Introduction

Years of in depth research have provided modern animal agriculture with a large body of knowledge that should logically lead to designing ruminant rations that repeatedly provide maximum productivity. Such is not the case, as nutritionists; regardless of locale continually face the dilemma of variable productivity given similar inputs and environmental conditions. The resulting difficulties invariably lead to the generalized comment of “If it works on paper, why does it not work in the field?”.

Unravelling this dichotomy is not easy. Nor is there a single answer that explains all ills, as biological processes are, by their nature, complex. Despite this obvious constraint, the near universal approach seems to be to try and find the one elusive answer. This approach has its merits in that it is usually inexpensive and simplistic enough to allow for easy interpretation. The downside is that undue faith is put in simplistic solutions and concerns about the nutritive value of feedstuffs cannot be answered nor can the dynamic aspects of rate and extent of digestion be examined.

The problem becomes one of understanding the sources of variability and gaining insight into which source of variability gives rise to the greatest cost, or leads to the greatest return. As may be seen from the following quote this concern was identified long ago.

“Meanwhile, experimental inquiry has been increasingly active: the laws of animal nutrition are getting to be better understood, the theories have been put to the test of actual experience. While their value to the farmer has been developed in ways where improvements have become apparent, we are constantly working towards a clearer understanding of the principles of feeding and a more successful application of them to the practice of the farm. It has thus become evident that to meet the demands of physiological chemistry and practical

feeding, the chemist must devise more accurate ways of estimating the nutritive value of feeding stuffs" W.O. Atwater 1891

Dimensions of the Problem

The variation in the rate and extent of disappearance for both fiber (Table 1) and starch (Table 2) that we have observed in our laboratories are large enough to cause major problems when designing dairy rations. To put it in perspective, it was estimated that a one percent (1%) increase in ruminal dietary fiber digestion will lead to a 0.57 kg increase in milk production in early lactation[1]. Looking at the problem from another angle, it has been estimated that economic value of testing feeds equates to $0.27 per cow per day if it is done correctly[2]. There is no question that information on ruminal digestion and nutritive value of feedstuffs is valuable for practicing nutritionists. However the problem becomes one of capturing the various characteristics of digestion in a manner that is practical in the field.

The possible options for estimation of ruminal digestion are *in vivo*, *in situ* or *in vitro* methods. While *in vivo* methods will probably provide the most accurate estimates of digestion it is both costly and not practical. Both the *in situ* and *in vitro* methods have practical benefits and drawbacks. The major drawback for both methods is that estimates of ruminal digestion characteristics are based on gravimetric measurements of substrate disappearance at set time points. Thus, an incorrect choice of time points can lead to incorrect interpretation of data. In addition, even if the choice of time points is correct, what happens between the points, or the kinetic aspects of digestion, must also be considered (Figure 1). Additional time points can be included in the analysis to provide information on the kinetics of digestion and improve the estimates but the additional samples rapidly turn the methods extremely labor intensive and impractical.

In vitro techniques that estimate digestion kinetics indirectly by measuring gas production are a more viable option. This approach becomes more powerful

when combined with measurements of substrate disappearance at the end of the incubation[3]. Gas production technology allows for a more usable collection of digestion kinetics data and has allowed for a growing body of knowledge that is directly applicable to the feeding programs that are in daily practical field use[4]. The range of data that can be acquired is broad and will no doubt grow over time. This technique also has the limitations intrinsic to an *in vitro* batch culture system; however it can be used to generate both qualitative and quantitative data on the rate and extent of digestion in a relatively practical and inexpensive manner. A review of the possibilities associated with this type of analysis is given below.

