Digestion and passage of fibre in ruminants

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Abstract

The objective of this chapter is to discuss the methods used for the estimation of digestion and passage kinetic parameters, intrinsic and extrinsic characteristics influencing the kinetic parameters and the reliability of kinetic parameters for predicting *in vivo* digestibility using dynamic rumen models. We focus mainly on digestion and passage of cell wall carbohydrates, since most of the variation in organic matter digestibility of ruminant diets can be attributed to concentration and digestibility of cell wall carbohydrates.

The extent and rate of NDF digestion are generally determined by *in situ* or *in vitro* methods, but estimates have seldom been validated using *in vivo* data. A method for estimating digestion rate from *in vivo* digestibility of potentially digestible NDF and assumed rumen residence time is suggested. Future work is required to estimate the intrinsic characteristics limiting rate and extent of cell wall digestion, and the quantitative effects of some extrinsic factors such as intake and diet composition.

Extensive data suggest that most of the compartmental retention time in cattle is pre-duodenal and that the reticulo-rumen is a system with selective retention of feed particles. However, models with selective retention have seldom been used to calculate NDF digestibility from kinetic parameters. This fundamental flaw in the model structure leads to serious underestimations of NDF digestibility unless unrealistically high digestion rates and/or low passage rates are used to correctly predict *in vivo* digestibility. To progress in understanding of NDF digestion, it is vital to develop useful mechanistic models for the prediction of digestibility and intake.

Keywords: cell wall carbohydrate, digestion kinetics, passage kinetics, intrinsic factor, extrinsic factor, modelling

Introduction

Animal performance depends on the intake of digestible and metabolisable nutrients. Although a large proportion (60-90%) of the variation in digestible energy (DE) intake is related to differences in intake (see Mertens, 1994), differences in diet digestibility also have a significant effect on nutrient supply. In addition to the direct effects on DE intake, digestibility also influences nutrient supply indirectly due to the close association between digestibility and intake in ruminants fed forage-based diets. In dairy cows fed grass silage based diets improvements in silage digestibility

were closely related to increased dry matter (DM) intake and animal performance (Rinne, 2000).

The concept of an ideal nutritional entity was initially proposed by H.L. Lucas. According to the Lucas principle, the true digestibility of a nutritional entity is determined as the slope of the regression between the amounts of nutrient digested (e.g. crude protein, ether extract, and cell solubles) against the intake of a given nutrient (Van Soest, 1994). The negative intercept of this regression represents faecal metabolic output. The true digestibility of cell solubles defined as DM - neutral detergent fibre (NDF) was found to be 0.98 and not significantly different from 1.00 (Van Soest, 1994). When cell solubles are defined as organic matter (OM) minus NDF, the true digestibility of cell solubles in grass silages from primary growths was complete, and the standard errors of both the slope and intercept were small (Nousiainen *et al.*, 2003a). Applying the Lucas principle to a larger data set with a wider range of diet composition from digestibility studies in sheep demonstrated a small variation in the true digestibility of cell solubles (Weisbjerg *et al.*, 2004a). Cell wall characteristics (dietary NDF concentration and digestibility) explained variation in OM digestibility with prediction errors of less than 10 g kg^{-1} within a study across three data sets (Nousiainen *et al.*, 2004; unpublished data). This indicates that OM digestibility, which is the key factor in determining the DE concentration of a feed, is primarily constrained by the cell wall characteristics. The primary importance of cell wall characteristics in assessing OM digestibility of the diet does not imply that other dietary components are not important. For example, starch digestibility is influenced by grain source and physical processing (Firkins *et al.*, 2001).

The availability and digestion passage kinetics of different carbohydrates are summarised in Table 1. Both soluble carbohydrates and soluble cell wall carbohydrates like β-glucans and pectins are readily degraded in the rumen, and only minor parts will escape for post-ruminal digestion except for some slowly degradable starch from e.g. maize. Most soluble carbohydrates are digestible in both the small intestine and the hind-gut, if they escape rumen degradation. Insoluble cell wall carbohydrates are generally slowly degraded in the rumen, and therefore the extent of rumen digestion is highly dependent on residence time of the fibre in the rumen. As digestion of both soluble and insoluble fibre is solely dependent on microbial fermentation, no digestion will take place in the small intestine; however fibre that escapes rumen digestion may be degraded in the hind-gut.

Digestion of dietary entities is a time-dependent process. The proportion of a nutrient that becomes available for absorption is determined by the rate of digestion relative to the rate of passage. For cell walls the rate of digestion in relation to passage is very slow compared with cell solubles, which explains the larger variability in cell wall digestibility. Digestion and passage in ruminants can be mechanistically described by compartmental models of varying complexity. Illius and Allen (1994) made a detailed comparison of the structure and assumptions of the models, which differed principally in the fractioning of feed and in the description of digestion and passage kinetics. Forage digestibility predicted by these models is generally within 15% of observed values (Illius and Allen, 1994), with R^2 values between observed and predicted in the range of 0.5 to 0.7. For practical feed evaluation, these models are simply not accurate enough. The bias in model predictions that frequently occur are more likely to result from a poor estimation of digestion

Table 1. Availability and digestion rate of different carbohydrate fractions.

	Rumen	Small intestine	Hind gut	Digestion rate
Cell solubles				
Sugars	High	(High)[1,2]	(High)[1]	Very fast
Starch	High (variable)	Variable	Variable	Fast
Soluble fibre				
Pectins, β-glucans	High	0	(High)[1)]	Fast?
Insoluble fibre				
NDF	Variable	0	Variable	Slow

[1] Only very little will reach post-duodenal digestive tract
[2] Some exceptions like sucrose

and passage kinetic parameters than from fundamental problems in model structure (Illius and Gordon, 1991). Much research has been conducted during the last decades to estimate digestion and passage kinetic parameters. However, most of the studies on digestion kinetics of cell walls (NDF) have compared parameter values between feeds, often estimating ruminal digestibility using simple dynamic rumen models. Curve fitting and the validity of kinetic methodology has also been extensively studied. However, the performance of models using digestion kinetic parameters has seldom been validated. In studies of passage kinetics the main focus has been in comparing markers, fitting marker concentration data to various compartmental models and particle size analysis, with less emphasis on validation of the data using dynamic rumen models. The lack of rigorous testing and evaluation of digestion and passage kinetic parameters in dynamic rumen models has probably prevented progress in developing useful models for accurate and reliable prediction of diet digestibility in ruminant animals. Experimental work and modelling should be carried out in harmony to reach satisfactory model performance.

The objectives of this chapter are to discuss the methods used for the estimation of digestion and passage kinetic parameters (1), intrinsic and extrinsic characteristics influencing the kinetic parameters (2) and the reliability of kinetic parameters for predicting *in vivo* digestibility using dynamic rumen models (3). Because most of the variation in OM digestibility can be explained by the characteristics of the cell wall fraction, the main focus will be placed on NDF digestion.

Site of digestion

In cattle the major proportion of cell wall digestion occurs in the forestomach. Paloheimo and Mäkelä (1959) examined the residence time in different digestion compartments in a slaughter study with dairy cows (n = 21) that consumed between 5-20 g DM per kg LW. Based on lignin pool sizes the mean fractional residence time of particulate matter was 0.76 in the reticulo-rumen, 0.10 in the omasum, 0.05 in the abomasum and small intestine and 0.10 in the caecum and colon. The proportion of NDF digestion in the forestomach (i.e. reticulo-rumen and omasum) is higher than indicated by the mean residence time because the potential digestibility [DNDF(digestible NDF)/NDF] of particulate matter decreases with time as digesta passes through successive compartments. Digestibility in the hindgut (i.e. caecum and colon) is therefore dependent on

the extent of forestomach NDF digestion. Sub-optimal rumen conditions decrease the digestion rate relative to that under optimal conditions, lower NDF digestibility in the forestomach and increase the amount of digestible NDF (DNDF) entering the hindgut. However, the efficiency of the hindgut microbial population to digest fibre seems to be lower than that of rumen microbes under normal rumen conditions. Michalet-Doreau *et al.* (2002) reported markedly lower cellulolytic activities in the caecum than in the rumen, suggesting that the rate of fibre digestion in the caecum and colon was lower than that in the rumen.

Flow studies have consistently indicated that the forestomach is the major site of NDF digestion in cattle (Tamminga, 1993). Based on three studies in duodenally cannulated cattle, the proportion of NDF digestion occurring in the forestomachs was on average 0.97 (Table 2). In seven other studies with lactating dairy cattle, NDF digestibility in the reticulo-rumen was determined based on the flow of NDF entering the omasal canal. The mean proportion of total tract NDF digestion in the reticulo-rumen was 0.93 in those studies. Because the role of the omasum appears to be larger than that of the hindgut in NDF digestion in cattle (Paloheimo and Mäkelä, 1959; Ahvenjärvi *et al.*, 2001) these results indicate that typically less than 0.05 of total NDF digestion takes place in the hindgut.

In order to illustrate the effects of suboptimal rumen conditions on NDF digestion occurring at different sites and the potential of hindgut digestion to compensate for decreased forestomach digestibility a simulation study on DNDF digestibility in the forestomach, hindgut and total tract was conducted. The model consisted of two rumen pools, a non-escapable pool with a second order gamma distribution of residence times, an escapable pool with a first order passage rate,

Table 2. Fractional proportion of NDF digestion in the intestines determined based on duodenal NDF flow and NDF digestion in the omasum and intestines determined based on omasal canal NDF flow.

Reference	Animal species	Diets, n	DM intake, kg^{-1} LW	Digesta sampling site	Fractional proportion of NDF digested proximal to the sampling site
Rinne *et al.*, 1997	Cattle	4	16	Duodenum	1.01
Huhtanen and Jaakkola, 1993	Cattle	6	18	Duodenum	0.93
Khalili and Huhtanen, 1991	Cattle	4	17	Duodenum	0.98
Stensig and Robinson, 1997	Cow	4	-	Duodenum	1.01
Volden, 1999	Cow	6	16 and 32	Duodenum	0.81 and 0.92
Lund, 2002	Cow	14	22	Duodenum	1.00
Ahvenjärvi *et al.*, 1999	Cow	4	26	Omasal canal	0.90
Korhonen *et al.*, 2002	Cow	4	30	Omasal canal	0.86
Ahvenjärvi *et al.*, unpublished	Cow	4	35	Omasal canal	0.89
Ahvenjärvi *et al.*, unpublished	Cow	4	31	Omasal canal	0.99
Ahvenjärvi *et al.*, unpublished	Cow	4	32	Omasal canal	1.00
Kuoppala *et al.*, unpublished	Cow	4	31	Omasal canal	0.92
Shingfield *et al.*, unpublished	Cow	4	30	Omasal canal	0.96

and a pool for the omasum (mixing pool), abomasum and small intestine (tubular flow), caecum and proximal colon (mixing pool) and distal colon (tubular flow). Further, the model assumed a total mean residence time of 46 h (Huhtanen and Hristov, 2001; Ahvenjärvi *et al.*, 2004), which was allocated between compartments based on the distribution between compartments reported by Paloheimo and Mäkelä (1959). Sub-optimal rumen conditions were assumed to decrease the rate of DNDF digestion from 0.075 to 0.030 h^{-1}, while the rate of DNDF digestion in the hindgut was assumed to be optimal (0.075), irrespective of rumen conditions. The results indicate that within the given range of the rate of digestion, DNDF digestibility in the forestomach decreased from 0.81 to 0.56 and that in the total tract from 0.85 to 0.66 (Figure 1).

The DNDF digestibility in the hindgut increased from 0.04 to 0.10 while the proportion of DNDF digestion in the hindgut as a proportion of total digestion increased from 0.05 to 0.16. These simulated results clearly suggest that due to limited residence time, the capacity of the hindgut to digest fibre is limited and can only partly compensate for lowered digestion in the forestomach. Consistent with flow measurements and modelling approaches, Huhtanen and Vanhatalo (1997) found using a combined rumen *in situ* incubation and mobile bag technique that the contribution of the hind-gut to the total NDF digestion was small. In the case that the hind-gut fermentation requires microbial colonisation (lag time), the extent of the cell wall digestion in the hindgut of ruminants would be further limited.

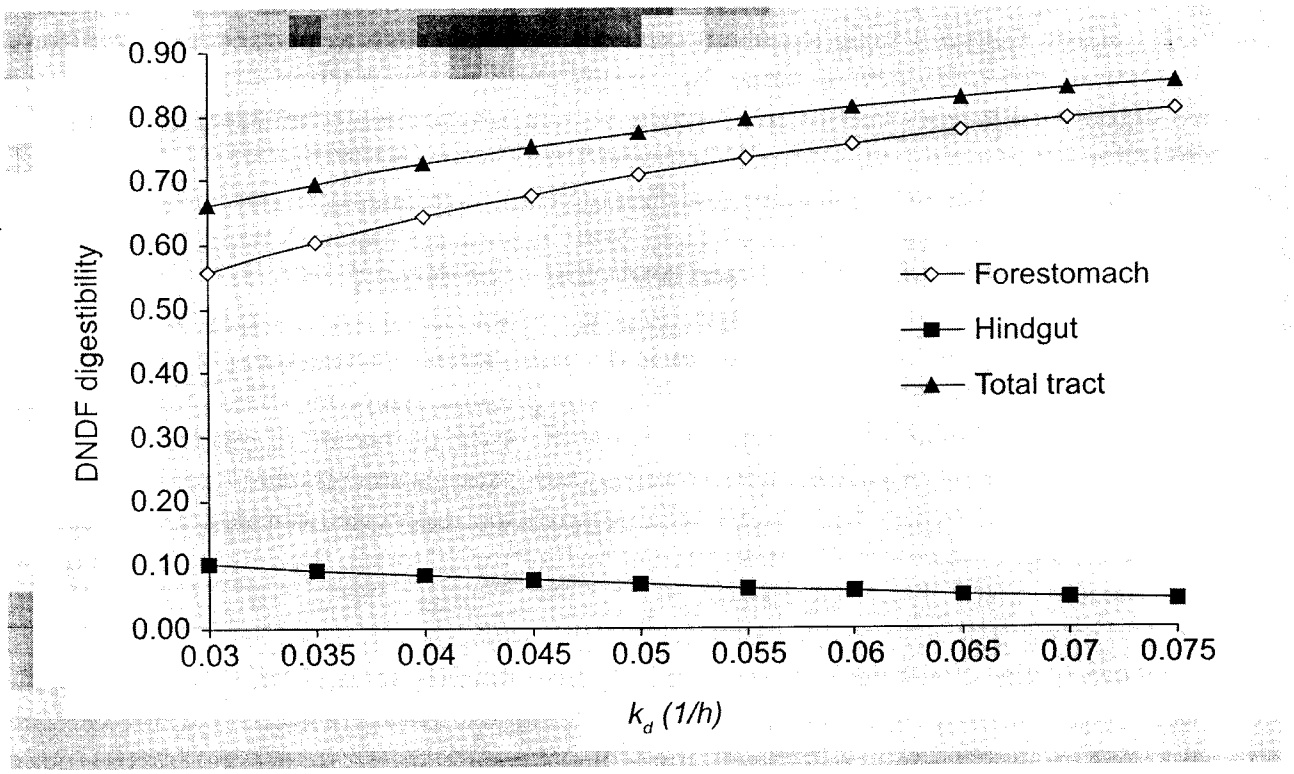

Figure 1. Simulated effects of digestion rate (k_d) in the forestomach on DNDF digestibility in the forestomach (reticulo-rumen and the omasum), hindgut (caecum and colon) and the total tract.

Digestion kinetics

The digestive system of ruminants is well adapted to the utilization of cell walls by microbial fermentation and the specialised ruminant stomach is comprised of four compartments (rumen, reticulum, omasum and abomasum). Fermentation of cell walls occurs in the first three compartments in a complex ecosystem that is influenced by interactions between feeds, microbial populations and the host animal. The rumen and reticulum form a large fermentation chamber (up to 20% of body weight) containing an active and diverse microbial population. Physical breakdown of large particles to small particles by mastication during ingestion and rumination is an important part of digestion process in ruminants. An optimal pH for microbial fermentation of cell wall carbohydrates is maintained by continuous salivary flow and absorption of volatile fatty acids (VFA) produced during fermentation. The role of the omasum, which is more developed in cattle than in sheep, is not completely understood. It appears to be related to the absorption and selective retention of feed particles in the rumen. In cattle, the omasum may have a greater role in NDF digestion than the intestines (Ahvenjärvi *et al.*, 2001). Microbial fermentation of carbohydrates is completed in the large intestine which behaves like a hybrid mixing-plug flow reactor.

