

## Modulation of the rumen microbiome



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**A combination of animal genetics and the unique, enlarged fore-stomach of ruminants (rumen) enable domesticated ruminants to be sustained on forages and fibrous feedstuffs that would be otherwise indigestible. Ruminants can also utilise more easily digestible, high energy plant material such as grain, to achieve rapid increases in weight gain, muscle bulk and in the case of dairy cows, high milk yields. Since the mid-1900s there has been a steady research effort into understanding the digestive processes of ruminants, striving to maintain animal health and nutrition whilst maximising the productivity and environmental sustainability of livestock production systems. This article describes strategies developed to modulate the rumen microbial ecosystem, enabling the utilisation of plant feedstuffs that may otherwise be toxic and enhancing feed utilisation efficiency or controlling populations of specific rumen microbes, such as those contributing to lactic acidosis and enteric methane emissions. It also traces advances in technologies that have enabled us to understand the underlying biological mechanisms involved in the modulation of the rumen microbiome.**

### The rumen microbial community

The rumen contains a dense microbial community that actively degrades plant material, providing the animal with energy via the end-products of fermentation (short chain fatty acids) and protein in the form of microbial protein, which flows from the rumen into the lower intestine<sup>1</sup>. Rumen microbes not only adhere to and degrade

plant material they may also utilise substrates produced by other microbes. The rumen microbial population includes bacteria that are predominantly strict anaerobes with the capacity to be highly fibrolytic and proteolytic (generally of the phyla Bacteroidetes, Firmicutes and Proteobacteria), methanogenic archaea (phylum Euryarchaeota) and anaerobic fungi (fungal division Neocallimastigomycota)<sup>2,3</sup>. These rumen microbes are predated on by populations of anaerobic protozoa (predominantly of the phylum Ciliophora)<sup>4,5</sup> and viruses (predominantly dsDNA bacteriophages of the order Caudovirales)<sup>6</sup>.

### Rumen microbes friend or foe? Strategies for reducing plant toxicity, acidosis and enteric methane

The modulation of rumen microbial populations has traditionally focussed on strategies to improve feed digestibility and consequently increase overall animal productivity, reducing the time taken for ruminant livestock to reach market-weight specifications. Microbiologists and animal nutritionists have sought to determine the impact of different diet formulations on rumen function and live-weight gains, investigating the effects of feedstuff pre-treatment employing either physical change (for example steam treatment, rolling or flaking of grain<sup>7-9</sup>) or physical and chemical changes through microbial and enzymatic pre-treatment (for example ensilage of fodder crops with or without the application of silage inoculants<sup>10-12</sup>). Research has also sought to increase the environmental sustainability of livestock production by investigating the

viability of alternative feedstuffs such as those that may also be a by-product of food production or industry (for example cotton seed meal<sup>13</sup>) or feeds that may be readily propagated on-farm (for example microalgae<sup>14,15</sup>). Strategies have also been developed to allow cattle to utilise plant feedstuffs that may otherwise be toxic to the animal, for example the leguminous shrub *Leucaena leucocephala* may be propagated on-farm as a high-protein fodder crop (Figure 1). *Leucaena* however, contains the toxic amino acid mimosine and normal rumen microbial degradation results in the formation of the toxin 3-hydroxy-4(1H)-pyridone (DHP). The accumulation of these toxins leads to negative health implications including hair loss, reduced live-weight gain and goitre. The solution to preventing the development of *Leucaena* toxicity arose from rumen microbes, as these toxins were first shown to be de-toxified by bacteria of the genera *Synergistes* isolated from the rumen of feral goats<sup>16</sup>. A mixed microbial drench containing *Synergistes jonesii* is currently produced in Queensland for the treatment of cattle grazing *Leucaena*<sup>17</sup>.

In addition to strategies to improve feed breakdown in the rumen, research has also been undertaken to specifically target and control certain rumen microbial populations including the rumen protozoa, amylolytic bacteria, and more recently, the methanogenic archaea. Rumen protozoa may positively contribute to ruminant feed breakdown<sup>18</sup>; however, as these eukaryotes actively graze on the rumen bacterial populations, their growth and proliferation within the rumen may also contribute to the inefficient, intra-ruminal recycling of microbial protein<sup>5</sup>. Strategies to reduce rumen protozoa have included the use of diet (high grain diets tend to reduce protozoal populations), dietary additives such as the clay bentonite, nitrates and vaccination<sup>19,20</sup>. While these strategies have been shown to

impact on protozoal populations, it has proven difficult to completely remove protozoa from the rumen.

Strategies have also been developed to control rumen populations of the amylolytic bacterium *Streptococcus bovis*. Amylolytic, lactic acid-producing bacteria such as *S. bovis*, may over-proliferate in the rumen when cattle are fed high concentrate or high grain diets, contributing to the development of a condition known as lactic acidosis. *S. bovis* has been targeted through the application of antibiotics such as monensin<sup>21</sup> and phage therapies<sup>22</sup>. The strategies developed to control *S. bovis* were largely undertaken between the 1970s and 1990s and the relative importance of controlling these organisms and their overall contribution to the development of lactic acidosis has been cause for debate<sup>23,24</sup>. Feeding practices avoiding sudden dietary changes to large quantities of fermentable carbohydrates can prevent the development of acidosis<sup>25</sup> and most of the novel strategies to prevent rumen acidosis reported in the literature<sup>26,27</sup> have not been either commercialised or implemented within the livestock production industry.

