

k_d = digestion rate (1/h), k_r = rate of release from the rumen non-escapable pool to the escapable pool (1/h) and k_p= passage rate of feed particles from escapable pool (1/h). Parameter k_r describes selective retention of particles in the rumen, i.e. the rate in which recently ingested feed particles become eligible for passage. These processes include particle size reduction as a result of chewing and increase in specific gravity as a result of reduced fermentation activity occurring simultaneously with time after ingestion. Using the values of 0.06 for digestion rate and 50 h MRRT (20 + 30 h) for non-escapable and escapable pools) pdNDFD would be 0.84, whereas with the simple model it would be 0.75. Two-pool model predicts higher pdNDFD with same MRRT, because recently after ingestion the particles become gradually eligible for passage but digestion takes place also in the non-escapable pool. The effect of digestion lag on the digestibility is less with the two-pool model compared with the one-pool model as the particles are not available for passage during the lag time. Allen and Mertens (1988) showed that the lag time will not affect digestibility, if there is a similar lag time for passage as for digestion.

Selective retention of feed particles in the rumen has been demonstrated by various techniques. The passage rate of feed particles from the rumen is inversely related to particle size (Poppi et al., 1980; Huhtanen et al., 2007a). Selective retention of feed particles in the rumen is also evident from duodenal marker excretion curves (Ellis et al., 1993; Huhtanen and Hristov, 2001; Lund, 2002), which clearly indicated an ascending phase. This challenges the interpretation of the rumen as a single compartment system, in which the probability of particles to escape is a random process. Also different particle size distribution in ruminal digesta and faeces demonstrates the mechanism of selective retention in the rumen.

Dynamic mechanistic models are excellent tools in understanding the mechanisms of fibre digestion, but to be useful in practical feed evaluation the predictions of feeding values

should be at least as accurate and precise as those with the empirical models based on *in vitro* and/or NIRS techniques. In addition to the theoretically correct model structure, accurate and precise parameter estimates are required to produce accurate estimates of feeding values. In the following methodologies to determine the parameter values for NDF digestion model are discussed.

Determination of model parameters

Potential NDF digestibility (pNDFD)

The concentration of iNDF was the most important parameter determining diet digestibility according to the Nordic Dairy Cow Model Karoline (Danfaer et al., 2006). Close empirical relationship between forage iNDF concentration and OMD also point out the importance to determine pNDFD. Potential NDFD can be described an intrinsic character of feed It should be determined in optimal conditions when only the plant characteristics – not e.g. the *in vitro* system - limit the digestion. Whether iNDF is determined either by *in vitro* or *in situ* incubations the animals should be fed a diet that ensures optimal environment for fibre digestion in the rumen.

The concentration of iNDF and pNDFD can be determined either as the asymptotic value of indigestible residue or digestibility by non-linear models or by long term incubations *in vitro* (Van Soest et al., 2004) or *in situ* (Huhtanen et al., 1994). Mertens (1993) recommended that at least three observations (time points) for each parameter to be estimated in the model. Most digestion models like a simple first-order model with discrete lag time have three parameters, indicating that nine time points are needed. However, in published literature the number of fermentation times seldom meets the minimum number, probably because of labour demand.

Long term *in vitro* or *in situ* incubations determine pNDFD and iNDF directly. The authors experience is that the variation in iNDF concentration between the animals and between replicates within animal is small and smaller than SD of *in vivo* OMD (see Page 6). In contrast, in the literature RSD is usually greater for potential DMD and NDFD digestion than for *in vivo* DMD/OMD and NDFD when *in situ* data determined using 5 – 6 fermentation times up to 72 – 96 h. In a Monte Carlo simulation study using the observed SD of NDF disappearance at six different fermentation times between 0 and 96 h (pNDFD = 0.80, k_d = 0.04/h and NDF = 550 g/kg DM) SD of calculated iNDF concentration was 11.7 g/kg DM. Increasing the number of

