A Mathematical Approach to Predicting Biological Values from Ruminal pH Measurements

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ABSTRACT

The use of continuous recording to monitor ruminal pH has received growing attention. Continuous ruminal pH data are usually summarized for each 24-h period for each cow by calculating mean pH, maximum pH, minimum pH, amount of time (min/d) below pH 5.6 and 6.0, and area (time $\times$ pH) below pH 5.6 and 6.0. In this study, a novel approach to analyzing ruminal pH is introduced. A database from 6 published studies encompassing 8 trials and 13 different treatment groups was used in a meta-analysis. Trials were selected on their ability to obtain daily pH measurements and diet analyses. A total of 613 records met the criteria for inclusion in the meta-analysis. The database was subdivided based on nonfiber carbohydrate (NFC, % of dry matter) level in the diet into low (32 to 36%, $n = 105$), moderate (37 to 39%, $n = 326$), and high NFC ($>40\%$, $n = 159$). From each day of recording and for each cow, the amount of time below multiple pH points from 5.0 to 7.6 using a 0.2-unit pH interval (i.e., 5.0, 5.2, ..., and 7.6) was calculated. Sigmoidal curves were constructed to summarize daily ruminal pH records using calculated time below (min/d) as the y -variate and pH cut-off point as the x -variate. The objectives of this study were to 1) collate continuously recorded ruminal pH data from studies that used a dietary regimen to induce pH depression, 2) assess mathematical equations and subject the collated data to analysis, 3) determine the most suitable equation or equations to describe the data, and 4) derive values from the selected equation or equations that may have biological implication across dietary treatments. The analysis was performed on pooled data in each category using nonlinear modeling. Trial effect was considered as fixed and also as random. Four growth functions were considered: spline lines, Morgan, Richards, and logistic. All models had 4 parameters except the logistic equation, which had 3. The logistic and the Richards equations gave a better fit

to the data than did the Morgan and spline lines. All parameter estimates were significant except for 1 parameter for the spline lines. The logistic equation uses the least number of parameters and consistently gave a better prediction. Therefore, the logistic is considered the best option to use in describing pH curves. Model-derived values that have biological interpretation such as curve inflection point, curve slope, and time and area below pH 5.6 and 6.0 were calculated for all models. Diets with higher NFC content resulted in greater depression in ruminal pH. Degree of drop in pH can be described by a shift of pH curve position toward the lower pH range, hence, by greater values of predicted time and area below most critical pH cut-off points. This shift can also be identified by a decrease in curve inflection point and curve slope. Therefore, we suggest using these model-derived biological values to summarize continuously recorded pH data. For example, the inflection points for high, moderate, and low NFC levels were 1.01, 1.17, and 1.28; respectively. This approach permits comparison of pH data across studies and helps quantify dietary effects on ruminal pH.

Key words: ruminal pH, continuous recording, logistic equation, nonfiber carbohydrate

INTRODUCTION

Ruminal pH depression caused by overfeeding of grains to meet requirements of cows during early lactation may lead to subacute ruminal acidosis (SARA). Determination of ruminal pH is considered the best method to diagnose this disorder. However, variation in pH measurement methods and interpretation thereof have contributed to inconsistency within the literature. Methods used to measure pH include rumenocentesis, oral stomach tubing, and rumen cannulation. Nordlund and Garrett (1994) suggested pH values of 5.2 and 5.6 as the critical points for diagnosis of acute and subacute ruminal acidosis, respectively. They suggested that samples obtained by rumenocentesis should be collected from a representative group of animals 5 to 8 h postfeeding in TMR-fed herds or 2 to 5 h postfeeding in component-fed herds. The use of continuously acquired ruminal pH data provides more frequent data points

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Table 1. List of previously published studies included in the meta-analysis¹

Study	Trial	Treatment groups, n	NFC ²		
			Low	Moderate	High
Keunen et al., 2002	1	2	35.5 (40)		43.6 (38)
	2	2	33.0 (20)		41.9 (18)
Mutsvangwa et al., 2002	1	1		38.4 (105)	
	2	1		38.8 (94)	
Keunen et al., 2003		2		38.4 (55)	45.4 (56)
Osborne et al., 2004		1		37.0 (60)	
Cottee et al., 2004		2	32.8 (56)		41.2 (47)
Rustomo et al., 2006a		2	35.3 (12)	38.6 (12)	

¹The number of continuous acquisition records (d) meeting the criteria for inclusion in the meta-analysis for each treatment group is given in parentheses.

