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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, R^2 = 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, R^2 = 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 ($\mathbf{k_d}$) 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 k_d 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).

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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_d 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-m^3 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, Pneutronics, 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 (MELSEC 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 CO_2-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 CO_2-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 CO_2. 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 > lag to ∞, 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 ($\mathbf{k_p}$), the digestibility can be evaluated by a simple algebraic equation [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 ($\mathbf{k_r}$) was calculated as: $k_r = (t \times \lambda^2)/[1 + (t \times \lambda)]$, where λ = the rate constant (λ = 2/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 = 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 [e.g., $k_{d1} = F_{fast}'(t)/F_{fast}(t)$].

The predicted dNDF (g/kg of DM) was calculated as pdNDF (g/kg of DM) × 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):

$$\text{Effective } k_d = \{-(k_p + k_r) + [(k_p + k_r)^2 + 4 \times \text{pdNDF digestibility} \times k_r k_p/(1 - \text{pdNDF digestibility})]^{0.5}\}/2.$$

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, Istaldsø, 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: RMSE = $\sqrt{\Sigma}$ (observed − 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).

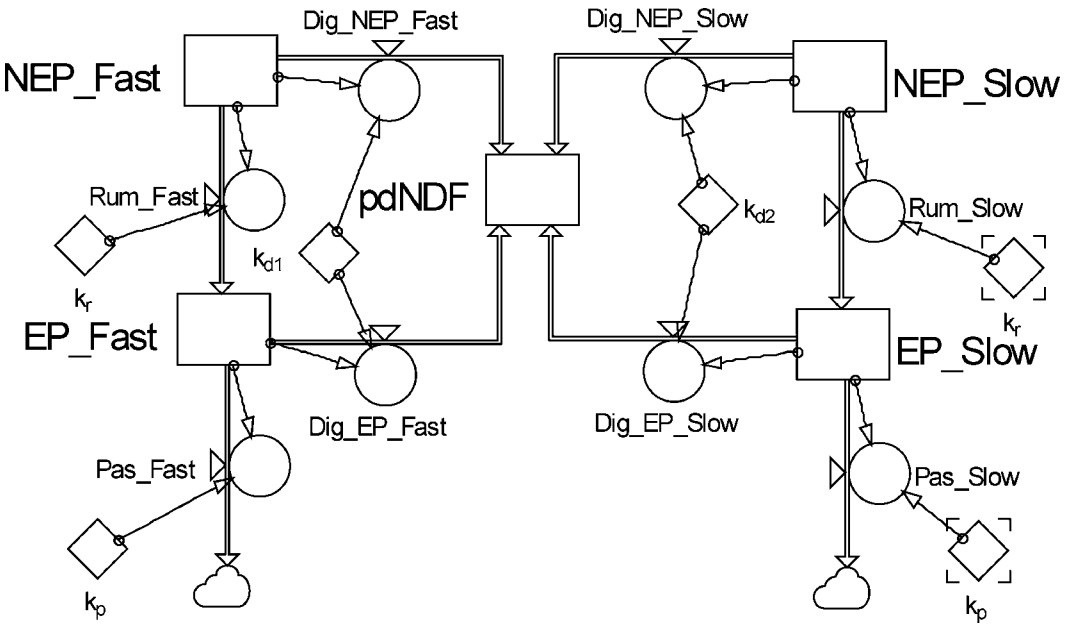

Figure 1. A graphical representation of the model of potentially digestible NDF (pdNDF) digestion in the rumen, incorporating selective retention of feed particles and rapidly (Fast) and slowly (Slow) digestible fractions of pdNDF. NEP = nonescapable pool (large recently ingested particles); EP = escapable pool (small ruminated particles available for passage); Rum = rumination (release from NEP to EP); Pas = passage; Dig = digestion; k_r = rate constant for Rum; k_p = rate constant for passage; k_{d1} and k_{d2} = rate constants for rapidly and slowly digestible NDF.

