

Influence of substrate and microbial interaction on efficiency of rumen microbial growth

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Summary. Microbial N produced in the rumen and flowing to the duodenum (N_i) is related to the total amount of OM fermented or apparently digested in the rumen (OM_f). This relationship, best expressed as microbial N yield ($gN_i/kgOM_f$), is affected mainly by the physical and chemical properties of feed carbohydrates and the amounts ingested. These factors influence yields at three levels of increasing complexity :

1) Bacterial fermentation within one compartment following the continuous culture model. Fermentation pattern as such does not seem to affect yields. High fermentation rates are associated with lactate production, low methane production and transient polysaccharide synthesis. These effects induce acidification and lower yields, partly compensated by faster growth.

2) Protozoal action, determined by the presence of sequestration spaces provided mainly by roughage diets. The presence of protozoa depresses microbial N yield but allows more complete fibre digestion.

3) Compartmentation and differential passage. With roughage diets, optimal microbial N yield seems to require well developed microbial compartmentation, involving a large proportion of microbes in a large-particle pool with a slow turnover, balanced by a small proportion in liquid, small-particle pools with a fast turnover. Such a situation is associated with long roughage feeding.

It is hypothesized that microbial N yields in the rumen may vary between two extremes which are associated with the feeding of long roughage on the one hand or with concentrate (starch) feeding on the other.

Rumen microbial growth : introductory remarks.

With most diets, at least half the protein reaching the ruminant duodenum is microbial protein, produced during the digestion of feed in the rumen. This amount can be related to the amount of organic matter fermented in the rumen (OM_f), determined as the net disappearance of OM between the rumen and duodenum and involving some assumptions (Egan, 1974). Both, N incorporated into microbial crude protein and leaving the rumen (N_i), and OM_f can be estimated *in vivo* using cannulated animals and markers for the determination of digesta flow and microbial N. Apart from differences in cell composition (Armstrong, 1980), the relationship of N_i to OM_f reflects the evident limitation of

microbial growth by energy liberated during rumen fermentation. However, the gross energy content of microbial OM (OM_m) is equal to that of feed carbohydrate and protein : the enthalpy change in the conversion of feed OM to OM_m is close to zero and the need for energy is mainly determined by a decrease in entropy. The energetic efficiency of microbial N incorporation (E) or microbial N yield as a measure of microbial growth yield is thus best expressed as $E = gN_i/kgOM_f$ (Demeyer and Van Nevel, 1976) and not as $E = gN_i/kg(OM_f + OM_m)$, a correction still used by some authors (Crawford *et al.*, 1980 ; Zinn *et al.*, 1981). Also, the error involved in the determination of N_i is introduced into both the numerator and denominator in the latter expression of E. It can be shown that $y = 80x/(x + 80)$, with $y = gN_i/kg(OM_f + OM_m)$ and $x = E = gN_i/kg OM_f$ and OM_m containing 0.08 N. Figure 1 shows that Y is less susceptible than E to changes in the efficiency of use of fermentation energy.

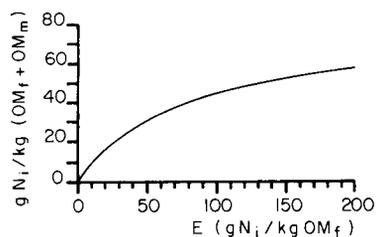


FIG. 1. — Relation between $E = gN_i/kgOM_f$ and $gN_i/kg(OM_f + OM_m)$ for OM_m containing 0.08 N.

Numerous determinations of E have been carried out *in vivo* and values vary between 10 and 70 (Van Nevel and Demeyer, 1977) (Cummins *et al.*, 1983) around a mean of about 30, a figure introduced in systems for ruminant feed protein evaluation (Jarrige *et al.*, 1978 ; Roy *et al.*, 1977). Although experimental error is an important factor in variability (Faichney, 1975 ; Sutton, 1979 ; Ling and Buttery, 1978), recent data have permitted the conclusion that, provided rumen degradable protein is not a limiting factor of microbial growth, diets largely based on concentrates and on silage give lower E values than mixed diets (Tamminga, 1983) or forage diets (Van Nevel and Demeyer, 1983 ; Van Soest, 1982). Contradictory results however have also been published (Cummins *et al.*, 1983) in relation to level (Tamminga, 1978 ; Zinn and Owens, 1983) and frequency (Hungate *et al.*, 1971 ; Brandt *et al.*, 1981) of feeding and protein supplementation (Redman *et al.*, 1980 ; McAllan and Smith, 1984). Even breed may affect E values (Kennedy, 1982). Table 1 summarizes some recent results (varying between the extremes of 66 and 13) obtained on concentrate (Cummins *et al.*, 1983) and tropical forage (Kennedy, 1982), respectively. It should be realized that in order to interpret differences, rumen microbial N yield, E, must be rationalized in models of increasing complexity. The continuous culture model of bacterial growth in one compartment has been used extensively (Harrison and McAllan, 1980), although it does not account for two important characteristics of the rumen emphasized in more recent work :

1) *The presence of protozoa as predators in the rumen* : The presence of protozoa in the rumen is responsible for bacterial turnover amounting to 20-50 % of bacterial N (Tamminga, 1980).

