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Rumen microbial ecology: Helping to change landscapes

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Abstract. Current land use in many parts of the world is unsustainable and we face critical issues of declining soil and water quality and loss of biodiversity. In addition, more consumers now demand agricultural systems that are clean, green and ethical, where high quality and safe products are produced efficiently with minimal impact on the environment. In ruminant livestock systems, many of the consumer concerns relate to issues associated with the rumen microbial ecosystem. For example: (i) animal emissions, including the greenhouse gas, methane, from rumen fermentation and nutrient waste in faeces; and (ii) the use of antibiotics, feed additives, and anthelmintics to alter gut health and function and hormones to improve animal performance. These concerns have left producers with the challenge of remaining profitable while, at the same time, meeting market demands for product specifications from healthy animals, without relying on chemicals or harming the environment; often in locations where feed availability is limited and variable. Consequently, there is an urgent need to identify new sources of feed, including those containing "natural bioactives", as safer alternatives to in-feed chemicals, to reduce methane transmissions and address animal health, production and welfare issues. With such a complex challenge, it is unlikely that any one alternative feed source will satisfy all of these necessary outcomes. A more likely scenario is that producers will need to provide animals with a greater variety of plants in polycultures. In ruminants, the success of this approach relies on the response of the ruminal microorganisms to the plants, the natural ability of the animal to select and self medicate and the management practices required by the producer to make it sustainable. In this paper we focus on *in vitro* screening for "natural bioactives" that manipulate ruminal microorganisms and how this information can be used to customise plant mixes appropriate to different land types and change landscapes.

Keywords. Rumen – Microbial ecology – Landscapes – Bioactives – Feed.

Ecologie microbienne du rumen: Contribution au changement des paysages

Résumé. *L'exploitation de la terre dans de nombreuses zones du monde n'est pas durable et on se heurte à des situations critiques relatives à la diminution de la qualité du sol et de l'eau et à une perte de la biodiversité. Par ailleurs, les consommateurs sont de plus en plus demandeurs de systèmes agricoles basés sur les notions de propreté, de verdure et d'éthique et qui permettent d'aboutir à des produits de qualité et sains avec le minimum d'impact négatif sur l'environnement. Dans les systèmes d'élevage, de nombreux soucis des consommateurs sont associés à l'écosystème microbien du rumen, tels que : (i) les émissions par les animaux y compris l'effet de serre, le méthane, résultant de la fermentation ruminale et de l'excrétion des nutriments dans les fèces ; et (ii) l'utilisation des antibiotiques, des additifs alimentaires, des hormones et des anthelmintiques qui affectent l'hygiène et la fonction intestinales. Ces soucis font que les producteurs doivent affronter le défi de la rentabilité, et en même temps, de couvrir les besoins du marché en produits répondant à des qualités spécifiques liées à la santé animale, sans compter sur les produits chimiques ou menacer l'environnement, souvent dans des endroits caractérisés par un déficit fourrager et une disponibilité variable des ressources alimentaires. En conséquence, il y a un besoin urgent d'identification de ressources alimentaires alternatives, en l'occurrence les bioactifs naturels, permettant d'éviter l'utilisation des produits chimiques, de réduire la transmission de méthane et de garantir une meilleure santé animale (production et bien-être). Avec ce défi complexe, il est difficile à priori de trouver une ressource alimentaire alternative permettant de satisfaire ces conditions. Le scénario le plus plausible est que les producteurs devraient présenter des animaux disposant d'une large gamme de plantes. La réussite de cette approche dépend de l'écologie microbienne du rumen, de la capacité naturelle de l'animal à sélectionner et à s'autotraiter, et des pratiques de gestion requises pour assurer*

sa durabilité. Dans cet article on s'est concentré sur les micro-organismes du rumen et le criblage in vitro *des "bioactifs naturels" et sur la façon de valoriser cette information pour définir des associations de plantes appropriées pour les différents types de sol et pour changer les paysages.*

Mots-clés. *Rumen – Ecologie microbienne – Paysages – Bioactifs – Aliment.*

I – Introduction

In many parts of the world, farming systems have not been in harmony with the natural resource base and land use practices are unsustainable. Furthermore, many practices rely on chemical inputs that have become unpopular with consumers. Consumers are no longer satisfied with easy access to abundant animal products; they want healthy and safe products derived from systems that are "clean, green and ethical" (Martin and Kadokawa, 2006). This means sustainable production systems, where high quality and safe products are produced efficiently with minimal pollution and erosion of the environment, limited loss of biodiversity, no impact on human health, minimal chemical inputs, and from animals that are well cared for. With globalization and the "livestock revolution", this pressure from consumers will not be limited to developed countries (Vercoe, 2003). It will also have an impact on developing countries, where livestock production is becoming more intensified and there is pressure to use marginal land for these systems, because priority is given to producing food for humans from the better quality and more suitable arable land.

In ruminants, it is possible to meet some of the consumer's demands by manipulating the rumen and its microbes. The efficiency of rumen fermentation determines the amount of methane and nitrogenous pollution expelled or excreted into the environment, the amount and quality of meat, milk or wool produced, animal health (e.g. incidence of lactic acidosis and bloat) and feed intake (Wallace, 2004). Until recently, antibiotics have been used to manipulate rumen fermentation, but since the ban on their use as growth promotants in Europe, and the review of their use in the US (Greathead, 2003) and Australia (JETACAR, 1999), there has been an urgency to find alternative ways to manipulate fermentation and satisfy consumer demands without decreasing productivity levels or livestock health. The alternatives have included changing management and husbandry practices and exploring the tremendous plant biodiversity in the world for plants, or their extracts, for "natural" alternatives to antibiotics and feed additives. However, it is unlikely that a single alternative feed source will satisfy the requirements of this complex task. A more likely scenario is that producers will need to provide animals with a greater variety of plants in polycultures. The success of this approach relies on the microbial ecology in the rumen, the natural ability of the animal to select and self medicate and the management practices required by the producer to make it sustainable. In this paper we focus on the ruminal microorganisms and *in vitro* screening for "natural bioactives", mainly from Australian native plants, and provide a brief vision of how we see assigning values to plants in this way could be used to customise plant mixes appropriate to different land types and change landscapes.

