

SYSTEMS ANALYSIS

Modeling Ruminant pH Fluctuations: Interactions Between Meal Frequency and Digestion Rate

R. E. PITT*¹ and A. N. PELL†

*Department of Agricultural and Biological Engineering and

†Department of Animal Science,
Cornell University, Ithaca, NY 14853

ABSTRACT

A steady periodic analysis of ruminal carbohydrate digestion was developed to predict the effects of diet and frequency of eating on ruminal pH fluctuation. Tests of the model against previous data showed that pH fluctuations were too large when previously published rates of carbohydrate digestion were used but were improved using rates from an *in vitro* gas production system, which were lower. With the original digestion rates, the minimum meal frequency to maintain steady-state conditions in the rumen increased from 4 to 12 meals/d as dietary effective neutral detergent fiber (NDF) decreased from 34 to 6% of dry matter (DM); with the revised rates, the minimum frequency was 3 to 6 meals/d, respectively. The minimum effective NDF to maintain a pH value above 6.0 increased from 14 to 23% of DM as meal frequency decreased from steady state to 2 meals/d using the original rates; with the revised rates, the minimum effective NDF was slightly smaller, increasing from 13 to 21% of DM, respectively. Effects of DM intake and body weight on pH fluctuation were minor, and dietary buffers, when used at rates less than 1%, did not reduce fluctuation. Different methods of calculating mean ruminal pH yielded different results for the effect of meal frequency on mean pH.

(**Key words:** ruminal pH, effective fiber, meal frequency, dietary buffers)

Abbreviation key: LR = low roughage used with 1 and 2 to indicate diet number, pK_d = dissociation constant.

INTRODUCTION

Frequency of eating affects ruminal and blood metabolites (31, 32), and less frequent meals may be

associated with lower milk fat concentration. This effect is related to fluctuations in ruminal production of VFA and is correlated with a larger fluctuation in ruminal pH as meal frequency decreases (20, 31). Predicting temporal variations in ruminal pH is a prerequisite to predicting acidosis and laminitis in dairy cattle (15). In continuous culture fermenters, fiber digestion rates decrease sharply when pH falls below 6.0 (12, 24, 27); however, the effects of short-term exposure to low pH on microbial digestion and yield are not well known.

A net carbohydrate and protein system was developed to use farm level information on cattle, intake, feed composition, and environment to evaluate animal performance (8, 17, 25). The model includes a ruminal submodel that predicts steady-state carbohydrate fermentation, protein digestion, microbial growth, passage, and production of VFA (19, 25, 28). In the model, intake, digestion, microbial growth, and passage are assumed to be constant and continuous in time. This assumption becomes less valid as meal frequency decreases (31), and, therefore, the model is limited to nearly ideal feeding practices.

The rate of carbohydrate digestion affects both ruminal digestibility in steady-state conditions and fluctuations in ruminal acidity between meals (20). Digestion rates in the net carbohydrate and protein system are typically 100 to 350%/h for sugars, fermentable organic acids, and short oligosaccharides and 10 to 50%/h for starch and pectic substances (28). A recent study (26) using the *in vitro* gas production system of Pell and Schofield (18) has indicated digestion rates that range from 3 to 21%/h for the neutral detergent soluble fraction of forages.

The purpose of this study was to develop a simple model to predict fluctuations in ruminal acidity and pH using the feed descriptions and inputs of the net carbohydrate and protein system and to test these predictions against literature data. A second objective was to examine the effects of meal frequency, carbohydrate digestion rates, dietary fiber concentration, and added buffers on the magnitude and rate of pH fluctuations.

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¹To whom correspondence should be addressed.

MATERIALS AND METHODS

Basic Assumptions

Of primary interest were the ruminal dynamics in the intervals between meals and the effect of meal frequency and diet on these dynamics. Because of the complexity of ruminal function, a number of simplifying assumptions were made. The time intervals between meals were assumed to be identical, and ruminal dynamics were assumed to be the same during each interval. Between meals, ruminal fermentation was assumed to operate as a semi-batch process, and carbohydrate digestion, passage, and acid absorption were assumed to occur continuously as first-order processes (19, 25, 28). This form of steady periodic analysis (9) gave criteria for initial conditions and yielded equations in closed form; therefore, numerical simulation was not needed. Subsequently, we discuss the limitations of the analysis.

The specific assumptions for the analysis were that 1) meals were of short duration relative to the intervals between meals, 2) all meals were identical in size and composition, 3) digestion and passage rate coefficients were constant between meals, and 4) the time interval between each meal was the same for all meals. The VFA were combined into a single pool without attempting to predict changes in VFA profiles between feedings. This simplification was made because of the difficulty in predicting VFA profiles (19) and because of the similarity in dissociation constants (pK_d) for the three principal VFA ($pK_d = 4.76$ for acetate, 4.87 for propionate, and 4.81 for butyrate). Ruminal absorption rate constants differed among the three VFA (19); therefore, a weighted mean for the absorption rate constant was used for the VFA pool. Lactate was treated as a separate pool because it has a substantially lower pK_d (3.86) and is metabolized to VFA by ruminal bacteria (5). A regression equation for saliva production (19) combined with a steady-state mass balance was used to predict buffering of ruminal fluid; saliva production was assumed to occur continuously both during feeding and between meals.

The basic method was to perform mass balances on ruminal carbohydrates, lactate, and VFA in which the rate of change of mass in the rumen equaled the difference between rates of production and disappearance. After solving these differential equations, we evaluated the constants of integration by imposing the steady periodic assumption.

Carbohydrate Fermentation

In the net carbohydrate and protein system (28), carbohydrates in feeds were divided into an A frac-

tion, representing sugars, fermentable organic acids, and short oligosaccharides; a B1 fraction, representing ruminally degradable starch and pectic substances; and a B2 fraction, representing digestible fiber. Digestion of carbohydrates produced microbial mass and fermentation end products including lactate and VFA for the A and B1 fractions but no lactate for the B2 fraction (19). In the present model, rates of disappearance of carbohydrates between meals were governed by digestion and passage, which were modeled as first-order processes. Therefore, the rates of change of A, B1, and B2 carbohydrates from each feed j in the rumen between meals were

$$\frac{dA_j}{dt} = -A_j(K_{pj} + K_{dj}^A), \quad [1]$$

$$\frac{dB1_j}{dt} = -B1_j(K_{pj} + K_{dj}^{B1}), \quad \text{and} \quad [2]$$

$$\frac{dB2_j}{dt} = -B2_j(K_{pj} + K_{dj}^{B2}) \quad [3]$$

where

A_j = amount of A carbohydrate in the rumen from feed j (grams),

$B1_j$ = amount of B1 carbohydrate in the rumen from feed j (grams),

$B2_j$ = amount of B2 carbohydrate in the rumen from feed j (grams),

t = time between meals (days),

K_{pj} = passage rate of feed j (per day), and

$K_{dj}^{A,B1,B2}$ = digestion rate of A, B1, or B2 carbohydrate, respectively, from feed j (per day).