²NFC level in the diet: low, 32 to 36%; moderate, 37 to 39%; and high, >40% DM.

that permit the assessment of diurnal fluctuation of ruminal pH in relation to dietary regimen (Keunen et al., 2002). However, continuous recording systems are presently considered technically and economically prohibitive. Thus, the development of user-friendly and relatively cheaper systems is expected to spread the use of continuously recorded ruminal pH (AlZahal et al., 2007; Krause et al., 2005; Penner et al., 2006). Continuous recording has enabled researchers to measure the amount of time spent below a critical pH point, which has added further complexity to the interpretation of ruminal pH data. Keunen et al. (2002) computed time below pH 5.6 and 5.8 to define SARA, whereas Krause and Combs (2003) used 5.8. Rustomo et al. (2006a,b) suggested using a wider range of pH points on a continuous scale from pH 5.0 to 7.2. None of these studies defined pH depression as the minimum amount of time spent below a critical pH point or suggested a methodology that permits integration of studies to define ruminal pH depression. Therefore, the objectives of this study were to 1) collate continuously recorded ruminal pH data from studies that used dietary regimens to induce pH depression; 2) select mathematical equations and analyze collated data; 3) determine the most suitable equation or equations that describe the data; and 4) derive values from selected equations that may aid in defining biological measures across dietary treatments.

MATERIALS AND METHODS

Data Sources

A database obtained from 6 published studies comprising 8 trials and 13 different treatment groups was used in the analysis (Table 1). Trials were selected based on the following criteria: 1) there was nutritionally induced ruminal pH depression, 2) ruminal pH was continuously recorded, 3) daily pH records were

available, and 4) studies reported chemical analysis of the diet and DM intake for each treatment group. All 6 published studies used in this analysis were conducted at the same facility (Physiology Wing of the Elora Dairy Research Centre, Elora, Ontario, Canada) between 1999 and 2005. The studies used the nutritional model described by Keunen et al. (2002) to induce ruminal pH depression, with the exception of the study of Rustomo et al. (2006a). Briefly, animals were fed a TMR twice a day at 0700 and 1300 h during wk 1. During wk 2, a portion of the offered TMR [25% in Keunen et al. (2002), Keunen et al. (2003), and Cottee et al. (2004); 15% in Mutsvangwa et al. (2002) and Osborne et al. (2004) on a DM basis] was replaced with mixed-grain pellets (50:50, wheat:barley) that were fed in multiple meals throughout the day. Rustomo et al. (2006a) induced ruminal pH depression by feeding a high-acidogenic-value (**HAV**) diet as a TMR twice a day at 0900 and 1300 h. The acidogenic value (**AV**) concept was first introduced by Wadhwa et al. (2001) to evaluate acid production from feedstuffs by measuring Ca dissociation from CaCO₃. Rustomo et al. (2006c) ranked common feedstuffs used in Ontario, Canada, based on AV. In subsequent studies (Rustomo et al., 2006a,b), high AV and low AV feedstuffs were used to formulate 2 distinct diets with high and low AV (**LAV**), respectively, to study the effect of acid load and its interaction with particle size on in vivo ruminal pH. All studies used in the meta-analysis employed the same continuous recording system (Keunen et al., 2002) to measure ruminal pH, and pH was recorded every minute for 3 to 5 d during the treatment week following 1 wk of dietary adaptation.

