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# Use of 'natural' products as alternatives to antibiotic feed additives in ruminant production

J.-P. Jouany† and D. P. Morgavi

*INRA, UR1213 Herbivores, Site de Theix, F-63122 Saint-Genès-Champanelle, France*

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*The banning in 2006 of the use of antibiotics as animal growth promoters in the European Union has increased demand from producers for alternative feed additives that can be used to improve animal production. This review gives an overview of the most common non-antibiotic feed additives already being used or that could potentially be used in ruminant nutrition. Probiotics, dicarboxylic acids, enzymes and plant-derived products including saponins, tannins and essential oils are presented. The known modes of action and effects of these additives on feed digestion and more especially on rumen fermentations are described. Their utility and limitations in field conditions for modern ruminant production systems and their compliance with the current legislation are also discussed.*

**Keywords:** enzymes, plant extracts, probiotics, production enhancers, ruminants

## Introduction

There is increasing public and scientific concern about the use of antibiotics as feed additives in animal production. This concern is fuelled by the emergence of antibiotic resistance in many human pathogenic bacteria (Manero *et al.*, 2006; Parveen *et al.*, 2006), the release of contaminating residues into the environment (water, soil, etc.) (Yang and Carlson, 2004) and the risk that growth-promoting antibiotic residues may occur in foods of animal origin. For all these reasons, the European Union (EU) decided that antibiotics used in livestock as production enhancers would be banned from 1 January 2006 (EU regulation no.1831/2003 of the European Parliament and of the Council of 22 September 2003). This ban effectively ends nearly 50 years of antibiotic use for non-therapeutic purposes. It covers all classes of antibiotics, including ionophores, a group of substances extensively used as coccidiostats in poultry production and as growth promoters or production enhancers in ruminants. Ionophores improve animal performance in both dairy and beef cattle (McDougall *et al.*, 2004; Gallardo *et al.*, 2005; Melendez *et al.*, 2006). In feedlot cattle in particular, their effects are well documented, with treated animals showing an increase in daily gain and feed efficiency averaging 1.6% and 7.5%, respectively (Goodrich *et al.*, 1984). Consequently, there is nowadays a real demand among animal producers for alternative feed additives, and among consumers for more natural and safe products in the human food supply chain.

Many substances, such as probiotics, prebiotics, some organic acids involved in metabolic patways, herbs and plant extracts, can offer some of the benefits that antibiotics provide. Here we will review current feeding and nutritional problems raised by modern ruminant production, and the availability of 'natural' substances and their potential efficacy in solving these problems. Possible limits to the use of these agents will also be discussed.

## Specificity of feed additives in ruminant production and consequences of the ban on antibiotics as production enhancers

Ruminants have the most differentiated and complex stomach system of all mammals. Three fermentative chambers (reticulum, rumen and omasum) precede the true stomach (abomasum). The reticulo-rumen is the largest (about 100 l in an adult cow) and plays a major role in digestion. About 10 kg of organic matter are digested every day in the rumen by a complex microbiota made of anaerobic bacteria ($\sim 10^{11}$ cells per ml), anaerobic protozoa ($\sim 10^{5}$ cells per ml), anaerobic fungi ($\sim 10^{3}$ cells per ml) and methanogen archaea ($\sim 10^{9}$ cells per ml) as the main groups of microorganisms involved in feed digestion and fermentation. This specialised digestive tract is well adapted to the digestion

† E-mail: jouany@clermont.inra.fr

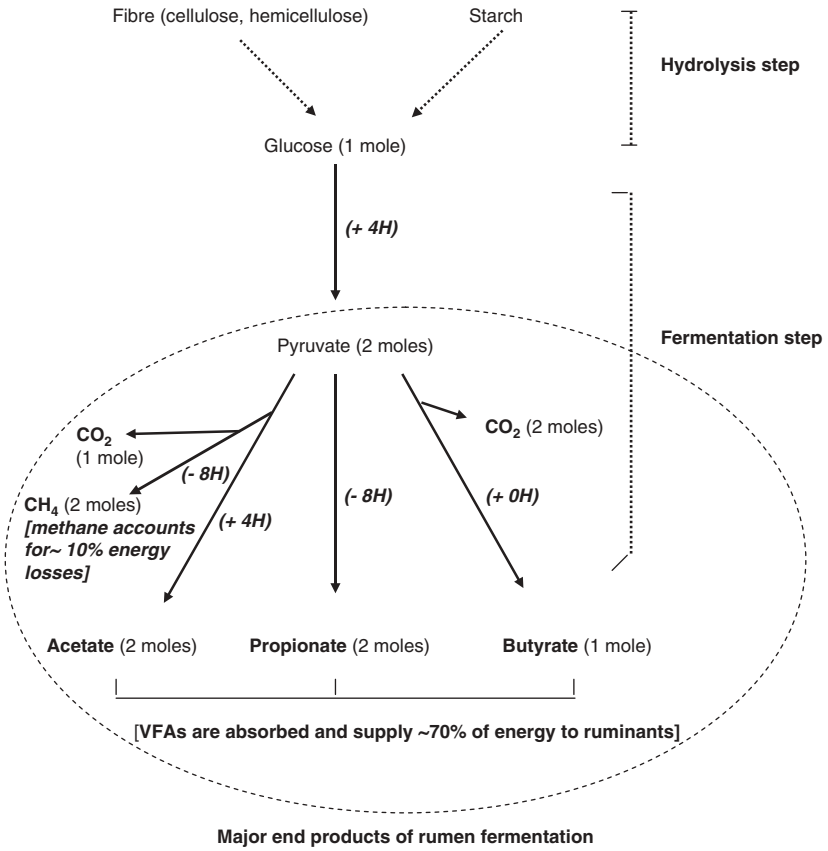


**Figure 1** Microbial hydrolytic and fermentation steps in the rumen; metabolic hydrogen (H) transfers are indicated.

of structural plant polysaccharides, and enables ruminants to feed exclusively on forages. Most of nutrients supplied to ruminants are produced in the rumen from microbial metabolism of carbohydrates (Figure 1) and protein or non-protein nitrogen (Figure 2).

Four main targets have been identified for feed additives to optimise some rumen functions for the benefit of ruminants (Figures 2 and 3): (i) decreased methane production in favour of propionate to improve the energy balance of animals; (ii) reduced feed protein degradation to increase bioavailability of amino acids in the small intestine; (iii) reduced degradation rate of rapidly fermentable carbohydrates (starch, sucrose) and control lactic acid concentration; and (iv) improved fibre digestion. Ionophore antibiotics covered most of these objectives in the rumen. When added continuously at low concentration in feeds (20–40 p.p.m.), they slowed the rate of acid production and prevented lactic acidosis (Osborne *et al*., 2004), mitigated methane production by redirecting metabolic H use towards propionate formation, decreased deamination of amino acids (Wallace *et al*., 1990), thus increasing peptide flow from the rumen into the small intestine (Gomez *et al*., 1991), and reduced frothy bloat in cattle grazing on legume pastures (Lowe *et al*., 1991). Post-ruminal effects of ionophores have also been reported. For example, they have been shown to be effective against coccidiosis, one of the most important and widespread parasitic diseases of ruminants (Stromberg *et al*., 1982). Hence, animals treated with feed antibiotics exhibited increased productivity.

One positive consequence of the ban is that standards of hygiene in livestock production had to be raised to reduce the risk of pathogen infection. But to maximise the efficiency of feed use and increase ruminant productivity through the manipulation of ruminal fermentation, alternative feeding strategies and new feed additives are needed. Here we review the main 'natural' feed additives that can potentially be used in ruminant production. Additives used as appetisers, to mask odour in faeces or manure, as feed preservatives or for specific purposes such as mycotoxin binders, antioxidants and immunostimulants, will not be considered in this review.

## Probiotics

The word 'probiotic', etymologically derived from the Greek 'for life', has the opposite meaning to the word 'antibiotic'. Fuller (1989) defined probiotics as 'live microbial feed supplements which beneficially affect the host animal by improving its microbial balance'. This definition stresses the need for a probiotic to be viable.

Probiotics have been recommended in young ruminants (Ellinger *et al*., 1978; Bruce *et al*., 1979) to prevent diarrhoea caused by enterotoxigenic bacteria in the gut, and

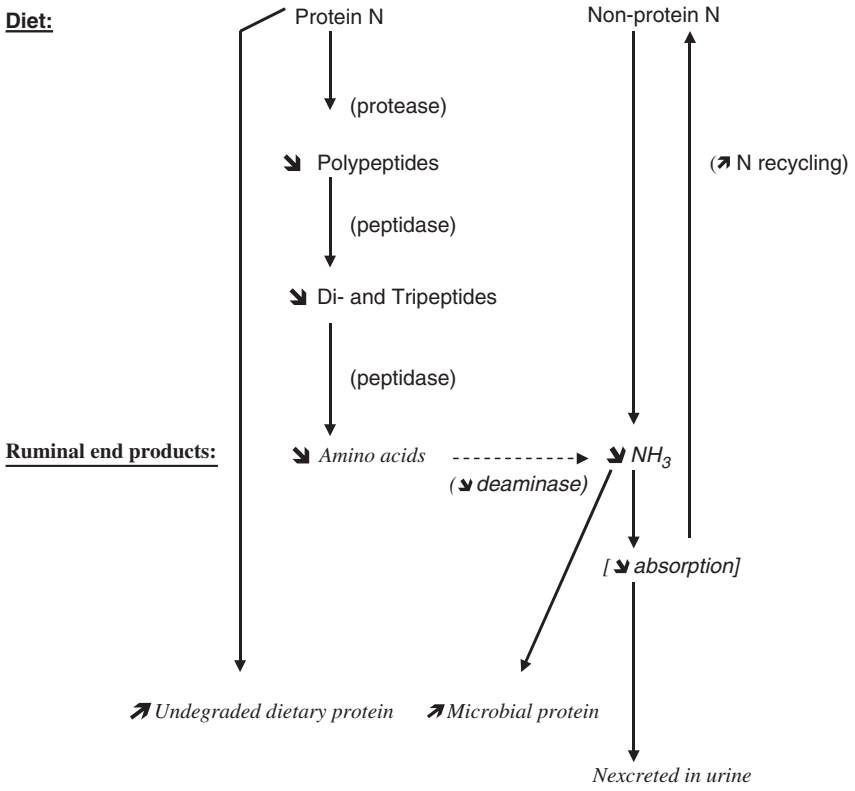


**Figure 2** Possible sites targeted by feed additives to improve nitrogen metabolism in the rumen (↗: increase of the function; ↘: decrease of the function).

