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Review article

Microbial protein supply from the rumen☆

R.J. Dewhurst*, D.R. Davies, R.J. Merry

Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK

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Abstract

This paper considers the theory, assessment and prediction of microbial protein synthesis in the rumen. The difficulties of techniques for assessing microbial protein synthesis, as well as the complexity of the rumen ecosystem, have limited progress. Inconsistencies in the literature undermine the value of incorporating advanced rumen models into rationing schemes and limit the exploitation of microbial protein as an important protein resource for ruminants. The paper gives examples of situations in which particular factors have significant effects on microbial protein synthesis, but moves on to discuss the development of new less-invasive approaches for estimating microbial protein synthesis. The latter approaches have the attraction of offering in-built technology transfer through the development of diagnostic tests, based on samples of milk or urine. Some of these techniques offer a description of rumen function that is less rigorously quantitative (in terms of microbial protein synthesis), but more usefully qualitative (in terms of microbial populations, substrates and interactions).

Keywords: Rumen; Microbial composition; Microbial synthesis; Odd-chain fatty acids; Purine derivatives

1. Introduction

The microbial processes of the rumen confer the ability to convert fibrous feeds and low-quality protein, even non-protein-nitrogen, into valuable nutrients for the ruminant animal. Other papers in this Symposium consider the use of alternative crops (Wilkins and Jones, 2000) or concentrates (Beerman et al., 2000) as protein resources for ruminants; both of these approaches can introduce new costs and risks to systems. Over

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* Corresponding author. Tel.: +44-0-1970-823072; fax: +44-0-1970-828357.
E-mail address: richard.dewhurst@bbsrc.ac.uk (R.J. Dewhurst)

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half of the amino acids absorbed by ruminants, and often two-thirds to three-quarters, derive from microbial protein (Agricultural and Food Research Council, 1992), so that microbial protein must be considered as an important protein resource. The *metabolisable protein* supply from microbial protein is similar to that from undegraded dietary protein from grass silage (64%; Agricultural and Food Research Council, 1992) and rumen microbes have a variable, but generally good amino acid profile (Storm and Ørskov, 1983; Clark et al., 1992).

Whilst the rumen presents advantages, particularly when animals are offered low-quality feeds, it can be a major cause of inefficiency of nitrogen utilisation in ruminants. For dairy cows offered a standard level and type of concentrates, the efficiency of conversion of feed-N to milk-N ranged from 23 to 32% across the 16 grass silages used by Dewhurst et al., 1996. When legume silages, with higher N content, were offered to dairy cows even lower efficiencies were measured (18% for lucerne silage; Dewhurst et al., 2000).

It is a basic premise of our approach that uncertainty about how the rumen is performing in a given situation is a major cause of inefficient use of dietary protein. It is essential to predict or assess how much microbial protein is produced in order both to correct any problems and to make the best use both of forage nitrogen and the more expensive protein sources.

Sustained research efforts over 30 years has sought to estimate microbial protein synthesis (MPS) in a wide range of feeding situations and to produce models for its prediction. This information has traditionally been used in rationing models and software that predict MPS as the basis for balancing rations with other protein sources. The usual link between the research efforts and commercial practice has been models of MPS, though invariably the variation explained by these models is disappointingly low (Agricultural Research Council, 1980, 1984; Agricultural and Food Research Council, 1992).

Whilst this paper will review the approach, its application will be limited without new understanding, particularly about the responses of microbial populations to certain combinations of dietary energy, protein and other co-factors. The current position on factors influencing microbial protein supply will be explored and the latter part of the paper will focus on more recent and potentially powerful approaches to assessing rumen function. Real-time monitoring of volatile components of breath, measurement of microbially-derived compounds in milk and other non-invasive techniques could offer a new approach to applying work on-farm, by spawning diagnostic tests.

2. Microbial protein synthesis: theoretical basis

The theory and mathematics describing microbial growth has been reviewed on a number of occasions (Stouthamer, 1969; Tempest and Neijssel, 1984; Russell and Wallace, 1997). Pirt (1975) defined the requirements for microbial growth in laboratory culture as a viable inoculum, an energy source, nutrients to provide the essential materials for growth, the absence of growth-preventing inhibitors, and suitable physico-chemical conditions.

Many studies have assessed microbial growth, using both pure and mixed cultures of rumen microbes (Russell and Baldwin, 1978, 1979; Hespell and Bryant, 1979; Cotta and Russell, 1982; Bates et al., 1985; Griswold et al., 1996). Russell and Baldwin (1978) studied the doubling times of a number of different rumen bacteria grown on a range of pure substrates. For example, with glucose as the substrate, doubling times ranged from 0.34 h for *Streptococcus bovis* to 1.78 h for *Butyrivibrio fibrisolvens*.