Since the proposal of the NDF analysis as a measure of insoluble fibre some 40 years ago (Van Soest, 1963), this analysis has gained popularity and is now generally accepted as the most appropriate analysis for the determination of cell wall content of ruminant feeds. Although the NDF fraction does not include certain cell wall materials such as pectins and β-glucans, measurement of this entity does separate the completely digestible fraction from insoluble and partially digestible nutrients. Pectins and β-glucans are rapidly fermented and almost completely digested in the ruminant digestive tract (Van Soest, 1994), whereas the digestibility of other cell wall carbohydrates is highly variable due to differential lignification.

Parameter estimates of intrinsic rate and extent of digestion

Accurate and precise predictions of the intrinsic digestion kinetic parameters are critical to the accurate prediction of NDF digestibility. However, complicated symbiotic interactions between rumen microbes, the diet and host animal are essential for the utilisation of nutrients from cell walls. In order to be useful in dynamic rumen models, the kinetic parameters should only be limited by the attributes of substrates, i.e. intrinsic characteristics of cell walls. Physical and chemical attributes of the digestion environment should not be limiting factors in the determination of the potential rate and extent of NDF digestion

Several reviews of digestion kinetics of cell wall carbohydrates (Mertens, 1993a, 1993b; Ellis *et al.*, 1994, 1999) have addressed the problems associated with the estimation of kinetic parameters. The following discussion will focus primarily on the problems related to the determination of kinetic parameters and the importance of both accuracy and precision of these measurements. Errors in kinetic variables used for data validation limit improvement in mechanistic models as much as for empirical models. The importance of the rate and extent of NDF digestion on OM and NDF digestibility, rumen NDF pool and microbial N flow can be demonstrated by the Nordic model of dairy cow metabolism "Karoline" (Danfær *et al.*, 2005a,b). Simulations were made for a 550

kg dairy cow consuming 15 kg d^{-1} of grass silage DM using a range of indigestible NDF (INDF) concentrations and rates of digestible NDF (DNDF) digestion (Table 3). Simulation results clearly demonstrate profound effects of these parameters on OM digestibility and consequently on the supply of energy and microbial protein.

The earliest attempts to describe the kinetics of digestion have been reviewed by Mertens (1993a, 1993b). The term "rate of digestion" appeared in the 1950s, but the assessments were mainly based on the visual interpretation of digestion curves. The major breakthrough was made by Waldo (1970), who suggested that digestion curves are a combination of indigestible and digestible material. He also suggested that if the indigestible residue was subtracted, digestion of potentially digestible cell walls might follow first-order kinetics. The hypothesis that some material is indigestible was based on earlier work of Wilkins (1969), who observed that some cellulose remained undigested after extended periods of fermentation. Smith *et al.* (1972) used 72 h *in vitro* fermentations to determine indigestible NDF content (INDF). The potentially digestible NDF residue at earlier fermentation times was estimated by subtracting INDF from total NDF residue. The regression between the natural logarithm of DNDF against time was linear supporting the hypothesis that DNDF follows the first-order digestion kinetics. Indigestible NDF is an ideal nutritional entity according to the Lucas principle, because by definition it is digested at a predictable rate of zero. According to Ellis *et al.* (1999) determination of INDF should be included in all basic feedstuff analysis because (1) it has a predictable digestibility; (2) it can be used for the estimation of DNDF as NDF-INDF and (3) it has an important role in contributing to rumen digesta load.

The in situ method

The *in situ* method is the most common method used to estimate NDF digestion kinetic parameters. Several excellent reviews (Nocek, 1988; Mertens, 1993b; Ellis *et al.*, 1994; 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. Regardless of the method used to generate kinetic data, the system should measure the intrinsic rate of digestion,

Table 3. The effects of INDF[1] concentration and fractional rate of DNDF digestion (k_d) on total digestibility, rumen NDF pool and microbial N flow simulated by the Nordic dairy cow model (Danfær et al., *2005a).*

	INDF (g kg^{-1} DM)			k_d (h^{-1})			
	60	**100**	**140**	**0.04**	**0.05**	**0.06**	**0.07**
Digestibility							
OM	0.733	0.700	0.667	0.663	0.692	0.712	0.727
NDF	0.727	0.673	0.620	0.615	0.663	0.697	0.720
DNDF	0.808	0.808	0.808	0.739	0.795	0.836	0.865
NDF pool (kg)	6.76	7.47	8.18	7.91	7.20	6.65	6.25
Microbial N flow (g d^{-1})	227	213	197	203	216	226	233

[1]INDF concentrations (60, 100 and 140 g kg^{-1} DM) correspond to potential NDF digestibility of 0.900, 0.833 and 0.767, respectively.

which requires that the system itself does not limit digestion. The number of data points collected should be sufficient, particularly at the beginning and end of fermentation, to establish the initial solubilisation/lag and potential extent of fermentation (Mertens, 1993b). However, most of the published data on NDF digestion kinetics have been determined using less sampling times than what Mertens (1993b) suggested being optimal.

Several kinetic models to describe NDF digestion kinetics have been proposed (Mertens, 1993a, 1993b; Ellis *et al.*, 1994). The models differ with respect to the assumptions of a partition between potentially digestible and indigestible fibre (1), the number of compartments having a homogenous rate of digestion (2), a discrete lag time vs. compartmental lag time (3), time-independent vs. time-dependent distribution of digestion rates in the compartment (4) and first-order vs. second-order digestion kinetics (5). Although the assessment of INDF is critical for the accurate estimation of kinetic parameters, too often the kinetic parameters are calculated without accounting for the indigestible residue or by using a value determined over too short a fermentation time (Mertens, 1993a) when using linear regression on natural logarithm transformed DNDF residue. Using a model with heterogeneous rates provided superior or at least as good a fit compared with first-order models (Ellis *et al.*, 1994). The use of this model may be justified by the heterogeneous nature of chemical entities and their physical distribution in diverse plant tissues (Van Soest *et al.*, 2000). However, Van Milgen *et al.* (1993) recommended the use of first-order models because they provide rates that are easily interpreted, in contrast to the parameters generated by models using heterogeneous rates. In addition, using heterogeneous rate parameters in dynamic mechanistic rumen models is more difficult than using a first-order rate parameter. These problems may be solved by using the mean rate for the heterogeneous rates models (Ellis *et al.*, 1994), but in this case the possible advantages of a better model fit of the data are lost in the prediction of digestibility.

A plot of NDF residues against fermentation time often exhibits a lag period before the onset of fermentation (Mertens, 1993a). Lag is assumed to represent processes such as hydration of feeds, the time for microbial colonization and occurrence of analytically detectable digestion. The biological mechanisms underlying the lag phenomena are discussed in more detail by Allen and Mertens (1988) and Firkins *et al.* (1998). Mertens (1977) modified the first-order digestion model by including a discrete lag time. However, in biological systems it is unlikely that first-order digestion would start instantaneously after the lag period. Allen and Mertens (1988) proposed a two-compartmental sequential model (lag compartment and digestion compartment) to describe the process involving attachment of microbes to the cell walls followed by microbial digestion of cell walls. Van Milgen *et al.* (1991) proposed mathematical models that can be used to estimate the parameters for the sequential two-compartment model. This model affords a method for describing a less abrupt initiation of digestion. However, more work is needed on the biological accuracy of lag parameters and their importance to the accuracy and precision of NDF digestibility predictions by dynamic mechanistic rumen models. Allen and Mertens (1988) demonstrated by mathematical analysis, that if the lag phenomenon affect both digestion and passage, then the lag term has no influence on digestibility. Digestibility is independent of lag because wetting of particle is a prerequisite for both digestion and passage. However, a lag time on both digestion and passage will severely increase rumen load and alter the prediction of feed intake.

Two main methods are used for fitting data to the first-order kinetic models: linear regression on logarithmic transformations of undigested residues (ln-linear) and nonlinear estimation of parameters. Nonlinear models estimate parameter values simultaneously and assume an equal error at each fermentation time, whereas the ln-linear models assume that error is proportional to the size of residue at each time point. Neither of these approaches seems reasonable, because random errors are typically the largest for medium (8-48 h) incubation times. In the ln-linear approach indigestible NDF must be determined experimentally using the data from the last incubation time, and therefore any error in the estimation potential digestibility can bias the values for other parameters. For further details of the calculations of model parameter values the reader is referred to the reviews of Mertens (1993a, 1993b) and Ellis *et al.* (1994). Numerous models describing digestion kinetics have been evaluated by comparing the fit of the data, whereas robust testing of the kinetic models by comparing model predictions of digestibility with reference *in vivo* measurements is extremely limited.

Digestion of NDF continues even after long incubation periods *in situ* (Robinson *et al.*, 1986) suggesting that extended incubations are necessary in order to estimate INDF. Prolonged incubations also present other problems such as mineral precipitation occluding bag pores, escape of small particles from the bag or influx of material into the bag. These problems can partly be avoided by determining INDF on an ash-free basis and using bags of small pore size. A close empirical relationship between silage INDF content and OM digestibility (Nousiainen *et al.*, 2003b) indicates that INDF is a useful entity for the prediction of the nutritive value of forages. Indigestible NDF was determined by 12 d ruminal incubations in nylon bags of small pore size (6 or 17 μm). The relationship between INDF and OM digestibility was uniform for the primary growth and regrowth silages, whereas the relationship between OM pepsin-cellulase solubility and OM digestibility were different for the two types of silages. Near infrared reflectance spectroscopy (NIRS) can potentially be used for a rapid and accurate estimation of INDF content from forage samples (Nousiainen *et al.*, 2004). Ideally both the rate and extent of NDF digestion should be estimated simultaneously.

In vitro methods

Digestion kinetics can be evaluated *in vitro* from the disappearance of NDF or by measuring the volume of gas produced during the fermentation. When the methods are used to determine the intrinsic characteristics of feeds, it is important that the system does not impose constraints on digestion. Essential nutrients (e.g. ammonia, amino acids, and trace elements), pH, redox potential, anaerobicity and microbial numbers should not be limiting when measuring intrinsic characteristics of cell walls (Grant and Mertens, 1992). Variation in the activity of inoculum has been reported to affect the rate of NDF digestion (Cherney *et al.*, 1993). Variation between animals, species of the donor animal, feeding management, time of inoculum collection relative to feeding and the diet fed to the donor animal all affect *in vitro* digestibility (Weiss, 1994). These are animal factors which could also influence digestion kinetic parameters, of which the effect of diet is probably the most important. *In vitro* methodology has been extensively reviewed elsewhere (Mertens, 1993a; Weiss, 1994; Firkins *et al.*, 1998).

Automated methods to measure gas production system have some advantages relative to other methods. Frequent measurements can be made by the use of electronic pressure sensors and datalogging equipment. Automated data collection from the same fermentation vessel allows the collection of a sufficient number of observations for accurate parameter estimation. The second advantage is that digestion rates of different feed fractions can be estimated by fractionation of the feed before incubation. The NDF fraction is relatively easy to deal with because NDF can be chemically isolated and digestion kinetics can be measured by the gas production system (Schofield and Pell, 1995). If the digestion curve of NDF is subtracted from the equivalent amount of intact feed, a gas production curve for neutral detergent (ND) solubles is obtained and the kinetic parameters for ND solubles can be estimated from the latter curve. Estimation of digestion kinetics for ND solubles by the *in situ* method is not possible, because most of ND solubles escape the bag either by solubilisation or by efflux as small particles. Attempts have also been made to relate the pools estimated by a three-pool model to chemical fractions of a feed (Cone *et al.*, 1997). Although some similarities were observed, the relationship was not consistent. The pools estimated by the multi-pool models should therefore be viewed as purely mathematical constructs that may or may not correspond to chemical entities (Schofield, 2000).

In their review, Firkins *et al.* (1998) referred to several problems of the gas production system including a correction for changes in fermentation stoichiometry (VFA ratio) over time, evolution of gas from the buffer, errors caused by small sample sizes, an inability of the system to distinguish between different substrates, the contribution of ammonia to the gas pool and problems related to the blank correction. Many of these problems can, however, be reduced by chemically isolating NDF and measuring its digestion behaviour *in vitro*. Comparative studies on NDF digestion in the whole forages and in isolated NDF suggest that both the extent and rate of NDF digestion are similar (Doane *et al.*, 1997). The systems measuring digestion kinetics from gas production profiles have many common sources of errors with systems based on substrate disappearance, and it is equally important that digestion rate is not limited by the system. Technical details of automated gas production systems have been described in detail (Pell and Schofield, 1993; Theodorou *et al.*, 1994; Cone *et al.*, 1996).

Rumen evacuation technique

In the steady state situation the flux of an entity in or out of the rumen is related to compartmental mass. Fractional rates of intake, passage and digestion for the entities can be estimated by dividing these flows by rumen pool size (Robinson *et al.*, 1987) using the following equations:

$$k_i \text{ (Rate of intake; } h^{-1}) = \text{Intake (kg } h^{-1}) \text{ / Rumen pool (kg)} \quad (1)$$

$$k_p \text{ (Rate of passage; } h^{-1}) = \text{Flow (kg } h^{-1}) \text{ / Rumen pool (kg)} \quad (2)$$

$$k_d \text{ (Rate of digestion; } h^{-1}) = k_i - k_p \quad (3)$$

For a meaningful interpretation, k_d must be estimated only for digestible NDF. Estimating k_d for total NDF, although reported for some rumen evacuation studies, is meaningless and of little value. On a biological basis, estimating k_d by rumen evacuation (flux method) for total NDF is

incorrect, because digestion rate is determined for a fraction that also contains INDF. Kinetically it is not correct, because the pools do not have homogenous kinetic characteristics. Different proportions of INDF and DNDF are present in the flux and rumen pool.

Theoretically, the rumen evacuation technique should be an ideal method for estimating digestion rate, but it does have some disadvantages. It is time-consuming, expensive and laborious precluding its use for routine analysis. An accurate estimation of rumen pool size requires steady-state conditions which are difficult to achieve even in *ad libitum* fed animals. This problem can be reduced, although not completely eliminated, by frequent rumen evacuations and careful selection of rumen evacuation times to represent the mean rumen pool size. Estimation of k_d and k_p for DNDF requires accurate duodenal DNDF flow measurements. This may not be a major problem since the contribution of post-ruminal compartments to total NDF digestion is small (see Table 2), especially when conditions in the rumen are not a limiting factor for digestion. Under these circumstances predicting duodenal DNDF flow from faecal output as suggested by Robinson *et al.* (1987) may not markedly increase the error of k_d estimates. Probably the single largest disadvantage of the technique is that only digestion parameters for the total diet rather than for individual feeds can be estimated. Rumen evacuation technique ignores omasal cell wall pools, which has some influence on estimated kinetic parameters. Assuming that INDF and DNDF pool sizes in the omasum represent proportionally 0.20 and 0.13 of that in the rumen, respectively (Ahvenjärvi *et al.*, unpublished data), passage rate would be overestimated by a factor of 1.25 for INDF, and by 1.15 for DNDF digestion and passage rates.

In vivo digestibility method

Digestibility coefficients measured in sheep fed at a maintenance level of feeding is still the basis of most feed evaluation systems. Because digestibility of DNDF is a function of digestion and passage rates, it might be possible to estimate digestion rate of DNDF if the values for the digestibility of DNDF and compartmental residence time were available. In digestion trials digestibility of DNDF can be calculated when INDF content of the feeds is determined. The DNDF digestibility can be calculated from the kinetic parameters using the two compartment model that incorporates selective retention of feed particles in the rumen (Allen and Mertens, 1988) as follows:

$$\text{DNDF digestibility } (D) = (k_d/(k_d + k_r)\,[1 + k_r/(k_d + k_p)] \quad (4)$$

where k_d, k_r and k_p are the rates of digestion, release from the non-escapable to the escapable compartment and passage to the lower tract. The rate of digestion can be solved from equation 4:

$$k_d = [-(k_p + k_r) + [(k_p + k_r)^2 + 4Dk_rk_p/(1 - D)]^{0.5}]/2 \quad (5)$$

To estimate the rate of digestion indirectly by this method, an estimate of total mean residence time in the fermentation compartments and the distribution of the residence time between the two compartments are required. A data set of 52 primary growth and regrowth grass silages harvested at different stages of maturity (Nousiainen *et al.*, 2003a; 2003b) was used to calculate digestion rate assuming a compartmental mean residence time (CMRT) of 50 h for sheep fed at

maintenance and a value of 0.30 for the proportion of total CMRT in the first compartment. The mean rate of DNDF digestion was 0.075 h^{-1} (s.d. 0.0163, range 0.050 - 0.117). The disadvantages of this approach are that it requires reliable estimates of the passage kinetic parameters and that the kinetic data are estimated retrospectively from end-point determinations.