time points to nine decreased SD to 10.4 g/kg DM. It could be more beneficial to include time points beyond 96 h rather than increase them between 0 – 96 h. In addition, one long term incubation is less labour intensive than time series of *in vitro* or *in situ* incubations. *In vitro* study (Van Soest et al., 2004) clearly demonstrated that digestible NDF pool can be divided into rapidly and slowly digestible fractions. Consequently, it is possible that *in vitro* and especially *in situ* techniques can underestimate pNDFD unless fermentation times longer than 96 h are used. Rinne et al. (2002) found that the difference between observed pNDFD in 12-d *in situ* incubation and asymptotic NDFD derived from fermentation times up to 96 h increased with advancing maturity of grass. Small variability in iNDF concentration with long term *in situ* incubation strongly supports this method is estimating pNDFD for mechanistic models.

Attempts to predict digestion kinetic parameters from chemical composition have been met with variable success. A close linear relationship between lignin and iNDF contents for a diverse population of forage samples was reported by Smith et al. (1972) and Mertens (1973). In the dataset of Huhtanen et al. (2006a) lignin concentration was moderately correlated to iNDF (R^2 = 0.50), but prediction errors of iNDF (28.4) and pNDFD (0.065) were relatively high. A large part of variability resulted from between forage type variation in lignin to iNDF ratio as indicated by much smaller (18.0 and 0.031) prediction errors within forage type. In a recent study with tropical forages (Gomes et al., 2011) lignin analysed by various methods was significantly correlated with iNDF concentration, but the prediction errors were relatively high (58.7 – 87.3 g/kg NDF). It can be concluded that although lignin certainly plays a role in the cell wall degradation, and consequently is closely correlated with iNDF concentration, measurements of lignin cannot be universally used for the determination of iNDF concentration and pNDFD.

Digestion rate (k_d)

Digestion rate of pdNDF is an intrinsic character of feed and as pNDFD, it should be determined in conditions when only plant characteristics restrict digestion. The *in situ* method is the most commonly used method to estimate digestion rate of pdNDF. Several excellent reviews (Mertens, 1993; Stern et al., 1997; Nozière and Michalet-Doreau, 2000) have been published that provide a detailed insight into the sources of variation and methodological aspects of the procedure.

Estimates of NDFD derived from the rumen models using *in situ* data are seldom validated against *in vivo* data. Usually the values for ruminal NDFD calculated from the *in situ* kinetic data are low compared to the observed or expected *in vivo* NDFD. Archimède (1992) used *in situ* digestion kinetic data to predict ruminal NDFD estimated from duodenal flow (cited by Nozière and Michalet-Dureau 2000). The model clearly underestimated ruminal NDFD. The slope between predicted and observed values was 1.00 and the proportion of variance accounted for was relatively high (R^2 = 0.65). Underestimation of *in vivo* digestibility was suggested to be due to the underestimation of digestion rate by the *in situ* technique. The use of single compartment rumen model could be one reason for the underestimation of *in vivo* NDFD from the *in situ* data, especially if the passage rate estimates are only based on descending phase of marker excretion curve. Post-ruminal digestion has been suggested to explain under-prediction of NDFD by models based on the *in situ* data. However, the contribution of post-ruminal digestion to the total NDF digestion of typical dairy cow diets is marginal (see Huhtanen et al., 2010a).

Digestion rates determined by *in situ* incubation have been lower than values derived from rumen evacuation in studies conducted in different laboratories (Aitchison et al., 1986; Tamminga et al., 1989; Rinne et al., 2002). These findings suggest that normal microbial colonization of samples within the bags was not achieved and/or that conditions in the bag were sub-optimal. Meyer and Mackie (1986) showed that the bacterial population inside the bags was smaller than in the surrounding digesta, particularly for the cellulolytic bacterial populations. Lower fibrolytic activities in particle-associated microbes in bag residues than in rumen digesta (Huhtanen and Khalili, 1992; Nozière and Michalet-Doreau, 1996) is consistent with lower microbial numbers within the bags. Recently Jančík et al. (2011) reported significantly slower digestion rates of pdNDF with the *in situ* method compared with incubations of isolated NDF with automated *in vitro* gas production system. The correlation between the methods was reasonably good, but both the mean and slope bias were significant (Figure 4). Digestion rates estimated by a gravimetric *in vitro* method were higher than those determined *in situ* (Bossen et al., 2005), which also suggests that the *in vitro* techniques may allow a more accurate estimation of the true intrinsic k_d than the in situ technique.