The microbial growth equation of Pirt (1965) gives a scientific basis to two of the clearer influences on MPS. The yield of microbial biomass is related to the amount of substrate available and the energy used for maintenance which, in turn, is a function of the maintenance requirement and the growth rate (or dilution rate). Energy within cells is used for either growth or maintenance and maintenance energy can be defined as the energy required to maintain cells in a live state. Harmeyer (1986) listed motility, cellular turnover, production of extracellular molecules, active transport, inefficient phosphorylation, uncoupling and lysis of cells as important maintenance costs of bacteria. The theoretical basis of maintenance first became apparent with the advent of laboratory techniques that enabled continuous cultures of micro-organisms to be grown, with growth rates carefully controlled by substrate supply and dilution rate. The maintenance energy requirement is dependent on the bacterial growth rate with the slower growth rates requiring proportionally more maintenance energy than faster growth rates, consequently cell yields are lower at slower growth rates. Maintenance energy has been described mathematically by a number of researchers (Marr et al., 1962; Pirt, 1965; Tempest and Neijssel, 1984). Most equations assume that maintenance is independent of growth rate, though Djavan and James (1980) apportioned microbial maintenance into growth-rate dependent and independent costs.

Having discussed the theoretical values for microbial cell yields in laboratory cultures, it is interesting to discuss the relevance of these factors in the rumen. The reason for doing this was succinctly pointed out by Russell and Hespell (1981), who noted that *Streptococcus bovis* is capable of doubling every 14 min. Since one cell would completely fill a 60-l rumen in <14 h and would equal the mass of the earth in ≈34 h, it is obvious that other factors must limit the growth of microbes in the rumen. In laboratory culture, the feed source is supplied to exactly match microbial requirements and environmental conditions are kept close to the optimum for growth. These are easy to achieve in a well-mixed homogenous culture in the laboratory, but in the rumen with semi-continuous supply of heterogeneous feed and saliva this is not possible. If we consider just one component of the diet namely nitrogen then, depending on the form of nitrogen, large differences in growth rate and, consequently, cell yield can occur. The ruminant animal is unique in its ability to survive on a diet consisting entirely of non-protein nitrogen. However, the efficiency of microbial growth is enhanced by both the addition of amino acids and peptides to their growth medium (Maeng et al., 1975; Cotta and Russell, 1982; Griswold et al., 1996). By manipulating the form of the supply of nitrogen under laboratory conditions, it is possible to alter microbial growth rates. However, when these manipulations are attempted in vivo, their effects can be masked by other factors, particularly those relating to interactions between the >200 species of rumen micro-organism. Most notably, protozoa have a crucial role in the turnover of microbial biomass. In a sheep's rumen, between 2.4 and 45 g of bacteria are digested by

protozoa per day (Russell and Hespell, 1981), leading to a direct decline in the apparent efficiency of microbial growth.

3. Problems of estimating microbial protein synthesis in vitro and in vivo

The literature concerning MPS has been reviewed on many occasions and yields few totally reliable predictors of MPS or microbial efficiency (EMPS; Agricultural Research Council, 1984; Broderick and Merchen, 1992; Firkins, 1996). This is both because of the complexity of the rumen system and the technical difficulties of estimating MPS, particularly in vivo. There are two distinct problems, namely estimating how much material leaves the rumen and then assessing what proportion of that material is microbial protein. These estimates have entailed the use of fistulated cows which has tended to add to these problems, through low replication. Titgemeyer (1997) reviewed the literature and showed that, on average, treatment groups of 12 animals are required in order to identify treatment differences of 10% in EMPS.

It has proved very difficult to design gut cannulae that minimise disturbance to normal function and which produce representative samples. Whilst the use of dual-phase markers (Faichney, 1975) theoretically allows for correction of unrepresentative sampling, they introduce additional complications and potential sources of error including the problem of markers migrating after sampling. These marker systems have been used with automated sampling of digesta from simple T-piece cannulae (Evans et al., 1981) or sampling of digesta through a rumen cannula and the reticulo-omasal orifice (Huhtanen et al., 1997). Recently, there has been a return to use of total diversion cannulae because of interest in the pattern of nutrient flows within the day (Gill et al., 1999).

The problems of making accurate estimates of microbial synthesis in vitro are similar to those in vivo. However, in vitro systems have the advantage that representative sampling is assured and the outflow of both particulate and liquid phases can be accurately controlled (Stern et al., 1978; Merry et al., 1987). The latter point, however, is not true of batch culture systems and a criticism of both batch and continuous culture models is that compared to the rumen where absorption occurs, substrate levels must be reduced and/or outflow rates raised to maintain physiological conditions. In vitro systems can be used to test hypotheses and examine mechanisms, but in vivo studies are essential to confirm results.

