Preventing rumen

For a number of years now, there have been groups dedicated to educating the world about the benefits of yeast in diets for ruminants, but until recently, their efforts were hampered by a relative lack of knowledge about why it worked. In the background, however, rumen microbiologists have been working hard to accumulate the data and now they believe they have filled in many of the gaps.

The transition to solid food is a stressful period for lambs. Failure to establish a good rumen microflora at this stage can lead to problems later on.

By Sarah Mellor

Ten years ago, Lallemand Animal Nutrition and the French national research institute, INRA's microbiology unit began a partnership to investigate the *in vivo* mode of action of yeast in the rumen and to look for potential new strains of yeast. The microbiology work at INRA is led by Dr. Frédérique Chaucheyras-Durand.

The project was divided into two parts: to study the interactions between yeast and the rumen microflora, using pure cultures and by developing in vivo animal models; and secondly, to look for ways to modulate digestive ecosystems, primarily the rumen and human colon in the interests of limiting gas production, especially methane production in cattle. To do this, it was necessary to gain a better understanding of the complex ecosystem that develops in the ruminant animal.

This has been helped by more recent developments in biotechnology- whereas in the past, only those microbes that could be grown in vitro could be identified, now, all the microorganisms can be identified and quantified by means of their DNA.

The rumen microflora

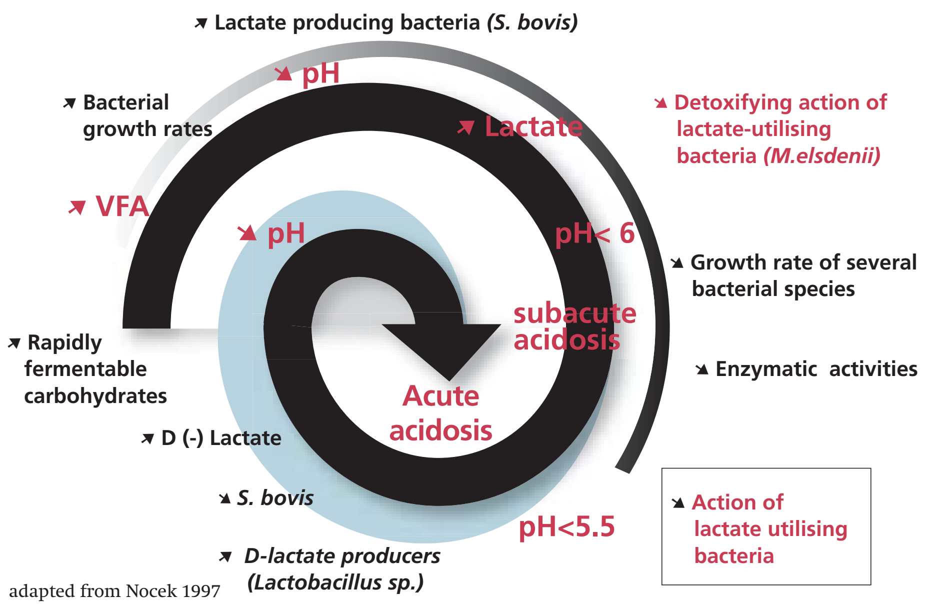
In ruminants, the rumen is the main site of degradation and fermentation of dietary components. Several anaerobic communities of microbes- bacteria, fungi and protozoa- are responsible for digestion, producing the volatile fatty acids acetate, propionate and butyrate, which are the ruminant's main source of energy; gases methane and carbon dioxide, which are eructated; and ammonia, which is partly excreted and partly used as a nitrogen source in microbial protein production. To date, more than 200 species of rumen bacteria have been isolated and are classified by their substrates. Most are associated with the solid phase, where adhesion of bacterium to substrate is an important step in the hydrolytic process. Rumen bacteria far outnumber the protozoa and fungi present in the rumen, but interrelationships between three types of microorganisms act to digest different types of feed components, through various interactions, such as synergy, cooperation, commensalism, antagonism and predation. The interaction between microorganisms is responsible for the rumen's high efficiency in digesting fibrous material, but because of its delicate balance, digestive problems do still occur, disrupting health and decreasing performance.