Nutritive Value of Feedstuffs

Without any doubt, gas production technology has been and continues to be predominantly used to examine and determine the nutritive value of feedstuffs. Gas production, in this *in vitro* system, arises directly from microbial substrate degradation or indirectly from the reaction of acid end products with the bicarbonate fraction of the buffering system. Therefore, gas production is highly correlated with substrate digestion and is a powerful and proven method to estimate the rate and extent of ruminal OM degradation[5]. Its application to the estimation of various OM fractions (fiber, starch, etc.) appears to be more limited, but it is still a practical approach. Pooled data from a wide variety of forage sources demonstrated a usable correlation between gas production and NDF digested (Gas yield = 0.35 ml/mg of NDF digested; R^2=0.92) [6]. Work on starch digestion revealed that a similar approach can be used when considering this fraction of OM[7]. The authors examined the fermentation of six starchy ingredients and eight corn silages and calculated a relationship that is of as much practical benefit as that determined for NDF (starch degradation (mg/g OM) = -191.6 + 0.303 x starch content + 1.648 x gas production at incubation time t; R^2 = 0.92).

Estimation of Volatile Fatty Acid Molar Proportions

Both the qualitative and quantitative aspects of ruminal VFA production are of keen importance to the nutrition of the ruminant. Measuring these aspects is another matter entirely especially when the constraints of a batch gas system are considered and the complexity of the problem has been identified in the literature[8] .The VFA produced *in vitro* are not subject to ruminal passage rate or absorption nor do VFA concentrations measured *in vitro* necessarily reflect production. Therefore, although valuable, information on VFA production and profile can be easily collected the data is often difficult to interpret. Additional concerns revolve around *in vivo* ruminal VFA fluctuations following feeding and the concomitant changes in the non gluconeogenic to gluconeogenic (NGR) ratio of VFA[9] as these effects cannot be measured *in vitro*. The potential relationships between VFA generated using *in vitro* gas production methods and *in vivo* has been reported in an experiment using mature weathers[10]. This study showed a correlation (R^2=0.61) between *in vivo* NGR and *in vitro* ATP production and time to maximum gas production. More importantly the total VFA, molar percentages of acetate, propionate, n-butyrate and the NGR ratio followed the similar patterns when varying levels of corn were added to the diet and compared both *in vitro* and *in vivo*. The fact that the two systems followed similar patterns is encouraging as gas production methods then offer a valuable and inexpensive alternative to predict ruminal VFA profiles.

Estimation of Ruminal Microbial Protein Synthesis

Microbial biomass is usually the major source of protein for ruminants hence its prediction is of paramount importance. Unfortunately this is not an easy task, as there are no rapid and simple laboratory techniques to estimate microbial protein synthesis[11]. However, the combination of gas production methods and end point substrate degradation measurements provide a reasonable and practical approach to this problem. This approach estimates microbial protein synthesis from the stoichiometric partitioning of degraded substrate between gas production, VFA and microbial biomass[12]. A simplified approach has recently been proposed where microbial biomass production is predicted with the

equation MBP = TSD - (gas volume x SF)[13]. In this equation, MBP represents microbial biomass production, TSD represents true substrate degradability as defined by Goering and Van Soest (1970)[14] and SF represents a stoichiometric factor[15]. Once the MBP figure is known the efficiency of microbial protein synthesis (EMP) can be calculated according to the equation EMP= (TSD - (gas volume x SF))/TSD.

Protein and Fat Affect *In Vitro* Gas Production Estimates

The fermentative characteristics of protein fractions must also be considered when reviewing total gas production. Fermentation of casein, for example produces only 32% of the gas amount produced by carbohydrates[16]. In addition, it is estimated that an increase in CP of one percent (1%) will reduce gas production by 2.48 ml/g of OM. Therefore, it is important to consider and correct for the CP content when comparing gas production from different feedstuffs. Fermentation of protein results in both amino acids and short chain peptides which can end up either in microbial biomass or in fermentation end products such as VFA, CO_2, or NH_3. As the breakdown of proteins takes place in the first few hours and is not linear, it is perhaps incorrect to draw inferences from gas production measurements relative to protein degradation rates or extents. One possible suggestion to get around this difficulty is to suppress amino acid incorporation in microbial protein through the use of hydrazine and chloramphenicol[17] but this technique is beyond the practical application of gas production technology as presented here.