TABLE 1

Rumen microbial N yields in vivo with different diets.

Main diet component	Animals	Microbial N yield (gN _i /kgOM _i)	Reference
Sugars.			
Sugar beet	dairy cows	24	Hvelplund & Moeller, 1978.
Molasses	sheep	26	Demeyer & Van Nevel, 1978.
Sucrose	sheep	23	Hart & Orskov, 1979.
Concentrates.			
Starch	sheep	24	Hart & Orskov, 1979.
Barley + flaked maize	sheep	15	Chamberlain & Thomas, 1979.
Corn	steers	66	Cummins <i>et al.</i> , 1983.
Corn L. I.	steers	24	Zinn & Owens, 1983.
H. I.		31	
Barley	steers	23	Whitelaw <i>et al.</i> , 1984.
Barley	sheep	23-27	Ling <i>et al.</i> , 1983.
Corn	steers	27	Zinn & Owens, 1983a.
Barley	sheep	30	Mathers & Miller, 1981.
Roughage.			
<i>Poor quality</i>			
Oaten chaff	steers	21	Redman <i>et al.</i> , 1980.
Paper	sheep	20	Offer <i>et al.</i> , 1978.
Tropical pasture hay	steers	13	Kennedy, 1982.
<i>Silage (unsupplemented)</i>			
Maize silage	calves	19	Cottrill <i>et al.</i> , 1982.
Wilted silage	cattle + sheep	22-27	Meggison <i>et al.</i> , 1979.
Grass silages	cattle + sheep	10-22	Chamberlain & Thomas, 1980.
Maize silage	dairy cows	21-22	Teller & Godeau, 1984.
<i>Forage</i>			
Ground brome grass	sheep	36-51	Kennedy & Milligan, 1978.
Alkali-treated straw	sheep	33	Hart & Orskov, 1979.
Alkali-treated straw	sheep	36	Harmeyer <i>et al.</i> , 1980.
Alkali-treated straw	dairy cows	30	Moeller & Hvelplund, 1982.
Fresh forage	sheep	37	Moeller & Hvelplund, 1982.
Alfalfa hay	steers	29	Kennedy, 1982.
	Brahman x Hereford	21	Kennedy, 1982.
Chopped hay	sheep	30	Chamberlain & Thomas, 1979.
Chopped hay	steers	28	Zinn & Owens, 1983a.
Lucerne hay	sheep	39	Mathers & Miller, 1981.
Mixed diets.			
Hay + corn (chopped)	steers	29	Cummins <i>et al.</i> , 1983.
(ground)		34	Cummins <i>et al.</i> , 1983.
Hay + barley	sheep	26	Whitelaw <i>et al.</i> , 1984.
Hay + barley + maize	sheep	33-36	Chamberlain & Thomas, 1979.
Hay + barley	sheep	27-34	Ushida <i>et al.</i> , 1984.
Lucerne hay + barley	sheep	36-43	Mathers & Miller, 1981.
Wheat starch + paper	sheep	48	Offer <i>et al.</i> , 1978.
Roughage + concentrate			
— High intake	dairy cows	24-49	Tamminga, 1978.
— Low intake	dairy cows	27-56	Tamminga, 1978.
Roughage + concentrate	dairy cows	35	Meggison <i>et al.</i> , 1979.
Roughage + concentrate	dairy cows	35	Harmeyer <i>et al.</i> , 1981.

2) *Compartmentation and differential retention of rumen contents* : The need of the microbes to attach to insoluble feed particles before onset of fermentation and during fermentation has emphasized the importance of the compartmentation of the rumen population (Cheng and Costerton, 1979). It is clear that on the average, microbes leave the rumen at fractional rates (k_m) which are less than liquid phase fractional outflow or dilution rate (k_l) (fractional rate = actual outflow in g or ml/h divided by rumen total pool size in g or ml), as shown by Hungate *et al.* (1971) and concluded by Mathers and Miller (1981) from data in the literature.

Obviously the composition and physical properties of feed determine E at three levels of complexity :

- 1) continuous or batch culture within each compartment ;
- 2) protozoal predation ;
- 3) microbial distribution over the compartments.