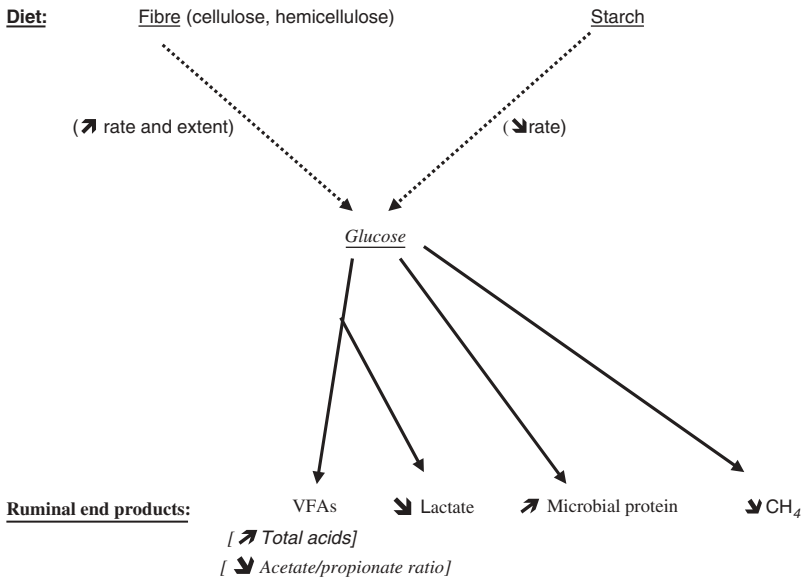


**Figure 3** Possible sites targeted by feed additives on carbohydrate fermentation in the rumen (↗: increase of the function; ↘: decrease of the function).

also during the weaning period to enhance the rate at which the rumen flora and fauna become established. *Lactobacillus acidophilus* alone or in combination with other lactobacilli has been shown to reduce scouring and increase the growth rate of calves in some trials (Bechman *et al.*, 1977; Beeman, 1985) but not in others (Jonsson and Olson, 1985).

In adult animals with a functioning rumen and a fully settled microbial population, probiotics are recommended when the microbial balance could be disturbed, e.g. during diet changes, especially during the transition from a forage-based to a cereal-rich diet. Bacterial probiotics have been predominantly promoted to prevent ruminal acidosis. Lactic acid-producing and lactic acid-utilising bacteria are used, sometimes in combination, to reduce the negative impact of the rapid fermentation of high-starch feeds in the rumen. Lactate-utilisers such as *Megasphaera elsenii* or

*Selenomonas ruminantium* has been reported to prevent the accumulation of lactate and alleviate the drop in rumen pH when animals are fed high-starch or high-sugar diets (Wiryawan and Brooker, 1995). Propionibacteria are also used for their lactate-utilising activity and for their high production of propionate. The rationale for the utilisation of lactate producers is that by maintaining a low and constant level of lactic acid, they sustain an active population of lactate utilisers that in turn will prevent lactate accumulation and pH drop in the rumen (Nocek *et al*., 2002). *Lactobacillus* and *Enterococcus* sp. are the most commonly used species of lactate producer. Krehbiel *et al*. (2003) reviewed the effects of these bacterial probiotics in beef and milk production. Reported results are encouraging and suggest the potential for this type of additives although the authors caution that the information available was limited, but recent papers will help to properly evaluate them (Nocek and Kautz, 2006; Stein *et al*., 2006; Emmanuel *et al*., 2007; Raeth-Knight *et al*., 2007).

Production of bacteriocin-like substances by some bacteria such as *Enterococcus faecium* has been tested as a way to control the growth of pathogens in the rumen (Marcinakova *et al*., 2004). *L. acidophilus* strains have also been shown to reduce shedding of *E. coli* O157 in cattle (Elam *et al*., 2003; Younts-Dahl *et al*., 2005; Peterson *et al*., 2007). Some bacteria with specific functions in the rumen, such as *Butyrivibrio fibrisolvens* which produces enzymes that isomerise linoleic acid to conjugated linoleic acid, have been proposed as probiotics for ruminants (Fukuda *et al*., 2006).

In spite of the increasing number of studies on bacterial probiotics, by far the most commonly used probiotics in adult ruminants are based on yeast preparations of *Aspergillus orizae* and (or) *Saccharomyces cerevisiae*. They are usually supplied as dried preparations of live cells with their spent growth medium in an inert matrix. Preparations containing dead cells and growth medium are commercially available, but the mode of action of these products containing non-living cells will be limited to supplying micronutrients to rumen microbes, and are not considered as probiotics.

The mechanisms governing the homeostasis of the indigenous microbiota living in the digestive tract are extremely complex and not easy to manipulate. In addition, abiotic factors of dietary origin or due to specific characteristics of the host such as rate of ingestion, flow rate of liquid- and solid-phase digesta and saliva secretion can cause large differences in ruminal ecosystems among individuals in the same herd (Raibaud, 1992). These ecological complexities may explain the inconsistent results often reported in the literature on the effects of probiotics (Fuller, 1989; Vanbelle *et al*., 1990; Newbold, 1995). Response variability can also be explained by differences in the microbial species or strains used as probiotics, in the viability rate of cells and in the doses administered. Recent reports describing the mode of action of probiotics help to understand some of the observed inconsistencies and supply evidence of the beneficial effects of probiotics in ruminants under certain feeding conditions. Among them, Jouany (2006) proposed a model to explain how yeast cells could interact with other rumen microbes in a 'micro-consortium structure' and improve their activity (Figure 4).

Yeasts are aerobic and cannot survive for long in an anaerobic environment such as the rumen. For this reason they must be supplied continuously in feeds to reach the minimum effective concentration, set at $10^5$ colony-forming units (c.f.u.) per g rumen content. Yeast cells in the rumen use the traces of available oxygen on the surfaces of freshly ingested feed to maintain their metabolic activity for a few hours. This is why yeast cells are more abundant in the solid matrix than in the liquid phase of rumen digesta (Jouany *et al*., 1991). The removal of oxygen by yeasts in the microenvironment of the solid matrix that takes place shortly after feed has arrived in the rumen was confirmed *in vivo* by Jouany *et al*. (1999a), who observed a significant decrease in redox potential in the rumen of treated animals of up to −20 mV. This change creates better conditions for the growth of anaerobic cellulolytic bacteria, stimulates their attachment to cellulose particles (Roger *et al*., 1990), increases the initial rate of cellulolysis and consequently improves voluntary feed intake by ruminants (Offer, 1990; Callaway and Martin, 1997; Doreau and Jouany, 1998). There is a high variability in oxygen-scavenging capacity among strains, and this factor has to be considered as a priority in the selection of probiotic yeasts for ruminants.

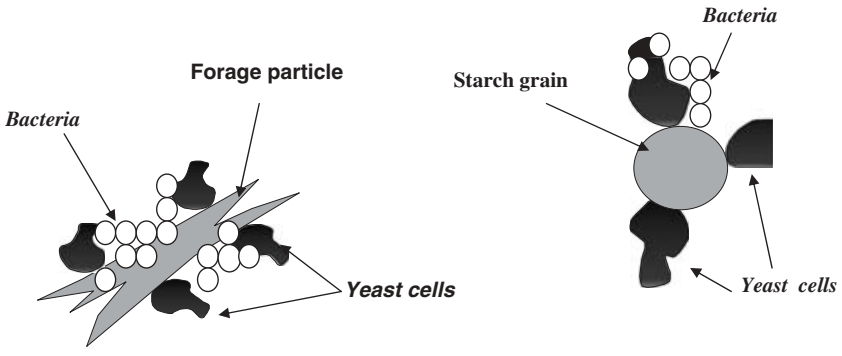


**Figure 4** Yeast cells can use the oxygen located within and immediately around freshly ingested solid particles; anaerobiosis is improved, thus benefiting bacteria closely associated with yeasts in a 'micro-consortium structure' (from Jouany, 2006).

The oxygen fugacity or partial pressure of $O_2$ [log $f(O_2)$] in the rumen content helps to regulate the lactate-propionate equilibrium according to the following redox reaction:

$$C_3H_6O_3\,(\text{lactate}) \leftrightarrow C_3H_6O_2\,(\text{propionate}) + \tfrac{1}{2}O_2, \qquad \text{(reaction 1)}$$

The [log $f(O_2)$] value, which is related to the lactate/propionate equilibrium, is calculated using the laws of thermodynamics by integrating the Gibbs free energy of these two compounds in the Nernst equation

$$\log f(O_2) = 64.59\,\text{Eh} + 4\,\text{pH} - 78.60 \qquad (1)$$

to give

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

as indicated by Marden and Bayourthe (2005). Equation (2) shows that the formation of propionate is favoured at the expense of lactate when $f(O_2)$ is decreased. On the contrary, increasing $f(O_2)$ in the rumen content limits the formation of propionate, thereby allowing accumulation of lactate and a fall in pH. This mechanism explains why live yeasts can regulate the rumen pH when ruminants are fed diets rich in fermentable carbohydrates (sugars, starch, pectins, etc.), which generate large amounts of acids, especially lactic acid, resulting in acidosis (Williams *et al*., 1991). Other mechanisms have been identified to explain the effect of yeasts on pH regulation: (i) they compete with *Streptococcus bovis* and lactobacilli for glucose use, and thus less lactic acid is produced (Chaucheyras *et al*., 1996; Goad *et al*., 1998); (ii) they release malate and small peptides, which stimulate L-lactate use by *M. elsdenii* and *Selenomas ruminantium* (Callaway and Martin, 1997) and (iii) they can promote an increase in protozoa concentration in the rumen (Jouany *et al*., 1999b), which regulates lactic acid concentration, since protozoa compete with *S. bovis* for glucose uptake and are able to metabolise lactic acid (Chamberlain *et al*., 1983; Newbold *et al*., 1985). Acid-sensitive bacteria such as the cellulolytics (Stewart, 1977) recover and cellulase activities are improved at more appropriate ruminal pH after yeasts are added to a grain-hay (50 : 50) diet (Jouany *et al*., 1999a). As yeast cells are autolysed after few hours in the rumen, proteins and other cellular components are released and become available to rumen microbes living in the yeast's micro-environment (Jouany *et al*., 1998a and 1998b). The 'microconsortium' concept explains why such small amounts of nutrients extracted from yeasts can be efficiently used by associated bacteria without dilution in the rumen fluid. Taken together, the effects described above explain why yeasts can improve rumen bacterial growth and protein synthesis, bacterial enzymatic activities, digestion of fibre, voluntary feed intake and animal production (Figure 5).

Using a meta-analysis of published data, Sauvant (2005) examined the effects of yeast on rumen fermentation and animal production. *In vitro* data on rumen fermentation collected from 49 experiments showed that yeasts increased the pH ($P = 0.028$) but had no effect on volatile fatty acid (VFA) ($P = 0.62$), methane ($P = 0.576$) or $NH_3$ ($P = 0.5275$) productions. The numbers of total viable and cellulolytic bacteria were significantly increased ($P = 0.009$ and 0.002, respectively), microbial protein synthesis and the efficiency of microbial growth tended to rise ($P = 0.107$ and 0.099, respectively), and *in vitro* neutral-detergent fibre degradability was improved ($P = 0.062$) in the presence of yeasts. The same statistical analysis applied to *in vivo* data collected from 186 experiments indicates that yeasts had no effect on the end products of ruminal fermentations including lactic acid concentration, and tended only to increase rumen pH ($P = 0.086$). Finally, the data collected from 122 *in vivo* treatments showed that yeasts improved milk yield (+1.3 kg per cow per day; $P = 0.08$) and acid-detergent fibre digestibility (+2.8%; $P = 0.15$) when all stages of lactation were considered.

