Perspectives on ruminant nutrition and metabolism

I. Metabolism in the Rumen

E. F. Annison and W. L. Bryden

Department of Animal Science, University of Sydney, Camden N.S.W. 2570, Australia

Abstract

Advances in knowledge of ruminant nutrition and metabolism during the second half of the twentieth century have been reviewed. Part I is concerned with metabolism in the rumen: Part II discusses utilization of nutrients absorbed from the rumen and lower tract to support growth and reproduction. The time frame was prompted by the crucial advances in ruminant physiology which arose from the work of Sir Jospeh Barcroft and his colleagues at Cambridge in the 1940s and 50s, and by the brilliant studies of Robert Hungate on rumen microbiology at much the same time.

In reviewing the growth of knowledge of the role of bacteria, protozoa, fungi and bacteriophages in the rumen, outstanding developments have included the identification and characterization of fungi and the recognition that the utilization of polysaccharides in the rumen is accomplished by the sequential activities of consortia of rumen microorganisms. The role of protozoa is discussed in relation to the long standing debate on whether or not the removal of protozoa (defaunation) improves the efficiency of ruminant production. In relation to nitrogen (N) metabolism, the predation of bacteria by protozoa increases protein turnover in the rumen and reduces the efficiency of microbial protein production. This may account for the beneficial effects of defaunation where dietary N intakes are low and possibly rate limiting for growth and production.

Current approaches to the measurement of rates of production of short chain fatty acids (SCFA) in the rumen based on the mathematical modelling of isotope dilution data are outlined. The absorption of SCFA from the rumen and hindgut is primarily a passive permeation process.

The role of microorganisms in N metabolism in the rumen has been discussed in relation to ammonia and urea interrelationships and to current inadequacies in the measurement of both protein degradation in the rumen and microbial protein synthesis. The growth of knowledge of digestion and absorption of dietary lipids has been reviewed with emphasis on the antimicrobial activity of lipids and the biohydrogenation of unsaturated fatty acids. The protection of unsaturated dietary fats from ruminal biohydrogenation is an approach to the manipulation of the fatty acid composition of meat and dairy products.

Discussion of the production of toxins in the rumen and the role of microorganisms in detoxification has focused on the metabolism of oxalate, nitrate, mycotoxins, saponins and the amino acid mimosine. Mimosine occurs in the tropical shrub leucaena, which is toxic to cattle in Australia but not in Hawaii. Tolerance to leucaena stems from the presence of a bacterium found in the rumen of Hawaiian cattle, which when transferred to Australian cattle survives and confers protection from mimosine. The genetic modification of rumen microorganisms to improve

https://doi.org/10.1079/NRR19980014 Published online by Cambridge University Press

their capacity to ultilize nutrients or to detoxify antinutritive factors is an attractive strategy which has been pursued with outstanding success in the case of fluoroacetate. A common rumen bacterium has been genetically modified to express the enzyme fluoroacetate dehalogenase. The modified organism has been shown to survive in the rumen at metabolically significant levels and to confer substantial protection from fluoroacetate poisoning.

Introduction

The considerable advances in the volume and efficiency of ruminant production that have taken place in the past half century have been beneficial both to the producer and the consumer (see Armstrong, 1993). These have been achieved largely by genetic improvement, more effective disease control and improved nutrition. Improved feeding standards, in particular, owe much to the growth in knowledge of ruminant physiology and digestion that has occurred during the past four to five decades. This is an appropriate time for an overview of ruminant nutrition and metabolism, since it is generally recognized that the foundations of modern ruminant physiology and nutrition were laid by Barcroft, Phillipson, Elsden, McDonald and others at Cambridge in the 1940s. At much the same time the brilliant studies of Robert Hungate on rumen microorganisms stimulated the rapid development of rumen microbiology (see Hungate, 1966). The Barcroft school confirmed the presence of short chain fatty acids (SCFA) in rumen contents and were the first to recognize that the extent of SCFA absorption from the rumen was "sufficient to supply an appreciable part of the energy requirements of the animal" (Barcroft *et al.* 1944). Evidence that ruminal SCFA are the end products of the fermentation of dietary carbohydrates and other fermentable substrates, a process which provides the energy for microbial cell synthesis, was recognized by the mid 1950s (see Hungate, 1966). A few years later, at Cambridge, McDonald (1948, 1952, 1954) showed that dietary protein is degraded to a varying extent in the rumen and that the ammonia produced may be absorbed and returned to the rumen as salivary urea. These findings were crucial to the subsequent development of protein nutrition in ruminants. The main features of the fate of dietary lipid in the rumen also became known during the 1950s (see Garton, 1961). Ingested lipids had been shown to be rapidly hydrolysed by lipases of microbial and plant origin, with the biohydrogenation of unsaturated free fatty acids.

It is difficult not to reflect on the advances made in this field since a monograph with the same title as the subtitle of this review was published nearly 40 years ago (Annison & Lewis, 1959). The rumen ecosystem is now largely defined and a great deal is known qualitatively of the metabolic fate of the major classes of feedstuffs in the rumen. The intrinsic complexity of rumen metabolism, however, typified by the fluctuations in the numbers, types and activities of microorganisms that occur in response to nutrient supply, makes it extraordinarily difficult to construct effective simulation models of this system (see Baldwin, 1995). In particular, the variations in the efficiency of microbial protein synthesis and the difficulty of measuring the extent of dietary protein breakdown in the rumen continue to thwart intensive efforts to devise feeding systems able to predict with acceptable accuracy the supply of essential amino acids to the small intestine.

The increased understanding of rumen metabolism in the past half century, which is discussed below, has been accompanied by a corresponding growth in knowledge of the utilization

of nutrients absorbed from the rumen and lower alimentary tract to support growth and reproduction. These advances in ruminant metabolism are discussed in Part II of this review (Annison & Bryden, 1999).

Rumen microbiology

An excellent account of the studies which started in the eighteenth century and culminated in the recognition of the main features of digestion in the rumen appears in the monograph of Hungate (1966). Robert Hungate, whose influence on the development of modern rumen microbiology cannot be overstated, elaborated techniques in the 1950s and 1960s for the isolation and cultivation of the main classes of anaerobic bacteria found in the rumen. The adoption of these techniques by workers in USA, Europe, South Africa, Australia and New Zealand, in particular, led to a rapid increase in the knowledge of rumen microbiology during that period (see Hungate, 1966). Comparative aspects of the evolution of microbial digestion in the alimentary tract of ruminants were discussed by Moir (1968), Hume & Warner (1980) and recently by Baker (1997).

The pace of growth of knowledge of rumen microbiology has been maintained and more than 200 species of rumen bacteria, for example, have been identified since Robert Hungate's seminal studies in the 1940s (see Theodorou & France, 1993). A continuing stimulus to research in this area has been the need to define the effects of ruminal fermentation on the supply of nutrients that become available to the tissues of animals receiving diets of known composition. This information is crucial to the development of feeding systems for ruminants, as emphasized by Baldwin (1995) in his scholarly account of his continuing efforts, with colleagues, to use available knowledge of ruminant metabolism to construct computer based models able to predict responses to nutrients in the rumen and in the whole animal.

In recent decades the most surprising discovery was the presence of metabolically significant numbers of anaerobic fungi in the rumen (Orpin, 1975). This development and many other advances in this field have contributed to current understanding of the interactions and interdependence of the microorganisms that constitute the inevitably complex rumen ecosystem (Theodorou & France, 1993). The digestion of complex polysaccharides, for example, depends on the sequential enzymic activities of a range of organisms which may include fungi (Flint & Forsberg, 1995). These considerations reinforce the view that the health and productivity of ruminants is dependent on a well maintained rumen.

Rumen bacteria

The continuing efforts of microbiologists and nutritionists to manipulate rumen fermentation to improve livestock productivity have highlighted the need for effective procedures to assess changes in the microbial population. The advent of methods based on molecular biology has revealed that the diversity of rumen bacteria has been greatly underestimated (Krause & Russell, 1996).

The attachment of bacteria to plant material in the rumen was observed by Henneberg (1919) and confirmed by Baker (1943). The role of adherent rumen bacteria in the digestion of plant material was comprehensively discussed by Cheng & Costerton (1980), who used scanning electron microscopy to demonstrate the attachment of large numbers of bacteria to partly digested food particles. The kinetics of microbial colonization have been defined by

Sauvant & van Milgen (1995). The attachment is effected by polysaccharide glycocalyces (Costerton *et al.* 1978) produced by adherent bacteria. Examination of washed rumen epithelium by scanning electron microscopy led to the unexpected discovery that considerable numbers of bacteria adhere to the epithelium (Bauchop *et al.* 1975). These bacteria grow in a matrix of carbohydrate glycocalyx fibres and form series of adherent microcolonies at the tissue surface. The bacteria, which are exposed to oxygen which diffuses through the tissue, are facultative anaerobes and their main functions are to remove oxygen to protect oxygen sensitive anaerobes, to digest dead epithelial cells and to hydrolyse urea diffusing into the rumen (see Cheng & Costerton, 1980). Direct examination by electron microscopy of the bacteria in rumen fluid revealed that most cells were surrounded by polysaccharide glycocalyces (Cheng & Costerton, 1980).

The role of the association of bacteria and other rumen microorganisms in the digestion of feed and developments in feed processing to enhance microbial attachment and improve digestion have been discussed by McAllister *et al.* (1994).

Rumen protozoa

The early literature on the discovery, isolation and classification of protozoa was reviewed by Hungate (1966). Although earlier workers had shown that bacteria are a food source for certain protozoa, the extent and significance of the predation of bacteria by protozoa was first revealed in the early 1970s by the comprehensive studies of Coleman (1975). The uptake of bacteria by protozoa reduces the efficiency of nitrogen utilization in the rumen, largely by increasing protein turnover (see Ushida *et al.* 1991). The numbers and proportions of protozoa in the rumen are influenced by the nature of the diet and the level and frequency of feeding, which in turn dictate the most important determinant of rumen metabolism, rumen pH (Franzolin & Dehority, 1996).

Recognition of the potentially adverse effects of protozoa on nitrogen utilization in the rumen revived interest in the 1980s in the removal of protozoa (defaunation) from livestock. The relative ease of defaunation had led to many studies in which the performance of normal and protozoa-free livestock had been compared (see Hobson & Jouany, 1988). Varied results were reported, but the most clearcut beneficial effects of defaunation were demonstrated by Bird & Leng (1985) in studies with lambs reared by faunated or defaunated dams under grazing conditions. A feature of this work was that N intakes by the dams were low and probably rate limiting for milk production. In this situation improved efficiency of microbial protein production in the rumen of the dams would have accounted for the improved performance of defaunated lambs.

In some situations the presence of protozoa may be beneficial. Demeyer (1989) and Ushida *et al.* (1991) in their reviews of published work on the effects of defaunation on rumen fibre digestion concluded that in most cases defaunation inhibits plant cell wall digestion. The experimental evidence is not conclusive, however, in view of the wide variation in the nature and amounts of diets fed, in the feeding regimes used and in the methods of defaunation used in different experiments.

Most defaunating agents, which are toxic to some extent to all rumen microorganisms, depress feed intake, and even if reinoculated with rumen contents rumen function may be permanently affected (Bird, 1989). These uncertainties highlight the need for more research with animals either reared protozoa-free from birth, or selectively defaunated. The use of defaunated animals in livestock production is unlikely to be widely trialled, however, until a

potent, specific defaunating agent safe to both livestock and producer and residue free becomes available. Certain natural products containing saponins have shown promise as potential defaunating agents (see Wallace, 1997).

Defaunation reduces methanogenesis in the rumen (see Ushida *et al.* 1991). In faunated animals 10–20 % of methanogenic bacteria are attached to the surface of entodiniomorphic protozoa, particularly *Entodinium* spp. (Stumm *et al.* 1982). The methanogens take up hydrogen, which appears to inhibit *Entodinium* spp. activity (Wolin, 1975; Hino, 1983). Methanogenic bacteria have been shown to form stable cocultures with rumen fungi (Bauchop & Mountfort, 1981) which degrade cellulose more rapidly than monocultures of the fungi (Mountford & Asher, 1985).

Rumen fungi

The demonstration by Orpin (1975) that certain flagellated organisms isolated from the rumen were zoospores of obligately anaerobic fungi introduced a new dimension to rumen ecology, since these fungi play an important role in the digestion of plant cell wall polysaccharides (Theodorou *et al.* 1996). At about the same time Bauchop (1979) was using scanning electron microscopy to show that large populations of anaerobic fungi colonize plant fragments in the gut of ruminants and other herbivorous animals.

Current knowledge of the distribution, biochemistry and ecology of anaerobic fungi has been comprehensively reviewed by Trinci *et al.* (1994), Theodorou *et al.* (1996) and Gordon & Phillips (1998). The ability of fungi to penetrate and colonize highly fibrous plant materials (see Fonty *et al.* 1990; Stewart *et al.* 1995) has been shown to stem from their ability to produce a wide range of enzymes which include cellulases, a range of hemicellulases, various disaccharidases, pectin lyase, various esterases, amylases and amyloglycosidases and proteinases (see Theodorou *et al.* 1996).