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 NH_3-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-

Table 1. Composition and digestibility of the 15 silages

Item	Mean	SD	Minimum	Maximum
DM, g/kg	200	16.9	229	171
g/kg of DM				
OM	927	8.6	909	940
CP	161	38.3	112	239
NDF	586	82.0	402	669
Indigestible NDF	81	47.6	17	158
Lignin	36	10.3	20	55
Potentially digestible NDF	426	33.7	349	469
Water-soluble carbohydrates	43	16.8	73	17
Lactic acid	46	11.9	72	32
Acetic acid	22	9.9	49	11
Butyric acid	0.2	0.6	2.2	0
pH	4.10	0.13	3.79	4.31
NH_3-N, g/kg of total N	47	13.0	28	82
In vivo digestibility				
OM	0.725	0.073	0.613	0.840
NDF	0.737	0.085	0.597	0.869
Potentially digestible NDF	0.844	0.035	0.791	0.907

predicted in vivo k_d both accurately (mean bias = 0.004/h) and precisely (R^2 = 0.86). The MSPE resulted mainly from bias (0.51) and random errors (0.47) with the slope error being minimal (0.02).

DISCUSSION

GP Models

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

			95% confidence limits	
Item[1]	Mean	SE	Lower	Upper
V_1, mL	57.1	0.78	55.6	58.6
k_1, 1/h	0.100	0.0016	0.097	0.103
L_1, h	4.60	0.060	4.49	4.72
V_2, mL	65.2	0.75	63.7	66.7
k_2, 1/h	0.027	0.0003	0.027	0.028
L_2, h	5.71	0.239	5.24	6.18

[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).

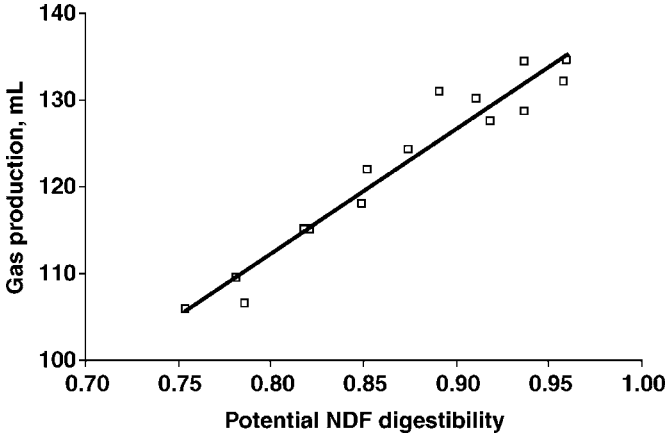

Figure 2. The relationship between potentially digestible NDF estimated by 12-d in situ incubation and asymptotic gas production from isolated NDF. Gas production = –3 (±8.9) + [144 (±10) × potential NDF digestibility], R^2 = 0.937, and the root mean square error = 2.6 mL.

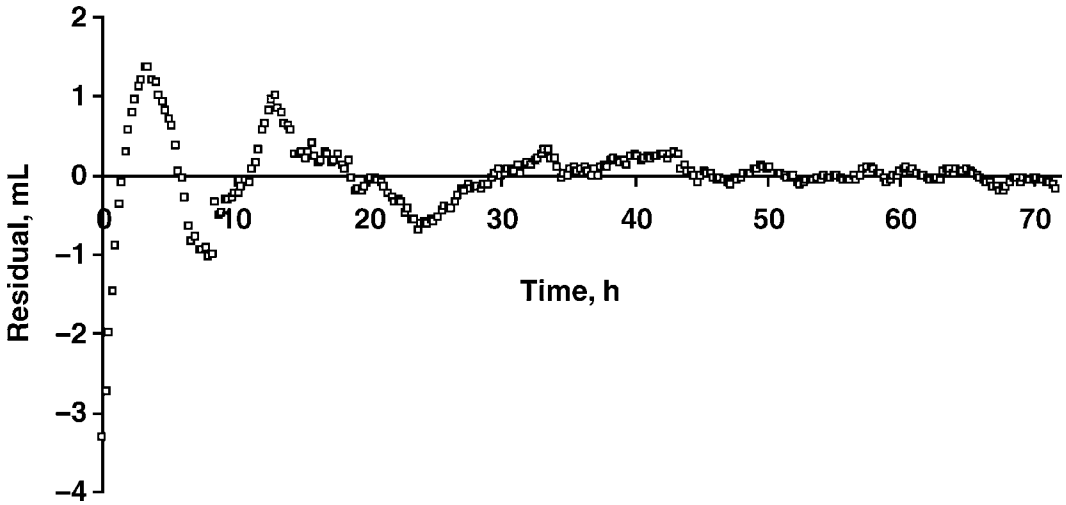

Figure 3. The residuals (observed – predicted) of gas production from isolated NDF of silage 9. The residual crossed the x-axis 24 times, and the root mean square error was 0.22 mL.