1. Microbial growth of mixed rumen bacteria within one compartment.

This relatively simple model has allowed comparisons of theoretical growth yields $Y_{ATP}^{theor.}$ (g cell DM/mol ATP) with practical values of Y_S (g cell DM/mol substrate fermented). Calculation of n (moles ATP/mol substrate fermented) from known biochemical pathways allows calculation of Y_{ATP} , and such values are always lower than $Y_{ATP}^{theor.}$ This difference can be explained since a significant proportion of ATP is required for maintenance and varies with fractional growth rate, μ , following the Pirt-Shouther equation (Shouther and Bettenhausen, 1973). It is not always clear however if energy costs are to be incorporated in the calculation of $Y_{ATP}^{theor.}$ or to be defined as maintenance. Energy cost for nutrient transport (Hespell and Bryant, 1979) or the degree of coupling between energy yield and biosynthesis (Thauer and Kröger, 1983) are examples of this controversy. Furthermore, as bacteria are known to vary the degree of coupling as well as ATP yield in response to environmental conditions (Thauer and Kröger, 1983), any calculation of Y_{ATP} is bound to be at best a reasonable estimate. Application of the Pirt-Stouther model indicates however that a double reciprocal plot of Y_S vs μ is linear when ATP yield does not change with μ (Hespell and Bryant, 1979).

1.1. *Fermentation pattern and Y_{ATP} estimation with mixed rumen bacteria.*
 — Mixed rumen bacteria are considered to be mainly strict anaerobes generating energy from carbohydrate fermentation through substrate level phosphorylation (SLP), giving 4 mol ATP/mol hexose monomer completely fermented to acetate. Removal of the electrons in methanogenesis and eventual phosphorolytic cleavage of the disaccharides generate additional ATP. Disposing of reducing equivalents (2H) in end-products other than methane may result in lower ATP yields because acetate precursors are removed from SLP and used as electron acceptors in lactate or ethanol production (Wolin and Miller, 1983). In mixed rumen microbes, 2H may be disposed of in propionate production at the expense of methanogenesis (Demeyer and Van Nevel, 1975), and it is now

accepted that propionate production following the succinate pathway generates 1 mol ATP/mol propionate by anaerobic electron transport phosphorylation (ETP) (Thauer and Kröger, 1983). ETP may be a special example of energy generation by the separation of electrons and protons across membranes, generating a charge separation utilized for ATP synthesis by a proton translocating ATPase. A similar electrochemical gradient may result from the outflow of fermentation acids coupled with a proton flux (Erfle *et al.*, 1984 ; Thauer *et al.*, 1977).

The importance of anaerobic ETP in the fermentation of mixed rumen bacteria may be assessed from a comparison of total microbial growth efficiency determined in short-time batch incubations using carbohydrate and pyruvate as substrates. Yields (E) are calculated from $^{32}\text{PO}_4^{3-}$ incorporation and are not affected by cell lysis brought about, *e.g.*, by protozoal ingestion of bacteria (Van Nevel and Demeyer, 1977 ; Harmeyer and Güldenhaupt, 1980).

From known biochemical pathways it is obvious that molar ATP yields from ETP (ATP_{ETP}) are equal with both substrates, where as we can calculate molar ATP yield from SLP (ATP_{SLP}) for both substrates with reasonable accuracy (Tamminga, 1979). The difference in total microbial N yields (calculation in Van Nevel and Demeyer, 1977) between the two substrates obviously occurs because of a different SLP only. This allows calculation of $\text{gN}_i/\text{mol ATP}$ (table 2). In table 2, this difference is $47-27 = 20\text{gN}_i/\text{kg OM}_f$ derived from 12.5 moles ATP/kg OM_f generated by SLP. From these values it can be calculated that $\text{gN}_i/\text{mol ATP} = 20/12.5 = 1.6$. The latter value allows calculation of the total ATP yield (SLP + ETP) from both substrates as $(\text{gN}_i/\text{kg OM}_f) / (\text{gN}_i/\text{mol ATP})$, giving 29.4 and 16.9 for carbohydrates and pyruvate, respectively. Subtracting SLP ATP-yield from total ATP yield gives an estimated yield of 5.9 moles of ATP from ETP.

The data are obviously affected by differences in cell composition (*e.g.* differences in polysaccharide synthesis) but do suggest that ETP is less important than SLP (table 2). Assuming 8 % N in bacterial DM, $Y_{\text{ATP}} = 20$ can be calculated (table 2) in reasonable agreement with the value $Y^{\text{max}} = 25$, corrected for the energy cost of nutrient transport and monomer synthesis (Hespell and

TABLE 2

Estimation of ATP yield from ETP and of Y_{ATP} in mixed rumen bacteria¹.

Substrate	ATP yield (moles/kgOM _f) from		Total growth efficiency	
	SLP ²	ETP ⁴	$\text{gN}_i/\text{kgOM}^1$	Y_{ATP}^3
Cellobiose + maltose	23.5	5.9	47	
Pyruvate	11.0	5.9	27	
Difference	12.5 (1)	—	20 (2)	20

¹ Data from Demeyer and Van Nevel, 1979. ² Calculated from biochemistry (Tamminga, 1979) from carbohydrate monomer Mol. Wt. = 162 and molar acetate, propionate and butyrate proportions of 70, 20 and 10, respectively. ³ Calculated from (2) and (1), assuming that bacterial DM contains 0.08 N. ⁴ Calculated from $\text{gN}_i/\text{kg OM}_f$, and SLP as described in the text.