II – Plants with "bioactive" compounds

Plants produce an array of secondary compounds as a chemical defence against herbivory and microbial and insect attack (Cowan, 1999). These compounds have been screened extensively for bioactive properties useful in human medicine, particularly for those with antimicrobial properties. Cowan (1999) provides an extensive review on the history, major groups, screening and activity of antimicrobial compounds from plants for medical applications. What is highlighted in the paper is the variety of plants that have specific or broad activities against all classes of microorganisms, including bacteria, protozoa, fungi and viruses. Considering the microbial ecology in the rumen includes all of these types of microorganisms, as well as archaeal organism, the medical literature has provided animal scientists with a solid basis for screening plants for bioactives that may influence rumen fermentation and a degree of excitement about the likelihood of success. It is not

surprising that there are now several major collaborative research projects where large collections of plants are being screened for natural alternatives to antibiotics, anthelmintics and feed additives for livestock. These include "Rumen-up" (Wallace *et al*., 2002) and "Replace", the EC Projects; and the "Enrich" project in Australia. There are many other groups not linked to these projects but with similar objectives, some of which are mentioned in this paper.

Cowan (1999) grouped antimicrobial compounds from plants into five classes: phenolics, terpenoids/essential oils, alkaloids, lectins and polypeptides and polyacetylenes. There is good evidence that secondary compounds from at least some of these classes affect microorganisms found in the rumen (Woodward *et al*., 2001; Wallace *et al*., 2002; Kamra *et al*., 2006; Durmic *et al*., 2007). The evidence is based mainly on *in vitro* studies, where plant or plant extracts have been added to rumen fluid, or pure cultures, and end-products of targeted fermentation pathways or microbial growth are measured (Wallace *et al*., 1994, 2002; Kamra *et al*., 2006; Bodas *et al*., 2007). In ruminants, the secondary compounds that have received the most attention are the essential oils, saponins and tannins (McSweeney *et al*., 2001; Wallace *et al*., 2002; Greathead, 2003; Kamra *et al*., 2006).

The secondary compounds have been tested mainly for their antimicrobial properties and potential to influence N metabolism, methane production, fibre utilisation, biohydrogenation, bloat, lactic acidosis and feed intake. Many of the results are promising. For example, essential oils are found to inhibit *Clostridium stricklandii* and *Peptostreptococcus anaerobius* (hyper-ammonia-producing bacteria, HAP) and decrease NH_3 production from amino acids in rumen fluid *in vitro* (Wallace *et al*., 2002). Reducing the rate of ammonia production by targeting the HAP bacteria would benefit the animal by improving the efficiency of nitrogen utilisation. Saponins have attracted attention because of their antiprotozoal properties (Makkar *et al*., 1998). Protozoa influence nitrogen retention in the rumen (Wallace and McPherson, 1987) and methane production, because of their association with methanogens. Saponins and tannins, amongst other secondary compounds, for example essential oils and spices, are found to reduce methane production *in vitro* (Mohammed *et al.*, 2004; Cardozo *et al*., 2005; Busquet *et al.*, 2006). Recently, Bodas *et al*. (2007) completed the most comprehensive study of plant species screened for antimethanogenic properties. They screened 450 plant species *in vitro* in batch cultures of mixed rumen microorganisms and compared their effects on methane production to a "standard". There was a normal frequency distribution of classes of methane production around the standard, with the majority of plants grouped within ±10% of the standard. Thirty-five (8% of the total number of plants) reduced methane production (mmol/g DM incubated) by more than 15% compared with the standard and 12 (3% of total) reduced methane production by more than 20% without affecting other fermentation patterns that were measured [DM digestibility, volatile fatty acid (VFA) production; molar proportions of VFA]. Six of the most promising plants were selected for further analysis and their effects on methanogensis are now patent protected (Bodas *et al*., 2007).

Considering the total plant biodiversity available for screening, the variation in the effects on methane production they found from only 450 plants is promising and indicates that the likelihood of successfully finding plant bioactives that will be useful in livestock production and health is high. However, there are two important issues that still need to be addressed. First, many of the plants that have been identified as possessing desirable bioactive compounds are likely to be new to agriculture. Most of our agricultural plants, at least in developed countries, have been through extensive plant selection and breeding, often to reduce the concentration of particular secondary plant compounds. This means that we are perhaps more likely to find "bioactive plants" from species that have not undergone extensive selection programmes. In turn, this requires research into how these novel plants can be used in agricultural systems, including and "duty of care" issues relating to factors like animal health and weed risk (Revell and Revell, 2007). The second issue that requires effort to resolve is the transfer of *in vitro* findings to *in vivo*. There are very few examples where specific bioactivities observed *in vitro* have been demonstrated *in vivo* and this was identified as one of the major weaknesses, currently, of this field at an international workshop organised jointly by the IAEA, the British Society of Animal Science and Writtle College, UK in 2006

("Alternative Feed Resources: A Key to Livestock Intensification in Developing Countries"). An obvious reason for this is the expense of screening large numbers of plants using animal house experiments. However, when a good candidate has been screened *in vivo*, often the results are not as clear or as significant as the effect *in vitro* (Wallace *et al*., 2002). There are examples where plants, known to have high levels of a particular class of secondary compound, have been associated with significant effects on rumen fermentation (e.g. Puchala *et al*., 2005; Mayberry *et al*., 2009) or numbers of specific bacterial species *in vivo* (Min *et al*., 2002; Mayberry *et al*., 2009), but there is a need to establish closer relationships between *in vitro* and *in vivo* screening systems. This may involve focussing more on *in vitro* continuous culture systems, where end products are removed, rather than batch cultures. It is also possible that the problem lies in the delivery of the plants or plant extracts *in vivo* and more attention and funding needs to be focussed on this once a promising plant has been identified *in vitro*. This topic needs to be debated by experts in the field to identify the most efficient way to proceed, which may be through a global collaboration.