The ability of some anaerobic fungi to produce cellulases able to degrade highly ordered, or crystalline, cellulose (Wood *et al.* 1986) is of considerable interest. The activity of the cellulase from *Neocallimastix frontalis* was enhanced when in coculture with a rumen methanogen, the activity exceeding that of the most active cellulase previously identified (Wood *et al.* 1986). As pointed out by Theodorou *et al.* (1996), anaerobic fungi may well have the capacity to be grown commercially in continuous culture to produce highly active cellulases. An obvious use for such preparations would be the supplementation of animal feeds (see Armstrong & Gilbert, 1991).

Rumen bacteriophages

Rumen bacteriophages, which were first isolated from the bovine rumen by Adams *et al.* (1966), are usually present in sufficient numbers (Ritchie *et al.* 1970; Klieve & Bauchop, 1988; Klieve & Swain, 1993) to suggest that they could cause enough bacterial lysis to reduce the efficiency of feed utilization (Nolan & Leng, 1972; Firkins *et al.* 1992). The development of a procedure for measuring phages in rumen fluid based on DNA analysis (Klieve & Swain, 1993) has made it possible to investigate the diversity of rumen phages and the factors that influence their population size (Swain *et al.* 1996; Klieve *et al.* 1996). The new procedure will make it possible to study the factors responsible for the occasional spontaneous lysis of a large proportion of rumen bacteria (Nolan & Leng, 1972). An exciting development in the genetic

modification of rumen bacteria is the possible use of rumen phages as vectors for gene transfer by transduction (Morrison, 1996).

Polysaccharide digestion

The degradation in the rumen of plant structural and storage polysaccharides, which account for most of the energy intake of ruminants, is achieved by the microbial flora. Rates of polysaccharide breakdown are influenced by the numbers and proportions of carbohydrate splitting microorganisms and by the amounts of other fermentable substrates (Akin & Benner, 1988; Flint & Forsberg, 1995).

Cellulose digestion

The major component of plant cell walls is cellulose, which is linked to hemicelluloses and closely associated with glycoproteins (Flint & Forsberg, 1995). Lignin, which is crosslinked to polysaccharides and protein (Lam *et al.* 1990; Iiyama *et al.* 1994), is largely nondegradable in the rumen (Akin & Benner, 1988) and a negative correlation between lignin concentration and organic matter digestibility in the rumen has been reported (Susmel & Stefanon, 1993). Phenolic esters (Hartley *et al.* 1992) and condensed tannins (McAllister *et al.* 1994) also inhibit the digestion of plant carbohydrates.

There is increasing evidence that the microbial degradation of complex polysaccharides in the rumen is accomplished by the cooperative efforts of a range of cellulolytic and non-cellulolytic microorganisms (Cheng *et al.* 1991; Flint & Forsberg, 1995). Synergy between microorganisms involving end product utilization or crossfeeding has been clearly demonstrated in studies using cocultures of cellulolytic fungi or bacteria, and non-cellulolytic species (Dehority, 1991; Williams *et al.* 1991). Plant materials entering the rumen are rapidly colonized with bacteria and fungi (Cheng *et al.* 1991) and adhesion provides close contact for enzyme activity and ready uptake of liberated substrates. There is evidence that adhesion of bacteria to plant material is effected by a binding protein, or adhesin, but the significance of these nonenzymic binding proteins is not known (see Flint & Forsberg, 1995). The use of antibodies to these proteins to block the adhesion of cells to substrate should prove useful in the elucidation of the complex interactions of consortia of microorganisms involved in polysaccharide degradation (Flint & Forsberg, 1995).

Flint & Forsberg (1995) have pointed out that the cellulolytic enzyme systems of certain nonrumen fungi and bacteria have received more intensive study than those of rumen microorganisms. Several different mechanisms of cellulose degradation have been identified in studies with aerobic fungi and a number of highly cellulolytic bacteria (see Flint & Forsberg, 1995). Sequence data for genes coding for plant cell wall degrading activities have revealed many similarities between cellulolytic organisms (Gilkes *et al.* 1991; Henrissat & Bairoch, 1993). The distribution and characteristics of polysaccharidase activities among rumen bacteria and fungi were reviewed by Cheng *et al.* (1991) and more recently by Flint & Forsberg (1995). The latter authors also discussed the possible genetic modification of rumen microorganisms to enhance the efficiency of digestion in the rumen, an issue discussed earlier by Russell & Wilson (1988). Increased capacity to degrade cellulose and other plant cell wall polysaccharides is an obvious target and Russell & Wilson (1996) have recently addressed the possible genetic modification of ruminal cellulolytic bacteria to allow them to digest cellulose at lower than

usual rumen pH. This would overcome the depression in cellulose/hemicellulose utilization which may occur in grain fed cattle when rumen pH levels fall below 6.0 (Russell & Wilson, 1996).

Progress in the difficult area of gene transfer into anaerobic bacteria has been encouraging (see Morrison, 1996), but an important question is whether a genetically altered microorganism can survive in the rumen (see Teather, 1985). Gregg & Sharpe (1991) have stressed that a new genetic trait will be maintained only if it is of selective advantage to the organism. Further, the energy required for the expression of a particular gene must be at least equalled by the benefits arising from that expression. A major problem with the enhancement of cellulase/hemicellulase activity in specific organisms is that, as outlined earlier, the degradation of complex polysaccharides in the rumen involves a complex set of microbial enzymes acting in an ordered sequence. Identification of both the rate limiting step in the breakdown of polysaccharides, and the organism involved, must precede the selection of the organism for genetic improvement. In the short term, the modification of cellulolytic bacteria to improve their tolerance of lower rumen pH would appear to be a more achievable objective (Russell & Wilson, 1996). The improvement of fibre digestibility by a range of biotechnological approaches has been outlined by Wallace (1997). The genetic modification of plants to increase fibre digestibility, if not achieved at the expense of other desirable agronomic properties, has obvious attractions (Chesson *et al.* 1995).

Starch digestion

The main features of starch digestion in the rumen were recognized by the 1960s (Hungate, 1966). The major amylolytic bacteria, particularly *Streptococcus bovis*, had been isolated and characterized and it had been shown that grain feeding results in higher proportions of ruminal propionate and increased rumen acidity (Hungate *et al.* 1961). Whereas little starch or other α-linked glucose polymers reached the small intestine on high forage diets (Heald, 1952) there was increasing evidence that on high grain diets, particularly if the grain was ground, considerable amounts of starch escaped ruminal digestion (see Lindsay, 1970). Subsequent work has confirmed and extended these early observations (Sutton, 1985; Ørskov, 1986; Huntington, 1997).

The primary cause of the increased acidity in the rumen associated with high starch diets is the accumulation of lactic acid, which if severe may cause illness and death (Dunlop & Hammond, 1965). Both D- and L-lactic acid are produced in the rumen but the D isomer, which is cleared more slowly from the rumen, is mainly responsible for lactacidosis (Rowe & Pethick, 1994); this may be largely avoided by adapting the rumen microbial population to high starch diets by their gradual introduction over 5–10 days.

The bacteria mainly responsible for lactic acid production in the rumen are *Streptococcus bovis* and *Lactobacillus* spp. The antibiotic virginiamycin has been shown to control the proliferation of these organisms in sheep (Rowe *et al.* 1989) and cattle (Thorniley *et al.* 1994). Virginiamycin prevents lactic acid accumulation in both the rumen and hindgut, even when high starch diets are fed to animals without prior adaptation (Rowe & Pethick, 1994).

The importance of maintaining the rumen at pH 6.5 or above was clearly demonstrated by Kaufmann *et al.* (1980), who stressed that rumen pH was the dominant influence on the type and number of microflora. Below pH 6.0, lactic acid-producing bacteria may proliferate, but of equal significance is that at lower rumen pH the growth of cellulolytic bacteria is inhibited.

Huntington (1997), in his stimulating review of starch utilization, examined the reported effects of buffers such as sodium bicarbonate, calcium carbonate and magnesium oxide which have been used to ameliorate the effects of high starch diets on digestive disorders associated with low rumen pH. In most reports these ruminal buffers had no measurable effect on rumen pH or site of starch digestion (see Huntington, 1997). Russell & Chow (1993) had concluded from theoretical considerations that the influence of dietary buffers on rumen pH was of minor importance relative to the predominant effect of the transfer of CO_2 from blood.

The factors that influence the rate and extent of starch digestion in the rumen include source of starch, diet composition, amount of feed consumed per unit time, grain processing, chemical alterations (degree of gelatinization), degree of adaptation by rumen microorganisms and dietary additives (see Owens & Goetsch, 1986; Huntington, 1997). The most important determinants are feed particle size and feed consumption, which influence ruminal outflow rates. Huntington (1997) has collated total tract digestibility coefficients for dietary starch reported since 1986. The range of values for corn and sorghum (91·2–98·9 and 87·2–98·0) were somewhat lower than those for barley (94·3–98·2), wheat (98·2–98·6) and oats (98·3–98·8).

Short chain fatty acid metabolism

Measurement of short chain fatty acid production in the rumen

This topic was comprehensively reviewed by France & Siddons (1993), who also outlined the metabolic pathways by which dietary carbohydrates are fermented to SCFA *via* pyruvate. Nonisotopic methods for the measurement of SCFA production (Annison, 1965; Hungate, 1966; France & Siddons, 1993), which were inevitably imprecise (France & Siddons, 1993), have largely been superseded by isotope dilution procedures (Leng, 1970; Morant *et al.* 1978; Bruce *et al.* 1987). In their discussion of isotopic dilution procedures, France & Siddons (1993) have outlined other mathematical and modelling approaches to data generated by the single injection or constant infusion of isotopically labelled SCFA into the rumen.

A major advantage of isotope dilution procedures is that the extent of interconversions of the major SCFA in the rumen may also be measured (Bergman *et al.* 1965; France & Siddons, 1993). Most measurements of SCFA production rates are made on continuously fed animals in which steady-state conditions in the rumen are assumed to apply. France & Siddons (1993) suggest that the methods can be adapted to the nonsteady-state conditions which apply in normally fed animals. An alternative approach is to assume that the contribution of each individual SCFA to total SCFA production is reflected in their relative concentrations in the rumen (Weston & Hogan, 1968). These relationships appear not to apply when low forage diets are fed (Esdale *et al.* 1969) almost certainly because rumen pH values are somewhat lower and more varied on these diets and the relative rates of absorption of ruminal SCFA are influenced by rumen pH (Kaufmann, 1976).

Transport of short chain fatty acids in the rumen and hindgut

The proportions of SCFA found in the rumen and hindgut are roughly similar and SCFA are readily absorbed from both parts of the alimentary tract. Rechkemmer *et al.* (1995) in their recent review pointed out that the histology and structural organization of the ruminal and large

intestinal epithelium are quite different, posing the question of whether the transport of SCFA across the epithelium is similar in both tissues.

A great deal of research by von Engelhardt and his colleagues (see Rechkemmer *et al.* 1995) has revealed surprising similarities in the mechanisms of uptake of SCFA from the rumen and hindgut. The linear relationship between concentration and net absorption indicates that SCFA transport is primarily a passive permeation process in both organs, as suggested by Stevens (1970). Studies *in vitro*, however, suggest that nonspecific anion transporters also participate in transepithelial passage of SCFA in the rumen and intestine (Rechkemmer *et al.* 1995; Gäbel & Sehested, 1997).

In recent studies Sehested *et al.* (1997) have examined the effects of feeding patterns in dairy cows on the transport of butyrate across rumen epithelium *in vitro*. Feeding strategies that resulted in raised ruminal SCFA levels for relatively short periods increased butyrate transport. Epithelial surface area and structure were unchanged, suggesting that there is an SCFA transport mechanism in epithelial cells which is influenced by ruminal SCFA concentrations (Sehested *et al.* 1997).

Nitrogen metabolism

Protein, ammonia and urea interrelationships

Current understanding of nitrogen metabolism in the rumen stems from the pivotal discovery by McDonald (1952, 1954) that dietary protein is degraded by microbial activity with the formation of ammonia, a proportion of which is recycled into the rumen as urea in saliva. The nutritional significance of protein degradation was confirmed by Chalmers *et al.* (1954) who showed that casein, which is rapidly degraded in the rumen (McDonald, 1954), is utilized more efficiently when administered postruminally. Further, the nutritive value of dietary casein was much improved by heat denaturation to reduce solubility. This finding led to many attempts to protect dietary protein from ruminal degradation by chemical and physical treatments (see Broderick *et al.* 1991). The most successful of these, formaldehyde treatment (Ferguson *et al.* 1967; Ferguson, 1975), was cleverly adapted by Scott *et al.* (1971) to protect dietary unsaturated fat from ruminal biohydrogenation (see p. 185).