Fats have long been added to ruminant diets as a method of increasing the energy density of the diet. Debate as to the negative effects of fats on ruminal digestibility is found throughout the literature[18] [19]. The addition of palmitate, stearate, or oleate triglycerides to *in vitro* incubations did not affect total VFA production, acetate, propionate, and the acetate to propionate ratio [20] hence it may well be possible to uses a gas system to demonstrate either positive or

negative VFA changes derived from fat additions. Recent experiments have reviewed the addition of fat on VFA, IVTD, and ammonia-N concentrations using an *in vitro* gas production system[21]. The fat sources utilized were corn oil, tallow or yellow grease provided as triglycerides or potassium soaps. Triglycerides had no major effects on gas production, digestion or VFA production, however all potassium soaps reduced gas production digestion and VFA production. The results suggest that the suspected negative effects of fat on ruminal fermentation and digestion cannot be generalized and are dependent on the form supplied with triglycerides having smaller effects than the corresponding free fatty acids.

Estimation of Dry Matter Intake

One of the primary limitations to the estimated nutritive value of forage is the constraint of intake; hence its accurate prediction has long been of interest. Some researchers have reported on the use of gas production technology to predict DMI[22, 23]. The gas production of various portions of plant tissue have been examined and it was concluded that a combination of gas produced between 4-8 hours when taken in conjunction with substrate degraded from 24 hours on results in a better prediction then that of using gas production alone. Restriction of gas production to the NDF fraction alone explained 82% of the variation in intake.

Managing Reality Through Practical Application of Gas Production Technology

As discussed above, gas production technology combined with substrate degradation measurements may be used as a valuable and practical tool to evaluate ruminant diets. In our experience, the greatest value is obtained when the system is used on a risk assessment basis on the total mixed ration (TMR) rather than the individual dietary ingredients. The fact that it is a batch system precludes the correct interpretation of shifts in microbial yields but when viewed from the point of applicability in the field the speed of analysis and lower cost makes up for this limitation. Data from TMR samples (n=100) from Ontario,

Quebec, British Columbia, Pennsylvania, Massachusetts, and New York are presented in Table 3. The gas production profile data was analyzed using a two pool logistic equation[24] and demonstrates that fermentations can be broken into a fast and slow fraction. Neither of these two pools is chemically homogeneous as fermentation of all OM constituents occurs simultaneously. In general our experience indicates that the fast fraction contains mainly starch and soluble fiber while the slow fraction contains cellulose, hemicellulose and slow starch. While these observations may annoy those looking for an analysis that reflects the fermentation of chemically identifiable and measurable feed fractions it does approximate the nature of ruminal fermentation. Therefore it represents a practical means to evaluate rations, predict the productive response and make sound nutrition decisions that affect both animal productivity and ultimately economic costs and profitability.

As was pointed out earlier, the cost of ignoring the inherent sources of variation in the ration can be substantial. Theoretical economic costs have been cited as being $0.28 [2]. Our experience has shown that this cost is in fact higher. As may been seen in Table 4 only the variation in the rate of NDF disappearance can give rise to variations in cost in the CDN$0.68 (USD$0.58) range. Given today's low milk prices, these are not insignificant numbers and practicing nutritionists would be wise to address the inherent problems.

Table 1 Variation in NDF Disappearance levels

Crop	n	OHr NDF	6Hr NDF	48hr NDF
Haylage	525	46 +/-7.2	36.8 +/- 8.1	17.9 +/- 5.2
Grass Silage	26	55.5 +/- 7.1	45.7 +/-7.9	21.6 +/- 7.4
Rye Silage	25	51.7 +/- 6.6	40.3 +/- 10.4	17.0 +/-8.5
Corn Silage	838	42.2 +/-8.6	35.9 +/-8.0	18.8 +/-4.8
Hay	22	53.0 +/- 9.8	41.7 +/-12.3	23.8 +/-8.4

Table 2 Variation in *In Situ* Starch Disappearance

Ingredient	N	6 Hr	24 Hr
Flaked Corn	20	6.8 – 59.9%	49.1 – 93.3%
H.M Corn	100	7.1 – 89.9%	28.4 – 97.8%
Corn Meal	30	12.9 – 70.2%	33.7 – 93.8%
Corn Silage	350	20.4 – 50.2%	33.7 – 98.4%