Since about 17 to 34% of administered yeasts remain alive during transit along the gut of ruminants (Durand-Chaucheyras *et al*., 1998), yeasts may also exert an effect in the post-rumen digestive compartments. The positive effect on the intestinal mucous layer, the proliferation of epithelial cells and mucosal macrophages observed in piglets (Bontempo *et al*., 2006) may also take place in young ruminants. Sougioultzis *et al*. (2006) showed that *S. boulardii* produces a soluble anti-inflammatory factor that have beneficial effects on human intestinal epithelial cells and monocytes. Such effect could also be found in animal colonocytes and monocytes. Also, several recent studies indicate that *S. boulardii* has a preventive effect in decreasing *Salmonella* pathogenesis (Mahzounieh *et al*., 2006), *Escherichia coli* endotoxin production (Buts *et al*., 2006) and *Clostridium difficile* diarrhoea (Katz, 2006).

Probiotics are recommended whenever there is a risk of rumen dysfunction to improve anaerobiosis, stabilise pH and supply nutrients to microbes in their microenvironment. However, allowance must be made for large between-strain differences in their ability to control the digestive microbial ecosystem (Newbold *et al*., 1993).

## Dicarboxylic acids

Dicarboxylic acids (aspartate, fumarate and malate) are considered to be naturally occurring substances since they are major metabolic intermediates of the citric acid cycle and are therefore commonly found in plant and animal tissues. They are used as electron acceptors in the succinate–propionate pathway (Figure 6) and so play a major role in ATP production in some rumen microbial cells. Propionogenesis resulting from the metabolism of dicarboxylic acids competes with rumen methanogenesis for metabolic hydrogen use. In agreement with the stoichiometry of rumen fermentation, supplementation of dicarboxylic acids in the diet of ruminants will therefore reduce rumen methane production (Lopez *et al*., 1999; Carro and Ranilla,

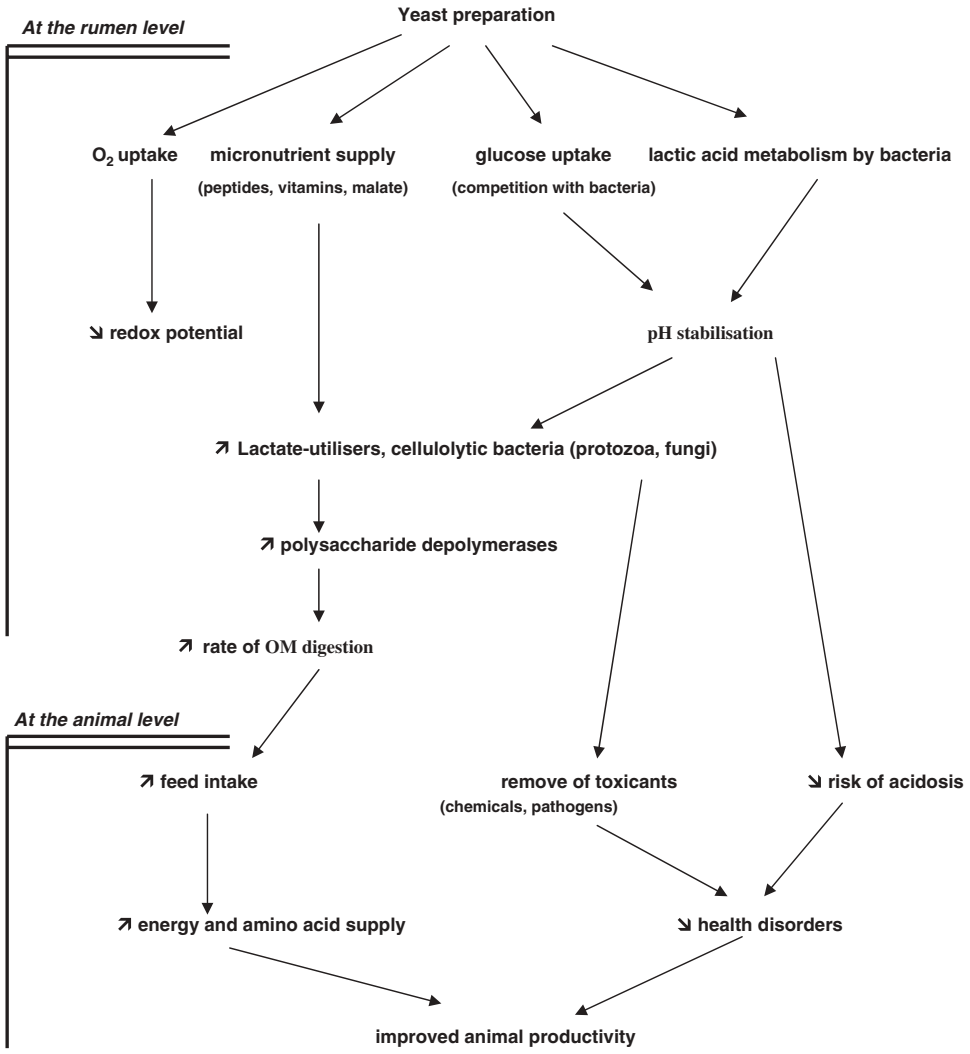


**Figure 5** Proposed model to describe the action of yeast in the rumen and consequences for ruminants.

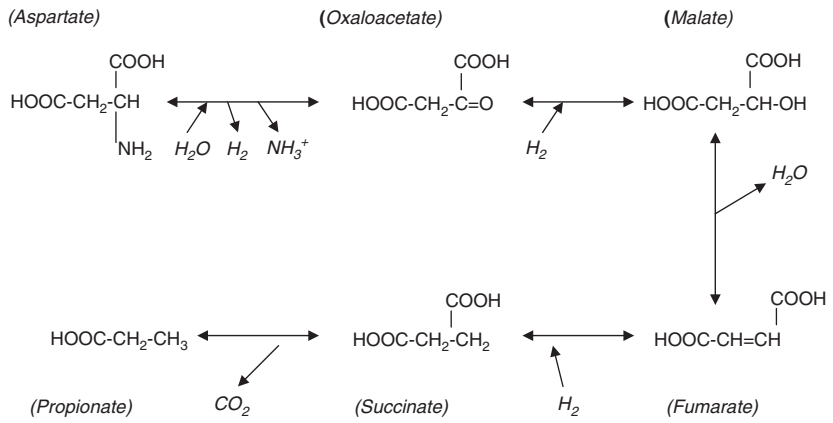


**Figure 6** Succinate–propionate metabolic pathways of the dicarboxylic acids aspartate, malate and fumarate.

2003; Newbold *et al.*, 2005) and improve the animal energy balance.

Newbold *et al.* (2005) calculated from *in vitro* tests that as much as 2.9 kg sodium fumarate must be added daily to a dairy cow diet to decrease by 10% its methane production. The authors also indicated that free acids are more efficient than their salts. Direct addition of large amounts of pure dicarboxylic acids to animal diets is not practical because of the undesirable drop in rumen pH and reduced intake, and also because of cost. The effect on pH has been

prevented by using an encapsulated form of fumaric acid made of a mixture of fumaric acid (85%) and partially hydrogenated vegetable oil (15%) (Wallace *et al.*, 2006). This allowed a decrease in methane production of up to 75%, accompanied by an increase in feed conversion efficiency, in lambs supplemented with fumaric acid given at a dose of 10% of the diet. The reduction in methane production in this experiment was greater than expected based on stoichiometric calculations. The authors hypothesised that the fumaric acid slowly released for a long time might adversely affect methanogen populations by depriving them of $H_2$ substrate. Such supplementation is not cost-effective in current ruminant production systems, but considering that methane is a major greenhouse gas implicated in climate change, mitigation strategies of this kind deserve further investigation. In particular, the persistence of the effect of fumaric acid on methanogenesis needs to be assessed.

Dicarboxylic acids can also be effective in preventing the drop in ruminal pH usually observed 1 to 2 h after feeding high-concentrate diets by redirecting fermentation towards propionate formation at the expense of lactate (Figure 6). In agreement with the stoichiometric reaction (1), Martin (1998) showed that accumulation of $H_2$ inhibited the conversion of lactate to pyruvate by *S. ruminantium* lactate dehydrogenase. Therefore, dicarboxylic acids provide an electron sink for $H_2$ that increases lactate use by this predominant ruminal bacterium. According to Nisbet and Martin (1994), lactate uptake by *S. ruminantium* due to malate supplementation is an inducible pathway. Linehan *et al.* (1978) suggested another possibility for dicarboxylic acids to stimulate the growth of *S. ruminantium* on lactate-enriched medium by overcoming the oxaloacetate deficiency associated with gluconeogenesis through the phosphoenolpyruvate pathway. Fumarate and malate are used directly as precursors of oxaloacetate (Figure 6), and aspartate is used for glucose synthesis instead of oxaloacetate, which therefore remains available as a substrate for *S. ruminantium*.

The minimal effective dose of malate that acts on rumen metabolism and reduces the risk of acidosis has been estimated at 40 to 80 g/day for steers weighing 450 kg (Martin *et al.*, 1999), i.e. 55 to 115 g/day for a cow weighing 650 kg. Like fumaric acid, addition of pure malate is not cost-effective and can lower pH when added in the acid form. Malate can be supplied naturally through organic acid-rich forages such as selected alfalfa cultivars. Callaway *et al.* (1997) indicated that the desired efficient dose of malate could be obtained simply by incorporating 6.0 kg of alfalfa at 42 days of maturity into dairy cow diets.

## Plants and plant extracts

Plants synthesise a broad range of secondary metabolites that are not directly involved in their growth, development or reproduction (Figure 7). These compounds defend plants against predators, parasites and diseases, protect them from interspecies competition and can attract insects involved in their reproductive process. They were used extensively as medicines before antibiotics were discovered. Some of them have been extracted and concentrated for use in animal nutrition (i) for their aromatic value as flavouring agents in feeds and (ii) for their antimicrobial activity and ability to influence the digestion of some ingredients (Elgayyar *et al.*, 2001). Plant secondary metabolites are a complex group of substances, and their description lies outside the scope of this review (for review see Bruneton, 2005). Only the major bioactive products that are already being used or could potentially be used as additives in ruminant feeds will be considered here.

### *Tannins*

The first reviews on tannins in feed and food published in the 1960s and 1970s treated them as toxic xenobiotics, despite many reports indicating that their toxicity was generally low (Dollahite *et al.*, 1962; Armanious *et al.*, 1973). Tannins are frequently added to drinks and foods for their astringent properties (Santos-Buelga and Scalbert, 2000) and occur naturally in many legumes and fruits. Their chemical nature and dose are the deciding factors for their safe use as feed or food additives.

Tannins can be defined by their physical or biological activities, or by their chemical structure. Horvath (1981) proposed the following general definition of tannins: 'water-soluble polymeric phenolics of sufficiently high molecular weight containing sufficient phenolic hydroxyls and other suitable groups (e.g., carboxyls) to form effectively strong complexes with protein and other macromolecules under the particular environmental conditions being studied'. Reed (1995) added that they were equally able to complex minerals, starch and cellulose as well as proteins. Chemically, tannins fall into two classes: (i) condensed tannins (CTs) or proanthocyanidins, which are polymers of flavonoid units joined by highly stable covalent carbon–carbon bonds and (ii) hydrolysable tannins (HTs), which contain a carbohydrate with hydroxyl groups partially or totally esterified with phenolic acids such as gallic acid (gallotannins) or ellagic acid (ellagitannins).