A wide range of approaches have been used to identify microbial protein in rumen contents (both in vitro and in vivo) and in digesta flowing at the abomasum, omasum or duodenum, though all have limitations. Early work used protein-free diets and this still can provide a useful base-line against which to assess other markers (Arambel et al., 1987). Other early studies tried to distinguish feed and microbial protein on the basis of their amino acid content: the absence of lysine in zein (McDonald, 1954); amino acid profiles (Offer et al., 1978) or unusual amino acids (D-alanine; Garrett et al., 1982). Endogenous or exogenous markers to label microbial material, including: ^{35}S (Roberts and Miller, 1969), ^{15}N (Brandt et al., 1980), ^{3}H-leucine (Bruce et al., 1985; Sinclair et al., 1993); ^{32}P (Smith et al., 1978), diaminopimelic acid (DAPA; Hutton et al., 1971), RNA (Smith and McAllan, 1970) and purine and pyrimidine bases (Jackson et al., 1977; Zinn

and Owens, 1986) have been most popular. Each of these markers has its own problems, including safety (radioisotopes), cost (^{15}N and amino acid profiles), difficulty of analysis (RNA and DNA; McAllan and Smith, 1969) and presence within feeds, thereby lacking specificity to the microbial fraction (DAPA; Dufva et al., 1982; nucleic acids and their bases; McAllan, 1982). More recently, Lebzien and Paul (1997) successfully used near-infrared reflectance spectroscopy as a quick approach to distinguish the microbial content of duodenal digesta.

The term microbial protein can be misleading since estimates are generally made using marker ratios solely for bacteria that were isolated from rumen contents. Thus, they are really an estimate of 'bacterial' protein and other microbes contributing to protein flow are not included, except, to a degree, where marker may have passed via bacteria through another microbial pool. An exception is where AEP (amino ethyl phosphonic acid) was used to mark the microbial fraction leaving the rumen, in combination with a bacterial marker (Hutton et al., 1971). It has, however, been argued that protozoa are largely sequestered in the rumen of animals fed high fibre diets and may not make a major direct contribution to microbial protein flow to the small intestine (Weller and Pilgrim, 1974; Coleman et al., 1980). The other major microbial fraction leaving the rumen is the anaerobic fungi, but to our knowledge no estimates have been made of their contribution and this will vary according to the biomass on a particular diet, although this may be up to 8% (Orpin, 1983/84). Work carried out with nucleic acids and their component bases reveals one particular problem, which is variation in marker content in bacteria. A wide range of values have been reported for the ratio purine-N:Total-N in experiments conducted both in vivo and in vitro, and most are in the range 0.08 to 0.12 (Dewhurst, 1989). Early work by Smith and McAllan (1974) indicated that ruminant species and time of sampling after feeding altered the RNA-N:Total-N ratio. More recent studies have confirmed these findings (Bates et al., 1985; Craig et al., 1997). The former authors found that RNA:protein ratios in pure cultures of rumen bacteria increased with rise in their specific growth rates and in mixed bacteria harvested from sheep fed a high concentrate diet the ratio was highest in 'free-floating' (rapidly growing) bacteria. It is this 'free floating' or liquid-associated bacterial fraction that is most often harvested in order to establish the marker/N ratios for calculating the amount of microbial protein in a digesta sample.

A number of studies have now demonstrated large differences in the composition of rumen microbes according to whether they were isolated from rumen liquids (liquid-associated bacteria; LAB) or solids (solid-associated bacteria; SAB). The stimulus for this work was the potential for inaccurately calculating microbial synthesis if marker/N ratios were derived from non-representative samples of rumen bacteria. The content of RNA and purine bases and DAPA (usually expressed in relation to N content) tends to be greater in the LAB (Merry and McAllan, 1983; Legay-Carmier and Bauchart, 1989; Volden and Harstad, 1998). Some authors have also reported differences in the enrichment of isotopic markers in LAB and SAB (Merry and McAllan, 1983; Martin et al., 1994). Since our current research interest is in the use of microbially derived fatty acid in milk as indices of rumen function, it is useful to consider variation in microbial lipids. Differences are very apparent when considering the total lipid content of bacteria, with a consistent trend towards higher values for SAB: 12.4 (LAB) vs. 24.5% DM (SAB;

Merry and McAllan, 1983); 16 vs. 21% OM (1 h after feeding) Craig et al., 1997; 9.9 vs. 22.0 DM (Legay-Carmier and Bauchart, 1989); 11.2 vs. 22.7% DM (Bauchart et al., 1990); 10.4 vs. 15.7 DM (O'Kelly and Spiers, 1990); and 17.5 vs. 27.7% DM (Volden and Harstad, 1998).

It is relatively easy to isolate LAB and the composition of bacteria that are isolated directly from the liquid phase is quite similar to that obtained by gentle washing of the solids (Legay-Carmier and Bauchart, 1989). However, the problem of variation in bacterial composition as described above is further accentuated by an inability to isolate more than a proportion of bacteria associated with rumen solids using a range of approaches including detergents, chilling and stomaching (Merry and McAllan, 1983). Lack of knowledge about the relative contributions of different classes of microbe to rumen efflux is a further complication. Nevertheless, analytical developments mean that it is becoming possible to distinguish different classes of microbes. This identification might be on the basis of, for example, distinctive fatty acid profiles (SAB vs. LAB; Lee et al., 1999; see below) or discrimination of stable isotopes in certain enzymatic pathways (Wattiaux and Reed, 1995).