Table 3 TMR Characteristics from Gas Analysis N=100

Measurement	Ave	Std. Dev.
Dry Matter %	48.02	5.49
Starch %	28.95	4.67
NDF %	32.3	3.16
Ash %	7.26	1.03
Size of fast pool ml	41.19	18.63
Rate of fast pool %/hr	24.1	7.08
Lag time hr.	0.013	0.002
Size of second pool ml.	48.32	12.36
Rate of second pool %/hr	4.2	0.01

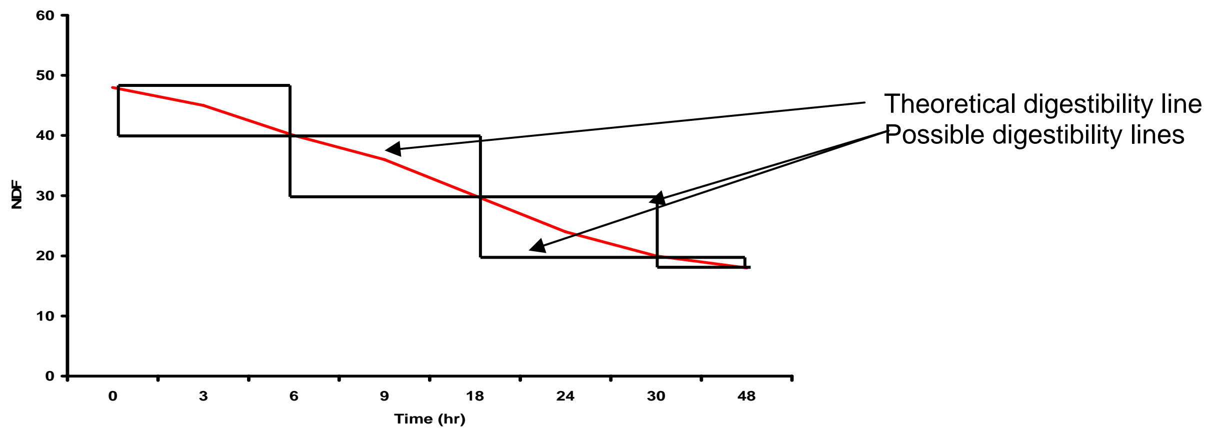
Table 4 Feeding cost per cow per day for test farm using corn silage with either 3 or 7%/hr C:B2 digestion rate

Date	Feed cost (CA$/cow/day)
September[1]	3.14
October[1]	3.07
November[2]	3.63
December[2]	3.75
January[2]	3.71

[1]Ration based on the corn silage with C:B2 digestion rate of 7%

[2]Ration based on the corn silage with C:B2 digestion rate of 3%

Figure 1 Interpreting NDF Disappearance Data



References

[1] Allen, M., Oba,M., 1996 Fiber digestibility from forages> Proc 57th Minnesota Nturition Conf. and Provita Technical Symposium. Extension Special Programs, Univ of Minnesota. P.O. Box 64780, St Paul MN 55164-0780

[2] St. Pierre N.R., Harvey, W.R., 1986. Uncertainty in composition of ingredients and optional rates of success for a maximum profit total mixed ration. J Dairy Sci. 76:3160-3178.

[3] France J., Dijkstra J., Dhanoa M. S., Lopez S., Bannink A. 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: derivation of models and other mathematical considerations. Br. J. Nutr. 83, 143-150.

[4] Pitt, R.E., Cross, T.L., Pell,A.N., Scholfield,P., Doane, P.H., 1999. Use of in vitro gas production models in ruminal kinetics. Mathematical Biosciences 159: 145-163.

[5] Dijkstra J., Kebreab E., Bannink A., France J., and Lopez S. 2005. Application of the gas production technique to feed evaluation systems for ruminants. Anim. Feed Sci. Technol. 123-124, 561-578.

