In vitro gas production profiles to estimate extent and effective first-order rate of neutral detergent fiber digestion in the rumen

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ABSTRACT: An automatic in vitro gas production technique was evaluated for predicting in vivo fiber (NDF) digestibility and effective first-order digestion rate of potentially digestible NDF (pdNDF) of 15 grass silages. Observed in vivo NDF digestibility of the silages harvested at different stages of maturity during 3 yr was determined by the total fecal collection in sheep fed at the maintenance level of intake. Isolated grass silage NDF was incubated for 72 h in the presence of rumen fluid and buffer to determine the pdNDF digestion kinetics based on cumulative gas production profiles. The digestion kinetic parameters were estimated by a 2-pool Gompertz function. The estimated parameter values were then used in a 2-compartment mechanistic rumen model to predict the in vivo digestibility of pdNDF. A total compartmental mean residence time of 50 h was used in the model, and a further assumption of the distribution of the residence time between the rumen nonescapable and escapable pools in a ratio of 0.4:0.6 was made. To make a distinction between potentially digestible and indigestible NDF, the potential extent of NDF digestion was determined by a 12-d ruminal in situ incubation. The model-predicted in vivo NDF digestibility accurately and precisely (root mean square error = 0.013 units, R² = 0.99). Effective first-order digestion rate was estimated from the predicted pdNDF digestibility, and the values were compared with those calculated from the in vivo pdNDF digestibility using the same passage kinetic parameters. The predicted effective first-order digestion rate was strongly correlated with digestion rate estimates derived from in vivo data (root mean square error = 0.006/h, R² = 0.86). It can be concluded that a simple first-order digestion rate can be estimated from a complicated gas production kinetic model including 6 parameters. This rate constant can be used in continuous steady-state dynamic mechanistic rumen models predicting the nutrient supply to the host animal.

Key words: effective digestion rate, fiber, gas production, mathematical model, rumen digestion

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INTRODUCTION

The in vitro gas production (GP) technique has widely been used during the last decades by ruminant nutritionists to study feed degradation (Menke et al., 1979; Rymer et al., 2005). One of the advantages of the in vitro GP technique is that it can be automated to obtain a large number of data points allowing more accurate parameter estimation than the gravimetric in vitro techniques or in situ methods. Mathematical description of the data has been accomplished by fitting a variety of nonlinear models (France et al., 1993, 2000; Schofield et al., 1994; Cone et al., 1996). Most of the new models are non first-order models [i.e., digestion rate (kd) is variable during fermentation]. A limitation of these models is that the non first-order kinetic parameters cannot be used in steady-state rumen models. Moreover, the parameter values of these models do not always permit unequivocal comparison of feedstuffs due to the strong correlations between parameters. To overcome these problems, Pitt et al. (1999) derived equations to estimate effective first-order kd from non first-order models assuming that the rumen is a 1-compartment system with random passage of feed particles. However, the feed particles are retained selectively in the rumen (Allen and Mertens, 1988), and without the mechanisms of selective retention in the rumen, it would be impossible to accurately predict in vivo digestibility of potentially digestible NDF with realistic parameter values (Ellis et al., 1994; Huhtanen et al., 2006).
The first objective of the current study was to test an automatic in vitro GP technique by comparing NDF digestibility predicted from GP parameters using a mechanistic 2-compartment rumen model and in vivo NDF digestibility determined in sheep by the total fecal collection method. The second objective was to derive effective first-order $k_f$ from model-predicted potentially digestible NDF (pdNDF) digestibility and to compare these values to estimates derived from in vivo pdNDF digestibility.

**MATERIALS AND METHODS**

All animals were managed according to legislation documented in the Finnish Animal Welfare Act (247/96), the order of using vertebrate animals for scientific purposes (1076/85), and the European convention for the protection of vertebrate animals used for experimental and other scientific purposes, appendix A and B, implemented under the auspices of the local animal use and care committee.