The problem with determination of the digestion rate of pdNDF has been difficulties in validating the data. Comparing the accuracy and precision of two *in vitro* methods or *in vitro* vs. *in situ* almost worthless unless the model predicted values are compared with *in vivo* data.

Huhtanen et al. (2006b) proposed a method estimating *in vivo* k_d from the in vivo digestibility of pdNDF by solving the equation of Allen and Mertens (1988):

$$k_d = [-(k_p + k_r) + [(k_p + k_r)^2 + 4Dk_rk_p/(1 - D)]^{0.5}]/2,$$

where D = *in vivo* pdNDFD and other parameters as described before. To estimate the rate of digestion indirectly by this method, an estimate of total mean retention time in the fermentation compartments and the distribution of the residence time between the two compartments are required. We have used an estimate of 50 h for the MRRT that is distributed as 40:60 between the two compartments, i.e. values of 0.05 and 0.033/h are used for k_r and k_p, respectively.

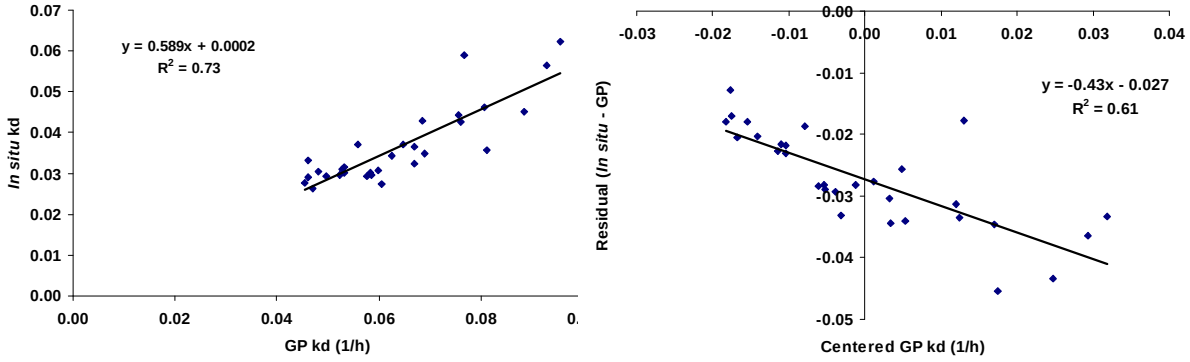

Figure 4. Relationship between pdNDF digestion rate determined by in vitro gas production (GP) method or in situ (left) and residual analysis (right). The data points with GP method are centered by subtracting the mean value of GP data from each data point. From Jančík et al. (2011).

Huhtanen et al. (2008a) validated this model by using 15 primary growth silages, of which *in vivo* digestibility was determined in sheep, iNDF by 12-d in situ incubation and k_d of isolated NDF by in vitro gas production technique. The two-pool Gompertz model was fitted to gas production data, ruminal pdNDFD was simulated by a two-compartment dynamic rumen model and first-order k_d determined by solving the previous equation. *In vivo* NDFD and k_d were predicted accurate and precisely (NDFD: R^2 = 0.99, RSD = 13 g/kg; k_d: R^2 = 0.86; RSD = 0.005/h) providing support for the *in vitro* gas production system to determining kinetic data.

