*e-planet* **15** (2): 117-126 (December 2017)

# Modelling options for analysing ruminal ammonia-N concentration profiles

**M.S. DHANOA[1], R. SANDERSON[2], R.C. SIDDONS[3], S. LOPEZ[4] AND J. FRANCE[1*]**

[1] *Centre for Nutrition Modelling, Dept. of Animal Biosciences, University of Guelph, Guelph ON, N1G 2W1, Canada.*

[2] *Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Gogerddan, SY23 3EB, UK*

[3]*Formerly at Institute of Grassland and Environmental Research, Hurley, Berkshire, SL6 5LR, UK*

[4] *Instituto de Ganadería de Montaña, Departamento de Producción Animal, Universidad de León, León, Spain*

**jfrance@uoguelph.ca*

Date of receipt: 23.09.2017 Date of acceptance: 17.10.2017

**ABSTRACT**

In this communication we descuss mathematical models that can be used to describe ammonia-N concentration profiles in the rumen of animals post-feeding. The unimodal pattern of ammonia-N profile following a single meal is modelled and analysed. Animals fed twice daily show bimodal patterns where each component is separated due to the time interval between feeding times. Thus the selected model can be fitted to each peak separately. Various modelling options are explained and illustrated. There are three categories of models discussed: (1) summarising models to generate data for treatment comparisons, (2) kinetics-based models that furnish parameter estimates and meaningful functions thereof which can be used for treatment comparisons and (3) empirical models to generate quantitative information for further analysis. More complete information was obtained while analysing ammonia-N profiles using kinetics-based models such as the critical exponential and summarising models such as the single Gaussian.

**Key words:** Gaussian function, lactation curve, Michaelis-Menten equation, modelling options, rumen ammonia-N concentration profiles, saturation kinetics

## INTRODUCTION

The food eaten by ruminant animals undergoes physical and chemical changes due to fermentation processes in the rumen. As a consequence of rapid degradation of feed, the concentration of ammonia-N increases above a baseline to a maximum and thereafter decreases to baseline level if animals are fed once a day (Fig. l) or will have a second peak in animals fed twice daily. This unimodal or bimodal profile of ammonia-N concentration in the rumen is dependent on the composition of the food eaten, *e.g.* Roffler and Satter (1975) derived a quadratic prediction equation for ruminal ammonia-N in terms of dietary protein (CP) which was improved when quadratic terms of total digestible nutrient (TDN) were added. It was also shown that non-protein (NPN) supplements lead to higher levels of ruminal ammonia-N concentration (Roffler and Satter, 1975; Ciszuik and Eriksson, 1973). The concentration decline from the peak may be explained in terms of respiratory losses, absorption across the rumen wall, microbial protein synthesis and excretion through urine. Thiago et al. (1992) observed myoelectric activity associated with rumen opening in once fed and eight times fed steers and found dependence on the composition of feed. They also found that activity increased in once fed animals presumably in anticipation of the feeding time.

Ammonia-N profiles are similar to the lactation curve in dairy cows and digesta-flow marker patterns in ruminant and non-ruminant animals. So models used in these fields should be applicable to describe ammonia-N profiles. Once food is offered to the animal it takes some time before the satiety signal is activated and the animal stops eating. Thereafter, food mass undergoes particle size reduction, saliva incorporation, bacterial attachment and bacterial growth. Thus, there may be some lag-time before food degradation commences and digestion by-products accumulate while subjected to absorption and dissipation processes. The objective of this study was to evaluate distribution-based, kinetics-based and empirical models most appropriate for the analysis of rumen ammonia profiles in order to generate meaningful information for treatment comparisons.

## MATERIALS AND METHODS

### Ammonia-N data

Example data come from an experiment described in Sanderson et al. (in preparation). Steers were offered one of two second-cut grass silages prepared from herbage differing in dry matter content and the diets as fed *ad libitum* were: wetter silage once daily (W); drier silage once daily (D); drier silage 24×daily *i.e.* hourly (DC). The statistical design comprised two 3×3 Latin squares balanced for carry over effects. Rumen fluid samples were taken over a 24 h period (09:00 - 14:00 at half-hourly intervals, 14:00 - 20:00 at hourly intervals and then at 00:00, 07:00, 08:00 and 09:00). Samples were withdrawn manually via a weighted filter immersed in the digesta matrix, acidified to pH 2.0 using concentrated sulphuric acid and stored frozen at –18°C until analysed for ammonia-N content. Ammonia-N content was determined by an enzymatic method that employed Test Kit No. 66-50 (Sigma-Aldrich Co. Ltd., Poole, Dorset, UK) for determination of urea nitrogen.