Passage rates and carbohydrate digestion rates at normal ruminal pH were given by Sniffen et al. (28). Adjustment of digestion rate for reduced pH was described by Pitt et al. (19).

Solution of Equations [1] through [3] produced an exponential decay in ruminal carbohydrate masses through time; a single constant of integration was used for each carbohydrate fraction for each feed. The constants were evaluated by imposing the steady periodic assumption in which the disappearance of carbohydrate caused by digestion and passage between meals was equal to the intake of carbohydrate during a single meal. The following equations were obtained for the quantity of A, B1, and B2 carbohy-

drates in the rumen for each feed j as a function of time between meals:

$$A_j = \frac{\text{Intake}_{A_j} \times t_f}{1 - e^{-(K_{pj} + K_{dj}^A)t_f}} e^{-(K_{pj} + K_{dj}^A)t}, \quad 0 \leq t \leq t_f; \quad [4]$$

$$B1_j = \frac{\text{Intake}_{B1_j} \times t_f}{1 - e^{-(K_{pj} + K_{dj}^{B1})t_f}} e^{-(K_{pj} + K_{dj}^{B1})t}, \quad 0 \leq t \leq t_f; \quad [5]$$

and

$$B2_j = \frac{\text{Intake}_{B2_j} \times t_f}{1 - e^{-(K_{pj} + K_{dj}^{B2})t_f}} e^{-(K_{pj} + K_{dj}^{B2})t}, \quad 0 \leq t \leq t_f \quad [6]$$

where

Intake_{A,B1,B2j} = intake of A, B1, or B2 carbohydrate, respectively, from feed j (grams per day), and
 t_f = meal interval (days).

Lactate

Sources of ruminal lactate were the intake of fermented feeds and ruminal fermentation of A and B1 carbohydrates; disappearance of lactate was due to ruminal digestion of lactate, liquid passage from the rumen, and absorption through the ruminal epithelium. The rate of change of the amount of lactate in the rumen between meals was equal to the net sum of production and disappearance rates as follows:

$$\frac{dL}{dt} = \sum_{j=1}^{n\text{feeds}} \left[\begin{matrix} f_{\text{acid}}^A f_L^A (1 - Y_j^A) (K_{dj}^A) A_j + \\ f_{\text{acid}}^{B1} f_L^{B1} (1 - Y_j^{B1}) (K_{dj}^{B1}) B1_j \end{matrix} \right] - (K_{\text{abs,L}} + K_1 + K_d^L)L \quad [7]$$

where

- L = mass of lactate in the rumen (grams);
- nfeeds = number of feeds in the diet;
- $f_{\text{acid}}^{A,B1}$ = mass fraction of end products, which are acids from digestion of A or B1 carbohydrate, respectively (grams per gram);
- $f_L^{A,B1}$ = mass fraction of acid end products, which are lactate from digestion of A or B1 carbohydrate, respectively (grams per gram);
- $Y_j^{A,B1}$ = microbial yield on A or B1 carbohydrates from feed j (grams per gram);
- $K_{\text{abs,L}}$ = ruminal absorption rate constant of lactate (per day);

- K_1 = liquid passage rate constant (per day); and
- K_d^L = digestion rate constant of lactate (per day).

Liquid passage rate was predicted from DMI and BW (19). Ruminal absorption rate, digestion rate, and fraction of acids that are lactate were all functions of ruminal pH (19).

Equations [4] through [6] for carbohydrate masses in the rumen were substituted into Equation [7], which was then solved. Again, the steady periodic assumption was imposed to evaluate the constant of integration, in this case by requiring that the difference in lactate masses in the rumen at times 0 and t_f (immediately after and before a meal) be equal to the amount of lactate taken in from silages during a single meal. The result was

$$L = (C_1) e^{-(K_{\text{abs,L}} + K_1 + K_d^L)t} + \sum_{j=1}^{n\text{feeds}} \left\{ (C_{A_j}) e^{-(K_{dj}^A + K_{pj})t} + (C_{B1_j}) e^{-(K_{dj}^{B1} + K_{pj})t} \right\}, \quad 0 \leq t \leq t_f \quad [8]$$

The coefficients in Equation [8] were

$$C_{A_j} = \frac{(1 - Y_j^A)(K_{dj}^A)(A_{0j})(f_{\text{acid}}^A)(f_L^A)}{K_{\text{abs,L}} + K_1 + K_d^L - K_{dj}^A - K_{pj}}, \quad [9]$$

$$C_{B1_j} = \frac{(1 - Y_j^{B1})(K_{dj}^{B1})(B1_{0j})(f_{\text{acid}}^{B1})(f_L^{B1})}{(K_{\text{abs,L}} + K_1 + K_d^L - K_{dj}^{B1} - K_{pj})}, \quad \text{and} \quad [10]$$

$$C_1 = \frac{\text{Intake}_L \times t_f - \sum_{j=1}^{n\text{feeds}} \left[(C_{A_j}) (1 - e^{-(K_{dj}^A + K_{pj})t_f}) + (C_{B1_j}) (1 - e^{-(K_{dj}^{B1} + K_{pj})t_f}) \right]}{1 - e^{-(K_{\text{abs,L}} + K_1 + K_d^L)t_f}} \quad [11]$$

where

- Intake_L = intake of lactate from all fermented feeds (grams per day),
- A_{0j} = $A_j(0)$ = mass of A carbohydrate in the rumen at time 0 after feeding (grams), and

$B1_{0j} = B1_j(0) =$ mass of B1 carbohydrate in the rumen at time 0 after feeding, (grams).

$$f_{VFA,u} = 1/[1 + 10^{(pH - pK_{VFA})}] \quad [14]$$

where

- A_{Ru} = ruminal surface area (square meter),
- V_{Ru} = ruminal liquid volume (cubic meter),
- $K_{u,VFA}$ = absorption rate constant for undissociated VFA (meters per day),
- $K_{d,VFA}$ = absorption rate constant for dissociated VFA (meters per day),
- $f_{VFA,u}$ = fraction of VFA in undissociated form, and
- $pK_{VFA} = 4.80$.

