Journal of Animal and Feed Sciences, 16, Suppl. 2, 2007, 145–150

Estimating fractional rate of NDF degradation from *in vivo* digestibility

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ABSTRACT

Fractional rate of degradation (k_d) of potential degradable NDF (dNDF) was estimated based on *in situ* degradation profiles. *In situ* values were compared to values for k_d which were calculated based on *in vivo* NDF digestibility and dNDF concentration for 61 forage samples including both young and very mature grasses and legumes. The calculation only failed for three samples, and *in situ* estimates correlated reasonably well to calculated values. Calculated k_d for dNDF has a large potential for use in practice as a low resource method.

KEY WORDS: nylon bag, *in situ*, *in vivo* validation, NDF

INTRODUCTION

Values for fractional rate of degradation (k_d) of potential degradable NDF (dNDF, % of dry matter (DM)) have so far mainly been based on NDF degradation profiles obtained by nylon bag or *in vitro* methods. However, for practical feed evaluation these methods are far too resource demanding (time, labour, costs) and dependent on access to fistulated cows. Therefore alternative methods are necessary for practical use. For forage NDF, it is essential that NDF degradation parameters can be analysed or estimated on the actual sample. This means that a simple method is needed for estimation of k_d based on biological/chemical laboratory methods. Further, only little information is available on *in vivo* validation of *in situ* values. The aim of the present paper is to compare k_d for dNDF estimated from *in situ* incubations with k_d which were calculated based on NDF digestibility measured in sheep fed at maintenance.

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MATERIAL AND METHODS

Material and analysis

Sixty one forage samples including grasses, legumes, whole crop cereals, whole crop maize and straw were analysed for *in vivo* NDF digestibility in sheep fed at maintenance (four sheep per forage sample) and for rumen degradability *in situ* (nylon bags). NDF degradation was performed using the *in situ* method according to Weisbjerg and Hvelplund (2005). Samples were freeze-dried, milled through 1.5 mm screen and incubated in nylon bags with pore size 37 µm (for 288 h 12 µm) for 0, 2, 4, 8, 24, 48 and 96 h in three dry cows fed a standard ration. As estimates for potential NDF degradability (pdNDF, % of NDF), degradabilities obtained after either 288 or 504 h rumen incubation were used. The reason for two methods was a change in the standard used in our laboratory.

After incubation residues were transferred quantitatively for NDF analysis (ash free) including amylase treatment.

Degradability was calculated relatively to 0 h residue to account for eventual particle losses, except for 288 h incubations where particle loss was negligible due to the small pore size in the nylon bag. Degradation profile parameters were estimated using incubation times up to 96 h by PROC NLIN in SAS, using a simple exponential model including degradability asymptote and rate of degradation. Further, a model including a lag time was also run.

Calculation of k_d

In vivo dNDF digestibility (D) can be found by combining NDF digestibility at maintenance and pdNDF. If D is known and assumed equal to rumen digestibility, and the rate of passage of dNDF is either measured or assumed, then the rate of dNDF degradation can be calculated. The simple one pool models for rumen passage behaviour, which do not account for the selective retention of newly ingested particles, underestimate effective degradation, and is therefore not suitable for this 'backwards' calculation. The two compartment model (Figure 1) by Allen and Mertens (1988) takes the selective retention of feed particles in the rumen into account:

$$\text{dNDF digestibility (D)} = [k_d /(k_d + k_r)]\,[1 + k_r/(k_d + k_p)] \qquad \text{(Equation 1)}$$

This equation can be solved according to k_d (Huhtanen et al., 2006):

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

where k_r and k_p are fractional rates of passage from the non-escapable and escapable pool, respectively. Estimation of k_d using this principle thus requires information on the rate of passage (k_r and k_p), pdNDF and *in vivo* NDF digestibility in sheep fed at maintenance.