### Some modelling options

The product of multi-stage processes can sometimes result in an outcome which leads to a gamma distribution (Kendall, 1948, 1952). The gamma distribution has probability density function

$$f(x) = (x/b)^{c-1}[\exp(-x/b)]/[b\Gamma(c)];\ 0 \le x < \infty$$

with scale parameter $b > 0$, shape parameter $c > 0$ and distribution mean $bc$. Here $\Gamma(c)$ denotes the complete gamma function

$$\int_0^\infty z^{c-1}\mathrm{e}^{-z}dz, \mathrm{g}{>}0$$

The gamma distribution when shape parameter $c$ is an integer is also known as the Erlang distribution (Evans et al., 2000).

Wood (1967) used a version of the gamma function to model the lactation curve (daily milk yield, $y \ge 0$) of a dairy cow

$$y(t) = At^b\mathrm{e}^{-ct} \quad (1)$$

where $t$ denotes days of lactation, $A$ is scale parameter, $b$ is a shape parameter, and $c$ (per d) is the relative rate of decline in milk production. To reduce parameter collinearity, Dhanoa (1981) modified the above equation

$$y(t) = At^{mc}\mathrm{e}^{-ct} \quad (2)$$

Now $m$ (d) is the mode or peak position (an important parameter in lactation studies), and $A$ and $c$ as in the Wood equation. Further information is given in Dhanoa and Le Du (1982). These authors also proposed a partial adjustment model as an alternative that accounts for high levels of autocorrelation which can be encountered when analysing lactation curves

$$y_T = \lambda(b_0 - b_1T) + (1-\lambda)y_{T-1};\ T \ge 1,\ 0 \le \lambda \le 1 \quad (3)$$

Here $T$ is discrete time, $y_T$ is the current value, $y_{T\text{-}1}$ the preceding value, $\lambda$ is the fraction by which a value adjusts to the next level and $(1 - \lambda)$ the fraction by which a value persists at the previous level. The parameter $b_1$ is the rate of decline and $b_0$ is a constant. For integration of the Wood model see France and Dhanoa (1984). Other lactation models

are discussed in Rook et al. (1993) and Thornley and France (2007). Ammonia-N profiles show similarity to the shape of lactation curve except that relative to the timeframe, the profile mode is little earlier and the declining phase is shorter.

Rather than considering the aggregate process as when applying the Wood equation, France et al. (1985), following Kendall (1948, 1952), considered $g + 1$ pools of a process with same rate constant $k$ (per unit time) applying to flow between each pool and showed that the aggregate process can be described by the equation

$$y(t) = \frac{t^{g-1}\mathrm{e}^{-t/m}}{m^{g}\Gamma(g)} \qquad (4)$$

where $m = k^{-1}$. France et al. (1985) also described $g$ parallel sub-processes with same rate constant $k$ ($= m^{-1}$) as a Yule process. The overall process completes as soon as the sub-processes complete and take time $\tau$. Rahn (1932) showed $\tau$ in such a system is distributed according to the Yule distribution with probability density function

$$f(\tau) = \frac{g}{m}\mathrm{e}^{-\tau/m}(1-\mathrm{e}^{-\tau/m})^{g-1}$$

and distribution mean

$$E(\tau) = m\left(1+\frac{1}{2}+\frac{1}{3}+\ldots+\frac{1}{g}\right)$$

Dhanoa et al. (1985) and France et al. (1985) proposed a multi-compartmental or double exponential model to describe a variety of unimodal profiles

$$y(t) = A\mathrm{e}^{-k_1 t}\exp[(-(g-2)\mathrm{e}^{-(k_2-k_1)t}] \qquad (5)$$

where $A$ is the scale parameter, $k_1$, and $k_2$ are the two smallest rate constants (per unit time), and $g$ is the number of pools or compartments. This function (Equation 5) does not have the limitation of three of the previous functions (Equations 1, 2 and 4) that $y(0) = 0$. However, it is convenient to fit this model in its log form (see Dhanoa et al., 1985)

$$\log(y) = \alpha + \beta\mathrm{e}^{-kt} + \lambda t \qquad (6)$$

where $\alpha = \log(A)$, $\beta = -(g-2)$, $k = (k_2 - k_1)$ and $\lambda = -k_1$.