The acidosis spiral

To support the high growth rates and milk production levels of beef and dairy cattle, respectively, it has become common to feed these animals on high energy, high protein concentrate diets, based on high starch ingredients, such as barley. Unfortunately, high levels of barley in the diet are associated with acidosis, due to the high content of rapidly fermentable carbohydrates. Nocek (1997) described the sequence of events leading to latent, subacute and acute acidosis. This is represented diagrammatically in *Figure 1*. The rapid rate of fermentation promoted by the presence of highly soluble carbohydrates such as starch promotes rapid production of volatile fatty acids (VFA's), reducing the pH and encouraging growth of the bacterial species that thrive under the more acidic conditions. Of these are the lactate producing bacteria, such as *Streptococcus bovis*, further decreasing rumen pH. The increase in lactate concentrations promotes growth of lactate utilising bacteria such as *Megasphaera elsdenii*. Once the pH has dropped below 6, the

problems with yeast

Figure 1 - Step-by-step mechanism for the occurrence of acidosis (adapted from Nocek, 1997).



adapted from Nocek 1997

Figure 2 - The pH drop induced by concentrate feeding is somewhat reduced by feeding yeast.

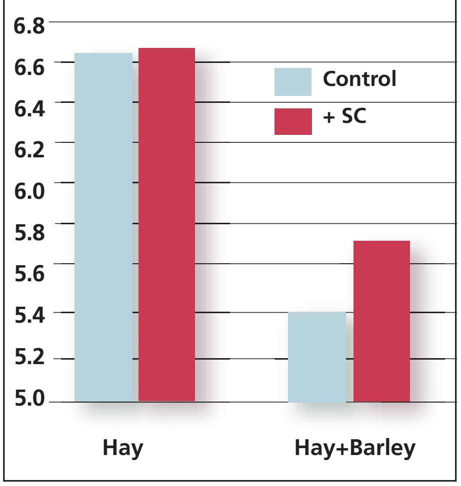
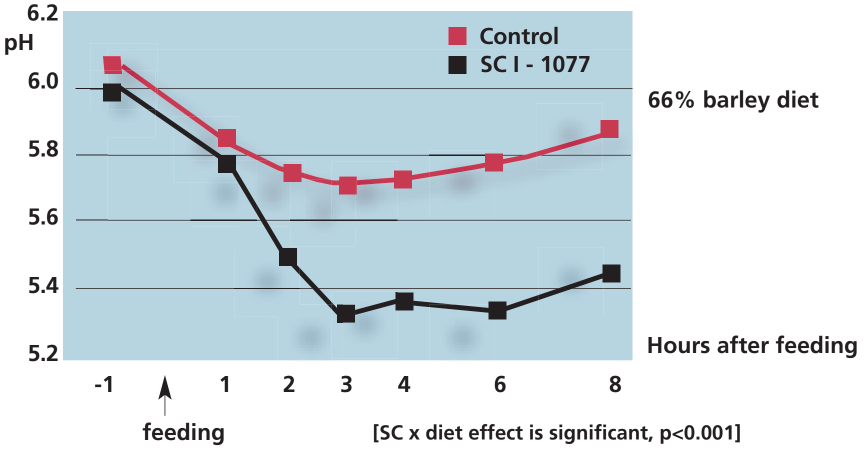


Figure 3 -Yeast stabilises the daily variation in rumen pH.



[SC x diet effect is significant, p<0.001]

growth rates of several species of bacteria falls, reducing enzymatic bacterial digestion of the feed and the detoxifying activity of *M. elsdenii*. This condition is subacute acidosis. If this condition is not corrected, the pH can drop further. Below pH 5.5, *S. bovis* activity decreases, but conditions are now ideal for the producers of D-lactate- the lactobaciili. Production of D-lactate decreases pH yet further, to acute acidosis.