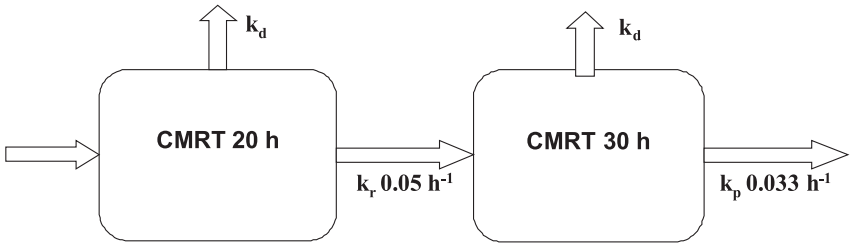

Figure 1. Model of two compartment passage of fibre through the rumen with values for compartmental mean retention times (CMRT) and resulting passage rates used to calculate k_d

Assuming a total mean retention time (MRT) in the rumen of the sheep fed at maintenance level of 50 h with 40% of the retention time in the non-escapable pool (Huhtanen et al., 2006) gives the values for k_r and k_p shown in Figure 1. However, this further relies on the assumption that rumen mean retention time and distribution of retention time between compartments are the same for all feed types, which can be questioned, but the assumption will probably only introduce a minor error.

Using the values from Figure 1 with total MRT of 50 h for obtaining k_r and k_p, Equation 2 result in the Equation 3. To illustrate the effect of MRT, calculations were also performed using a total MRT of 60 h with the same compartmental distribution, resulting in Equation 4. Equations 3 and 4 have been used for k_d calculations in the present paper.

$$k_d = -0.041667 + [0.006944 + 0.0066667D/(1 - D)]^{0.5}/2 \quad \text{(Equation 3)}$$
$$k_d = -0.034722 + [0.004823 + 0.0046296D/(1 - D)]^{0.5}/2 \quad \text{(Equation 4)}$$

RESULTS

From the total of 61 samples, calculated k_d could not be estimated for three samples (two red clover and one lucerne) due to higher measured *in vivo* NDF digestibility than measured *in situ* pdNDF. For the 58 samples with calculated k_d, pdNDF varied from 0.410 to 0.936. Calculated k_d varied from 0.019 to 0.211 per h, *in situ* degradation parameters for the model without lag time varied for k_d from 0.011 to 0.186 per h and for the asymptote from 0.394 to 0.919. For the model

with lag time *in situ* k_d varied from 0.013 to 0.377 per h, asymptote from 0.394 to 0.910 and lag time from 0 to 6.8 h.

Plots of *in situ* k_d against calculated k_d are shown in Figure 2. For three very young grass/grass-clover crops very high *in situ* k_d (0.16-0.19 per h, model without lag) were obtained. For calculated k_d six samples of young crops obtained very high k_d above 0.15 per h, as measured pdNDF was only slightly higher than measured *in vivo* digestibility of NDF for these samples. One sample of early cut grass-clover resulted in a very high *in situ* k_d of 0.38 when estimated with a model including lag.

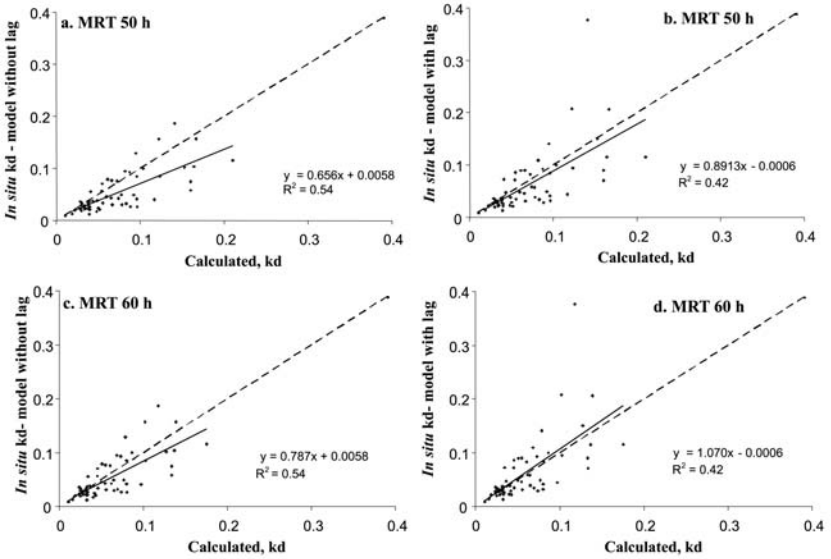

Figure 2. Plot and regression of *in situ* k_d from model without (a, c) or with (b, d) lag time against calculated k_d. Total rumen MRT 50 h (a, b) and 60 h (c and d). Dotted line indicates y=x

The *in situ* k_d from a model without lag (Figure 2a) were generally lower than calculated k_d. Further, the variation around the regression line increased with increasing k_d. *In situ* k_d values from a model including lag time were more of the same magnitude as the calculated k_d, with a regression coefficient of 0.89 and an intercept of -0.0006 very close to zero. Increasing MRT from 50 to 60 h generally increased the regression coefficient for both model types without changing R^2.