However, two questions arise; (1) are the differences between individual feed passage kinetics important enough to be taken into account and (2) how accurately can they be determined using current methods compared with simplifying using a constant value or deriving empirical relationships between intake and residence time. Estimates of k_d values are not markedly influenced by small variations in CMRT. For example, an increase in CMRT from 50 to 55 h would decrease the mean k_d value from 0.060 to 0.055 h^{-1}. Also the distribution of CMRT between the two compartments has a relatively small influence on calculated k_d value when the proportion of total CMRT is within a range of 0.20 - 0.40 (0.058 - 0.065 h^{-1}).

In conventional feed evaluation the digestibility of OM in sheep at maintenance is routinely estimated using laboratory methods. Using the Lucas principle does allow the estimation of ND solubles digestibility which combined with OM digestibility enables NDF digestibility to be calculated (Weisbjerg *et al.*, 2004b). If this approach is combined with INDF determination, estimates of k_d could be obtained from conventional feed analysis.

Effect of intrinsic characteristics on digestion kinetics

Plant species and maturity are the two most important sources of variation in digestion kinetics (Mertens, 1993a). Both the extent and rate of NDF digestion decrease with advancing maturity of grasses and legumes (Smith *et al.*, 1972). Close positive correlation between the indigestible residue and growing days was observed in the data of Nousiainen *et al.* (2003a) for 27 grass silages (mixtures of timothy and meadow fescue) harvested across seven years at different stages of maturity. A similar negative relationship was observed between growing days and the rate of DNDF digestion estimated using equation 5 (Figure 2). Cone *et al.* (1999) also reported close ($R^2 > 0.90$) relationships between growing days and both the extent and rate of digestion of grass silage. Digestion rates of legumes are higher compared with grasses (Smith *et al.*, 1972; Grenet, 1989; Van Soest, 1994) and the difference in digestion rates between red clover and timothy is much higher for stems than leaves (Rinne and Nykänen, 2000).

Digestion rates reported in the literature are highly variable between feeds, and surprisingly also within plant species. This raises the question whether these differences always reflect true differences in the digestion rate between feeds and dietary treatments and to what extent do the differences reflect the experimental techniques used. Smith *et al.* (1972) were the first to measure digestion kinetic parameters for a wide range of forage samples. The average DNDF digestion rate for grass samples was 0.096 h^{-1} and the range for early and late harvested samples varied from 0.140 to 0.053 h^{-1}. Although some grasses were harvested at a very early stage, the high values may also reflect the short incubation time (72 h) used to estimate the indigestible fraction. The average INDF concentration was 190 g kg^{-1} DM, which was more than 2-fold higher compared with that in primary growth grass silages (78 g kg^{-1} DM) harvested at various stages of growth (Nousiainen *et al.*, 2004). Nousiainen *et al.* (2004) determined INDF by 12 d *in situ* incubations using nylon

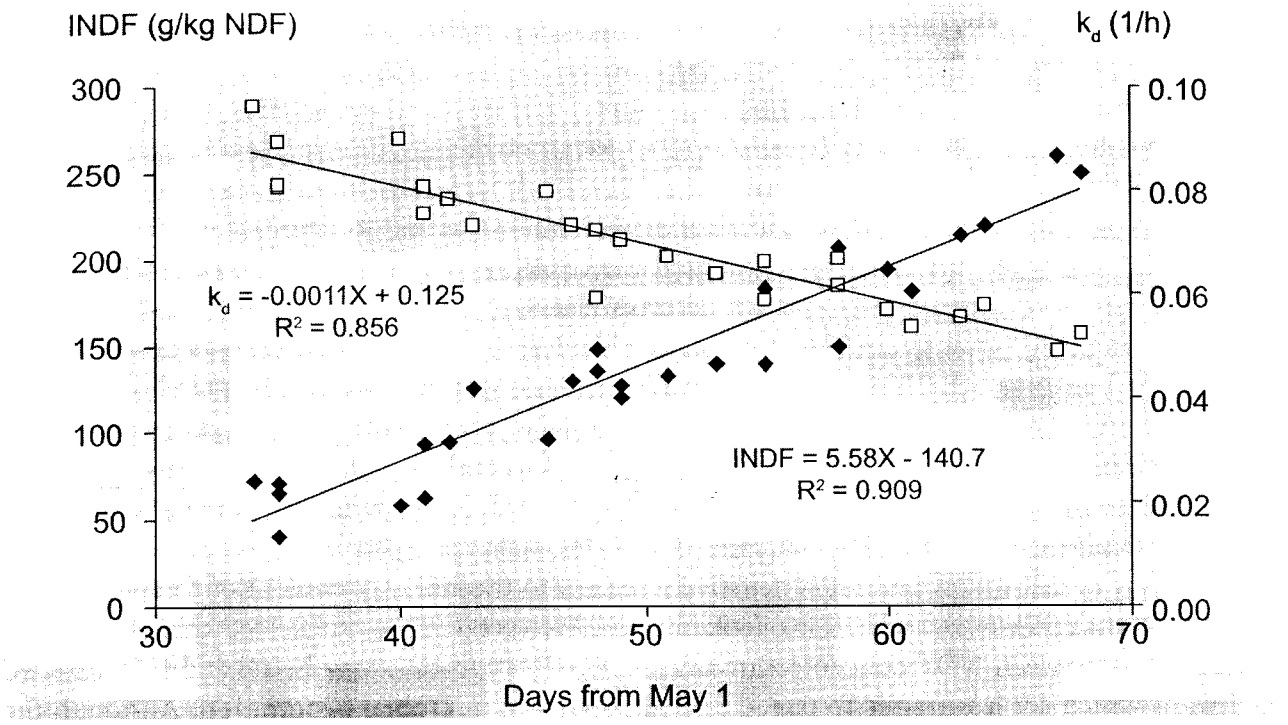

Figure 2. Effects of maturity of primary-growth timothy-meadow fescue silages on the proportion of indigestible NDF (g INDF/kg NDF) and the rate of NDF digestion. The values are adjusted for a random year effect. (Source: data from Nousiainen et al., *2003b).*

bags of a small pore size. The average DNDF digestibility of grasses calculated from the data of Smith *et al.* (1972) was 0.686, which was markedly lower than *in vivo* NDF digestibility (0.754) measured in sheep (Nousiainen *et al.*, 2004). The rate of digestion of silages estimated by equation 5 from *in vivo* DNDF digestibility was markedly lower (0.070 h^{-1}) than reported by Smith *et al.* (1972). Although the comparison of two different data sets is problematic, it is probable that the contrasting differences of digestion parameters are more likely related to methodological differences than a reflection of true differences in digestion kinetics.

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). The correlation between permanganate lignin and INDF concentrations in the data of Nousiainen *et al.* (2004) were also high both for primary growth and regrowth silages (0.86 and 0.91) but pooling this data together resulted in a weaker relationship (r = 0.61) suggesting that the association between lignin and INDF is not uniform. Regrowth silages contained more INDF than primary growth silages at the same lignin concentration. Furthermore, INDF estimated by *in situ* incubation predicted faecal NDF output (g NDF per kg DM intake) better than lignin (r = 0.91 vs. 0.79), and the relationship was more uniform between primary and regrowth silages with INDF than lignin. Satter *et al.* (1999) presented data demonstrating no relationship between lignin content and *in vitro* NDF degradability. We conclude 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 used universally for the estimation of INDF concentration or potential NDF digestibility.

Predicting the rate of digestion from chemical parameters may be even less successful than predicting the extent of digestion. Smith *et al.* (1972) reported high correlations between the rate of cell wall digestion and some chemical measurements within forage species or type (grass vs. legume) but when the data was combined the relationships were much weaker. Weisbjerg *et al.* (2003) found using barley and whole crop wheat forages that both NDF and ADF, but not ADL, were significantly and negatively correlated with the rate of NDF digestion. Wilman *et al.* (1996) reported that the rate of cell wall digestion was negatively correlated (r = -0.81) with silage NDF concentration and proposed that leafier crops were digested more quickly. A similar inverse relationship between NDF concentration and the rate of DNDF digestion was observed for primary growth silages (r =-0.80), but not for regrowth silages (r =-0.14) in the data of Nousiainen *et al.* (2004). Sauvant *et al.* (1995) also showed based on a large data set that the rate of cell wall digestion is negatively related to the cell wall content of forages, but no such relationships were observed for concentrates. Mertens (1993a) suggested that the inverse relationship between NDF concentration and NDF digestion rate may be related to thickened cell walls which are less fragile to particle breakdown and microbial penetration. Although the concentration and digestion rate of NDF can be closely related for certain types of forages such as primary growth grasses, the relationship is not uniform across a wide range of feeds.

Effect of extrinsic characteristic on digestion kinetics

The intrinsic rate and extent of cell-wall digestion set the upper limit for the utilisation of forages by ruminants. Extrinsic factors are independent of intrinsic factors, and may down regulate or decrease the rate, such that the intrinsic rate is not achieved, if rumen conditions are not ideal. Dietary components have different effects on rumen microbes, and interactions between dietary components in rumen digestion can occur. In addition to accurate estimates of digestion kinetic parameters, the effects of various extrinsic factors on digestion kinetic parameters should be understood to predict digestibility properly by dynamic mechanistic rumen digestion models. For low quality forages, limitations in the rate and extent of digestion can be attributed to a deficiency in the supply of essential nutrients such as N, S or in some cases branched-chain VFA (Hoover, 1986). In contrast, in high producing ruminants fed mixed diets, the rate of cell wall digestion in particular can be strongly retarded by substrates which inhibit the growth of rumen cellulolytic bacteria. In the following sections we will briefly discuss extrinsic factors, which may influence the intrinsic digestion kinetic parameters of cell-wall carbohydrates. A detailed discussion of the possible mechanisms behind these is beyond the scope of this review.

Carbohydrate supplementation

Increasing the concentration of non-structural carbohydrates (mainly starch and sugars) in the diet has frequently been shown to decrease fibre digestion. Decreases in the rate of cell wall digestion with increased supply of non-structural carbohydrates has been attributed mainly to lower ruminal pH, because cellulolytic bacteria are more sensitive to low pH than those utilising starch (Hungate, 1966; Russel and Dombrowski, 1980). *In vitro* (Grant and Mertens, 1991) and

in situ data (Mould *et al.*, 1983) suggest that rumen pH affect digestion kinetics in a biphasic manner. Above pH 6.2, the effects of pH on ruminal cell wall digestion are relatively small, but at a lower pH the effects are much stronger. Huhtanen and Jaakkola (1993) studied the effect of increased concentrate supplementation (barley + rapeseed meal) on cell wall digestion in cattle fed grass silage or barn dried hay as the basal forage. Total NDF digestibility decreased much more when the proportion of concentrate was increased from 0.50 to 0.75 than for increases from 0.25 to 0.50. Rumen pH decreased linearly from 6.43 at the lowest concentrate level to 6.21 and 6.03 with the medium and high concentrate diets, respectively. The mean rate of NDF digestion for the two forages determined by the rumen evacuation technique was depressed in a biphasic manner from 0.081 (low) to 0.075 (medium) and 0.047 (high), respectively. These results also suggest that a threshold pH for rumen cellulolysis is approximately 6.2, below which the degree of decrease in the rate of NDF digestion is much higher. The effect of increased concentrate on *in situ* NDF digestion rate was smaller than that observed by rumen evacuation, probably because the extent of *in situ* NDF digestion also tended to decrease. A reduced rate of NDF digestion estimated by rumen evacuation has been reported by Khalili and Huhtanen (1991) with sucrose supplements, by Stensig *et al.* (1998) with sucrose and starch and by Oba and Allen (2003) with dietary starch supplementation.

Lindberg (1981) studied the effects of oats fed proportionately at 0, 0.30 and 0.70 of diet DM on *in situ* digestion kinetics of forages. It was noticeable that increasing the amount of oats in the diet was associated with a decrease in both the rate and extent of NDF digestion. Similar effects were later reported by Huhtanen and Jaakkola (1994), who incubated six grasses in the rumen of cattle fed grass silage or barn dried hay with proportionally 0.25, 0.50 or 0.75 concentrates of diet DM. However, care should be exercised in interpreting these findings. It is possible that the longest incubation periods were too short to allow an accurate estimation of the extent of NDF digestion.

Grant and Mertens (1991) reported that the effect of rumen pH on *in vitro* cell wall digestion varied with substrate. Digestion of legumes was less sensitive to lower rumen pH than that of grasses. The results of *in situ* studies by Mould *et al.* (1983) and Huhtanen and Jaakkola (1994) indicate that digestion of low quality forages is influenced to a greater extent by increased concentrate supplementation.

Mould *et al.* (1983) differentiated the adverse effects of non-structural carbohydrates on cell-wall digestion between a 'pH effect' and a 'carbohydrate effect'. The depression in cell wall digestion that could not be alleviated by increasing rumen pH with buffers was designated the 'carbohydrate effect' and the depression related to low pH was designated as the 'pH effect'. When the supply of rapidly degradable substrates such as sugars and starch is excessive, the bacteria using these substrates will predominate in the rumen. Under these circumstances cell wall digestion could be impeded due to high acid production or the use of limiting nutrients by these bacteria. Although the evidence suggests that the depression in cell-wall digestion is associated with reduced rumen pH, there is little evidence that it is the sole causative factor (Mertens, 1993a). Intraruminal infusion studies (Rooke *et al.*, 1987; Huhtanen, 1987) indicated that cell wall digestion can be depressed by a continuous supply of rapidly degradable carbohydrates without decreasing rumen pH. Mertens and Loften (1980) observed that when pH was maintained at 6.8, digestion rate of

forage NDF was reduced slightly and the lag time increased markedly when starch was added *in vitro*. Reduced cell wall digestion with continuous infusion of sucrose was associated with lower particle-associated enzyme activities in rumen digesta (Huhtanen and Khalili, 1992). Studies using continuous cultures allowing for independent changes in pH and level of rapidly degradable carbohydrates showed that the level of the rapidly degradable carbohydrates was the most important for fibre digestibility (Weisbjerg *et al.*, 1999). *In vitro* work (Groleau and Forsberg, 1981; Williams and Withers, 1982) has indicated that the activity of cell-wall degrading enzymes depends on the carbon source.

Protein supplementation

When N supply becomes limiting, cell-wall digestion is retarded. Therefore it is essential that N supply is not a limiting factor when intrinsic digestion parameters are determined. Hoover (1986) suggested a minimum ammonia concentration of 3.6 mmol L^{-1} when dietary crude protein (CP) concentration exceeds 60 g kg^{-1} DM. Ellis *et al.* (1999) reported that protein supplementation increases the rate of digestion of cell-wall carbohydrates of forages which contain less than 80 g CP kg^{-1} DM. Mertens (1993a) postulated that the minimum amount of available N depends on the digestibility of forages, and is relatively higher for highly digestible forages. The concentration of dietary N needed to optimise cell-wall digestion is also a function of ruminal protein degradability (Ørskov, 1982). Positive effects of protein level on cell-wall digestion in dairy cows were reported by Oldham (1984) at a much higher level than the suggested minimum for optimal digestion. An increase of 7.3 g kg^{-1} in NDF digestion per 10 g kg^{-1} DM increase in dietary CP was estimated from a data set (N = 182) of studies conducted in lactating dairy cows (Huhtanen *et al.*, unpublished). Dietary CP was increased by replacing energy supplements with protein supplements such as rapeseed, soybean and fish meals. However, the mechanisms of protein responses in dairy cows are not completely clear, and the effects may partly be mediated through changes in the intrinsic characteristics of cell walls and partly through the effects of protein on microbial activity in the rumen. Improved digestibility of DNDF with protein supplementation reported by Shingfield *et al.* (2003) suggests that an increased availability of amino acids in the rumen improved cell wall digestion that may have been mediated through increases in the rate of digestion. However, the possible effects of reduced starch content in the diet can not be ruled out as a mechanism for improved digestion rate with increased dietary CP. The singular effects of degradable protein can be determined using urea as a N source. Weisbjerg *et al.* (1998) added 260 g urea d^{-1} per cow to a ration highly deficient in rumen degradable protein and increased dietary CP from 112 to 144 g kg^{-1} DM resulting in an enhanced rate of DNDF degradation from 0.019 to 0.031 h^{-1}, although *ad libitum* feed intake increased also considerably.