Most of the new models are non-first-order models, i.e., the digestion rate is variable during fermentation. A limitation of the non-first-order kinetic parameters is that they cannot be used in steady-state rumen models. Moreover, the coefficients in gas production models may be difficult to interpret, because the models with several parameters do not permit straight forward

comparisons among different feeds (Pitt et al., 1999). To overcome these problems, Pitt et al. (1999) derived equations to estimate the effective first-order k_d from non-first-order models assuming that the rumen is one-compartment system with random passage of particles. Huhtanen et al. (2008b) extended this approach by including selective retention. A number of one- two- and three-compartment models were fitted to gas production data and pdNDFD in the two-compartment rumen system was simulated. Interestingly, parameters from rather simple models such as first-order exponential with discrete lag predicted *in vivo* NDFD and k_d as accurately and precisely as more complicated models with 6 – 9 parameters despite the latter gave much better fit of gas production data. This was probably because the residuals between observed and predicted gas data was mainly during early fermentation, when the particles still remain in the rumen. Anyway, non-first-order digestion rates must be converted to single first-order rates for steady-state rumen models.

As for pNDFD there has also been interest to predict k_d from chemical composition of the diet. These attempts have not been very successful; within a feed type there can be reasonable correlation between lignin and k_d, but overall relationship is poor. In the dataset of Huhtanen et al. (2006a) comprising 86 forages (primary and regrowth grass, red clover and whole crop) the correlation between lignin and k_d derived from *in vivo* data was not significant. For a homogenous set of forages such as primary growth grass from the same field in the same year (Jančík et al., 2011) the logarithmic relationships between ADL and k_d determined by *in situ* or automated *in vitro* gas production system were moderately high (R^2 = 0.61 and 0.67), and even higher between iNDF and k_d (0.75 and 0.90, respectively).

Passage rate

In addition to parameters for pNDFD and k_d, mechanistic dynamic models require estimates of passage kinetic parameters. Passage rate is influenced by intrinsic feed characteristic and extrinsic animal and diet factors. Details of the models, methodology and interpretation of passage kinetics are described in reviewed in detail by Ellis et al. (1994) and Huhtanen et al. (2006b). For predictions of digestibility at the maintenance level of feeding a constant value of passage kinetic parameters may be used. Our experience is that using 50 h MRTT divided as 40:60 between rumen non-escapable and escapable pools and k_d determined by the gas production technique gives NDFD estimates that are similar to observed *in vivo* values. Using an

appropriate rumen model incorporating selective retention is much more important than small differences in MRRT; for example, 34 and 50 h MRRT result in the same NDFD with one-pool and two-pool models (k_d = 0.06/h). Marker kinetic data indicate that the release of feed particles is a time-dependent process (e.g. Ellis et al., 1994); the rate of release of feed from non-escapable to escapable pool increases with time. In steady-state rumen models this process can be described by dividing the non-escapable pool (20 h) into two pools (10 + 10 h) in the case of gamma2 time-dependency. Model simulations (Huhtanen et al., 2006b) suggest that ruminal NDFD would increase about 0.02 units by including time-dependency in the first rumen compartment. Distribution of MRRT between the two rumen pools will also influence the simulated NDFD estimates but within the range from 30:70 to 70:30 the effect is not more than 0.01 units.

Passage rate is influenced by diet composition and feed intake in addition to intrinsic feed characteristics. In a recent meta-analysis (Krizsan et al., 2010) NDF intake (in g/kg of BW) was the most important dietary factor influencing the passage rate of iNDF estimated by rumen evacuation technique. Interestingly, observed iNDF passage rates were much slower than those predicted by NRC (2001) and the Cornell Net Carbohydrate and Protein System (CNCPS; Fox, 2004). The reason for the high passage rate estimates of these models is because the estimates are based on the passage data derived from the descending phase of marker excretion curve, and the retention in the non-escapable pool is ignored.