Russell *et al.* (1991) pointed out that by the 1970s it was generally accepted that protein solubility was the rate limiting step in protein degradation. For most proteins solubility and rumen degradability are closely related (Ferguson, 1975) but the molecular structure of the protein may also be involved (Annison, 1956; Mangan, 1972). By the mid 1970s there was evidence that free amino acids and peptides are released into the rumen after a protein feed, but that insignificant amounts of amino acids are absorbed from the rumen (see Smith, 1975).

Quantitative data on nitrogen metabolism in the rumen and in the whole animal were sparse until Mathison & Milligan (1971) and Nolan & Leng (1972) used isotope dilution techniques based on ^{15}N to measure ammonia and urea exchanges in the rumen and, in later studies, in the whole animal (see Nolan, 1975). A decade later the regulation of nitrogen metabolism in the ruminant and the available data on nitrogen recycling were comprehensively reviewed by Egan *et al.* (1986). The results of subsequent research in this area have been used to construct models of nitrogen metabolism in the rumen (Obara *et al.* 1991; Firkins *et al.* 1992). A possible limitation of some models based on isotope dilution data is that the high cost of the experiments inevitably limited the numbers of animals studied. Further, models which require data on rates of dietary protein degradation and on the efficiency of microbial protein

synthesis must take into account the limitations of current methods for the measurement of these indices, as discussed below.

The extent of urea recycled to the rumen, both in saliva and by transfer across the rumen wall, is markedly influenced by the nature of the diet (see Obara *et al.* 1991). In ruminants given readily fermentable carbohydrate there is usually a significant increase in the proportion of urea recycled to the rumen (see Obara *et al.* 1991). This increase in urea transfer stems from an increase in permeability of the rumen wall and is independent of both plasma urea concentration, urea input *via* saliva and rumen ammonia concentration (Norton *et al.* 1982; Obara *et al.* 1991). Although the increased permeability of the rumen wall to urea is associated with increased production and absorption of the end products of fermentation (CO_2 and SCFA), the mechanisms involved remain to be elucidated.

Role of microorganisms in nitrogen metabolism

Progress of research into the nitrogen metabolism of ruminal microorganisms in the 1960s was reviewed by Blackburn (1965) and Allison (1970). A crucial discovery in this period was that ammonia is the preferred nitrogen source for most rumen bacteria and is essential for the growth of several important species (Bryant & Robinson, 1962, 1963). The success achieved by Virtanen (1966) in maintaining lactating cows on diets containing nonprotein nitrogen but negligible amounts of amino acids or peptides was consistent with these findings. Milk yields in these studies were relatively low and Maeng & Baldwin (1976) later demonstrated a marked increase in microbial protein yield in response to amino acid supplements in a cow maintained on a purified diet supplemented with nonprotein nitrogen. On normal diets ruminal degradation contributes to amino acid supply; the peptides and free amino acids liberated during the breakdown of the rumen degradable fraction supply the needs of nonautotrophic microorganisms (Cotta & Hespall, 1986; Wallace & Cotta, 1988) and permit the maintenance of a more diverse and effective rumen ecosystem.

Many species of organisms are involved in proteolysis (Wallace & Cotta, 1988). A major development in this field was the recognition that protein degradation in the rumen often results in a build up of peptides (Chen *et al.* 1987). Most studies on the uptake of amino acids and peptides have indicated that mixed populations of rumen organisms preferentially incorporate peptides rather than free amino acids (see Wallace, 1996). Pure culture studies with the organism *Prevotella ruminicola* supported this general view but this organism, unlike other common microbial species so far identified, has dipeptidyl peptidase activity (see Wallace, 1996). Ling & Armistead (1995) demonstrated that free amino acids were the preferred amino source for *Streptococcus bovis* and several other organisms and that peptides were the preferred source only for *Prevotella ruminicola*. Wallace (1996), in his comprehensive review of peptide metabolism by ruminal organisms, has argued convincingly that the observed preference for peptides by mixed populations of rumen organisms in most studies probably reflected the presence of large numbers of *Prevotella ruminicola*. The key role of this organism in peptide metabolism in the rumen makes it an obvious target for attempts to manipulate ruminal activity and indeed the effects of ionophores are in part mediated *via* their effects on *Prevotella ruminicola* (see Wallace, 1994).

Measurement of protein degradation in the rumen

The long recognized significance of the metabolic fate of dietary proteins has led to sustained interest in the development of methods for measuring rates of ruminal degradation (see Bro-

derick *et al.* 1991). Indeed, the most widely used feeding systems, the Cornell Net Carbohydrate and Protein System (Sniffen *et al.* 1992; Chalupa & Sniffen, 1994) and the U.K. Metabolizable Protein System (Agricultural and Food Research Council, 1992; Beever & Cottrill, 1994) require estimates of dietary protein degradation in the rumen. The most widely used method was developed by Ørskov and his colleagues (Mehrez & Ørskov, 1977; Ørskov & McDonald, 1979) and involves suspending the test protein in a nylon bag in the rumen. This *in situ* procedure, which has proved an effective method for comparing the relative degradability of different proteins, has severe limitations (see Broderick *et al.* 1991). The most important of these are the potential contamination of the sample with microbial protein and the loss of soluble proteins which may not be degraded.

Efforts to measure directly the extent of dietary protein degradation and the amounts of microbial protein and other nitrogenous materials passing out of the rumen began in the 1960s. Extensive use was made of cannulation procedures (MacRae, 1975) supported by comprehensive digesta marker techniques (Faichney, 1975, 1986). Methods for assessing the contributions of undegraded dietary protein and microbial protein to digesta entering the duodenum were reviewed by Smith (1975), who commented on the limitations of methods based on the use of microbial markers, such as α,ε-diaminopimelic acid (DAPA) and nucleic acids, which are present only in microbial cells and absent from the remainder of the digesta. Errors associated with the use of marker substances stem largely from variations in the ratios of marker to nitrogen in mixed populations of rumen microorganisms. Even if estimates of microbial protein concentrations are valid, the difference between this value and total nonammonia nitrogen in duodenal digesta would overestimate levels of undegraded feed protein in the absence of information on the amounts of endogenous protein in digesta (see Smith, 1975). Clark *et al.* (1992), although aware of the problems associated with using DAPA : N and purine : N ratios discussed above, used mean literature values for these ratios to calculate the microbial protein synthesis and flows of nitrogen fractions to the duodenum of large numbers of dairy cows. An important finding was that microbial N accounted for some 59 % of the nonammonia nitrogen that flowed to the duodenum, although it was pointed out that more data were required for cows at the higher levels of feed intake more representative of current feeding practice.

Measurement of microbial protein synthesis

Bauchop & Elsden (1960) first introduced the concept of the relationship between the energy derived from anaerobic fermentation and microbial cell growth, expressed as cell yield/mol ATP (γATP). The relationship may be used to calculate microbial protein synthesis in the rumen from the amounts of organic matter digested, as discussed by Walker (1965). Smith (1975) reviewed much published data and concluded that the wide variations in calculated γATP values made estimates of microbial protein yield from the amounts of organic matter digested in the rumen of doubtful validity. Subsequent research has confirmed this conclusion.

The use of markers to measure microbial protein in the digesta flowing into the duodenum continues to attract attention. A recent critical evaluation of the use of DAPA and RNA as markers reported that although DAPA gave "quantitatively reasonable" results, values based on RNA were clearly incorrect (Robinson *et al.* 1996). Studies of this type are difficult to interpret in the absence of comparative data obtained at the same time by other procedures.

The measurement of urinary purines derived from microbial nucleic acids may prove to be an acceptable method for assessing microbial protein synthesis (see Stangassinger *et al.* 1995). Furthermore, studies in dairy cows have shown that outputs of allantoin in milk are closely correlated with the amount of microbial protein passing to the duodenum (Lebzien *et al.* 1993).

Lipid metabolism

Digestion and absorption of dietary lipids

The key features of the digestion and metabolism of lipids in the rumen were elucidated in the 1950s (Garton, 1961, 1965). Complex lipids are rapidly hydrolysed in the rumen with the liberation of free long chain fatty acids (LCFA), a high proportion of which are unsaturated. Lipolysis is accomplished almost entirely by rumen bacteria (Garton, 1977). Free unsaturated LCFA undergo extensive biohydrogenation, as discussed below.

The complex mixture of free LCFA produced in the rumen by lipolysis and biohydrogenation pass out of the rumen adsorbed on to the solids of the digesta (Lough, 1970). The uptake of lipid by rumen bacteria has been reported by Bauchart *et al.* (1990) and the uptake and incorporation into microbial lipid of volatile fatty acids and amino acids is well established (see Harfoot & Hazlewood, 1988). Lipid balance across the rumen has been examined in 15 studies with sheep and cattle fed supplements of fat (see Jenkins, 1993). Net losses or gains were small and varied but lipid losses were more common for diets with added fat (11 out of 15) than for control diets (see Jenkins, 1993). There is no evidence of either significant absorption of LCFA from the rumen, omasum or abomasum, or of appreciable degradation of LCFA (see Jenkins, 1993). Protozoa, mainly holotrichs, ingest LCFA for direct incorporation into cellular lipids, but there is little catabolism of LCFA by protozoa or by other rumen microorganisms (see Jenkins, 1993). There is some evidence of an inverse relationship between lipid intake and microbial lipid synthesis (Klusmeyer & Clark, 1991).

Lipid absorption from the small intestine is essentially similar to that in nonruminants, except that the amphiphile in micellar fat is lysophosphatidyl choline and not monoacylglycerols (see Garton, 1977). There is no selectivity either in the absorption of the *cis*- and *trans*-isomers from the small intestine, or in their incorporation into triacylglycerols in intestinal epithelium. During uptake there is some desaturation of stearic acid, with the formation of *cis*-Δ9-oleic acid (Bickerstaffe *et al.* 1972).

Antimicrobial activity of dietary lipids

The inhibitory action of dietary fat on forage digestibility was recognized in the late 1950s (Brethour *et al.* 1958; Grainger *et al.* 1961) and the capacity of calcium and iron to overcome the adverse effects of fats by the formation of insoluble soaps had also been established (Grainger *et al.* 1961). Subsequent studies confirmed that antimicrobial activity was confined to surface active amphiphilic lipids, exemplified by the LCFA liberated by the lipolysis of lipids in the rumen (Czerkawski *et al.* 1966*a*; Galbraith *et al.* 1971). Galbraith *et al.* (1971) showed in studies with nonrumen organisms that the antimicrobial activity of LCFA with eighteen carbon atoms (C18) increased with degree of unsaturation and that *cis*-isomers are more inhibitory than *trans*-isomers. A similar range of inhibitory effects was observed later with rumen bacteria (Chalupa *et al.* 1984).

Gram-positive bacteria are more susceptible than Gram-negative bacteria to the adverse effects of LCFA (Galbraith *et al.* 1971). Two of the three major cellulolytic bacteria in the rumen, *Ruminococcus flavifaciens* and *Ruminococcus albus* and the main methanogenic genera *Methanobacterium* spp., are susceptible to lipid (Maczulac *et al.* 1981). Rumen protozoa and rumen fungi are even more sensitive to the adverse effects of amphiphilic lipids (Ushida *et al.* 1992).

The presence of calcium ions and, to a lesser extent, magnesium ions is known to reduce the antimicrobial action of LCFA (Galbraith & Miller, 1973). Inclusion of calcium hydroxide in ruminant diets makes it possible to increase rates of fat inclusion substantially without adverse effects on fibre digestibility (Palmquist, 1988; Ohajuruka *et al.* 1991). The encapsulation of dietary fats to reduce biohydrogenation in the rumen (see below) also protects rumen micro-organisms from the antimicrobial activity of the fat.

Biohydrogenation

It was only in the 1950s that an explanation emerged for three features of ruminant carcass fat that had long puzzled food scientists. In contrast to the situation in nonruminants, sheep and beef fat are characterized by their relative hardness, which was correctly ascribed to high levels of stearic acid (Banks & Hilditch, 1931). The other features were the inability to increase the degree of unsaturation of ruminant fat by inclusions of unsaturated oils in the diet and the presence in carcass and milk fat of *trans*-unsaturated fatty acids (see Garton, 1961). The explanation for these unique aspects of ruminant carcass fat lies in the biohydrogenation of dietary unsaturated fatty acids by microorganisms in the rumen, first reported by Reiser (1951). Shorland *et al.* (1957) confirmed this finding and demonstrated the formation of *cis*- and *trans*-positional isomers of octadecenoic acid.