Bryant, 1979). The data are in line with the contention that maximal fermentation energy yield is obtained when methanogenesis, as electron sink not involving acetate precursors, is maximal (Hungate, 1963). Lowered E values when methanogenesis is inhibited in batch incubations of mixed rumen bacteria with corresponding shifts to propionate production (Van Nevel and Demeyer, 1981) also support this theory.

However, rumen contents from different sheep with various propionate proportions in the end-products of short-term batch incubations showed no difference in microbial N yields (Demeyer and Van Nevel, 1975a). Increasing μ (fractional outflow rate) in continuous cultures of mixed rumen bacteria (absence of protozoa) also changes the fermentation pattern, but linear plots of $\frac{1}{Y}$ vs $\frac{1}{\mu}$ are apparent (fig. 2) (Van Nevel and Demeyer, 1979; Isaacson *et al.*, 1975; Hoover *et al.*, 1982). This indicates a constant ATP yield although large differences in the relative proportions of methane and propionate are observed.

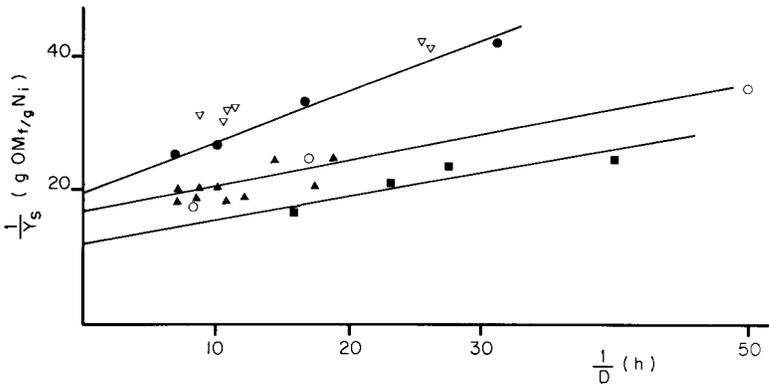


FIG. 2. — Double reciprocal plot between growth yield Y_s (gN_i/gOM_F) and growth rate or dilution rate (h⁻¹).

Data from continuous cultures: ● Van Nevel & Demeyer (1979); ○ Isaacson *et al.* (1975); ■ Hoover *et al.* (1982). Data were recalculated and corrected for 15% overestimation of microbial RNA. Particle passage rates are used.

Data obtained in vivo: ▽ Harrison *et al.* (1975); ▲ Kennedy *et al.* (1976), Kennedy and Milligan (1978). Both liquid and particle outflow rates are shown.

From the mean intercepts with the ordinate in figure 2, a mean value for $Y_s^{\max} = 62.8$ g N_i/kg OM_F can be calculated. Assuming ATP yield to be 29.4 moles/kg OM_F (table 2) and that bacterial DM contains 0.08 N, it can be calculated that $Y_{\text{ATP}}^{\max} = 27$, a reasonable estimate (Hespell and Bryant, 1979).

Results suggest that short-term inhibition of methanogenesis with a shift to propionate production lowers ATP yield. A mixed flora seems to be able to adapt to a high propionate fermentation however, without loss of energetic efficiency: lowered energy yield through acetokinase and methanogenesis is effectively replaced by energy yield from propionate production. The intercellular succinate transport involved in the two-organism system of rumen propionate production (Wolin, 1979), as well as methylmalonyl-coA decarboxylation, may be adapted systems for extra energy generation (Erfle *et al.*, 1984). The relative unimportance

of fermentation pattern in the energetic efficiency of rumen fermentation is also suggested by the finding that propionate as well as acetate fermentation types can be associated with higher yields of microbial N *in vivo* (Harisson *et al.*, 1975 ; Kennedy *et al.*, 1976). Propionate production following the acrylate pathway would not generate ATP however and this pathway prevails at low pH values (Durand, 1982), whereas changes in the degree of coupling as well as changes in ATP yield with alterations in the fermentation pattern can be involved (Thauer and Kröger, 1983). Because of the latter effects, the use of Y_{ATP} in rumen fermentation is to be discouraged.

A last but important point is the question of rumen anaerobiosis. Rumen metabolism is normally regarded as being extremely anaerobic (Russel and Hespell, 1981) but mixed rumen microbes can consume O_2 with stoichiometric and reversible inhibition of methanogenesis (Demeyer *et al.*, 1972 ; Scott *et al.*, 1983).

Recently, Czerkawski and Clapperton (1984) reported that rumen contents may consume 100 ml O_2 /day/l. Similar quantities were consumed in short-term batch incubations : reduced endproduct formation was inhibited according to stoichiometry, but substrate disappearance and total microbial N yield was not affected (Demeyer *et al.*, 1984). In this respect, it may be significant that facultative anaerobic *Bacillus* sp. may contribute to rumen fibre digestion, *e.g.* by the presence of α -L-arabinofuranosidase (Williams and Withers, 1983).