Molecular technologies have received much interest over the last decade and the genetics of rumen bacteria has recently been reviewed (Teather et al., 1997). The bulk of the research has been on the potential for genetically manipulating rumen bacteria and on the use of DNA probes for studies of molecular ecology. This has highlighted the problems of current culture techniques in the isolation of the entire range of microbes present in the rumen. These technologies have also been used in better methods for the phylogenetic classification of bacteria and fungi (Brownlee, 1994; Maidak et al., 1994). Molecular techniques that enable a quantitative assessment of microbial biomass and, hence, rumen microbial protein outflow, are still a distant dream. Problems exist in that there are a large number of rumen microbial species that currently do not have DNA probes and further developments are required to make current techniques robustly quantitative. However, the biggest advance required before these techniques could make an impact is the need for the coming together of minds between ruminant nutritionists and the molecular biologists.

4. Dietary effects on microbial protein synthesis

The literature concerning microbial protein synthesis (MPS) is confusing and often contradictory, both as a result of the complex factors involved as well as the difficulties of measuring it. There are a number of treatments that have shown clear effects that can be related to components of the Pirt model, and these will be discussed before dealing with more general dietary effects. Maintenance costs can be reduced by defaunation, leading to increased EMPS (Meyer et al., 1986). Feeding of free oils does not contribute energy to the microbes, but can increase EMPS by a defaunating action (see below). The dissipation of trans-membrane potential when ionophores are fed increases maintenance costs and thereby reduces EMPS (Allen and Harrison, 1979; Hoeller et al., 1985; Dewhurst and Webster, 1992). The clearest example of manipulation of rumen dilution rates is a series of experiments conducted in Canada to investigate the effects of low temperatures on

digestion (Kennedy et al., 1976). Increasing levels of intake generally result in increased rumen dilution rates (Evans, 1981), though this need not relate simply to microbial growth rates, because of the complexity of the rumen ecosystem.

4.1. Level of feeding

Increasing the level of feeding of ruminants is expected to reduce maintenance costs of microbes because they spend less time within the rumen. Some experiments (Robinson et al., 1985) have shown clear effects of level of feeding on EMPS, though other evidence is less clear (Agricultural and Food Research Council, 1992). Some diets that lead to high intakes also lead to reduced EMPS, for example through energy spilling to maintain cellular pH when rumen pH is low, perhaps through feeding high levels of starchy concentrates. The level of feeding effect appears to hold true for maximum EMPS, since there are no occurrences of high EMPS at low intakes. A level of feeding effect has been incorporated into the UK metabolisable protein system (Agricultural and Food Research Council, 1992).

4.2. Rumen synchrony

Before describing the effects of specific energy or protein fractions/supplements, it is useful to consider the rumen synchrony concept, which has been proposed as a conceptual framework to simplify the description of energy and protein supply to rumen microbes (Johnson, 1976).

It is envisaged that MPS will be maximised by synchronising the availability of fermentable energy and degradable nitrogen in the rumen. Rooke et al. (1987) demonstrated the principle of increased MPS when glucose with or without casein was infused into the rumens of cows consuming grass silage-based diets, particularly when casein was included. Since then there have been many other attempts to test the 'synchrony' hypothesis and a summary of some of the results, in relation to the way in which synchrony and asynchrony were achieved experimentally, is presented in Table 1.

It is possible to alter the synchronicity of diets, either by changing dietary ingredients, or by altering the relative times of feeding ingredients or dosing specific forms of energy and N into the rumen, or a combination of both approaches. It is not possible to identify whether an increase in MPS through feeding different ingredients (Herrera-Saldana et al., 1990; Aldrich et al., 1993; Sinclair et al., 1993, 1995) is an effect of synchrony or a factor associated with the manipulation of the ingredients (level and type) themselves. Another potential shortcoming of experiments where the degradation/fermentation rates of the protein and carbohydrate fractions are pre-determined by in sacco studies (Sinclair et al., 1993, 1995; Henderson et al., 1998) in order to calculate the 'synchronicity index' is that drying and grinding of substrates alters the characteristics and, thus, availability of dietary components (Davies et al., 1998) which are used to derive the index. Feeding the same ingredients according to different meal patterns or nutrient infusion, directly into the rumen, is the most robust test of the concept. However, even in this type of experiment little conclusive evidence has been presented which shows positive effects on MPS

Table 1
Summary of data from experiments examining the effect of synchronising dietary N and carbohydrate supply on N utilisation or microbial N synthesis and efficiency using different experimental approaches