**Feeds**

In vitro GP was measured from isolated neutral detergent residue of 15 grass silage samples. Incubation of isolated NDF allows estimation of digestion kinetic parameters for the fiber fraction alone so that GP from the neutral detergent soluble fraction does not interfere. The silages were prepared in Jokioinen, Finland, from primary growth timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) swards at different stages of maturity over 3 yr, and ensiled unwilted into 3-m³ experimental silos using 4 L of formic acid/ton as a preservative. The feeds were dried (60°C) and milled through a 1-mm screen. Fiber (NDF) was extracted from the feeds by a modified method of Van Soest et al. (1991), as follows: feed samples (3 g) were boiled for 1 h in nylon bags (pore size 38 μm, open surface area 28%) in neutral detergent solution, and the bags were first rinsed with water and then twice with acetone (150 mL of acetone per bag at each time) to remove the detergent. Chemical analysis of the silage samples and in vivo digestibility experiments were conducted as described by Nousiainen et al. (2003a).

In vivo digestibility was determined by the total fecal collection method in sheep fed at maintenance level of intake. Indigestible NDF (iNDF) concentration of the feeds was assessed by 12-d in situ incubations as described by Huhtanen et al. (1994) but was expressed as ash-free. Small (6 μm) pore-size bags and long incubation period were used to avoid inflow and outflow of particles from the bags and to ensure that the potential extent of digestion was obtained with this cloth type. The concentration of pdNDF (g/kg of DM) was then calculated as NDF (g/kg of DM) – iNDF (g/kg of DM). In vivo digestibility of pdNDF was calculated as [(pdNDF intake (g/d) – pdNDF output (g/d))/pdNDF intake (g/d)]. The concentration of digestible NDF (dNDF, g/kg of DM) was calculated as [(NDF intake (g/d) – fecal NDF (g/d))/DMI (kg/d)].

**In Vitro GP Measurements**

In vitro GP measurements were made by an automated system, which consists of 39 serum bottles with a volume of 120 mL each. Tubes connect the fermentation bottle to a pressure transducer (142PC05D, Honeywell Inc., Minneapolis, MN) and to a solenoid valve (11-15-1-SV-24Q70, Pneutechnics, Hollis, NH). The contents of the fermentation bottles are stirred intermittently, with 15 s of stirring and 30-s pauses using a magnetic stirring plate. The stirrers are designed to also stir the surface layer, thereby keeping the substrate within the liquid phase. To release the accumulated gas (approximately 1.1 mL), magnetic valves are adjusted to open when the differential pressure in the bottle reaches 2.8 kPa. A modular, programmable logic controller (MESEC AnS, Mitsubishi Electric Europe GmbH, Industrial Automation, Ratingen, Germany) and FactoryLink software (United States Data Corporation, Richardson, TX) automatically control the valve openings, recordings, and calculations. The system is maintained at 39°C in a thermostatically controlled (CAL 3200, CAL Controls Ltd., Hitchin, UK) room.

Samples (500 mg) of isolated NDF were weighed into the serum bottles. Sixty milliliters of CO₂-saturated buffer solution containing a reducing reagent (Goering and Van Soest, 1970) was dispensed into the serum bottles. The feed samples were allowed to hydrate for 1 h before inoculation. Rumen fluid (1 L) was collected from 2 ruminally fistulated Finnish Ayrshire heifers fed a forage-based (20% concentrate on DM basis) diet. Rumen fluid was maintained at 39°C until strained through 2 layers of cheesecloth into a CO₂-filled flask. The solid residue was homogenized for 60 s in a blender with 500 mL of Goering and Van Soest (1970) buffer (0°C) to detach particle-associated microbes and strained through 2 layers of cheesecloth into the same flask. Inoculum was warmed to 39°C, and the bottles were inoculated with 20 mL of inoculum under a continuous stream of CO₂. Cumulative GP was recorded every 15 min for the 72-h incubation periods (288 recordings per sample). All samples were incubated in triplicate runs.