Other possibilities for describing unimodal behaviour include the sum of two exponentials (Blaxter et al., 1956), although a slow rise in ammonia-N concentration in the early stages might require a sigmoidal function to represent the ascending stage. One such function, in addition to standard growth functions such as the Gompertz and logistic (Thornley and France, 2007), is the saturation kinetics function (Morgan et al., 1975; Phillips, 1982)

$$y(t) = (bK + y_{\max}t^n)/(K + t^n)$$

where $b$, $K$, $y_{\max}$ and $n$ are parameters. This model is capable of modification to incorporate a declining phase (Atkins, 1983). The saturation kinetics model was developed to describe physiological response caused by nutrient input, *e.g.* infusion of a nutrient and its concentration in blood plasma. It is essentially a generalised Michaelis-Menten equation (Lopez et al., 2000). To make it independent of input, Dhanoa et al. (2016) gave an alternative form of this model that gives a horizontal baseline which intersects the $y$-axis, *viz.*

$$y(t) = b + (y_{\max} - b)/(1 + Kt^{-n})$$

A more general form of the digesta-flow model by Blaxter et al. (1956) is the function

$$y(t) = \alpha + \beta\mathrm{e}^{-k_1 t} + \lambda\mathrm{e}^{-k_2 t} \qquad (7)$$

where $\alpha$, $\beta$, and $\lambda$ are parameters and $k_1$ and $k_2$ are rate constants ($> 0$). A limiting case of this double exponential function is the critical exponential equation (Ross, 1990; VSN International, 2015)

$$y(t) = \alpha + (\beta + \gamma t)\mathrm{e}^{-kt} \qquad (8)$$

This multiplicative form can be approximated by using an exponential plus a linear trend

$$y(t) = \alpha + \beta e^{-kt} + \lambda t$$

For empirical description of single-peaked curves, inverse polynomials (Nelder, 1966) are another option.

If the ammonia-N profile is multi-modal, the Gaussian function may be applied (Ross, 1990). Gauss curves can be implemented in statistical packages such MLP (Ross, 1990) and Genstat (VSN International, 2015) and fitted using the relevant directives, *e.g.* FIT CURVE in MLP and FITCURVE in Genstat, with relevant OPTIONS to produce parameter estimates. For a unimodal profile the single Gaussian can be used

$$y(t) = \alpha + \frac{\beta}{\sqrt{2\pi\sigma^2}} \exp\left(\frac{-(t-\mu)^2}{2\sigma^2}\right) \quad (9)$$

whereas for a (overlapping) bimodal (twice-fed) profile, the double Gaussian is required

$$y(t) = \alpha + \frac{\beta}{\sqrt{2\pi\sigma^2}} \exp\left(\frac{-(t-\mu)^2}{2\sigma^2}\right) + \frac{\lambda}{\sqrt{2\pi\sigma^2}} \exp\left(\frac{-(t-\nu)^2}{2\sigma^2}\right) \quad (10)$$

where $t$ is time since the first feed, α is the baseline, ß the area under the first peak (above the baseline) with mean $\mu$, $\lambda$ is the area under the second peak with mean $\nu$ and $\sigma$ is the standard deviation.

If the data profiles to be analysed are not consistent with any of the models described above, then numerical calculations can be used to peel off individual features that may be distorting the underlying main response signal. For example, a simple exponential curve may become different because of the presence of a positive or negative linear trend. In such situations components of the overall profile can be separated using curve peeling methods (Jacques, 1972).

## RESULTS AND DISCUSSION

### Data summary and diet effects

Diets W and D have unimodal profiles whilst Diet DC showed a non-peaked response to hourly feeding (Fig. 1). There is some decline up to 15:00 h in ammonia-N concentration under DC feeding, perhaps due to smaller intake being digested differently. To compare all three diets, the area under the curve (AUC) was calculated and these values subjected to ANOVA according to the experimental design. Calculated AUC was similar for D and DC but greater for W ($P < 0.05$).

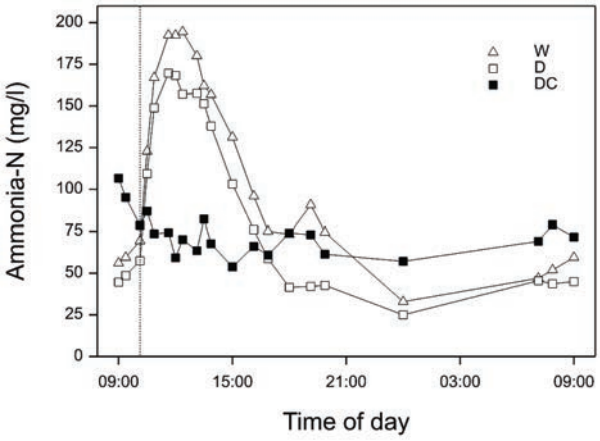


**Fig. 1.** Mean diurnal rumen fluid ammonia-N profiles for each of three diets (W, D and DC). Vertical line indicates fresh feed distribution for once-daily fed animals