Experimental approach/authors	Diet or energy/N source	Experimental system	Findings[d]
1. *Alteration of ingredients*			
Herrera-Saldana et al. (1990)	Hay and cottonseed hulls plus concentrates	Lactating cows	Synchronisation of dietary supplements for rapid fermentation (more degradable starch and protein) gave highest MN flows and EMPS than asynchronous or slow fermentation synchronous diets.
Aldrich et al. (1993)	Alfalfa silage+concentrates	Lactating cows	Synchronisation of rumen available carbohydrate and protein for rapid degradation gave highest MN flows.
Lee et al. (1997)	Straw+concentrates	In vitro	MN flow and EMPS higher with a synchronous diet.
Henderson et al. (1998)	*No information*	Lactating cows	Highest MN flow and EMPS with an asynchronous diet
2. *Same dietary ingredients* (*changed feeding pattern or pulse dosing into rumen*)			
Henning et al. (1991)	Glucose/urea/trypticase	In vitro	Synchrony between energy and N supply lowered ammonia concentrations and fluctuation, but no improvement in EMPS or microbial DM production. A single pulse dose of glucose improved both microbial DM production and EMPS.
Newbold and Rust (1992)	Glucose/urea	In vitro	Asynchronous supply of N and energy yielding substrates only had short-term effects on bacterial growth.
Henning et al. (1993)	Wheat straw/fishmeal/molasses[a]	Sheep	Ruminal ammonia lower and more stable with synchrony, but MN flow and EMPS unaffected. Continuous infusion of sugar improved MN flow
Kolver et al. (1998)	Grass/clover – timed concentrate feeding	Lactating cows	Ruminal ammonia concentrations consistently lower with synchronous diet.
Kim et al. (1999b)	Grass silage only[b]	Dry cows	No improvement in MN flow with synchronous conditions where sucrose infused
Kim et al. (1999a)	Grass silage+concentrates[c]	Dry cows	Marked increase in MN flow when malto-dextrin infused synchronously

[a] Infusion or pulse dosing of sugar and urea/casein.
[b] Infusion or pulse dosing of sucrose at different times.
[c] Infusion or pulse dosing of maltodextrin at different times.
[d] Synchronous, balanced supply of N and energy; and Asynchronous, unbalanced supply of N and energy, achieved by approaches 1and 2; MN, microbial-N; and EMPS, efficiency of microbial protein synthesis.

(Newbold and Rust, 1992; Henning et al., 1993; Henderson et al., 1998) or milk production (Kolver et al., 1998). Henning et al. (1993) showed that continuous infusion of sugar alone increased the efficiency of microbial growth and they concluded that dietary manipulation should aim at providing an even supply of energy whilst supplying the appropriate quantity of rumen degradable N. On the other hand, Henning et al. (1991) showed that, under in vitro conditions, a single dose of glucose was superior to other energy supply patterns in increasing microbial synthesis and efficiency. Workers at our Institute also offered diets formulated to be either highly synchronous or highly asynchronous, but in a rumen simulation continuous culture system (Lee et al., 1997). The synchronous diet gave higher microbial yields, despite the asynchrony effect being eliminated by continuous feeding in this system.

Clearly effects which are attributed to synchrony may be specific effects of individual nutrients, particularly protein and energy fractions. In this context it is useful to consider the 'synchrony' hypothesis and its interpretation from the standpoint of two quite different forage-based feeding systems, grazing of fresh forage and feeding of conserved forages such as silage and hay. Chamberlain and Choung (1995) presented the case for 'asynchrony' with conserved forage-based feeds, where an extreme imbalance can arise, with little readily-available energy at a time when there is an abundance of protein degradation products (peptides, amino acids and ammonia; McDonald et al., 1991). Indeed, in contrast to most of the findings discussed above, Kim et al. (1999a) observed an increase in microbial N flow in dairy cows when synchronous conditions in the rumen were created by infusion of sugars at different times and the basal diet was silage plus concentrates. However, in another similar experiment where silage was fed alone (Kim et al., 1999b), there was no effect of the infusion treatment. The authors suggested that the degree of synchrony will only influence MPS with diets already containing high levels of readily fermentable carbohydrate, although this is only likely if the capacity of the microbes to store starch is exceeded. It is difficult to explain these contradictory findings as a positive response would seem more likely with cows offered silage alone.

On the other hand, during grazing of fresh grass relatively high levels of soluble sugar and protein degradation products will be made available over extended periods, by the action of plant (Zhu et al., 2000) and microbial (Attwood and Reilly, 1996) enzymes. Moreover, if excess sugar is available during grazing and there is a transitory N shortage, rumen bacteria can synthesise and accumulate starch (up to 75% of cell dry matter — see Stewart et al., 1981), which can be stored for later use when N supply is resumed. Thus, asynchrony between energy and N supply may not present as great a problem during grazing of grass as when diets are based on grass silage. Factors such as level, type and balance of different carbohydrate sources may assume greater importance, particularly when variations in soluble sugar content (and other nutrients) in spring-, summer- and autumn-grazed grass are taken into account (see below). We would thus propose that in the case of silage the term 'balance' which embraces all of these requirements, should replace 'synchrony' when describing ruminal N and energy supply. Indeed, Chamberlain et al. (1993) have demonstrated that the soluble sugars sucrose, lactose and fructose are superior to starch (cereals are usually chosen for practical supplementation, rather than sucrose) as an energy sources for fixation of microbial nitrogen in the rumen. This finding may have significance for microbial synthesis in grazing animals, as

although starch levels are very low in grasses the storage polysaccharide fructan (a polymer of fructose), can form up to 70% of the grass WSC content (McGrath, 1988). Little has been reported on the fermentation characteristics and use of this sugar by rumen microbes (Ziolecki et al., 1992). However, the rate and extent of gas production from fructan under simulated ruminal in vitro conditions has been shown to be similar to that of starch and considerably slower than for sucrose and glucose (A.C. Longland, personal communication).