Fat supplementation

Supplementation of a diet with fats or fatty acids can affect ruminal metabolism and reduce fibre digestion. This is due to the toxic effects of fatty acids on rumen bacteria, particularly unsaturated and to a lesser extent medium chain fatty acids which can reduce fibre digestion (Weisbjerg and Børsting, 1989; Demeyer and Van Nevel, 1995; Doreau and Chilliard, 1997), but these negative effects are not always seen (Ueda *et al.*, 2003). Use of more rumen inert (protected) fat sources such as saturated fatty acids or calcium soaps of unsaturated fatty acids reduce negative effects of

the fat supplementation on rumen metabolism. Generally negative effects on fibre digestibility will not be manifested until fat supplementation exceeds 40-50 g kg^{-1} DM, but this limit depend on whether the fat is inert, on fatty acid concentration and composition of the basal ration and on the physical structure of the diet (Doreau and Ferlay, 1994; Lewis *et al.*, 1999).

The results of Tesfa (1992, 1993) indicate that high levels of rapeseed oil (67 g kg^{-1} DM) decreased the rate of NDF digestion markedly when determined by *in situ* or rumen evacuation techniques. Reduced particle-associated enzyme activities in rumen digesta and undigested *in situ* residues suggested that decreased cell wall digestion was associated with the adverse effects of oil supplementation on the activity of rumen cellulolytic bacteria rather than from the oil coating fibre particles.

Due to the high energy content, isoenergetic supplements of fat can be used to replace large amounts of rapidly degrading carbohydrates (starch) in concentrates. Therefore fat with a low iodine value can have a positive effect when added on an isoenergetic basis, due to the substitution with more problematic starch.

Since fat supplementation can alter rumen metabolism, it could also be expected to affect rumen passage kinetics. Although adverse effects have been seen in some studies, Doreau and Ferlay (1995) concluded from an analysis of 18 studies, that neither liquid or solid phase turnover was affected by fat supplementation.

Feeding level

Diet digestibility decreases with increased feed intake. Reduced digestibility has mainly been attributed to decreased rumen residence time allowing less complete digestion of DNDF. The effects of feeding level on the rate of NDF digestion have not been extensively studied. Reduced rates of NDF digestion have been reported by Staples *et al.* (1984), Robinson *et al.* (1987) and Okine and Mathison (1991) when DM intake increased. Staples *et al.* (1984) used an *in situ* technique, while the others used rumen evacuations. One reason for the adverse effect of feed intake on cell-wall digestion is that rumen VFA concentrations increase, with a concomitant decrease in rumen pH (Tamminga and van Vuuren, 1988; Volden, 1999), to which cellulolytic bacteria are sensitive. Huhtanen *et al.* (1995) fed growing cattle at 8.5 or 17.0 g DM kg^{-1} LW and reported that feeding level had no effect on rate of NDF digestion as estimated by rumen evacuation. The estimates based on *in situ* data suggested a trend towards a decrease in the extent, but an increase in the rate of digestion with increased feed intake. The decrease in total NDF digestibility from 0.758 to 0.707 could be entirely due to higher passage rates. Indirect comparison of digestion rates estimated by rumen evacuation data in growing cattle and dairy cows fed similar diets (Rinne *et al.*, 1997, 2002) suggest a reduced rate of digestion with increased feeding level. In both studies animals were fed four grass silages harvested at one week intervals. The silages had similar differences in INDF concentration (cattle: 143-228; cow: 149-217 g kg^{-1} NDF) and cows and young cattle were both fed the same proportion (0.30) of a similar concentrate (cereal grain and rapeseed meal). Feeding level was markedly higher for dairy cows than for growing cattle (32 vs. 17 g DM kg^{-1} LW). Estimated rates of DNDF digestion were clearly higher in growing cattle than in cows (0.073 vs. 0.056 h^{-1}). Digestion parameters of the silage estimated by the *in situ*

method were similar, but rumen pH was clearly lower in cows than cattle fed comparable diets, which may be the reason for the lower digestion rate observed in cows. The results of Llano and DePeters (1985), Huhtanen *et al.* (1995) and Volden (1999) suggest that reduced OM digestibility with increased DM intake can almost entirely be attributed to lower NDF digestibility. However, for some diet types e.g. rations rich in slowly degradable maize starch, an increase in DM intake can also significantly reduce the digestibility of cell solubles (Colucci *et al.*, 1982).

Validity of digestion kinetic methods

Information about the rate and extent of cell wall digestion has been increased by the use of *in situ* and *in vitro* techniques. However, *in vivo* validation of the results has seldom been carried out. Due to the lack of reliable and widely accepted reference methods, the merits and demerits of different digestion kinetic methods can not be verified. Ellis *et al.* (1994) suggested that the *in situ* method is preferable because aspects of rumen environment are more faithfully simulated. It has further been argued that the *in situ* method also measures the combined effects of both the animal and the diet on digestion, which can be considered as a disadvantage in the determination of intrinsic digestion kinetic parameters. The advantages related to the applicability of *in vitro* and *in situ* methods with respect to obtaining meaningful kinetic data have been discussed in detail (Mertens 1993a, 1993b). Mertens presented several critical aspects of the *in situ* method: kinetic results may be biased because of non steady-state conditions in the rumen (1), suboptimal conditions in the rumen may put an upper limit on the rates (2) and inflow and outflow of particles to the bag (3). These aspects are probably more critical for the determination of the intrinsic rate of digestion than for the determination of the extent of digestion. The close relationship between *in vivo* digestibility and the potential extent of digestion (Nousiainen *et al.*, 2003b) suggests that using prolonged incubations and bags with a small pore size may allow the extent of NDF digestion to be accurately measured.

Digestion rates determined by *in situ* incubation have been lower than values derived from rumen evacuation in studies conducted at different laboratories (Aitchison *et al.*, 1986; Tamminga *et al.*, 1989; Huhtanen and Jaakkola, 1993; 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 lower 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. The pH in the bags has also been lower than in rumen digesta (Nozière and Michalet-Doreau, 2000), which points towards the sub-optimal conditions within the bag. The differences in microbial activity may be explained by a shorter residence time of feed particles in bags compared with rumen digesta, the lack of mastication of forage particles placed in the bags or limiting conditions within the local bag environment (Nozière and Michalet-Doreau, 2000). The highest particle-associated enzyme activities within the bags were proportionally less than 0.50 of those found in rumen digesta (Huhtanen *et al.*, 1998) indicating that colonization of the cell degrading bacteria is constrained within the bags. In their study particle-associated enzyme activities in bag residues and NDF disappearance were greatly reduced with smaller pore size and/or smaller open surface area of the bags. Disappearance

of NDF from bags of different cloth types incubated for 6, 12, 24 and 96 h was closely related to the logarithmic transformed cumulative area under enzyme activity curves. This suggests that enzyme activity, rather than intrinsic characteristics of forages, limit cell wall digestion of feeds incubated in nylon bags.

If the rate of NDF digestion *in situ* is constrained by reduced microbial colonization and/or low pH in the bags, digestion kinetics determined *in vitro* may describe the intrinsic digestion kinetics more accurately than the *in situ* method. Cone *et al.* (1998) reported that *in situ* rates of NDF and OM digestion were closely related to the rate of digestion estimated from the second sub-curve of the total gas production curve, which corresponds to the degradation of non-soluble OM (Cone *et al.*, 1997). However, the estimated rates were much higher with the gas production than with the *in situ* technique. The relationship between digestion rates estimated from gas production profiles or by the *in situ* technique was weak for hays (Khazaal *et al.*, 1993) and straws (Blümmel and Ørskov, 1993). The gas production profiles were fitted to a single phase exponential model, and the gas production from the soluble fraction is included which is not the case for the *in situ* method. Firkins *et al.* (1998) suggested that the accuracy of determination of the rates of gas production can never be greater than the method of the verification (*in situ* or *in vitro* substrate depletion kinetics). However, comparing different kinetic methods (*in vitro, in situ*) with each other may be of little value before the accuracy of these methods has been improved.

Huhtanen *et al.* (2001) measured gas production kinetics of NDF isolated from 15 samples of grass silages harvested at different stages of maturity. The first-order NDF digestion rate estimated from gas production profiles was closely related with the rate of NDF digestion derived from *in vivo* measurements (see equation 5). However, the rate of NDF digestion estimated for a sub-set of six of the silages by ruminal *in situ* incubation was clearly underestimated compared with *in vivo* measures, but a strong linear relationship between the estimates based on *in situ* and gas production kinetics was observed (Figure 3).

Passage kinetics

Microbial digestion of cell walls is a relatively slow process. To achieve effective cell wall digestion, ruminant animals have developed large fermentation chambers in the fore-stomach, where they retain feed particles substantially longer than fluids. A long retention time in the reticulorumen improves the utilization of cell walls but it may restrict feed intake. The ruminal digestibility is determined by the rates of digestion and passage, i.e. digestion and passage are considered to be competitive processes. Unlike digestion kinetics that can be measured by *in vitro* or *in situ* methods, passage kinetics must be determined *in vivo* due to the interaction between diets and animals. Methods for the estimation of passage kinetics are laborious and expensive. The advantages and disadvantages of different markers and mathematical models applied are still debatable, such that the biological interpretation of estimated parameters is often uncertain and the parameter values are often erroneously used in rumen models to estimate digestibility. Different aspects related to the techniques used to estimate digesta passage have been the subject of numerous reviews (Lechner-Doll *et al.*, 1991; Faichney, 1993; Mertens, 1993a; Ellis *et al.*, 1994, 1999). This section will focus on the aspects of digesta passage kinetics relevant to the estimation of kinetic data for

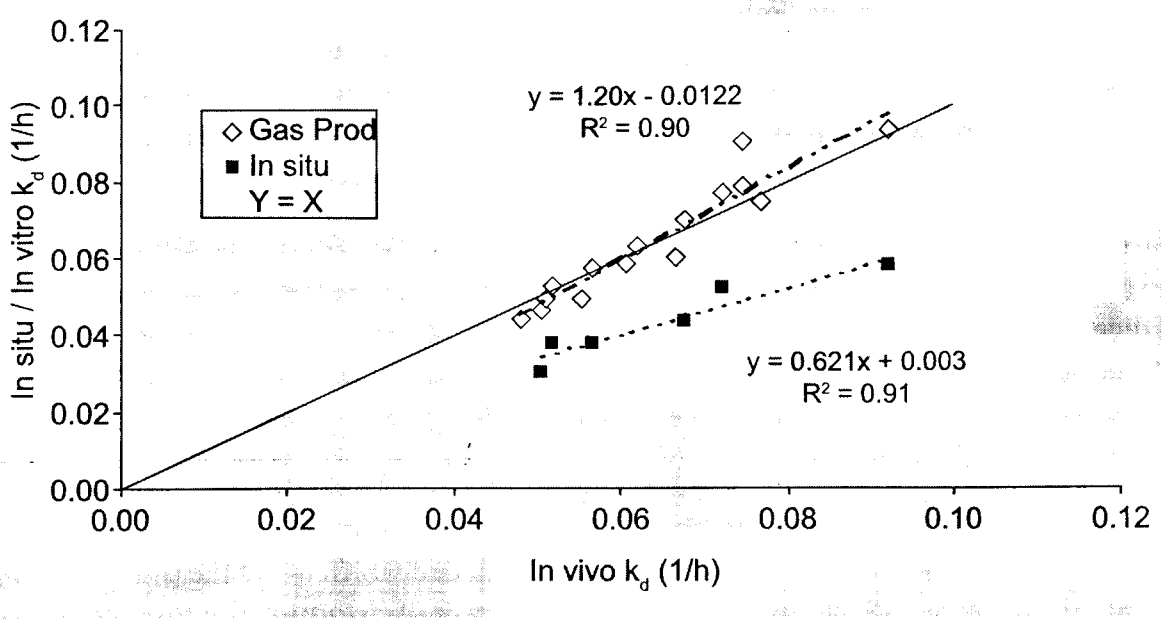

Figure 3. Digestion rate (k_d) estimated from in vivo data or by using in vitro gas production and in situ techniques (Data from Huhtanen et al., *2001; Nousiainen* et al., *2004; unpublished in situ data).*

mechanistic rumen models. Simulation results presented in Table 4 highlight the importance of accurate determinations of rumen residence time on the predictions of nutrient supply.

Methodology

Markers

In parallel with digestion kinetic methodology, the progress in developing an effective and accurate passage kinetic methodology has been retarded by the lack of a reliable, non-laborious and simple reference method to validate and interpret the kinetic data.

Table 4. The effects of rumen residence time on digestibility, rumen NDF pool and microbial N flow simulated by the Nordic dairy cow model (Source: Danfær et al., *2005a).*

	Rumen residence time (h)				
	30	**35**	**40**	**45**	**50**
Digestibility					
OM	0.657	0.676	0.691	0.703	0.713
NDF	0.608	0.637	0.659	0.678	0.693
DNDF	0.730	0.764	0.791	0.814	0.832
NDF pool (kg)	6.01	6.59	7.11	7.59	8.03
Microbial N flow (g d^{-1})	200	205	210	213	217

INDF 100 g kg^{-1} DM, DNDF 500 g kg^{-1} DM, k_d of DNDF 0.05 h^{-1}

Different markers, sampling procedures and methods of compartmental analysis have been applied, but the biological interpretation of compartmental analysis is debatable.

To estimate digesta passage rate, a limited amount of the marker is administered, usually as a single pulse-dose, followed by digesta sampling. Various external markers have been used to describe passage kinetics. Ideal markers are indigestible, i.e. have no effect or be little affected by the microbial population, and must be associated with undigested nutrients, or flow through the digestive tract at an identical rate, and do not separate from the respective labelled fraction (Kotb and Luckey, 1972; Ellis *et al.*, 1994). Probably none of the current passage kinetic markers satisfy all these criteria. The chromium mordanted fibre method, as described by Udén *et al.* (1980), has been criticized because it renders the fibre indigestible and tends to increase particle density (Ehle *et al.*, 1984), which may increase passage raté. In contrast to the generally observed relationship between density and passage rate (Lechner-Doll *et al.*, 1991), Lirette and Milligan (1989) reported a shorter total mean retention time (TMRT) for particles labelled at a low compared with high levels of Cr (0.2 vs. 5 g kg^{-1} DM), probably because the lower degree of mordant had less of an effect on the digestibility of labelled particles. In their study, TMRT was 13-14 h longer for 10 mm compared with 1-2 mm particles, demonstrating the importance of particle size in passage kinetic determinations. The particle size of the labelled feed should be similar to that of unlabelled feed.

Rare earths are probably the most commonly used passage kinetic markers. Rare earths are indigestible and are resistant to replacement from feed residues within the normal pH range in the rumen (Ellis *et al.*, 1994). Rare earths have been criticised since they migrate to rumen fluid (Beauchemin and Buchanan-Smith, 1989; Combs *et al.*, 1992) and are preferentially bound to small rather than large particles (Siddons *et al.*, 1985). A longer CMRT of Cr-mordanted fibre compared with Yb-labelled fibre (Beauchemin and Buchanan-Smith, 1989; Huhtanen and Kukkonen, 1995) suggests that at least one of these markers does not behave as an ideal marker. Removing unbound or loosely-bound rare earths by washing with a mild acid solution may solve the problems related to the dissociation of the marker from low affinity binding sites in labelled feed particles to rumen microbes and liquid phase or from large to small particles (Ellis *et al.*, 1994), which have a faster passage rates. The validity of the marker system may be tested by comparing the marker retention time to that estimated using the rumen evacuation technique for an internal marker naturally included in the feed such as INDF or lignin. Ellis *et al.* (2002) reported a similar CMRT estimated from the passage kinetics of rare earths or from the turnover of INDF from the rumen. They postulated that migration of rare earths from labelled particles, which has sometimes been observed, is probably a result of applying rare earths in excess of their binding capacity and failure to remove excess or unbound rare earths. Huhtanen and Kukkonen (1995) compared the CMRT estimated from duodenal Yb and Cr concentrations to that estimated by rumen evacuation technique and INDF turnover. A mean CMRT of 67, 57 and 63 h was calculated for Cr-mordanted fibre, Yb-labelled fibre and INDF, respectively, in cattle fed at two levels of intake. These results indicate that Cr slightly overestimated, and Yb underestimated CMRT based on ruminal INDF turnover. Lund (2002) also found that Yb-labelled fibre underestimated CMRT compared with INDF and rumen evacuation, particularly for diets with the highest CMRT of INDF. Earlier conclusions (e.g. Tamminga *et al.*, 1989; Huhtanen and Kukkonen, 1995) that Cr-mordanted fibre underestimates rumen retention time (overestimates

the passage rate) were mainly due to the estimation of passage rate from the descending faecal marker excretion curve and ignoring the ascending phase in marker concentrations.