Daily pH records were examined individually, outliers were omitted (pH <4 or pH >7.6), and only records that contained ≥1,200 data points (approximately 20 h/d of recording time) were included in the analysis. As a result, 613 records met the criteria for inclusion in the meta-analysis. The database was divided based on

Figure 1. An example of a constructed pH curve for one of the continuous pH daily records. The symbol (●) represents accumulated time below (y -axis, min/d) a pH cut-off point (top x -axis). The bottom x -axis represents pH - 5; thus, 0 was considered as the starting point when fitting candidate functions. The dotted lines indicate the time spent (215 min) below pH 5.8 during that day.

dietary level of NFC (% of DM) into low (32 to 36%, $n = 105$), moderate (37 to 39%, $n = 326$), and high NFC (>40%, $n = 159$; Table 1). Amount of accumulated time (min/d) spent below each pH cut-off point (5.0, 5.2, ..., 7.6) was calculated for each pH record in the database. Accumulated time spent below (y -variate, 0 to 1,440 min/d) was plotted against the range of the pH cut-off points (x -variate, pH 5 to 7.6) for each daily pH record to generate a sigmoidal shape (Figure 1) that will be referred to as a “pH curve” throughout this article. pH curves for each level of NFC were fitted to candidate functions. The sigmoidal curves represent the relationship between ruminal pH and dietary NFC taking into account accumulated times spent below 14 pH cut-off points rather than just the few cut-off points that are used in conventional approaches; for example, pH 5.6 and 5.8. Because all functions fitted were growth functions, pH minus 5 was considered the starting point on the x -axis, and a small positive value of 10^{-10} was used instead of zero as a starting point on the y -axis when applicable (Figure 1).

Nonfiber carbohydrate contents were calculated as follows:

$$\text{NFC} = 100 - (\text{CP} + \text{NDF} + \text{E})$$

where E is the average content of ash and fat; E equals 10, 10.4, and 4.7 for mixed feed, mixed hay, and grain mix, respectively, in this region of Ontario (Agri-Food Laboratories, Guelph, Ontario, Canada).

Candidate Functions

The 4 equations used to describe pH curves are presented in Table 2. For more details, refer to chapter 5 of Thornley and France (2007). The 4 equations were growth functions that have been used to describe growth of animals and plants. The first equation, spline lines, is a simple 4-parameter model (y_0 , y_f , x_0 , and x_f). The spline lines equation is a piecewise-linear equation that fits the data with 3 straight lines. The second equation, Morgan, is also a 4-parameter function (y_0 , y_f , x_h , and n). The parameter x_h refers to the pH cut-off point under which the animal spent half of the day. The third equation, logistic, has the least number of parameters (y_0 , y_f , and k). The fourth equation, Richards, has an extra parameter, n . The Richards equation encom-

Table 2. Equations used to describe ruminal pH curves

Equation	Functional form ¹	Inflection point (x^* , y^*)
Spline lines	$y(x) = y_0, 0 \leq x \leq x_0$ $y(x) = y_0 + k(x - x_0), x_0 < x < x_f$ $y(x) = y_f, x \geq x_f$ $k = \frac{y_f - y_0}{x_f - x_0}$	Not applicable
Morgan	$y(x) = \frac{y_0 x_h^n + y_f x^n}{x_h^n + x^n}$	$x^* = x_h \left(\frac{n-1}{n+1} \right)^{1/n}$ $y^* = \frac{(1 + 1/n)y_0 + (1 - 1/n)y_f}{2}$ $n \geq 1$
Logistic	$y(x) = \frac{y_0 y_f}{[y_0 + (y_f - y_0)e^{-kx}]}$	$x^* = \frac{1}{k} \ln \left(\frac{y_f - y_0}{y_0} \right)$ $y^* = \frac{1}{2} y_f$
Richards	$y(x) = \frac{y_0 y_f}{[y_0^n + (y_f^n - y_0^n)e^{-kx}]^{1/n}}$	$x^* = \frac{1}{k} \ln \left(\frac{y_f^n - y_0^n}{ny_0^n} \right)$ $y^* = y_f \left(\frac{1}{n+1} \right)^{1/n}$ $n > -1$

¹ $y(x)$ = time spent below cut-off point x (min/d), x = pH - 5, and $y_0, y_f, x_0, x_f, x_h, k$ (all >0 except $n > -1$ in the case of Richards) are parameters that define the scale and shape of the curves.

passes the Gompertz, logistic, and monomolecular (diminishing returns) equations when n has a value of 1, 0 and -1, respectively.