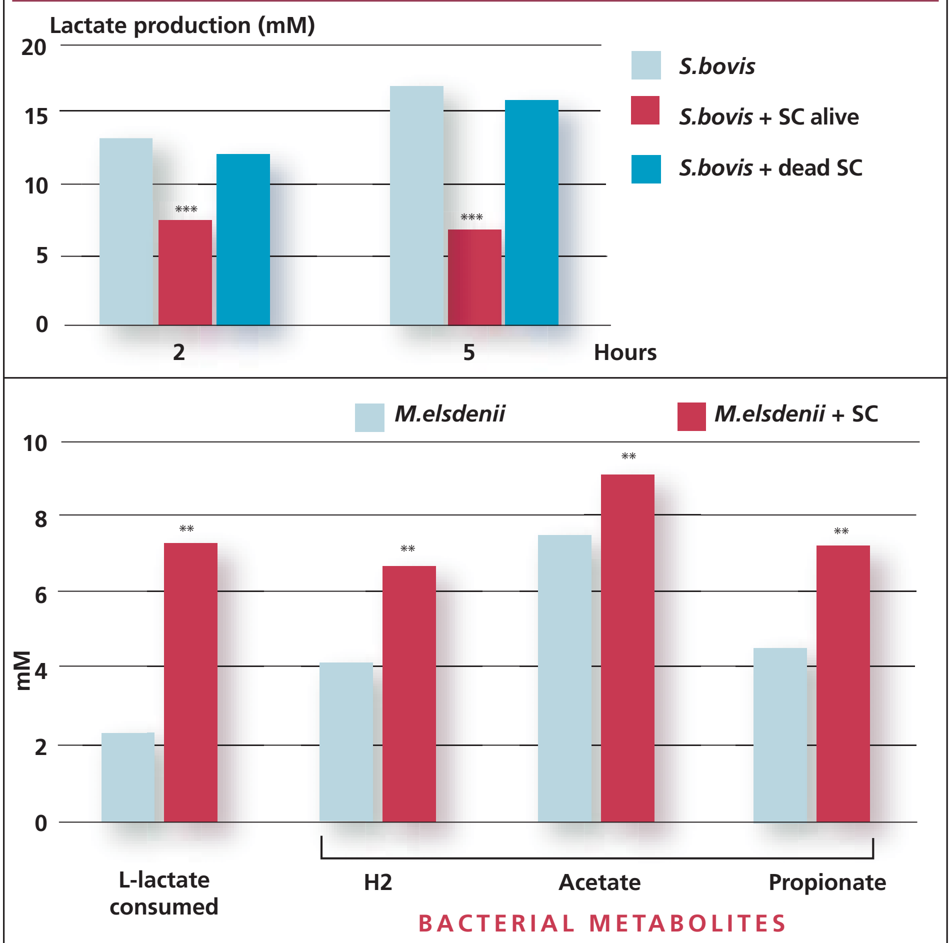
Yeast maintains rumen stability

Dr. Chaucheyras-Durand's group at INRA undertook work to look at the stabilising effects during adaptation to concentrate diets of the yeast *Saccharomyces cerevisiae* strain I-1077 (SC I-1077) (Levucell SC, Lallemand Animal Nutrition, France) on the rumen microflora *in vivo*, using fistulated sheep. Both groups (two sheep per group) were given the same dietary regime: the initial diet contained 90% hay and 10% soybean meal; the barley content was increased weekly at the expense of the hay in the diet, from 20 to 66% barley inclusion. One group received, in addition, 10^7 cfu of SC I-1077 per ml rumen fluid each day before the morning meal. Samples of rumen fluid were taken to measure rumen pH, lactate concentration, activity of fibrolytic microbes and cellulolytic bacteria (by oligonucleotide probes targeting 16SrRNA).

The high barley diet (66% barley) was associated with a decrease in mean rumen pH for both groups, though this drop was less severe in the sheep fed the yeast (*Figure 2*). Over the course of a day, it was found that supplementing the high starch diet with yeast reduced the pH drop significantly and smoothed the pH change compared to the control group (*Figure 3*). Including yeast as part of the feeding regime also limited the increase in ruminal lactate associated with the high barley diet. Furthermore, the yeast supplement was also attributed to a decrease in the variation between animals. This stabilising effect of the yeast on ruminal pH and hence the rumen microflora was attributed to the positive impact seen on the activity of the fibre degrading microbial enzymes avicelase (cellulose 1,4-?-cellobiosidase), xylanase, ?-glucosidase and ?-xylosidase. The relative proportions of ribosomal RNA (an indicator of enzyme production by the ruminal microbes) for the cellulolytic bacteria *F. succinogenes*, *R. flavefaciens* and *R. albus* were increased in the presence of the yeast, indicating improved fibre degradation.