We have extended the global search for "bioactive" plant compounds by establishing projects to screen Australian native plants and their extracts for antimicrobial properties and the ability to influence biohydrogenation, methane production and acidosis. Currently, our results are also limited mainly to *in vitro* analyses.

1. Australian native plants and rumen fermentation

Australian native plants, especially woody species, have evolved to produce an array of secondary compounds as chemical defenses against indigenous herbivores (Cork and Foley, 1991): for example, compounds in Eucalyptus leaves that affect diet selection and the gut microbial population of koalas (Cork and Foley, 1991; Moore *et al*., 2004). However, the great diversity in Australian flora and its phytochemistry remains largely unexplored for bioactive chemicals that could be used in animal production. Indigenous people of Australia are known to have used many Australian plants for medicinal purposes (Hammer *et al.*, 1999; Palombo and Semple, 2001). The use of Acacia species for medicinal purpose by indigenous Australians has been summarised by Wickens and Pennacchio (2002), who collated information on 26 species of Acacia that were used for treating an enormous range of ailments. It has also been noted that extracts from many Australian native plants possess a wide spectrum of antimicrobial activities (Palombo and Semple, 2001), and potent growth inhibiting activity towards specific bacteria (Carson *et al*., 1996; Min *et al*., 2002). Palombo and Semple (2001) demonstrated that extracts from Australian plants inhibit the growth of one or more species of human pathogenic bacteria, with five extracts showing broad spectrum antibacterial activity. Extracts from the leaves of *Eremophila* species (Myoporaceae) were the most active, with *Eremophila duttonii* exhibiting the greatest antimicrobial potency. Encouraged by this and a preliminary experiment, where we tested the effect of 30 Australian native plants (some with their extracts) on *in vitro* gas production (Durmic *et al*., 2006), we collected a larger number of Australian native plants and screened them *in vitro* for "bioactive" effects on methane production, acidosis and biohydrogenation and gas production.

A. In vitro *gas and methane production*

We have screened 62 woody shrub species of Australian native plants for their effects on total gas production and percentage of methane produced in *in vitro* batch cultures using rumen fluid from sheep fed a lupin/oaten chaff-based diet. The *in vitro* performances of the test plants were compared to a standard (oaten hay) substrate. The gas production of approximately half of the plants screened was the same or better than the positive control, and 22 of those resulted in methane levels that were similar or lower than the control (Fig. 1). The frequency distribution of classes of methane production around the positive control demonstrates that over half of the plants reduced the proportion of methane in the gas phase (Fig. 2) and about one quarter also showed comparable total gas production to the control. This is almost the opposite of what has been found in the "Rumen-up" project, where there was a normal distribution of methane production around the control (Bodas *et al*., 2007). It is more likely that it is the type, rather than the difference in the number (62 *vs* 450), of plants we have screened in comparison to Bodas *et al*. (2007) that is the

reason for this difference. The plants we screened were all woody shrubs adapted to the harsh Australian climate, which are likely to have high levels of secondary compounds and be very different to the oaten hay control. It is possible that as we screen a broader range of Australian native plant types we may change the frequency distribution.

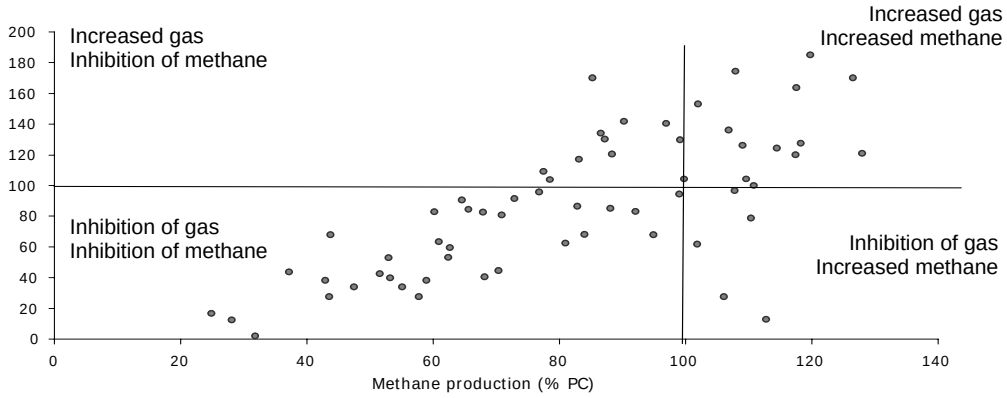

Fig. 1. *In vitro* gas and methane produced from sheep rumen fluid incubated with selected Australian native plants expressed as a percentage of standard (oaten hay).

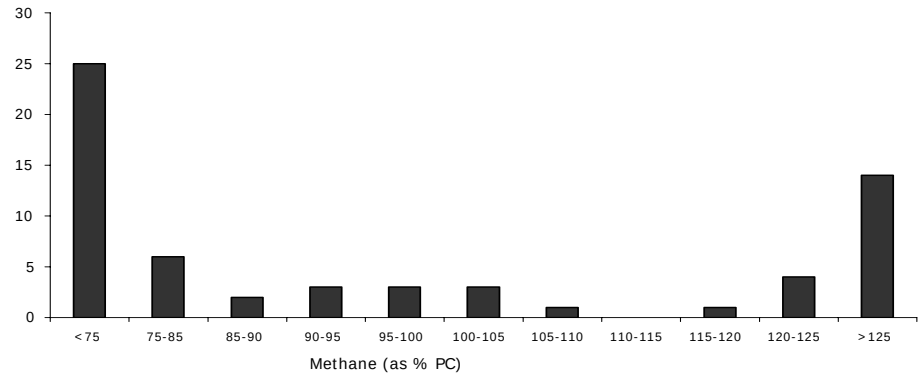

Fig. 2. Frequency distribution of classes of methane production from sheep rumen fluid during *in vitro* fermentation of 62 Australian native plants, expressed as a percentage of the standard substrate.