**Models and Curve Fitting**

For each NDF residue, a mean cumulative GP curve was calculated from the 3 replicates. The 2-pool Gompertz function (Schofield et al., 1994) was fitted to GP data by the NLIN procedure (SAS Inst. Inc., Cary, NC), as follows:

$$V_t = V_1 \times \exp\{-\exp[1 - k_1 \times (t - L_1)]\} + V_2 \times \exp\{-\exp[1 - k_2 \times (t - L_2)]\},$$

where $V_t$ = the measured gas volume at time $t$; $V_1$, $k_1$, and $L_1$ = the asymptotic cumulative gas volume, rate,
and lag parameters for pool 1; and \( V_2, k_2, \) and \( L_2 \) = the respective parameters for pool 2. For both pools, \( t \) is time (h). This model was chosen because it fit the data better (data not shown) than the commonly used (France et al., 2000) 1-pool models. Furthermore, the choice of the kinetic model had only minor effects on predicted NDF digestibility (data not shown). The compartmental interpretation of the function is described in detail by France et al. (2000).

**Rumen Digestion Model**

A dynamic, mechanistic rumen model was developed to predict ruminal pdNDF digestibility by incorporating a time-dependent \( k_d \) estimated from the GP profiles for a single batch of feed. The predicted pdNDF digestibility was used to calculate the effective first-order \( k_d \) (i.e., the effective \( k_d \)). Pitt et al. (1999) demonstrated that the function derived to estimate the effective \( k_d \) is independent of intake, which permits the use of this parameter in continuous steady-state rumen models. The relationship of GP to substrate digested was assumed to be constant throughout the incubation period. The graphical presentation of the model, including rapidly and slowly digestible NDF pools, is shown in Figure 1. The model estimates pdNDF digestibility for a single meal. Initial amount of pdNDF in the rumen at the beginning of simulation was 1 kg, which was divided between the fast and slow pools on the basis of estimated gas volumes of the pools.

Digestibility of cell wall carbohydrates in the rumen is a function of competition between digestion and passage. The total mass disappearing by digestion will be the integral over time from \( t > \text{lag to} \infty \), and the total extent of digestion is defined as total amount degraded divided by the total amount entering the system. For the simple exponential digestion kinetics model and the 1-compartment system with first-order passage rate (\( k_p \)), the digestibility can be evaluated by a simple algebraic equation \( \text{digestibility} = k_d/(k_d + k_p) \), but for the Gompertz function with time-dependent \( k_d \), the integrals are nonanalytical (France et al., 2000) and must be evaluated numerically. The \( k_d \) at each time point may be estimated as the mass digested as a proportion of the mass in the rumen. If the function of cumulative GP is described as \( F(t) \) and the derivative of the function \( F'(t) = dF/dt \) describes the mass disappearing at time \( t \), the rate of digestion at each time point \( t \) can be described as follows: \( k_d = F'(t)/F(t) \).

The model estimating the amount of pdNDF digested in the rumen is further complicated by incorporating the selective retention of feed particles in the rumen (Allen and Mertens, 1988). Large forage particles entering the rumen need to be comminuted below a threshold size before they can escape from the rumen. Specific gravity also influences the probability of particles escaping from the rumen. Processes describing particle size reduction and changes in specific gravity releasing particles from the rumen nonescapable pool to the escapable pool are described as Rum_Fast and Rum_Slow (Figure 1). Marker kinetic studies (Pond et al., 1988; Lund et al., 2006) have demonstrated that the release of feed particles from the nonescapable pool to the escapable pool is a time-dependent process, and therefore a gamma time-related function \( (G_2G_1) \) was used to describe this process. The release rate constant from the nonescapable to escapable pool (\( k_p \)) was calculated as: \( k_r = (t \times \lambda^2)/(1 + (t \times \lambda)) \), where \( \lambda = \text{the rate constant} \) \((\lambda = 2 \text{residence time in the compartment}) \) estimated by a passage model including a time-dependent flow from the nonescapable to the escapable pool (Ellis et al., 1994) and \( t = \text{time} \). The \( k_p \) from the escapable pool was assumed to be a first-order process. The mean residence time in the rumen was assumed to be 50 h, and the distribution between the nonescapable and escapable pools was assumed to be 0.4:0.6 (Lund et al., 2006; Huhtanen et al., 2007). For comparing the effects of the mechanisms of selective retention of the digesta in the rumen, the simulations were also made assuming the rumen as a 1-compartment system (i.e., that the distribution of 50 h of residence time between the 2 compartments was 0:1).