The main features of the hydrolysis of complex lipids in the rumen and of the biohydrogenation of their constituent unsaturated fatty acids were elucidated in the next decade (see Dawson & Kemp, 1970). At that time the reported correlation between intakes of saturated fats and cardiovascular disease observed in some countries, although shown later to be of doubtful validity (Blaxter, 1991), led health authorities to recommend reduced consumption of ruminant products. In an innovative effort to increase the proportions of unsaturated fatty acids in ruminant fats and increase consumer acceptance Scott *et al.* (1970) developed a technique for the protection of dietary fat from ruminal biohydrogenation. The inclusion in ruminant diets of protected unsaturated oils greatly increased the proportions of polyunsaturated fatty acids in milk and carcass fat (Scott *et al.* 1970, 1971). At that time products with increased proportions of unsaturated fatty acids were more prone to oxidation, which led to the development of unacceptable off flavours, but more recently Ashes *et al.* (1997) have controlled autoxidation in milk fat by including vitamin E in the diet of the lactating cow. Scott and his colleagues have also used protected lipids to manipulate the fatty acid composition of membrane lipids, a development with potential value in future efforts to regulate cellular metabolism (Ashes *et al.* 1995).

The bacteria involved in biohydrogenation in the rumen and the biochemistry of it have been discussed by Harfoot & Hazlewood (1988) in their comprehensive review of lipid metabolism in the rumen. Suggestions that the function of biohydrogenation is to dispose of surplus reducing capacity or to eliminate toxic unsaturated fatty acids (Harfoot & Hazlewood, 1988) have not been supported by subsequent studies. A prerequisite of biohydrogenation in the rumen would appear to be adsorption of the substrate on to the surface of finely divided food particles (Harfoot & Hazlewood, 1988). A review of published data obtained with cattle and sheep by Doreau & Ferlay (1994) showed that the extent of ruminal biohydrogenation of dietary linolenic and linoleic acids was 85–100 % and 70–93 % respectively. The lower value for linoleic acid was attributed to the uptake of this acid by bacteria (Bauchart *et al.* 1990). Bickerstaffe *et al.* (1972) showed that in the goat about 90 % of dietary linolenate, linoleate and

oleate were hydrogenated in the rumen, with the formation of a range of *cis*- and *trans*-positional isomers of octadecenoic acid.

Methane production

The use of respiration chambers to measure energy balances in ruminants in the 1950s and 1960s (see Blaxter, 1962) generated accurate data on methane outputs. The significant energy losses associated with methane production led Blaxter and his colleagues (Blaxter & Clapperton, 1965; Czerkawski *et al.* 1966*b*) to examine the interrelationships of feed intake, the composition and digestibility of the diet and methane production. These studies and later work showed that the fibre content of the diet was the major determinant of methane production (see Kirchgessner *et al.* 1995).

The intracellular biochemistry of methanogenesis in the rumen had been largely elucidated by the early 1970s (see Wolfe, 1971) and the stoichiometric relationship between methane and SCFA production established (Demeyer & van Nevel, 1975). The latter authors reviewed efforts to channel the energy normally associated with methanogenesis into assimilable nutrients by the use of additives. Simple halogenated compounds such as chloral hydrate were shown to inhibit methanogenesis and stimulate propionate production but *in vivo* the effects were not sustained for more than a few weeks (see Demeyer & van Nevel, 1975). Somewhat later ionophores, now widely used in ruminant diets to improve the efficiency of food utilization, were also shown to reduce methane production substantially for the first few days of treatment but again the effects are not sustained (Johnson *et al.* 1994). Nevertheless, Kirchgessner *et al.* (1995) have made the point that increased feed efficiency stemming from the use of ionophores results in a decrease in methane production proportionate to the reduction in food intake.

Interest in methane production by ruminants has been stimulated in recent years by the belief that atmospheric methane contributes both to global warming and to the destruction of the stratospheric ozone layer (Crutzen, 1995). Some two thirds of this methane originates from human activities and of this about a quarter arises from animal production, with ruminants the major contributors. Apart from the reduction in total methane output associated with the use of ionophores, no additives which suppress methane production over long periods have come into general use.

In his review of current knowledge of methanogenesis in the rumen, Miller (1995) has also noted that studies of anaerobic fermentation in the lower gut of other species have revealed that most colonic ecosystems do not produce large amounts of methane. In nonmethanogenic fermentations, H_2 is used to reduce CO_2 to acetate and this process, acetogenesis, has been identified in humans, rats, guineapigs and rabbits (see Miller, 1995). Earlier studies had revealed that concentrations of acetogens in the rumen were similar to those of methanogens (Greening & Leedle, 1989), but there is no evidence of significant acetate formation from CO_2 when rumen contents are incubated in an atmosphere of CO_2 and H_2 (see Miller, 1995). The basis for this dominance of methanogenesis over acetogenesis in the rumen is unknown and its importance warrants increased research effort. Miller (1995) has obtained promising results using cocultures of a cellulolytic bacterium and a methanogen. Wallace (1997) has suggested that another type of acetogenesis identified in pig digesta (De Graeve *et al.* 1994) might be adapted to reduce methanogenesis in the rumen.

Detoxification and production of toxins

Pasture plants, shrubs and trees contain many chemical constituents in addition to nutrients. Some of the compounds are allelochemicals which may be toxic, or in some instances converted by rumen microorganisms into metabolites with greater or less antinutritional effects or toxicity (see James *et al.* 1975; Dawson & Allison, 1988; Cheeke, 1998).

Degradation of toxins

Many plant toxins are eliminated by their degradation in the rumen. Adaptation to toxic materials may occur when normal microbial activities cannot immediately detoxify high levels of poisonous substances in the diet, an example of which is oxalate. The bacterium responsible for oxalate decarboxylation, *Oxalobacter formigenes*, increases in numbers as dietary oxalate levels rise (see Barry & Blaney, 1987; Dawson & Allison, 1988). Over a 3–4 day period adaptation may occur to levels of oxalate that would otherwise be lethal (James *et al.* 1975).

Mycotoxins are secondary fungal metabolites that have a broad range of toxic effects (Bryden, 1998). Ochratoxin is hydrolysed in the rumen to less toxic ochratoxin α and phenylalanine (Kiessling *et al.* 1984; Westlake *et al.* 1989; Xiao *et al.* 1991*a*,*b*). The hydrolysis of ochratoxin is much greater in animals fed high roughage diets (Xiao *et al.* 1991*a*,*b*). The trichothecene mycotoxins, T-2 toxin, HT-2 toxin, deoxynivalenol and diacetoxyscirpenol are degraded to varying extents in the rumen by both enzymic reduction and ester hydrolysis (Westlake *et al.* 1987*a*,*b*). *Butyrivibrio fibrisolvens* plays an important role in reducing trichothecene toxicity in ruminants (Westlake *et al.* 1987*b*).

Production of toxins

Microbial metabolism of plant compounds may lead to the production of toxins. For example, formation of 3-methylindole from tryptophan by *Lactobacillus* spp. may result in acute pulmonary emphysema caused by the products of 3-methylindole metabolism in the liver (see Carlson & Breeze, 1984). Another example, first described by Bennetts *et al.* (1946), is oestrogen-induced infertility in sheep grazing pasture legumes which is caused by the conversion of phyto-oestrogens to oestrogenic metabolites (Davies, 1987; Adams, 1995).

Nitrate poisoning has long been recognized in ruminants (Lewis, 1951). Accumulation of the toxic intermediate nitrite during the reduction of nitrate to ammonia in the rumen is the cause of toxicity. The relative rates of nitrate and nitrite reduction are critical factors in the development of toxicity but adaptation to high nitrate concentrations greatly increases rates of nitrate and nitrite detoxification (Allison & Rasmussen, 1992). Adaptation to nitrate also increases the rate of detoxification of other naturally occurring nitrotoxins (see Anderson *et al.* 1998). Recently a novel nitrotoxin metabolizing bacterium has been isolated and a new genus and species is needed to accommodate the unique properties of this bacterium (Anderson *et al.* 1998). The study of this organism, which uses anaerobic respiration or dissimilatory metabolism to survive in the rumen (see Rasmussen & Anderson, 1998), should contribute greatly to our understanding of this intriguing aspect of rumen metabolism.

A number of photosensitization diseases characterized by the deposition of crystalline microliths in the bile ducts and surrounding tissues occur throughout the world (Low *et al.* 1994; Flaoyen & Froslie, 1997). The diseases occur when steroidal saponin-containing plants

form a major part of the diet and the nature of the crystalline deposits, and their relationship to the plant saponins has been elucidated by Miles *et al.* (1994*a*,*b*). Ingested saponins are rapidly hydrolysed in the rumen, releasing free sapogenins which are converted to espismilagenin and episarsasapogenin. The crystals are primarily calcium salts of glucuronides which arise from the hepatic metabolism of these metabolites.

An intriguing feature of ruminant toxicity has involved the amino acid, mimosine, which is found in the tropical shrub legume, *Leucaena leucocephala* (leucaena). Cattle in Hawaii thrive on leucaena but in Australia cattle fed leucaena show poor weight gains and exhibit hypothyroidism. Jones (1981) suggested that differences in microbial flora could explain the tolerance of cattle in Hawaii. Mimosine is converted in the rumen to 3-hydroxy-4(1H)-pyridone, a potent goitrogen (Hegarty *et al.* 1976) which is destroyed by further ruminal activity in ruminants in Hawaii, but not in Australia. Jones & Lowry (1984) showed that susceptible Australian livestock may be protected by inoculation of the rumen with bacteria from ruminants tolerant to leucaena. Not only were inoculated animals unaffected by leucaena but protection could be passed to uninoculated animals, probably *via* faeces (see Hammond, 1995). The bacterium which confers protection, *Synergistes jonesii* (Allison *et al.* 1992) is unusual in that it is the only rumen bacterium so far identified that utilizes arginine and histidine as major energy substrates (McSweeny *et al.* 1993). This organism has been shown to protect cattle grazing leucaena in several countries (see Jones, 1994).

Rumen dysfunction

Some plant chemicals influence animal performance by altering the activities of rumen microbial populations. As described above (p. 179), acute and chronic acidosis are conditions that can occur following the ingestion of excessive amounts of readily fermentable carbohydrate. Not only are there changes in the microbial population but also in the acidity and osmolality of rumen contents. These changes predispose animals to the development of laminitis, polioencephalomalacia and liver abscesses (Owens *et al.* 1998). Ruminal lesions resulting from acidosis allow *Fusobacterium necrophorum*, a ruminal anaerobe, to penetrate and colonize the ruminal epithelium and infect the liver (see Nagaraja & Chengappa, 1998).

Polyphenols are probably the most widely distributed allelochemicals in plant legumes and have been the subject of much research (see Mehansho *et al.* 1987; Mangan, 1988; Reed, 1995). Proanthocyanidins (condensed tannins) and hydrolysable tannins are the two major classes of polyphenols. The latter are potentially toxic to ruminants following microbial production of pyrogallol in the rumen but condensed tannins are generally considered to improve protein digestion and metabolism. The widely accepted explanation for this effect is that complexing with tannin protects protein from ruminal degradation (see Reed, 1995).

Other compounds that alter rumen metabolism (see Dawson & Allison, 1988; Cheeke, 1998) include essential oils and aflatoxins and perloline produced by fungal endophytes of tall fescue and perennial ryegrass. Fluoroacetate which occurs in native plants in Australia, Africa and South America (Barry & Blaney, 1987) is cumulatively toxic in sheep (Annison *et al.* 1960) and most other animals, by blocking the tricarboxylic acid cycle (Peters *et al.* 1953). In an attempt to reduce stock losses associated with fluoroacetate poisoning, the rumen bacterium *Butyrivibrio fibrisolvens* has been genetically modified to express the enzyme fluoroacetate dehalogenase (Gregg *et al.* 1997). The modified organism was shown to survive at metabolically significant levels in the rumen (10^6–10^7/ml) and to confer substantial protection from fluoroacetate poisoning. This remarkable achievement will encourage further efforts to enhance

useful metabolic activities of rumen microorganisms, such as fibre digestion, and may well be the key to the future control of toxins and other antinutritive factors.

Manipulation of rumen fermentation

The manipulation of rumen fermentation to improve the utilization of feedstuffs must take into account the mechanisms by which rumen bacteria acquire nutrients. The complex polymers that comprise most feed ingredients must be degraded by extracellular enzymes to low molecular weight substances before transport into bacterial cells. The transport mechanisms in rumen bacteria have been outlined by Russell *et al.* (1990) and Martin (1994). The assimilation of the major energy yielding and nitrogenous nutrients by ruminal bacteria has been reviewed by McSweeney *et al.* (1994) in their evaluation of the potential use of genetic engineering to enhance microbial efficiency. They concluded that application of the technology that has proved so effective in the area of industrial fermentation will require more information on the metabolism of predominant ruminal bacteria.

The use of dietary ionophores, antibiotics and microbial feed to enhance productivity by manipulating rumen fermentation has been fully discussed by Wallace (1994). Monensin, first used in the 1970s, remains the most widely used ionophore. The nutritionally significant effects of monensin are to increase the proportion of propionate in ruminal SCFA and to control excessive ammonia production by inhibiting peptide metabolism and suppressing the growth of highly active deaminating bacteria (see Wallace, 1996).