VFA

Sources of ruminal VFA were the intake of fermented feeds, digestion of carbohydrates, and digestion of lactate. Disappearance of VFA was due to liquid passage and ruminal absorption. The rate of change in VFA in the rumen was the net sum of production and disappearance rates between meals, yielding

$$\begin{aligned} \frac{d(VFA)}{dt} = & \frac{K_d^L(1 - Y^L)}{1 + \alpha f_A^L} L - (K_{abs,VFA} + K_1)(VFA) \\ & + \sum_{j=1}^{nfeeds} [f_{acid}^A(1 - Y_j^A)(1 - f_L^A)(K_{dj}^A)A_j \\ & + f_{acid}^{B1}(1 - Y_j^{B1})(1 - f_L^{B1})(K_{dj}^{B1})B1_j \\ & + f_{acid}^{B2}(1 - Y_j^{B2})(K_{dj}^{B2})B2_j] \end{aligned} \quad [12]$$

where

VFA = total mass of VFA in the rumen (grams);

α = mass of CO₂ per mass of acetate produced from lactate digestion (grams per gram = 0.733 g/g);

f_{acid}^{B2} = mass fraction of end products, which are acids from digestion of B2 carbohydrate (grams per gram);

Y_j^{B2} = microbial yield on B2 carbohydrate from feed j (grams per gram);

Y^L = microbial yield on lactate (grams per gram);

f_A^L = mass fraction of acetate produced from digestion of lactate (grams per gram); and

$K_{abs,VFA}$ = absorption rate constant for VFA (per day).

Calculation of microbial yield and mass fractions as functions of pH was described by Pitt et al. (19). The absorption rate constants for dissociated and undissociated VFA were computed as a weighted mean of the constants for acetate, propionate, and butyrate (19); the weighting factors used were the steady-state mass fractions of individual VFA in the rumen (19). The overall absorption constant as a function of ruminal pH was then

$$K_{abs,VFA} = \frac{A_{Ru}}{V_{Ru}} [K_{u,VFA}f_{VFA,u} + K_{d,VFA} (1 - f_{VFA,u})] \quad [13]$$

Ruminal volume was a function of BW, and ruminal surface area was a function of ruminal volume and dietary NDF concentration (19).

Equations [4] to [6] and [8] to [11] for carbohydrate and lactate masses in the rumen were substituted into Equation [12], which was then solved. The constant of integration was evaluated by setting the difference in ruminal VFA masses at times 0 and t_f equal to the VFA intake from fermented feeds per meal. The resulting equation for VFA mass in the rumen was

$$\begin{aligned} VFA = & \sum_{j=1}^{nfeeds} \left[(D_{Aj})e^{-(K_{dj}^A + K_{pj})t} \right. \\ & + (D_{B1j})e^{-(K_{dj}^{B1} + K_{pj})t} \\ & \left. + (D_{B2j})e^{-(K_{dj}^{B2} + K_{pj})t} \right] \\ & + (D_1)e^{-(K_{abs,VFA} + K_1)t} \\ & + (D_L)e^{-(K_{abs,L} + K_1 + K_d^L)t}, \quad 0 \leq t \leq t_f \end{aligned} \quad [15]$$

The coefficients in Equation [15] were

$$D_{Aj} = \frac{f_{acid}^A(1 - Y_j^A)(1 - f_L^A)(K_{dj}^A)A_{0j} + \frac{K_d^L(1 - Y^L)}{1 + \alpha f_A^L} C_{Aj}}{K_{abs,VFA} + K_1 - K_{dj}^A - K_{pj}}, \quad [16]$$

$$D_{B1j} = \frac{f_{acid}^{B1}(1 - Y_j^{B1})(1 - f_L^{B1})(K_{dj}^{B1})B1_{0j} + \frac{K_d^L(1 - Y^L)}{1 + \alpha f_A^L} C_{B1j}}{K_{abs,VFA} + K_1 - K_{dj}^{B1} - K_{pj}}, \quad [17]$$

$$D_{B2j} = \frac{f_{acid}^{B2}(1 - Y_j^{B2})(K_{dj}^{B2})B2_{0j}}{K_{abs,VFA} + K_1 - K_{dj}^{B2} - K_{pj}}, \quad [18]$$

$$D_L = \frac{\frac{K_d^L(1 - Y^L)}{1 + \alpha f_A^L} C_1}{K_{abs,VFA} - K_{abs,L} - K_d^L}, \quad \text{and} \quad [19]$$

$$D_1 = \left\{ \text{Intake}_{\text{VFA}} \times t_f - \sum_{j=1}^{\text{nfeeds}} \left[\left(D_{Aj} \right) \left(1 - e^{-(K_{dj}^A + K_{pj})t_f} \right) + \left(D_{B1j} \right) \left(1 - e^{-(K_{dj}^{B1} + K_{pj})t_f} \right) - \sum_{j=1}^{\text{nfeeds}} \left(D_{B2j} \right) \left(1 - e^{-(K_{dj}^{B2} + K_{pj})t_f} \right) - \left(D_L \right) \left(1 - e^{-(K_{\text{abs,L}} + K_1 + K_d^L)t_f} \right) \right] \right\} \times \left\{ \frac{1}{1 - e^{-(K_{\text{abs,VFA}} + K_1)t_f}} \right\} \quad [20]$$

where

$$B_{20j} = B_{2j}(0) = \text{mass of B2 carbohydrate in the rumen at time 0 after feeding (grams) and} \\ \text{Intake}_{\text{VFA}} = \text{intake of VFA from fermented feeds (grams per day).}$$

pH Change

Equations [4] to [6], [8] to [11], and [13] to [20] were used to predict ruminal lactate and VFA as a function of time between meals. The pH change associated with this fluctuation in acidity was calculated as follows. Pitt et al. (19) modeled the buffering capacity of ruminal fluid from saliva production and the buffering capacity of saliva. Saliva production was calculated from DMI and effective NDF (19):

$$D_s = -177.2 + 0.01638(\text{DMI}) + 4.706(\text{eNDF}) \quad [21]$$

where

$$D_s = \text{saliva production (liters per day) and} \\ \text{eNDF} = \text{effective NDF as a percentage of dietary DM.}$$

The DMI was expressed in grams per day. Effective NDF was determined from the coefficients given by Sniffen et al. (28). The ruminal concentration of saliva was obtained from a mass balance yielding

$$C_s = \frac{D_s}{V_{\text{Ru}} K_1} \quad [22]$$

where C_s = saliva concentration (liters per liter). The buffering capacity of saliva as a function of pH was given by Pitt et al. (19). The buffering capacity of ruminal fluid was the product of saliva concentration and saliva buffering capacity.