proximately 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

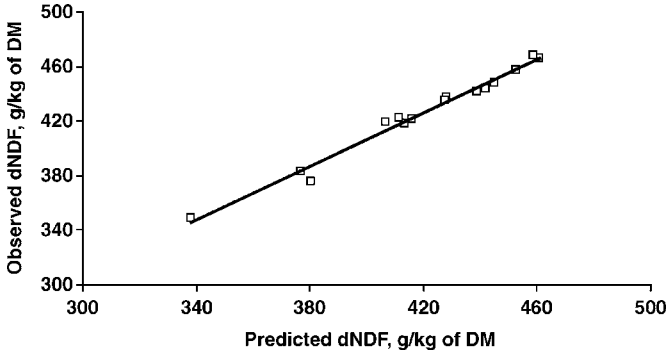

Figure 4. The relationship between predicted and observed digestible NDF (dNDF) concentrations. dNDF (g/kg of DM) = –14 (±15) + [0.98 (±0.036) × predicted dNDF], R^2 = 0.983, and the root mean square error = 7.3.

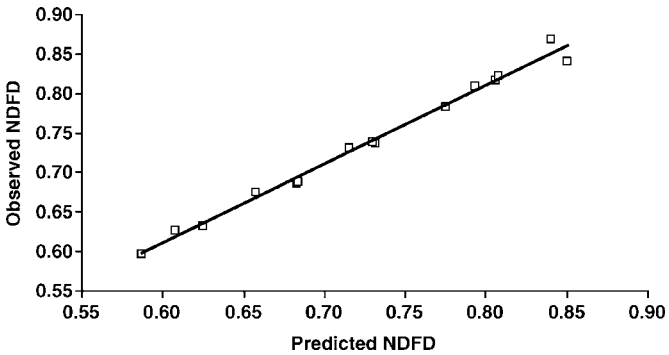

Figure 5. The relationship between predicted and observed NDF digestibility (NDFD). NDFD = –0.014 (±0.021) + [0.99 (±0.029) × predicted NDFD], R^2 = 0.989, and the root mean square error = 0.013.

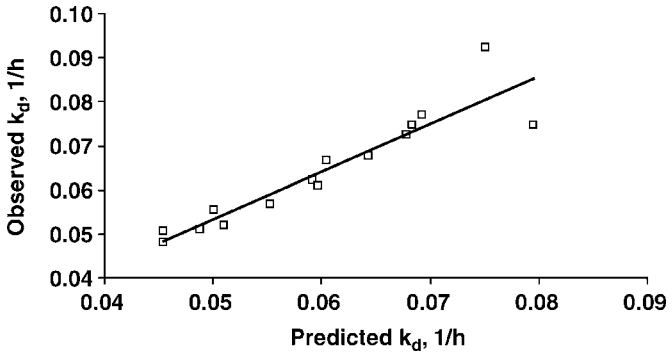

Figure 6. The relationship between the model-predicted and the observed in vivo potentially digestible NDF digestibility-derived digestion rate (k_d) of potentially digestible NDF. Observed (k_d, 1/h) = –0.0008 (±0.021) + [1.08 (±0.12) × predicted k_d], R^2 = 0.862, and the root mean square error = 0.0050.

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 empiri-

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

Allen, M. S., and D. R. Mertens. 1988. Evaluation constraints of fiber digestion by rumen microbes. J. Nutr. 118:261–270.

Barry, T. N., F. J. Faichney, and C. Redekopp. 1985. Gastro-intestinal tract function in sheep infused with somatostatin. Aust. J. Biol. Sci. 38:393–403.

Bernard, L., J. P. Chaise, R. Baumont, and C. Poncet. 2000. The effect of physical form of orchardgrass hay on the passage of particulate matter through the rumen of sheep. J. Anim. Sci. 78:1338–1354.

Bibby, J., and H. Toutenburg. 1977. Prediction and Improved Estimation of Linear Models. John Wiley & Sons, London, UK.