Depression in digestibility with increased intake can be predicted either by empirical models as described by NRC (2001) and Huhtanen et al. (2009) or by mechanistic models (e.g. Danfær et al., 2006). The NRC (2001) model predicts much greater depression in digestibility with increased intake than the model of Huhtanen et al (2009), probably because of the differences in diet composition in the data from which the models were derived. Using the passage rate estimates of Krizsan et al. (2010) in a two-pool rumen model predicted accurately in *vivo* pdNDFD and depression in NDFD with increased feed intake. This provides evidence of the accuracy of passage data determined by rumen evacuation technique, whereas to account for *in vivo* pdNDFD with passage rate values of the NRC and CNCPS had required unrealistically high k_d – even with the two-pool rumen model.

Forage protein value

The primary function of feed protein in the diet is to provide the ruminants with absorbed amino acids (AA), often denoted as metabolizable protein (MP) in the form of α-amino nitrogen. The MP requirement of ruminants is met from two sources: microbial protein synthesized in the rumen and feed protein that escapes microbial degradation in the rumen. Determination of the protein value of ruminant diets is complex, because the dietary supply of AA is modified both quantitatively and qualitatively by microbial fermentation in the fore-stomachs before digestion in the small intestine. The main emphasis in feed protein evaluation has been the determination of effective protein degradability in the rumen (EPD) and intestinal digestibility of rumen undegraded protein. Protein degradability has most commonly been determined by ruminal in situ incubations and digestibility of undegraded feed protein by the mobile nylon bag method. The merits and demerits of these methodologies have been discussed in several excellent reviews (Broderick, 1994; Noziere and Michalet-Doreau, 2000; Hvelplund and Weisbjerg, 2000). However, the data derived using these methods have seldom been validated against in vivo measurements of post ruminal nutrient flow or milk protein yield responses.

In meta-analysis of large datasets from North-American and North European production experiments marginal milk protein yield responses to increased microbial MP were 5-fold compared to feed MP (Huhtanen and Hristov, 2009). This is consistent with the reports of the regression coefficient (0.77) between predicted (NRC, 2001) and observed post-ruminal feed protein flow (Broderick et al., 2010) indicating that the true differences in EPD for diets of variable composition are smaller than predicted. On the other hand, the corresponding regression coefficient for microbial N flow was 1.26 suggesting that the contribution of microbial protein is greater than predicted.

The inconsistency in production and flow data between microbial and feed MP can partly relate to methodological problems of the *in situ* technique in determining EPD: In situ determinations of protein degradation are flawed because of several inherent problems, including 1) the assumption that proteins, peptides, and amino acids in the soluble fraction are completely degraded in the rumen, 2) escape of small feed particles from the bags without being degraded, 3) the physical restriction of feeds within the bag from microbial interaction and digestion, 4) the imprecision to quantify the microbial contamination of the undigested residues, and 5) incorrect

kinetic models used to calculate protein degradability in the rumen (Broderick and Cochran, 2000; Huhtanen and Hristov, 2009; Broderick et al., 2010).

Various experimental techniques have demonstrated an escape of soluble non-ammonia N (SNAN). Depending on the kinetic model, 13-15% of SNAN fraction escaped ruminal degradation, when a single dose of intrinsically ^{15}N labelled silage buffer-soluble N was given to dairy cows (Ahvenjärvi et al., 2007). According to generally used Ørskov and McDonald (1979) model SNAN is completely degradable, since it disappears at 0-h washing from the nylon bags. The proportion of SNAN of silage N had no influence on milk protein yield in meta-analysis of the data (253 diets) from milk production trials, when the concentration of silage MP was computed using a constant EPD irrespective of the proportion of soluble N (Huhtanen et al., 2008c). The only variable within a was silage fermentation quality (acids, ammonia N and soluble N), In a meta-analysis based 547 diets conducted to evaluate the effects of different forage variables on milk protein yield (Huhtanen et al., 2010b) forage DM intake and D-value (concentration of digestible OM) were the most important forage parameters influencing milk protein yield (Table 1). Ammonia N had a negative effect on milk protein yield, but the effect of SNAN was not significant. Calculated marginal efficiency of the utilization of incremental CP from forage was only 3.7%. This analysis clearly demonstrates that forage intake potential and OMD are much more important determinants of production responses than CP concentration or degradability.

