Feedstuffs, December 13, 2010 **Feedstuffs** Reprint

Gas fermentation: A promising diagnostic tool

Bottom Line

with
BILL MAHANNA*

ADJUSTING rations to account for digestibility differences among new-crop forages can be a humbling experience. Single-time-point neutral detergent fiber (NDF) digestibility (NDFD) values (e.g., 24-hour NDFD, % of NDF) can provide some comparative direction for nutritionists but have limited value in modern ration-balancing software that require digestion rates (Kd) rather than NDFD as feed library inputs.

A series of seminars delivered at the October World Dairy Expo unveiled a new laboratory method called the Fermentrics Gas Fermentation System, which is being offered by Dairyland Laboratories Inc. in Arcadia, Wis., in conjunction with RFS Technologies in Ottawa, Ont.

This method utilizes a rumen-fluid, batch culture, gas fermentation system to which mathematical curve-peeling techniques are applied to differentiate rapidly from slowly fermenting carbohydrate pools. This allows for a more direct approach to estimating carbohydrate (B_1, B_2, B_3) digestion rates.

This column will review the history of *in vitro* gas fermentation methods and share some practical experience in utilizing this tool to troubleshoot dairy feeding challenges.

History

In vitro and *in situ* methods have been employed to characterize rumen fermentation kinetics of starch and fiber by analyzing incubation residues following various incubation times. However, due to their laborious and expensive nature, these methods typically provide a limited number of data points (Chai et al., 2004).

The major disadvantage 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 data interpretation, conclusions and decisions. 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 (Johnston and Tricarico, 2007).

Most laboratories that currently provide NDF digestion rates calculate them from NDF, lignin and single-time-point NDFD values inputted into what is commonly referred to as the Van Amburgh Rate Calculator (Van Amburgh et al., 2003; CVAS, 2010).

Alternatively, the rate and extent of organic matter degradation, employing hundreds of data points, can be determined with *in vitro* gas production systems based on monitoring gaseous fermentation products (carbon dioxide and methane) of microbial metabolism and the additional carbon dioxide produced upon buffering microbial-produced short-chain fatty acids (SCFA) — primarily acetate and butyrate.

Its application to the estimation of various organic matter fractions (NDF, starch, soluble carbohydrates) does have limitations. However, forage NDF research (Doane et al., 1997) exemplifies the strong correlation between gas production and NDF digested (gas yield = 0.35 mL/mg of NDF digested; R^2 = 0.92; Johnston and Tricarico, 2007).

Researchers who work with *in situ* methods and continuous-flow fermenters are typically critical of batch fermentation systems. Yet, with meal-fed ruminants, rumen fermentation could be considered a repeated batch fermentation system where both avoiding rapid fermentation and minimizing an excessive delay of fermentation of slowly fermented feeds are important. Those factors should be as reliably estimated with a gas fermentation system as with a continuous fermentation system (Owens,

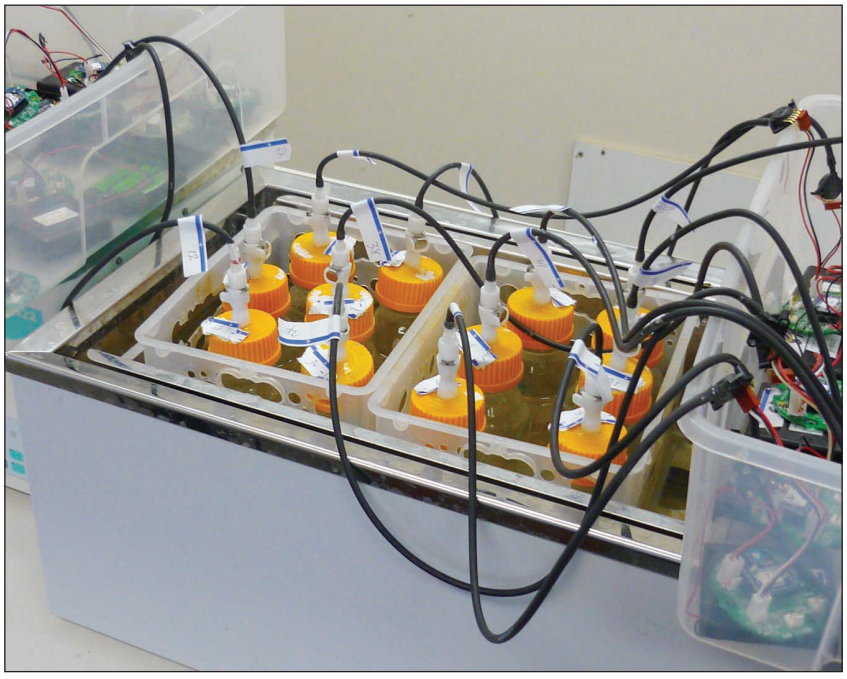


Photo: Robin Johnston.