To calculate change in pH, the slope of the relationship between buffering and pH (the buffer index) was needed. Using the equation for the buffering capacity of saliva (19) and taking the derivative yielded

$$\frac{d(\text{BC}_1)}{d(\text{pH})} = -C_s \frac{381.8 \times 10^{(\text{pH} - 6.4)}}{[1 + 10^{(\text{pH} - 6.4)}]^2} \quad [23]$$

where BC_1 = buffering capacity of ruminal liquid (milliequivalents per liter). The change in acidity associated with fluctuations in ruminal lactate and VFA masses was calculated relative to the steady-state ruminal masses of these acids obtained from the equations of Pitt et al. (19):

$$\Delta C_{\text{VFA}} = \frac{\text{VFA} - \text{VFA}_{\text{ss}}}{62V_{\text{Ru}}} 1000, \quad [24]$$

$$\Delta C_L = \frac{L - L_{\text{ss}}}{90V_{\text{Ru}}} 1000, \quad [25]$$

$$\Delta C_{\text{acid}} = \Delta C_{\text{VFA}}(1 - f_{\text{VFA,u}}) + \Delta C_L(1 - f_{\text{Lu}}), \text{ and} \quad [26]$$

$$f_{\text{Lu}} = 1/(1 + 10^{(\text{pH} - 3.86)}) \quad [27]$$

where

ΔC_{VFA} = deviation in ruminal VFA concentration from the steady state (millimoles per liter),

ΔC_L = deviation in ruminal lactate concentration from the steady state (millimoles per liter),

ΔC_{acid} = deviation in ruminal acidity from the steady state (milliequivalents per liter),

f_{Lu} = fraction of lactate in undissociated form, VFA_{ss} = steady-state VFA mass in the rumen (grams), and

L_{ss} = steady-state lactate mass in the rumen (grams).

Finally, fluctuations in ruminal pH were calculated from the deviation in ruminal acidity from the steady state and from the ruminal buffer index:

$$\text{pH} = \text{pH}_{\text{ss}} + \frac{\Delta C_{\text{acid}}}{d(\text{BC}_1)/d(\text{pH})}. \quad [28]$$

TABLE 1. Dietary characteristics in the studies used to test the model and predict ruminal pH fluctuations.

Study and diet	NDF	CP	Effective NDF ¹	DMI
Sutton et al. (31)				
Control	40.0	13.7	25.2	16.3
LR1 ²	24.2	13.1	11.6	12.0
LR2	34.2	16.4	14.4	15.5
Robinson and McQueen (22)				
Slow ³	28.5	15.7	21.6	25.3
Fast	28.3	15.6	21.2	25.6
Stokes et al. (29)				
Control	25.1	18.7	15.9	19.1
Added buffer ⁴	24.8	18.8	16.1	18.9

¹Calculated using values of Sniffen et al. (28).

²Low roughage diets 1 and 2.

³Rate of ruminal degradation of protein.

⁴0.7% NaHCO₃.

Steady-state pH, when not known, was calculated from effective NDF (19).

RESULTS

Model Comparisons

Temporal pH fluctuations between meals were compared with results from studies by Sutton et al. (31) and Robinson and McQueen (22) in which diets, meal frequency, and frequent pH measurements were reported. Table 1 summarizes the diets and DMI for those studies.

For each study, two sets of predictions were generated: one using the original digestion rates in the net carbohydrate and protein system (28) and one using revised rates independently obtained from the in vitro gas production system, chemical fractionation, and data analysis procedures of Hall et al. (11), Pell and Schofield (18), and Schofield and Pell (26). Table 2 gives the original rates and a representative set of revised rates obtained from digestion experiments with alfalfa, grass, corn gluten meal, wheat grain, and high moisture ear corn; values for other feeds were approximated from these rates. Differences between original and revised rates were minor for the B2 carbohydrate (digestible fiber), but revised rates

TABLE 2. Original and revised digestion rates for A, B1, and B2 carbohydrates at normal ruminal pH and solid passage rates for dietary ingredients in the evaluation studies.

Diet and ingredient	A		B1		B2		Passage rate
	Original	Revised	Original	Revised	Original	Revised	
	(%/h)						
Sutton et al. (31)							
Grass hay	250	15	30	8	3	5	3
Barley grain	300	20	30	18	5	9	5
Soybean meal	300	17	35	17	4	11.5	5
Flaked corn	175	40	25	10	7	7	4
Robinson and McQueen (22)							
Haylage	250	18.5	25	10.8	5.5	5.4	4
Barley	300	20	30	18	5	9	7
Corn grain	150	40	15	10	5	7	7
Soybean meal	300	17	25	17	6	11.5	8
Corn gluten meal	300	17.3	50	17.3	5	3	8
Stokes et al. (29)							
Haylage	250	15	25	8	5.5	5	4
Corn grain	150	40	15	10	5	7	6
Soybean meal	300	17	25	17	6	11.5	8
Ground oats	300	20	35	18	3	9	5
Potato meal	175	40	25	10	7	7	6

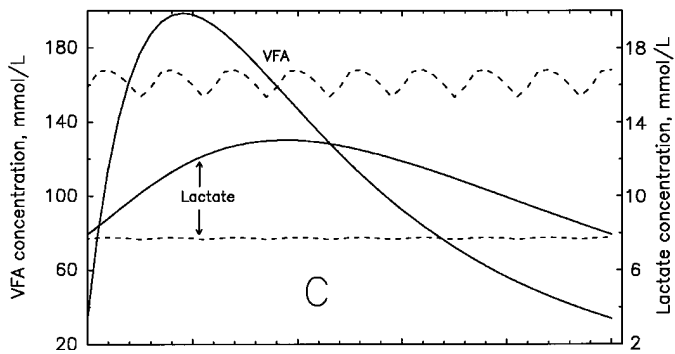
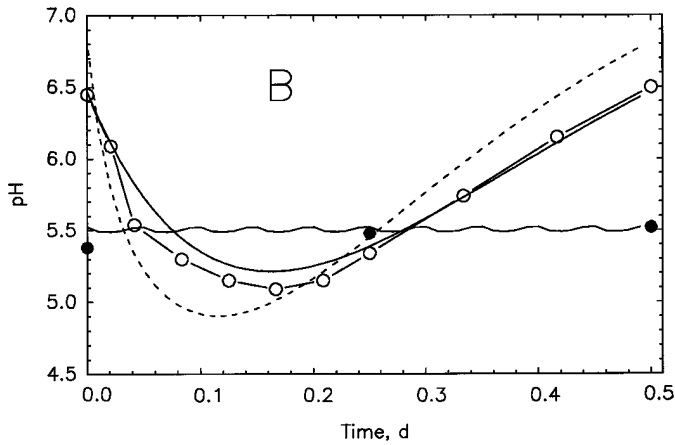
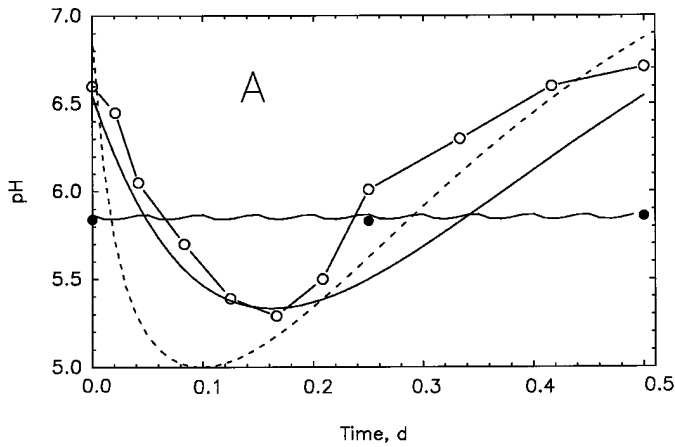