Blümmel, M., and E. R. Ørskov. 1993. Comparison of in vitro gas production and nylon-bag degradability in predicting feed intake in cattle. Anim. Feed Sci. Technol. 40:109–119.

Bossen, D., D. M. Mertens, and M. R. Weisbjerg. 2005. Influence of fermentation method on NDF degradation parameters. J. Dairy Sci. 88(Suppl. 1):188. (Abstr.)

Chesson, A. S., S. Stewart, K. Dalgarno, and T. P. King. 1986. Degradation of isolated grass mesophyll, epidermis and fiber cell walls by cellulolytic bacteria in axenic culture. J. Appl. Bacteriol. 60:327–336.

Cone, J. W., A. H. Van Gelder, G. J. W. Visscher, and L. Oudshoorn. 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics estimated with a fully automated time related gas production apparatus. Anim. Feed Sci. Technol. 61:113–128.

Danfær, A., P. Huhtanen, P. Udén, J. Sveinbjörnsson, and H. Volden. 2006. Karoline – a Nordic cow model for feed evaluation – model description. Pages 407–415 in Nutrient Digestion and Utilization in Farm Animals: Modelling Approaches. E. Kebreab, J. Dijkstra, A. Bannink, J. Gerrits, and J. France, ed. CAB Int., Wallingford, UK.

Deaville, E. R., and D. I. Givens. 2001. Use of the automated gas production technique to determine the fermentation kinetics of carbohydrate fractions in maize silage. Anim. Feed Sci. Technol. 93:205–215.

Dhanoa, M. S., S. Lopez, J. Dijkstra, D. R. Davies, R. Sanderson, A. B. Williams, Z. Zileshi, and J. France. 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: Comparison of models. Br. J. Nutr. 83:131–142.

Dijkstra, J., J. A. N. Mills, and J. France. 2002. The role of modeling in understanding the microbial contribution to rumen functions. Nutr. Res. Rev. 15:67–90.

Doane, P. H., P. Schofield, and A. N. Pell. 1997. Neutral detergent fiber disappearance and gas and volatile fatty acid production during the in vitro fermentation of six forages. J. Anim. Sci. 75:3342–3352.

Ellis, W. C., J. H. Matis, T. M. Hill, and M. R. Murphy. 1994. Methodology for estimation digestion and passage kinetics of forages. Pages 682–756 in Forage Quality, Evaluation and Utilization. G. C. Fahey Jr., M. Collins, D. R. Mertens, and L. E. Moser, ed. Am. Soc. Agron., Madison, WI.

France, J., M. S. Dhanoa, M. K. Theodorou, S. J. Lister, D. R. Davies, and D. Isac. 1993. A model to interpret gas accumulation profiles associated with in vitro degradation of ruminant feeds. J. Theor. Biol. 163:99–111.

France, J., J. Dijkstra, M. S. Dhanoa, S. Lopez, and A. Bannink. 2000. Estimating the extent of degradation of ruminant feeds

from a description of their gas production profiles observed in vitro: Derivation of models and other mathematical considerations. Br. J. Nutr. 83:143–150.

García-Rodriquez, A., N. Mandaluniz, G. Flores, and L. M. Oregui. 2005. A gas production technique as a tool to predict organic matter digestibility of grass and maize silage. Anim. Feed Sci. Technol. 123–124:267–276.

Goering, K. H., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Applications). Agric. Handb. No. 379. ARS-USDA, Washington, DC.

Hall, M. B., A. N. Pell, and L. E. Chase. 1998. Characteristics of neutral detergent soluble fiber fermentation by mixed ruminal microbes. Anim. Feed Sci. Technol. 70:23–39.

Hetta, M., J. W. Cone, G. Bernes, A. M. Gustavsson, and K. Martinsson. 2007. Voluntary intake of grass silages in dairy cows depending on chemical composition and in vitro gas production characteristics. Livest. Sci. 106:47–56.

Huhtanen, P., S. Ahvenjärvi, M. R. Weisbjerg, and P. Nørgaard. 2006. Digestion and passage of fiber in ruminants. Pages 87–135 in Ruminant Physiology. K. Sejrsen, T. Hvelpund, and M. O. Nielsen, ed. Wageningen Acad. Publ., Wageningen, the Netherlands.