The single Gaussian (Equation 9) was able to describe profiles from Diets W and D including estimating the baseline (Fig. 2). Parameters from the fit of this model to each animal dataset were compared by analysis of variance for Diets W and D only (Table 1). Although the single Gaussian fits well to Diet W and D replicates, there is suggestion of the apparent presence of two overlapping peaks in some of the individual animals' fitted curves (see Fig. 3). This effect is presumably due to the pattern of intake when a large initial meal or feeding bout is followed by another large bout. In Fig. 3, the acceptable double Gaussian fit

(Equation 10) is for Animal 1 to 4 in Period 1, Animal 1, 5 and 6 in Period 2, and Animal 3 and 4 in Period 3 (Fig. 3a). Animal 2 in Period 2 showed data upturn before next feed which distorted the fit quite badly (Fig. 3b). There was no improvement in fitting the double Gaussian for Animal 6 in Period 2 and Animal 5 and 6 in Period 3.

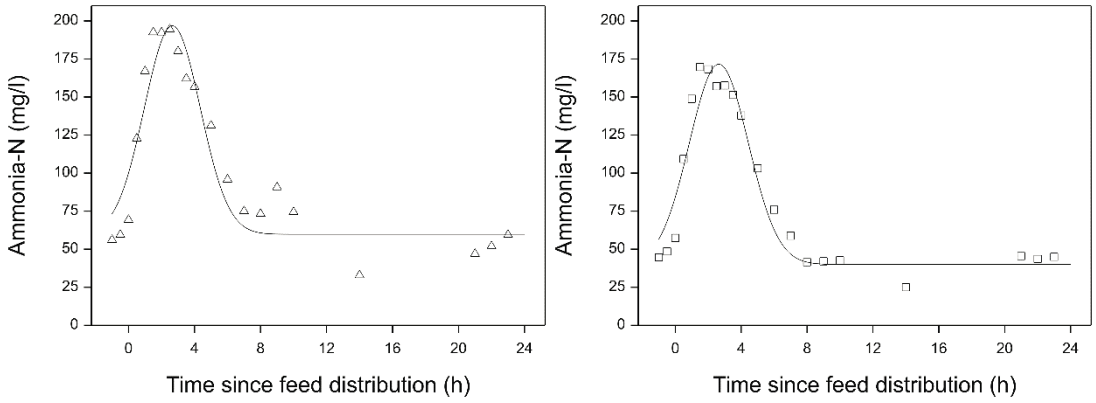


**Fig. 2.** Single Gaussian equation fitted to mean profiles for Diet W (left) and D (right)

**Table 1.** Diet parameters means and their significance

| Estimates | Diet | | | SED | Probability |
|---|---|---|---|---|---|
| | W | D | DC | | |
| Area under the curve | | | | | |
| AUC | 1905[b] | 1513[a] | 1606[a] | 94.8 | 0.008 |
| Single Gaussian model parameters | | | | | |
| Baseline ($\alpha$) | 58.0 | 37.9 | - | 10.72 | 0.158 |
| Peak area ($\beta$) | 613 | 627 | - | 199.5 | 0.948 |
| Mean ($\mu$) | 2.51 | 2.65 | - | 0.460 | 0.776 |
| Standard deviation ($\sigma$) | 1.58 | 1.76 | - | 0.305 | 0.604 |

[a, b] Different superscripts indicate means differ ($P < 0.05$) based on Fisher's protected least significant difference test (VSN International, 2015). AUC was compared between all diets whilst only diets W and D in terms of Gaussian parameters

Both the double and critical exponential functions (Equations 7 and 8, respectively) and the multi-compartment model in log form (Equation 6) were also fitted to the Diet W and D profiles for each animal. The double exponential only fitted 2 of the 12 profiles, but the critical exponential fitted much better. Using curve peeling, this fitted curve was subtracted from the associated data profile but departures from the fitted curve seemed to have no strong apparent trend. The parameter estimates obtained for the critical exponential are shown in Table 3 and those for the multi-compartment model in Table 4. Data beyond 20 h were omitted when applying the multi-compartment model so as to prevent the curve flipping over when fitting Equation (6).