Table 1. The best-fit mixed model regression equation of milk protein yield responses to forage variables (RMSE adjusted for random study effect = 16.0)

Effect	Unit	Estimate	Error	P-Value	SD[1]	Response per SD unit
Intercept		-310	46.8	<0.001		
CMP[2]	kg/d	790	66.7	<.0001		
CMP × CMP	kd/d	-192	38.7	<.0001		
Forage DMI[3]	kg/d	27.7	1.42	<0.001	1.53	42.4
D-Value[4]	g/kg DM	0.490	0.069	<0.001	40.9	20.1
Forage CP	g/kg DM	0.417	0.114	<0.001	22.4	9.3
Ammonia N	g/kg N	-0.217	0.067	0.001	21.5	4.7

[1] SD = standard deviation
[2] CMP = Concentrate metabolizable protein
[3] DMI = dry matter intake
[4] D-value = Digestible organic matter in dry matter

Theoretically, forage rumen degradable protein (RDP) can be calculated using the following model:

$$RDP = CP \times ISP \times (k_d\text{-ISP} / ((k_d\text{-ISP} + k_r) [1 + k_r / (k_d + k_p)] + CP \times SNAN \times ((k_d\text{-ISP} / (k_d\text{ -SNAN} + k_l) + CP \times \text{Ammonia N},$$

where ISP = proportion of insoluble CP, k_d-ISP = digestion rate of ISP, k_r = rate of release of ISP from non-escapable to escapable pool, k_p = rate of passage of ISP from escapable pool, SNAN = proportion of soluble NAN, k_d -SNAN = digestion rate of SNAN, k_l = liquid passage rate and Ammonia N = proportion of ammonia N of total N. This model takes into account selective retention of feed particles and escape of SNAN in the liquid phase. According to model simulations the effects of CP solubility on RDP and EPD are much smaller than the Ørskov and McDonald (1979) model predicts. However, it is questionable if the current experimental methodologies are sufficiently accurate to justify more complicated models. Evidence from the meta-analysis of the data from milk production trials suggests that marginal, if any, improvements in forage protein evaluation can be expected from using more complicated methodologies and models compared with using constant values for both ruminal CP degradability and intestinal digestibility of undegraded protein. Considering inaccuracies of the current methods, the benefits of determination forage protein degradability in practical ration formulation is questionable.

According models by Huhtanen and Hristov (2009), marginal milk protein yield responses increased MP supply derived from reduced ruminal protein degradability (constant energy and CP intake) was only 6 – 8%. This corresponds to about 1 g/d increase in milk protein yield per 1 %-unit decrease in forage EPD (forage dry matter intake (DMI) = 12 kg/d and CP 150 g/kg DM). The action of polyphenol oxidase in red clover polymerizes with proteins (Jones et al., 1995) and reduces proteolysis (Hatfield and Muck, 1999). Reduced proteolysis of red clover silage has increased protein flow from the rumen (Dewhurst et al., 2003; Vanhatalo et al., 2009). However, incremental protein supply to the small intestine was not utilized for milk protein synthesis in these studies. One reason for the poor efficiency of incremental protein flow with diets based on red clover silage could be low intestinal digestibility of undegraded protein. In the dataset of Huhtanen et al. (2006a) faecal CP output in sheep was much higher for red clover than primary growth grass silages (56 vs. 39 g/kg DMI; n = 19 vs. 33).

Forage intake

Accurate prediction of DMI has a central role in the low cost ration formulation, since most of the variation in total nutrient intake is related to differences in intake rather than digestibility. However, energy intake, DMI and digestibility are interrelated, due to the close relationship between diet digestibility and DMI. Regulation of feed intake in ruminants involves multiple mechanisms related to dietary and animal factors that are poorly understood (Mertens, 1994). Despite extensive effort over the past 30-40 years, no generally accepted intake model has been developed. Limited success in this field is, at least in part, due to the complicated interactions between the animal and diet characteristics, and the difficulties in distinguishing and quantifying these factors.