Intrinsically labelled plant cell walls should be ideal markers because they undoubtedly flow with undigested feed residues. The problem of internal markers such as ^{13}C and ^{14}C is that the label is incorporated into both the digestible and indigestible cell wall fractions. Digestible components should therefore be removed before dosing (Smith, 1989), or alternatively the marker concentration could be measured in the INDF fraction. Intrinsically labelled forage ADF-bound ^{15}N was used as a passage kinetic marker by Huhtanen and Hristov (2001). Ahvenjärvi *et al.* (2004) compared ADF-^{15}N in grass silage to Cr-mordanted and Yb-labelled silage as passage kinetic markers in dairy cows. They observed that the CMRT and total mean residence time (TMRT) were similar for ADF^{15}N and Cr-mordanted but shorter for Yb. The use of intrinsic markers is too laborious and expensive for routine use, but it may be a useful tool for evaluating the reliability of external markers.

Compartmental analysis

Similarly as for digestion kinetics, various mathematical models have been proposed for the estimation of passage kinetics. The earliest compartmental model to describe digesta flow based on faecal marker excretion data was described by Blaxter *et al.* (1956). The model consisted of two sequential age-independent mixing compartments with a discrete time delay. Grovum and Williams (1973) proposed that the two sequential compartments represent the retention of feed particles in the rumen and the caecum-proximal colon. Matis (1972) proposed a two compartmental model with sequential age-dependent and age-independent compartments and a time delay. He assigned an age-dependent distribution of residence times to the faster compartment, which describes processes such as hydration, microbial colonization and fragmentation of feed particles by rumination. The flow from the second, age-independent compartment is described by simple first-order kinetics. Details of the age-dependent models are described by Pond *et al.* (1988) and Ellis *et al.* (1994). The models of Dhanoa *et al.* (1985) and France *et al.* (1985) also describe age-dependent processes but use different mathematical approaches.

Estimates of rumen passage kinetics can be verified by the rumen evacuation technique or by slaughter studies. The mean residence time (MRT) in each segment of the digestive tract using indigestible NDF as a marker is calculated as: MRT (h) = INDF (g) in the segment / INDF intake (g/h). Paloheimo and Mäkelä (1959) used this method to estimate turnover time of lignin in different sections of the digestive tract of cows. Residence time in different sections of the digestive tract can also be estimated by marker dosing and sampling digesta from different sites of the digestive tract. Only small differences in the CMRT estimated from duodenal or faecal samples have been observed in cattle when different external markers (Pond *et al.*, 1988; Huhtanen and Kukkonen, 1995; Wylie *et al.*, 2000; Lund, 2002) or an internal marker (Huhtanen and Hristov, 2001) were used. These observations suggest that most of the residence time in the first compartment is pre-duodenal. Ellis *et al.* (2002) proposed that the proportion of the total compartmental residence time due to the mixing flow in the rumen is relatively constant (in order of 0.9), and that CMRT in the rumen could be predicted from faecal marker profiles. Indeed, faecal sampling may be even more accurate in predicting pre-duodenal CMRT due to the inability of collecting representative

samples of rumen digesta and problems in obtaining representative samples of duodenal or ileal digesta. Dosing particle markers in the abomasum (Wylie *et al.*, 2000) or duodenum (Huhtanen and Kukkonen, 1995; Mambrini and Peyraud, 1997) and sampling faeces also indicated that post-ruminal residence time in the mixing compartments is relatively short, representing less than 0.10 of total CMRT estimated from faecal sampling. In the slaughter study (Paloheimo and Mäkelä, 1959), the proportion of retention in the hind-gut of that in the total tract for lignin was 0.10 which is entirely consistent with the marker kinetic data.

Interpretation of marker kinetic data obtained from duodenal sampling supports the suggestion of Hungate (1966), who proposed that there are two different compartments: a rumination pool of large particles and a passage pool of small particles, where the passage of initially large particles from the rumen is a result of two sequential first-order processes. Actually, the models of Matis (1972) and his co-workers (Pond *et al.*, 1988; Ellis *et al.*, 1994) proposed an age-dependency to the rumination pool of the large particles. Faichney (1986) seriously questioned this approach. First, the model does not take into account the entry of small particles produced by chewing during eating, and secondly, the abomasum and caecum/proximal colon act as mixing compartments. However, as previously discussed the contribution of post-duodenal segments to total CMRT is relatively small, at least in cattle. When faecal particles labelled with rare earths were dosed into the rumen followed by duodenal or faecal sampling, the CMRT in the age-dependent compartment was about 9 h and not different between the sampling sites (Wylie *et al.*, 2000). Dosing labelled faecal particles into the abomasum, and estimating total CMRT from duodenal and faecal sampling indicated residence times of 1.1 and 3.0 h in the abomasum and hind-gut, respectively, indicating that these sites contribute relatively little to the total residence time in mixing compartments.

The models with gamma time-dependency have improved the fit of the data compared with the two-compartment model with first-order passage from both compartments (Pond *et al.*, 1988; Ellis *et al.*, 1994; Huhtanen and Kukkonen, 1995; Lund, 2002). However, this does not necessarily imply that these models describe the distribution of the total retention time in different segments of the gastrointestinal tract any better than other models. Increasing the degree of age-dependency in the first compartment changes the partitioning between the time delay and the age-dependent residence time. However, estimates of time delay are more consistent with observed data (first appearance of marker at the sampling site) with age-dependent models (Pond *et al.*, 1988; Huhtanen and Kukkonen, 1995). Conversely, estimates of CMRT should be more consistent with actual data, but validation is more difficult. In the study of Huhtanen and Kukkonen (1995), CMRT estimated from duodenal sampling was 52, 52, 56 and 60 h with increasing age-dependency in the first-compartment. The last two values are in better agreement with INDF turnover time estimated by rumen evacuation (63 h).

Most of the experimental data indicate that the passage of feed particles in ruminants is a multi-compartmental process. When marker excretion data is fitted with a two-compartment model, only the residence time in two compartments (or aggregated compartments) can be described (Mertens, 1993a). However, if the passage kinetic parameters are estimated simultaneously from duodenal and faecal sampling, fairly accurate representations of the residence time of particles in different compartments may be obtained. We estimated the passage kinetic parameters from

simulated duodenal and faecal marker concentration data using passage kinetic models with increasing age-dependency in the first compartment. The following residence times in different sections of digestive tract were used: rumen large particle (lag-rumination) pool 10 h (with an exponential or 2nd order gamma distribution of residence times), rumen pool of small particles 25 h (exponential distribution of residence times), omasum 4 h (mixing flow), abomasum and small intestine 4 h (tubular flow), caecum and proximal colon 5 h (mixing flow), distal colon 4 h (tubular flow). The parameter values estimated by the best fit model of series of the models with age-dependency in the first compartment are shown in Table 5. When the rumen lag-rumination (non-escapable) pool had gamma two age-dependency, G_3G_1 model (see Ellis *et al.*, 1994) resulted in the best fit of simulated duodenal marker concentration, and the G_4G_1 model was the best for faecal sampling data. Similarly, Lund (2002) found that a higher order gamma function was needed to fit faecal sample data compared with duodenal measurements, suggesting the existence of an additional post duodenal compartment (Huhtanen and Hristov, 2001). The best fit models estimated the 'true' time delay and CMRT correctly. Interestingly, the residence times in the omasum (duodenal sampling) and omasum + caecum + proximal colon (faecal sampling) were realised as an increase in the residence time in the lag-rumination compartment. This suggests that the model does not correctly describe the biological processes of digesta passage. However, this does not mean the parameter values estimated by the passage models would be of little value for mechanistic rumen digestion models. The best fit models estimated accurately both the pre-duodenal and total residence time in the mixing compartments, and consequently the residence time in the post-ruminal mixing compartments was correctly estimated by difference. When more than two mixing compartments exist and/or the residence time in the lag-rumination pool (the first compartment) is age-dependent, a two-compartment model with an exponential distribution of residence times also provides an adequate description of the data. When the parameters are estimated by the two-compartment model with no time dependency, the TMRT may be correctly estimated, but the distribution of the TMRT between the time delay and CMRT can be seriously biased. This bias can result in considerable errors in estimates of cell wall digestibility.

Particle dynamics

Ruminant animals have developed a strategy to take full advantage of digestible energy in forages by selective retention of digesta particles in the rumen. The large particles and particles containing a high proportion of digestible material are selectively retained in the rumen, whereas the particles containing less digestible material have a higher probability of escaping from the rumen. Several excellent reviews of particle kinetics have been published (Faichney, 1986; Kennedy and Murphy, 1988; Kennedy and Doyle, 1993; Murphy and Kennedy, 1993). The following discussion will briefly encompass the mechanisms of selective retention, and the determination of kinetic parameters related to the release of feed particles from the rumen lag-rumination compartment to the escape pool.

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; McLeod and Minson, 1988). Many authors have suggested that the critical particle size is approximately 1-2 mm because only a small proportion of particles appearing in faeces are retained on these screens. The distribution of particle length and width in rumen contents and faeces does

Table 5. Mean compartmental residence time and time delay estimated from synthetic marker excretion data by using the models with increasing time dependency in the first compartment (Source: see Pond et al., *1988).*

Sampling site	Model	$CMRT_1$	$CMRT_2$	CMRT	TD	TMRT	EMS
Duodenum [D]	G_1G_1	13.3	20.0	33.3	4.7	38.0	32.7
	G_2G_1	12.6	24.0	36.6	2.1	38.7	2.5
	G_3G_1	**14.0**	**25.1**	**39.0**	**0.0**	**39.0**	**0.0**
	G_4G_1	**13.2**	**27.0**	**40.2**	**0.0**	**40.2**	**17.0**
Rectum [R]	G_1G_1	15.4	20.1	35.5	16.4	51.9	52.5
	G_2G_1	15.9	22.8	38.8	12.7	51.4	6.8
	G_3G_1	17.4	24.3	41.7	10.1	51.8	0.8
	G_4G_1	19.0	25.1	44.1	8.0	52.1	0.0
Difference (R-D)[1]		5.1	0.0	5.1	8.0	13.0	

$CMRT_1$ = Mean residence time in the first mixing compartment (lag—rumination pool, $CMRT_2$ = Mean residence time in the slower turnover compartment, CMRT = Total mean compartmental residence time, TD = Time delay, TMRT = Total mean retention time, EMS = Error mean square).
The 'true' residence times in the different segments of gastrointestinal tract were: rumen large particle (lag-rumination) pool 10 h (with an exponential or 2nd order gamma distribution of residence times), rumen pool of small particles 25 h (exponential distribution of residence times), omasum 4 h (mixing flow), abomasum and small intestine 4 h (tubular flow), caecum and proximal colon 5 h (mixing flow), distal colon 4 h (tubular flow).
[1] Calculated for the best fit models

not provide evidence supporting the critical particle size controlling the flow of particles from the rumen of cows fed grass silage (Nørgaard and Sehic, 2003). It is more likely that the probability of particles to leave the rumen decreases with increases in particle length and width. The concept of critical particle size has been questioned since a large proportion of rumen DM and particulate matter is below the suggested critical size (Ulyatt *et al.*, 1986; Lechner-Doll *et al.*, 1991). Lechner-Doll *et al.* (1991) postulated that specific gravity was twice as important as particle length in determining the likelihood of particles escaping the reticulo-rumen. Sutherland (1988) suggested that particles separate into those having buoyant properties attained via entrapped fermentation gases (newly ingested particles) and those having sedimentation properties after they have been depleted in fermentable substrates. Sutherland (1988) separated ruminal particles according to their buoyancy with warm artificial saline. Hristov *et al.* (2003) used a similar approach and observed that the sedimenting particles contained more INDF than the buoyant particles.

The passage rate estimated by rumen evacuation has been faster for INDF compared with DNDF for various diets (Tamminga *et al.*, 1989; Huhtanen and Jaakkola, 1993; Lund, 2002; Oba and Allen, 2003) demonstrating that digestible material is selectively retained in the rumen, in spite of the fact that digestible and indigestible fractions are contained in the same particles. Despite extensive efforts, the mechanisms controlling the separation and outflow of particles from the reticulo-rumen have not been unequivocally elucidated. Particle size reduction as a result of chewing and increase in specific gravity as a result of reduced fermentation activity occur

simultaneously with increased time after ingestion as indicated by a close negative correlation between particle size and specific gravity (Evans *et al.*, 1973; Hooper and Welch, 1985). Microbial degradation facilitates particle breakdown during mastication by increasing particle fragility (Kennedy and Doyle, 1993). A close positive relationship between potential NDF digestibility and the size of particles (Ahvenjärvi *et al.*, 2001) also supports the view that the density and size of particles are closely correlated. Gas production from an active fermentation decreases when fermentable substrates become depleted by increased residence time in the rumen. Because the particle size of digesta decreases concomitantly with increased residence time, it is difficult to interpret which mechanism, particle size or specific gravity, is more important in regulating the outflow of feed particles.

Selective retention of feed particles in the rumen is also evident from duodenal marker excretion curves (Pond *et al.*, 1988; Ellis *et al.*, 1994; Huhtanen and Hristov, 2001; Lund, 2002) which clearly indicate an ascending phase, which challenges the interpretation of the rumen as a single compartment system, where the probability of particles to escape is a random process. Whatever the mechanism underlying selective retention of feed particles, the process of selective retention should be incorporated into mechanistic dynamic rumen models to accurately predict cell wall digestibility. The total residence time in the reticulo-rumen fermentation compartments may be estimated by the rumen evacuation method or use of appropriate markers and compartmental models. With marker techniques, interpretation of rate constants related to specific compartments is difficult and highly dependent on the choice of model used. Information of residence time in the total tract may be more useful which is also less affected by the configuration of the model. As suggested by Ellis *et al.* (2002), the residence time in pre-duodenal fermentation compartments could be accurately estimated from faecal marker concentration data using appropriate kinetic models. However, the distribution of residence time between the two compartments in the forestomach (lag-rumination pool, escape pool) still remains debatable.

Kennedy and Doyle (1993) discussed the methods for measuring particle kinetics. One method is to estimate the decline of particle load by complete emptying of the rumen and based on the assumption of a linear or exponential decline, rate constants can be calculated. However, because the large particle load also disappears by digestion, the decline for indigestible fraction of the particle load should also be estimated to describe the rate of particle breakdown correctly. In the second method, the particle comminution rate is calculated from steady-state kinetics as [(input – escape (g h^{-1})) / load (g)]. The approach requires rumen evacuation data and an estimation of the large particle content of ingested feed. This method also requires that an allowance is made for digestion. Several marker techniques have been used to estimate the comminution rate of feed particles. The rate of particle comminution can be estimated by difference of ruminal or total retention time between the large labelled feed particles and faecal particles. Using this approach, very high comminution rates (0.1-0.3 h^{-1}) of feed particles have been reported by Bowman *et al.* (1991) and Cherney *et al.* (1991). Ellis *et al.* (1999) estimated residence time in the lag-rumination pool by fitting two compartmental models with age-dependency in the first compartment to marker data and concluded that the mean residence time in the lag-rumination pool is unaffected by dietary INDF level being relatively constant at around10 h.