1.2. *Effect of energy source.* — The major sources of fermentation-derived energy in the rumen are carbohydrates and proteins, lipids being relatively insensitive to microbial attack, apart from lipolysis, fatty acid hydrogenation and incorporation (Demeyer, 1973 ; Demeyer *et al.*, 1978).

1.2.1. *Carbohydrates.* — Feed carbohydrates can be broadly classified into plant cell wall or structural carbohydrates (β -glycosidic insoluble cellulose and hemicelluloses) and storage or non structural carbohydrates (α -glycosidic, partly soluble starches and soluble oligosaccharides or sugars) (Tamminga, 1979).

Non-structural carbohydrates are rapidly fermented in the rumen and the concomittant excess of pyruvate production shifts fermentation to propionate and, ultimately, to lactate production (see *e.g.* Silley and Armstrong, 1984). Individual sugars show small differences in fermentation pattern (Van Nevel *et al.*, 1972) but this pattern is mainly related to rate of fermentation and thus, to rate of substrate supply (Demeyer and Van Nevel, 1975). Fermentation rate, irrespective of fermentation pattern, reflects growth rate and its increase augments E (fig. 2) (Demeyer and Van Nevel, 1975a), unless the need to dispose rapidly of ATP increases lactate production, decreasing SLP and, thus, E (Van Nevel and Demeyer, 1977). Concomittant increases in polysaccharide synthesis and decreases in pH may also lower E. Microbial growth is very sensitive to changes in pH (Hoover *et al.*, 1984), possibly because of interference with the generation of proton motive force, a low pH reducing ETP (Erfle *et al.*, 1984). Such effects help to explain the low microbial N yields observed with high sugar and starch diets (table 1). Increasing the frequency of feeding could prevent these

fermentation bursts and result in higher E values (Hungate *et al.*, 1971), but this effect is not always observed (Brandt *et al.*, 1981). Differences in rates of starch fermentation between rolled barley and ground maize are probably responsible for differences in the microbial N yields observed with these diets (Oldham *et al.*, 1979).

Structural carbohydrate fermentation requires preliminary attachment of the microbes to dietary fibre, resulting in a time-lag before the onset of fermentation (Cheng *et al.*, 1977). Synthesis of the adhering glycocalyx fibres obviously requires energy and one would expect E to be lower on structural carbohydrates than on starches, when corrected for differences in growth rate. Stern *et al.* (1978) determined E *in vitro* with increasing inclusion of cellulose into a starch substrate. Other factors (fermentation and growth rate, fractional outflow rate, protozoal count and pH) being constant, the results clearly show that E is lowered with increasing cellulose inclusion (table 3). Clearly, this result is opposite to animal data showing that higher E values are obtained with roughage than with concentrate diets (table 1). Besides the effect of fermentation bursts with concentrate diets, effects at the level of protozoal predation and compartmentation are probably involved, as discussed further on.

TABLE 3

Effect of cellulose on rumen microbial N yield in continuous culture.

Substrate (% of diet)			
Corn starch	4.0	18.0	31.4
Solca floc.	29.9	15.0	0
OM _F (g/24 h) ²	23.4	24.9	24.4
N _i (g/24 h)	0.95	1.19	1.33
gN _i /kg OM _F	40.6	47.8	54.5

¹ Data from Stern *et al.* (1978). Average values for $k_1 = 0.060$, $k_s = 0.025$, pH = 6.60, protozoa = 10^4 /ml. ² Calculated from volatile fatty acid production.

1.2.2. *Protein.* — Many diets contain up to 20 % protein, 70 % of which is degraded in the rumen as a result of proteolysis, followed by oxidative deamination and transamination of the amino acids and fermentation of the resulting keto-acids, according to the stoichiometry of carbohydrate fermentation (Demeyer and Van Nevel, 1979). Carbohydrate availability does not affect proteolysis but lowers ammonia production (Russell *et al.*, 1982). In line with theoretical considerations (Tamminga, 1979), microbial N yields obtained using proteins as energy sources are comparable to those obtained with pyruvate and amount to only 0.50 of the yields obtained with carbohydrate as an energy source (Demeyer and Van Nevel, 1979). It is evident that the inclusion of apparently digested protein in OM_F is a source of variation in E. Therefore, and to overcome problems related to the inclusion of endogenous OM in digesta flow, Tamminga (1978) proposed to relate N_i to the total of crude fibre and N-free extractives apparently digested in the rumen. As only a limited part of fermentation energy is

derived from protein, such an expression may be more appropriate, although less straightforward because more empirical analysis is necessary.