Current *in vivo* studies on latent acidosis being performed at INRA are again using fistulated sheep models, but are also taking a more technological approach. A pH probe in the rumen fistula allows continuous ruminal pH measurement, whilst inserting a probe mounted on a cannula, attached to a data logger can be used for continuous measurement of blood parameters. So far, a study using a high wheat diet, with a starch content of 40% to induce latent acidosis suggest that feeding yeast dramatically reduces the time for which the rumen pH drops below pH 5.5 and also reduces the overall time spent below pH 6, thus increasing the mean ruminal pH significantly. The yeast has no effect on the total VFA concentration, neither does it alter the proportion of VFA's produced, but the numbers of ciliate protozoa are increased (only the entodiniomorphs which digest plant matter with cellulases and hemicellulases; not of the holotrichs that use mainly soluble sugars as their substrates). The data collected so far also support previous data on the increase in average ruminal pH by feeding yeast. An increase in feed intake has also been reported, supporting previous trials in cattle.

Figure 4 - Yeast (SC I-1077) was able to limit lactate production and stimulate lactate consumption *in vitro*.
Source: Chaucheyras *et al*. (1996).



SC effect is significant: *** $p< 0.001$; ** $p<0.01$

Modes of action in more detail

A number of possible modes of action have been put forward in the past for the activity of yeast on the microbial ecology of the rumen. It has been suggested that, because yeast is an aerobe, adding live yeast increases anaerobic activity by scavenging oxygen trapped between plant fibres, thus increasing the activity of anaerobic bacteria. A second suggestion is that the yeast and the rumen microflora compete for nutrients, thus slowing fermentative activity when a high concentration of rapidly fermentable carbohydrates (RFC) is fed. However, the yeast cell is only able to survive for around 24 hours in the anaerobic environment of the rumen, so the extent of its contribution in this capacity is limited. Thirdly, because the yeast cell is rich in nutrients, particularly amino acids, vitamins and organic acids, essential for the lactate fermenting bacteria, the presence of extra nutrient supply for these bacteria is responsible for their increased proliferation.

The group conducted some *in vitro* studies on the effects of the yeast strain on two predominant ruminal bacterial species (*Figure 4*). *Streptococcus bovis* is often responsible for the rapid acidification of the rumen contents when high starch diets are fed. The addition of live yeast was able to limit lactate production by *S. bovis*, but dead yeast cells had no effect. This would indicate that the yeast exerts its effect on *S. bovis* by competition for nutrients, rather than by merely supplying nutrients to the lactate fermenting species. Live yeast was also seen to stimulate lactate consumption by *Megasphera elsdenii*. *Figure 6* shows the postulated mode of action of the live yeast *Saccharomyces cerevisiae* in reducing the risk of acidosis when diets high in RFC's are fed.

Microbial plant fibre degradation

Over the past ten years, a number of studies have been published detailing the effects of yeast on fibre digestion under ruminal conditions. Initial studies used the *in vitro* model rumen simulation technique "rusitec" and found that inclusion of SC I-1077 improved degradation and digestibility of neutral detergent fibre (NDF); and degradation of cellulose, acid detergent fibre (ADF) and hemicellulose (Jouany *et al*., 1991; 1994). Later studies using fistulated sheep (Michalet-Doreau *et al*., 1997) and dairy cows (Jouany and Doreau, 1998) showed that adding yeast tended to increase the rate of NDF degradation (cows) and maintained fibrolytic activity and high levels of dietary barley (sheep). In beef cattle, professor F.J. Scwartz of the Technical University of Munich in Germany noted a 12% increase in fibre degradation in a field trial with beef cattle. More recently, Drs. Chaucheyras-Durand and Fonty (2001) at INRA studied the activity of cellulolytic bacteria *in vivo* and cellulolytic fungi *in vitro* with a view to investigate the potential benefits of feeding SC I-1077 on fibre degradation and to identify its possible modes of action. Gnotoxenic lambs, which were inoculated with just three strains of cellulolytic bacteria, were used as animal models. In those animals that also received a live yeast supplement, the bacteria were established more quickly and their populations maintained at a higher level, even during the stress imposed by cannulation, when the oxygen levels of the rumen increase. Moreover, the cellulolytic activity (cellulases and hemicellulases) was higher in those lambs receiving yeast supplementation and *in sacco* degradation of wheat straw was increased by around 10% (P<0.10).