FIELD TOOL: The RFS Technologies gas fermentation system has contributed to the practical, on-farm utility of gas fermentation data.

*Bill Mahanna (Ph.D., Dipl. ACAN) is a collaborative faculty member at Iowa State University and a board-certified nutritionist for Pioneer Hi-Bred, a DuPont business, based in Johnston, Iowa. To expedite answers to questions concerning this article, please direct inquiries to *Feedstuffs*, Bottom Line of Nutrition, 12400 Whitewater Dr., Suite 160, Minnetonka, Minn. 55343, or e-mail comments@feedstuffs.com.

2010).

Interest in gas fermentation as an analytical tool dates back to the early 1940s, when gas production from various feedstuffs was measured with a manometer connected directly to rumen-cannulated Merino sheep (Quin, 1943).

Menke et al. (1979) experimented with gas production measured in closed, calibrated syringes (the Hohenheimer Futterwert Test) and proposed feedstuff energy equations based on the assumption that accumulated 24-hour gas production is proportional to the amount of digested carbohydrate. One of the challenges with the Menke approach was the variability introduced from friction differences between syringes.

Pell and Schofield (1993) further refined gas fermentation methods by publishing research on computerized gas production using individual vessel pressure sensors to relay gas pressure data. A more controlled system in conjunction with digestion kinetics analyzed by two-pool logistic models (curve-peeling) techniques (Schofield et al., 1994; Schofield and Pell, 1995) further allowed the gas fermentation to be divided into a "fast pool" (primarily B_1-starch and B_2-soluble fiber) and a "slow pool" (primarily B_3-insoluble available fiber).

It should be noted that these pools are not homogeneous because there can be both slow and fast pools within each carbohydrate fraction (e.g., the slow pool may contain some slowly digested starch). This fact may annoy those looking for an analysis that reflects the fermentation of chemically identifiable and measurable feed fractions, but it does approximate the nature of ruminal fermentation and provides a practical means to evaluate rations, predict the productive response and make sound nutrition decisions that affect both animal productivity and, ultimately, economic profitability (Johnston and Tricarico, 2007).

Blummel et al. (1997) provided additional insight to this methodology by publishing research on the relationship between gas from SCFA production and microbial biomass yield — the other important fermentation end product.

He found an inverse relationship between gas production and microbial biomass yield when the variables were related to a given unit of truly degraded substrate. This is due to higher gas production when SCFAs such as acetate are produced compared to the adenosine triphosphate (ATP) energy available for microbial growth when propionate is produced.

Blummel et al. proposed a partitioning factor (PF), which is the ratio of truly degraded substrate to gas volume produced. Interestingly, forages with a high PF (e.g., low gas production per unit of truly degraded substrate) exhibited higher intakes. This dry matter intake prediction model included the rate and extent of 24-hour gas production along with PF and accounted for 84% of the variation in the intake of 54 forages in their study.

A limitation of the gas fermentation methods is that without knowing which volatile fatty acids (VFAs) are being produced, the energy coming from the feed cannot be accurately predicted. Several researchers have employed gas chromatography to detail VFA production and, in an attempt to predict ATP generation from silages, analyzed gas fermentation methods.

More recently, Chai et al. (2003) published equations for starch feed ingredients and corn silage describing the relationship between gas production and measured starch degradation. This allows for redefining the fast pool into B_1 (starch) and B_2 (soluble fiber) for better defining feedstuff kinetics in ration-balancing software.

ANKOM Technology further streamlined the method by releasing the ANKOM^{RF} Gas Production System, which included RF pressure sensor modules and computer interface/software (ANKOM, 2010). Gas fermentation systems, while popular among European researchers, exist in only three or four research laboratories in North America.

Jay Johnston, chief executive officer of RFS Technologies and Ritchie Feed & Seed in Ottawa, Ont., became intrigued with the gas fermentation system as a tool for evaluating rations and the variability among feed ingredients. He has spent the past 15 years developing, refining and field testing an online system (Photo), which, when combined with wet chemistry and gas chromatography, has contributed immensely to the practical, on-farm utility of gas fermentation data.