B. Acidosis

In recent studies in our laboratory, Australian native plants have been screened for their potential to prevent lactic acidosis *in vitro*. Lactic acidosis is a major rumen disorder that decreases productivity and occurs mainly in circumstances where animals are introduced to diets high in fermentable carbohydrates too quickly; for example, dairy production and feedlots. Acidosis is also a welfare issue because animals become sick and can die if left untreated. Under normal conditions, lactate in the rumen is an intermediate product that is degraded further to VFAs. This is due to the balance

between lactate-producing bacteria (*Streptococcus bovis* and *Lactobacillus* spp.) and lactate-utilising bacteria (*Megasphaera elsdenii* and *Selenomonas ruminantium*) (Klieve *et al*., 1999). However, when animals are introduced to large amounts of ruminally-fermentable carbohydrate quickly the microbial balance is disrupted (Nocek, 1997). The pH in the rumen drops because the lactate producers outnumber the lactate utilisers (Klieve *et al*., 1999). Other microbial changes amplify the problem and at pH 5.2 the animal develops acute acidosis (Nocek, 1997; Owens *et al*., 1998). Sub-therapeutic doses of antibiotics can be used to control acidosis because they inhibit the growth of lactate-producing bacteria without affecting normal rumen fermentation (Nagaraja and Taylor, 1987; Ives *et al*., 2002).

Over 100 Australian plants were screened *in vitro* for their effect on pH and gas production in a simulated acidosis environment (Hutton *et al*., 2006a,b). Acidosis was simulated *in vitro* by adding 5 g of glucose to bottles containing rumen fluid and either 0.5 g of oaten chaff (uncontrolled environment, UCE), 0.5 g oaten chaff + antibiotic [antibiotic-controlled environment, ACE (Eskalin500™)] or 0.5 g of plant candidate material (plant-controlled environment, PCE). The pH was measured every 5 hours as well as the accumulated gas pressure in the headspace, VFA concentrations and D-lactate at the end of the incubation. Two plants (A and B in Table 1) had similar effects to the antibiotic-controlled treatment on the parameters we measured (Table 1). Extracts from these plants selectively inhibited pure cultures of rumen bacteria associated with lactic acidosis. The results from Plant A in particular were very encouraging and we are now aiming to translate these results *in vivo*.

Table 1. The extent of control of acidosis (pH and D-lactate) and fermentation (gas and VFA) indicators by the addition of dried and ground Australian plants after 24-hour incubation in a simulated acute acidosis environment (from Hutton *et al.*, 2006a)

	UCE	ACE	PCE-Plant A	PCE - Plant B
pH	$4.31^{c} \pm 0.01$	$5.16^{a} \pm 0.04$	$5.15^{ab} \pm 0.03$	$4.95^{b} \pm 0.11$
D-lactate (mmol/l)	$0.3^{a} \pm 0.2$	$7^{b} \pm 1.7$	$28^{c} \pm 1.1$	$47^{d} \pm 1.3$
Accumulated gas pressure (kPa)	$119^{c} \pm 2.74$	$171^{a} \pm 1.36$	$134^{b} \pm 3.44$	$121^{c} \pm 0.3$
Total VFA (mmol/l)	$92^{d} \pm 2.94$	$153^{a} \pm 2.07$	$143^{b} \pm 1.92$	$119^{c} \pm 0.60$

^{a,b,c,d}Values are means ± SEM. Means with different superscripts on the same row are significantly different ($P < 0.05$).

C. Biohydrogenation

Improving the quality of meat and milk to satisfy consumer demands for safer and healthier products has been another one of our aims. Such properties have been assigned to the group of unsaturated fatty acids called conjugated linoleic acids (CLA; Ha *et al*., 1987; Bessa *et al*., 2000; Bhattacharya *et al*., 2006), that are formed in the rumen by bacterial isomerization of dietary linoleic acid, the first reaction in a multi-step process that ultimately produces the saturated fatty acid stearic acid (Harfoot and Hazlewood, 1997). CLA are absorbed from the small intestine and incorporated into animal tissues and milk, while vaccenic acid (VA), another intermediate of biohydrogenation of linoleic acid, can be converted back to CLA by a desaturase in mammalian tissue (Harfoot and Hazlewood, 1997; Griinari and Bauman, 1999). Griinari and Bauman (1999) indicated that the flow from the rumen of VA plays a more important role than CLA in determining CLA concentration in animal tissues.

Many ruminal bacteria species have been implicated in ruminal biohydrogenation, including species of the genera *Butyrivibrio*, *Ruminococcus*, *Treponema-Borrelia*, *Micrococcus*, *Megasphaera*, *Eubacterium*, *Fusocillus* and *Clostridium* (Polan *et al*., 1964; Harfoot and Hazlewood, 1997; Maia *et al*., 2007). The most active species are in the *Butyrivibrio* group, where all bacteria form CLA from linoleic acid (LA), while only *Clostridium proteoclasticum* (as previously isolated *Fusocillus* spp.) is

found to convert vaccenic acid to stearic acid (Polan *et al*., 1964; Kemp *et al*., 1975; Maia *et al*., 2007; Paillard *et al*., 2007). Selective suppression of *Cl. proteoclasticum*, without affecting *B. fibrisolvens*, would provide more unsaturated acids, including vaccenic acid and CLA, escaping the rumen to be absorbed and incorporated into animal tissues. Our objective has been to identify plants that could manipulate the bacteria and processes involved in ruminal biohydrogenation, without disrupting overall rumen fermentation efficiency, to improve the fatty acids profile of ruminant-derived food products. As with our other screening work, *in vitro* gas production was used as an indicator of the extent to which a plant or extract disrupted fermentation.