Historically, the property of tannins to form chemical complexes with proteins has been extensively used to convert fresh animal skin into rotproof leather. The same property was explored as a way to slow dietary protein breakdown in the rumen and enhance the amino-acid bioavailability in the small intestine, and reduce ruminal $NH_3$ production and nitrogen (N) excretion in urine (Aerts *et al.*, 1999; Barry and McNabb, 1999). This impairment of ruminal protein degradation improves the animal's nutritional status and reduces the amount of N released into the environment (Figure 2).

*In vitro* studies indicated that tannin–protein complexes are formed in the pH range 6 to 7 prevailing in the rumen, and are destroyed at pH $< 3.5$ in the abomasum and at $>7$ in the small intestine, thus making proteins available for

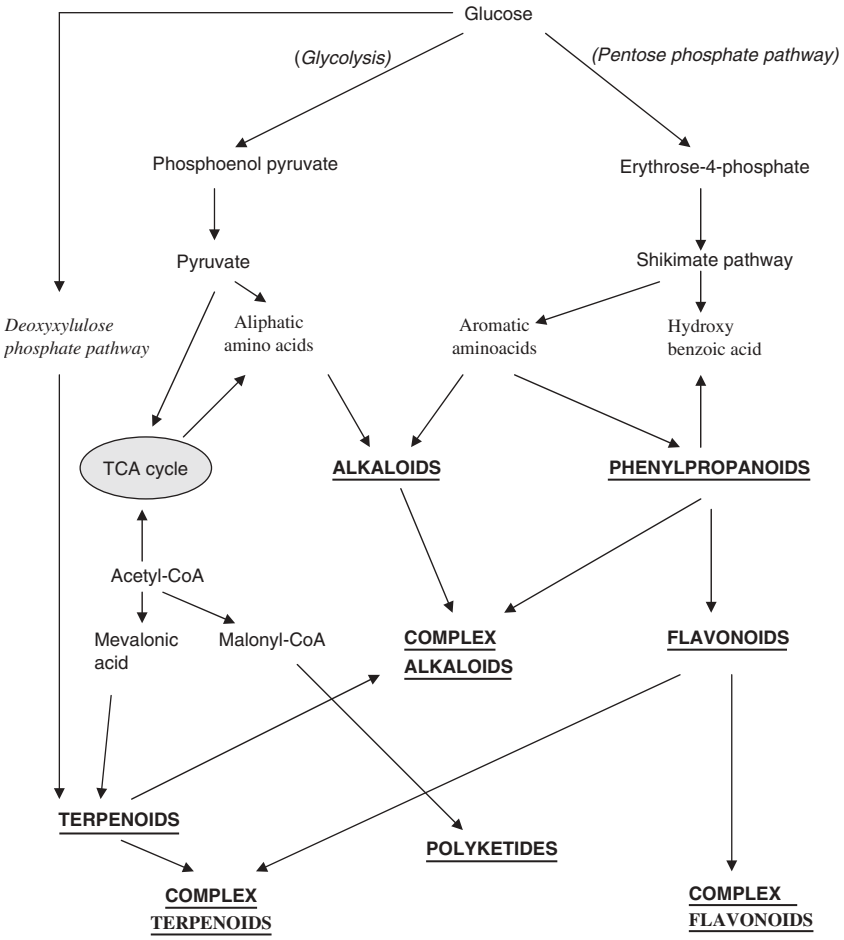


**Figure 7** The different plant metabolic pathways involved in the synthesis of secondary metabolites.

gastric and pancreatic digestion (Jones and Mangan, 1977). Proteins are linked to tannins by hydrogen bonds between the hydroxyl groups of tannins, and the amine and carboxyl groups of proteins. Aromatic cycles of tannins and amino acids can also interact to form van der Waals bonds. When the protein/tannin ratio is low, tannins cover the protein with their hydrophilic groups. The 'protein–tannin' complexes then become more hydrophobic in their outer layer, and precipitate in water solution. When the protein/tannin ratio is high, tannins form chemical bridges between proteins and precipitate them (Haslam, 1988). The nutritional effects of tannins vary widely owing to their great chemical diversity, and this variability has not been sufficiently appreciated in many animal feeding trials. In addition, inadequate analytical methods used to determine the type and concentration of tannins hinders the interpretation of results reported in the literature.

The 'ideal' evolution of the 'tannin–protein' complex stability according to pH in the digestive tract of animals, which has been observed with *Leucaena leucocephala*, does not occur with *Lotus corniculatus* or *L. pedunculatus*, for which the complexes are too stable, and remain undissociated and undigested in the small intestine (Mueller-Harvey, 2006). When released in the intestines, tannins can have detrimental effects if they bind endogenous animal proteins such as enzymes, mucus or intestinal mucosal cells. For example, quebracho (*Schinopsis lorentzii*) tannins have no effect on ruminal by-pass of proteins, but can dramatically reduce protein absorption in the small intestine of ruminants (Min *et al.*, 2003; Mueller-Harvey, 2006). The increase in faecal N usually observed with tanniferous plants may originate in undigested complexes formed with dietary and (or) endogenous proteins.

The effects of tannins are more pronounced when they are directly administered in the rumen rather than added to the feed. This stresses the capacity of some salivary proteins, especially proline-rich glycoproteins, to bind tannins (Shimada, 2006) and prevent their further activity in the rumen. This action on salivary proteins explains the sensation of astringency recognised during the ingestion of tannin-rich food or drinks. It also indicates that proteins with high proline content, rich in non-polar amino acids, with high molecular weight and with an open tertiary structure, are more susceptible to interaction with tannins (Mehansho *et al.*, 1987).

The possible antimicrobial effect of tannins, in both the rumen and the intestine, may modify feed digestion in ruminants. For example, *L. corniculatus* tannins lower the

concentrations of bacteria in the rumen and decrease the amounts of bacterial proteins flowing to the intestine (Waghorn *et al.*, 1987). Thus, the beneficial effect of tannins on the intestinal flow of ruminal undigested feed proteins can be cancelled by the negative effect on intestinal flow of microbial proteins. The fact that proteolytic bacteria are sensitive to *L. corniculatus* tannins (Min *et al.*, 2002), and that the binding strength of protein complexes formed with this source of tannins is high, explains why *L. corniculatus* can negatively affect the N digestion of ruminants.

Tannins can inactivate rumen microbial enzymes of proteic structure and adversely affect global ruminal digestion (Barry and Manley, 1986; Makkar *et al.*, 1988; Barry and McNabb, 1999). CTs have been shown to cause a shift in the partitioning of nutrients during fermentation in the rumen so that a higher proportion is redirected to microbial mass synthesis at the expense of VFA production (Makkar, 2003), thus increasing the efficiency of microbial protein synthesis. Several *in vivo* experiments have shown that the amount of methane produced per unit of digestible dry matter (DM) intake is reduced (20–30%) when ruminants are fed forages rich in CTs such as *L. pedunculatus* or *L. corniculatus* (Woodward *et al.*, 2001; Waghorn *et al.*, 2002; Pinares-Patino *et al.*, 2003), but no clear indication was given of the mechanisms involved. However, tannins were strongly suspected since addition of polyethylene glycol (PEG), a tannin-binding reagent, caused recovery of methane production. Tavendale *et al.* (2005) stressed that CTs extracted from *L. pedunculatus* have a direct inhibitory effect on archaea methanogens, which impairs ruminal fermentation. Toxicity of HTs and CTs towards methanogens was also demonstrated in methanogenic digesters (Field *et al.*, 1989).

CTs are effective against ruminal bloat caused by the capture of ruminal gases in a polysaccharide slime induced by the rapid release of soluble protein into ruminal fluid (Clarke and Reid, 1974). Severity of bloat legumes is thus lowered in the presence of CTs since gas formation and microbial protein degradation are both decreased (Jones and Lyttleton, 1971; Waghorn and Jones 1989; Min *et al.*, 2003, 2005). Tannins can also destabilise the structure of plant protein foams by altering surfactant forces in the ruminal liquid phase (Tanner *et al.*, 1995).

Regarding toxicity in animals, Mueller-Harvey (2006) indicate that CTs and HTs are not very different, although HTs have usually been considered more toxic than CTs by many authors (McSweeney *et al.*, 2001; Odenyo *et al.*, 2003; Waghorn and McNabb, 2003). They explain that the toxicity of HTs may be due to intestinal absorption of small molecular weight substances originating from their microbial degradation, unlike CTs, which cannot be extensively degraded by rumen microbes (Makkar *et al.*, 1995) and then absorbed (Terrill *et al.*, 1994). Optimal doses of tannins are difficult to assess since their activity depends on their molecular structure, and especially on their molecular weight (Meagher *et al.*, 2004). It is generally accepted that dietary concentrations of CTs must be less than 50 g/kg dietary DM to have beneficial effects (McMahon *et al.*, 2000; Hervás *et al.*, 2003; Min *et al.*, 2003). These values were set from *in vivo* trials carried out mainly with *Lotus* spp., but are not applicable to all sources of tannins. For example, large amounts of CTs ($\cong$80 g/kg DM) can promote animal production when supplied from sulla (*Hedysarum coronarium*) or sainfoin (*Onobrychis viciifolia*), whereas doses as low as 25 g/kg DM have a strong negative effect on animal growth rate when the tannins are from carob pulp (Mueller-Harvey, 2006). Tested at 21 g/kg soya bean (3.9% crude protein), chestnut tannins had no significant effect on feed intake, feed efficiency, daily gain or fattening period of lambs from 15 to 25 kg (Frutos *et al.*, 2004). The same tannins applied at 0.4% of crude protein of wilted grass silage significantly increased duodenal non-ammonia N flow and intestinal N digestibility (Decruyenaere *et al.*, 1996). Yet 80 g/kg of chestnut tannins sprayed onto hay significantly decreased DM digestibility in sheep and goats (Zimmer and Cordesse, 1996). These results indicate that an optimal dose, even for the same source of tannins, is difficult to assess, especially as the protein source varies. Also, comparisons between trials are difficult because doses of tannins are expressed relative to DM, N or protein feed content. Trials carried out on lactating dairy cows showed positive effects on production or live-weight gain when peanut skins (180 g CTs per kg) were added at 8 to 16% of the dietary DM (West *et al.*, 1993) or tamarind seed husks (140 g CTs per kg) at 2.5% of the dietary DM (Batta *et al.*, 2000). At the rumen level, the optimal concentration of CTs to reduce proteolysis *in vitro* has been set at about 400 μg CTs per ml and above (Aerts *et al.*, 1999). The same concentration reduced the growth of several bacterial strains from the rumen (Molan *et al.*, 2000).