1.3. *Effect of N source.* — It is obvious that rumen bacteria have to be supplied with sufficient N (as well as with other nutrients such as S, P and trace elements) to realize maximum growth under energy-limiting conditions. From $E = 30$ and a proportion of 0.70 OM_f in feed OM it can be calculated that feed OM should contain ca. 13 % of rumen-degradable crude protein, similar to other estimates (Satter and Roffler, 1981). Low quality roughage diets as well as high-concentrate diets often have N contents below that value, and E with such diets may therefore be limited by N and enhanced by non-protein nitrogen supplementation (Elliott and Armstrong, 1982). However, no effect on E was obtained with N supplementation of low N roughage diets by Kropp *et al.* (1977, 1977a), Amos and Evans (1976), Redman *et al.* (1980) or Moeller and Hvelplund (1982). With high-concentrate diets, results are contradictory: N supplementation either improves E (McAllan and Smith, 1984) or has no effect (Brandt *et al.*, 1981). The contradictory results may be explained by differences in urea recycling to the rumen according to the diet: long roughage diets promote urea recycling by intensive rumination and salivation, in contrast to concentrate diets. The availability of ammonia-N for rumen microbial growth is reflected in rumen ammonia concentration and it has been proposed that values below 50 mg NH₃-N/l indicate N-limitation of microbial growth. It is unlikely however that such concentrations limit growth as NH₃ K_s values (concentrations at which 0.50 of optimal growth is obtained) for rumen bacteria are around 1 mg/l (Hespell and Bryant, 1979). Also rumen ammonia concentration is not necessarily related to microbial intracellular ammonia concentration. Furthermore the concentrations reflect the balance of production and utilization: low values may reflect low turnover and efficient growth rather than limitation, as in the defaunated rumen discussed further on. Amino acid transport k_m values for several anaerobic bacteria are however similar to the low values often found in the rumen, and Hespell and Bryant (1979) suggest that rumen fermentation may be uncoupled due to impaired amino acid transport into bacteria. All amino acid carbon skeletons can be synthesized in the rumen, and ammonia-N is incorporated by rumen bacteria through the combined action of glutamine synthetase and glutamate synthase at low ammonia concentrations (Hespell, 1983) and through glutamate dehydrogenase. Tracer experiments *in vivo* indicate that rates of methionine and phenylalanine synthesis may limit bacterial growth with protein-free diets and rumen bacteria may use up to 0.80 N as peptide or amino acid N, depending on the dietary protein content (Smith, 1979; Demeyer and Van Nevel, 1980). This does not decrease the energy cost of cell formation, as the major cost is polymerization and not monomer synthesis (Hespell and Bryant, 1979), but it may decrease transport energy expenditure as an inverse function of amino acid polymer length (Hespell and Bryant, 1979; Demeyer and Van Nevel, 1980). These ideas are supported by the finding that free amino acids or protein stimulate E *in vitro* (Maeng *et al.*, 1976; Cotta and Russell, 1982). The initial observation of Hume (1970) that protein stimulates E in animals fed a synthetic diet has been

confirmed (Cottrill *et al.*, 1982 ; Elliott and Armstrong, 1982 ; McAllan and Smith, 1983, 1984) as well as contradicted (Brandt *et al.*, 1981 ; Kropp *et al.*, 1977, 1977a ; Amos and Evans, 1976 ; Redman *et al.*, 1980 ; Moeller and Hvelplund, 1982) in animals receiving natural diets. The absence of an effect in the animal may be related to long rumen retention of microbes with long roughage diets ensuring sufficient recycling of microbial protein to provide the necessary supply of amino acids and peptides (Kropp *et al.*, 1977). Also, sufficient protein should be added to prevent complete degradation of amino acids in the rumen, thus allowing their incorporation (Ben-Ghedalia *et al.*, 1978).

2. The presence of protozoa

The role of protozoa in the rumen is still debated (Hobson and Wallace, 1982) and their metabolic importance considered obscure (Van Soest, 1982). It is obvious however that their presence is associated with considerable turnover of microbial N because of the turnover of the population of holotrichous protozoa, itself, associated with molasses feeding (Leng, 1982 ; Leng and Nolan, 1982), or the turnover of bacteria ingested by populations largely composed of entodiniomorphid protozoa (Coleman and Sanford, 1979). Such predation is probably not accounted for by Pirt-like maintenance equations (Hobson and Wallace, 1982) as it necessitates the adaptation of mathematical models for continuous culture (Smouse, 1981). The detrimental role of protozoa in rumen microbial N yield was speculated upon in earlier work (review in Demeyer, 1981) and demonstrated by the determination of simultaneous total synthesis and degradation of microbial matter *in vitro* (Van Nevel and Demeyer, 1977) using faunated and defaunated mixed rumen microorganisms (Demeyer and Van Nevel, 1979). The absence of protozoa lowered degradation and slightly increased total synthesis, resulting in a considerable increase of net synthesis (*e.g.* total synthesis-degradation). The efficiency of net microbial N incorporation (E) was increased by defaunation, not only because of elimination of turnover due to protozoal lysis and predation, but also because of a shift to a faster-growing amylolytic flora (Kurihara *et al.*, 1978), as indicated by the increased efficiency of total synthesis. These results obtained *in vitro* have recently been confirmed in a number of animal experiments and it is clear that variability in microbial N yields may be determined to a large extent by variability in the number and activity of rumen protozoa, quite apart from energy yield in fermentation (Demeyer and Vervaeke, 1984). Dietary factors affecting the protozoal population have been reviewed by Demeyer (1981) and mainly relate to the physical form of feed particles, the proportion of starches and sugars and the level of feeding.