We tested ethanolic extracts and/or essential oils from 91 Australian plants for their Minimal Inhibitory Concentrations (MIC) of the main bacterial species involved in rumen biohydrogenation using an agar dilution method. We selected the most promising candidates based on their *in vitro* gas production and their ability to selectively inhibit target bacterial species, and examined their effects on LA biohydrogenation using an *in vitro* system with a mixed rumen bacterial population. A wide range of the plant extracts had selective inhibitory effect towards *Cl. proteoclasticum* (which forms stearate from LA) without affecting *B. fibrisolvens* (which forms CLA and vaccenic acid, but not stearate) (Durmic *et al*., 2007). Durmic *et al*. (2007) classified these plants into 4 groups according to their inhibitory effects towards specific bacterial species: (i) 37 plants selectively inhibited *Cl. proteoclasticum* P18 (Group A); (ii) 6 selectively inhibited *B. fibrisolvens* JW11 (Group B); (iii) 10 inhibited both bacterial species (Group C); and (iv) with the remaining 38 showing no inhibition of either bacteria (Group D). In Group A, 10 plants inhibited *Cl. proteoclasticum* P18 (MIC < 1 mg/ml) without inhibiting *B. fibrisolvens* JW11 (MIC > 10 mg/ml). Also, none of these 10 plants inhibited 8 other rumen bacterial species, not linked to biohydrogenation, at 1 mg/ml. *In vitro*, one plant (Accession W048; Fig. 3) improved the metabolism of linoleic acid and the amount of CLA produced after incubation for 6 hours when compared to the control. Other plants, including *Acacia iteaphylla* and *Kennedia eximia*, improved LA metabolism or inhibited stearate formation in the mixed bacterial community *in vitro* but we have not tested any of these effects *in vivo*.

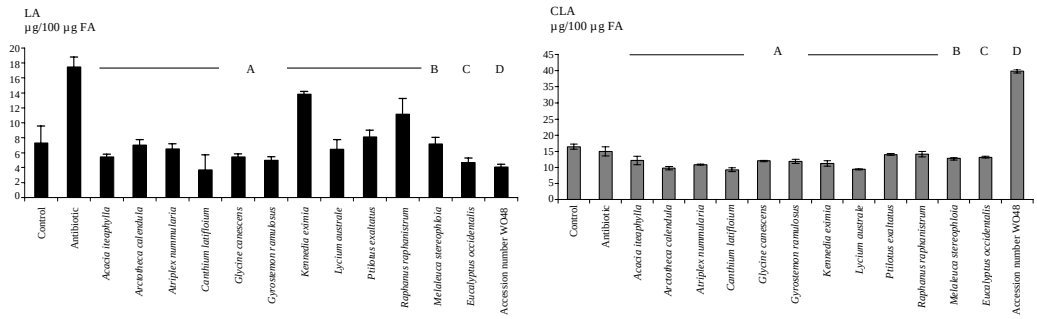

Fig. 3. Proportions of LA and CLA (% of total FA, mean ± SEM) in the liquid phase of the *in vitro* system after 6 h of incubation in the presence of 100 µg/ml of LA and 1 mg/ml of plant extract. [Note: "Control" in all above figures refers to the positive control rumen fluid + oaten chaff + LA + ethanol. A, B, C, D refers to plant groups as per text. Adapted from Durmic *et al.* (2007)].

Our results suggest that some Australian native plants have properties with the potential to manipulate rumen fermentation for specific purposes. The screening programme is still in its early stages; screening approximately 120 plants in total compared with the many thousands of species Australian native plants available can not be considered extensive, nor have any of the effects we have observed been measured *in vivo,* except for the effects of saltbush on methane production (Mayberry *et al.*, 2009). However, we have demonstrated that it is possible to rank and assign a value to a plant for characteristics other than just nutritive value, based on its ability to target specific fermentation pathways without disrupting overall *in vitro* fermentation. The ability to assign

different values to plants will be critical when selecting plants to build functional polycultures and change landscapes.

III – Scaling up: Building a functional system that incorporates plants with multiple benefits

The pressure and interest to find alternative approaches to manipulate the rumen ecosystem to improve animal production and/or animal health is coinciding with an increased desire by societies to improve the management of natural resources. This is manifest in a variety of ways around the world, and stems primarily from three main driving forces. The first is the increase in urbanization, which on the one hand is fuelling the increased demand for animal products especially in developing countries (the so-called Livestock Revolution; see Steinfeld, 2004), but on the other is displacing productive agricultural lands. The second driver is the decline in soil and water quality associated with unsustainable agricultural production; e.g., the extensive areas of salinization of agricultural landscapes in Australia (Australian Dryland Salinity Assessment, 2001; McFarlane *et al*., 2004), land degradation in Africa (Cooper *et al.*, 1996) and the Middle East (Portnov and Safriel, 2004) and reduced water quality in livestock farming areas (Hooda *et al.*, 2000). The third driver arises from factors that have been considered threats of the livestock revolution (Vercoe, 2003) and consequences of intensification of livestock production; for example, increased greenhouse gas emissions, concentration of nutrient wastes and pollution of waterways, increased chemical and drug use to overcome the increased risk of disease transmission (especially when in high-humidity regions; Steinfeld, 2004) and increased pressure on the local feed and reproduction management systems.

The alignment of multiple pressures facing livestock production could be overwhelming, but it also offers opportunities to trigger changes in land use to improve profitability and natural resource management. We contend that innovative approaches for more sustainable production systems are, in fact, more likely to succeed when multiple benefits are accrued than in circumstances where a single problem is addressed in isolation. This is because single-issue problems tend to be solved by making slight changes to the current system, often without addressing the related issues. In biological systems, simplification rarely leads to more sustainable systems. For example, the specialisation and intensification of production systems has lead to a breakdown in nutrient and an increase in problems with excessive nutrients in ecosystems. To demonstrate our proposition that a more holistic approach can simultaneously address multiple demands, we briefly describe a current research project in Australia – the "Enrich" programme – that is exploring the potential of incorporating native shrub species into mixed-farming systems. Our attention is less on the potential of plant extracts for improving animal health and production and more on the use of alternative plants *in situ* in mixed forage system.