To calculate cumulative GP at each time point, the parameters used were \( V_1, K_1, \) and \( L_1 \) for the rapid pool and \( V_2, K_2, \) and \( L_2 \) for the slow pool. The rates \( k_d1 \) and \( k_d2 \) at each time point were calculated by dividing the derivative of the cumulative GP by the cumulative GP at that time point \( [\text{e.g., } F_{d1} = F_{\text{fast}}'(t)/F_{\text{fast}}(t)] \).

The predicted dNDF (g/kg of DM) was calculated as pdNDF (g/kg of DM) \( \times \) predicted pdNDF digestibility. Total NDF digestibility was calculated as predicted dNDF (g/kg of DM)/NDF (g/kg DM).

Single, effective first-order \( k_d \) was calculated from the in vivo pdNDF digestibility, and passage kinetic parameters were calculated using the equation described by Huhtanen et al. (2006):

\[
\text{Effective } k_d = \{-(k_p + k_r) + [(k_p + k_r)^2 + 4 \times \text{pdNDF digestibility} \times k_r k_p/(1 - \text{pdNDF digestibility})^{2/5}\}/2.
\]

Values of 0.05 and 0.0333 were used for \( k_p \) and \( k_r \), respectively (i.e., the same rumen residence time was used for the in vivo data and for the rumen model).

The rumen models were constructed using Powersim software (Powersim AS, Ista, Norway). Simulations were run for 120 h with a time step of 0.0625 h and using the Euler integration method.

**Statistical Analysis**

Root mean squared errors (RMSE) between observed in vivo and model-predicted dNDF and NDF digestibility, and in vivo-derived and model-predicted effective \( k_d \) were calculated as: \( \text{RMSE} = \sqrt{\text{Σ} (\text{observed} - \text{predicted})^2/n} \). Mean squared prediction error (MSPE) was divided into the components resulting from mean bias, slope bias, and random variation around the regression line (Bibby and Toutenburg, 1977).
RESULTS

Composition of Feeds and In Vivo Digestibility

Mean composition and in vivo digestibility of the 15 experimental silages are shown in Table 1. Because silages were preserved unwilted, DM concentration was low, but fermentation quality was good, as indicated by low pH and concentrations of VFA and NH₃-N. Both the chemical composition and in vivo OM and NDF digestibility exhibited large variation related to the maturity of ensiled grass material. Variation both in the concentration and the digestibility were much smaller for the digestible fraction of NDF than for total NDF.

Curve-Fitting of Gas Production Data

The fit of the 2-pool Gompertz model to the data was good, as indicated by low RMSE of observed gas volumes (mean 0.26 mL; 0.21% of asymptotic volume) and high $R^2$ values (>0.99). Average parameter estimates with their standard errors and confidence intervals are shown in Table 2. Standard errors of all parameter values were small, ranging from 1.2 to 1.6% of the mean except for $L_2$ (4.2%). As a result of the small standard error, the 95% confidence interval for the parameter estimates was small. The potential NDF digestibility was closely related to the total volume of GP (Figure 2).

Residuals of GP from the fit of the 2-pool Gompertz model showed a distinguishable pattern during early fermentation times, but later they were randomly distributed around zero. On average, the residuals crossed the x-axis 24 times (range from 10 to 50). A typical pattern of residuals is shown in Figure 3.