The effects of ionophores are due to their capacity to disrupt membrane function, but microorganisms vary widely in their sensitivity. Gram-positive bacteria are more readily inhibited than Gram-negative bacteria (see Wallace, 1994). Indeed, the beneficial effects of ionophores on nitrogen metabolism in the rumen may stem from the resistance and proliferation of the Gram-negative organism *Prevotella ruminicola* (p. 182). The selective toxicity of antibiotics such as avoparcin is also due to interference with membrane function in responsive microorganisms (Wallace, 1994).

Live microbial feed additives, particularly of yeast cells, are attracting increasing attention since published data suggest that their use leads to improvements in ruminant productivity similar to those observed with ionophores (Wallace, 1994). The purported nutritional benefits stem from increased feed intake, in contrast to responses to ionophores, which enhance the efficiency of feed utilization. The increased feed intake is driven by an improved rate of fibre breakdown (Martin & Nisbet, 1992) and an increased flow of absorbable amino nitrogen to the duodenum (Williams *et al.* 1990; Erasmus *et al.* 1992). Both effects are ascribed to an increase in the numbers of viable bacteria in the rumen (Wallace, 1994). Possible reasons for this increase in rumen bacteria in animals receiving yeast supplements is the capacity of yeast cells to remove oxygen, which is toxic to cellulolytic organisms, and to the presence of unidentified metabolic activities or a heat labile nutrient (see Wallace, 1994). The paucity of published data confirming and extending the initial observations, however, would suggest that favourable production responses to microbial additives occur only in exceptional dietary circumstances.

Concluding comments

Hungate (1966) showed remarkable prescience when proposing possible reasons for the diversity of rumen microorganisms. His hypotheses that different organisms would vary widely

in their metabolic capacities and that diversity would stem from the principle of selection for maximum biochemical work have proved to be consistent with the recognized importance of synergic interactions in the rumen. The most striking of these is the breakdown of complex polysaccharides, the major energy source for ruminants, by consortia of bacteria and, in some instances, fungi (p. 178)

The outstanding success of Gregg *et al.* (1997) in genetically modifying a commonly occurring rumen bacterium to degrade fluoroacetate, and of equal importance, to survive for some months in the rumen will encourage more application of recombinant DNA technology to rumen microorganisms. Improved fibre digestion *via* enhanced enzymic activity is an obvious target but, as discussed earlier, the complexity of polysaccharide breakdown will pose major problems. More success is likely to be achieved by seeking to modify microorganisms known to be of metabolic significance in the rumen, such as *Prevotella ruminicola* (p. 182).

The rumen has the capacity to modify substantially all the major dietary nutrients except minerals, but the degradation of protein and concomitant production of microbial protein makes it difficult to evaluate the supply of essential amino acids to the small intestine. Current inadequacies in the measurement of the degradation of dietary protein in the rumen and of the extent of microbial protein production continue to hinder the development of feeding systems that predict production responses to nutrients in ruminants. This issue, and a review of the metabolism in tissues of amino acids and energy yielding nutrients absorbed from the rumen and small intestine, are discussed in Part II of this review (Annison & Bryden, 1999).

References

Adams, J. C., Gazaway, J. A., Brailsford, M. D., Hartman, P. A. & Jacobson, N. L. (1966). Isolation of bacteriophages from the bovine rumen. *Experientia* **22**, 717–718.

Adams, N. R. (1995). Detection of the effects of phytoestrogens on sheep and cattle. *Journal of Animal Science* **73**, 1509–1515.

Agricultural and Food Research Council (1992). Technical Committee on Responses to Nutrients. Report no. 9. Nutritive Requirements of Ruminant Animals: Protein. *Nutrition Abstracts and Reviews B* **62**, 787–835.

Akin, D. E. & Benner, R. (1988). Degradation of polysaccharides and lignin by ruminal bacteria and fungi. *Applied and Environmental Microbiology* **54**, 1117–1125.

Allison, M. J. (1970). Nitrogen metabolism of ruminal microorganisms. In *Physiology of Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 3, 1969)*, pp. 456–473 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.

Allison, M. J., Mayberry, W. R., McSweeney, C. S. & Stahl, D. A. (1992). *Synergistes jonesii*, gen. nov. sp. nov.: a rumen bacterium that degrades toxic pyridinediols. *Systematic and Applied Microbiology* **15**, 522–529.

Allison, M. J. & Rasmussen, M. A. (1992). The potential for plant detoxification through manipulation of the rumen fermentation. In *Poisonous Plants*, pp. 367–376 [L. F. James, R. F. Keeler, E. M. Bailey, P.R. Cheeke and M. Hegarty, editors]. Ames, IA: Iowa State University Press.

Anderson, R. C., Majak, W., Rasmussen, M. A. & Allison, M. J. (1998). Detoxification potential of a new species of ruminal bacteria that metabolize nitrate and naturally occurring nitrotoxins. In *Toxic Plants and Other Natural Toxicants*, pp. 152–158 [T. Garland and A. C. Barr, editors]. Wallingford: CAB International.

Annison, E. F. (1956). Nitrogen metabolism in the sheep. Protein digestion in the rumen. *Biochemical Journal* **64**, 705–714.

Annison, E. F. (1965). Absorption from the ruminant stomach. In *Physiology of Digestion in the Ruminant (International Symposium on Ruminant Physiology 2, 1964)*, pp. 185–197 [R. W. Dougherty, R. S. Allen, W. Burroughs, N. L. Jacobson and A. D. McGilliard, editors]. London: Butterworths.

Annison, E. F. & Bryden, W. L. (1999). Perspectives on ruminant nutrition and metabolism. II. Metabolism in ruminant tissues. *Nutrition Research Reviews* in press.

Annison, E. F., Hill, K. J., Lindsay, D. B. & Peters, R. A. (1960). Fluoroacetate poisoning in sheep. *Journal of Comparative Pathology* **70**, 145–155.

Annison, E. F. & Lewis, D. (1959). *Metabolism in the Rumen*. London: Methuen.

Armstrong, D. G. (1993). Quantitative animal nutrition and metabolism: a review. *Australian Journal of Agricultural Research* **44**, 333–345.

Armstrong, D. G. & Gilbert, H. J. (1991). The application of biotechnology for future livestock production. In *Physiological Aspects of Digestion and Metabolism in Ruminants (International Symposium on Ruminant Physiology*, **7**, 1989) pp. 737–761 [T. Tsuda, Y. Sasaki and R. Kawashima, editors]. New York: Academic Press.

Ashes, J. R., Fleck, E. & Scott, T. W. (1995). Dietary manipulation of membrane lipid and its implications for their role in the production of second messengers. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, pp. 373–385 [W. V. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke, editors]. Stuttgart: Ferdinand Enke Verlag.

Ashes, J. R., Galati, S. K. & Scott, T. W. (1997). Potential to alter the content and composition of milk fat through nutrition. *Journal of Dairy Science* **80**, 2204–2212.

Baker, F. (1943). Direct microscopical observations upon the rumen population of the ox. I. Qualitative characteristics of the rumen population. *Annals of Applied Biology* **30**, 230–239.

Baker, S. K. (1997). Gut microbiology and its consequences for the ruminant. *Proceedings of the Nutrition Society of Australia* **21**, 6–13.

Baldwin, R. L. (1995). *Modeling Ruminant Digestion and Metabolism*. London: Chapman & Hall.

Banks, A. K. & Hilditch, T. P. (1931). The glyceride structure of beef tallows. *Biochemical Journal* **25**, 1168–1182.

Barcroft, J., McAnally, R. A. & Phillipson, A. T. (1944). Absorption of volatile fatty acids from the alimentary tract of the sheep and other animals. *Journal of Experimental Biology* **20**, 120–129.

Barry, T. N. & Blaney, B. J. (1987). Secondary compounds of forages. In *The Nutrition of Herbivores*, pp. 91–120 [J. B. Hacker and J. H. Ternouth, editors]. New York: Academic Press.

Bauchart, D. F., Legay-Carmier, F., Doreau, M. & Gaillard, B. (1990). Lipid metabolism of liquid-associated and solid-adherent bacteria in rumen contents of dairy cows offered lipid-supplemented diets. *British Journal of Nutrition* **63**, 563–578.

Bauchop, T. (1979). The rumen anaerobic fungi: colonizers of plant fibre. *Annales de Recherches Vétérinaires* **10**, 246–248.

Bauchop, T., Clarke, R. T. J. & Newhook, J. C. (1975). Scanning electron microscope study of bacteria associated with the rumen epithelium of sheep. *Applied Microbiology* **30**, 668–675.

Bauchop, T. & Elsden, S. R. (1960). The growth of microorganisms in relation to their energy supply. *Journal of General Microbiology* **23**, 457–469.

Bauchop, T. & Mountford, D.O. (1981). Cellulose fermentation by a rumen anaerobic fungus in both the absence and the presence of rumen methanogens. *Applied and Environmental Microbiology* **42**, 1103–1110.

Beever, D. E. & Cottrill, B. R. (1994). Protein systems for feeding ruminant livestock: a European assessment. *Journal of Dairy Science* **77**, 2031–2043.

Bennetts, H. W., Underwood, E. J. & Shier, F. L. (1946). A specific breeding problem of sheep on subterranean clover pastures in Western Australia. *Australian Veterinary Journal* **22**, 2–12.

Bergman, E. N., Reid, R. S., Murray, M. G., Brockway, J. M. & Whitelaw, F. G. (1965). Interconversions and production of volatile fatty acids in the sheep rumen. *Biochemical Journal* **97**, 53–58.

Bickerstaffe, R., Noakes, D. E. & Annison, E. F. (1972). Quantitative aspects of fatty acid biohydrogenation, absorption and transfer into milk fat in the lactating goat, with special reference to the *cis*- and *trans*- isomers of octadecenoate and linoleate. *Biochemical Journal* **130**, 607–617.

Bird, S. H. (1989). Production from ciliate-free ruminants. In *The Roles of Protozoa and Fungi in Ruminant Digestion*, pp. 233–245 [J. V. Nolan, R. A. Leng and D. I. Demeyer, editors]. Armidale, NSW: Penambul Books.

Bird, S. H. & Leng, R. A. (1985). Productivity responses to eliminating protozoa from the rumen of sheep. In *Reviews in Rural Science 6*, pp. 109–117 [R. A. Leng, J. S. F. Barker, D. B. Adams and K. J. Hutchinson, editors]. Armidale, NSW: University of New England Publishing Unit.

Blackburn, T. H. (1965). Nitrogen metabolism in the rumen. In *Physiology of Digestion in the Ruminant (International Symposium on Ruminant Physiology 2, 1964)*, pp. 322–334 [R. W. Dougherty, R. S. Allen, W. Burroughs, N. L. Jacobson and A. D. McGilliard, editors]. Washington: Butterworths.

Blaxter, K. L. (1962). *The Energy Metabolism of Ruminants*. London: Hutchinson.

Blaxter, K. L. (1991). Animal production and food: real problems and paranoia. *Animal Production* **53**, 261–269.

Blaxter, K. L. & Clapperton, J. L. (1965). Prediction of the amount of methane produced by ruminants. *British Journal of Nutrition* **19**, 511–522.

Brethour, J. R., Sirny, R. J. & Tillman, A. D. (1958). Further studies concerning the effects of fats in sheep rations. *Journal of Animal Science* **17**, 171–179.

Broderick, G. A., Wallace, R. J. & Ørskov, E. R. (1991). Control of rate and extent of protein degradation. In *Physiological Aspects of Digestion and Metabolism in Ruminants (International Symposium on Ruminant Physiology 7, 1989)*, pp. 541–592 [T. Tsuda, Y. Sasaki and R. Kawashima, editors]. New York: Academic Press.

Bruce, L. A., Lobley, G. E. & MacRae, J. C. (1987). Measurement of volatile fatty acid production rates in sheep given roughage. *Research in Veterinary Science* **42**, 47–52.

Bryant, M. P. & Robinson, I. M. (1962). Some nutritional characteristics of predominant culturable ruminal bacteria. *Journal of Bacteriology* **84**, 605–614.

Bryant, M. P. & Robinson, I. M. (1963). Apparent incorporation of ammonia and amino acid carbon during growth of selected species of ruminal bacteria. *Journal of Dairy Science* **46**, 150–154.

Bryden, W. L. (1998). Mycotoxin contamination of Australian pastures and feedstuffs. In *Toxic Plants and Other Natural Toxicants*, pp. 464–478 [T.Garland & A. C.Barr, editors]. Wallingford, UK: CAB International.

Carlson, J. R. & Breeze, R. G. (1984). Ruminal metabolism of plant toxins with emphasis on indolic compounds. *Journal of Animal Science* **58**, 1040–1049.

Chalmers, M. I., Cuthbertson, D. P. & Synge, R. L. M. (1954). Ruminal ammonia formation in relation to the protein requirement of sheep. I. Duodenal administration and heat processing as factors influencing fate of casein supplements. *Journal of Agricultural Science* **44**, 254–262.