Current land use practices in many parts of Australia are unsustainable, including those in the livestock-cropping zones of southern Australia. The climate in these zones is temperate (or Mediterranean), characterised by seasonal and low rainfall leading to fluctuations in the quality and quantity of feed supply for livestock. The problem is compounded because the land in these zones is fragile and many of the soils throughout Australia are of low fertility due to weathering over millennia. Imposing inappropriate livestock systems on such fragile land has led to serious environmental problems, in particular salinity (Australian Dryland Salinity Assessment, 2001; McFarlane *et al*., 2004) and soil erosion (Prosser *et al.*, 2001). The natural flora in these regions has evolved in response to water- and nutrient-poor conditions. Two features of this evolution that are relevant here include: (i) the presence of secondary plant compounds as a defence against excessive herbivory, which may offer medicinal, therapeutic or microbial effects for improving animal health and production; (ii) the rich diversity of Australia's native flora (e.g. Erikson, 1973) that has not been exploited in agriculture.

We developed the "Enrich" programme in Australia to capitalise on these features and evaluate the potential for Australian native shrubs to be incorporated in livestock-crop farming systems. The

attention has been on forage shrub species because of their inherent strengths in ecosystem functions (El Aich, 1991) and potential to address key issues of production systems, including: (i) summer activity and a capacity to respond to out-of-season rainfall events, thereby contributing to feed when conventional annual pasture species are at their lowest quality and quantity; (ii) deep roots and capacity to access water deep in the soil profile, providing a more consistent and predictable feed supply; (iii) a perennial growth pattern, reducing the risk of dryland salinity (Bathgate and Pannell, 2002; Turner and Ward, 2002; Peck and Hatton, 2003; Ridley *et al.*, 2004); and (iv) minimising soil erosion and increasing soil organic matter (Young *et al.*, 1996; Whitbread *et al.*, 2000). Other potential advantages of incorporating the use of native shrub species into farming systems include: (i) enhancing biodiversity outcomes; (ii) improved animal welfare through provision of shade and shelter; (iii) addressing the growing resistance to chemical anthelmintic drugs (Hordegen *et al*., 2003); and (iv) development of new approaches to replace antimicrobial drugs in livestock production (Wegener, 2003). If any one of these issues were addressed in isolation, it is most unlikely that forage shrubs would be considered as a solution. However, the potential for Australian native shrub species to provide benefits in multiple areas means that, collectively, a new functional system can be envisaged. In the context of the data presented earlier in this paper, we are identifying Australian shrub species that have favourable effects on the rumen ecosystem and, for the first time, are putting a value on these traits that encompasses the health of both the livestock and the landscape.

IV – Conclusions

How we use land for livestock production in many parts of the world needs to change. The need to change stems from consumers demanding "clean, green and ethical" production systems and also from increasing demand for livestock products that, in some parts of the world, is pushing producers to use marginal land or unsustainable practices. Consumers want high quality and safe products that are produced efficiently with minimal impact on the environment. In ruminant livestock systems, some of the consumer concerns relate to issues associated with the rumen microbial ecosystem. There are now a number of projects where the rich plant diversity in the world is being screened for useful plants or compounds that could be used to manipulate the rumen to reduce methane emissions and nutrient waste in faeces, and replace the use of antibiotics, feed additives, and anthelmintics to alter gut health and function. Most of the screening so far has been done *in vitro* and there are some concerns that the *in vitro* results do not always translate well *in vivo* and this needs to be debated by experts in this field to consider the best way to proceed: a global collaboration may be most efficient. Our work has focussed on screening Australian native plants and their extracts *in vitro* for antimicrobial properties and the ability to influence biohydrogenation, methane production and acidosis. We have identified plants that influence all of these ruminal processes *in vitro*, which is exciting, but they need to be tested *in vivo* before we can be confident of their potential value in the field. So far our search has been focussed on forage shrub species because of the broader goals of the "Enrich" programme, which extends beyond their value for manipulating the rumen to their inherent strengths in ecosystem functions and other issues of production systems like the provision of year-round feed supply, water use and improving soil structure and biology. However, by assigning value to these plants based on their ability to manipulate the rumen ecosystem we are taking one step towards improving the likelihood of developing innovative systems where novel plants are incorporated into polycultures that enhance the health of both the livestock and the landscape.

References

Australian Dryland Salinity Assessment, 2001. National Land and Water Resources Audit. (Canberra).

Bathgate A. and Pannell D.J., 2002. Economics of deep-rooted perennials in Western Australia. In: *Agricultural Water Management*, 53. p. 117-132.

Bessa R.J.B., Santos-Silva J., Ribeiro J.M.R. and Portugal A.V., 2000. Reticulo-rumen byohydrogenation and the enrichment of ruminant edible products with edible products with linoleic acid conjugated isomers. In: *Livest. Prod. Sci.*, 63. p. 201-211.

Bodas R., López S., Fernández M., García-González R., Rodríguez A.B., Wallace R.J. and González J.S., 2007. *In vitro* screening of the potential of numerous plant species as antimethanogenic additives for ruminants. In: *Anim. Feed Sci. Technol.*, 145(1). p. 245-258.

Bhattacharya A., Banu J., Rahman M., Causey J. and Fernandes G., 2006. Biological effects of conjugated linoleic acids in health and disease. In: *J. Nutr. Biochem.*, 17. p. 789-810.

Busquet M., Calsamiglia S., Ferret A. and Kamel C., 2006. Plant extracts affect *in vitro* rumen microbial fermentation. In: *J. Dairy Sci.*, 89. p. 761-771.

Cardozo P.W., Calsamiglia S., Ferret A. and Kamel C., 2005. Screening for the effects of natural plant extracts at different pH on *in vitro* rumen microbial fermentation of a high-concentrate diet for beef cattle. In: *J. Anim. Sci.*, 83. p. 2572-2579.

Carson C.F., Hammer K.A. and Riley T.V., 1996. *In vitro* activity of the essential oil of *Melaleuca alternifolia* against *Streptococcus* spp. In: *J. Antimicrobial Chemotherapy*, 37. p. 1177-1178.

Cooper P.J.M., Leakey R.R.B., Rao M.R. and Reynolds L., 1996. Agroforestry and the mitigation of land degradation in the humid and sub-humid tropics of Africa. In: *Experimental Agriculture*, 32. p. 235-290.