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

[7] Chai,W.Z., van Gelder, A.H., Cone, J.W. 2004. Relationship between gas production and starch degradation in feed samples. Anim.Feed Sci. Technol. 114:195-204

[8] Dijkstra,J.,1994 Simulation of the dynamics of protozoa in the rumen. Br.J.Nutr. 72,679-699

[9] Robinson, P.H., Tamminga,S., Van Vuuren, A.M., 1986. Influence of declining level of feed intake and varying proportion of of starch in the concentrate on rumen fermentation in dairy cows. Livest. Prod. Sci. 15,173-189

[10] Rymer, C., Givens, D.I., 2002, Relationship between patterns of rumen femrentatio measured in sheep and in situ degradability and the in vitro gas production profile of the diet. Animal Feed Science and Technology 101: 31-44.

[11] Menke,K.H., Raab,L., Salewski,A.,Sreingrass,H., Fritz,D., and Schneider,W., 1979. The estimation f the digestibility and metabolisable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor. J.AgricSci. (Camb.) 93:217-222

[12] Blummel, M. Predicting the partitioning of fermentation products by combined in vitro gas volume-substrate degradability measurements:oppourtunities and limitations.In : Gas production: Fermentation Kinetics for Feed Evaluation and to assess Microbial activity. British Society of Animal Science, Penicuik,Midlothian, pp 48-58

[13] Grings E.E., M. Blummel. And K.H. Sudekum. 2005. Methodological considerations in using gas production techniques for estimating ruminal microbial efficiencies for silage based diets. Anim. Feed Sci. Technol. 123-124, 527-545.

[14] Goering, H.K.,Van Soest, P.J., 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). In: Agricultural Handbook No. 379. USDA-ARS, Washington, DC, USA.
[15] Blummel, M., 2000. Predicting the partitioning of fermentation products by combined in vitro gas volume–substrate degradability measurements: opportunities and limitations. In: Gas Production: Fermentation Kinetics for Feed Evaluation and to Assess Microbial activity. British Society of Animal Science, Penicuik, Midlothian, pp. 48–58.
[16] Cone. J.W., A.H. van Gelder, 1998 Influence of protein fermentation on gas production profiles. Animal Feed Science and Technology 76:251-264
[17] Broderick, G.A., 1987. determination of protein degradation rates using a rumen In Vitro system containing inhibitors of microbial nitrogen metabolism. Br. J. Nutr. 58: 463-476.
[18] Palmquist, D.L., Conrad, H.R., 1978. High fat rations in dairy cows. Effects of feed intake, milk, and fat production, and plasma metabolites. J.Dairy Sci. 61.890-901.
[19] Estrdige,M.L., Firkins,J.L., 2000. Feeding tallow triglycerides of different saturation and particle size to lactating dairy cows. Anim. Feed Sci. Tech. 83:249-259.
[20] Chalupa, W., Rickabaugh, B., Kronfeld, D.S. Sklan,D., 1984. Rumen fermentation in vitro as influenced by long chain fatty acids. J Dairy Sci. 67. 1439-1444.
[21] Getachew, G., DePeters, E.J., Robinson, P.H., Taylor, S.J. 2001. In vitro fermentation and gas production: influence of yellow grease, tallow, corn oil, and their potassium salts. Anim.Feed Sci. Tech. 93: 1-15.
[22] Blummel M., Orskov, E.R. 1993: Comparison of an in vitro gas production and nylon bag degradability of roughages in predicting feed intake in cattle. Anim. Feed Sci. Technol. 40:109-119.
[23]Blummel, J.W. Cone, A.H. Van Gelder, I. Nshalai, N.N. Umunna, H.P.S. Makkar, K. Becker. 2005. Prediction of forage intake using in vitro gas production methods: Comparison of multiphase fermentation kinetics measured in an automated gas test, and combined gas volume and substrate degradability measurements in a manual syringe system. Animal Feed Science and Technology. 123–124:517–526.
[24] Schofield P, R. E. Pitt, A. N. Pell. 1994. Kineticks of fiber digestion from in vitro gas production. J. Anim. Sci. 72:2980-2991.