Huhtanen, P., U. Asikainen, M. Arkkila, and S. Jaakkola. 2007. Cell wall digestion and passage kinetics estimated by marker and in situ methods or by rumen evacuations in cattle fed hay 2 or 18 times daily. Anim. Feed Sci. Technol. 133:206–227.

Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Anim. Feed Sci. Technol. 48:211–227.

Huhtanen, P., A. Vanhatalo, and T. Varvikko. 1998. Enzyme activities of rumen particles and feed samples incubated in situ with differing types of cloth. Br. J. Nutr. 79:161–168.

Kennedy, P. M., J. B. Lowry, and L. L. Conlan. 1999. Isolation of grass cell walls as neutral detergent fiber increases their fermentability for rumen micro-organisms. J. Sci. Food Agric. 79:544–548.

Lund, P., M. R. Weisbjerg, and T. Hvelplund. 2006. Passage kinetics of dairy cows obtained from duodenal and faecal ytterbium excretion. Effect of forage type. Anim. Feed Sci. Technol. 128:229–252.

Menke, K. H., L. Raab, A. Salewski, H. Staingass, D. Fritz, and W. Schneider. 1979. The estimation of the digestibility and metabolizable energy contents of ruminant feedstuffs from the gas production when they are incubated with rumen liquor. J. Agric. Sci. 92:499–503.

Mertens, D. R. 1973. Application of theoretical mathematical models to cell wall digestion and forage intake in ruminants. PhD Thesis. Cornell Univ., Ithaca, NY.

Mertens, D. R. 1993. Kinetics of cell wall digestion and passage in ruminants. Pages 535–570 in Forage Cell Wall Structure and Digestibility. H. G. Jung, D. R. Buxton, R. D. Hatfield, and J. Ralph, ed. Am. Soc. Agron., Madison, WI.

Nousiainen, J., M. Rinne, M. Hellämäki, and P. Huhtanen. 2003a. Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsin-cellulase solubility. Anim. Feed Sci. Technol. 103:97–111.

Nousiainen, J., M. Rinne, M. Hellämäki, and P. Huhtanen. 2003b. Prediction of the digestibility of the primary growth and regrowth grass silages from chemical composition, pepsin-cellulase solubility and indigestible cell wall content. Anim. Feed Sci. Technol. 110:61–74.

Pitt, R. E., T. L. Cross, A. N. Pell, P. Schofield, and P. H. Doane. 1999. Use of in vitro gas production models in ruminal kinetics. Math. Biosci. 159:145–163.

Pond, K. R., W. C. Ellis, J. H. Matis, H. M. Ferreiro, and J. D. Sutton. 1988. Compartmental models for estimating attributes of digesta flow in cattle. Br. J. Nutr. 60:571–595.

Rinne, M., A. Olt, J. Nousiainen, A. Seppälä, M. Tuori, C. Paul, M. D. Fraser, and P. Huhtanen. 2006. Prediction of legume silage digestibility from various laboratory methods. Grass Forage Sci. 61:354–362.

Rymer, C., J. A. Huntington, B. A. Williams, and D. I. Givens. 2005. In vitro cumulative gas production techniques: History, methodological considerations and challenges. Anim. Feed Sci. Technol. 123–124:9–30.

Schofield, P., and A. N. Pell. 1995. Validity of using accumulated gas pressure readings to measure forage digestion in vitro: A comparison involving three forages. J. Dairy Sci. 78:2230–2238.

Schofield, P., R. E. Pitt, and A. N. Pell. 1994. Kinetics of fiber digestion from in vitro gas production. J. Anim. Sci. 72:2980–2991.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Van Soest, P. J., M. E. Van Amburgh, J. B. Robertson, and W. F. Knaus. 2005. Validation of the 2.4 times lignin factor for ultimate extent of NDF digestion, and curve peeling rate of fermentation curves into pools. Pages 139–149 in Proc. Cornell Nutr. Conf. Feed Manuf., East Syracuse, NY. Cornell University, Ithaca, NY.

Wilman, D., G. R. Foulkes, and I. D. Givens. 1996. A comparison of four methods estimating the rate and extent of cell wall degradation in silages. Anim. Feed Sci. Technol. 63:99–109.