Prediction of Digestible NDF, NDF Digestibility, and Digestion Rate

Using a 1-compartment rumen model but the same compartmental mean residence time (50 h) clearly underestimated dNDF (mean bias 68.1 g/kg of DM) compared with the 2-compartment model (mean bias 4.4 g/kg of DM). Also, the slope of 1.18 was significantly ($P = 0.01$) greater than 1.00 for the 1-compartment model. Because the effects of using the 1-compartment rumen model on prediction bias were similar for all parameters, only the data based on the 2-compartment system is presented.

The relationships between predicted and observed dNDF are shown in Figure 4. In general, using the digestion kinetic parameters estimated from GP profiles resulted in very accurate (RMSE = 7.3 g/kg of DM) and precise ($R^2 = 0.98$) estimates of the in vivo dNDF values. The intercept was not significantly different from zero, and the slope was not significantly different from 1. The proportions of bias, slope, and random errors of MSPE were 0.64, 0.01, and 0.35, respectively.

The predictions of the in vivo NDF digestibility (Figure 5) reflected a pattern similar to dNDF predictions. Distribution of MSPE was similar to that for dNDF (0.58, 0.00, and 0.42 for bias, slope, and random error, respectively). The model-predicted first-order effective $k_d$ was closely related to digestion rate derived from the in vivo pdNDF digestibility (Figure 6). The model-
In vitro gas production and neutral detergent fiber digestion

Table 1. Composition and digestibility of the 15 silages

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, g/kg g/kg of DM</td>
<td>200</td>
<td>16.9</td>
<td>229</td>
<td>171</td>
</tr>
<tr>
<td>OM</td>
<td>927</td>
<td>8.6</td>
<td>909</td>
<td>940</td>
</tr>
<tr>
<td>CP</td>
<td>161</td>
<td>38.3</td>
<td>112</td>
<td>239</td>
</tr>
<tr>
<td>NDF</td>
<td>586</td>
<td>82.0</td>
<td>402</td>
<td>669</td>
</tr>
<tr>
<td>Indigestible NDF</td>
<td>81</td>
<td>47.6</td>
<td>17</td>
<td>158</td>
</tr>
<tr>
<td>Lignin</td>
<td>36</td>
<td>10.3</td>
<td>20</td>
<td>55</td>
</tr>
<tr>
<td>Potentially digestible NDF</td>
<td>426</td>
<td>33.7</td>
<td>349</td>
<td>469</td>
</tr>
<tr>
<td>Water-soluble carbohydrates</td>
<td>43</td>
<td>16.8</td>
<td>73</td>
<td>17</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>46</td>
<td>11.9</td>
<td>72</td>
<td>32</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>22</td>
<td>9.9</td>
<td>49</td>
<td>11</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>0.2</td>
<td>0.6</td>
<td>2.2</td>
<td>0</td>
</tr>
<tr>
<td>pH</td>
<td>4.10</td>
<td>0.13</td>
<td>3.79</td>
<td>4.31</td>
</tr>
<tr>
<td>NH3-N, g/kg of total N</td>
<td>47</td>
<td>13.0</td>
<td>28</td>
<td>82</td>
</tr>
</tbody>
</table>

In vivo digestibility

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean</th>
<th>SE</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM</td>
<td>0.725</td>
<td>0.073</td>
<td>0.613</td>
<td>0.840</td>
</tr>
<tr>
<td>NDF</td>
<td>0.737</td>
<td>0.085</td>
<td>0.597</td>
<td>0.869</td>
</tr>
<tr>
<td>Potentially digestible NDF</td>
<td>0.844</td>
<td>0.035</td>
<td>0.791</td>
<td>0.907</td>
</tr>
</tbody>
</table>

The main objective of the current study was to test the in vitro GP technique against in vivo data. Digestible NDF concentration and NDF digestibility were predicted by a dynamic mechanistic rumen model, and the values were compared with in vivo data.