Predicting actual intake is difficult due to strong animal effects that can mask differences in the intake potential of forages, especially if individual animal data and simple fixed regression models are used. To overcome these problems Huhtanen et al. (2002) proposed relative silage DMI index (SDMI index) derived from a mixed model regression analysis using treatment means data. The model included D-value, total acid and ammonia N concentration. The model was extended (Huhtanen et al., 2007b) to include additional factors [DM concentration, harvest: primary vs. regrowth, NDF concentration and forage type (grass, legume, whole crop)]. Mertens (1994) proposed a conceptual model of intake regulation, in which NDF was used as a factor to regulate the intake of fill limited diets. However, in the study of Huhtanen et al. (2007b) digestibility (D-value) was a much better predictor of DMI than NDF concentration. It seems that in addition to NDF concentration its digestibility and passage, and probably also particle breakdown characteristics influence intake. Bivariate model with iNDF and pdNDF predicted DMI as well as D-value. Negative coefficient of iNDF was much higher than that of pdNDF indicating the slower turnover of rumen iNDF pool. Increased NDF concentration at a constant D-value had a small negative effect on intake reflecting increased bulkiness of feed.

Reductions in intake of silage induced by fermentation end products have often been evaluated in comparisons with either grass or hay diets. However, the strong negative effect of silage total acid concentration on silage DMI (Huhtanen et al., 2007b) suggests that extent of in-silo fermentation rather than preservation method *per se* influence silage intake. Krizsan et al. (2007) addressed the intake reductions in growing cattle primarily to two patterns of in-silo fermentation; extensive lactic acid and secondary fermentation of the grass were negatively

related to silage DMI. Reductions were between 6 and 32% compared with the silage giving the highest intake. Moreover, a nine percentage unit increase in intake was observed of silage displaying restricted fermentation compared to hay of equal maturity produced from the same standing crop (Krizsan and Randby, 2007). Silage fermentation products provide less energy than WSC to the rumen microorganisms; microbial protein synthesis in the rumen can be reduced in cows fed grass silage-based diets (Jaakkola et al., 2006) and impact the ratio of amino acids to energy available at tissue level. An imbalance between amino acids and energy could explain a reduction in intake of extensively fermented silages, in line with positive intake responses to supplementary protein in cows fed grass silage-based diets (Oldham, 1984; Huhtanen et al., 2008d). The lower intake of secondarily fermented silages is most likely a consequence of reduced palatability, i.e. smaller meals and a lower frequency of meals. Attempts to isolate specific fermentation products involved in controlling silage intake have failed to pinpoint any particular product as responsible for reducing silage intake. Modelling the effect of fermentation end products on intake should rather be restricted to assess the relative importance among different fermentation quality parameters and at what concentrations they are detrimental to intake.

Silage DM concentration had a quadratic effect on DM intake with the maximum at 350 – 400 g DM/kg (Huhtanen et al. 2007b). Feeding mixtures of grass and red clover silages or grass and whole-crop cereal silages demonstrated positive associative effects on intake; i.e. the intake of mixtures was greater than the mean of both forages when fed separately. Intake of regrowth silage was 0.44 kg/d lower than that of primary growth silage even when other factors (D-value, fermentation, DM and NDF concentration) were taken into account. The reasons for this are unclear, but could be related to increases in the amount of dead material in offered silages.

In the British Feed into Milk system forage intake potential is predicted by NIRS (Keady et al., 2004a). Calibration was developed by feeding 136 farm silages to growing cattle (Agnew et al., 2001). NIRS analysis takes into account different factors influencing intake without specifying them. It is an attractive alternative that is inexpensive to use, but producing and maintaining calibration dataset is expensive. Relative prediction error of the NIRS model (7.6% residual variation) was greater than residual variation within a study for the SDMI index system (3.1%).

