A new concept to explain

FOR A BETTER UNDERSTANDING OF THE MODE OF ACTION OF LIVE YEAST, NEW MODELS HAVE BEEN DEVELOPED THAT USE THERMODYNAMIC CALCULATION TO EXPLAIN PH STABILISATION IN THE RUMEN. BY R. MONCOULON AND ERIC AUCLAIR.

Today, Emeritus Professor at the Ecole Nationale Supérieure Agronomique of Toulouse, for more than 25 years Professor Moncoulon headed a research team focused on the optimisation of rumen function in dairy cows.

Improved ruminant performance and production have been frequently associated with a similar increase in the energy concentration of the diet. So today, sub-acute ruminal acidosis (SARA) in dairy or beef cattle has become one of the major concerns for farmers.
This metabolic disorder, which affects the performance and welfare of the animals (pica, lameness, diarrhoeas etc.), generally results in a decrease in the pH of the ruminal milieu, due to an accumulation of lactate.
With a fibre-rich diet, i.e. not acidotic, the production of lactate (pka = 3.86) by *S. bovis* bacteria is counter-balanced by the activity of lactate-utilising bacteria such as *M. elsdenii* and *S. ruminantium*, which transform lactic acid into the less acidifying propionic acid (pka = 4.87).
When the ration includes a high proportion of fermentable sugars, the production of lactate is increased, and the lactic acid consuming bacteria are inhibited, resulting in a drop in the rumen pH.
The ability of live yeast to stabilise ruminal pH has often been reported. Williams and Newbold (1990) related this effect to a reduced production or accumulation of lactate when yeast was present in the rumen.
The ruminal milieu is anaerobic; however significant proportions of O_2 enter the rumen during feeding or by diffusion from blood.
The capacity of the yeast to consume O_2 was suggested by Newbold *et al.* (1996) to explain the strengthening of the anaerobic status in the rumen, and consequently the activity of strictly anaerobic bacteria. However, the link between oxygen scavenging and pH stabilisation remains unclear.

yeast's effects in ruminants

WHY THERMODYNAMICS?

How can a few grams of yeast influence ruminal fermentation? Many studies have tried to answer this question, but chemistry, biology or microbiology have their limits, especially in a complex anaerobic environment like the rumen. Oxygen uptake is a specificity of yeast and it reinforces the reducing power of the rumen. But, what is the link between concentration of O_2 and ruminal pH?

In 1982, Marounek and colleagues highlighted the existence of an inverse relationship between pH and Eh (redox potential) of the digestive content in goats: a low pH corresponds to a high Eh and vice-versa. By scavenging O_2 and thus reinforcing the anaerobic character of the rumen, we assume that the yeast has an indirect influence on the pH. For that purpose, measurement of the O_2 concentrations in the ruminal contents appears determinant.

Specific probes exist that enable detection of negligible traces of O_2, but the ruminal milieu is so anaerobic that the most powerful probes cannot quantify these traces precisely. As direct quantification of O_2 is difficult in practice; the originality of the proposed approach is to estimate the quantity of O_2 in the rumen (also called fugacity of oxygen) indirectly from pH and Eh measurements, according to the second law of thermodynamics.

Using the Nernst equation at a temperature of 39°C, the oxygen fugacity [log $f(O_2)$] can be expressed as:

$$\log f(O_2) = 64.59\ Eh + 4\ pH - 78.60 \quad (1)$$

WHAT IS REDOX POTENTIAL?

Oxidation and reduction reactions are defined as reactions in which electrons are transferred between reactants and products. All these reactions (and they are numerous in the rumen) involve an oxidising-reducing couple (Redox) whereby a species accepting electrons is reduced and that donating electrons is oxidised.

The redox potential, generally reported as Eh, represents the force of the system exchanging electrons. It is the potential (E in volts) generated between a platinum electrode and the Standard Hydrogen Electrode (E = 0.00 volts). Since in practical conditions, the latter is unstable, a reference electrode (Calomel or Ag/AgCl) is currently used and the E measured must be corrected to obtain the "true" Eh value. The Eh of an oxidising medium is > 0 and is < 0 for a reducing medium.

In normal conditions, the ruminal milieu is strictly anaerobic, with a markedly negative redox potential, generally between –160 mV and –220 mV. It reflects a strong reducing potential due to the quasi-absence of oxygen, conditions favourable to cellulolytic bacteria and thus fibre degradation.

MEASUREMENT OF PH AND EH IN THE RUMEN

The pH and Eh measurements were performed with specific electrodes connected to a digital pH meter. For thermodynamic considerations, it is important to preserve the ruminal conditions, i.e. the integrity of the gas mixture in equilibrium with the liquid phase.

A continuous measuring and sampling system was set up by Marden *et al.* (2005) requiring cows to be fitted with a permanent ruminal cannula. Rumen fluid is continuously pumped out by means of a peristaltic pump and collected in hermetic cells equipped with pH, Eh and temperature electrodes. This system prevents any gaseous (air) contaminating the ruminal sample. In addition, collection of ruminal liquid was performed at the outlet (*Figure 1*) for determination of volatile fatty acids and lactic acid.