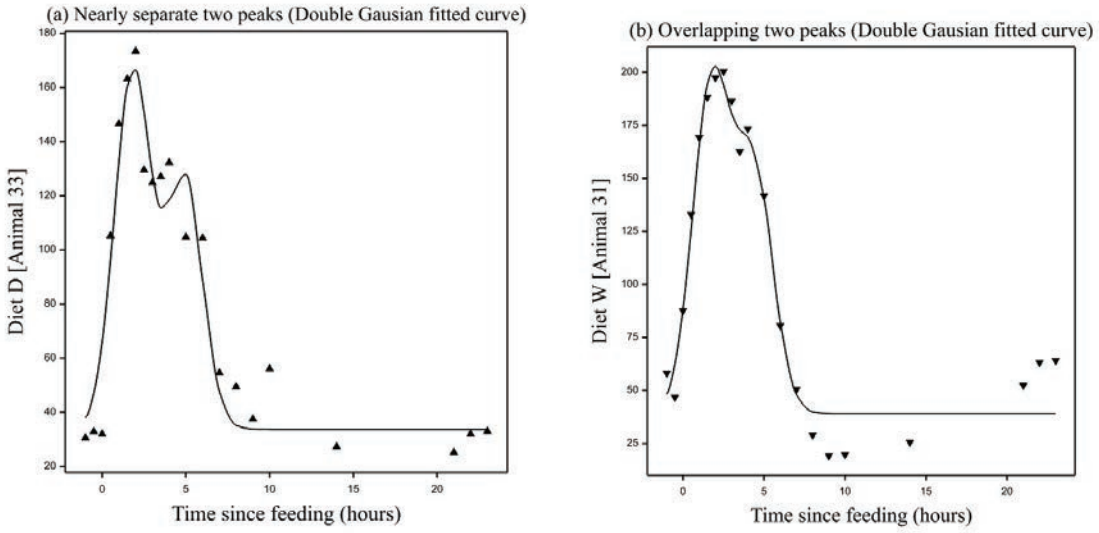


**Fig. 3.** Plots illustrating departure from a single peak (a) nearly separate two peaks and (b) mainly overlapping two peaks.

**Table 2.** Parameter values obtained for the double Gaussian (Equation 10)

| Animal | Period | Diet | $\sigma$ | $\mu$ | $v$ | $\beta$ | $\lambda$ | $\alpha$ | F prob.* | $R^2$ (%)§ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | W | 1.162 | 1.75 | 4.39 | 447 | 329 | 39.0 | 0.008 | 92.0; 95.2 |
| 2 | 1 | D | 0.812 | 1.31 | 3.26 | 219 | 138 | 45.3 | 0.011 | 89.5; 93.5 |
| 3 | 1 | W | 1.355 | 2.11 | 4.78 | 487 | 300 | 33.3 | 0.035 | 94.0; 95.7 |
| 4 | 1 | D | 1.116 | 1.81 | 4.07 | 307 | 184 | 43.1 | 0.057 | 91.6; 93.5 |
| 1 | 2 | D | 0.929 | 1.46 | 3.59 | 346 | 160 | 43.4 | 0.027 | 91.3; 93.9 |
| 2 | 2 | W† | 0.777 | 1.55 | 15.41 | 213 | -673 | 92.3 | 0.047 | 53.9; 65.3 |
| 5 | 2 | D | 1.462 | 2.38 | 5.22 | 672 | 245 | 34.8 | 0.045 | 95.3; 96.5 |
| 6 | 2 | W | 0.578 | 1.25 | 2.47 | 178 | 84 | 84.4 | 0.295 | 66.4; 67.6 |
| 3 | 3 | D | 1.089 | 1.83 | 4.88 | 359 | 254 | 33.7 | 0.001 | 76.2; 89.3 |
| 4 | 3 | W | 1.519 | 2.93 | 6.67 | 745 | 281 | 40.3 | <0.001 | 92.3; 97.6 |
| 5 | 3 | W | 0.673 | 1.67 | 3.14 | 240 | 236 | 75.0 | 0.667 | 65.4; 62.9 |
| 6 | 3 | D | 1.495 | 2.50 | 5.01 | 408 | 408 | 31.6 | 0.475 | 92.5; 92.3 |

*F-test probability that the double Gaussian is a better fit than the single Gaussian

§Adjusted $R^2$: 1st value is for the single Gaussian and 2nd value is for the double Gaussian