2.1. Roughage diets. — Long roughage sustains populations largely composed of entodiniomorphid protozoa in the rumen by providing sequestering spaces for the swimming protozoa. Low quality roughage is probably less beneficial to protozoa because of a shortage of easily degradable starches and sugars (Jouany, 1978). Grinding the roughage impairs sequestration, lowers protozoal count and also

promotes retention by reducing salivation and k_f . The large protozoal populations associated with silage diets (Chamberlain and Thomas, 1980) may be due to dietary promotion of sequestration and they could be responsible for low E values (table 1), although the same argument holds for long roughage diets which do not promote low E values.

2.2. Concentrate diets. — Restricted feeding of starch diets sustains large rumen populations of entodiniomorphid protozoa (Eadie *et al.*, 1970) that are possibly responsible for the low E values observed with such diets (Ling *et al.*, 1983 ; Whitelaw *et al.*, 1984). Higher feeding levels and/or grinding concentrate diets lowers protozoal count through acidification and increased tonicity associated with intensive fermentation, reduced salivation and lower k_f values, although other factors may be involved (Lyle *et al.*, 1981). Many animals on high-concentrate feeds are defaunated (Johnson, 1976) and high concentrate feeding is in fact an efficient defaunation procedure (Whitelaw *et al.*, 1984a). The detrimental effects on E values of fermentation bursts and lowered salivation associated with such diets may be partly balanced by the defaunating effect, as illustrated by a comparison of some data in the literature (table 4). Liquid diets containing large amounts of sugars sustain large populations of holotrichous protozoa because limited intake restricts fermentation intensity (Coleman, 1979).

TABLE 4

Microbial N yield in faunated and defaunated animals fed high-concentrate diets.

Animal	Diet	Level of Feeding (kg/d)	Microbial N-yield (gN _i /kgOM _t)
<i>Sheep</i>			
Faun. ¹	Flaked maize + dried grass	0.4	24
Faun. ²	Starch	0.6	24
Defaun. ³	Rolled barley	0.6	35-40
<i>Steers</i>			
Faun. ⁴	Oaten chaff + molasses + starch	3.1-3.2	16-22
Defaun. ⁵	Starch + straw	2.3-2.5	22-29 ⁶

¹ Harrison *et al.*, 1975 ; ² Hart and Orskov, 1979 ; ³ Mercer *et al.*, 1980 ; ⁴ Redman *et al.*, 1980 ; ⁵ McAllan and Smith, 1984 ; ⁶ Only data for N supplemented diet were used.

A special case is the inclusion of fat in the diet which has been observed to increase E in sheep (Demeyer, 1981) and possibly in cattle (Tamminga *et al.*, 1983), probably as a result of the defaunating effect of the fat, as recently confirmed by Czerkawski and Clapperton (1984). Increased microbial N yields in the rumen result in better animal performance under some conditions (Bird *et al.*, 1979 ; Demeyer *et al.*, 1982), but animal response depends on the multiple interrelated effects of defaunation such as :

- inhibition of rumen fibre degradation (Demeyer, 1981) ;
- increase in fractional particle passage rate (Kayouli *et al.*, 1983/84) ;
- inhibition of rumen protein degradation (Kayouli *et al.*, 1983) ;

- inhibition of rumen methanogenesis (Whitelaw *et al.*, 1984a)
- elimination of protozoal contribution to duodenal microbial protein normally ranging between 0.14 and 0.55 (Demeyer and Vervaeke, 1984) ;
- lowered microbial incorporation of higher unsaturated fatty acids (Demeyer *et al.*, 1978).

2.3. *Compartmentation and differential retention of microbes.* — Czerkawski (1984) and Owens and Goetsch (1984) distinguish three rumen compartments referred to here as A, B and C with particle dimensions of $< 200\mu$, between 200 and 1200μ , and $> 1200\mu$, respectively. Microbes in A and B leave the rumen at respective fractional rates k_A and k_B , whereas microbes in C do not leave the rumen (Owens and Goetsch, 1984). Their retention with associated lysis and recycling within C may be necessary for maximal fibre degradation as suggested for bacteroides (Cheng *et al.*, 1983/84). It is clear that $k_m = C_A k_A + C_B k_B$, where C_A and C_B represent the proportion of total microbes in A and B, respectively (Oldham, 1984).