Chalupa, W., Rickabaugh, B., Kronfeld, D. S. & Sklan, D. (1984). Rumen fermentation *in vitro* as influenced by long chain fatty acids. *Journal of Dairy Science* **67**, 1439–1444.

Chalupa, W. & Sniffen, C. J. (1994). Carbohydrate, protein and amino acid nutrition of lactating dairy cattle. In *Recent Advances in Animal Nutrition–1994 (University of Nottingham Feed Manufacturers' Conference 28, 1994)*, pp. 265–275 [P. C. Garnsworthy and D. J. A. Cole, editors]. Nottingham: Nottingham University Press.

Cheeke, P. R. (1998). *Natural Toxicants in Feeds, Forages and Poisonous Plants*, 2nd edn. Danville, USA: Interstate Publishers.

Chen, G., Sniffen, C. J. & Russell, J. B. (1987). Concentration and estimated flow of peptides from the rumen of dairy cattle: effects of protein quantity, protein solubility and feeding frequency. *Journal of Animal Science* **70**, 983–992.

Cheng, K.-J. & Costerton, J. W. (1980). Adherent rumen bacteria—their role in the digestion of plant material, urea and epithelial cells. In *Digestive Physiology and Metabolism in Ruminants (International Symposium on Ruminant Physiology 5, 1979)*, pp. 227–250 [Y. Ruckebush and P. Thivend, editors]. Lancaster: MTP Press.

Cheng, K.-J., Forsberg, C. W., Minato, H. & Costerton, J. W. (1991). Microbial ecology and physiology of feed degradation within the rumen. In *Physiological Aspects of Digestion and Metabolism in Ruminants (International Symposium on Ruminant Physiology 7, 1989)*, pp. 595–624 [T. Tsuda, Y. Sasaki and R. Kawashima, editors]. New York: Academic Press.

Chesson, A., Forsberg, C. W. & Grenet, E. (1995). Improving the digestion of plant cell walls and fibrous feeds. In *Recent Developments in the Nutrition of Herbivores*, pp. 249–277 [M. Journet, E. Grenet, M. H. Farce, M. Theriez and C. Demarquilly, editors]. Paris: INRA.

Clark, J. H., Klusmeyer, T. H. & Cameron, M. R. (1992). Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. *Journal of Dairy Science* **75**, 2304–2323.

Coleman, G. S. (1975). The relationship between rumen ciliate protozoa and bacteria. In *Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 4, 1974)*, pp. 149–164 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, NSW: University of New England Publishing Unit.

Costerton, J. W., Geesey, G. G. & Cheng, K.-J. (1978). How bacteria stick. *Scientific American* **238(1)**, 86–95.

Cotta, M. A. & Hespell, R.B. (1986). Protein and amino metabolism of rumen bacteria. In *Control of Digestion and Metabolism in Ruminants (International Symposium on Ruminant Physiology 6, 1984)*, pp. 122–136 [L. P. Milligan, W. L. Grovum and A. Dobson, editors]. Englewood Cliffs, NJ: Prentice-Hall.

Crutzen, R. J. (1995). The role of methane in atmospheric chemistry and climate. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, pp. 291–313 [W. V. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke, editors]. Stuttgart: Ferdinand Enke Verlag.

Czerkawski, J. W., Blaxter, K. L. & Wainman, F.W. (1966*a*). The effect of functional groups other than carboxyl on the metabolism of C_{18} and C_{12} alkyl compounds by sheep. *British Journal of Nutrition* **20**, 495–508.

Czerkawski, J. W., Blaxter, K. L. & Wainman, F. W. (1966*b*). The metabolism of oleic, linoleic and linolenic acids by sheep with reference to their effects on methane production. *British Journal of Nutrition* **20**, 349–362.

Davies, H. L. (1987). Limitations to livestock production associated with phytoestrogens and bloat. In *Temperate Pastures: their production, use and management*, pp. 446–456 [J. L. Wheeler, G. C. J. Pearson and G. E. Robards, editors]. Melbourne, Australia: CSIRO.

Dawson, K. A. & Allison, M. J. (1988). Digestive disorders and nutritional toxicity. In *The Rumen Microbial Ecosystem*, pp. 445–459 [P. N. Hobson, editor]. London: Elsevier Applied Science.

Dawson, R. M. C. & Kemp, P. (1970). Biohydrogenation of dietary fats in ruminants. In *Physiology of Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 3, 1969)*, pp. 504–518 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.

De Graeve, K. G., Grivet, J. P., Durand, M., Beaumatin, P., Cordelet, C. & Hannequart, G. (1994). Competition between reductive acetogenesis and methanogenesis in the pig large-intestinal flora. *Journal of Applied Bacteriology* **76**, 55–61.

Dehority, B. A. (1991). Effects of microbial synergism on fibre digestion in the rumen. *Proceedings of the Nutrition Society* **50**, 149–159.

Demeyer, D. I. (1989). Effect of defaunation on rumen fibre digestion and digesta kinetics. In *The Roles of Protozoa and Fungi in Ruminant Digestion*, pp. 171–180 [J. V. Nolan, R. A. Leng and D. I. Demeyer, editors]. Armidale, NSW: Penambul Books.

Demeyer, D. I. & van Nevel, C. J. (1975). Methanogenesis, an integrated part of carbohydrate fermentation, and its control. In *Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 4, 1974)*, pp. 366–382 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, NSW: University of New England Publishing Unit.

Doreau, M. & Ferlay, A. (1994). Digestion and utilisation of fatty acids by ruminants. *Animal Feed Science and Technology* **45**, 379–396.

Dunlop, R. H. & Hammond, P. B. (1965). D-Lactic acidosis of ruminants. *Annals of the New York Academy of Sciences* **119**, 1109–1132.

Egan, A. R., Boda, K. & Varady, J. (1986). Regulation of nitrogen metabolism and recycling. In *Control of Digestion and Metabolism in Ruminants (International Symposium on Ruminant Physiology 6, 1984)*, pp. 386–402 [L. P. Milligan, W. L. Grovum and A. Dobson, editors]. Englewood Cliffs, NJ: Prentice-Hall.

Erasmus, L. J., Botha, P. M. & Kistner, A. (1992). Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. *Journal of Dairy Science* **75**, 3056–3065.

Esdale, W. J., Broderick, G. A. & Satter, L. D. (1969). Measurement of ruminal volatile fatty acid production from alfalfa hay or corn silage rations using a continuous infusion isotope dilution technique. *Journal of Dairy Science* **51**, 1823–1830.

Faichney, G. J. (1975). The use of markers to partition digestion within the gastro-intestinal tract of ruminants. In *Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 4, 1975)*, pp. 277–291 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, NSW: University of New England Publishing Unit.

Faichney, G. J. (1986). The kinetics of particulate matter in the rumen. In *Control of Digestion and Metabolism in Ruminants (International Symposium on Ruminant Physiology 6, 1984)*, pp. 173–195 [L. P. Milligan, W. L. Grovum and A. Dobson, editors]. Englewood Cliffs, NJ: Prentice-Hall.

Ferguson, K. A. (1975). The protection of dietary proteins and amino acids against microbial fermentation in the rumen. In *Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 4, 1975)*, pp. 448–464 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, NSW: University of New England Publishing Unit.

Ferguson, K. A., Hemsley, J. A. & Reis, P. J. (1967). Nutrition and wool growth. The effect of protecting dietary protein from microbial degradation in the rumen. *Australian Journal of Science* **30**, 215–217.

Firkins, J. L., Weiss, W. P. & Piwonka, E. J. (1992). Quantification of intraruminal recycling of microbial nitrogen using nitrogen-15. *Journal of Animal Science* **70**, 3223–3233.

Flaoyen, A. & Froslie, A. (1997). Photosensitization disorders. In *Handbook of Plant and Fungal Toxicants*, pp. 191–204 [J. P. F. D'Mello, editor]. Boca Raton, FL: CRC Press.

Flint, H. J. & Forsberg, C. W. (1995). Polysaccharide degradation in the rumen: biochemistry and genetics. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, pp. 43–63 [W. V. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke, editors]. Stuttgart: Ferdinand Enke Verlag.

Fonty, G., Bernalier, A. & Gouet, P. H. (1990). Degradation of lignocellulosic forages by anaerobic fungi. In *Advances in Biological Treatment of Lignocellulosic Materials*, pp. 253–268 [M. P. Coughlan and M. T. Collaco, editors]. London: Elsevier.

France, J. & Siddons, R. C. (1993). Volatile fatty acid production. In *Quantitative Aspects of Ruminant Digestion and Metabolism*, pp. 107–121 [J. M. Forbes and J. France, editors]. Wallingford: CAB International.

Franzolin, R. & Dehority, B. A. (1996). Effect of prolonged high-concentrate feeding on ruminal protozoa concentrations. *Journal of Animal Science* **74**, 2803–2809.

Gäbel, G. & Sehested, J. (1997). SCFA transport in the forestomach of ruminants. *Comparative Biochemistry and Physiology* **118A**, 367–374.

Galbraith, H. & Miller, T. B. (1973). Effects of metal cations and pH on the antibacterial activity and uptake of long-chain fatty acids. *Journal of Applied Microbiology* **36**, 635–646.

Galbraith, H., Miller, T. B., Paton, A. M. & Thompson, J. K. (1971). Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. *Journal of Applied Bacteriology* **34**, 803–813.

Garton, G. A. (1961). Influence of the rumen on the digestion and metabolism of lipids. In *Digestive Physiology and Nutrition of the Ruminant (University of Nottingham Easter School in Agricultural Science 7, 1960)*, pp. 140–153 [D. Lewis, editor]. London: Butterworths.

Garton, G. A. (1965). The digestion and assimilation of lipids. In *Physiology of Digestion in the Ruminant (International Symposium on Ruminant Physiology 2, 1964)*, pp. 390–398 [R. W. Dougherty, R. S. Allen, W. Burroughs, N. L. Jacobson and A. D. McGilliard, editors]. Washington: Butterworths.

Garton, G. A. (1977). Fatty acid metabolism in ruminants. In *Biochemistry of Lipids*, vol. 2, pp. 337–370 [T. W. Goodwin, editor]. Baltimore, MD: University Park Press.

Gilkes, N. R., Henrissat, B., Kilburn, D. G., Miller, R. C. & Warren, R. A. J. (1991). Domains in microbial β-1,4-glycanases: sequence conservation, function, and enzyme families. *Microbiological Reviews* **55**, 303–315.

Gordon, G. L. R. & Phillips, M. W. (1998). The role of anaerobic gut fungi in ruminants. *Nutrition Research Reviews* **11**, 133–168.

Grainger, R. B., Bell, M. C., Stroud, J. W. & Baker, F.H. (1961). Effect of various cations and corn oil on crude cellulose digestibility by sheep. *Journal of Animal Science* **20**, 319–322.

Greening, R. C. & Leedle, J. A. Z. (1989). Enrichment and isolation of *Acetitomaculum ruminis*, gen. nov., sp. nov.: acetogenic bacteria from the bovine rumen. *Archives of Microbiology* **151**, 399–406.

Gregg, K., Hamdorf, B., Henderson, K., Kopecny, J. & Wong, C. (1997). Genetically modified rumen bacteria protect sheep from fluoroacetate poisoning. In *Recent Advances in Nutrition in Australia*, pp. 63–67 [J. L. Corbett, M. Choct, J. V. Nolan and J. B. Rowe, editors]. Armidale, NSW: University of New England Publishing Unit.

Gregg, K. & Sharpe, H. (1991). Enhancement of rumen microbial detoxification by gene transfer. In *Physiological Aspects of Digestion and Metabolism in Ruminants (International Symposium on Ruminant Physiology 7, 1989)*, pp. 719–735 [T. Tsuda, Y. Sasaki and R. Kawashima, editors]. New York: Academic Press.