The commercial release of Fermentrics by Dairyland Laboratories (2010) and RFS Technologies (2010) marks the first time field nutritionists have had ready access to gas fermentation data for use as a diagnostic tool.

Using gas fermentation data

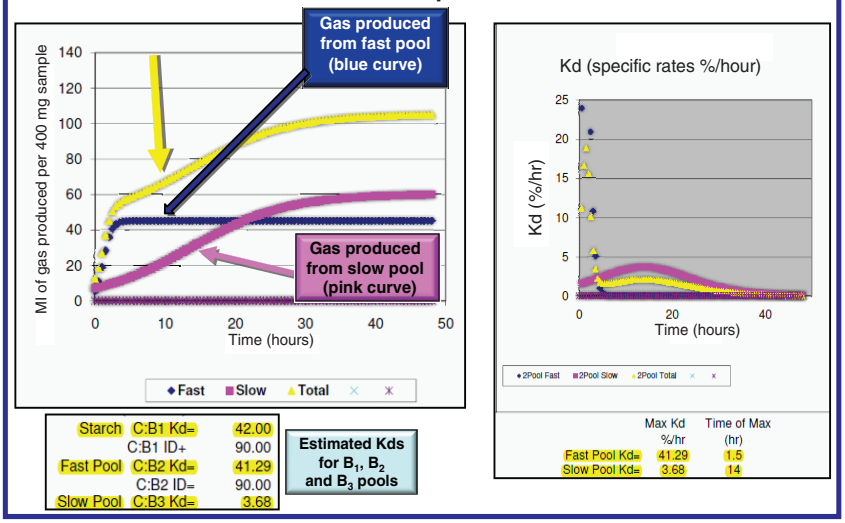
Johnston and Tricarico (2007) reported

Effect of year on ruminal fermentative characteristics of corn silage samples (± standard error) determined using *in vitro* gas production

	Year			
Item	2003	2004	2005	2006
Number	62	17	101	41
Fast pool size, mL	40.9 ± 1.12[a]	35.9 ± 2.14[ab]	39.5 ± 0.88[a]	32.0 ± 1.34[b]
Fast pool rate, mL/hour	25.9 ± 1.26	25.6 ± 2.40	27.0 ± 0.99	24.0 ± 1.51
Slow pool size, mL	50.1 ± 1.41	46.9 ± 2.70	49.3 ± 1.10	45.0 ± 1.67
Slow pool rate, mL/hour	4.37 ± 0.10[a]	4.52 ± 0.19[a]	4.13 ± 0.08[ab]	3.65 ± 0.13[b]

[a,b]Means within a row with a different superscript differ (P < 0.05).
Source: **Johnston and Tricarico (2007).**

Example of Fermentrics gas fermentation data from a total mixed ration for a group of high-producing dairy cows experiencing milk fat depression



gas production data (Table) of corn silages harvested across the northeastern U.S., Ontario and Quebec from 2003 to 2006. Their data clearly illustrate the effects genetics and the growing environment can have on corn silage digestion kinetics.

I have several years of experience using gas fermentation data generated by RFS Technologies. Perhaps the first lesson learned was that differences among feedstuffs do not reside only in the total amount of gas produced but in the relative shifts in pool sizes, specific rates and the relative time for the two pools to reach maximum rates.

The Figure shows Fermentrics output from a total mixed ration (TMR) for a high-producing dairy cow group experiencing milk fat depression while being fed 2009 forage and high-moisture corn.

Field experience has shown that herds are typically in an acidosis situation, with all of the expected outcomes, including hoof problems, manure inconsistency and milk fat depression when B_1 Kd rates exceed 25% per hour, B_3 rates are less than 5% per hour and "time-to-max" rates for the slow versus fast pools exceed 10 hours (Johnston, 2009).

The nutritionist for this herd had an idea of how to correct the situation, but the information in the Fermentrics report gave him added confidence to reduce levels of the highly fermentable high-moisture corn (to slow down the B_1 rate) and supplement the ration with a highly digestible soluble fiber source (to increase the B_3 rate). These changes resulted in increasing milk fat levels from 3.2% to 3.7%.

Field experience with hundreds of herds experiencing production challenges indicates that the vast majority of problem herds are fed a TMR with an excessively fast "fast pool" and a relatively slow "slow pool" (Johnston, 2009).