A more general equation states $k_m = \sum C_i k_i$, where C and k are the proportion of microbes and the fractional outflow rate, respectively, of i compartments. This complexity is further increased by changes in C and k with time after feeding because of decreasing particle size with progressive digestion and changes in saliva flow. Compartment B may serve as a transport or shuttle compartment between A and C (Czerkawski, 1984). Within each compartment, E increases with k following the Pirt-Stouthamer model, but k_m is obviously determined by the magnitude of C_A and k_A relative to C_B and k_B (e. g. an increase in k_B may be offset by a decrease in k_A and C_B/C_A). In continuous cultures, bacterial growth rate (μ) equals k_i or the dilution rate (D) and their increase augments E (fig. 2). Similar relations between k_i and E hold for the rumen, when particle (k_p) and liquid (k_l) fractional outflow rates are similar in the relatively large compartments A and B. Such conditions are found when ground and pelleted or concentrate-rich diets are fed continuously (Kennedy *et al.*, 1976 ; Kennedy and Milligan, 1978 ; Harrison *et al.*, 1975) (fig. 2). Fractional outflow rates of bacteria, reflecting bacterial growth rate, are lower than k_l however and better approximated by k_p when $k_l > k_p \approx \frac{k_A + k_B}{2}$.

Changes in k_p should therefore affect k_m more than changes in k_l , but also because $k_p < k_l$. Indeed, the hyperbolic relationship between μ and E predicts a greater response at low values of μ (Van Soest, 1982). Crawford *et al.* (1980) in their *in vitro* system determined the effect of independently varied k_l (0.07-0.15) and k_p (0.034 - 0.070) values on E, obtained using RNA as a microbial marker. Recalculation of their data after correction for dietary RNA contamination (0.15 of RNA) (Smith, 1979) allows calculation of the regression equation $E = -49 + 965k_p + 423K_l$ ($n = 9$, $R^2 = 0.73$) in line with the relative importance of k_p and k_l .

With high-concentrate or ground and pelleted diets rumination and salivation is reduced, resulting in lower k_l values. Small feed-particle size on the other hand increases k_p . Whereas microbes present in compartments B and C or in the

rumination pool (Hungate *et al.*, 1971) turn over much more slowly than k_1 in roughage-fed animals, this difference may diminish when concentrates or ground and pelleted diets are fed continuously (Faichney and Griffiths, 1978). With larger feed-particle size and lower feeding frequency, as in animals fed chopped or long roughage twice daily, k_1 is very different from k_p and there is no apparent relation between E and k_p or k_1 (Kennedy *et al.*, 1982; Mathers and Miller, 1981; Hadjipanayiotou *et al.* 1982). In roughage-fed animals however, a major part of the microbial matter, including protozoa, is associated with slow turnover in a large compartment C. Compartments A and B show rapid and thus efficient growth, with small particles continuously supplied from an efficiently drained compartment C. Because of the fibrous nature of the substrate growth in C is slow, resulting in low E values, also because protozoa are present. This may be balanced however by an energetically efficient acetate-type fermentation and the provision of necessary peptides and other growth factors by extensive recycling. It would seem that overall microbial N yield in the animal benefits from the increased rumen compartmentation with roughage diets (table 1), in spite of the lower growth yields with fibrous substrates within one compartment (table 3). Fractional outflow rates of forage (k_f) and concentrate (k_c) particles, as affected by level of intake with high and low-forage diets, were determined by Colucci *et al.* (1982). Table 5 shows that level of intake affects k_c more with low-forage (high-concentrate) diets than it does k_f with high-forage (low-concentrate) diets. Similar effects were also reported by Owens and Goetsch (1984) and Van Vuuren (1984).

TABLE 5

Effect of diet and intake level on rumen retention of forage and concentrate particles¹.

Diet	Level of intake	Fractional outflow rate (k) of	
		Forage ²	Concentrate ²
Low-forage	Low	0.023	0.038
	High	0.041	0.070
High-forage	Low	0.041	0.053
	High	0.049	0.068

¹ From Colucci *et al.* (1982); ² Determined using Cr-mordanted material.

Such data help to explain contradictory results on the effects of level of feeding on microbial N yield (table 1): increased level of feeding would be expected to increase k_p , and thus μ and E, with concentrate diets, as found by Zinn and Owens (1983). With mixed diets, an opposite effect was reported (Tamminga, 1978). In general, it would seem that increased duodenal flow is associated with higher yields of microbial N (Teller and Godeau, 1984; Zinn and Owens, 1983a).

Conclusion.

It is hypothesized (Demeyer and Vervaeke, 1984) that microbial N yields in the rumen may vary between two extremes:

1) Long particle retention time, extensive rumination and salivation and high liquid turnover rates associated with long roughage feeding : net microbial N yield is depressed by extensive recycling of bacteria due to the presence of an active protozoal population sequestered in the particle phase. This is balanced however by an energetically efficient acetate-type bacterial fermentation.

2) Short particle retention time, less rumination and salivation and low rumen liquid turnover associated with concentrate (starch) feeding : net microbial N yield is enhanced by the absence of protozoa, resulting in the presence of a fast growing flora. This is balanced however by an energetically less efficient propionate-lactate type bacterial fermentation.

An optimal situation, occurring when feeding mixed diets, is associated with a mean microbial N yields of 35 gN_i/kg OM_f.

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