Biological Values

Values that possess biological interpretation were derived from the models. The predicted values were intended to provide a numerical differentiation of pH curves. Biological values predicted from models accounted for the trial effect. Point of inflection (**IP**) of a pH curve, the point at which a curve changes direction from acceleration to deceleration, was calculated as given in Table 2. Average slope of the pH curve between 2 given points about IP for Morgan, logistic, and Richards was calculated as follows:

$$\Delta = \frac{y(x_2) - y(x_1)}{x_2 - x_1},$$

where $x_1 = \text{IP} - 0.4$ and $x_2 = \text{IP} + 0.4$.

The slope for spline lines was equal to k (Table 2). Models could predict time spent below any pH cut-off point and area under the curve (**AUC**), taking into account the trial effect. The AUC between 2 points, x_3 and $x_3 + \delta$, was calculated as follows:

$$\text{AUC} = \int_{x_3}^{x_3 + \delta} y(x) dx, \text{ where } x = \text{pH} - 5.$$

Statistical Analysis

The data were pooled by each NFC category, and statistical analyses were performed using the nonlinear mixed procedure (PROC NLMIXED; v. 9.1; SAS Institute, 2004). Trial effect was considered not only as fixed but also as random because the data came from different trials. Model performance was evaluated using the significance level of the parameters estimated, variance of error estimate, and its standard error. Bayesian information criteria (**BIC**; Leonard and Hsu, 2001) were used to compare models. Bayesian information criteria are model-order selection criteria based on parsimony; BIC favor simpler models by imposing penalty on models that include additional parameters. Bayesian information criteria combine both maximum likelihood and (log) maximum likelihood penalized with a term related to model complexity as follows:

$$\text{BIC} = -2\log(\hat{J}) + K\log(N),$$

where $\hat{J}$ is the maximum likelihood, K is the number of independent parameters in the model, and N is the

sample size. When comparing models, a smaller numerical value of BIC indicates a better fit. Accuracy of models was assessed by computing the mean square prediction error (MSPE; Bibby and Toutenburg, 1977):

$$\text{MSPE} = \frac{1}{M} \sum_{i=1}^M (P_i - A_i)^2,$$

where $i = 1, 2, \dots, M$, M is the number of pairs of model-predicted and actual (observed) values being compared and P_i and A_i are the i th model-predicted and actual values; respectively. The MSPE has limitation in assessing model performance in that it removes the negative sign and weighs the deviations by their squares, yielding more influence to larger deviations (Mitchell and Sheehy, 1997). Comparisons of model accuracy were based on the root of MSPE (RMSPE) and RMSPE as a percentage of the observed mean (%RMSPE). Mean square prediction error was decomposed into error due to overall bias of prediction, error due to deviation of the regression slope from unity, and error due to disturbance or random variation (Bibby and Toutenburg, 1977). Concordance correlation coefficient (Lin, 1989) was used to assess model precision and accuracy. The first component is the correlation coefficient (r) that measures precision. The second component (C_b) is the bias correction factor that assesses model accuracy. If C_b equals 1, this indicates that no deviation from a 45° slope had occurred. The multiplication of both values gives both precision and accuracy at the same time. A t -test was performed to determine whether biological values calculated across functions for each level of NFC were similar, and also to determine whether biological values predicted using the same model were different across levels of NFC.