Hammond, A. C. (1995). Leucaena toxicosis and its control in ruminants. *Journal of Animal Science* **73**, 1487–1492.
Harfoot, C. G. & Hazlewood, G. P. (1988). Lipid metabolism in the rumen. In *The Rumen Microbial Ecosystem*, pp. 285–322 [P. N. Hobson, editor]. London: Elsevier Applied Science.
Hartley, R. D., Morrison, W. H., Borneman, W. S., Rigsby, L. L., O'Neill, M., Hanna, W. W. & Akin, D. E. (1992). Phenolic constituents of cell wall types of normal and brown midrib mutants of pearl millet (*Pennisetum glaucum*(L) R Br) in relation to wall biodegradability. *Journal of the Science of Food and Agriculture* **59**, 211–216.
Heald, P. J. (1952). The assessment of glucose-containing substances in rumen micro-organisms during a digestion cycle in sheep. *British Journal of Nutrition* **5**, 84–93.
Hegarty, M. P., Court, R. D., Christie, G. S. & Lee, C.P. (1976). Mimosine in *Leucaena leucocephala* is metabolised to a goitrogen in ruminants. *Australian Veterinary Journal* **52**, 490.
Henneberg, W. (1919). [The ruminal and gut flora of sheep.] *Berliner Klinische Wochenschrift* **56**, 693–694.
Henrissat, B. & Bairoch, A. (1993). New families in the classification of glycosyl hydrolases based on amino acid similarities. *Biochemical Journal* **293**, 781–788.
Hino, T. (1983). Influence of hydrogen on the fermentation in rumen protozoa, Entodinium species. *Japanese Journal of Zootechnical Science* **54**, 320–328.
Hobson, P. N. & Jouany, J.-P. (1988). Models, mathematical and biological, of the rumen function. In *The Rumen Microbial Ecosystem*, pp. 461–511 [P. N. Hobson, editor]. London: Elsevier Applied Science.
Hume, I.D. & Warner, A. C. I. (1980). Evolution of microbial digestion in mammals. In *Digestive Physiology and Metabolism in Ruminants (International Symposium on Ruminant Physiology 5, 1979)*, pp. 665–684 [Y. Ruckebush and P. Thivend, editors]. Lancaster: MTP Press.
Hungate, R. E. (1966). *The Rumen and its Microbes*. New York: Academic Press.
Hungate, R. E., Mah, R. A. & Simesen, M. (1961). Rates of production of individual volatile fatty acids in the rumen of lactating cows. *Applied Microbiology* **9**, 554–561.
Huntington, G. B. (1997). Starch utilization by ruminants: from basics to the bunk. *Journal of Animal Science* **75**, 852–867.
Iiyama, K., Lam, T. B. T. & Stone, B. A. (1994). Covalent cross-links in the cell wall. *Plant Physiology* **104**, 315–320.
James, L. F., Allison, M. J. & Littledike, E. T. (1975). Production and modification of toxic substances in the rumen. In *Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 4, 1974)*, pp. 576–590 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, NSW: University of New England Publishing Unit.
Jenkins, T. C. (1993). Lipid metabolism in the rumen. *Journal of Dairy Science* **76**, 3851–3863.
Johnson, D. E., Abo-Omar, J. S., Saa, C. F. & Carmean, B. R. (1994). Persistence of methane suppression by propionate enhancers in cattle diets. In *Energy Metabolism of Farm Animals (EAAP Publication* no. 76), pp. 339–342 [J.F. Aguilera, editor]. Granada, Spain: CSIC.
Jones, R. J. (1981). Does ruminal metabolism of mimosine explain the absence of *Leucaena* toxicity in Hawaii? *Australian Veterinary Journal* **57**, 55.
Jones, R. J. (1994). Management of anti-nutritive factors—with special reference to leucaena. In *Forage Tree Legumes in Tropical Agriculture*, pp. 216–231 [R. C. Gutteridge and H.M. Shelton, editors]. Wallingford: CAB International.
Jones, R. J. & Lowry, J. B. (1984). Australian goats detoxify the goitrogen 3-hydroxy-4(1H)pyridone (DHP) after rumen infusion from an Indonesian goat. *Experientia* **40**, 1433–1436.
Kaufmann, W. (1976). Influence of the composition of the ration and the feeding frequency on pH-regulation in the rumen. *Livestock Production Science* **3**, 103–114.
Kaufmann, W., Hagemeister, H. & Dirksen, G. (1980). Adaptation to changes in dietary composition, level and frequency of feeding. In *Digestive Physiology and Metabolism in Ruminants (International Symposium on Ruminant Physiology 5, 1979)*, pp. 587–602 [Y. Ruckebush and P. Thivend, editors]. Lancaster: MTP Press.
Kiessling, K-H., Pettersson, H., Sandholm, K. & Olsen, M. (1984). Metabolism of aflatoxin, ochratoxin, zearalenone and three trichothecenes by intact rumen fluid, rumen protozoa and rumen bacteria. *Applied and Environmental Microbiology* **47**, 1070–1073.
Kirchgessner, M., Windisch, W. & Muller, H. L. (1995). Nutritional factors for the quantification of methane production. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, pp. 333–345 [W. V. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke, editors]. Stuttgart: Ferdinand Enke Verlag.
Klieve, A. V. & Bauchop, T. (1988). Morphological diversity of ruminal bacteriophages from sheep and cattle. *Applied and Environmental Microbiology* **54**, 1637–1641.
Klieve, A. V. & Swain, S. A. (1993). Estimation of ruminal bacteriophage numbers by pulsed-field gel electrophoresis and laser densitometry. *Applied and Environmental Microbiology* **59**, 2299–2303.
Klieve, A. V., Swain, R. A. & Nolan, J. V. (1996). Bacteriophages in the rumen; types present, population size and implications for the efficiency of feed utilization. *Proceedings of the Australian Society of Animal Production* **21**, 92–94.
Klusmeyer, T. H. & Clark, J. H. (1991). Effects of dietary fat and protein on fatty acid flow to the duodenum and in milk produced by dairy cows. *Journal of Dairy Science* **74**, 3055.
Krause, D. O. & Russell, J. B. (1996). How many ruminal bacteria are there? *Journal of Dairy Science* **79**, 1467–1475.
Lam, T. B.-T., Iiyama, K. & Stone, B. A. (1990). Primary and secondary walls of grasses and other forage plants: taxonomic and structural considerations. In *Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants*, pp. 43–69 [D. E. Akin, L. G. Ljungdahl, J. R. Wilson and P. J. Harris, editors]. New York: Elsevier.

Lebzien, P., Giesecke, D., Wiesmayr, S. & Rohr, K. (1993). [Measurement of microbial protein synthesis in the rumen of cows by determinins ^{15}N in duodenal digesta and isolating allantoin from milk. *Journal of Animal Physiology and Animal Nutrition* **70**, 82–88.

Leng, R. A. (1970). Formation and production of volatile fatty acids in the rumen. In *Physiology of Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 3, 1969)*, pp. 406–421 [A.T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.

Lewis, D. (1951). The metabolism of nitrate and nitrite in the sheep. I. The reduction of nitrate in the rumen of the sheep. *Biochemical Journal* **48**, 175–180.

Lindsay, D. B. (1970). Carbohydrate metabolism in ruminants. In *Physiology of Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 3, 1969)*, pp. 438–451 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.

Ling, J. R. & Armstead, I.P. (1995). The *in vitro* uptake and metabolism of peptides and amino acids by five species of rumen bacteria. *Journal of Applied Bacteriology* **78**, 116–124.

Lough, A. K. (1970). Aspects of lipid digestion in the ruminant. In *Physiology of Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 3, 1969)*, pp. 519–528 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.

Low, S. G., Jephcott, S. B. & Bryden, W. L. (1994). Weaner illthrift of cattle grazing signal grass (Brachiaria decumbens) in Papua New Guinea. In *Plant Associated Toxins*, pp. 567–571 [S. M. Colegate and P. R. Darling, editors] Wallingford, UK: CAB International.

MacRae, J. C. (1975). The use of re-entrant cannulae to partition digestion function within the gastro-intestinal tract of ruminants. In *Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 4, 1974)*, pp. 261–276 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, NSW: University of New England Publishing Unit.

Maczulac, A. E., Dehority, B. A. & Palmquist, D. L. (1981). Effects of long-chain fatty acids on growth of rumen bacteria. *Applied and Environmental Physiology* **42**, 856–862.

Maeng, W. J. & Baldwin, R. L. (1976). Factors influencing rumen microbial growth rates and yields: effect of amino acid additions to a purified diet with nitrogen from urea. *Journal of Dairy Science* **59**, 648–655.

Mangan, J. L. (1972). Quantitative studies on nitrogen metabolism in the bovine rumen. The rate of proteolysis of casein and ovalbumin and the release and metabolism of free amino acids. *British Journal of Nutrition* **27**, 261–283.

Mangan, J. L. (1988). Nutritional effects of tannins in animal feeds. *Nutrition Research Reviews* **1**, 209–231.

Martin, S. A. (1994). Nutrient transport by ruminal bacteria: a review. *Journal of Animal Science* **72**, 3019–3031.

Martin, S. A. & Nisbet, D. J. (1992). Effect of direct-fed microbials on rumen microbial fermentation. *Journal of Dairy Science* **75**, 1736–1744.

Mathison, G. W. & Milligan, L. P. (1971). Nitrogen metabolism in sheep. *British Journal of Nutrition* **25**, 351–366.

McAllister, J. A., Bae, H. D., Jones, G. A. & Cheng, K. J. (1994). Microbial attachment and feed digestion in the rumen. *Journal of Animal Science* **72**, 3004–3018.

McDonald, I. W. (1948). The absorption of ammonia from the rumen of the sheep. *Biochemical Journal* **42**, 584–587.

McDonald, I. W. (1952). The role of ammonia in ruminal digestion of protein. *Biochemical Journal* **51**, 86–90.

McDonald, I. W. (1954). The extent of conversion of food protein to microbial protein in the rumen of the sheep. *Biochemical Journal* **56**, 120–125.

McSweeny, C. S., Allison, M. J. & Mackie, R. I. (1993). Amino acid utilization by the ruminal bacterium *Synergistes jonesii* strain 78–1. *Archives of Microbiology* **159**, 131–135.

McSweeney, C. S., Mackie, R. I. & White, B. A. (1994). Transport and intracellular metabolism of major feed compounds by ruminal bacteria: the potential for metabolic manipulation. *Australian Journal of Agricultural Research* **45**, 731–756.

Mehansho, H., Butler, L. G. & Carlson, D. M. (1987). Dietary tannins and salivary proline-rich proteins: interactions, induction, and defense mechanisms. *Annual Review of Nutrition* **7**, 423–440.

Mehrez, A. Z. & Ørskov, E. R. (1977). A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. *Journal of Agricultural Science* **88**, 645–650.

Miles, C. O., Wilkins, A. L., Erasmus, G. L. & Kellerman, T. S. (1994*a*). Photosensitivity in South Africa. VIII. Ovine metabolism of *Tribulus terrestris* saponins during experimentally induced geeldikkop. *Onderstepoort Journal of Veterinary Research* **61**, 351–359.

Miles, C. O., Wilkins, A. L., Erasmus, G. L., Kellerman, T. S. & Coetzer, J. A. W. (1994*b*). Photosensitivity in South Africa. VII. Chemical composition of biliary crystals from a sheep with experimentally induced geeldikkop. *Onderstepoort Journal of Veterinary Research* **61**, 215–222.

Miller, T. L. (1995). Ecology of methane production and hydrogen sinks in the rumen. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, pp. 317–329 [W. V. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke, editors]. Stuttgart: Ferdinand Enke Verlag.

Moir, R. J. (1968). Ruminant digestion and evolution. In *Handbook of Physiology, Section 6: Alimentary canal*, vol. V. *Bile, Digestion; Ruminal Physiology*, pp. 2673–2694 [C.F. Code, editor]. Washington: American Physiological Society.

Morant, S. V., Ridley, J. L. & Sutton, J. D. (1978). A model for the estimation of volatile fatty acid production in the rumen in non-steady-state conditions. *British Journal of Nutrition* **39**, 451–462.

Morrison, M. (1996). Do ruminal bacteria exchange genetic material. *Journal of Dairy Science* **79**, 1476–1486.

Mountford, D. O. & Asher, R. A. (1985). Production and regulation of cellulase by two strains of the rumen anaerobic fungus *Neocallimastix frontalis*. *Applied and Environmental Microbiology* **49**, 1314–1322.

Nagaraja, T. G. & Chengappa, M. M. (1998). Liver abscesses in feedlot cattle. A review. *Journal of Animal Science* **76**, 287–298.

Nolan, J. V. (1975). Quantitative models of nitrogen metabolism in sheep. In *Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 4, 1974)*, pp. 416–431 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, NSW: University of New England Publishing Unit.

Nolan, J. V. & Leng, R. A. (1972). Dynamic aspects of ammonia and urea metabolism in sheep. *British Journal of Nutrition* **27**, 177–194.

Norton, B. W., Mackintosh, J. B. & Armstrong, D. G. (1982). Urea synthesis and degradation in sheep given pelleted-grass diets containing flaked barley. *British Journal of Nutrition* **48**, 249–264.

Obara, Y., Dellow, D. W. & Nolan, J. V. (1991). The influence of energy-rich supplements on nitrogen kinetics in ruminants. In *Physiological Aspects of Digestion and Metabolism in Ruminants (International Symposium on Ruminant Physiology 7, 1989)*, pp. 515–539 [T. Tsuda, Y. Sasaki and R. Kawashima, editors]. New York: Academic Press.

Ohajuruka, O. A., Wu, Z. G. & Palmquist, D. L. (1991). Ruminal metabolism, fiber and protein digestion by lactating cows fed calcium soap or animal-vegetable fat. *Journal of Dairy Science* **74**, 2601–2609.

Orpin, C. G. (1975). Studies on the rumen flagellate *Neocallimastix frontalis*. *Journal of General Microbiology* **91**, 249–262.