Cork S.J. and Foley W.J., 1991. Digestive and metabolic strategies of arboreal mammalian folivores in relation to chemical defenses in temperate and tropical forests. In: Pala R.T. and Robins C.T. (eds). *Plant Defenses against Mammalian Herbivory*. Boca Raton, USA: CRC Press. p. 133-166.

Cowan M.M., 1999. Plant products as antimicrobial agents. In: *Clinical Microbiological Reviews*, 12. p. 564-582.

Durmic Z., Hutton P.G., Kafilzadeh F. and Vercoe P.E., 2006. The effect of Australian plants on gas production by rumen microbes *in vitro*. In: *Proceedings of the 26th Biennial Conference of the Australian Society of Animal Production*, Perth (WA, Australia), 10-14 July 2006. Short. Comm. p. 54.

Durmic Z., McSweeney C.S., Kemp G.W., Hutton P., Wallace R.J. and Vercoe P.E., 2007. Australian plants with potential to inhibit bacteria and processes involved in ruminal biohydrogenation of fatty acids. In: *Anim. Feed Sci. Technol.*, 145(1). p. 271-284.

El Aich A., 1991. Role of shrubs in ecosystem functions. In: *Options Méditerranéennes, Series A*, 16. p. 43-46.

Erikson R., 1973. *Flowers and Plants of Western Australia.* Sydney, Australia: Reed.

Greathead H., 2003. Plants and plant extracts for improving animal productivity. In: *Proc. Nutr. Soc.*, 62. p. 279-290.

Griinari J.M. and Bauman D.E., 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: Yurawecz M.P., Mossoba M.M., Kramer J.K.G., Pariza M.W. and Nelson G.J. (eds). *Advances in Conjugated Linoleic Acid Research*. Champaign, IL, USA: AOCS Press. p. 180-185.

Ha Y.L., Grimm N.K. and Pariza M.W., 1987. Anticarcinogens from fried ground beef: Heat-altered derivatives of linoleic acid. In: *Carcinogen*, 8. p. 1881-1887.

Hammer K.A., Carson C.F. and Riley T.V., 1999. Antimicrobial activity of essential oils and other plant extracts. In: *J. Applied Microbiol.*, 86. p. 985-990.

Harfoot C.G. and Hazlewood G.P., 1997. Lipid metabolism in the rumen. In: Hobson P.N. and Stewart C.S. (eds). *The Rumen Microbial Ecosystem*. London and New York: Elsevier Applied Science. p. 382-426.

Hooda P.S., Edwards A.C., Anderson H.A. and Miller A., 2000. A review of water quality concerns in livestock farming areas. In: *The Science of the Total Environment*, 250. p. 143-167.

Hordegen P., Hertzberg H., Heilmann J., Langhans W. and Maurer V., 2003. The anthelmintic efficacy of five plant products against gastrointestinal trichostrongylids in artificially infected lambs. In: *Veterinary Parasitology*, 117. p. 51-60.

Hutton P., White C., Durmic Z. and Vercoe P.E., 2006a. Australian plants control induced acidosis *in vitro.* In: *Ethnoveterinary Medicine Conference: Harvesting Knowledge, Pharming Opportunities.* Paper 24. Chelmsford, UK: Writtle College.

Hutton P., White C., Durmic Z. and Vercoe P.E., 2006b. Australian plants have the potential to replace antibiotics in the control of lactic acidosis in ruminants*.* In: *Dairy Research Foundation Symposium: Dairying to Be Different.* Camden, Australia: University of Sydney.

Ives S.E., Titgemeyer E.C., Nagaraja T.G., del Barrio A., Bindel D.J. and Hollis L.C., 2002. Effects of virginiamycin and monensin plus tylosin on ruminal protein metabolism in steers fed corn-based finishing diets with or without wet corn gluten feed. In: *J. Anim. Sci.*, 80. p. 3005-3015.

JETACAR, 1999. *The Use of Antibiotics in Food-producing Animals: Antibiotic-resistant Bacteria in Animals and Humans*. Commonwealth Department of Health and Aged Care, Commonwealth Department of Agriculture, Fisheries and Forestry, Australia.

Kamra D.N., Agarwal N. and Chaudhary L.C., 2006. Inhibition of ruminal methanogenesis by tropical plants containing secondary compounds. In: *International Congress Series*, 1293. p. 156-163.

Kemp P., White R.W. and Lander D.J., 1975. The hydrogenation of unsaturated fatty acids by five bacterial isolates from the sheep rumen, including a new species. In: *J. General Microbiology*, 90. p. 100-114.

Klieve A.V., Heck G.L., Prance M.A. and Shu Q., 1999. Genetic homogeneity and phage susceptibility of ruminal strains of *Streptococcus bovis* isolated in Australia. In: *Letters in Applied Microbiology*, 29. p. 108-112.

Maia M.R., Chaudhary L.C., Figueres L. and Wallace R.J., 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. In: *Antonie Van Leeuwenhoek*, 91. p. 303-314.

Makkar H.P.S., Sen S., Blümmel M. and Becker K., 1998. Effects of fractions containing saponins from *Yucca schidigera*, *Quillaja saponaria* and *Acacia auriculoformis* on rumen fermentation. In: *J. Agric. Food Chem.*, 46. p. 4324-4328.

Martin G.B.M. and Kadokawa H., 2006. "Clean, green and ethical" animal production. Case study: Reproductive efficiency in small ruminants. In: *J. Reprod. Develop.*, 52. p. 145-152.

Mayberry D.E., Masters D.G. and Vercoe P.E., 2009. Saltbush (*Atriplex nummularia*) reduces efficiency of rumen fermentation in sheep. *Options Méditerranéennes, Series A*. This volume.

McFarlane D.J., George R.J. and Caccetta P.A., 2004. The extent and potential area of salt-affected land in Western Australia estimated using remote sensing and digital terrain models. In: *Proc. of the 1st National Salinity Engineering Conference*, Perth, Western Australia. Canberra, Australia: Institute of Engineers Australia. p. 55-60.