RESULTS AND DISCUSSION

Figures 2 and 3 show the fit of each model to the pH data. Table 3 summarizes the key statistical measures used to compare performance of the models. Each model was evaluated separately for low, moderate and high levels of NFC. All models for all levels of NFC gave similar variance of error. For low and moderate levels of NFC, all models had significant parameter estimates and the Richards equation gave the lowest BIC followed by the logistic, spline lines, and Morgan equations. For high NFC level, all parameter estimates were significant except for 1 parameter estimate for the spline lines. The BIC value was the lowest for the spline lines followed by Richards, logistic, and Morgan equations. For low and moderate NFC, the logistic equation gave the lowest values for RMSPE and %RMSPE, whereas the Richards model gave the lowest values for RMSPE

Figure 2. pH curves for low, moderate, and high levels of dietary NFC level (% DM basis). The y-axis represents the accumulated time (min/d) spent below each corresponding pH cut-off point on the x-axis. The scale from 0 to 2.6 on the x-axis represents pH 5 to 7.6. Symbols indicate average observed values for low NFC ($\square$), moderate NFC ($\triangle$), high NFC ($\bullet$), and point of inflection ($\blacksquare$). Lines were obtained by fitting candidate functions: spline lines (a) and Morgan (b). Dashed lines (---) represent slopes of pH curves. Error bars represent SE of the mean of observed values ($n = 128, 326$, and 159 ; respectively).

and %RMSPE for high NFC (Table 4). Despite the fact that MSPE was small for the Richards model, decomposition of MSPE especially for low and moderate NFC, showed greater mean bias and smaller random error. This was caused by the Richards equation slightly but consistently overpredicting values, which in turn caused a significant difference between the predicted and observed means. However, concordance correlation coefficient values were >0.99 for all models (data not shown), which indicated high precision and accuracy. The logistic and Richards equations gave a better fit to the data than the Morgan and spline lines. Although Richards gave a slightly better fit than the logistic, it proved to be an overparameterized model. Therefore,

Figure 3. pH curves for low, moderate, and high levels of dietary NFC (% DM basis). The y-axis represents the accumulated time (min/d) spent below each corresponding pH cut-off point on the x-axis. The value 0 to 2.6 on the x-axis represents pH 5 to 7.6. Symbols indicate average observed values for low NFC ($\square$), moderate NFC ($\triangle$), high NFC ($\bullet$), and point of inflection ($\blacksquare$). Lines were obtained by fitting candidate functions: logistic (a) and Richards (b). Dashed lines (---) represent slopes of pH curves. Error bars represent SE of the mean of observed values ($n = 128, 326$, and 159 ; respectively).

the logistic was considered the optimal choice to use in describing pH curves.

Table 5 summarizes biological values derived using the models. The values calculated within each level of NFC using different models were significantly different. Slopes and IP for different levels of NFC predicted by the same model are significantly different ($P < 0.05$; data not shown).

Researchers have shown that ruminal pH was highly influenced by the dietary level of fermentable carbohydrates (Krause and Combes, 2003; Rustomo et al., 2006a,b). Furthermore, an investigation using the same studies (Table 1; data not shown) revealed that in vivo ruminal pH has a higher correlation with dietary NFC content (% DM) than with total daily intake (kg) of NFC, daily NDF intake (kg), NDF content (% DM), AV

content (g of Ca/kg of DM), or total AV intake (g of Ca/d). Acidogenic values were calculated based on Rustomo et al. (2006c). Thus, data were pooled according to NFC content into low, moderate, and high. The effect of increasing NFC content on ruminal pH depression in the analysis across all studies translated into a shift of position of the pH curve toward a lower pH range, which in turn increased time below and AUC across all critical pH points. Depression of ruminal pH is also indicated by decrease in the x coordinate of the inflection point (IP), IP(x), and slope of the curves. For example, the IP of the logistic for high NFC (Figure 3a) was [1.01, 729; IP(x), IP(y)]. This indicated that the animals spent 729 min (approximately 12 h) below pH 6.0. The model can also predict the amount of accumulated time spent below any given pH; for example, the animals spent 475 and 283 min/d below the critical pH points 5.8, and 5.6, respectively. In other words, a high level of NFC content (>40% DM) across trials with an IP of 1.01 indicated pH depression of 12.0, 8.0, and 4.7 h below pH 6.01, 5.8 and 5.6, respectively. For moderate NFC content, the IP was (1.17, 7.16); therefore, it can be determined that animals spent 716, 284, and 148 min/d below critical pH points of 6.17, 5.8, and 5.6, respectively. Studies combined in this analysis used either moderate or high NFC content diets to induce SARA. Hence, SARA could be defined as a daily drop of pH for 148 to 283 min/d and 284 to 475 min/d below 5.6 and 5.8, respectively. Alternatively, SARA could be defined as IP(x) of 1.01 to 1.17, which is equivalent to spending approximately 12 h below a pH of 6.01 to 6.17.

