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In the 1990s, Diamond V scientists responded to a critical challenge: How could they isolate and study anaerobic fermentation and other complex microbiological processes that take place in the rumen of the dairy cow using a simple, sensitive, and repeatable bench-top procedure?

In response, Dr. Ilkyu Yoon, Joan Butler, and scientists at the Diamond V Research and Innovation Center developed the Rumen Activity Modifier Model (RAMM). Our scientists have used RAMM for nearly 20 years now, going on to refine and adapt the model for many applications.



Both RAMM and Diamond V's Intestinal Activity Modifier Model (IAMM) for monogastric application share the goal of mimicking anaerobic

fermentation and other microbiological activity *in vitro* – in the lab, not in animals – thereby controlling for critical variables that affect the gut environment. We use these models to isolate and study the effects of current and prototype Diamond V products as well as to support manufacturing quality assurance and to answer nutritional health questions from customers.

Batch culture *in vitro* system

RAMM is a batch culture *in vitro* system using rumen fluid from target species of ruminants. It is specifically developed to simulate ruminal anaerobic fermentation activity and to evaluate the effect of rumen modulators. The "benchmark" or standard by which we make comparisons in RAMM is the

Processing & Innovation

RAMM: Modeling rumen fermentation



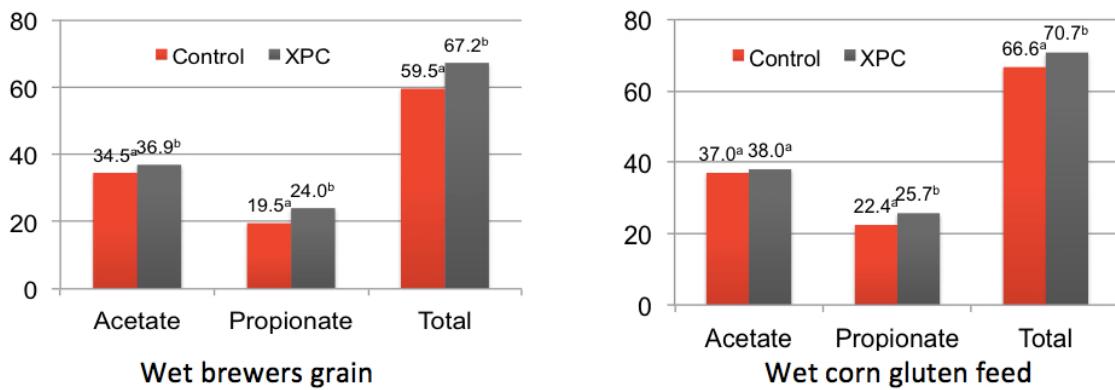
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measurement of production of volatile fatty acids (VFA) using Diamond V Original Products – Original XP™ and Original XPC™.



Initially used for dairy cattle, RAMM also is useful for simulations of microbial fermentation in other ruminants, including beef cattle, goats, sheep, and deer. RAMM is not a digestion assay. Rather, it evaluates the impact of Diamond V products for ruminants on VFA production as a result of manipulated ruminal microbial fermentation, as for example in Figure 1.

Figure 1. Effect of Original XPC on ruminal VFA production (mM) in RAMM on wet brewers grain and wet corn gluten feed as substrates.



Model validation and options

The RAMM procedure was validated by demonstrating VFA shifts similar to those achieved in animal studies using the well-documented rumen modulator monensin. In addition, West Virginia University reported very similar results to RAMM when using a dual-flow continuous culture fermenter, which is a well-accepted rumen model (Miller-Webster, 2002).

Since then, Diamond V scientists have added a wide range of options to the "base model" of RAMM. One of the most useful is applying polymerase chain reaction (PCR) technology to determine the levels of various microbial populations involved in ruminal fermentation.

PCR is a molecular biology tool that can detect and quantify microorganisms in a sample by DNA sequence. Using PCR, we can determine the concentration of specific organisms and total bacteria within a sample, which gives us relative abundance of those organisms in a sample.

When used in conjunction with RAMM, PCR can measure how Diamond V products affect certain microbial populations, such as fiber digesters and lactate utilizers, as well as other organisms of importance. For example, we can compare the performance of Original XPC and our Next Generation dairy technology, NutriTek®, in supporting growth of both fiber digesters and lactate utilizers against control samples (Brainard et al., 2014).

Product testing

When obtaining rumen fluid for RAMM, the diet of the source animal is controlled to match that of a specific feeding strategy (e.g. dairy, beef, deer, or other), which ensures the microbial inoculum has been adapted to a specific forage-to-concentrate ratio and combination of ingredients. In RAMM, studies may be completed with purified substrates like starch and cellulose. However, the use of conventional feed ingredients, including customer TMR (total mixed rations) offers the benefit of testing with more practical diets, resulting in more realistic digestive conditions and more widely accepted results. The challenge with TMR and combinations of typical feedstuffs is that as substrates become more complex, the variability of results increases.

RAMM also is a valuable tool for quality control and assurance of our manufacturing processes and products. For example, RAMM can evaluate shelf life of Diamond V products, function as a screening tool for new product development, and assess the relative effects of current and prototype products during new product development.

Keep in mind...

Ask your Diamond V representative for more information about RAMM so you can understand the model. Like other in vitro models, results from RAMM should be considered as an indication of treatment effect and should not be used to predict in vivo responses.

References

Brainard, A., V. Nsereko, I. Yoon, J. Butler, and M. Scott. 2014. Effects of *Saccharomyces cerevisiae* fermentation products on fiber digesting and lactate utilizing rumen bacteria at neutral and low pH in vitro. Symposium on Gut Health in Production of Food Animals, St. Louis, MO.

Miller-Webster, T., W.H. Hoover, M. Holt, and J.E. Nocek. 2002. Influence of yeast culture on ruminal microbial metabolism in continuous culture. *J. Dairy Sci.* 85:2009-2014.



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