Different versions of simple exponential equations have been applied in GP studies. Sometimes a parameter describing a rapidly or immediately degradable fraction applied to in situ data has also been used in models describing GP kinetics. As discussed by France et al. (2000), this often results in negative values at zero time. However, negative gas volumes at zero time are biologically meaningless artifacts of the procedure, and the usefulness of such parameter values in rumen models is questionable.

Small RMSE between observed and predicted gas volumes and the high number of the crossings of residuals over the x-axis indicate that the 2-pool Gompertz model fit the data well and that the residuals were randomly distributed. The Gompertz function has an inherent error in that predicted gas volumes are positive at time zero, which is not biologically possible, because the initial gas volume should be zero. However, when these models were applied assuming that pdNDF is comprised of rapidly and slowly degradable fractions as described by Schofield et al. (1994) and Schofield and Pell (1995), the performance of the model is markedly improved. With the 2-pool Gompertz function, the positive intercept was 2.9 mL (maximum gas volume ap-

Table 2. Average parameter estimates to fit the gas production profile of 15 grass silages by the 2-pool Gompertz model

<table>
<thead>
<tr>
<th>Item(^1)</th>
<th>Mean</th>
<th>SE</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>(V_1), mL</td>
<td>57.1</td>
<td>0.78</td>
<td>55.6</td>
<td>58.6</td>
</tr>
<tr>
<td>(k_1), 1/h</td>
<td>0.100</td>
<td>0.0016</td>
<td>0.097</td>
<td>0.103</td>
</tr>
<tr>
<td>(L_1), h</td>
<td>4.60</td>
<td>0.060</td>
<td>4.49</td>
<td>4.72</td>
</tr>
<tr>
<td>(V_2), mL</td>
<td>65.2</td>
<td>0.75</td>
<td>63.7</td>
<td>66.7</td>
</tr>
<tr>
<td>(k_2), 1/h</td>
<td>0.027</td>
<td>0.0003</td>
<td>0.027</td>
<td>0.028</td>
</tr>
<tr>
<td>(L_2), h</td>
<td>5.71</td>
<td>0.239</td>
<td>5.24</td>
<td>6.18</td>
</tr>
</tbody>
</table>

\(^1\)\(V_1\), \(k_1\), and \(L_1\) are the asymptotic cumulative gas volume, rate, and lag parameters for pool 1 (the rapid pool); \(V_2\), \(k_2\), and \(L_2\) are the respective parameters for pool 2 (the slow pool).
The residuals (observed – predicted) of gas production from isolated NDF of silage. The residual crossed the x-axis 24 times, and the root mean square error was 0.22 mL.

approximately 120 mL), and the time to reach zero residual was on average only 1.5 h. Chesson et al. (1986) reported that mesophyll and epidermis of isolated cell walls were completely digested in situ at 12 h, whereas proportionally only 0.40 of cell walls were digested. In vitro digestion of various forages also demonstrated a fast- and a slow-digesting pool (Van Soest et al., 2005).

Gradual development of particle-associated fibrolytic enzyme activities during ruminal in situ incubation (Huhtanen et al., 1998) and evidence that cell wall fractions may comprise fractions degraded at different rates (Mertens, 1973; Van Soest et al., 2005) support the concept of variable fractional digestion rates.

Model Assumptions

A value of 50 h was used as the mean residence time in the forestomachs, which is consistent with the data of Barry et al. (1985), who used the slaughter technique and lignin as a marker to estimate digesta residence time in various segments of gastrointestinal tract of sheep. In studies using the rumen evacuation technique with lignin as a marker in sheep (Bernard et al., 2000) or iNDF as a marker in cattle (Huhtanen et al., 2007), rumen residence times were 47.5 and 50 h using forage-alone diets. Dhanoa et al. (2000) used a value of 0.02/h indicative of maintenance level of intake in a single compartment system. They concluded that using different passage rate constants does not affect the ranking of models.