The slope of a pH curve was shown to be negatively correlated with dietary NFC. This can be explained by the differences in pH range covered during the day. High NFC content gave a greater drop in pH than low NFC and thus, a greater range of pH (i.e., 5.3 to 6.7 vs. 5.7 to 6.9, respectively) and subsequently a smaller slope. Times below critical pH 6.0 and 5.6 have been widely reported by researchers because they provide significant biological information. However, model-derived values reported in Table 5 account for the study effect. Area under curve between pH 5 and 5.6, AUC between pH 5 and 6, and AUC between pH 5 and 7.6 (total area) have been shown to have considerable potential in differentiating between curves. A greater value for area indicated a greater shift to the left namely, toward lower pH ranges.

The trial effect across all models and levels of NFC was significant. Seasonal variation in forage quality and carbohydrate fermentation might be the greatest contributor to this difference. On the other hand, study effect would contribute to a relatively greater numerical difference between model-predicted and observed values, hence a greater random error of prediction. This

Table 3. Parameter estimates and other measures when models were fitted to low, moderate, and high NFC levels in the diet¹

NFC	Item ²	Logistic		Richards		Morgan		Spline lines	
		Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Low	y_0	7.2	0.9	26.5	4.2	71.3	11.4	68.4	11.0
	y_f	1,463	10.4	1,464	9.8	1,528	13.6	1,451	11.0
	k	4.2	0.1	5.9	0.4				
	n			2.2	0.3	5.4	0.1		
	x_h					1.3	0.0		
	x_0							0.7	0.0
	x_f							1.9	0.0
	Var1 ³	18,006	350	18,001	352	18,007	345	18,005	345
	Var2 ⁴	330	72	300	64	342	77	326	72
	BIC ⁵	24,440		24,406		24,516		24,515	
Moderate	y_0	16.4	1.4	50.2	6.8	88.7	15.8	65.9	15.1
	y_f	1,431	8.7	1,457	10.6	1,526	16.7	1,416	14.8
	k	3.8	0.1	5.1	0.3				
	n			2.1	0.2	4.5	0.1		
	x_h					1.2	0.0		
	x_0							0.5	0.0
	x_f							1.8	0.0
	Var1	22,006	262	22,003	262	22,023	258	22,006	259
	Var2	1,002	326	1,000	336	1,004	363	1,003	366
	BIC	63,400		63,356		63,583		63,548	
High	y_0	42.1	3.0	27.4	6.0	41.8	11.4	27.5	11.4 ^{NS}
	y_f	1,457	9.8	1,458	9.7	1,530	14.0	1,412	9.8
	k	3.5	0.1	3.1	0.2				
	n			0.7	0.1	3.4	0.1		
	x_h					1.0	0.0		
	x_0							0.3	0.0
	x_f							1.7	0.0
	Var1	18,700	441	18,700	441	18,704	433	18,702	441
	Var2	250	45	251	46	303	60	280	54
	BIC	28,953		28,947		29,007		28,941	

¹NFC level in the diet: low, 32 to 36%; moderate, 37 to 39%; and high, >40% DM.² y_0 , y_f , x_0 , x_f , x_h , and k (all >0 except n >−1 in the case of Richards) are parameters that define the scale and shape of the curves.³Var1 = variance (δ^2) of “error.”⁴Var2 = variance (δ^2) of trial effects.⁵BIC = Bayesian information criteria.^{NS} $P > 0.05$.**Table 4.** Comparison of model performance using mean squared error of prediction (MSPE), root MSPE (RMSPE), and RMSPE as a percentage of the observed mean (%RMSPE)