Many tropical forages can contain very high levels of tannins, and ruminants may spend most of their grazing time on them. In these conditions, rumen microbes can develop defence mechanisms to resist the toxic effects of tannins. They can protect their membrane proteins through external deployment of lipid layers (Pell *et al.*, 2000) and they can secrete extracellular polysaccharides (Brooker *et al.*, 2000) and (or) produce glycoproteins with high tannin affinity, as shown for phytopathogenic fungi by Nicholson *et al.* (1986). Micro-organisms able to degrade HTs (Ephraim *et al.*, 2005) and even CTs (Odenyo *et al.*, 1999) have been found in the rumen of ruminants in tropical countries. Differences between ruminants in their ability to degrade tannins have been evidenced by McKie *et al.* (2004). However, microbial detoxifying capacity sometimes cannot overcome the tannin toxicity, and preventive treatments of forages are necessary to eliminate or degrade tannins. These include oxidation of phenolics at alkaline pH (Makkar and Becker, 1996a), storage of dried chopped plants or foliage (Makkar and Singh 1991), chemical treatments (Makkar and Becker, 1996a) and PEG incorporation (Makkar *et al.*, 1998). Polyetyleneglycol, and to a lesser extent polyvinylpyrrolidone, compete with proteins to complex tannins. The higher affinity of tannins for PEG than

for proteins, which protects proteins from an excessive complexation, has been extensively used in tropical and arid countries (Getachew *et al*., 2001), but the higher the level of dietary proteins, the weaker is the effect of these tannin-complexing agents (Makkar and Becker, 1996b).

Owing to their astringent taste, feeds rich in tannins have poor palatability. The lower feed intake, associated with a lower rate of digestion observed in some tannin-rich plants (Waghorn *et al*., 1994; Stienezen *et al*., 1996) can offset the positive effects of tannins on N digestion, resulting in a net negative effect on animal production.

Among the postruminal effects of CTs, recent research has demonstrated that they can be an alternative to chemical anthelmintics to control gastro-intestinal parasitic nematodes (Butter *et al*., 2000; Athanasiadou and Kyriazakis, 2004; Hoste *et al*., 2006) in grazing production systems. Farmers have traditionally used plants for deworming animals, and there is some evidence that animals select certain plants for self-medication during grazing (Hutching *et al*., 2003). Tannins have been shown to act through a direct anti-parasitic activity, but they may also stimulate host resistance as a result of an increase in intestinal protein supply (Coop and Kyriazakis, 2001). The anthelmintic efficacy of tannins is closely related to their chemical structure (Hoste *et al*., 2006). Grazing pastures with mixtures of grasses and legumes rich in tannins could be a practical way of preventing nematode parasitism in ruminants (Ramirez-Restrepo and Barry, 2005; Aufrere *et al*., 2006).

*Saponins*

Saponins are glycosides found in many plants. They contain a sugar moiety glycosidically linked to a hydrophobic aglycone called 'sapogenin', which may be terpenoid or steroid in nature. The great variety of saponins arises from the multiplicity of the aglycone structure, the nature of some existing side chains and the positions where the sugar moieties are attached on the aglycone. The ability of a saponin to foam is due to the combination of the non-polar sapogenin unit and the water-soluble sugar. Active components extracted from either *Yucca shidigera* (steroid saponins called also sarsaponins) or *Quillaya saponaria* and *Sapindus* sp. (triterpenoid saponins) are the most common commercial source of saponins. Lucerne and soya beans are the main examples of saponin-rich plants that are extensively used in ruminant diets, and could be implicated in some aspects of animal nutrition (Sen *et al*., 1998).

Saponins have considerable potential as pharmaceutical and (or) nutraceutical agents. They have been shown to have hypocholesterolaemic, anticoagulant, anticarcinogenic, hepatoprotective, hypoglycaemic, immunomodulatory, neuroprotective, anti-inflammatory and anti-oxidant activities (Milgate and Roberts, 1995; Cheeke, 1999). In ruminants, dietary supplementation with saponins has been claimed to improve growth, feed efficiency and health (Mader and Brumm, 1987). These effects have been explained partly by the action of saponins on ruminal microbes, resulting in a decrease in rumen degradability of feed proteins and an increase in microbial protein synthesis in the rumen, which both increase the intestinal flow of amino acids (Makkar and Becker, 1996b). As indicated previously for tannins, saponin administration will therefore improve the assimilation of feed N by animals because less $NH_3$ is produced in the rumen and less urea is eliminated in urine (Santoso *et al*., 2004a) (see Figure 2). Two mechanisms have been considered to explain the effect of saponins on N metabolism in the rumen: (i) saponins extracted from leaves of *Sesbania sesban* or from lucerne roots have been shown significantly to reduce the numbers of protozoa (Lu and Jorgensen, 1987; Klita *et al*., 1996; Newbold *et al*., 1997), which play a major role in ruminal feed protein degradation (Jouany, 1996) and (ii) $NH_3$ resulting from microbial protein degradation can be bound by saponins in a balanced chemical reaction regulated by $NH_3$ concentration (Headon *et al*., 1991). Thus, an adequate amount of $NH_3$ is continuously supplied for microbial protein synthesis in the rumen (Hussain and Cheeke, 1995).

The detrimental effect on protozoa is caused by the reaction of triterpenoid or steroid saponins with the sterols located in the ciliate cell membrane (Assa *et al*., 1975; Pacheco-Soares and de Souza, 2000). Although the toxicity of saponins towards protozoa seems to be widespread and non-specific (Valdez *et al*., 1986; Diaz *et al*., 1993; Hristov *et al*., 2003), some authors found no effect on protozoal number (Sliwinski *et al*., 2002; Hristov *et al*., 2003), or even observed an increase (Abreu *et al*., 2004; Eryavuz and Dehority, 2004). This may be due to the capacity of some rumen bacteria to hydrolyse saponins into free glycosyl and sapogenin fractions (Newbold *et al*., 1997), thus removing their toxicity against protozoa. When incubated *in vitro* with non-adapted rumen micro-organisms, no degradation of Quillaja saponins was observed during the first 6 h and about 50% of saponins disappeared during the next 6 h (Makkar and Becker, 1997). Saponins may also be degraded or 'inactivated' by some still unidentified salivary components (Odenyo *et al*., 1997; Teferedegne, 2000). This explains why the effects of saponins are more pronounced when they are directly added to the rumen rather than mixed with the diet (Odenyo *et al*., 1997). To avoid adaptation of bacteria and maintain the activity of saponins in the rumen, Thalib *et al*. (1995) advocate administering them every 3 days.

Saponins can alter the cell wall structure of Gram-positive bacteria such as *Ruminococcus flavefaciens* and *R. coccus albus,* and impair the digestion of filter paper by these bacteria, but had no effect on the cellulolytic activity of Gram-negative *Fibrobacter succinogenes* (Wina *et al*., 2006). The ruminal fungi *Neocallimastix frontalis* and *Piromyces rhizinflata* were totally inhibited by 2.25 μg saponins per ml. Using the membrane hybridisation technique with oligonucleotide probes, Wina *et al*. (2005) observed that a marked decrease in protozoal RNA offset an increase in bacterial RNA concentration when *S. rarak* saponins were added *in vitro* at 1 mg/ml. Methanogen RNA concentration was also affected and bottomed at the saponin concentration of

4 mg/ml. Because of the strong inhibiting effect of saponins on *S. cerevisiae* (Killeen *et al.*, 1998), it is strongly recommended that saponins not be used in association with yeast-based probiotics.

Some of the antimicrobial compounds have been identified as three butanol-extractable 5β-spirostan-3β-ol saponins (Killeen *et al.*, 1998), which are thought to alter bacterial membranes by increasing their porosity (Schulman *et al.*, 1955; Wang *et al.*, 2000). The foaming property of saponins increases the surface tension of the bulk solution and accelerates lysis of microbial cells with weakened membranes. In addition, bacterial growth inhibition may be caused by complexation of essential minerals and steroids with saponins, thus limiting their bioavailability for bacterial metabolism (West *et al.*, 1978; Simons *et al.*, 2006).

The effects of saponins on ruminal fermentation have been extensively studied *in vivo* and *in vitro* (Table 1). They significantly decrease ruminal $NH_3$ concentration and increase that of propionic acid at the expense of acetate and butyrate (Abreu *et al.*, 2004; Santoso *et al.*, 2004b; Hristov *et al.*, 1999). In agreement with the stoichiometry of ruminal fermentations, they significantly decrease methane production either *in vitro* (Lila *et al.*, 2003; Hu *et al.*, 2005) or *in vivo* (Santoso *et al.*, 2004a).

The strong bioactivity of saponins, associated with the possibility for rumen microbes to deglycosylate them, explain why their effect on animal performances is rather inconsistent (Mader and Brumm, 1987; Wu *et al.*, 1994; Calsamiglia *et al.*, 2005), and generally low when animals have been treated over a long period (Table 2).

In view of their foaming properties, dietary saponins have often been suspected of favouring rumen bloat in ruminants, but clear evidence is lacking (Cheeke, 1996).

Saponins can also act in the distal part of the digestive tract. They increase the permeability of intestinal cells through their ability to complex sterols in mucosal cell membranes (Johnson *et al.*, 1986), which enables the uptake of non-absorbable substances (Gee *et al.*, 1997). This property has been exploited to enhance absorption of orally administered drugs (Chao *et al.*, 1998). However, saponins fed at levels as low as 0.15% in the diet can alter the intestinal mucosa integrity (Bureau *et al.*, 1998), and affect the active transport of nutrients by lowering transmural potential difference across the brush border membrane of intestinal cells (Gee *et al.*, 1989).

Optimal doses for a positive effect of saponins on rumen fermentation or ruminant production are difficult to assess since saponins are supplied either as extracts or as ground saponin-rich plants, and doses are given variously per unit of animals' live weight, per head, per unit of dietary DM weight, or per unit of liquid volume in *in vitro* experiments. Several saponin sources have been tested (yucca, quillaja, *S. saponaria*, *S. rarak*, alfalfa root, tea) on various species of animals in various physiological states. Also, Cardozo *et al.* (2005) demonstrated that the effect of yucca saponins on rumen fermentations was highly dependent on rumen pH. They showed that saponins were much more active in the rumen at pH 5.5, corresponding to high-starch diets, than at pH 7.0, corresponding to low-digestible forage-based diets. This aspect has also to be considered in *in vitro* comparative tests, where the pH values are usually set between 6 and 7. Nevertheless, the results summarised in Table 2 indicate that optimal doses are generally lower for steroidal saponins than for triterpenoid saponins. They range from 1.5–8 to 20–60 g per cow or heifer per day for yucca extracts, depending on the mode of extraction. A dose as low as 280 mg per sheep per day of yucca showed significant effects on rumen fermentation in adult sheep (Santoso *et al.*, 2004b), whereas a dose of 30 g/sheep per day of a product called 'yucca extract' had no effect (Eryavuz and Dehority, 2004). Optimal doses for sheep were set at 12 to 50 g/day and 24 g/day of saponins from *Saponaria* sp. and alfalfa root, respectively. *In vitro*, the $NH_3$ concentration, acetate/propionate ratio, methane production and protozoa population were dose-dependently decreased by sarsaponin (Lila *et al.*, 2003). A negative effect on DM digestibility appeared at a dose of 1.8 g/l of sarsaponin in the liquid of fermentors.

Dietary supplementation with yucca extracts has also been recommended for non-nutritional purposes. They are used to reduce environmental pollution due to odoriferous manures from animal sheds by decreasing release of $NH_3$ to the air through the mechanism already described for the rumen (Killeen *et al.*, 1998). Also, the antiprotozoal property of saponins could be exploited in the treatment of protozoal infections in ruminants such as *Giardia* (McAllister *et al.*, 2001) and *Plasmodium* (Traore *et al.*, 2000).