† Not acceptable because of distortion due to the end point data

**Table 3.** Parameter values obtained for the critical exponential (Equation 8)

| Animal | Period | Diet | $k$ | $\beta$ | $\gamma$ | $\alpha$ | $R^2$ (%)§ |
|---|---|---|---|---|---|---|---|
| 1 | 1 | W | 0.3974 | 101.43 | 114.44 | 28.34 | 75.2 |
| 2 | 1 | D | 0.4926 | 71.29 | 80.97 | 37.91 | 73.4 |
| 3 | 1 | W | 0.3613 | 89.87 | 105.15 | 20.99 | 77.0 |
| 4 | 1 | D | 0.4140 | 65.24 | 86.55 | 35.76 | 76.7 |
| 1 | 2 | D | 0.4685 | 100.73 | 105.04 | 30.66 | 74.7 |
| 2 | 2 | W | 0.4040 | 65.46 | 74.12 | 62.47 | 61.0 |
| 5 | 2 | D | 0.3466 | 113.37 | 118.09 | 15.11 | 75.4 |
| 6 | 2 | W | 0.3944 | 70.14 | 73.15 | 59.75 | 57.8 |
| 3 | 3 | D | 0.3547 | 67.72 | 85.85 | 21.74 | 80.0 |
| 4 | 3 | W | 0.2909 | 90.10 | 105.03 | 20.29 | 74.8 |
| 5 | 3 | W | 0.3494 | 57.48 | 104.62 | 54.30 | 50.9 |
| 6 | 3 | D | 0.2996 | 66.01 | 92.03 | 16.94 | 72.5 |

§ Adjusted $R^2$

**Table 4.** Parameter values obtained for the multi-compartment function (Equation 6)

| Animal | Period | Diet | $(k_2 - k_1)$ | $(g - 2)$ | $k_1$ | ln($A$) | $R^2$ (%)§ |
|---|---|---|---|---|---|---|---|
| 1 | 1 | W | 0.3386 | 3.435 | 0.5262 | 8.040 | 93.9 |
| 2 | 1 | D | 0.7505 | 0.968 | 0.2361 | 5.516 | 86.5 |
| 3 | 1 | W | 0.3534 | 3.245 | 0.4590 | 7.669 | 89.4 |
| 4 | 1 | D | 0.6555 | 1.374 | 0.2305 | 5.763 | 72.1 |
| 1 | 2 | D | 0.9651 | 0.659 | 0.1877 | 5.397 | 53.2 |
| 2 | 2 | W | 1.1424 | 0.373 | 0.0661 | 5.154 | 44.1 |
| 5 | 2 | D | 0.2856 | 3.803 | 0.5050 | 8.411 | 89.9 |
| 6 | 2 | W | 0.9374 | 0.427 | 0.0779 | 5.205 | 32.5 |
| 3 | 3 | D | 0.5369 | 1.855 | 0.2412 | 6.026 | 74.7 |
| 4 | 3 | W | 0.2037 | 5.467 | 0.5412 | 9.905 | 88.8 |
| 5 | 3 | W | 0.7875 | 1.085 | 0.0997 | 5.508 | 39.6 |
| 6 | 3 | D | 0.2724 | 4.786 | 0.5145 | 8.813 | 86.7 |

§ Adjusted $R^2$

Treatment DC with its hourly feeding created an ammonia-N profile showing no specific peak, and therefore the models described above generally cannot be used for analysis. However, parameter estimates obtained by fitting the partial adjustment model (Equation 3) can yield some useful informa-

tion, e.g.

Diet D: $\lambda = 0.159$; $b_1 = 6.48$; $b_0 = 137.7$, Adjusted $R^2 = 84.5\%$

Diet DC: $\lambda = 0.575$; $b_1 = 0.0243$; $b_0 = 69.39$, Adjusted $R^2 = 20.1\%$

These fits are illustrated in Fig. 4.