Because the total DMI is strongly influenced by the amount and composition of supplementary feed, the SDMI index was extended to total DMI (TDMI) index (Huhtanen et al., 2008d). The following factors are included in the TDMI-index: linear and quadratic effects of the amount and CP concentration of the concentrate, linear effects NDF and fat concentration of the concentrate and interaction between the amount of concentrate and SDMI index. The model predicts greater depressions in silage DMI with increased concentrate supplementation for high intake potential silages. Prediction error of the model within a study was 0.37 kg/d.

Although the TDMI index model predicts the differences in intake potential between the diets within a study, it is not useful alone to predict actual DMI due to strong animal influence. Many intake models use milk yield (MY) as an input variable (Vadiveloo and Holmes, 1979; Dulphy et al., 1989; NRC, 2001; Keady et al., 2004b), but the usefulness of such models in ration formulation may be questioned, as the final output (MY) is used to predict input (feed intake). These models are primarily useful in predicting intake required to sustain a given level of MY, as stated by Keady et al. (2004b). It should be noted that MY is not known at the time of prediction (Ingvartsen, 1994). To overcome this problem animal and diet factors were integrated by using standardised energy corrected milk yield (sECM) instead of actual observed yield in the model (Huhtanen et al., 2011). The idea is to standardize the current yield according to days in milk, the intake potential (TDMI-index) and MP concentration of the current diet, i.e. how much the cows would produce on given day (150) with a standard diet. Residual MSE adjusted for the random study was 0.36 kg/d and RMSE with simple regression model 0.91. An advantage of this model is that information is available at the time ration formulation, not only retrospectively as is the case with models using observed yield.

Conclusions

Accurate and precise prediction of OMD or D-value is of fundamental importance in forage evaluation. This is not only because of digestibility is closely related to the ME concentration of forage, but also because forage digestibility is the main determinant of intake potential and synthesis of microbial protein that is the major source of MP in forages. Empirical models based on chemical composition are usually not accurate enough for practical feed evaluation due to lack of causal relationships between composition and digestibility. Within forage types and limited datasets these models may be acceptable, but over a wider range of forage types and

environmental conditions the accuracy of predictions is not acceptable for practical feed evaluation and ration formulation.

In vitro methods usually predict OMD more accurately than empirical equations based on statistical relationships between chemical composition and digestibility. Even though values determined *in vitro* may approach digestibility coefficients *in vivo*, it is important to calibrate *in vitro* methods using *in vivo* data to derive reliable prediction equations. Generally there are no consistent differences in the performance of methods based on rumen fluid or commercial enzymes. However, most of the comparisons between the methods are based on narrow range of forage types. Our experience is that the relationship between cellulase solubility and OMD varies between forage types, and even between grass species from the same harvest. Determination of iNDF concentration by a long term in situ incubation in the rumen is a promising tool for predicting OMD by an empirical regression; iNDF (or pNDFD) is also an essential parameter of dynamic models. Because of long incubation times (12 d) the variability between the replicates is small (SD = 5 g/kg), much less than in digestibility trials in sheep (0.010 – 0.02 digestibility units). The relationship between iNDF and OMD is more uniform than that between cellulase solubility and OMD.

Mechanistic dynamic models of ruminal NDF digestion are excellent tools to understand mechanisms of fibre digestion, but whether they are useful in practical feed evaluation is questionable at the moment. Except for pNDFD, the estimation of the feed specific values of model parameters (digestion rate, MRRT, distribution of MRRT between the two compartments, variation in faecal metabolic OM) is less accurate than the current *in vitro* / NIRS methods in predicting OMD. For practical feed evaluation NIRS can be an excellent tool, but the success depends on the quality of calibration. Good OMD calibrations need a large number and a wide range of samples. Because *in vivo* calibration is not a practical option, the reference *in vitro* method should properly be calibrated against *in vivo* method.

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