Miller et al. (1999) have used novel grass varieties bred for elevated WSC content with the aim of balancing energy supply to the rumen and improving milk production from dairy cows. This approach may tackle the problem of energy supply from both the standpoint of level and type of sugar, as the majority of the sugar in these grass lines is in the form of the polymer fructan. These authors found increased milk yields when cows were fed grass of elevated sugar content, compared to a lower sugar control grass and suggested that the main cause was probably an increase in digestible dry matter intake. However, rumen digestion studies were not carried out and an effect on microbial N synthesis is also possible. Manipulating forage traits for expression of characteristics that lead to elevated water soluble carbohydrate and reduced protein degradability is a way forward and has the potential to tailor plant material for optimal ruminal requirements of sugar, N and many other nutrients.

4.3. Forage quality

The yield and efficiency of synthesis of microbial protein has frequently been recorded as high (30–45 g microbial-N per kg OM apparently digested in the rumen), when high-quality grass is grazed (Beever et al., 1986; Dove and Milne, 1994; Carruthers et al., 1997; Jones-Endsley et al., 1997; Elizalde et al., 1998) or zero-grazed (Beever et al., 1978; O'Mara et al., 1997). Much lower microbial efficiencies (<20) have been noted with lower-quality autumn-grass, though in these experiments season was confounded with physiological state of the animals (Dove and Milne, 1994; Carruthers et al., 1997).

Preservation of forages as silages involves fermentation of carbohydrates in the silo and so it is not surprising that in silage-fed animals yields of microbial protein are lower than for fresh forages (Agricultural Research Council, 1984). Recent studies using the urinary purine derivative technique (see below) have demonstrated this effect using fresh and ensiled lucerne (Vagnoni and Broderick, 1997). Jaakkola and Huhtanen (1993) found higher MPS with a silage compared to hay made from the same grass; this unusual result may reflect the restricted fermentation (high residual sugar and true protein) from use of a high level of formic acid as additive.

There are no consistent effects of silage additives on MPS and it seems likely that those observed are mediated by effects on levels of residual sugars or protein and its breakdown products, which may be important to rumen microbes in specific feeding situations. Jacobs and McAllan (1992) found that feeding rapeseed meal increased MPS when offered alongside an enzyme-treated silage, but not a formic acid-treated silage.

There are also no consistent effects of cutting date or stage of maturity of silages on MPS, so that effects in individual experiments are probably related to other factors, including interactions with level and type of concentrate, and not maturity per se.

McAllan et al. (1994) attributed higher MPS (and efficiency) with a grass silage prepared in early June (compared with one prepared in late-June), to higher levels of amino acids and peptides. On the other hand, Rinne et al. (1997) found no effect of stage of maturity on MPS, whilst Hart and Leibholz (1990) only found an effect with silages made from kikuyu grass. Conversely, Thomas et al. (1980) found higher EMPS with more mature silage, when comparing autumn-cut silages.

4.4. Composition of supplements

MPS is often increased by supplementing silage-based diets with moderate levels of readily-fermented carbohydrates (Thomas et al., 1980; Harstad and Vik-Mo, 1985; Rooke et al., 1985). The inclusion of starch in ruminant diets can affect the rumen microbes in a variety of ways and so it is not easy to predict its overall effect, though moderate inclusions invariably benefit the rumen microbes through increased substrate and growth rates for liquid-associated bacteria. Starch can have negative effects through decreased rumen pH, decreased fibre degradation, increased energy spilling (Russell and Wallace, 1997) and a higher requirement for pre-formed amino acids (Russell et al., 1992). Microbial protein synthesis is sometimes, but not always, increased by replacing maize starch with barley starch (see discussion by Overton et al. (1995)). Chamberlain et al. (1993) showed that sugars increase MPS from grass silage-based diets more than starch supplements, though Oh et al. (1999) were unable to attribute this to the more stringent requirements of amylolytic bacteria, commenting that one (*Ruminobacter amylophilus*) uses very largely ammonia-N. This type of observation shows the importance of further studies to elucidate the organisms and substrates involved in microbial processes in the rumen (see discussion of odd-chain fatty acid and isotopic discrimination techniques below).

The effect of protein sources on MPS is more complicated than a synchrony effect or a limitation imposed by ERDP supply. In some situations, the pursuit of diets that are high in non-degradable protein leads to a deficiency of fermentable energy (or end-products of fermentation) for rumen microbes and, consequently, reduced MPS (Siddons et al., 1985; Rooke et al., 1986; Cecava et al., 1991; Cecava and Parker, 1993; Christensen et al., 1993). This negative effect of protein supplements was not noted in work with grazed grass (Elizalde et al., 1998), where fermentable OM was not limiting. Dewhurst et al. (1999) noted different MPS responses to protein supplements according to the type of energy supplied in concentrates.