It is believed that rumen fungi play a major role in physically breaking up fibrous material through the growth of hyphae, which can enter stoma and surface damage on plant matter, grow, and thus break it down into smaller particles, increasing the surface area for growth of cellulolytic bacteria. But these anaerobic fungi also have cellulolytic activity. To assess the effect *in vitro* of SC I-1077 on this activity, the rumen fungus *Neocallimastix frontalis* was grown under anaerobic conditions in the presence or absence of SC I-1077 and a nutrient source. In the presence of yeast, dry matter degradation increased by more than 50% and formate production more than doubled, while H_2 production was increased only slightly. The authors believe that yeast provides valuable nutrients for fungal metabolism, including thiamine (rich in yeast cells), which is important for fungal zoosporogenesis. Because yeast metabolism utilises oxygen and soluble sugars, it creates conditions more favourable to the growth of cellulolytic bacteria and fungi.

Figure 5 - Degradation of rapidly fermentable carbohydrates, lactate metabolism and the effect of yeast (SC I-1077) in the rumen.
Source: Chaucheyras *et al*. (1996).

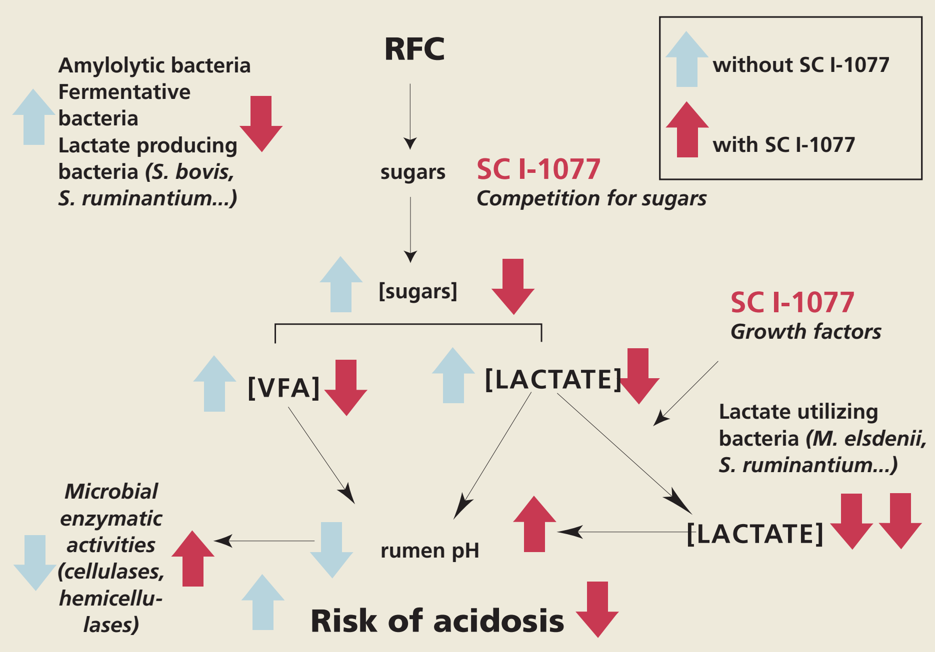
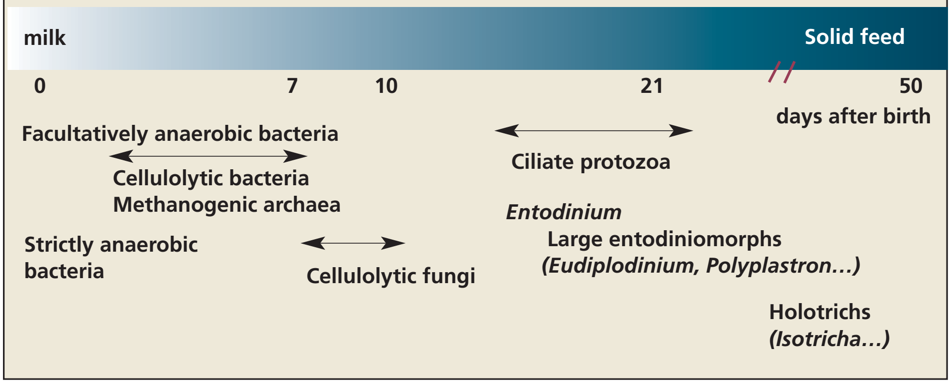