Figure 1. Observed pH fluctuations with meal frequencies of 2 (○) and 24 (●) meals/d from Sutton et al. (31) for the control (A) and a low roughage diet 2 (LR2) (B); pH fluctuations were predicted by the model using original (----) and revised (—) rates of carbohydrate digestion. Predicted fluctuations in ruminal VFA and lactate concentrations are shown for diet LR2 (C) using meal frequencies of 24 (----) and 2 (—) meals/d.

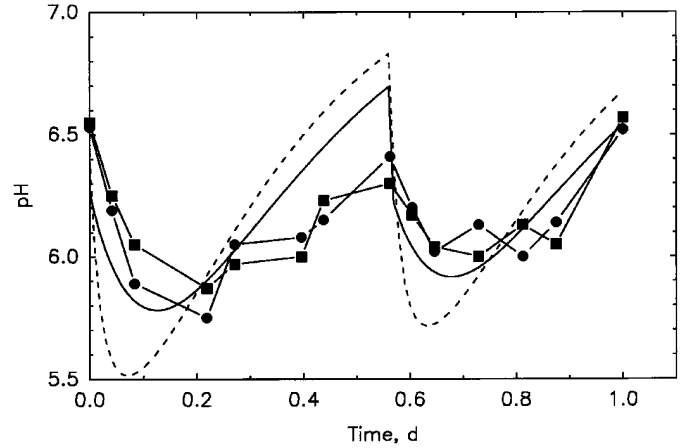


Figure 2. Observed pH fluctuations for slowly (■) and rapidly (●) degraded protein sources from Robinson and McQueen (22); fluctuations predicted by the model were calculated using original (----) and revised (—) rates of carbohydrate digestion.

for the A fraction (sugars, fermentable organic acids, and short oligosaccharides) were greatly reduced. The discrepancy was intermediate for the B1 carbohydrate (starch and pectic substances).

Figure 1, A and B, shows the pH data from the study of Sutton et al. (31) and the model projections for a control and a low roughage (LR) diet (LR2), respectively. A feeding frequency of 24 meals/d was predicted to be essentially steady state, and the three data points indicated little variation. For cows fed 2 meals/d, the pH fluctuations predicted by the model using the original rates were substantially larger than those of the data and showed a rate of decline that was too rapid. When the revised digestion rates were used, the predicted fluctuations were more accurate, exhibiting a smaller overall change, a slower initial drop after feeding, and a longer time for pH to reach a minimum. The observed time of minimum pH (approximately 0.16 d) was reasonably predicted when the revised digestion rates were used. For cows fed diet LR1 [temporal data were not published (31)], the mean pH fluctuation was 1.60; the model predicted a fluctuation of 2.53 with the original digestion rates and 1.58 with the revised rates.

Figure 2 shows predicted and observed ruminal pH fluctuations for two diets of Robinson and McQueen (22). Because the 2 daily feedings were not evenly spaced, the model was run with a meal frequency of 1.78 meals/d for the longer time interval, and 2.29 meals/d for the shorter time interval. With the original digestion rates, the model again predicted a pH fluctuation that was too large and rapid after feeding, but the accuracy was improved with the revised rates. For the longer time interval, the model predicted a

greater rise in pH before the next feeding than was shown in the data; this result was probably caused by the unequal time intervals between feedings, which was not consistent with the steady periodic assumptions.

The shape of the pH time course between meals was directly related to predicted acid concentrations. Figure 1C shows the concentrations of lactate and VFA for the two feeding frequencies using the revised digestion rates for diet LR2 in the study of Sutton et al. (31). Lactate is a stronger acid than the VFA; however, because the concentration of VFA was so much larger than that of lactate, the pH curve essentially mirrored the VFA curve (Figure 1B).

Effects of Meal Frequency and Effective NDF

The control and LR1 diets from Sutton et al. (31) (Table 1) were used to study the effects of meal frequency and effective NDF on ruminal pH fluctuation. To vary effective NDF in the model, the dietary NDF content and the effectiveness of NDF as a percentage of dietary NDF were adjusted for all four dietary ingredients listed in Table 2. Alteration of effective NDF had several effects on the model: mean ruminal pH, saliva production rate, ruminal buffering, fiber digestion rates, and ruminal absorption rates of acids were all directly or indirectly related to effective NDF.

Figure 3, A and B, shows the range of pH fluctuation as a function of meal frequency, effective NDF, diet, and carbohydrate digestion rate. The plots are on a log-log scale because the pH fluctuations varied so widely. Fluctuation in pH increased as meal frequency or effective NDF decreased. As shown earlier, the predictions were influenced by whether original or revised digestion rates were used. For example, the pH fluctuations reported by Robinson and McQueen (21) were 0.35 units for a diet with approximately 30% effective NDF; Figure 3A predicts a fluctuation of 0.55 units with the original digestion rates and 0.40 units with the revised rates.

Figure 3 can be used to predict the meal frequency required to maintain ruminal steady state. For example, if it is assumed that steady state is equivalent to a pH fluctuation of less than 0.2 units, then for the control diet with original digestion rates (Figure 3A), the meal frequency would have to be at least 4 meals/d with an effective NDF of 34% of DM and at least 12 meals/d with an effective NDF of 6% of DM. With the revised rates, the minimum meal frequencies were lower: at least 3 and 5.5 meals/d, respectively. For diet LR1 from Sutton et al. (31), pH fluctuations were higher at a given effective NDF, meal frequency, and digestion rate (Figure 3B). For example, at 6%