NFC level ¹	Equation	MSPE	RMSPE	%RMSPE	Components of MSPE, %		
					Mean bias	Regression bias	Random error
Low	Logistic	334	18.3	2.5	0.23	3.45	96.32
	Richards	745	27.3	3.67	97.32	0.04	2.65
	Morgan	1,803	42.5	5.7	35.64	0.00	64.36
	Spline lines	1,823	42.7	5.7	37.57	0.00	62.43
Moderate	Logistic	266	16.3	2.1	6.42	0.33	93.24
	Richards	1,773	42.1	5.3	97.96	0.02	1.98
	Morgan	2,347	48.5	6.2	50.69	0.17	49.14
	Spline lines	1,526	39.1	5.0	36.17	0.00	68.83
High	Logistic	691	26.3	3.1	45.57	2.14	52.23
	Richards	400	20.0	2.3	0.25	0.51	99.24
	Morgan	914	30.2	3.5	0.12	0.00	99.88
	Spline lines	342	18.5	2.2	0.46	0.00	99.54

¹NFC level in the diet: low, 32 to 36%; moderate, 37 to 39%; and high, >40% DM.

Table 5. Biological values derived using each model for low, moderate, and high levels of dietary nonfiber carbohydrates (NFC, % DM)

NFC level ¹	Item ²	Equation			
		Logistic	Richards	Morgan	Spline lines
Low	IP(x*), pH – 5	1.28	1.36	1.21	
	IP(y*), min/d	732	864	666	
	Slope	1,245	1,000	1,010	1,214
	Time < pH 5.6, min/d	83	132	93	68
	Time < pH 6.0, min/d	350	377	358	419
	AUC < pH 5.6, pH min/d	19	40	46	42
	AUC < pH 6.0, pH min/d	97	135	123	122
	AUC < pH 7.6, pH min/d	1,941	2,017	2,004	2,010
Moderate	IP(x), pH – 5	1.17	1.25	1.07	
	IP(y), min/d	716	853	648	
	Slope	1,152	922	938	1,091
	Time < pH 5.6, min/d	148	212	153	135
	Time < pH 6.0, min/d	497	530	543	572
	AUC < pH 5.6, pH min/d	37	69	61	41
	AUC < pH 6.0, pH min/d	158	210	189	186
	AUC < pH 7.6, pH min/d	2,058	2,181	2,154	2,129
High	IP(x), pH – 5	1.01	0.97	0.87	
	IP(y), min/d	729	680	565	
	Slope	1,098	875	889	1,001
	Time < pH 5.6, min/d	283	269	243	301
	Time < pH 6.0, min/d	718	717	737	702
	AUC < pH 5.6, pH min/d	80	69	56	56
	AUC < pH 6.0, pH min/d	275	262	251	258
	AUC < pH 7.6, pH min/d	2,317	2,287	2,263	2,269

¹NFC level in the diet: low, 32 to 36%; moderate, 37 to 39%; and high, >40% DM.

²Items: IP(x*, y*) = inflection point of pH curve; slope of the pH curve (slope between x_1 and x_2 is $\Delta = [y(x_2) - y(x_1)]/(x_2 - x_1)$, where $x_1 = x^* - 0.4$ pH and $x_2 = x^* + 0.4$ pH), where (x*, y*) is the inflection point; predicted time below pH 5.6 and 6.0 (min/d); and area under the pH curve (AUC) between pH 5 and pH 5.6, 6.0, and 7.6 (total area). Area under curve between 2 points, x_3 and $x_3 + \delta$, is calculated as follows:

$$\text{AUC} = \int_{x_3}^{x_3 + \delta} y(x)dx, \text{ where } x = \text{pH} - 5.$$

might explain the elevated random error encountered in this analysis. However, there is no known method to separate the effect of study or covariate when evaluating the performance of a given model.

In conclusion, the logistic can be used to describe curves constructed from continuous pH data. Values derived from this model, such as curve IP and slope, can be used to differentiate between pH curves. Time below critical pH cut-off points and area under the curve between any 2 given points can also be predicted. This approach accounts for study effect and could contribute greatly to our understanding of dietary effects on ruminal pH.

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