In the past decade, investigations to modulate rumen microbial populations have focused on strategies to reduce the amount of the potent greenhouse gas methane generated by livestock production. Normal rumen microbial fermentation results in the accumulation of hydrogen. This hydrogen may be utilised by acetogenic bacteria (for example of the genera *Acetitomaculum*, *Eubacterium* and *Blautia*), however the majority of hydrogen is consumed by populations of methanogenic archaea belonging to the genera *Methanobrevibacter*, *Methanobacterium*, *Methanococcus*, *Methanomicrobium* and *Methanoseta*, with the methane produced lost to the animal via eructation<sup>28</sup>. Strategies currently in development to control rumen methanogen populations include specific diets



Figure 1. Cattle herd grazing the leguminous shrub *Leucaena leucocephala* propagated on-farm in Northern Australia.

(high grain and/or oil), dietary additives (for example naturally-occurring plant-derived compounds and synthetic anti-methanogenic compounds), animal breeding, phage-based therapies and vaccination<sup>29–32</sup>. While several of these novel approaches have been shown to be effective in reducing rumen methanogen populations, the majority are not yet at the stage of commercial application and adoption by the livestock production industry.

## Feed supplements, probiotics and direct fed microbials

The Australian agricultural feed industry produces many feed supplements designed and marketed to improve ruminant production efficiency, particularly for the dairy industry. Australian law requires that all agricultural and veterinary chemical products sold in Australia be registered by the Australian Pesticides and Veterinary Medicines Authority (APVMA, <http://apvma.gov.au>) and are listed on the permits and PubCRIS database (<https://portal.apvma.gov.au/pubcris>). These feed supplements include direct fed microbials (DFM) or probiotics and may also incorporate additional enzymes (amylases, proteases), minerals and salts (selenium, potassium). There are approximately 30 formulations of probiotic microbes available for use within Australia to enhance the overall digestive efficiency of ruminants incorporating bacteria such as *Bifidobacterium* (including the species *bifidum*, *longum* and *thermophilum*), *Lactobacillus* (species *acidophilus*, *delbrueckii* subspecies *bulgaricus*, *plantarum* and *rhamnosus*) and *Enterococcus faecium*. In addition, a further 11 registered products are available that are exclusively based on the yeast *Saccharomyces cerevisiae*. While the survival and proliferation of these organisms in the rumen and the effect of these probiotic bacteria on rumen digestive processes has been largely under-represented in the scientific literature, the probiotic effects of yeasts (*S. cerevisiae*) has been more extensively assessed<sup>33,34</sup>. Results can be highly variable between studies, however several investigations have established that the provision of these commercially available probiotics is most useful when applied to young ruminants to accelerate the establishment of a healthy gastrointestinal microflora<sup>35,36</sup>. Probiotics may be used to exclude undesirable zoonotic pathogens such as *Escherichia coli* O157 from establishing in the ruminant gastrointestinal tract and may also impact on the ruminant host immune system and feed breakdown efficiency<sup>36–38</sup>.

While bacterial strains of rumen origin would be anticipated to survive and proliferate in the rumen and therefore have a selective advantage over microbes of non-rumen origin<sup>36</sup>, there are currently no commercial formulations of rumen-derived probiotic bacteria registered for use in Australia. The mixed microbial drench for Leucaena toxicity is the only APVMA approved rumen-derived microbial treatment. There have however been several reports in the scientific literature of rumen-derived bacterial isolates being examined for application as potential probiotics, for example, *Megasphaera eldenii*, *Ruminococcus* sp., *R. flavefaciens*, *Prevotella bryantii*, *Butyrivibrio fibrisolvens*<sup>37,39</sup>. These strains were

selected for further testing in order to increase starch utilisation (by the genera *Ruminococcus*, *Prevotella* and *Butyrivibrio*) and to assist with the prevention of lactic acid accumulation contributing to acidosis (*M. elsdenii*) in feedlot cattle.

## Future directions

The success of any strategies developed to modulate the microbial population of the rumen will always need to be underpinned by fundamental research efforts to understand how the strategies impact on the baseline or ‘normal’ functioning of the rumen microbial ecosystem<sup>40</sup>. Addressing the gaps in current understanding of the rumen microbial ecosystem<sup>41</sup> is therefore key to the development of new strategies to control the microbial populations. The rumen contains a very dense microbial ecosystem, end-products of microbial digestion, salts and plant material including partially digested fibre, carbohydrate, and phenolic compounds and as such, samples of rumen material often present unique technical challenges. Rumen microbiologists have therefore always been quick to adopt new developments in technology in order to more fully understand the complex microbial ecosystem of the rumen. Early research efforts relied on microbial cultivation and although the study of cultivated rumen microbes is important for characterising microbial genera and elucidating their specific genetic and metabolic traits, culture-independent studies for the detection of specific microbes (real-time PCR assays) and community analysis based on the comparative phylogeny of the prokaryote 16S rRNA gene are often employed to ascertain the extent to which probiotic microbes survive and proliferate within the rumen<sup>31</sup>.

Rapid advances in high-throughput sequencing technologies have also facilitated investigations into how probiotics can influence both the community composition and functional gene capacity of the rumen. Metagenomic studies of the rumen have progressed current understanding of the functional gene potential of the rumen microbial population enabling the *in silico* identification of enzymes involved in feed breakdown<sup>42</sup>.

In the future, a greater reliance on gene sequence-based technologies or ‘omics’ will lead to an increased understanding of the interactions occurring between probiotics and the microbial populations indigenous to the ruminant gastrointestinal tract. This is of particular interest for the development and optimisation of new and more effective strategies for the modulation of the rumen microbiome. Development of new strategies, treatments and probiotics to enhance rumen feed utilisation efficiency, represents an area of great potential for the Australian livestock industries and will enable the production of quality products to meet global demands.

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