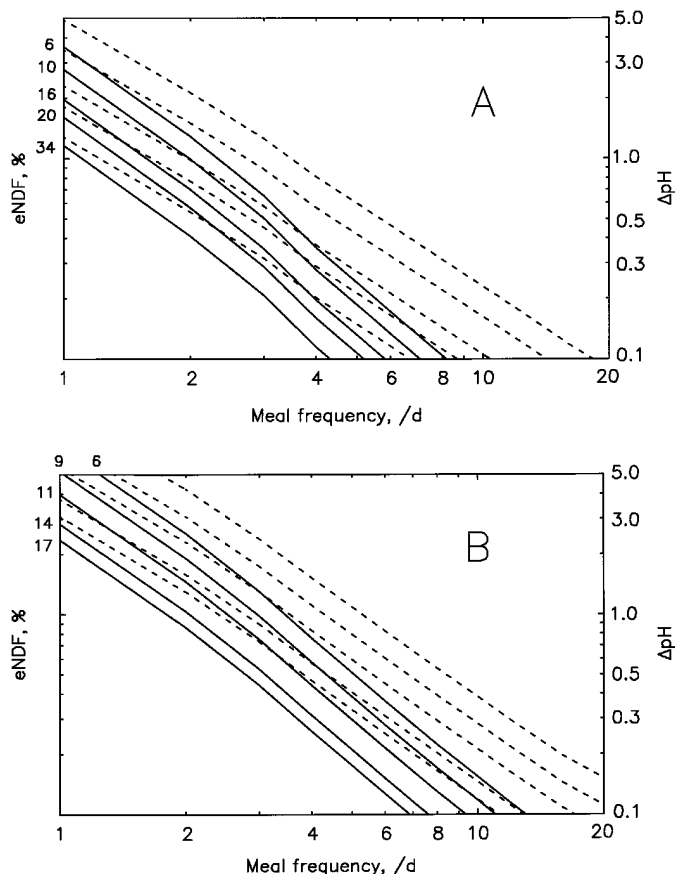


Figure 3. Magnitude of predicted pH fluctuation (ΔpH) as a function of meal frequency and effective NDF (eNDF) for the control diet (A) and low roughage diet 1 (B) from Sutton et al. (31) using original (---) and revised (—) rates of carbohydrate digestion.

effective NDF, the minimum meal frequency with the revised rates was 5.5 meals/d with the control diet and 8.5 meals/d with diet LR1.

Another view of these results was obtained by plotting minimum, maximum, and mean ruminal pH versus effective NDF. Figure 4A shows the limits of ruminal pH for the control diet (31) with original digestion rates; when the revised rates were used (Figure 4B), the limits were closer to mean pH. If it is assumed that ruminal pH should be maintained above a set level [e.g., 6.0 (2)], the minimum effective NDF requirement can be read from these graphs along a horizontal line at pH 6.0. With the original rates (Figure 4A), effective NDF would have to exceed 23% of DM with 2 meals/d, 18% of DM with 4 meals/d, and 14% of DM with steady-state feeding. With the new rates, the corresponding effective NDF requirements were slightly less restrictive: 21, 16, and 13% of DM, respectively. The results were also

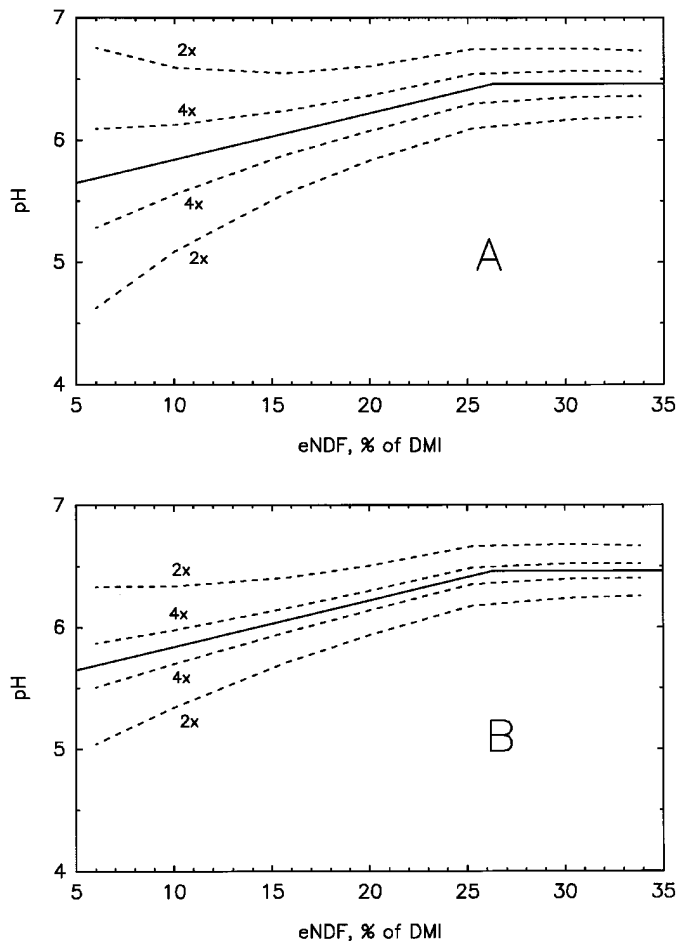


Figure 4. Predicted mean ruminal pH (—) and limits of predicted pH fluctuations (---) for various meal frequencies versus effective NDF (eNDF) using the control diet from Sutton et al. (31) with original (A) and revised (B) rates of carbohydrate digestion.

dependent on diet. For the LR1 diet (31), the minimum effective NDF using revised digestion rates with 4 meals/d was slightly higher (17 vs. 16% of DM).

Overall, the model predicted an interaction among meal frequency, effective NDF, and carbohydrate digestion rate. The minimum meal frequency needed to maintain ruminal steady state increased as effective NDF decreased. Conversely, the minimum effective NDF needed to maintain ruminal pH above a set level increased as meal frequency decreased. Use of the revised digestion rates made these requirements less stringent.

Effects of DMI and BW

Two variables that affected the predicted ruminal pH fluctuation were DMI and BW. Changing DMI

affected the total rate of carbohydrate digestion, solid and liquid passage rates, and saliva production. Table 3 shows the effect of a 20% variation in DMI on the range of pH fluctuation (difference between minimum and maximum pH between meals). The control and LR1 diets from Sutton et al. (31) were used with revised digestion rates (Table 2). For the control diet, DMI had essentially no effect on pH fluctuation. For the LR1 diet, higher DMI was associated with a slightly smaller pH fluctuation. Thus, DMI was less of a factor in governing pH fluctuation than was meal frequency, digestion rate, or effective NDF.

Changing BW in the model affected ruminal volume (19), which in turn influenced acid concentrations and ruminal absorption rate because of changes in the surface to volume ratio (Equation [13]). Solid and liquid passage rates were also affected (28). Table 4 shows the effects of a 10% variation in BW. Larger BW resulted in slightly smaller pH fluctuations. However, the effect of BW on pH fluctuation was minor compared with the effects of meal frequency and diet.