The kinetics of pH and Eh in the ruminal milieu was established in cows receiving either a fibre-rich or a starch-rich diet. The period of measurement began one hour before feeding until eight hours post-feeding. In dry dairy cows (*Figure 2*), the fibre-rich diet is characterised by high pH values and low Eh values of the ruminal content while a low pH and a high Eh are observed with the starch-rich diet. In order to study the mode of action of live yeast (as Sc 47, Lesaffre, France), the cows were fed a

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FIGURE 1- CONTINUOUS SAMPLING AND MEASUREMENT IN THE ABSENCE OF ANY GASEOUS CONTAMINATION FROM MARDEN *ET AL* (2004)

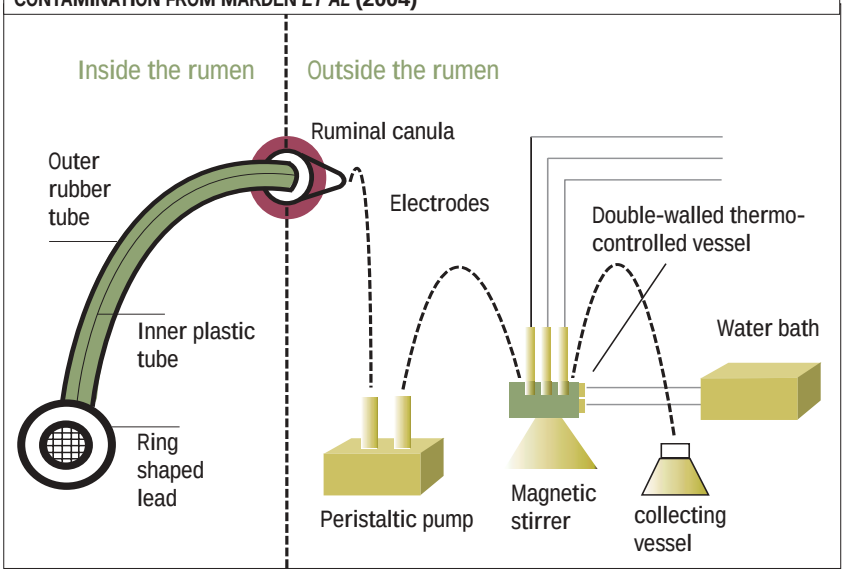
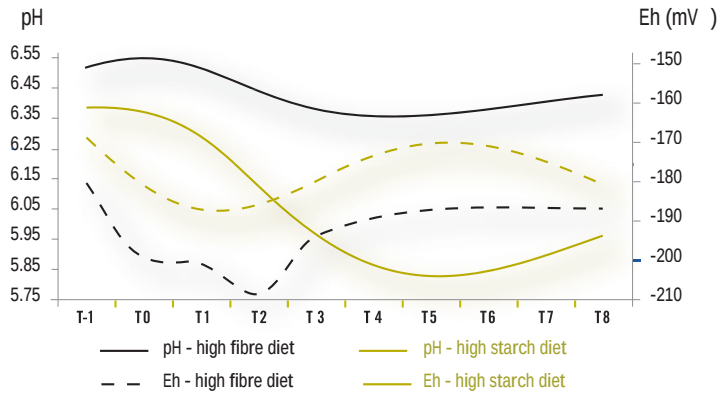


FIGURE 2- DIET DEPENDENT KINETICS OF pH AND eH IN THE RUMEN



high-starch diet, inducing latent acidosis and a treatment diet, consisting of the control diet supplemented with yeast. The experimental design can allow the study of diet, dose and strain effects.

LIVE YEAST STABILISES RUMINAL PH

As mentioned previously, the oxygen fugacity [log $f(O_2)$] can be calculated from simultaneous measurements of pH and Eh. So, the comparison between log $f(O_2)$ values obtained from the two diets (supplemented or not with live yeast), confirms O_2 consumption by the yeast. The great interest of developing this thermodynamic concept is to establish a link between the O_2 concentration in the rumen and the lactate/propionate ratio, which controls SARA. In the rumen, lactate transformation to propionate is dependent upon the activity of lactic acid-utilising bacteria. Furthermore, it involves two molecules of NAD+ coenzyme that participate in a conjugated redox couple for exchange of hydrogen ions and electrons. The reaction illustrating the stoichiometry of lactate conversion to propionate via the acrylate pathway is shown below:

$$\underset{\text{(Lactic acid)}}{C_3H_6O_3} \rightleftharpoons \underset{\text{(Propionic acid)}}{C_3H_6O_2} + {}^1/_2\, O_2 \qquad (2)$$

The log $f(O_2)$ value, necessary to maintain lactate and propionate in equilibrium (39°C and 1 atm) can be calculated by using lactate and propionate concentrations in the rumen, the law of mass action and by integrating the Gibbs free energy of these compounds, according to the equation:

$$\log f(O_2) = 2 \log \{ [\text{lactate}] / [\text{propionate}] \} - 50.16 \qquad (3)$$

From equation (3), at equilibrium log $f(O_2) = -50.16$. When decreasing the $f(O_2)$ in the ruminal milieu (more reducing power), the formation of propionate from lactate is favoured. On the other hand, increasing the $f(O_2)$ in the rumen (less reducing power) limits the formation of propionate thereby allowing lactate accumulation. Consequently, if live yeast affects the oxygen fugacity, it will influence first of all the Eh, then the lactate/propionate ratio and finally the pH.

FERMENTATION EFFECTS

The results obtained in recent studies in dairy cows confirm this concept and clearly show the aptitude of the live yeast to favour the transformation of lactate into propionate and in doing so, demonstrate its capacity to stabilise the ruminal pH. This will be presented in an upcoming issue of *Feed Mix*.
This approach illustrates the importance of developing new animal tools for studying the mode of action of feed additives. The interest of this work is not only restricted to the yeast because pH and Eh measurements and thermodynamic calculations can be used also for a better understanding of the mysterious elements of ruminal metabolism. <-

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