*Essential oils*

The term 'essential oils' (EOs) includes a wide variety of products extracted from plants by the general process of steam distillation carried out with either water or aqueous alcohol. EOs are complex mixtures chemically made up of alcohol, ester or aldehyde derivatives of phenylpropanoids or terpenoids (Greathead, 2003). Only a few preparations have been standardised and marketed specifically as feed additives for ruminants.

Most of the biologically active molecules in EOs have antimicrobial activities that protect plants from pathogens and herbivores. EOs are lipophilic, and thus interact with the cell membrane of bacteria, which accounts for their toxicity and antimicrobial effects, particularly against Gram-positive bacteria. The external capsule of Gram-negative bacteria can protect them against the EOs (Griffin *et al.*, 1999; Chao *et al.*, 2000), but some molecules of EOs are small enough to gain access to the inner membrane of Gram-negative bacteria and damage it. EOs can also cause coagulation of cytoplasmic material (Burt, 2004). The same biological effects of EOs can impair the growth of fungi and protozoa (Davidson and Naidu, 2000; Lee *et al.*, 2005; Tabanca *et al.*, 2006) and viruses (Chao *et al.*, 2000; Schnitzler *et al.*, 2001; Farag *et al.*, 2004).

The substances listed in Table 3, except perhaps for the complex patented commercial blend of EOs (CBEO) called

**Table 1** *Influence of various saponin-containing plants on rumen microbes, and their hydrolytic and fermentative activities*

| Origin | Doses | Animals (or *in vitro*) | Rumen VFAs | $NH_3$-N | $CH_4$ | Rumen microbes | Enzymatic activities | Authors |
|---|---|---|---|---|---|---|---|---|
| Sarsaponin | 0;35;55;77 mg/kg feed | *In vitro* | nd | nd | nd | ↓prot.;↑bact. | ↑ADF digestion | Valdez *et al.* (1986) |
| | 0;77 mg/kg feed | Dairy cows | = | = | nd | nd | nd | |
| *S. saponaria* | 0;25;50 g/day | Adult sheep | nd | nd | nd | ↓prot.;↑bact. (dose 50 g) | nd | Diaz *et al.* (1993) |
| Yucca extract | 0;2;4;6;8 g/day | Dairy cows | = | = | nd | nd | nd | Wu *et al.* (1994) |
| Yucca extract | 0;250 mg/kg feed | Steers | ↓C2;↑C3 | ↓ | nd | nd | nd | Hussain and Cheeke (1995) |
| Alfalfa root extracts | 0;10;20;40 g/kg DMI | Adult sheep | ↓C2/C3 | nd | = | nd | nd | Klita *et al.* (1996) |
| *S. rarak* extract | 0;0.70 g/kg BW | Adult sheep | nd | ↓ | nd | ↓prot.;↑bact. | nd | Thalib *et al.* (1996) |
| Powdered yucca | 0;20;60 g/day | Heifers | ↓C2/C3 | = | nd | ↓prot. | ↓CMCase; xylanase; amylase | Hristov *et al.* (1999) |
| Sarsaponin | 0;1.2;1.8;2.4;3.2 g/l | *In vitro* | ↓C2/C3 | ↓ | ↓ | nd | nd | Lila *et al.* (2003) |
| *S. saponaria* extract | 0;1 g/kg $BW^{0.75}$ | Adult sheep | ↓C2, C4; ↑C3 | = | nd | ↑prot | nd | Abreu *et al.* (2004) |
| Yucca extract | 0;5;10;20;30 g/day | Adult sheep | nd | nd | nd | = or ↑ prot.;=bact.,fungi | nd | Eryavuz and Dehority (2004) |
| *S. saponaria* | 0;25;50 g/day | Adult sheep | = | = | ↓ | nd | = | Santoso *et al.* (2004a) |
| Yucca | 0;240 mg/kg feed | Adult sheep | ↓C2/C3 | ↓ | nd | nd | nd | Santoso *et al.* (2004b) |
| *S. rarak* | 0;4;8;12 g/day | Adult sheep | nd | nd | nd | nd | nd | Wina *et al.* (2004) |
| Tea saponin | 0;0.2;0.4 mg/ml | *In vitro* | = | ↓ | ↓ | nd | nd | Hu *et al.* (2005) |
| Mangosteen peel | 0;100;150 g/day | Cattle | = | = | = | ↓prot.; ↑bact.;=fungi | nd | Ngamsaeng *et al.* (2006) |
| *S. rarak* | 0;0.48;0.72 g/kg BW | Adult sheep | nd | ↓ | nd | ↓prot. | ↓cellulase; xylanase | Wina *et al.* (2006) |

↑: significant increase ($P < 0.05$); ↓: significant decrease ($P < 0.05$); =: no significant effect; nd: no data; DMI: dry-matter intake; ADF: acid-detergent fibre; $NH_3$-N: ammonia nitrogen; BW: body weight; $BW^{0.75}$: metabolic BW; C2: acetate; C3: propionate; C4: butyrate; prot.: protozoa; bact.: bacteria; CMCase: carboxymethyl cellulase.

**Table 2** *Effect of saponins on animal performance, ADF digestability, rumen end products, N digestion and blood urea*

| Saponin | Doses | Animals (*n*) | Diets | DMI | Milk prod. | Milk comp. | Growth | ADF dig. | $NH_3$-N | $CH_4$ | VFAs | pH | Blood urea | N retention | Authors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sarsaponin | 44 mg/kg feed | Dairy cows (4) | Sorghum silage | = | nd | nd | nd | (1) | nd | nd | nd | ↓ | nd | nd | Goetsch and Owens (1985) |
| Steroidal | 77 mg/kg feed | Dairy cows (16) | 55/45 c/f | = | = | = | nd | = | = | = | = | = | nd | nd | Valdez *et al.* (1986) |
| Yucca | 150 mg/day | Steer calves (224) | 77/33 f/c | = | – | – | = | nd | nd | nd | nd | nd | nd | nd | Mader and Brumm (1987) |
| Yucca | 2;4;6;8 g/day | Dairy cows (5) | TMR | = | = | = | – | nd | nd | nd | nd | nd | nd | nd | Wu *et al.* (1994) |
| Yucca | 250 mg/kg feed | Steers (4) | 4 roughage diets | = | – | – | nd | nd | ↓ | nd | nd | nd | ↓ | nd | Hussain and Cheeke (1995) |
| | | | 4 concentrate diets | = | – | – | nd | nd | ↓ | nd | nd | nd | ↓ | nd | |
| Alfalfa | 200;400;800 mg/kg BW | Wethers (4) | Grass hay | nd | – | – | – | nd | nd | nd | nd | ↓ | nd | nd | Klita *et al.* (1996) |
| Yucca | 20;60 g/day | Heifers (6) | 61/39 c/f | = | – | – | – | = | nd | nd | ↑C3 | = | nd | nd | Hristov *et al.* (1999) |
| Sarsaponin | 1.2;1.8;2.4;3.2 g/l | (*In vitro*) | – | – | – | – | – | ↓ | ↓ | nd | ↑C3 | ↓ | nd | nd | Lila *et al.* (2003) |
| GraniPrep | 60 ml/t barley grains | Steers (138) | 50/50 c/f | = | – | – | ↓FCR | = | nd | nd | nd | nd | nd | nd | Wang *et al.* (2003) |
| Sapindus | 1 g/kg $BW^{0.75}$ | Sheep (6) | Grass | ↑ | – | – | – | ↓ | = | nd | ↓(C2/C3) | nd | nd | = | Abreu *et al.* (2004) |
| Yucca | 5;10;20;30 g/day | Sheep (20) | 50/50 c/f | nd | – | – | – | nd | nd | nd | nd | ↑ | nd | = | Eryavuz and Dehority (2004) |
| Yucca | 120 mg/kg feed | Wethers (4) | 70/30 c/f | nd | – | – | – | = | ↓ | ↓ | = | = | nd | = | Santoso *et al.* (2004a) |
| Yucca | 240 mg/kg feed | Wethers (4) | 60/40 f/c | nd | – | – | – | nd | ↓ | nd | = | = | nd | = | Santoso *et al.* (2004b) |
| Tea saponin | 0.2;0.4 g/l | (*In vitro*) | Pure substrates | – | – | – | – | = | ↓ | ↓ | = | = | = | = | Hu *et al.* (2005) |
| Yucca | 1;2;4 g/l | (*In vitro*) | 70/30 f/c | – | – | – | – | nd | ↓ | (↓) | ↑C3 | = | nd | nd | Wina *et al.* (2005) |
| Yucca | 2;4;6 ml/l | (*In vitro*) | 50/50 f/c | – | – | – | – | nd | ↓ | ↓ | ↑C3 | = | – | – | Pen *et al.* (2006) |
| Quillaya | 2;4;6 ml/l | (*In vitro*) | 50/50 f/c | – | – | – | – | nd | (↓) | = | ↑C3 | ↑ | – | – | |
| Yucca | 240 mg/kg feed | Wethers (3) | 85/15 f/c | nd | – | – | – | nd | ↓ | nd | ↓C2 | = | nd | ↓urinary N | Santoso *et al.* (2006) |
| Sapindus | 0.6 g/kg BW | Goats (4) | 65/35 f/c | – | – | – | – | nd | nd | nd | nd | nd | nd | nd | Wina *et al.* (2006) |

(*n*): number of animals used in the experiment; ↑:significant increase ($P < 0.05$); ↓: significant decrease ($P < 0.05$);=: no effect; ( ): a tendency was evidenced ($0.05 < P < 0.10$); nd: no data; c/f: concentrate/forage ratio; FCR: feed conversion ratio; BW: body weight; $BW^{0.75}$: metabolic BW; DMI: dry-matter intake; ADF: acid-detergent fibre; N: nitrogen; $NH_3$-N: ammonia nitrogen; $CH_4$: methane; VFAs: volatile fatty acids; C2: acetate; C3: propionate.