DISCUSSION

The results show that it is possible to obtain reasonable degradation data from *in vivo* digestibility of NDF combined with dNDF concentration. Only for very young

crops, where *in vivo* digestibility is close to pdNDF, random and method errors can induce situations where measured *in vivo* digestibility become higher than measured pdNDF and k_d therefore cannot be calculated. Generally, k_d estimated *in situ* using a model including lag time is more in accordance with k_d calculated from *in vivo* data in a model without digestion lag time, indicating that the lag time found in nylon bags might be a shortcoming of the *in situ* method. Further, 60 h compared to 50 h for total rumen MRT used for calculating k_d are in closer accordance with *in situ* data.

The calculation method for k_d is limited by its very indirect approach where analytical and prediction errors can add up. Further, and maybe most critical, the *in vivo* digestibilities are often very close to the potential degradabilities, and therefore only small 'errors' on either dNDF or *in vivo* NDF digestibility have large influence on the estimated fractional rate of degradation. This is especially true for feed samples with high fractional rate of dNDF degradation, where only small analytical errors can result in dNDF digestibilities above 100%, and then no solution for k_d can be found.

Sensitivity tests have shown that feed samples with high k_d are very sensitive to an 'erroneous' overestimation of digestibility of dNDF, but less sensitive to underestimation. Sensitivity to 'errors' in ash and NDF concentration was found to be rather low. Samples with less degradable NDF are generally much less sensitive to 'errors' in analyses.

As mentioned above, the nylon bag method has been widely used to obtain NDF degradation parameters for modelling. There are, however, several indications showing that k_d for dNDF found using the nylon bag method underestimate k_d found using *in vitro* or *in vivo* methods (Huhtanen et al., 2006). This could be due to a less favourable micro-environment for NDF degradation in the nylon bags than in the surrounding rumen environment.

At the onset of NDF degradation there is often a delay (lag time), both with the nylon bag method and the *in vitro* method. Whether these *in situ* lag times are real or shortcomings of the *in situ* method or a mixture of both are not known. However, it is very important for the interpretation of degradation profiles to establish whether the lag times are real or not, as it highly affects the magnitude of the estimated k_d.

Further, the fractional rate for dNDF degradation is not constant, as the name 'degradation rate constant' indicates. Besides the lag time, it seems, after the lag time has ended, that k_d decreases as incubation time increases. However, this decrease is probably less problematic than the lag time for the interpretation of degradation profiles. But it is necessary with increased knowledge in this area to correctly interpret degradation profiles, and this implies that the k_d found from *in situ* data cannot be taken as the absolute truth.

PRACTICAL IMPLICATIONS

For the use in feed evaluation on samples from practice, sheep digestibility experiments are far too resource-demanding. Using the Lucas principle, a true NDS (Neutral Detergent Solubles) digestibility of 101% and an endogenous loss of 90 g NDS per kg ingested dry matter (DM) were found (Weisbjerg et al., 2004a). When OM digestibility is known, this allows a prediction of NDF digestibility, as digested OM is equal to the sum of digested NDS and digested NDF. An evaluation of 2,337 observations showed an acceptable prediction of digested NDF in % of feed DM with no bias (Weisbjerg et al., 2004b). This principle enables an estimation of NDF digestibility from OM digestibility and NDF concentration, which both are routine analysis made in practical feed evaluation using *in vitro* methods and wet chemistry or near infra-red reflectance spectroscopy (NIRs). When NDF digestibility and dNDF concentration are known, dNDF digestibility can be calculated. The advantage of this approach for estimation of fractional rate of degradation of dNDF is that it relies only on laboratory methods already used in practice. The method is at present used in practice in Denmark for estimation of k_d for forages, where OM digestibility, and NDF and dNDF concentrations, are estimated using NIRS.

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