Fat supplements can have a major effect on rumen MPS either directly by not supplying fermentable energy for rumen microbes or indirectly through effects on the composition of rumen microbes and, in particular, rumen protozoa. One of the major ‘costs’ to the rumen microbes is the energy lost through protozoal predation on bacteria. Feeding high levels of free oils (unsaturated fat) to ruminants often leads to defaunation and a marked increase in MPS (Knight et al., 1978; Ikwuegbu and Sutton, 1982; Tesfa, 1993). Broudiscou et al. (1994) suggested that the increase in MPS in response to infusion of linseed oil was only partially explained by the defaunation. Murphy et al. (1987) fed full-fat rapeseed meal to dairy cows and noted an increase in EMPS, though it was not clear whether defaunation was involved.

5. Modelling microbial processes

The absence of consistent effects on MPS (Agricultural Research Council, 1984) led to adoption of relatively simple equations for predicting MPS as a function of organic matter apparently digested in the rumen (Agricultural Research Council, 1980, 1984) or digested carbohydrates (Madsen, 1985). More recent systems have improved these by incorporating levels of feeding effects (Agricultural and Food Research Council, 1992) and more corrections for nutrients which do not yield energy to rumen microbes (fat, bypass protein, bypass starch, fermentation acids; Tamminga et al., 1994).

The Cornell model (Russell et al., 1992) takes rumen modelling to a further level of complexity by addressing some of these issues. However, it requires a quantum leap in the level of diet characterisation required. The good fit claimed for the rumen sub-model (slope=0.94; r^2=0.88) is not unexpected because it was fitted to only five experiments and covered a wide range of microbial yields (80–320 g per day) in lactating cows and steers.

A number of workers have developed mechanistic models of the rumen and these have been thoroughly reviewed (see Dijkstra and Bannink, 1999 and the references therein). Models have undoubtedly advanced our ability to estimate microbial protein synthesis, but as yet there are still areas that need further research and improvement. Factors of particular importance in improving our ability to predict microbial protein synthesis were highlighted by Dijkstra et al. (1998) as follows: the variation in our ability to predict microbial protein synthesis; effects of substrate type and concentration; microbial interactions; and the amino acid profile of microbial protein. Most if not all these factors will, with time, be resolved under the conditions prevailing in a well run animal experiment and with the aid of mechanistic models will be of great benefit to the ruminant agricultural sector. However, we must not extrapolate the situation of experimental animal to farm herd animal too far without examining how our experimentally derived models fit on farm. On farm many more variables occur, animals are likely to be under more stresses, there will be greater competition for feed and, not least, the analysis of the feed can be subject to a great deal of variability. Secondly, a great deal of the work to develop the models has used surgically modified animals. Therefore, it would be of great benefit to develop systems that could monitor the efficiency of rumen function on an intact animal on farm, be this either at an individual cow or whole herd level and here we refer to systems based ideally on milk samples. However, information gained should feed back to the modellers to enable the further improvement of models used in ration formulation because, as pointed out by Forbes and France (1993), ‘‘they [mechanistic models] play a useful role in hypothesis evaluation and in the identification of areas where knowledge is lacking, leading to less ad hoc experimentation.’’

6. Rumen diagnostics

Recent interest in less-invasive techniques has been fuelled by welfare concerns about the use of fistulated animals in developed countries as well as the ease of application of these techniques in developing countries. Less invasive experimental approaches also

offer potential for the development of novel diagnostics that could predict microbial synthesis in an individual feeding situation.

Rumen diagnostics are based on the measurement of a compound that derives from rumen microbes and which appears in urine or milk. Early efforts focussed on urinary allantoin, which is derived from the purine bases within nucleic acids, and so builds on experience of using RNA or purine bases as a marker for microbial content in duodenal digesta (Smith and McAllan, 1970; Zinn and Owens, 1986).

Early studies showed a significant relationship between urinary allantoin excretion and intake of digestible DM or OM in cattle and sheep (e.g. Vercoe, 1976; Antoniewicz et al., 1981). More recent studies have investigated allantoin excretion in other ruminant species (e.g. Liang et al., 1994), demonstrating the value of this approach that can be easily applied in the field. More recent studies have identified effects for a range of supplements on urinary allantoin excretion: (ionophores and sodium, Dewhurst and Webster, 1992; sugars: Chamberlain et al., 1993; and N supplements: Susmel et al., 1994).

The urinary allantoin techique is based on assumptions about the endogenous contribution from cell turnover as well as the proportion of allantoin flux that is excreted in urine rather than lost to excretion in milk, saliva or other enteral losses. Studies with animals maintained solely on intra-gastric infusions incorporating varying levels of exogenous purines (Chen et al., 1990 for sheep; Verbic et al., 1990 for cattle) identified the endogenous contribution. These experiments were open to criticism about the abnormal digestion and metabolism processes in these animals, a situation which was partially remedied using normally-fed animals with replacement of duodenal contents (Balcells et al., 1991). Recently, Pérez et al. (1998) used a stable isotope technique to confirm the endogenous contribution to allantoin excretion in normally-fed sheep and described the variation in response to level of feeding.