**Table 3** *Effects of various pure and mixed EOs on rumen microbial population, deaminase activity, DM digestibility and the end products of fermentation*

| | Blend* | Garlic oil | Thyme oil | Cinnamon oil | Oregano oil | Artenisia oil | Anise oil | Capsidium oil | Cinnamaldehyde | Eugenol | Allicine | Anethol | Limonene | Gaiacol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total bacteria | – | – | – | – | – | ↓1 | – | – | – | – | – | – | – | – |
| Cellulolytic bacteria | – | =10 | ↓10 | – | – | – | – | – | – | – | – | – | – | – |
| Protozoa | =4 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Deaminase activity ($NH_3$ prod./mg protein/h) | ↓2;4 | – | – | – | – | – | – | – | – | ↑13 | – | – | ↑13 | ↑13 |
| DM digestibility | =8;9 | =10 | ↓10 | – | – | – | – | – | – | – | – | – | – | – |
| PH | =4;9 | – | ↑13 | – | – | – | – | – | – | ↑13 | – | – | ↑13 | ↑13 |
| TVFAs | =4;9;↑8 | ↓7 | ↓13 | – | – | – | – | ↓7 | ↓7 | ↓13 | =6 | ↓7 | ↓13 | ↓13 |
| Acetate | =4;8;9 | ↓5;6;11 | – | ↓7 | ↓7 | – | ↓7;11;12 | – | ↓5;7 | ↓13 | =6 | ↓11 | – | – |
| Propionate | =4;8;9 | ↑5;6;11 | – | ↑7 | ↑7 | – | ↑7;11;12 | – | ↑5;7 | ↑13 | =6 | ↑11 | – | – |
| Butyrate | =4;8;9 | ↑5;6;11 | – | – | – | – | – | – | ↑5 | – | =6 | – | – | – |
| BVFAs | =4;8;9 | ↓5 | – | – | – | – | – | – | ↓5 | ↓13 | =6 | – | – | |
| $CH_4$ | =8;9 | ↓6;11 | – | – | – | – | – | – | – | – | – | – | – | |
| $NH_3$-N | =4;9 | =5;↓7 | ↓3 | ↓7;11 | ↓7;11 | – | ↑7;↓12 | ↓11 | ↓7;11 | ↑7;↓7;13 | – | ↑7 | – | – |

*: commercial blend of essential oil (CBEO) compound (CRINA®); =: no effect; ↑: significant increase ($P < 0.05$); ↓: significant decrease ($P < 0.05$); –: no data; EO: essentials oils; $NH_3$: ammonia; DM: dry matter; TVFAs: total volatile fatty acids; BVFAs: total sum of branched-chain volatile fatty acids; $CH_4$: methane; $NH_3$-N: ammonia nitrogen.
References: 1 – Naguy and Tengerdy (1968); 2 – McIntosh *et al.* (2003); 3 – Macheboeuf *et al.* (2004); 4 – Newbold *et al.* (2004); 5 – Busquet *et al.* (2005a); 6 – Busquet *et al.* (2005b); 7 – Cardozo *et al.* (2005); 8 – Castillejos *et al.* (2005); 9 – Fernandez *et al.* (2005); 10 – Garcia-Gonzales *et al.* (2005); 11 – Busquet *et al.* (2006); 12 – Cardozo *et al.* (2006); 13 – Castillejos *et al.* (2006).

'CRINA®', are not characterised in terms of purity, and the active chemical entities are not precisely quantified. EO content varies widely among various cultivars of the same species or various organs of a plant (leaves, stems, roots, flowers and fruits), and according to growing conditions, age or physiological stage of the plant, and extraction and processing methods. Different plants of the same species or genus that externally appear identical but present a variation in their chemical constituents, often due to climatic, altitude or soil conditions, are termed chemotypes. For instance, Sivropoulou *et al.* (1996) indicate that the concentrations of thymol and carvacrol in oregano oil ranges, depending on its origin, from 0.44 to 31.8% and from 0.43 to 79.6%, respectively. Comparisons between chemotypes are very instructive: chemotype 1 of *Thymus vulgaris L.* is rich in linalol, whereas chemotypes 2, 4 and 6 are composed mainly of geraniol, thuyanol and thymol, respectively. Thus, efficacy and toxicity will vary widely among different chemotypes of the same plant species or genus. Furthermore, synergetic or antagonist effects among components in a mixture make it difficult to estimate effective doses of bioactive compounds.

McIntosh *et al.* (2003) and Newbold *et al.* (2004) report that CBEO rich in thymol, gaiacol, eugenol, vanillin and limonene strongly impairs protein metabolism in the rumen through two additive mechanisms: (i) by reducing protein degradation to peptides and (ii) by specifically inhibiting some microbes such as the 'hyper ammonia-producing bacteria' (HAP) (McEwan *et al.*, 2002a) and their deaminase activity (Newbold *et al.*, 2004). However, the effectiveness of CBEO depends on the protein source. Molero *et al.* (2004) observed that the *in sacco* degradation rate of sunflower meal and peas was reduced by addition of 700 mg/day of CBEO to the diet of growing heifers, but had no effect on soya-bean meal, fish meal or lupin seeds. McEwan *et al.* (2002b) found that the degradation of peas and rapeseed meal was reduced, whereas that of fish meal, soya-bean meal and sunflower meal remained unchanged after CBEO addition. The authors hypothesised that EOs impaired the attachment of bacteria to the protein substrates in the rumen, but offered no explanation for the absence of any effect of EOs on some protein sources. Surprisingly, Newbold *et al.* (2004) observed no effect on total bacterial growth or ammonia concentration or on the other end products of rumen fermentation in adult sheep treated with 110 mg/day of CBEO. Similarly, Fernandez *et al.* (2005) obtained no response to CBEO dosed at 40 mg/day in semi-continuous flow fermenters.

With the exception of Castillejos *et al.* (2005 and 2006), who found a stimulating effect of CBEO on total VFAs, most of the other authors observed no response on VFA production (Table 3). Molar percentage of VFA mixtures has often been altered by CBEO and many EOs, with a decrease in acetate in favour of propionate and sometimes butyrate. Garlic oil, cinnamaldehyde, eugenol, carvacrol and thymol, used as crude extracts or as purified forms, are the EOs more particularly active on rumen fermentations (Table 3).

As noted for CBEO, most of them depress $NH_3$ and methane production, and improve propionate production at the expense of acetate. Busquet *et al*. (2005b) indicated that garlic oil and diallyl sulphide decreased by 70% methane production in *in vitro* tests, whereas allyl mercaptan impaired methane production by only 20%. The authors hypothesised that garlic oil, more especially the organosulphur compounds it contains, could have a specific inhibiting effect on methanogenic archaea through an inhibition of the HMG-CoA reductase. This enzyme is necessary for the synthesis of isoprenid units found in lipidic structure of the archaea membrane. Thus, garlic oil could have the potential to specifically inhibit methanogens without affecting other rumen micro-organisms.

As already stated for other natural bioactive compounds, optimal doses of EOs are difficult to assess, mainly because of the large differences in chemical composition that occur between preparations. The results of several tests conducted in well-controlled *in vitro* conditions indicate that the optimal doses vary over a wide range according to EOs. Some authors also indicated that the optimal doses varied depending on rumen pH (Cardozo *et al*., 2005) whereas no interaction with diet was observed (Castillejos *et al*., 2005). The mean effective dose of 300 mg/l has been obtained for anethol (Busquet *et al*., 2006) and eugenol (Cardozo *et al*., 2005). Macheboeuf *et al*. (2006a) showed that diallyl disulphide at 75 mg/l decreased methane and acetate production by 90 and 50%, respectively, but did not alter propionate production or DM digestibility. Mixtures of feed additives for ruminants have been studied *in vitro* by means of a simplex centroid model (Macheboeuf *et al*., 2006b). This approach enables us to observe potential beneficial interactions between compounds and to design specific combinations for a particular objective. For instance, when the criteria defined in the model were to keep DM digestibility and total VFA production at the same level as in controls without additives, but to reduce methane and ammonia production, the ideal mixture was composed of malate (60%), cinnamaldehyde (32%), carvacrol (2%) and diallyl disulphide (6%).

Even for the same oil, differences in optimal doses for rumen fermentation end products can be significant. For example, the lowest effective dose of cinnamon oil for reducing ruminal ammonia concentration was set at 0.3 and 3.000 mg/l by Cardozo *et al*. (2005) and Busquet *et al*. (2006), respectively. Castillejos *et al*. (2006) reported that the mean concentration of 5 mg/l of CBEO in semi-continuous fermentors increased VFA production and reduced deamination, whereas the inhibiting effect on HAP bacteria appeared only at concentrations in the range 35 to 360 mg/l. Newbold *et al*. (2004) noted only slight effects in the rumen fermentation characteristics of sheep fed 110 mg/day, corresponding to a calculated maximum ruminal concentration of about 14 mg/l. Castillejos *et al*. (2006) states that rumen microbes need to be exposed to EOs for at least 6 days to observe changes in VFAs and for 28 days to detect changes in N metabolism. Differences in efficacy of the same EO mixture may be explained by the ability of EOs to adsorb on the surface of some dietary ingredients and more specifically affect the microbes attached to them (Duval *et al*., 2004). Thus, ration composition may modulate the response of rumen microbes to EO addition. More research must be carried out to evaluate this hypothesis.

The simplest and cheapest method of delivering bioactive plant secondary metabolites would be to feed the animal with the relevant fresh or dried plant. Raw plants rich in EOs have thus been tested for their potential activity. Calsamiglia *et al*. (2005) indicate that *Lavandula officinalis*, *Solidago virgaurea* and *Achillea millefolium* activate rumen fermentation, whereas *Equisetum arvense* and *Salvia officinalis* inhibit methanogenesis when tested *in vitro*. Ando *et al*. (2003) observed that a daily dose of 200 g of sun-dried peppermint given to rumen-cannulated steers significantly decreased the population of rumen protozoa and the $NH_3$ concentration in rumen fluid.

Only a few experiments have been carried out on the effects of EOs on ruminant production (Table 4). Of the five reported experiments carried out with CBEO, four gave a significant positive effect on milk production. In contrast, the other tested plant extracts showed no efficacy on lactating dairy cows.

### *Main hurdles for the practical use of plant extracts as feed additives*

The adoption (or widespread use) of plant extracts as performance enhancers for ruminants and other animals in practical applications is limited for several reasons. First, the quality and quantity of the active compound(s) are extremely variable and difficult to standardise. Other factors that influence the secondary metabolite composition are the vegetative stage, the part of the plant used to prepare the extract, and whether the plant was stimulated (intentionally or not) to synthesise defensive compounds. Environmental conditions and cultivar variety can partly explain the variation in secondary metabolite production by plants.

Some of the bioactive compounds are antioxidants, and this property can be lost during storage. Dosage for the benefit of animals depends on the whole chemical composition of the plant preparation, since there can be additive, synergetic and antagonistic effects among bioactive compounds in a complex mixture of secondary metabolites (Cordell, 2000; Burt, 2004).