Poppi *et al.* (2001) suggested an alternative interpretation of marker kinetics in the rumen, namely a raft model. Their reversible flow model based on marker kinetics data from dorsal and ventral rumen digesta had the following features: a relatively slow age-dependent transfer from the raft pool to the ventral rumen pool and a very rapid first-order exit from the ventral rumen-reticulum. It is important to note that the sequence of the rate constants: (slow raft, fast small particle) differ from earlier particle kinetic models (fast large particle, slow small particle). The high proportion of total rumen DM in the raft (0.75 – 0.89) was consistent with the escape from the raft being the rate limiting step. The proportion of raft material to total rumen digesta appears to be related to feed intake. The proportion of raft in total digesta increased from 0.42 to 0.95 when DM intake increased from 6 to 24 kg/d (Robinson *et al.*, 1987).

In animals fed *ad libitum*, the high proportion of raft and absence of a distinct mat and liquid phases may prevent free particle movement by sedimentation and flotation. Under these circumstances the capacity of the mat to entrap potentially escapable small particles may be an important mechanism to maximise fibre digestibility. A similar particle size distribution and potential NDF digestibility within each sieve size in the dorsal and ventral sacs and reticulum (Ahvenjärvi *et al.*, 2001) indicates that flotation and sedimentation may not be the main mechanisms influencing the escape potential of digesta particles as suggested by Sutherland (1988). Observations of the particle size distribution and potential NDF digestibility within each sieve size is consistent with the solid mat filling both the dorsal and ventral rumen. The high proportion (>0.50) of large particles (>2.5 mm) in rumen particulate OM in the dorsal and ventral sacs suggest that digesta passage is more limited by the release of particles from the raft pool into the escape pool. The results from a recalculation of data from Rinne *et al.* (2002) are also consistent with the raft model concept. The mean residence time of the large particle pool estimated as [(input – flow) / load] by making an allowance for digestion was on average 28 h for the four grass silage based diets. This estimate is almost three times higher than the residence time assigned to the lag-rumination pool based on two pool models with an age-dependent and age-independent residence time (Ellis *et al.*, 1999). The corresponding total rumen INDF residence time estimated by rumen evacuation was 41 h. When the entrapment of small particles by the mat is taken into account, the results can also be interpreted by the raft model, i.e. the retention time in the lag-rumination (raft) compartment was markedly longer than that in small particle escape compartment. The large particles (>2.5 mm) comprised proportionally 0.49 of particles in the rumen. These observations are consistent with those of Bruining *et al.* (1998) who found that rumen digesta contained more large particles than small particles. In their study the rate of particle comminution determined by steady-state kinetics ranged from 0.034 h^{-1} (grass silage based diet) to 0.049 h^{-1} (lucerne silage based diet).

The sequence of rumen compartments is difficult to determine from duodenal marker profiles. Assuming that the two rumen compartments consist of two sequential exponential pools, the order of the pools (fast-slow vs. slow-fast) has no effect on the marker profile. If the raft pool is gamma age-dependent, the marker profile would change, when the sequence of the pools is changed (Figure 4). However, a model with an appropriate age-dependency estimated the parameter values correctly even when the sequence of the pools was switched, but this will inevitably affect the estimated digestibility as discussed later.

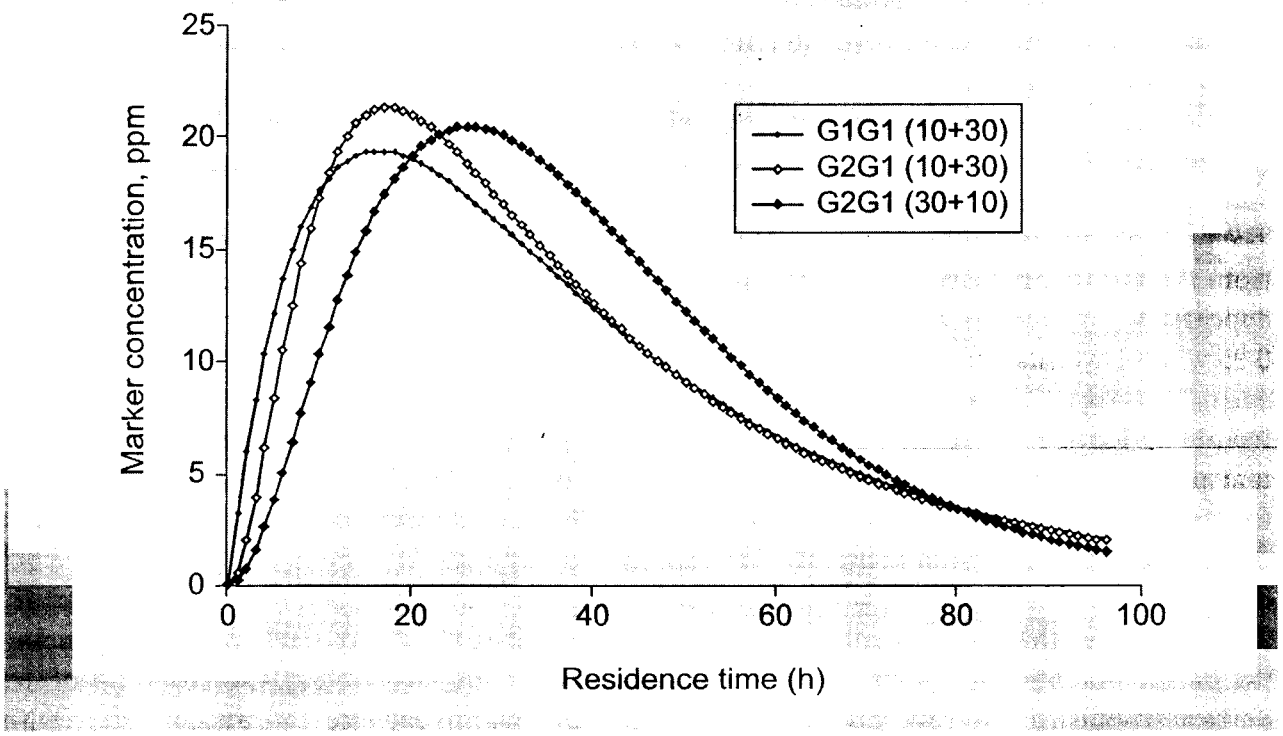

Figure 4. Simulated duodenal marker concentrations when the residence time in the two rumen compartments are 10 + 30 h or 30 + 10 h with model of no time dependency (G_1G_1) or gamma two time dependency in the first compartment (G_2G_1). Note that the two curves with the G_1G_1 model are exactly similar and only 10 + 30 h is shown.

Intrinsic and extrinsic factors influencing passage kinetics

The importance of passage rate on intake and digestibility was clearly outlined by Blaxter *et al.* (1956) and Waldo *et al.* (1972). It is often difficult to conclude whether intrinsic characteristics of particles or diet type have a greater influence on passage kinetics. Vega and Poppi (1997) addressed this question by labelling small (0.5-1.2 mm) grass and legume particles which had either been extensively digested (faecal particles) or not digested (ground feed particles) and inserted the particles into the rumen of sheep fed four different diets. The passage rate of particles was similar within a diet, irrespective of the type (grass vs. legume) or status (undigested vs. digested) of particles. Rumen conditions affected by diet type had the most influence on particle passage rate. It is therefore possible that the passage kinetic parameters are often a reflection of the effects of feed intake rather than the intrinsic plant characteristics. Welch (1982) assessed the effects of diet and feeding level on rumen raft consistency by measuring the rate of vertical penetration through the raft under a constant force. The rumen raft was more tightly packed with steers offered grass hay compared with maize silage or high levels of concentrate. Increases in feeding level from 0.40 to 1.0 of *ad libitum* intake increased rumen raft consistency.

Accurate passage kinetics data are a prerequisite for any nutritional model which attempts to predict the relationship between diet and nutrient supply. Many empirical models have been developed to predict passage kinetic parameters from dietary and animal data (e.g. Owens and

Goetch, 1986; Sniffen *et al.*, 1992; Cannas *et al.*, 2003). However, the predictions have not been very accurate, which may, at least in part, be explained by aspects of the methodologies used. In addition to the true intrinsic and extrinsic factors involved, factors such as marker type, kinetic model, sampling site and physical form of the marker can markedly influence the passage kinetic values obtained. To predict the actual passage kinetics it is important that the labelled particles simulate the passage of natural feed components. Without an accurate description of the particle size distribution of markers it is difficult to interpret the passage kinetics parameters from different studies. As an example, increasing the particle size of mordanted hay from <0.3 mm to 0.6-1.0 mm decreased passage rate from 0.041 to 0.021 h^{-1} (Bruining and Bosch, 1992). Both values may describe relative differences due to diet and animal on passage kinetics, but not necessarily indicate the intrinsic kinetic properties of natural feeds.

The wide range of methods and procedures that have been used to quantify particle kinetics and the biological mechanisms have been reviewed by Kennedy and Murphy (1988) and Kennedy and Doyle (1993). It was postulated that the intrinsic characteristics of plant cell walls influencing passage kinetics are mainly associated with the resistance of cell walls to comminution and particle-size reduction.

Although the resistance to comminution might be expected to be lower for early harvested forages, there is evidence that rumen retention time decreases with increasing cell wall content and maturity. Gasa *et al.* (1991), Bosch *et al.* (1992) and Bosch and Bruining (1995) observed that late cut grass silage with a higher NDF concentration resulted in a faster passage rate of Cr-mordanted fibre than grass silage harvested earlier. Rinne *et al.* (2002) found using rumen evacuation technique a significantly increased passage rate with the most delayed harvest time in dairy cows offered diets based on grass silage harvested at different stages of maturity. The proportion of large particles (>2.5 mm) of rumen particulate DM (particles >0.08 mm) decreased from 0.56 for the earliest cut to 0.43 with the latest cut. The slower breakdown of large particles in the early compared with late harvested silage suggests either a preferential removal of stems from the large-particle pool to the small-particle pool, or that the fragility of both leaves and stems increase with advancing maturity. Whether the increase in passage rate with advancing maturity of grass is an intrinsic property or a reflection of differences in intake, remains unclear. In the study of Rinne *et al.* (2002), DM intake decreased with greater maturity, but NDF intake increased due to the proportionally higher increase in silage NDF concentration which more than compensated for the reduction in DM intake. Similarly, Lund (2002) reported a faster passage rate of INDF with increased maturity using the rumen evacuation method when comparing silages harvested at 3 week intervals when forage was the sole feed, but this was not the case when the diets were supplemented with concentrate. In contrast to the studies referred above, Ellis *et al.* (2000) found that CMRT increased with increasing NDF concentration, but they did not define the details of the forages fed. However, based on INDF concentration, these forages were of lower quality than grass silage.

Kuoppala *et al.* (2004) compared the passage kinetics of primary and re-growth grass silages each harvested at two maturities using the rumen evacuation technique in dairy cows. Within harvests, the passage rate of INDF was not influenced by maturity, but it was markedly slower for the re-growth than primary growth silages (0.027 vs. 0.021 h^{-1}) despite the higher proportion of leaves

in the re-growth grass. Because the intake of cell wall and DM were higher for primary-growth silages, no definite conclusions on the cause and effect can be made: was the lower intake of re-growth silages mediated by metabolic constraints that reduced passage rate or did the intrinsically slower passage rate of re-growth grass constrain intake. A slower passage rate of silage with a higher leaf proportion is in contrast with the observations of Poppi *et al.* (1981) and Cherney *et al.* (1991), who reported that retention time was shorter for leaves than stems for various forage species. More research is needed to assess the relative importance of the intrinsic properties of forages (e.g. potential NDF digestibility, leaf to stem ratio, legume *vs.* grass, particle size) and animal/diet factors in the regulation of passage kinetics.

Comparison of the passage kinetics of markers dosed either as large or small (ground feed or faeces) particles have clearly shown differences in total retention time (Cherney *et al.*, 1991; Mambrini and Peyrand, 1997; Wylie *et al.*, 2000; Ahvenjärvi *et al.*, 2004). The differences in total mean retention time have been approximately 10 h. However, very little is known about the effects of forage harvesting techniques on passage kinetics. Differences in the particle size of forages are distinctly smaller than differences in the particle size of markers used in these studies. It is possible that within the range of chop lengths on-farm the effect on intrinsic passage kinetic parameters is likely to be small. However, as demonstrated by Vega and Poppi (1997), the diet fed to animals often has a much stronger effect on passage rate than the properties of the labelled feed itself. It could be speculated that with fine chopping of forages the ability of the mat to entrap small particles may be reduced resulting in lowered residence time in the rumen. Bernard *et al.* (2000) replaced chopped orchard-grass with ground and pelleted orchard-grass. Grinding and pelleting of hay clearly decreased the mean residence time of lignin in the rumen when the proportion of ground hay was 0.50. No further decreases were observed at higher inclusion rates of ground pelleted hay. Shaver *et al.* (1986) fed pre-bloom lucerne hay in long, chopped or ground and pelleted form (60:40 forage to concentrate ratio DM basis) to dairy cows at three stages of lactation. Total mean residence time of labelled concentrates and forages decreased with increasing feed intake, but there were no effects of chopping or grinding on ruminal or total residence time. An absence of a difference between the long and chopped (mean particle length 7.8 mm) hay indicates that within the normal range achieved in practice, the effects of chop length on digesta passage kinetics is insignificant. Depression of digestibility associated with grinding was related to reduced ruminal digestion rate. In published studies the effects of grinding on rumen or total retention time have been variable (see Bernard *et al.*, 2000). Effects of forage conservation methods on passage kinetics are likely to be small. Udén (1984) reported similar values using Cr-mordanted fibre for the passage kinetic parameters of the cows fed silage or hay. Huhtanen and Jaakkola (1993) used the rumen evacuation technique to study passage kinetics of grass silage and hay made from the same sward. The differences between the forages, although sometimes significant, were relatively small. Particle passage kinetics of lucerne hay and silage measured using ^{15}N enrichment of acid detergent fibre bound nitrogen as an internal marker were found to be similar (Huhtanen and Hristov, 2001).

Rumen residence time of concentrate particles is shorter than that of forages (e.g. Shaver *et al.*, 1988; Colucci *et al.*, 1990) reflecting the smaller particle size and higher specific gravity. Offer and Dixon (2000) compiled data in the literature and concluded that the effects of supplement composition on passage rates appear to be small. Robinson *et al.* (1987) observed decreased

passage rates using both Cr-straw and rumen evacuation techniques, when the starch content in concentrates increased. However, these effects may be more related to the extrinsic effects of the diet on passage kinetics, since concentrates were not labelled. Stensig *et al.* (1998) also found that supplementation of a starch rich low fibre concentrate decreased passage rate. Huhtanen *et al.* (1993) compared the passage kinetics of Yb-labelled barley, barley fibre, rapeseed meal and soybean meal in cattle. Despite the large differences in chemical composition of the feeds with respect to starch, NDF and protein content, compartmental residence times, estimated from duodenal or faecal marker profiles, were similar. Duodenal marker profiles of labelled concentrate feeds have clearly shown an ascending phase in the marker excretion curve (Huhtanen *et al.*, 1993; Mambrini and Peyraud, 1997) indicating that the passage kinetics can not be described by a first-order single pool model. The diurnal pattern of duodenal amino acid (Robinson *et al.*, 2002) and starch (Tothi *et al.*, 2003) flow are consistent with marker kinetic data, and clearly indicate that the passage kinetics of solid feed components cannot be described using a single compartment first-order model. Excluding the ascending phase of the marker excretion curve will markedly underestimate the retention time of concentrates in the rumen fermentation compartments.