In the current study, the 2-compartment rumen model incorporating selective retention of feed particles in the rumen was used. The passage kinetics model had no influence on precision of predictions, but the 1-compartment model clearly underestimated dNDF concentration and NDF digestibility. The first-order $k_d$ of pdNDF should have been on average 0.115/h to achieve the observed in vivo NDF digestibility within the 50-h rumen residence time, or with the 1-compartment system, the rumen residence time should have been on

Figure 4. The relationship between predicted and observed digestible NDF (dNDF) concentrations. dNDF (g/kg of DM) = $-14 \pm 15 + [0.98 \pm 0.036 \times \text{predicted dNDF}]$, $R^2 = 0.983$, and the root mean square error = 7.3.

Figure 5. The relationship between predicted and observed NDF digestibility (NDFD). NDFD = $-0.014 \pm 0.021 + [0.99 \pm 0.029 \times \text{predicted NDFD}]$, $R^2 = 0.989$, and the root mean square error = 0.013.
average 88 h to attain the in vivo NDF digestibility using \( k_d \) values calculated from in vivo pdNDF digestibility. These examples are consistent with Ellis et al. (1994), who argued that without the mechanisms of selective retention in the rumen, it would be impossible to attain observed in vivo pdNDF digestibility with realistic parameter values. Mertens (1993) concluded that assuming the rumen as a single compartment is not an adequate mathematical or biological representation of rumen functions. Including the second degree of gamma time-dependency in the flow from the nonescapable to the escapable pool increased the predicted pdNDF digestibility by 0.02 to 0.03 units, but higher degrees of time-dependency had only minor effects on the simulated pdNDF digestibility (Huhtanen et al., 2006).

We used isolated NDF to estimate the digestion kinetic parameters of cell walls from GP curves. This approach is based on the assumption that extraction does not influence digestion of cell walls. Kennedy et al. (1999) found that isolated NDF was fermented more readily than the cell walls in intact grasses. In contrast, Doane et al. (1997) and Hall et al. (1998) reported that NDF digestibility was largely unaffected by extraction. Extraction slightly increased (0.817 vs. 0.781) the extent of in situ NDF digestion of grass silages, but the rate was slightly faster for intact silages (Wilman et al., 1996). In the current study, the accurate prediction of the in vivo NDF digestibility and \( k_d \) suggests that extraction of NDF did not markedly influence digestion characteristics of the silage samples.

**Relationship Between pdNDF Digestibility and Gas Production**

Although there was a close relationship between potential NDF digestibility and total GP (Figure 2), extended ruminal in situ incubation is likely to be a more reliable method for estimation of potentially digestible fraction of NDF. In the current study, the relationship between GP and potential extent of NDF digestion was good, in agreement with Deaville and Givens (2001), but differences in rumen VFA fermentation pattern may affect relationships. A close empirical relationship between iNDF concentration in grass or legume silages and in vivo OM digestibility (Nousiainen et al., 2003b; Rinne et al., 2006) further supports using the in situ method for estimation of the potentially digestible NDF.

Isolated NDF from grass silages is a relatively homogenous substrate, and therefore, it may be assumed that the proportions of VFA as well as the ratio between carbon used for VFA production and microbial synthesis are relatively constant among the feeds. The close relationship between the extent of potential NDF digestibility and GP supports this suggestion. The intercept of the regression between total GP and potential NDF digestibility was not different from zero (\( P = 0.72 \)), which also indicates a similar VFA pattern from fermentation of isolated NDF from the silages used.