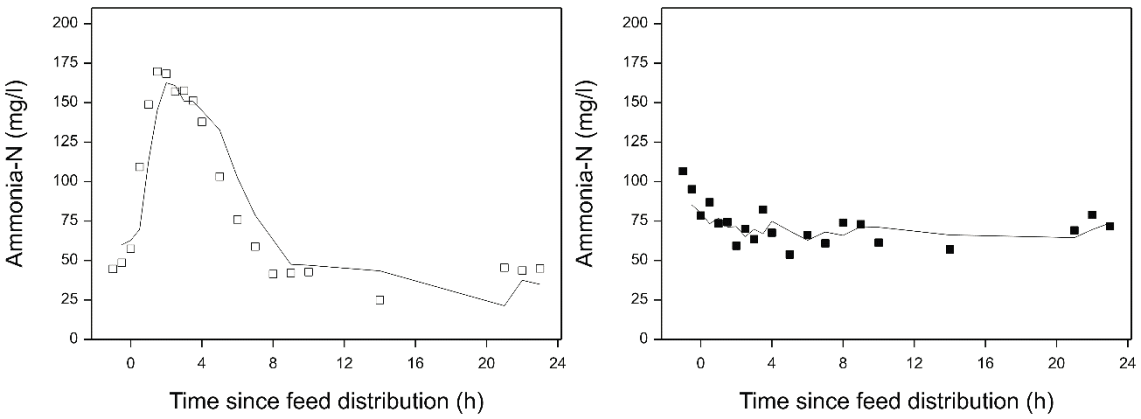


**Fig. 4.** Partial adjustment model (Equation 3) fitted to mean ammonina-N profiles for Diet D (left) and DC (right). Time since feeding refers to the once-daily feeding regime only as DC animals were fed hourly.

Ammonia is produced in the rumen by microbial degradation of nitrogenous compounds (both protein and non-protein) of feed, endogenous and microbial origin. The rate of production is a function of the quantity and degradability of the N sources and microbial activity, which in turn is a function of the availability of fermentable organic non-N compounds. $NH_3$ is lost from the rumen by microbial assimilation, outflow in the digesta and absorption. The concentration of $NH_3$ in the rumen liquor at any given time reflects the balance between production and loss. Typically in animals fed once a day, the concentration increases soon after feeding to a peak and subsequently declines whilst in frequently fed animals the concentration remains relatively constant. The purpose of the present study was to examine the suitability of various mathematical functions to describe the changes in rumen $NH_3$ levels under such circumstances.

Rumen function and N cycling in ruminants is a complex system and to understand it knowledge-based mechanistic models have been constructed (see Dijkstra et al., 1998). For many sub-components in these models we need to summarise various profiles of fermentation outputs such as ammonia-N, methane, volatile fatty acids, etc. However, time profiles (since feeding) of the fermentation end-products in the rumen depend on the attributes of any diet and its interaction with the feed intake processes. Interactions of these attributes, when undergoing actions of degrading agents, will be reflected in the profiles of by-products. Therefore, to discriminate between diets we need to model and quantify the relevant profiles. In this communication we have discussed and illustrated options for analysing ammonia-N profiles. The choice of model and analysis will depend on the purpose of the study. Here, we have illustrated three categories of model. (1) Summary models to generate measures for treatment comparisons. The single Gaussian (Equation 9) was suitable for this

purpose in comparing Treatments W and D. AUC (area under the curve), used herein to compare all three treatments (W, D and DC), can be regarded as a numerical surrogate for the Gaussian. (2) Kinetics-based models that furnish parameter estimates and meaningful functions thereof which can be used for treatment comparisons. Among various options available are the critical exponential (Equation 8) and the multi-compartment model (Equation 6) which provided more complete information. (3) More empirical models to generate quantitative information for further analysis. For unimodal profiles, models based on the gamma function (*e.g.* Equations 1 and 2; results not included), inverse polynomials (Nelder, 1966; results not included) and feed-back or partial adjustment models (*e.g.* Equation 3) are relevant options. All three categories of model allow us to understand the data in hand more precisely. The inferences thus gained help us form conclusions and develop enhanced and new hypotheses. This modelling approach can be applied not just to ammonia but to other fermentation end-products (*e.g.* methane, volatile fatty acids) following a similar post-feeding trend.

## CONCLUSION

In this communication we have outlined some of the modelling choices when analysing ruminal profiles of ammonia-N concentration. The typical shape of such profiles is that following infrequent feeding it increases from its baseline to a peak and thereafter declines back to the baseline position. As described above, choice of model depends upon the purpose of the study. Success of the modelling will depend on the quality of selected curve fit and unbiased parameter estimates. For this purpose models need to be parsimonious and robust, and models may have to be refined to remove any ill-conditioning.

## ACKNOWLEDGEMENT

Funding, in part, was provided by the National Science and Engineering Council of Canada, Ottawa.

## REFERENCES

Atkins, G.L. 1983. A comparison of methods for estimating the kinetic parameters of two simple types of transport process. *Biochim. Biophys. Acta Biomembr.* **73**: 455-463.

Blaxter, K.L., Graham, N.M. and Wainman, F.W. 1956. Some observations on the digestibility of food by sheep and related problems. *Br. J. Nutr.* **10**: 69-91.