Most of the studies have shown a decrease in CMRT with increased feed intake. Intake is used in many feed evaluation systems (e.g. AFRC, 1993; NRC, 2001, Sniffen *et al.*, 1992) to predict passage rate. These relationships are based mainly on marker kinetic data. We estimated the relationship between intake and diet parameters from Danish and Finnish dairy cow studies. The data included 41 treatment means with a wide range of diets (DM intake 8.2 – 23.7 kg d^{-1}, NDF concentration 238 – 638 g kg^{-1} DM, proportion of concentrate 0.00 – 0.70). The passage rate of INDF was estimated using the rumen evacuation technique, i.e. it describes the passage rate of the total diet. Intake, rather than faecal output of INDF was used to estimate the passage rate. When analysed with a single regression model, NDF intake predicted INDF passage rate much better than DM intake (R^2 0.68 vs. 0.31). Accounting for the random effect of study in a mixed model regression analysis did not change the parameter values, but the model did account for more of the observed variation (Figure 5). When NDF intake was segregated to forage and concentrate NDF the variation explained increased to 0.73 with the single regression model but was not further improved with the mixed model. The slope of the INDF passage rate was significantly higher for NDF from concentrates than forages (mixed model: 0.00034 vs. 0.00023 per kg NDF intake). This indicates that the passage rate of concentrate INDF was faster than that of forage INDF, which is consistent with the data from studies comparing the passage kinetics of labelled forages and concentrates (Shaver *et al.*, 1986; Colucci *et al.*, 1990; Mambrini and Peyraud, 1997). It seems that the passage rate of INDF for diets based on maize or lucerne silage is much higher compared with diets based on grass silage in relation to NDF intake. The mean NDF intake and INDF passage rate for 8 diets in the studies of Oba and Allen (2003) and Voelker and Allen (2003) were 5.7 kg d^{-1} and 0.035 h^{-1}, respectively. The passage rate of INDF was markedly higher than a value of 0.020 h^{-1} predicted by the equation derived from our dataset. Lund (2002) also found a markedly higher INDF passage rate for maize silage compared with other forages. There may be differences in the consistency of the rumen raft due to intrinsic differences between the forages that explain this finding. Bruining *et al.* (1998) estimated using a steady-state procedure that comminution rates for diets based on maize or lucerne silages (0.043 and 0.049 h^{-1}) were clearly higher than for a diet based on grass (0.034 h^{-1}).

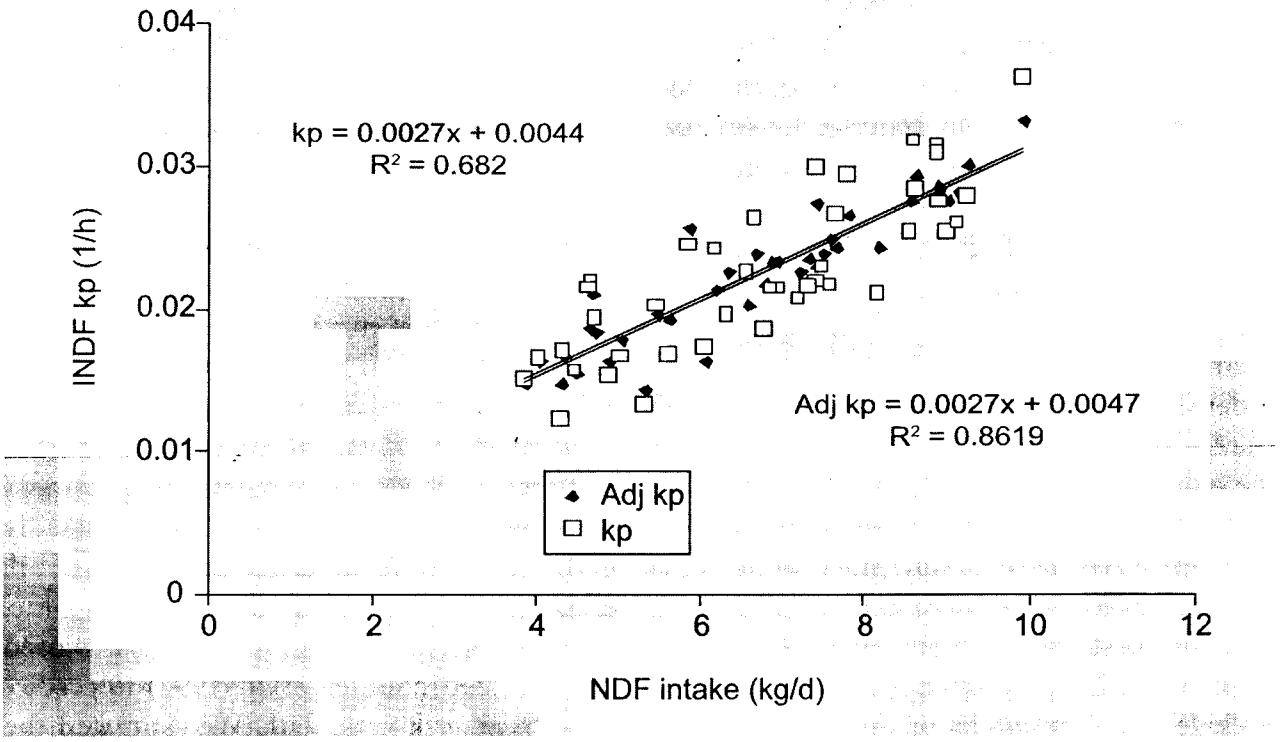

Figure 5. The relationship between NDF intake and passage rate of INDF estimated by rumen evacuation technique. The data from the Danish and Finnish studies was analysed either by single regression or by a mixed model with random study effect. Adj kp: Values are adjusted for a random study effect.

The relationship between NDF intake and INDF passage rate in our data was linear. Cannas *et al.* (2003) observed that the relationship between lignin turnover and NDF intake was best described by a concave curve (NDF intake was transformed by the natural logarithm). However, because the inverse of passage rate represents turnover, the relationship between NDF intake and NDF rumen turnover time are consistent by Cannas *et al.* (2003). Cannas and Van Soest (2000) showed that forage NDF passage rate, estimated by external markers, was best described by a convex curve, i.e. at high levels of intake passage rate increased to a lower extent than at low levels of intake. If this relationship were true, the rumen NDF pool would increase exponentially with feed intake unless the rate of digestion increased, but this does not appear to be the case (Robinson *et al.*, 1987). Tamminga *et al.* (1989) estimated passage rate using both Cr-mordanted straw as an external marker and the rumen evacuation technique in dairy cows fed at different levels of intake. Passage rate of INDF increased linearly (or even slightly curvilinearly) with increased intake, whereas the pattern was not so clear with Cr-mordanted straw. It is possible that the relationships between intake and passage rate in this experiment were influenced by the problems related to marker kinetics data, the most serious problem being that the passage rate estimated from marker profiles did not account for the residence time represented by the ascending phase of the marker-excretion curve, and the inverse of marker passage rate therefore underestimates rumen turnover.

Reducing effects of increased concentrate in the diet on passage rate were reported by Colucci *et al.* (1990), Gasa *et al.* (1990), Bosch *et al.* (1992) and Huhtanen and Jaakkola (1993). In the study

of Colucci *et al.* (1990) increasing the proportion of concentrate showed a significant negative relationship with passage at a low level of feeding, but at a higher level of feeding the effects were small and non-significant. Huhtanen and Jaakkola (1993) used the rumen evacuation technique to estimate passage kinetics of cell walls in cattle fed at a fixed DM intake of diets supplemented with three levels of concentrate. Increasing the level of concentrate in the diet decreased the passage rate of INDF linearly, even though the proportion of concentrate NDF of intrinsically higher flow characteristics increased.

The rumen evacuation technique appears to be a useful tool for estimating passage kinetics of INDF. More detailed analysis of larger sets of data might be useful for the estimation the effects of diet, animal and feed characteristics on INDF passage kinetics. The problem with this method relates to the fact that it estimates the passage rate for the total diet and not for individual ingredients. A combination of marker and rumen evacuation techniques may be useful to separate the effects due to intrinsic and extrinsic factors on passage kinetics. For the estimation of intrinsic feed characteristics, the labelled feed should be fed in the same physical from as it is fed in the diet. Extrinsic effects on passage kinetics can be estimated by using one common marker for all diets in a study or by the rumen evacuation technique.

Integrated models of cell wall digestion in the rumen

Random passage models

The original model with random passage of Waldo *et al.* (1972) has been the basis of dynamic rumen models predicting cell wall digestibility. The model involved a concept of potential digestibility, fractional rates of digestion and passage and that digestibility is a competition between digestion and passage. The simple model has been modified, but most of the published data on predicted NDF digestibility estimated from the kinetic parameters are still based on this concept. The use of this model has been extended to estimate the effective protein degradability in the rumen (Ørskov and McDonald, 1979), and is probably more widely used for that purpose than predicting NDF digestibility.

Integrated models of cell wall digestion have been extensively reviewed (Allen and Mertens, 1988; Mertens, 1993a; Illius and Allen, 1994; Ellis *et al.*, 1994). The models have considerable differences in substrate fractionation and in the structures applied to describe digestion and passage kinetics. The abilities of the published models to predict digestibility have not been particularly reassuring (Illius and Allen, 1994). To be useful for practical feed evaluation and ration formulation purposes, integrated models should predict digestibility at least with the same accuracy as models based on empirical relationships between digestibility and selected chemical components. Our objective here is to discuss the effects of model structure on the prediction of NDF digestibility and the possible mechanisms and processes that relate to the model structure that should be used.

Although the original model of Waldo *et al.* (1972) has been widely used to calculate NDF digestibility in many studies, it has not been extensively validated against *in vivo* data based on a large number of measurements. Archimède (1992) used *in situ* digestion kinetic data to predict

ruminal NDF digestibility estimated from duodenal flow (cited by Nozière and Michalet-Doreau 2000). The model clearly underestimated ruminal NDF digestibility, but the slope between predicted and observed values was 1.00 and the proportion of variance accounted for by this model 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. This model probably results in the correct ranking of NDF digestibility, since the empirical relationship between INDF concentration and in *vivo* OM digestibility is particularly strong (Nousiainen *et al.*, 2003b). In addition to the underestimation of the rate of digestion, too high passage rates from ignoring the ascending phase of the marker excretion curve can lead to an underestimation of NDF digestibility with this model.

Knowledge of *in vivo* digestibility of DNDF can be used to validate the feasibility of a model structure. The mean DNDF digestibility of 52 grass silages harvested at different stages of maturity was 0.87 (range 0.79 – 0.93) (Nousiainen *et al.*, 2004), such that using a passage rate of 0.02 h^{-1}, indicates that digestion rate should be 0.075, 0.128 and 0.260 h^{-1} to achieve the observed minimum, mean and maximum *in vivo* DNDF digestibility. Accordingly, using a digestion rate of 0.06 h^{-1}, a passage rate of 0.0094 h^{-1} would be required to achieve the observed mean *in vivo* DNDF digestibility. These simple calculations indicate that unrealistic values for the digestion and/or passage rates have to be used in order to predict *in vivo* DNDF digestibility correctly, when inappropriate models are used. It has sometimes been argued that the hind-gut digestion compensates for the difference between predicted and observed digestibility (Moore *et al.*, 1990). However, in animals fed forage diets the contribution of the hind-gut to total NDF digestion cannot be that large as discussed earlier in this chapter. Another explanation for the underestimation of NDF digestibility is that passage rates used in various models are often estimated from the descending phase of marker excretion curves. It can be concluded that although the model has many basic elements of the dynamics of cell wall digestion, cell wall digestion can not be correctly estimated by a simple mathematical function based on random passage and the model is not biologically sound either.

Selective retention models

Ruminants have evolved an effective system of selective retention to maximise the intake of digestible energy by retaining the newly ingested digestible and large particles in the rumen and allowing the passage of aged particles, which are small and depleted of digestible material. Although the mechanisms of selective retention are well described in the literature (Kennedy and Murphy, 1988; Sutherland, 1988; Allen and Mertens, 1988; Kennedy and Doyle, 1993) and have been incorporated in many integrated rumen models (Illius and Allen, 1994), it has not been integrated in feed evaluation systems and very seldom in the calculation of NDF digestibility from kinetic data (e.g. Sniffen *et al.*, 1992; AFRC, 1993; NRC, 2001).

A two-compartment model incorporating indigestible and digestible NDF is illustrated in Figure 6. From the first compartment (lag-rumination pool) DNDF disappears by digestion and comminution to the second compartment (small particle pool). From the second compartment DNDF disappears both by digestion and passage. By definition INDF disappears from the first compartment only by release to the second compartment and from that by passage to the small

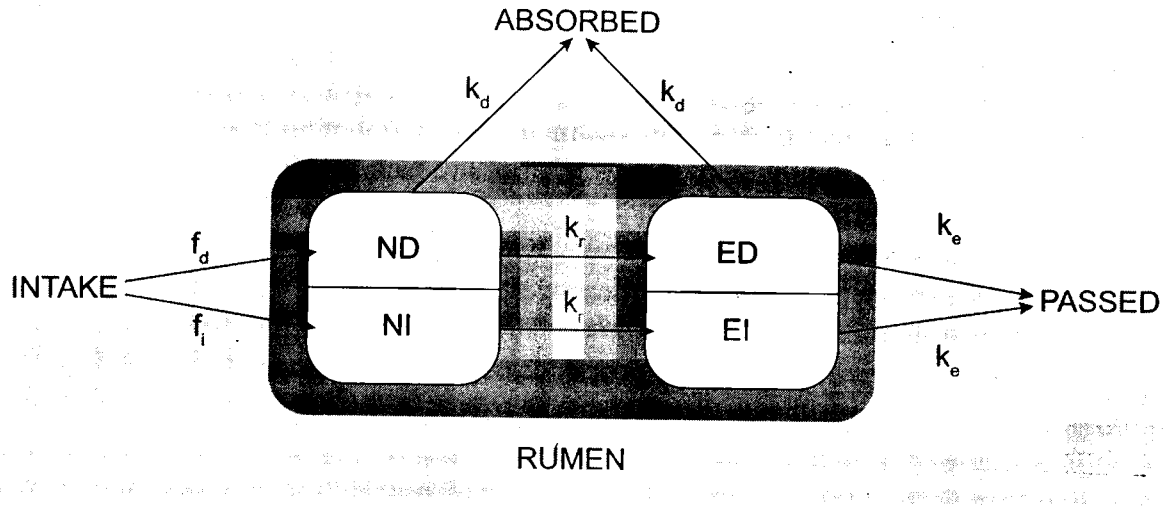

Figure 6. Model of ruminal cell wall digestion incorporating selective retention of potentially digestible (D) and indigestible (I) fractions in non-escapable (N) and escapable (E) pools. Cell wall fractions and rates are represented as follows: digestible NDF (f_d), indigestible NDF (f_i), rate of digestion (k_d), rate of release from N to E (k_r) and rate of escape from E (k_e) (Allen and Mertens, 1988).

intestine. Derivation of digestibility was presented by Allen and Mertens (1988) (equation 4 in this chapter). The use of this model results in much more realistic values of *in vivo* DNDF digestibility than the single compartment model.

An example of the effects of selective retention and time dependency of the flow in the first compartment (Ellis *et al.*, 1994) on ruminal DNDF digestibility is shown in Figure 7. Inclusion of the selective retention of particles in the model increased ruminal DNDF digestibility from 0.69 to

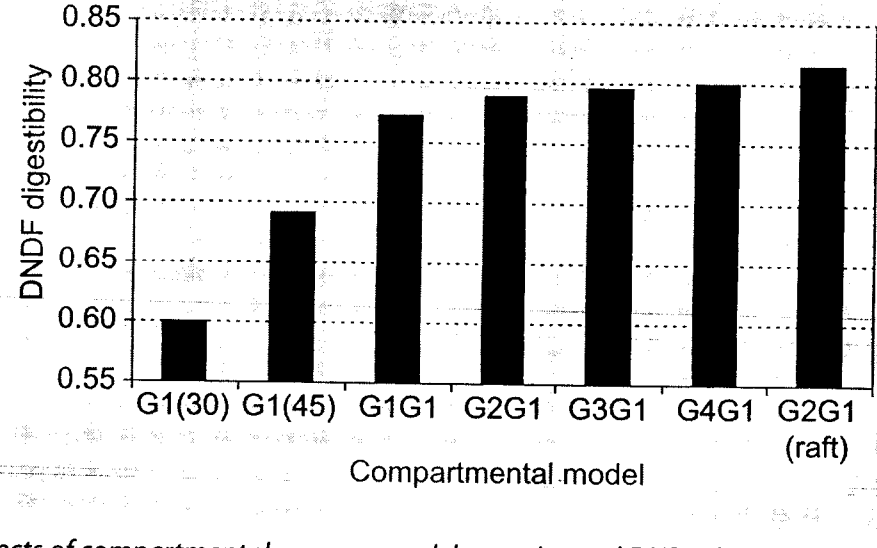

Figure 7. The effects of compartmental passage model on estimated DNDF digestibility. One compartment models (G1) assume either the same compartmental residence time (45 h) or that it was estimated from the ascending phase of the marker excretion curve (30 h). A value of 0.05 h^{-1} was used for the rate of digestion.