**Prediction of dNDF and NDF Digestibility**

In general, the models predicted in vivo dNDF concentration and NDF digestibility both accurately and precisely, suggesting that an automated in vitro GP system can be a powerful tool for estimating \( k_d \) of pdNDF for mechanistic dynamic models (Dijkstra et al., 2002; Danfær et al., 2006). Good performance of the model can be attributed to the following 3 factors: (1) distinction between iNDF and pdNDF, (2) digesta passage models including selective retention of feed particles in the rumen, and (3) an accurate and precise prediction of \( k_d \) from GP. As discussed earlier, the close empirical relationship between in vivo OM digestibility and iNDF concentration in grass or legume silages and the biologically meaningful in vivo digestibility of pdNDF (mean 0.844) suggest that the 12-d in situ incubation in small-pore nylon bags is a reliable method to divide feed NDF between potentially digestible and indigestible components. This study demonstrated that the variation in pdNDF digestibility can be predicted accurately by applying GP profiles to estimate \( k_d \) at each time point and integrating pdNDF digestibility. For example, the RMSE of dNDF predicted mechanistically using parameter values estimated from GP profiles was much smaller (7.3 g/kg of DM) compared with the SD of dNDF (16.0 g/kg of DM) derived from the mean pdNDF digestibility (0.844). However, it should be noted that the variation in pdNDF digestibility was relatively small, and therefore, very accurate estimations of \( k_d \) are needed to predict dNDF more accurately than assuming a constant pdNDF digestibility.

According to our knowledge, this was the first study in which the kinetic parameters estimated from GP profiles were used in mechanistic models, and model predictions were tested against in vivo data. Previously, the data on GP kinetics have been used mainly to estimate in vivo digestibility or to predict intake empirically.
cally using multiple regressions (Blümmel and Ørskov, 1993; García-Rodriquez et al., 2005; Hetta et al., 2007).

**Prediction of the Effective First-Order Digestion Rate**

Another objective of the present work was to validate the in vitro GP system in estimating the $k_d$ of pdNDF to be used in the mechanistic dynamic rumen models. Because of many methodological problems of the in situ technique and strong evidence of underestimation of the in vivo rates of pdNDF digestion (Huhtanen et al., 2006), we used $k_d$ estimates calculated from in vivo pdNDF digestibility for validation of the method. Digestion rates estimated by a gravimetric in vitro method were higher than those determined in situ (Bossen et al., 2005), which also suggests that the in vitro techniques may allow a more accurate estimation of the true intrinsic $k_d$ than the in situ technique.

The estimated effective $k_d$ can be used in steady-flow rumen models, and using it will result in the same pdNDF digestibility as estimated for a single feed batch using time-related $k_d$ estimated from the GP curves. Pitt et al. (1999) presented the formal derivation for several models with variable digestion rates for estimating the effective $k_d$ including the effects of digestion lag. They assumed the rumen as a 1-compartment system without selective retention of particles, and both passage rate and lag time had an influence on the estimated effective $k_d$. In the 2-compartment system, the proportion of dNDF disappearing by passage is smaller during the early residence times compared with the 1-compartment 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 on digestibility in the 2-compartment system than in the 1-compartment system (Ellis et al., 1994).

The effect of rumen residence time on the effective $k_d$ was investigated using rumen residence times of 30, 40, 50, and 60 h and GP parameters estimated by the 2-pool Gompertz model. The estimated mean effective $k_d$ values for the 15 silages were 0.0623, 0.0599, 0.0575, and 0.05388 (SEM = 0.0002; $P < 0.001$) for 60-, 50-, 40-, and 30-h rumen residence times, respectively. With decreasing residence time, the feed particles become eligible for passage faster, which increases the effect of digestion lag on the effective $k_d$. Evidence from the literature also indicates reduced $k_d$ with increased feed intake, although the data is not very conclusive (Huhtanen et al., 2006).

Automated in vitro GP technique is widely used to estimate digestion kinetic parameters, and it has several advantages compared with traditional in vitro or in situ systems in terms of efficiency and cost. Our objective was to test the accuracy of the system in predicting digestion kinetic parameters against in vivo data and to use those parameters in mechanistic dynamic rumen models to predict dNDF, NDF digestibility, and effective first-order digestion rate. The in vivo dNDF, NDF digestibility, and effective $k_d$ could be predicted accurately and precisely using the kinetic parameters estimated from the GP curves using the 2-pool Gompertz function and iNDF determined by 12-d in situ incubation. Further studies using a wider range of forages are needed to confirm the current observations.

**LITERATURE CITED**