Ørskov, E. R. (1986). Starch digestion and utilization in ruminants. *Journal of Animal Science* **63**, 1624–1633.

Ørskov, E. R. & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science* **92**, 499–503.

Owens, F. N. & Goetsch, A. L. (1986). Digesta passage and microbial protein synthesis. In *Control of Digestion and Metabolism in Ruminants (International Symposium on Ruminant Physiology 6, 1984)*, pp. 196–223 [L. P. Milligan, W.L. Grovum and A. Dobson, editors]. Englewood Cliffs, NJ: Prentice-Hall.

Owens, F. N., Secrist, D. S., Hill, W. J. & Gill, D. R. (1998). Acidosis in cattle: A review. *Journal of Animal Science* **76**, 275–286.

Palmquist, D. L. (1988). The feeding value of fats. In *Feed Science*, pp. 293–311 [E. R. Ørskov, editor]. Amsterdam: Elsevier.

Peters, R. A., Wakelin, R. W., Buffa, P. & Thomas, L. C. (1953). Biochemistry of fluoroacetate poisoning. The isolation and some properties of the fluorotricarboxylic acid inhibitor of citrate metabolism. *Proceedings of the Royal Society, B* **140**, 497–507.

Rasmussen, M. A. & Anderson, R. C. (1998). Dissimilatory metabolism by ruminal microbes: impact on ruminant toxicoses. In *Toxic Plants and Other Natural Toxicants*, pp. 73–77 [T. Garland and A. C. Barr, editors]. Wallingford: CAB International.

Rechkemmer, G., Gäbel, Diernaes, L., Sehested, J., Møller, P. D. & von Engelhardt, W. (1995). Transport of short chain fatty acids in the forestomach and hindgut. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, pp.95–116 [W. V. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke, editors]. Stuttgart: Ferdinand Enke Verlag.

Reed, J. D. (1995). Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science* **73**, 1516–1528.

Reiser, R. (1951). Hydrogenation of polyunsaturated fatty acids by the ruminant. *Federation Proceedings* **10**, 236.

Ritchie, A. E. I., Robinson, I. M. & Allison, M. J. (1970). Rumen bacteriophage: survey of morphological types. In *Microscopie Electronique*, pp. 333–334 [P. Favard, editor]. Paris: Société Française de Microscopie Electronique.

Robinson, P. H., Fadel, J. G. & Ivan, M. (1996). Critical evaluation of diaminopimelic acid and ribonucleic acid as markers to estimate rumen pools and duodenal flows of bacterial and protozoal nitrogen. *Canadian Journal of Animal Science* **76**, 587–597.

Rowe, J. B., Brown, G., Ralph, I. G., Ferguson, J. & Wallace, J. F. (1989). Supplementary feeding of young Merino sheep, grazing wheat stubble, with different amounts of lupin, oat or barley grain. *Australian Journal of Experimental Agriculture* **29**, 29–35.

Rowe, J. B. & Pethick, D. W. (1994). Starch digestion in ruminants—problems, solutions and opportunities. *Proceedings of the Nutrition Society of Australia* **18**, 40–52.

Russell, J. B. & Chow, J. M. (1993). Another theory for the action of ruminal buffer salts: decreased starch fermentation and propionate production. *Journal of Dairy Science* **76**, 826–830.

Russell, J. B., Onodera, R. & Hino, T. (1991). Ruminal protein fermentation: new perspectives on previous contradictions. In *Physiological Aspects of Digestion and Metabolism in Ruminants (International Symposium on Ruminant Physiology 7, 1989)*, pp. 681–697 [T. Tsuda, Y. Sasaki and R. Kawashima, editors]. New York: Academic Press.

Russell, J. B., Strobel, H. J. & Martin, S. A. (1990). Strategies of nutrient transport by ruminal bacteria. *Journal of Dairy Science* **73**, 2996–3012.

Russell, J. B. & Wilson, D. B. (1988). Potential opportunities and problems for genetically altered rumen microorganisms. *Journal of Nutrition* **118**, 271–279.

Russell, J. B. & Wilson, D. B. (1996). Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH. *Journal of Dairy Science* **79**, 1503–1509.

Sauvant, D. & van Milgen, J. (1995). Dynamic aspects of carbohydrate and protein breakdown and the associated microbial matter synthesis. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, pp. 71–91 [W. V. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke, editors]. Stuttgart: Ferdinand Enke Verlag.

Scott, T. W., Cook, L. J., Ferguson, K. A., McDonald, I. W., Buchanan, R. A. & Loftus Hills, G. (1970). Production of poly-unsaturated milk fat in domestic ruminants. *Australian Journal of Science* **32**, 291–293.

Scott, T. W., Cook, L. J. & Mills, S. C. (1971). Protection of dietary polyunsaturated fatty acids against microbial hydrogenation in ruminants. *Journal of the American Oil Chemists' Society* **48**, 358–364.

Sehested, J., Basse, A., Andersen, J. B., Diernæs, L., Møller, P. D., Skadhauge, E. & Aaes, O. (1997). Feed-induced changes in transport across the rumen epithelium. *Comparative Biochemistry and Physiology* **118A**, 385–386.

Shorland, F. B., Weenink, R. O., Johns, A. T. & McDonald, I. R. C. (1957). The effect of sheep-rumen contents on unsaturated fatty acids. *Biochemical Journal* **67**, 328–333.

Smith, R. H. (1975). Nitrogen metabolism in the rumen and the composition and nutritive value of nitrogen compounds entering the duodenum. In *Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 4, 1974)*, pp. 399–415 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, NSW: University of New England Publishing Unit.

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G. & Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. *Journal of Animal Science* **70**, 3562–3577.

Stangassinger, M., Chen, X. B., Lindbergh, J. E. & Giesecke, D. (1995). Metabolism of purines in relation to microbial production. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, pp. 387–408 [W. V. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke, editors]. Stuttgart: Ferdinand Enke Verlag.

Stevens, C. E. (1970). Fatty acid transport through the rumen epithelium. In *Physiology of Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 3, 1969)*, pp. 101–112 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.

Stewart, C. S., Fèvre, M. & Prins, R. A. (1995). Factors affecting fermentation and polymer degradation by anaerobic fungi and the potential for manipulation of rumen function. In *Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction*, pp. 251–270 [W. V. Engelhardt, S. Leonhard-Marek, G. Breves and D. Giesecke, editors]. Stuttgart: Ferdinand Enke Verlag.

Stumm, C. K., Gijzen, H. J. & Vogels, G. D. (1982). Association of methanogenic bacteria with ovine rumen ciliates. *British Journal of Nutrition* **47**, 95–99.

Susmel, P. & Stefanon, B. (1993). Aspects of lignin degradation by rumen microorganisms. *Journal of Biotechnology* **30**, 141–148.

Sutton, J. D. (1985). Digestion and absorption of energy substrates in the lactating cow. *Journal of Dairy Science* **68**, 3376–3393.

Swain, R. A., Nolan, J. V. & Klieve, A. V. (1996). Natural variability and diurnal fluctuations within the bacteriophage population of the rumen. *Applied and Environmental Microbiology* **62**, 994–997.

Teather, R. M. (1985). Application of gene manipulation to rumen microflora. *Canadian Journal of Animal Science* **65**, 563–574.

Theodorou, M. K. & France, J. (1993). Rumen microorganisms and their interactions. In *Quantitative Aspects of Ruminant Digestion and Metabolism*, pp. 145–163 [J. M. Forbes and J. France, editors]. Wallingford: CAB International.

Theodorou, M. K., Zhu, W. Y., Rickers, A., Nielsen, B. B., Gull, K. & Trinci, A. P. J. (1996). Biochemistry and ecology of anaerobic fungi. In *The Mycota. VI. Human and Animal Relationships*, pp. 265–295 [D.H. Howard and J.D. Miller, editors]. Berlin: Springer Verlag.

Thorniley, G. R., Boyce, M. D. & Rowe, J. B. (1994). Dose of virginiamycin required to control lactic acid accumulation in rumen and caecal digesta. *Proceedings of the Australian Society of Animal Production* **20**, 448.

Trinci, A. P. J., Davies, D. R., Gull, K., Lawrence, M. I., Nielsen, B. B., Rickers, A. & Theodorou, M. K. (1994). Anaerobic fungi in herbivorous animals. *Mycological Research* **98**, 129–152.

Ushida, K., Jouany, J. P. & Demeyer, D. I. (1991). Effects of presence or absence of rumen protozoa on the efficiency of utilization of concentrate and fibrous feeds. In *Physiological Aspects of Digestion and Metabolism in Ruminants (International Symposium on Ruminant Physiology 7, 1989)*, pp. 625–654 [T. Tsuda, Y. Sasaki and R. Kawashima, editors]. New York: Academic Press.

Ushida, K., Umeda, M., Kishigami, N. & Kojima, Y. (1992). Effect of medium chain and long chain fatty acid calcium salts on rumen microorganisms and fibre digestion in sheep. *Animal Science and Technology (Japan)* **63**, 591–597.

Virtanen, A. I. (1966). Milk production of cows on protein free diets. *Science* **153**, 1603–1604.

Walker, D.J. (1965). Energy metabolism and rumen microorganisms. In *Physiology of Digestion in the Ruminant (International Symposium on Ruminant Physiology 2, 1964)*, pp. 296–310 [R. W. Dougherty, R. S. Allen, W. Burroughs, N. L. Jacobson and A. D. McGilliard, editors]. Washington: Butterworths.

Wallace, R. J. (1994). Ruminal microbiology, biotechnology and ruminant nutrition: progress and problems. *Journal of Animal Science* **72**, 2992–3003.

Wallace, R. J. (1996). Ruminal microbial metabolism of peptides and amino acids. *Journal of Nutrition* **126**, 1326S–1334S.

Wallace, R. J. (1997). Rumen microbiology and efficency of digestion: opportunities and impact of biotechnology. In *Milk Composition, Production and Biotechnology*, pp. 465–487 [R. A. S. Welch, D. J. W. Burns, S. R. Davis, A. I. Popay and C. G. Prosser, editors]. Wallingford: CAB International.

Wallace, R. J. & Cotta, M. A. (1988). Metabolism of nitrogen-containing compounds. In *The Rumen Microbial Ecosystem*, pp. 217–249 [P. N. Hobson, editor]. London: Elsevier Applied Science.

Westlake, K., Mackie, R. I. & Dutton, M. F. (1987*a*). T-2 toxin metabolism by ruminal bacteria and its effect on their growth. *Applied and Environmental Microbiology* **53**, 587–592.

Westlake, K., Mackie, R. I. & Dutton, M. F. (1987*b*). Effects of several mycotoxins on specific growth rate of *Butyrivibrio fibrisolvens* and toxin degradation *in vitro*. *Applied and Environmental Microbiology* **53**, 613–614.

Westlake, K., Mackie, R. I. & Dutton, M. F. (1989). *In vitro* metabolism of mycotoxins by bacterial, protozoal and ovine ruminal fluid preparations. *Animal Feed Science and Technology* **25**, 169–178.

Weston, R. H. & Hogan, J. P. (1968). The digestion of pasture plants by sheep. I. Ruminal production of volatile fatty acids by sheep offered diets of ryegrass and forage oats. *Australian Journal of Agricultural Research* **19**, 419–432.

Williams, A. G., Withers, S. E. & Joblin, K. N. (1991). Xylanolysis by cocultures of the rumen fungus *Neocallimastix frontalis* and ruminal bacteria. *Letters in Applied Microbiology* **12**, 232–235.

Williams, P. E. V., Walker, A. & MacRae, J. C. (1990). Rumen probiosis: the effects of addition of yeast culture (viable yeast (*Saccharomyces cerevisiae*) plus growth medium) on duodenal protein flow in wether sheep. *Proceedings of the Nutrition Society* **49**, 128A.

Wolfe, R. S. (1971). Microbial formation of methane. *Advances in Microbial Physiology* **6**, 107–146.

Wolin, M. J. (1975). Interactions between the bacterial species of the rumen. In *Digestion and Metabolism in the Ruminant (International Symposium on Ruminant Physiology 4, 1974)*, pp. 135–148 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, NSW: University of New England Publishing Unit.

Wood, T. M., Wilson, C. A., McCrae, S. I. & Joblin, K. N. (1986). A highly active extracellular cellulase from the anaerobic rumen fungus *Neocallimastix frontalis*. *FEMS Microbiology Letters* **34**, 37–40.

Xiao, H., Marquardt, R. R., Frohlich, A. A., Phillips, G. D. & Vitti, T. G. (1991*a*). Effect of a hay and a grain diet on the rate of hydrolysis of ochratoxin A in the rumen of sheep. *Journal of Animal Science* **69**, 3706–3714.

Xiao, H., Marquardt, R. R., Frohlich, A. A., Phillips, G. D. & Vitti, T. G. (1991*b*). Effect of a hay and a grain diet on the bioavailability of ochratoxin A in the rumen of sheep. *Journal of Animal Science* **69**, 3715–3723.