0.77 when the same rumen residence time of 45 h (15+30) was used. Assuming an age-dependent flow from the first to the second compartment resulted in a further 0.02-0.03 unit improvement in DNDF digestibility. If the passage rate obtained from the slower compartment of the two compartment model (30 h) had been used in a single compartmental model, estimated DNDF digestibility would have been only 0.60. However, this is the approach used in most published data and also in feed evaluation systems.

In a single compartment system with first-order digestion and passage rates, the proportions of DNDF disappearing via digestion per definition remain constant throughout a range of residence times. In a two compartment system the proportion of DNFD disappearing by digestion is much higher during earlier residence times when the particles are mainly in the lag-rumination pool and not eligible for passage (Figure 8). The proportion of DNDF disappearing by passage in the two compartment system is smaller during the early residence time compared with a single compartmental system, but for later residence times the reverse is true. Due to the slow disappearance via passage during early residence times a digestion lag time would have a smaller effect than in a single compartmental system (Ellis *et al.*, 1994). Allen and Mertens (1988) showed mathematically that if the lag phenomena influence both digestion and passage, cell wall digestibility is independent of lag. They also derived an equation to calculate simple first-order passage rate from the three kinetic parameters:

$$k_p = k_r \times k_e / (k_d + k_r + k_e) \quad (6)$$

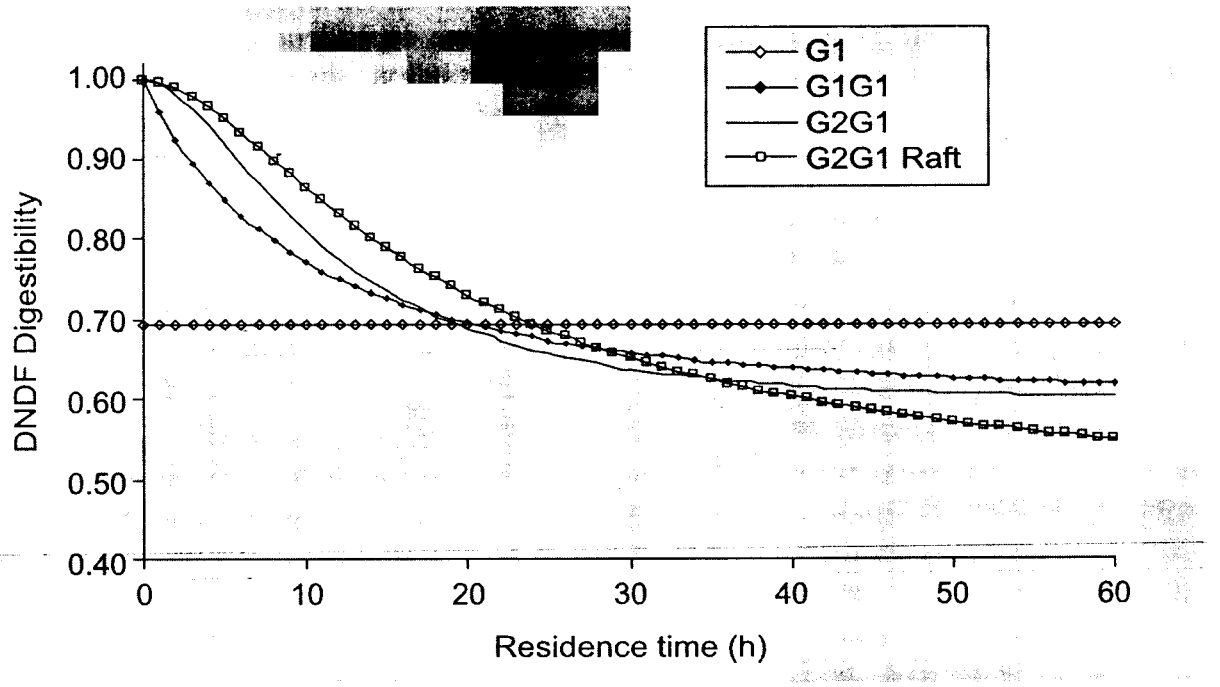

Figure 8. Digestibility of DNDF (proportion of DNDF disappearing by digestion) at different rumen residence times assuming rumen as a single compartment (G_1) or two compartment system. In the two compartment systems the flow followed first-order kinetics (G_1G_1) or the flow from the first compartment was age-dependent (G_2G_1). The distribution of the residence time was 15 + 30 h for the G_1G_1 and G_2G_1 systems and 30 + 15 h for the G_2G_1 raft system. Digestion rate was assumed to be 0.05 h^{-1}.

where k_d, is the rate of digestion, k_r is release from the non-escapable to the escapable compartment and k_e is passage from the escapable compartment to the lower tract. The first-order passage rate is not only a function of the passage kinetic parameters but also of the fractional digestion rate. At a constant CMRT, the higher the rate of digestion, the lower the first-order passage rate would be. It might be argued that estimating parameter values of the model is difficult. However, for the determination of digestibility, an accurate estimate of total residence time in the two compartments is much more important than the distribution of the residence time between the two compartments. If the distribution of the total residence time of 45 h were 10+35, 15+30 or 20+25, the calculated DNDF digestibility would be 0.758, 0.771 and 0.778, respectively. This example suggests that digestibility is relatively insensitive to small changes in the distribution of the residence time between the two compartments. However, with large changes the model will approach a single compartment model and have a large impact. The raft model recently suggested by Poppi *et al.* (2001) would result in a distinctly higher DNDF digestibility provided that passage from the raft pool to the passage pool is a time dependent process, as their data suggest. When the same digestion rate (0.05 h^{-1}) and total compartmental retention time (45 h) were used in the model but assuming a gamma two time dependency in the raft pool with a distribution of 35+10 h in the two compartments, DNDF digestibility was predicted to be 0.811 (see Figure 7). This suggest that the raft model is more effective in maximising the efficiency of ruminal DNDF digestion than models assuming a shorter retention time in the first than second compartment. More efficient digestion is related to the greater proportion of DNDF disappearing by digestion during early residence times when the particles become more slowly available for escape (See Figure 8). The data of Ahvenjärvi *et al.* (2001) support the concept of a raft model. In this study, cows were fed grass silage as the sole feed and digestibility of DNDF was shown to be very high (0.89) despite the relatively high NDF intake (12 g/kg LW). The similar particle size distribution and potential NDF digestibility within each particle size fraction in the rumen ventral and dorsal sacs are suggestive of a raft model concept rather than of selective retention based on particle flotation and sedimentation.

The validity of the two compartment model has not been extensively tested against *in vivo* data. Ellis *et al.* (1994) discussed that without the mechanisms of selective retention in the rumen it would be impossible to attain observed *in vivo* DNDF digestibility with realistic parameter values. Mertens (1973) concluded that assuming the rumen as a single compartment is not an adequate mathematical or biological representation of rumen functions. Huhtanen *et al.* (1995) and Rinne *et al.* (1997) predicted *in vivo* NDF digestibility using digestion rates derived from *in situ* incubations or from rumen evacuation and passage kinetic parameters estimated from the marker profiles. The mean NDF digestibility predicted using a two compartmental system and digestion rates based on rumen evacuation or in situ were 0.725 and 0.581, respectively, but only 0.424 when estimates were based *on situ* digestion rate and passage rate assuming the rumen to be a single compartment. All systems ranked the diets correctly, but the latter models clearly underestimated NDF digestibility (0.728). The data suggests two obvious reasons for the underestimation of digestion: first, the *in situ* method underestimates the rate of digestion and second, the assumptions of a single rumen compartment were not correct. Huhtanen *et al.* (2001) estimated NDF digestibility of 15 grass silages in sheep fed at maintenance with a two compartment rumen model assuming a total residence time 50 h (20+30 h). Digestion rate was estimated by the gas production technique from isolated NDF and potential NDF digestibility by

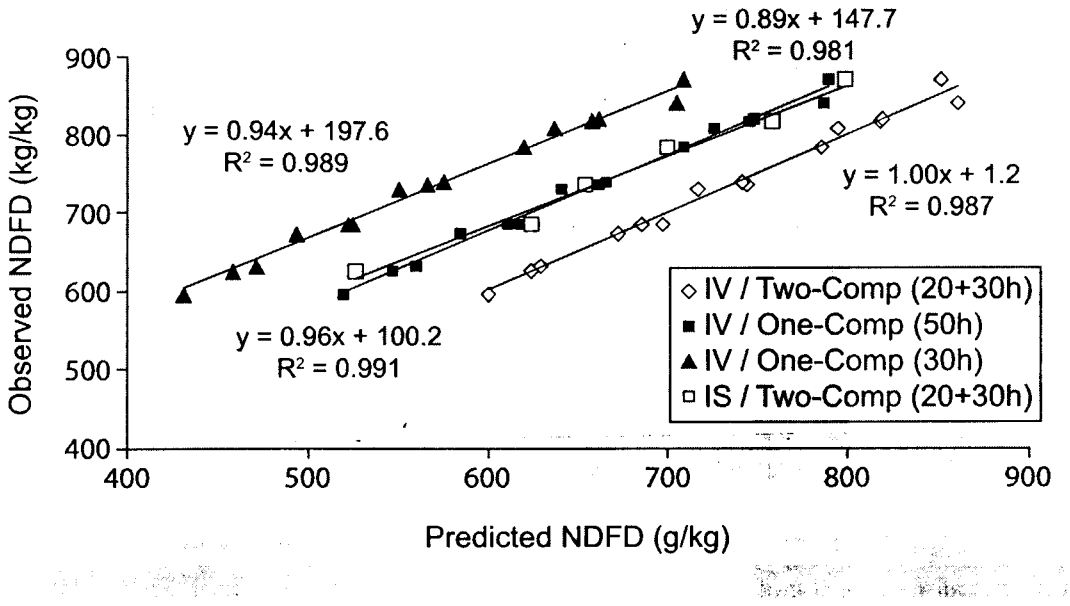

Figure 9. The relationship between predicted and observed NDF digestibility (NDFD) in sheep fed at maintenance. NDFD values were predicted using digestion rates determined by in vitro gas production (IV) or by in situ incubation (IS) using a two compartment rumen model (residence time 20+30h) or one compartment rumen model (residence time 50 or 30 h). (Data from Huhtanen et al., *2001 and unpublished data).*

12 d *in situ* incubations. The model predicted NDF digestibility accurately without any mean or slope bias (Figure 9). Using a single compartmental model with the same residence time clearly underestimated NDF digestibility, but did not change the proportion of variance accounted for by the model. *In situ* rate of digestion was determined for six of the 15 silages. Again, the method ranked the feeds correctly, but the lower predicted NDF digestibility suggests that the *in situ* method underestimated the rate of digestion.

The basal model structure in the Nordic dairy cow model (Danfær *et al.*, 2005a) is a two compartment rumen system and a single hind-gut compartment. Cell walls are fractioned into digestible and indigestible forage and concentrate NDF. Digestion of cell walls is assumed to be a first-order process. Intrinsic ruminal DNDF digestion is regulated by the ratio of non-structural carbohydrates and NDF, which models the adverse effects of rapidly degradable carbohydrates on cell wall digestion. Cell wall digestion is assumed to take place both in the rumen and in the hind-gut. Passage rate is regulated by feed intake in terms of NDF per unit live weight. Higher passage rates are used for concentrates than for forages. The proportion of the lag-rumination (non-escapable) compartment of the total rumen residence time is assumed to be 0.30 and 0.20 for forages and concentrates, respectively. Preliminary validation of the model indicates that this approach provides an accurate prediction of ruminal and total NDF digestibility with minimal mean and slope bias (Danfær *et al.*, 2005b). The relationship was much stronger when the values were adjusted for the random effects of experiment (Figure 10). This indicates that the model predicted the differences between the diets within experiment very accurately, and that a large proportion of the variance in the simple regression analysis arises from methodological

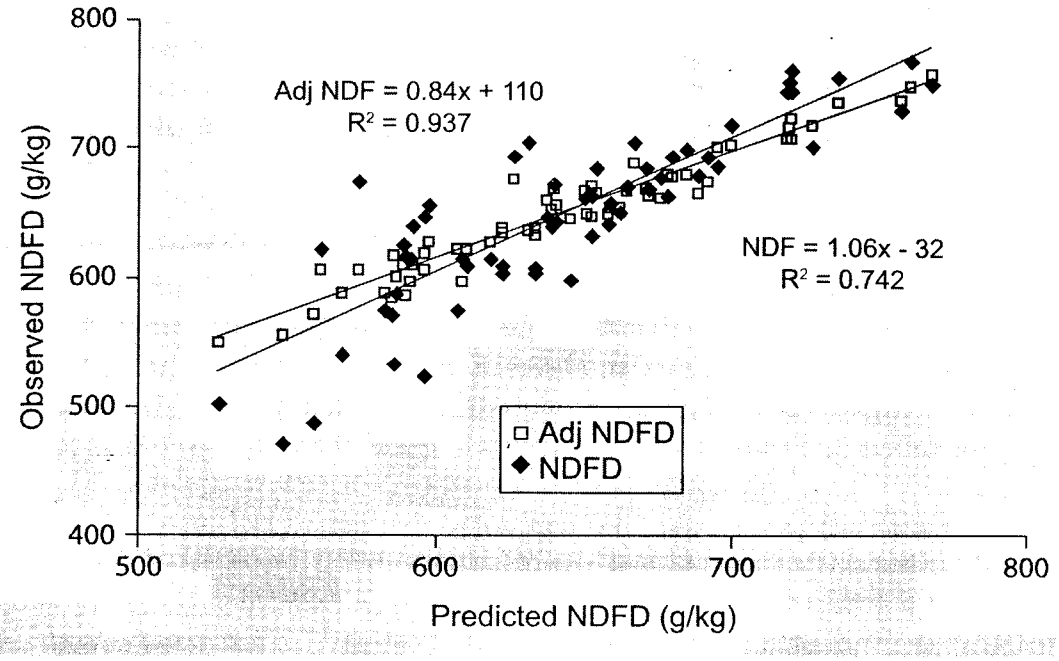

Figure 10. Relationship predicted (Nordic Dairy Cow Model; Danfær et al., *2005a) and observed NDF digestibility (NDFD) with or without adjustments for random experimental effects.*

differences in the determination of digestion kinetic parameters. The close relationship between digestion rates determined by different methods support this suggestion.

Conclusions

Development of useful mechanistic models for estimating digestibility and intake require both an accurate estimation of the parameter values and appropriate model structure. More work is required to validate the methods used to estimate digestion kinetic parameters. Most of the validation has been conducted by comparing two systems (e.g. *in vitro* vs. *in situ*) without validation against reliable *in vivo* data. Most of the systems appear to rank the feeds reasonably well, but that is not a satisfactory criterion to be of use for mechanistic rumen models. It appears that none of the present methods fulfil the requirements of an ideal method. It is important that only the intrinsic characteristics of cell walls limit the rate and extent of digestion, i.e. that the system itself is not a limiting factor. Future work is also required to estimate quantitative effects of some extrinsic factors such as intake and diet composition on the intrinsic rate of cell wall digestion.

A better understanding of the effects of, and interactions between, intrinsic and extrinsic factors on passage kinetics are required. In many cases estimated passage kinetic parameters represent interactions between animal and feed characteristics. In the future, more attention should be paid to distinguishing between animal (e.g. intake, diet composition) and intrinsic feed characteristics. Also more validation of the current marker systems against preferred reference methods (slaughter, rumen evacuation, appropriate internal markers) needs to be conducted.

Even though the mechanisms of selective retention of feed particles have been unequivocally described, it has seldom been used to calculate NDF digestibility from kinetic parameters. This fundamental flaw in the model structure, used extensively in most feed evaluation systems, leads to serious underestimations of NDF digestibility. When the mechanisms of selective retention of feed particles in the mechanistic rumen models are ignored, unrealistically high digestion rates and/or low passage rates have to be used to correctly predict *in vivo* digestibility. For an accurate prediction of NDF, and consequently OM digestibility, reliable estimates of intrinsic digestion kinetics and an adequate description of the underlying digestion and passage processes are required, but it is also essential that the regulation of intrinsic digestion rate and rumen residence time due to diet composition and level of intake are taken into account. For further progress in developing useful mechanistic models for the prediction of digestibility and intake, it is vital that modellers and ruminant biologists work in harmony.

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