Effect of Dietary Buffers

The effect of buffer addition on ruminal pH fluctuation was studied using data from dairy cows in the experiment of Stokes et al. (29). Dietary characteristics and digestion rates are given in Tables 1 and 2, respectively. With the addition of 0.7% NaHCO_3 , ruminal volume increased from 56.0 to 60.4 L, liquid passage rate increased from 17.1 to 18.3%/h, and mean ruminal pH increased from 5.77 to 5.81. The implementation of these changes in the model had virtually no effect on ruminal pH fluctuation. When the buffering associated with the NaHCO_3 concentration in the rumen, predicted using a mass balance, was added to the buffering capacity of ruminal liquid, the effect was still small (Table 5). Increasing the level of supplementation to 1.4 or 5.0%, assuming no further changes in ruminal volume, liquid passage rate, or mean ruminal pH, reduced the pH fluctuation more substantially (Table 5).

Effect of Method of Calculating Mean Ruminal pH

Mean ruminal pH is affected by method of calculation. Use of logarithmic pH values instead of hydrogen ion concentrations affects mean pH (14). Another factor is how mean pH is calculated from temporal data when the sampling times are unevenly spaced. Using the present model, three different methods of calculating mean pH were compared. The simplest method (method 1) was to average the minimum and maximum values. Method 2 was to average all read-

TABLE 3. Predicted ruminal pH fluctuations as affected by DMI, diet, and meal frequency using revised digestion rates.¹

Meal frequency (/d)	Control diet			LR1 ²		
	0.8 DMI	DMI	1.2 DMI	0.8 DMI	DMI	1.2 DMI
2	0.58	0.57	0.58	3.13	1.69	1.37
4	0.16	0.16	0.16	0.94	0.51	0.42
8	0.05	0.05	0.05	0.12	0.15	0.12
12	0.02	0.02	0.02	0.06	0.08	0.06
24	0.01	0.01	0.01	0.02	0.03	0.02

¹Observed DMI are presented in Table 1.

²Low roughage diet 1 from Sutton et al. (31).

ings, ignoring the fact that the intervals between measurements may be variable. Method 3, which was most correct mathematically, was to find the area under the pH versus time curve and divide by the total time period. For diet LR1 from Sutton et al. (31) with 2 meals/d, method 1 yielded a mean pH of 5.8, and methods 2 and 3 yielded slightly lower values (5.65 and 5.68, respectively).

Figure 5 shows mean pH as a function of meal frequency for the control diet of Stokes et al. (29). Method 3 yielded a nearly constant mean that was equal to the steady-state pH in the model. Method 1 was an unreliable estimator of mean pH when meal frequency was less than 8 meals/d. Thus, averaging minimum and maximum pH could either overestimate or underestimate true mean pH, depending on meal frequency and magnitude of pH fluctuation.

DISCUSSION

The model predicted an interactive effect among meal frequency, effective fiber, and carbohydrate digestion rate on the magnitude and rate of pH fluctuations. Lower meal frequencies in the model in-

creased the quantity of feed consumed per meal, resulting in a greater release of fermentation end products between meals and a larger fluctuation in ruminal pH. Higher digestion rates had a similar effect, and, thus, an increase in meal frequency would mathematically offset an increase in digestion rates. Effective fiber has been defined as the fraction of NDF in a feed that contributes to rumination and buffering and accounts for particle size effects on the usage of fiber by ruminants (1, 28). Total effective NDF of a diet is a consequence of both the NDF concentration in the diet and the effectiveness of that NDF (1). Reduction in dietary effective NDF in the model, either because of a reduction in dietary NDF or particle size, had an inhibitory effect on saliva production and ruminal buffering; thus, higher meal frequencies or lower digestion rates were predicted to offset a low effective NDF. To maintain steady-state conditions in the rumen, higher meal frequency was required when effective NDF was lowered.

The revised digestion rates of Table 2 are considered to be more accurate than the original rates because the revised rates were obtained from digestion experiments (18, 26) independently of the modeling in this paper. In comparisons with litera-

TABLE 4. Predicted effect of BW on ruminal pH fluctuations using revised digestion rates.¹

Meal frequency (/d)	Control diet		LR1 ²	
	0.9 BW	1.1 BW	0.9 BW	1.1 BW
2	0.60	0.54	1.77	1.63
4	0.17	0.15	0.54	0.49
8	0.05	0.04	0.16	0.14
12	0.02	0.02	0.08	0.07
24	0.01	0.01	0.03	0.03

¹Observed BW was 550 kg.

²Low roughage diet 1 from Sutton et al. (31).

TABLE 5. Effect of added NaHCO₃ at three concentrations on predicted pH fluctuation using revised digestion rates presented in Table 2.

Meal frequency (/d)	Control	0.7% ¹	1.4%	5.0%
2	1.66	1.62	1.55	1.05
4	0.67	0.66	0.65	0.44
8	0.31	0.30	0.30	0.20
12	0.21	0.21	0.21	0.14
24	0.12	0.12	0.12	0.08

¹Diet and level of supplementation used by Stokes et al. (29).

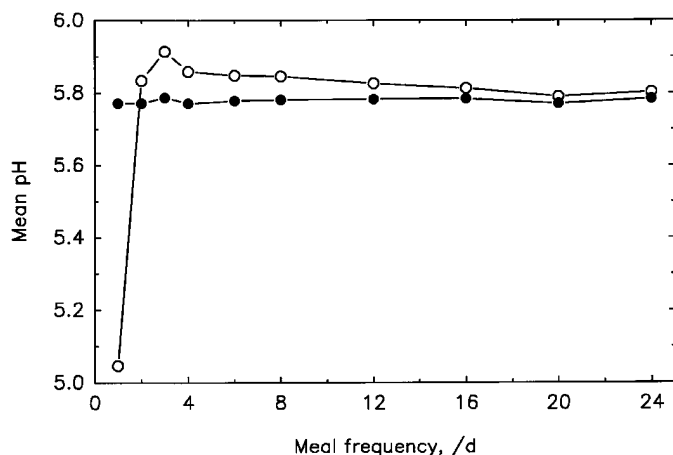


Figure 5. Effect of meal frequency on mean ruminal pH calculated by two methods: average of minimum and maximum values (○) and area under the curve (●).

ture data, the revised rates produced a better prediction of the magnitude and rate of pH fluctuations between feedings. However, the variability in ruminal pH measurements, which would be obtained by replication of ruminal fluid sampling, has not been reported. Therefore, it is difficult to assess in statistical terms how close our predictions should be to the actual data. Ruminal pH measurements depend on site of sampling within the rumen (36), a factor that also contributes uncertainty to our tests of the model.

Use of revised digestion rates decreased the required effective NDF in the diet by 1 to 2 percentage points. However, given the statistical uncertainty about ruminal pH, the unknown sensitivity of ruminal bacteria that digest fiber to temporary exposure to low pH conditions, and the lack of consensus about methods to measure effective NDF in the field, a recommendation to reduce effective NDF from that of current guidelines would probably not be advisable.