McSweeney C.S., Palmer B., McNeill D.M. and Krause D.O., 2001. Microbial interactions with tannins: Nutritional consequences for ruminants. In: *Anim. Feed Sci. Technol.*, 91. p. 83-93.

Min B.R., Attwood G.T., Reilly K., Sun W., Peters J.S., Barry T.N. and McNabb W.C., 2002. *Lotus corniculatus* condensed tannins decrease *in vivo* populations of proteolytic bacteria and affect nitrogen metabolism in the rumen of sheep. In: *Canadian J. Microbiol.*, 48. p. 911-921.

Mohammed N., Ajisaka N., Lila Z.A., Hara K., Mikuni K., Kanda S. and Itabashi H., 2004. Effect of Japanese horseradish oil on methane production and ruminal fermentation *in vitro* and in steers. In: *J. Anim. Sci.*, 82. p. 1839-1846.

Moore B.D., Wallis I.R., Wood J.T. and Foley W.J., 2004. Foliar nutrition, site quality, and temperature influence foliar chemistry of tallowwood (*Eucalyptus microcorys*). In: *Ecological Monographs*, 74. p. 553-568.

Nagaraja T.G. and Taylor M., 1987. Susceptibility and resistance of ruminal bacteria to antimicrobial feed additives. In: *Applied Environmental Microbiology*, 53. p. 1620-1625.

Nocek J.E., 1997. Bovine acidosis: Implications on Laminitis. In: *J. Dairy Sci.*, 80. p. 1005-1028.

Owens F.N., Secrist D.S., Hill W.J. and Gill D.R., 1998. Acidosis in cattle: A review. In: *J. Anim. Sci.*, 76. p. 275-286.

Paillard D., McKain N., Chaudhary L.C., Walker N.D., Pizette F., Koppova I., McEwan N.R., Kopecny J., Vercoe P.E., Louis P., Wallace R.J., 2007. Relation between phylogenetic position, lipid metabolism and butyrate production by different Butyrivibrio-like bacteria from the rumen. In: *Antonie Van Leeuwenhoek*, 91. p. 417-422.

Palombo E.A. and Semple S.J., 2001. Antibacterial activity of traditional Australian medicinal plants. In: *Journal of Ethnopharmacology*, 77. p. 151-157.

Peck A.J. and Hatton T., 2003. Salinity and the discharge of salts from catchments in Australia. In: *Journal of Hydrology*, 272. p. 191-2002.

Polan C.E., McNeill J.J. and Tove S.B., 1964. Biohydrogenation of unsaturated fatty acids by rumen bacteria. In: *Journal of Bacteriology*, 88. p. 1056-1064.

Portnov B.A. and Safriel U.N., 2004. Combating desertification in the Negev: Dryland agriculture versus urbanization. In: *Journal of Arid Environments*, 56. p. 659-680.

Prosser I.P., Rutherfurd I.D., Olley J.M., Young W.J., Wallbrink P.J. and Moran C.J., 2001. Large-scale patterns of erosion and sediment transport in river networks, with examples from Australia. In: *Marine and Freshwater Research*, 52. p. 81-99.

Puchala R., Min B.R., Goetsch A.L. and Sahlu T., 2005. The effect of a condensed tannin-containing forage on methane emission by goats. In: *Journal of Animal Science*, 83. p. 182-186.

Revell C.K. and Revell D.K., 2007. Meeting "duty of care" obligations when developing new pasture species. *Field Crops Research*, 104. p. 95-102.

Ridley A.M., Mele P.M. and Beverly C.R., 2004. Legume-based farming in Southern Australia: Developing sustainable systems to meet environmental challenges. In: *Soil Biology and Biochemistry*, 36. p. 1213-1221.

Steinfeld H., 2004. The livestock revolution – A global veterinary mission. In: *Veterinary Parasitology*, 125. p. 19-41.

Turner N.C. and Ward P.R., 2002. The role of Agroforestry and perennial pasture in mitigating water logging and secondary salinity: Summary. In: *Agricultural Water Management*, 53. p. 271-275.

Vercoe J.E., 2003. The livestock revolution: A pathway out of poverty? In: Brown A.G. (ed.). *The Livestock Revolution: A Pathway out of Poverty*? Parkville, Victoria, Australia: The ATSE Crawford Fund. p. 81-85.

Wallace R.J., 2004. Antimicrobial properties of plant secondary metabolites. In: *Proc. Nutr. Soc.*, 63. p. 621-629.

Wallace R.J., Arthaud L. and Newbold C.J., 1994. Influence of *Yucca shidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. In: *Applied and Environmental Microbiology*, 60. p. 1762-1767.

Wallace R.J., McEwan N.R., McIntosh F.M., Teferedegne B. and Newbold C.J., 2002. Natural products as manipulators of rumen fermentation. In: *Asian-Australasian J. Anim. Sci.*, 15. p. 1458-1468.

Wallace R.J. and McPherson C.A., 1987. Factors affecting the rate of breakdown of bacterial protein in rumen fluid. In: *Brit. J. Nutr.*, 58. p. 313-323.

Wegener H.C., 2003. Ending the use of antimicrobial growth promoters is making a difference. In: *American Society for Microbiology*, 69. p. 443-448.

Whitbread A.M., Blair G.J. and Lefroy R.D.B., 2000. Managing legume leys, residues and fertilisers to enhance the sustainability of wheat cropping systems in Australia. 2. Soil physical fertility and carbon. In: *Soil and Tillage Research*, 54. p. 77-89.

Wickens K. and Pennacchio M., 2002. A search for novel biologically active compounds in the phyllodes of *Acacia* species. In: *Conservation Science Western Australia*, 4. p. 139-144.

Woodward S.L., Waghorn G.C., Ulyatt M.J. and Lassey K.R., 2001. Early indication that feeding lotus will reduce methane emission from ruminants. In: *Proc. New Zealand Soc. Anim. Prod.*, 61. p. 23-26.

Young W.J., Marston F.M. and Davis J.R., 1996. Nutrient export and land use in Australian catchments. In: *J. Environ. Manage.*, 47. p. 165-183.