Ciszuik, P. and Eriksson, S. 1973. Ammonia formation in the rumen of sheep fed on grass, clover or lucerne preserved in various ways. *Swedish J. Agric. Res.* **3**: 13-20.

Dhanoa, M.S. 1981. A note on an alternative form of the lactation model of Wood. *Anim. Prod.* **32**: 349-351.

Dhanoa, M.S. and Le Du, Y.L.P. 1982. A partial adjustment model to describe the lactation curve of a dairy cow. *Anim. Prod.* **34**: 243-247.

Dhanoa, M.S., Siddons, R.C., France, J. and Gale, D.L. 1985. A multicompartmental model to describe marker excretion patterns in ruminant faeces. *Br. J. Nutr.* **53**: 663-671.

Dhanoa, M.S., Sanderson, R., López, S. and France, J. 2016. Reduction of parameter multicollinearity in a saturation kinetics model. *Can. J. Anim. Sci.* **96**: 634-635.

Dijkstra, J., France, J. and Tamminga, S. 1998. Quantification of the recycling of microbial nitrogen in the rumen using a mechanistic model of rumen fermentation processes. *J. Agric. Sci.* **130**: 81-94.

Evans, M., Hastings, N.A.J. and Peacock, J.B. 2000. *Statistical Distributions*, 3rd edn. John Wiley and Sons, New York.

France, J. and Dhanoa, M.S. 1984. On estimating lactation yield. *J. Agric. Sci.* **103**: 245-247.

France, J., Thornley, J.H.M., Dhanoa, M.S. and Siddons, R.C. 1985. On the mathematics of digesta flow kinetics. J. *Theor. Biol.* **113**: 743-758.

Jacques, J.A. 1972. *Compartmental Analysis in Biology and Medicine*. Elsevier, Amsterdam.

Kendall, D.G. 1948. On the role of variable generation time in the development of a stochastic birth process. *Biometrika* **35**: 316-330.

Kendall, D.G. 1952. On the choice of mathematical model to represent bacterial growth. *J. Royal Stat. Soc. Series* B **14**: 41-44.

Lopez, S., France, J., Gerrits, W.J.J., Dhanoa, M.S., Humphries, D.J. and Dijkstra, J. 2000. A generalized Michaelis-Menten equation for the analysis of growth. *J. Anim. Sci.* **78**: 1816-1828

Morgan, P.H., Mercer, L.P. and Flodin, N.W. 1975. General model for nutritional responses of higher organisms. *Proc. Natl. Acad. Sci. USA* **72**(11): 4327-4331.

Nelder, J.A. 1966. Inverse polynomials, a useful group of multi-factor response functions. *Biometrics* **22**: 128-141.

Phillips, R.D. 1982. Modification of the saturation kinetics model to produce a more versatile protein assay. *J. Nutr.* **112**: 468-473.

Rahn, O. 1932. A chemical explanation of the variability of the growth rate. *J. Gen. Physiol.* **15**: 257-277.

Roffler, R.E. and Satter, L.D. 1975. Relationship between ruminal ammonia and nonprotein nitrogen utilization by ruminants. II. Application of published evidence to the development of a theoretical model for predicting nonprotein nitrogen utilization. *J. Dairy Sci.* **58**: 1889-1898.

Rook, A.J., France, J. and Dhanoa, M.S. 1993. On the mathematical description of lactation curves. *J. Agric. Sci.* **121**: 97-102.

Ross, G.J.S. 1990. *Nonlinear Estimation*. Springer-Verlag, New York.

Sanderson, R., Gill, M., Dhanoa, M.S. and Romney, D.L. 2018. Effects of ensiled herbage dry matter content on intake and digestion of grass silages by growing steers (in preparation).

Thiago, L.R.L., Gill, M. and Sissons, J.W. 1992. Studies of method of conserving grass herbage and frequency of feeding in cattle. 2. Eating behaviour rumen motility and rumen fill. *Br. J. Nutr.* **67**: 319-336.

Thornley, J.H.M. and France, J. 2007. *Mathematical Models in Agriculture: Quantitative Methods for the Plant, Animal and Ecological Sciences*, 2nd edn. CAB International, Wallingford, Oxon, UK.

VSN International. 2015. Genstat for Windows, 18th edn. VSN International, Hemel Hempstead, UK. Web page: Genstat.co.uk

Wood, P.D.P. 1967. Algebraic model of the lactation curve in cattle. *Nature* **216**: 164-165.