Although the revised digestion rates had a major effect on ruminal pH predictions with low meal frequencies, the effect of digestion rate was minimal in steady state or with frequent feeding. For example, with the control diet of Sutton et al. (31), the steady-state concentration of VFA was predicted to be 111 mmol/L using the original rates and 106 mmol/L using the revised rates. The actual concentration was 110 mmol/L with 24 meals/d. Ruminal degradation of carbohydrates was predicted to be 5925 g/d with the original rates and 5890 g/d with the revised rates. The minimal effect of digestion rate in steady state can be explained by the fraction $K_d/(K_d + K_p)$, which is the fraction of feed carbohydrate ruminally

digested. For the A and B1 carbohydrates, digestion rates were much larger than passage rates (Table 2); thus, the fraction was largely unaffected by the changes in digestion rate. For example, the fraction of A carbohydrate digested in flaked corn was 0.98 with the original digestion rate and 0.91 with the revised rate. For the B2 carbohydrate, for which digestion and passage rates were of comparable magnitude, the original and revised rates were similar (Table 2).

The trends regarding meal frequency in the model are in general agreement with the literature. Robinson (20) found that increased feeding frequency reduced diurnal variations in ruminal metabolites including VFA and lactate. Inclusion of more slowly fermented carbohydrates were noted to decrease fluctuations (20), which was equivalent to decreasing the carbohydrate digestion rates in the current model. Robinson (20) suggested that diets containing high fiber and low concentrate would be insensitive to increased feeding frequency. Figure 3 provides theoretical support for that hypothesis. For example, if effective NDF is 20% of DM, meal frequencies greater than 4 meals/d are predicted to result in steady-state conditions in the rumen (Figure 3A); thus, increasing meal frequency from 4 to 24 meals/d would have little additional effect on the constancy of ruminal function.

The implications of reduced fluctuations in ruminal acidity and pH are unclear. Sutton et al. (30) showed that increased feeding frequency tended to ameliorate milk fat depression in dairy cows, but the actual effects on molar proportions of individual VFA were small or inconsistent (31). The present model gives no insight into the relationship, if any, between VFA profiles and meal frequency, because no attempt was made to predict these profiles. Possible effects of meal frequency on DMI (20) also were not included in the model. In a study with sheep, Charmley et al. (4) found that increased feeding frequency had no effect on DMI or digestibility of DM. Although the model can predict time spent below a given pH (6), actual effects on fiber digestion and microbial yield are not well known outside of steady-state conditions (2).

The steady periodic assumption underpinning this analysis limits the application of the model. Robinson and McQueen (22) examined the effect of synchronizing protein and energy sources on ruminal fermentation. In the steady periodic analysis, all feeds were required to be fed at the same time; thus, the present model provides no avenue to examine questions about synchronization. The requirement that all meals be identical is counter to the data of Dado and Allen (6) in which cows averaging 11.6 meals/d consumed 26 to 31% of the DMI in the single largest meal.

Inherent in the steady periodic analysis was that solid and liquid passage rates, ruminal volumes, and saliva production remained constant over time. Charmley et al. (4) observed no effect of feeding frequency on overall passage rates or ruminal volumes of sheep; however, diurnal variations were not studied. Solid passage rates and saliva production rate are likely to deviate from constancy as frequency of eating decreases, because particle size reduction occurs during and between feedings (20). Beauchemin (1) found that saliva production was two to four times greater during feeding and rumination than during resting. Another problem is that solid passage rates used in the net carbohydrate and protein system were intended to describe steady-state conditions; however, mean passage rates may vary when frequency of eating decreases. The present model neglects such effects.

Dietary buffers have been shown to increase mean ruminal pH, liquid passage rate, and ruminal liquid volume (29). In the present study, use of added buffers at concentrations less than 1% of dietary DM were predicted to have little effect on ruminal pH fluctuations, but addition of buffers at much higher rates compensated for lower meal frequencies by damping ruminal pH fluctuations. Erdman (7) cited several studies in which dietary buffers increased the minimum pH observed at 4 to 8 h after feeding. West et al. (35) observed pH fluctuations of 0.4 units without buffer and 0.3 units with 2% buffer added to a diet with 20% effective NDF fed twice daily. Ghorbani et al. (10) measured pH fluctuations of 0.53 units with twice daily feeding, with or without addition of 1% NaHCO₃; with an effective NDF of 18% (estimated from tabular values), the present model predicts a fluctuation of 0.65 units (Figure 3) with or without the buffer (Table 5).

We presumed in this study that the mechanism by which ruminal buffers function was to increase the buffering capacity and buffer index of ruminal fluid. Le Ruyet and Tucker (13) detected changes in the titration curves of ruminal fluid when incubated with added buffer. However, Russell and Chow (23) proposed that ruminal buffers act mainly by increasing liquid passage rate, a mechanism we did not model for levels greater than 0.7% (29, 33).

In this study, meal frequency refers to the frequency with which cows actually consume feed, not to the frequency with which cows are presented with fresh feed. Numerous studies (20) have attested to the uncoupling of these two variables. Cassida and Stokes (3) provided 4 meals/d to lactating dairy cows but found that the cows actually consumed approxi-

mately 10 meals/d. In two other studies with twice daily feeding (6, 34), cows consumed a mean of approximately 12 meals/d. Nocek and Braund (16) offered feed to cows once daily, but the cows consumed only 10% of the feed in the 1st h after feeding, indicating that feed consumption was distributed over a much greater period. The present model suggests that meal frequencies in excess of 10 meals/d are tantamount to steady-state conditions, except with rapidly digestible carbohydrates and low effective NDF in the diet.

The restrictive assumptions for the steady periodic analysis led to closed form solutions that were nevertheless very complicated. Incorporation of temporal variations in meal size, digestion, passage, saliva production, and ruminal buffering, assuming these variations are known, will add more complexity. The present model, although limited in scope, provides the first computational basis for considering the effects of meal frequency and its interactions with diet, digestion rate, and animal characteristics on ruminal function. More work is needed to broaden the applicability of the model to more complex feeding strategies and realistic eating patterns.

CONCLUSIONS

Use of revised digestion rates obtained from an *in vitro* gas production system substantially improved the predictions of the magnitude and rate of ruminal pH fluctuations. Meal frequency, diet, effective NDF, and digestion rate had an interactive effect on predicted fluctuation of ruminal pH. The minimum meal frequency needed to maintain ruminal steady state increased as dietary effective NDF decreased; conversely, the minimum effective NDF needed to maintain pH above a set level increased as meal frequency decreased. Both DMI and BW were predicted to have relatively minor effects on the magnitude of pH fluctuations. Use of dietary buffers at levels less than 1% of DM was predicted to have little effect on pH fluctuation. The relationship between meal frequency and mean pH was influenced by the method of calculating mean pH from temporal data.

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