The allantoin technique also makes assumptions about bacterial composition and the degradability of purines that are contained within feed. It has already been noted that variation in the purine content of bacteria can be difficult to overcome since it is not clear what proportion of microbial flow from the rumen is associated with different microbial populations. Whilst early studies showed that nucleic acids in feed are rapidly degraded (McAllan and Smith, 1973a), it is less clear whether the constituent purine and pyrimidine bases are degraded. In vitro studies by McAllan and Smith (1973b) showed that guanine and, in particular, adenine are not degraded immediately and might contribute to an overestimation of microbial protein flows using these techniques. This may explain the observation (R.J. Dewhurst, unpublished) that over 20% of adenine flowing to the duodenum is in a free water-soluble form.

Our current work focuses on developing on-farm diagnostics to indicate what is going on in the rumen. The objective is a milk-based test that will indicate the status of the rumen microbes in order to guide feeding decisions to maximise MPS and use protein supplements most efficiently. This work concentrates on compounds which are derived from rumen microbes and which appear in milk.

Milk allantoin is derived from rumen microbial nucleic acids and showed promise as a diagnostic. Rosskopf et al. (1991) and Giesecke et al. (1994) found significant correlations between milk yield and allantoin output in milk. However, this regression involves a large element of auto-correlation and it was not clear whether milk allantoin

explained any useful variation. More recent studies have shown that milk allantoin makes up only a proportion of total allantoin excretion (Shingfield and Offer, 1998). Milk allantoin excretion was only weakly related to urinary allantoin output (r=0.36 in Dewhurst et al., 1996; r=0.36 in Martín-Orúe et al., 1996; and r=0.42 in Vagnoni et al., 1997). We have looked for other components of rumen microbes that might transfer to milk and thereby act as a marker of rumen function and microbial synthesis. Rumen bacteria contain significant amounts of lipid (see above). Odd-chain fatty acids are not found in plant material (Diedrich and Henschel, 1990), whilst they can comprise over 25% of fatty acids in rumen microbes (Lee et al., 1999) and so they have potential for use as a microbial diagnostic. Distinctive fatty acid profiles in microbial lipids have been used to identify particular classes of bacteria (e.g. Basile et al., 1995; Gram-type), or to look at the degree of homogeneity of different strains of bacteria (e.g. Dzierzewicz et al., 1996). Individual fatty acids have been used as markers of bacteria and fungi in soil (Bardgett and Hobbs, 1996). Odd-chain fatty acids, particularly the isomers of pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) provide a distinctive signature to fats in ruminants (Polidori et al., 1993 (cows); (Jenkins, 1995; sheep) and Rojas et al., 1994 (goats)) and other animals with symbiotic fermentation (e.g. beavers; Kakela et al., 1996). Levels of C15:0 and C17:0 in subcutaneous adipose tissue (Wolk et al., 1998) and plasma (Smedman et al., 1999) have been used as markers of the intake of ruminant fat by humans. We (Lee et al., 1999) have recently shown that bacteria which are associated with different fractions of rumen contents (solids or liquids) have distinctive patterns of odd-chain isomers

Three isomers of C15:0 (and three corresponding isomers of C17:0) can readily be separated in freeze-dried milk using conventional gas chromatography (C15:0; *iso* C15:0 and *anteiso* C15:0). Bovine milk contains measurable quantities of both pentadecanoic acid (C15:0; 0.96–1.65% of total milk fatty acids) and heptadecanoic acid (C17:0; 0.59–0.89%) as well as branched chain isomers *iso* C15:0 (0.14–0.34%), *anteiso* C15:0 (0.90–1.52%), *iso* C17:0 (0.15–0.24%) and *anteiso* C17:0 (0.33–0.45%; R. J. Dewhurst, unpublished observations for cows offered grass silage based diets). Patterns of these fatty acids are different when cows are fed diets based on grass (R. J. Dewhurst, unpublished observations), suggesting that they are useful at least to provide a qualitative description of the rumen function.

Our current studies are developing the non-invasive approach to studying rumen function by utilising new and highly sensitive equipment (selective ion flow tube mass spectroscopy; Španěl et al., 1999) to monitor microbially-derived compounds in expired rumen gases, such as ammonia, sulphides and volatile fatty acids.

7. Concluding remarks

Technical difficulties and the complexity of the rumen ecosystem have together limited progress in understanding how to optimise the rumen function. Further effort in basic studies and modelling is required to understand the effects of specific nutrients on individual micro-organisms and their interactions. Progress in applying research findings to provide improvements in rumen efficiency will be aided by development of rumen

diagnostic tests that can be applied on individual farms. Some of these techniques might also contribute to a development of descriptions of the rumen that are less rigorously quantitative (in terms of microbial protein synthesis), but more usefully qualitative (in terms of microbial populations, substrates and interactions).

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