Figure 6 - Establishment of microbial communities in the lamb rumen.
Source: Fonty *et al*. (1987).



Establishing a good start

Ruminants are born without a rumen microflora, but this begins to establish within a few hours of birth, when anaerobic bacteria begin to multiply. Between two and four days later, cellulolytic bacteria and methanogenic archaea appear. Anaerobic fungi begin to colonise the rumen during the second week and the ciliate protozoa in the third week (*Figure 6*). Once the rumen ecosystem has stabilised, the young ruminant is ready for the transition to a solid diet. The microbial inoculate originates mainly from the mother's saliva, so early removal of the young, as is common practice in dairy herds and early weaning is not ideal for rapid establishment of a healthy rumen microflora. Therefore, perturbation of the microbial balance, less sub-optimal development of the rumen (in terms of rumen volume and the rumen wall epithelial structure), digestive pathologies are common at this time and all result in reduced performance, which can continue into later life.

When Chaucheyras and Fonty (2002) looked at the effect of SC I-1077 on the establishment of microbial communities in the rumen of newborn lambs, which were reared under conventional conditions (suckling, followed by a hay/concentrate diet offered *ad libitum* from 21 days, then weaned at six weeks of age), they found the establishment of several microbial populations was accelerated in those lambs supplemented with live yeast. Analysis of rumen parameters (VFA, NH3, pH and redox potential) and weight also revealed positive effects on rumen fermentation: less rumen ammonia was produced, indicating increased ammonia uptake by the microflora (and hence increased microbial protein production); the redox potential was reduced, indicating a stronger state of anaerobiosis; and VFA concentrations were higher when live yeast was fed. Furthermore, the average daily gain was increased by 4.5% in the lambs supplemented with yeast. This increase was attributed to more rapid microbial colonisation of the rumen, enabling the lambs to digest solid feed earlier and with fewer digestive problems.

The next steps

Work at INRA is continuing on the mechanisms of yeast action in the rumen. This project also encompasses topics in ruminant nutrition that reflect consumer concerns.

Firstly, the group is conducting a study into the interrelationships between the rumen microflora and *E. coli*. The most common origin of food-borne *E. coli* infections is through cattle products (beef and dairy products). The rumen and colon of cattle appear to serve as a reservoir for potentially pathogenic bacteria. Laboratory research is now underway to characterise pathogenic strains and study the factors that affect their survival in the digestive tract. Furthermore, since yeast has such a stabilising effect on rumen microbial dynamics, especially during times of nutritional stress, the group is investigating whether it may have a role in preventing pathogenic *E. coli* growth in the ruminant digestive tract.

A second ongoing project is looking at the influence of yeast on rumen nitrogen metabolism. Ruminant nitrogen metabolism is usually regarded as inefficient and so in high producing cattle, rumen-protected protein supplements are often fed to keep up with demand. The studies already done in gnotoxenic and conventional lambs both saw a decrease in ruminal ammonia concentrations when SC I-1077 was fed. However, it is not yet clear whether this occurs as a result of lower ammonia production or higher microbial ammonia uptake.

Finally, an issue of great environmental importance. Under anaerobic fermentation, a large amount of hydrogen is produced. This is re-used by the methanogenic organisms and others to prevent it accumulating in the rumen and the resulting methane is eructated. The daily methane production of cattle is between 400 and 500 litres. This not only increases environmental methane, which has been implicated in global warming, it also represents a loss of 10-15% of the animal's metabolisable energy intake. By investigating the microbial pathways of methanogenesis in the rumen, possible strategies can be developed to limit methane emissions. ●

References are available upon request.