Comparing ruminal activity measurements performed with Gas Endeavour **with literature data**

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The challenge

Disruptive scientific theories and methods usually face the same reaction when first presented to the scientific community: they sometimes seem to contradict the "established" literature.
Measuring ruminal activity by means of the gas production method is no exception: the method has long been established and a large quantity of data on many ruminant feedstock already exists. Measures performed with Gas Endeavour are by far more precise and accurate than those obtained with other instruments. In spite of the higher precision and accuracy provided by Gas Endeavour, some users point out that the values measured with it "are underestimated" when compared to "literature averages". The present application note shows that there is no underestimation at all, but just a difference in conventions to express gas volumes.

How to compare measurements taken with different instruments

In spite of the existence of specific norms on assessing the uncertainty margin of laboratory measures, a constant in all the literature is the lack of such kind of analysis. Most of the papers on gas production from ruminal fluids provide tables reporting the individual values of several measures and their dispersion. On one side, such values represent the "raw" gas production, which is unrealistic because it includes the moisture. From a metrological point of view, checking only the standard deviation of a set of values is not fully correct, since it is a measure of the precision (i.e. repeatability and reproducibility) of the test, but not of its accuracy. The accuracy is especially important when the measured values must be employed for further elaboration –e.g. the energy value of the feedstock as a function of the gas production- since calculations amplify the error of the final result, according to rules derived from theorems. Furthermore, it is common knowledge that gasses vary their volume with temperature and pressure. While the reference temperature is always 39 °C in all ruminal fermentation tests, the atmospheric pressure is highly variable and not controllable. The following example shows how to elaborate correctly "raw" data from the literature, in order to compare them with normalized data obtained with Gas Endeavour. As example of literature data, we took the average of ten measures of gas production from corn silage, 228.4 ml gas / g DM, having a standard deviation equal to ± 20 ml. Such value was published by P.H. Robinson and G. Getachew in A Practical Gas Production Technique to Determine the Nutritive Value of Forages: The UC Davis Approach - California Chapter of the American Society of Agronomy (2005). The paper states that the tests were performed according to Menke and Steingass (1988), using 100 ml glass syringes filled with 30 ml of ruminal fluid and 200 mg DM of sample. The paper does not state which the atmospheric pressure was when the test was performed. We will assume Patm = 101.3 kPa. The following procedure allows to calculate the error or uncertainty of said measures.

1. Uncertainty of measure induced by the gas moisture at 39 °C (312 K).
   From the thermodynamic tables of water vapor:

   $P_{sat.vap.}$ at 310K = 6.230 kPa
   $P_{sat.vap.}$ at 315K = 8.143 kPa

   By linear interpolation to 312 K:

   $\Delta P = (8.143 - 6.230)/5 \times 2 = 0.765$ kPa
   $P_{sat.vap.}$ at 312K = 6.230 + 0.765 = 6.995 kPa

   The percentage of the gas volume occupied by water vapour, G%, is equal to the quotient of the partial pressures:

   $G_{\%} = (6.995 \text{ kPa})/(101.3 \text{ kPa}) = 6.9\%\ \ 7\%$

Effects of added fat on feed degradation. Fats are common supplements used in the diets of lactating dairy cows. Gas Endeavour's high accuracy and repeatability ensure that any difference between the fat-added sample and the control sample is exclusively caused by the effect of fat addition, and not by instrumental uncertainties (e.g. variations of the room temperature and pressure when using syringes, calibration offsets, weak batteries and thermal drift when using pressure sensors).

Conclusion

Gas Endeavour is able to produce in short time large amounts of accurate (1% error margin) and repeatable (3%<C.V.<8%) GP data from *in vitro* tests, providing scientists with easily analysable Excel tables for their research, and commercial dairies with the most reliable information to optimize the ration's composition, and hence the feed cost and milk productivity.

Anti-nutritive factors. Some feeds, such as forage legumes and cottonseed, contain phenols, alkaloids and saponins that have negative biological effects on microbes and reduce microbial growth in rumen. Gas Endeavour's high repeatability, low detection limit and independence of external factors like room temperature and pressure, ensure the measurement of anti-nutritive levels that syringes and barometric instruments are not able to detect.

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Rumen modifiers. Monasin, yeast and yeast fermentation by-products are added often to the diet in order to modify the bacterial populations in the rumen. Gas Endeavour makes it easy to study their effects, by simply incubating feeds in the presence or absence of such compounds. The data from such tests are crucial for optimizing milk production costs and overall productivity in commercial dairy farms.

Feed associative effects. Rations are mixtures of individual feeds, with a multitude of possible combinations. Because of positive associative effects on *in vitro* GP, it is not correct to sum the individual ME of the ration's components. Gas Endeavour allows performing triplicate tests on five different rations at a time.

Monitoring rumen microbial change. In addition to rates and extents of digestion, Gas Endeavour can be used to study substrate-related factors that influence microbial populations in the rumen. This enables manipulation of rumen microflora to increase the utilization of feeds through degradation of fibre and lignin, increasing the efficiency of nitrogen utilization or allowing the degradation of anti-nutritional and toxic components of feeds.

Nutrient synchronization. Carbohydrate and nitrogen sources must be available simultaneously in order to maximize microbial growth. Ruminal ammonia concentrations can be influenced by the degradation rates of carbohydrates and nitrogen-containing compounds. Gas Endeavour offers an opportunity to easily study microbial requirements for nitrogen and carbohydrate in order to enable efficient fermentative activity and accumulation in the rumen.

Plant breeding, biotechnology. Several forage and cereal crops have been genetically modified to increase yield, or produce chemical constituents normally deficient in a particular plant. Forage plants are selected for rapid fibre digestibility. Plants have also been genetically engineered to produce human lysozyme, but it is unclear what effect lysozyme has on microbes in the rumen. Although many genetically engineered plants are intended for human consumption, their by-products will be fed to animals as a means of disposal. Gas Endeavour, having 15 reactors and measurement channels, allows to evaluate large numbers of samples, in order to select in short times those e with the highest feeding values.

Environmental degradation. More than half of the nutrients consumed by ruminants leave the animal unutilized and undigested, and are excreted in faeces, urine and gases. This increases animal production costs as well as environmental impacts, by contaminating surface- and groundwater and contributing to air pollution. Gas Endeavour allows the quick and easy selection of the rations featuring the highest efficiency of feed utilization, which in turn reduces the amount of unutilized nutrients leaving the animal hence, lower feed costs and environmental burden.

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