Interpretation of gas fermentation data should not be limited to reviewing only the Kd values of the B_1 and B_3 pools. I recently worked with a herd that had just started feeding new-crop 2010 corn silage and was experiencing low intakes, stiff manure and reduced milk production.

The Fermentrics analysis showed extremely high gas production in the TMR, indicating that the cause of the excessively fast fast pool was primarily due to the B_2 (soluble fiber) pool producing lots of methane and carbon dioxide gas (along with acetate) from rapidly digested soluble fiber rather than from excessively fermentable starch (B_1).

Supplementing this TMR with additional soluble fiber sources would only result in the production of more gas. In this situation, more energy from propionate (whose pathway does not produce gas) was needed to drive energy for improving milk production.

This TMR also displayed more than a 10-hour difference in "time to max" between the fast pool and the remaining slow (B_3) pool, further suggesting that the ration needed a more intermediate source of energy from either dry corn or, perhaps, highly digestible alfalfa hay.

The herd did respond to the addition of starch and the removal of some mature, high-dry matter alfalfa silage. A possible explanation for the success of this ration change is that the soluble fiber in the ration (relatively new-crop corn silage) was more available than expected, and conversely, the starch was not as available as assumed. Conducting a fecal starch analysis might be further warranted to determine if excess starch was escaping digestion from either poor corn silage processing or too short a time in fermented storage.

In challenging field situations, Fermentrics provides consulting nutritionists with data they can show producers to help them understand why they are experiencing production issues and also to help convince the producer why the recommended course of action must be implemented. It has also proven helpful to conduct a TMR gas fermentation analysis when cows are performing up to expectations to create a benchmark for future reference in case production should falter.

The Bottom Line

Gas fermentation data, like those available from the Fermentrics Gas Fermentation System, provide a directly measured estimate of the carbohydrate (B_1, B_2, B_3) digestion rates needed to more accurately populate feed libraries in newer ration-balancing software.

Insight can also be gained (and benchmarks established) to avoid the nutritional perils of excessively rapid or excessively low rates (and extents) of carbohydrate fermentation.

Now that gas fermentation has exited the research lab and is available to consulting nutritionists, interpreting data and relating conclusions to practical on-farm solutions will require time and experience no different from what's required following the introduction of other analyses such as physically effective NDF, soluble protein, NDFD or kernel processing scoring.

My field experience with gas fermentation suggests that it can be a powerful diagnostic tool to assist nutritionists in making data-driven ration adjustments.

References

ANKOM. 2010. www.ankom.com/product/ankom-rfs-gas-production-base-kit.aspx.

Blummel, M., H. Steingass and K. Becker. 1997. The relationship between *in vitro* gas production, *in vitro* microbial biomass yield and 15N incorporation and its implications for the prediction of voluntary feed intake of roughages. Br. J. Nutrition. 77:911-921.

Chai, W.Z., A.H. van Gelder and J.W. Cone. 2004. Relationship between gas production and starch degradation in feed samples. Animal Feed Science & Technology. 114:195-204.

CVAS. 2010. www.foragelab.com/resources/feedReports.cfm.

Dairyland Laboratories Inc. 2010. www.dairylandlabs.com.

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

Johnston, J. 2009. Personal communication.

Johnston, J.D., and J.M. Tricarico. 2007. Practical implications of fiber in dairy rations: Making use of forage fiber. Proceedings of the 22nd Annual Southwest Nutrition & Management Conference. Feb. 22-23. Tempe, Ariz.

Menke, K.H., L. Raab, A. Salewski, H. Steingass, D. Fritz and W. Schneider. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor. J. Ag. Sci. 93:217-222.

Owens, F.N. 2010. Personal communication.

Pell, A.N., and P. Schofield. 1993. Computerized monitoring of gas production to measure forage digestion *in vitro*. J. Dairy Sci. 76:1063-1073.

Quin, J.L. 1943. Studies on the alimentary tract of merino sheep in South Africa, 7: Fermentation in the forestomachs of sheep. Onderspoor J. of Vet. Sci. & Animal Ind. 2:91-117.

RFS Technologies. 2010. www.ritchiefeed.com/Lab_%20Research/Lab_Research.html.

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

Van Amburgh, M.E., P.J. Van Soest, J.B. Robertson and W.F. Knaus. 2003. Corn silage neutral detergent fiber: Refining a mathematical approach for *in vitro* rates of digestion. Proceedings of the 65th Cornell Nutrition Conference for Feed Manufacturers. Oct. 21-23. ■