It is commonly accepted that plant extracts are safe because they are produced naturally. However, it must be remembered that these secondary metabolites are part of the defence mechanism used by plants against pathogens, herbivores and hostile environment conditions. Thus, an excessive dosage can be toxic to animals or at least induce a negative response when consumed. Toxicity mechanisms and toxic doses for animals are not well known. Little information is available on the transfer of these substances into edible animal products and their possible toxicity for consumers. Until evidence-based data are available their

**Table 4** *Effect of various pure and mixed EOs on animal performance variables*

| Product | Animals | Diet | Dose | Treatment duration | DMI | Growth | FCR | Milk production | Milk compos. | Authors |
|---|---|---|---|---|---|---|---|---|---|---|
| CRINA® | 357 ewes | Alfalfa hay + concentrate *ad libitum* | 1 mg/kg BW per day | 11 weeks | nd | Ewes: +36 g/day at W + 8 ($P < 0.01$) | nd | nd | nd | Blanluet) *et al.* (2002) |
| | | | | | nd | Lambs: +18 g/day at W + 6 ($P < 0.01$) | nd | nd | nd | |
| Cyanarin + boldin + sylmarin + romarinic acid | 69 dairy cows | Maize silage + hay + concentrate | 150 g/day | 20 days | = | nd | nd | = | nd | Durand *et al.* (2002) |
| CRINA® | 4 dairy cows | TMR *ad libitum* | 2 g/day | 21 days | = | nd | nd | = | = | Benchaar *et al.* (2003) |
| CRINA® | Dairy cows ($n = ?$) | TMR: alfalfa silage + maize silage + hay + conc. | 0.6 g/day | 14 days | +1.9 kg/d | nd | nd | +2.7 kg/day ($P < 0.05$) | = | Schmidt *et al.* (2004) |
| CRINA® | 170 dairy cows | TMR | 1.2 g/day | nd | nd | nd | nd | +1.6 kg/day ($P < 0.05$) | = | Varga *et al.* (2004) |
| Garlic bulbs | 80 lambs | Alfalfa hay +concentrate *ad libitum* | 30–60 kg/t conc. | 70 days | = | = | = | nd | nd | Bampidis *et al.* (2005a) |
| Garlic husks | 80 lambs | (same diet) | 50–100 kg/t conc. | 70 days | = | = | = | nd | nd | |
| Oregano leaves | 45 lambs | (same diet) | 144–288 mg/kg conc. | 70 days | = | = | = | nd | nd | Bampidis *et al.* (2005b) |
| Origanum oil (+bambermycin) | 20 dairy cows | nd | 1.5 g/day | nd | nd | nd | nd | ↑(*P*?) | nd | LiGuo and HengMin (2005) |
| CRINA® | 16 dairy cows | Grass silage *ad libitum* + protein concentrate | From 0.5 to 2 g/day | 28 days | = | nd | nd | 2.0 kg/day ($P = 0.03$) | = | Offer *et al.* (2005) |
| CRINA® | 4 dairy cows | Grass silage + maize silage + maize grain + soya-bean meal | 2 g/day | 28 days | = | nd | nd | = | = | Benchaar *et al.* (2006a) |
| Vertan® * | 5 steers | Grass and legume silage + rolled barley | 2–4 g/day | 28 days | ↑ | = | nd | nd | nd | Benchaar *et al.* (2006b) |

*Commercial mixture of essential oils (EOS) consisting of thymol, eugenol, vanillin and limonene.
=: no effect; nd: no data; t: ton; TMR: total mixed ration; FCR: feed conversion ratio; BW: body weight; ↑: significant increase ($P < 0.05$).

use requires caution. Owing to the strong odoriferous properties of EOs, their transfer could alter the organoleptic quality of animal products (Viallon *et al*., 2000; Ando *et al*., 2001; Burt, 2004).

Their observed beneficial and harmful effects are in fact associated. If we consider that plant secondary metabolites can be toxic, their effects on animals can be explained by the hormesis concept recently proposed in toxicology (Calabrese and Baldwin, 2003), including studies on phytochemicals (Mattson and Cheng, 2006). Unlike the linear or threshold models, the hormetic model considers that at low levels a bioactive compound has a beneficial effect, while toxic effects are induced at higher levels of exposure. The curve will have the shape of an inverted 'J' where the upper part represents a positive effect on the measured trait. This positive effect is explained by induction of a mild stress on specific targeted cells, which respond by enhancing their ability to cope with more severe stress and resist injury and disease. In the case of ruminants, the effects can be on the rumen microbial ecosystem and on the animal itself. The threshold of cell resistance is defined by the tip of the J-shaped curve, and the toxic effects appear at higher concentrations when the slope is negative.

## Exogenous enzymes

The application of enzymes as feed additives for ruminants has been under development for the last 10 years. Although exogenous enzymes may be a promising way to improve the productive efficiency of ruminants, their practical use has not been widely adopted. The implementation of this technology has been hampered by the variability of the animal response obtained with existing products. Another no less important drawback is the relatively high cost of enzymes compared with other growth promoters available to producers. However, the trend to limit the use of antibiotics and hormone-derived growth enhancers in animal production (already banned in the European Union), coupled to the present improvements in enzyme production technology, offers a renewed opportunity for the use of exogenous enzymes in ruminants' diets.

Most studies conducted on exogenous enzymes were performed in producing dairy cattle. In a review of 20 experimental trials consisting of 41 treatments, enzyme feed additives increased milk production on average by 1.1 $\pm$ 1.5 kg/day (Beauchemin *et al*., 2003). This figure is fairly close to the 1.1 kg/day average milk production increase obtained with yeast supplements (mean of 32 lactation studies as reviewed by Kung, 1998), a feed additive largely used in dairy farms. Although there are fewer data, a positive response of exogenous enzymes in weight gain of growing animals has also been reported in beef cattle (reviewed by Beauchemin *et al*. (2001 and 2004)), and in goats and sheep (Titi, 2003, Titi and Lubbadeh, 2004).

Exogenous enzymes are used mainly to increase the digestion of cell wall carbohydrates in forages. Although under most common feeding conditions poor starch digestion is not considered a problem (Krause *et al*., 2003), amylases have also been used in some studies with mixed results on animal performance (DeFrain *et al*., 2005; Rojo *et al*., 2005; Tricarico *et al*., 2005). Owing to the scarcity of available information, amylases as enzyme additives will not be further discussed here. The positive effect that exogenous enzymes have on forage digestion is mediated through the enhancement of the rate of fibre degradation. In contrast, they have little effect on the extent of fibre degradation. An understanding of this mechanism is useful for identifying feeding situations where addition of enzymes may be profitable. The grazing ruminant and its rumen microbiota have co-evolved for millions of years to make use of the abundant cellulosic resources present in grasslands. The relatively slow rate of fibre degradation has been offset by the animal by the enlargement of the pre-gastric chamber (the reticulo-rumen) and an increased residence time for microbial fermentation to proceed. Fibrolytic rumen micro-organisms possess one of the most efficient and complete arrays of enzymes able to hydrolyse plant cell wall polysaccharides, and as a result digestion of this recalcitrant cellulosic material in the rumen can be as high as 70%. Considering the short residence time of the substrate, the rumen is one of the most efficient fermentation systems in nature. However, the economic viability of the modern livestock industry depends on high-producing animals. To meet their requirements for production, these animals must consume large amounts of energy-dense diets. The increase in the amount of readily fermentable carbohydrates in the diet, which is often accompanied by a higher ingestion and higher rate of feed turnover, leads to suboptimal rumen conditions for fibre digestion. Under these feeding regimes, cell wall digestion may be as low as 35% in the rumen (Beauchemin *et al*., 2001), and in these situations where digestion is less efficient, feed enzymes are expected to improve feed digestion and increase animal performance.

The rumen ecosystem normally harbours about $10^{10}$ micro-organisms, and is composed of several hundred species (Edwards *et al*., 2004; Larue *et al*., 2005). These species possess a vast array of enzymes acting concertedly to degrade the plant substrates present in feeds. The complexity and diversity of both the plant fibres present in ruminants' diets and the diversity of the enzymes required for their degradation explain why the search for a single critical enzymatic activity that increases digestibility has so far proved elusive. Notwithstanding, enzymes hydrolysing hemicelluloses are often positively correlated to increases in substrate degradation, e.g., xylanase and esterase activities (Nsereko *et al*., 2000b; Colombatto *et al*., 2003b; Yu *et al*., 2005). Exogenous ferulic acid esterase and arabinofuranosidase have been shown to act in synergy with xylanases and cellulases (Yu *et al*., 2003; Koukiekolo *et al*., 2005). Similarly, the synergy of exogenous and rumen-derived enzymes has been shown to be one important mode of action of feed enzyme additives (Morgavi *et al*., 2000a). Enzymes not involved directly in fibre hydrolysis can also

have a positive effect on plant digestion, probably by removing structural barriers that hinder microbial attachment (Nsereko *et al*., 2000a). A protease from *Bacillus licheniformis* has been shown to have such an effect (Colombatto *et al*., 2003a and 2003b) when applied to alfalfa hay.

The mechanism by which exogenous enzymes improve fibrous feed digestion is complex. Several modes of action have been identified, including the effects reported on the feed before ingestion, and ruminal and post-ruminal effects (reviewed by Beauchemin *et al*., 2004). The effects on rumen metabolism are considered to be the most important, for instance the synergy with endogenous microbial enzymes, stimulation of bacterial colonisation of feeds and the increment in total microbial numbers. However, the relative weight of each of these mechanisms of action may vary depending on the enzyme product, the diet and the target animal. These interrelated factors will ultimately dictate whether the application of a feed enzyme product results in increased animal performance. The dose–response effect of enzymes is usually quadratic with decreasing positive responses at doses higher than the optimum. However, and unlike feed additives derived from plants, high doses do not generally have a negative effect on production. The physiological status of the animal, the type of enzymatic activity, the level and way of applying the enzyme, and the correct matching of the enzyme product with the feed target must all be considered for this technology to be applied successfully (Beauchemin *et al*., 2003). The difference in enzyme products and experimental conditions tested are in part responsible for the variable response reported in published studies.

Enzyme products present on the market originate mostly from *Trichoderma longibrachiatum, Aspergillus* sp. and *Bacillus* sp., and contain a complex mixture of enzyme types and activities (Pendleton, 2000). While these products would work in some on-farm situations, further research is needed to gain a better understanding of the conditions necessary to obtain a consistent positive response to the application of this technology. The notion that enzymes are not effective because the proteolytic activity of the rumen inactivates them has been dispelled by several studies demonstrating that enzymes are stable in the rumen for a long enough time to exert their action (Hristov *et al*., 1998; Morgavi *et al*., 2000b; Morgavi *et al*., 2001). The development of products specifically tailored for ruminants and targeting a particular diet seem to be a compulsory step in achieving this objective. The combination of chemical treatment and enzymes seems a promising way to improve the use of poor-quality forages such as straw by ruminants (Wang *et al*., 2004; Eun *et al*., 2006).

Feed enzymes have the potential to increase animal performance in ruminants with high nutritional requirements. As more information is available on their mode of action, the effectiveness of commercial products will improve, facilitating their adoption by farmers. Though still unexplored, a new generation of enzyme products targeting specific applications such as the inactivation of toxins or the elimination of antinutritional compounds that may be present in ruminant feeds could expand the utility of feed enzymes in ruminant nutrition.

## Conclusion

Probiotics, dicarboxylic acids, enzymes and plant-derived products are able to act positively on rumen feed digestion and ruminant production. These additives are diverse and exert their action through different mechanisms. Despite their diversity, they all ultimately affect the fermentation metabolic pathways and (or) the digestive microbial ecosystem. However, unlike antibiotics, which have a specific microbial target, these additives generally have multiple, subtler modes of action. Their efficacy is influenced by the type of feed and physiological status of the animal. The interactions that occur between additives, feeds and host are complex, and more research is needed to improve our understanding of these processes. This knowledge will help us to predict when a given feed additive may be useful, thus reducing inconsistencies in response observed in the field.

Natural plant products are a particular case among the additives reviewed. Owing to the diversity of bioactive components in these natural, non-purified preparations, their effective doses are difficult to determine and the effects on animals are not totally controlled. Farmers and consumers alike generally perceive 'natural plant extracts' to be less toxic than antibiotics or other chemical products. However, this perception is unsound as there are many examples of dangerous natural toxins. The use of plant extracts as feed additives must therefore obey the same general rules as non-natural products, e.g. they must be safe for both the animal and the handler of the product, they must not be found as residues in animal products, and they must not be a hazard to the environment. The dose–response relationship must be precisely established from well-designed experiments for a specific allegation in animal production, and risks associated with the use of these native natural preparations must be robustly tested in carefully designed clinical studies.

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