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Invited commentary

Is the synthesis of rumen bacterial protein limited by the availability of pre-formed amino acids and/or peptides?

The main amino acid supply to ruminant animal tissues originates from bacterial protein produced in the rumen. Amino acids of microbial protein may be synthesised *de novo* using NH_3-N and C chains, derived from a variety of pathways and characteristic for the complexity of the rumen microbial ecosystem (Wallace *et al.* 1997). C chains are provided by endproducts of carbohydrate or protein fermentation. NH_3-N is derived from endogenous sources (rumino–hepatic recycling) or feed urea, and from deamination of amino acids. Amino acids are produced during: proteolysis of feed protein by both bacteria and protozoa; proteolysis of bacterial, protozoan and fungal protein released from lysed cells (intra-ruminal recycling); excretion (mainly as alanine) by bacteria as well as protozoa and degradation of sloughed rumen epithelial cells. On the other hand, peptides or amino acids, may be taken up intact by bacterial cells and incorporated directly or after transamination reactions into protein.

Obviously, the diversity of the micro-organisms present, mainly changing with both the level and nature of the feed, determines the relative importance of the pathways of bacterial amino acid formation. Moreover, intricate regulatory mechanisms involve interactions between peptides, amino acids and NH_3 uptake, and require further study (Morrison, 2000). Nevertheless, the classic work of Virtanen (1966) clearly established that the rumen bacterial population can maintain itself in animals fed protein-free diets and most rumen bacteria can use NH_3 as sole N source (Allison, 1969). However, the results of ^{15}N-labelled ammonium salt infusion experiments *in vivo* clearly indicated that in sheep fed hay diets, up to 50 % of rumen bacterial-N was not derived from NH_3 (Mathison & Milligan, 1971). Later work *in vitro* suggested that the proportion of such direct incorporation increases with dietary protein content and may reach 80 % (Blake *et al.* 1983).

Amino acids and/or peptides are released by proteolysis of feed protein as well as of bacteria (Wallace & McPherson, 1987) and fungi (Newbold & Hillman, 1990) ingested by mainly *Entodiniomorph* protozoa. These protozoa indeed excrete amino acids (and/or peptides) far in excess of the amounts they ingest (Williams & Coleman, 1988). In addition, protozoa themselves are largely retained in the rumen, subject to considerable proteolytic recycling after lysis (Williams & Coleman, 1992). Such 'turnover' of microbial protein easily exceeds 50 % bacterial-N formed (Leng & Nolan, 1984).

Russell & Strobel (1993) pointed out that the use of intact amino acids does not provide bacteria with an energetic advantage, as the saving of energy needed for *de novo* synthesis is compensated for by the energy need for active uptake and transport of amino acids and peptides. However, bacteria generally grow faster upon addition of amino acids and hence more efficiently, because of a diluted maintenance requirement. At high fermentation rates, the provision of intact amino acids or peptides probably allows for rates of protein synthesis during growth to keep up with fermentation rate (Russell, 1998). A stimulatory effect is therefore apparent with rapidly degradable energy sources, but not when using slowly degraded fibre (Russell *et al.* 1992). In the mixed rumen microbial population, slowly degraded fibre may also sustain a protozoal population of sufficient activity to provide an ample supply of free amino acids and/or peptides by turnover of microbial protein.

From these arguments, the synthesis of rumen bacterial protein may be limited by amino acid supply in the rumen of animals at high production levels, receiving diets that are enriched in starch and in protein subject to limited rumen degradation. Such diets are indeed known to lower rumen pH and are often associated with relatively small particle sizes. Both these factors have an inhibitory effect on rumen protozoan populations (Lyle *et al.* 1981), whereas feed protein degradation is inhibited by pH values $<6{\cdot}0$ (Erfle *et al.* 1982). A combination of these effects may lead to very low values of free amino acid and peptide levels in rumen contents. This is in agreement with the general lowering of free amino acid and peptide levels observed by experimental removal of protozoa (Ivan *et al.* 1991). Hence, optimisation of rumen bacterial protein production in ruminant animals fed at high production levels, may require knowledge of the nature of the limiting free amino acids.

In earlier work, no single amino acid or subgroup produced the same stimulation of net bacterial growth as addition of the complete mixture (Maeng *et al.* 1976) and both stimulatory and inhibitory effects have recently been reported (Kajikawa *et al.* 2002). An alternative approach involves determination of *de novo* synthesis of amino acids from $^{15}NH_3$. Limiting amino acids may then be identified as those with low incorporation of label or whose *de novo* synthesis is lowered most by the addition of amino acids and/or peptides. Results of such studies *in vivo* suggested phenylalanine and methionine (Salter *et al.* 1979) and proline and phenylalanine for non-cellulolytic and cellulolytic bacteria respectively (Wallace *et al.* 2001). Such studies deal with the N part of some amino acids only, although direct incorporation of the C skeleton or its precursor may be the limiting factor, as is the case for

branched-chain fatty acids and cellulolytic bacteria (Bryant & Robinson, 1962). The paper by Atasoglu *et al.* (2004) in the present issue of the *British Journal of Nutrition* provides a tool to study *de novo* amino acid synthesis as well as amino acid-N and possibly C turnover in mixed rumen micro-organisms. Changes in amounts, as well as labelling of NH_3, amino acids and microbial protein, during separate but simultaneous incubations of mixed rumen microbes with $^{15}NH_3$, ^{15}N-labelled protein hydrolysate and ^{13}C-labelled protein hydrolysate are interpreted using classic concepts of tracer dilution. The use of GC–MS allows interpretation down to individual amino acids. The results of the preliminary experiment presented again point to phenylalanine, leucine, isoleucine and valine as microbial amino acids mainly derived from preformed amino acid-C. A different sequence is obtained, however, for amino acid-N where lysine and proline become very important.

It is clear that the technique described could be used to identify limiting amino acids for different feeding conditions. Such information is not only important for practical feeding purposes, but also for further refinement of feed evaluation schemes incorporating the complexities of rumen metabolism (Dijkstra *et al.* 2002). A major prerequisite for its application may be the use of a representative sample of rumen contents within an environment allowing protozoan activity. Animals notoriously vary in the proportions of bacteria and protozoa maintained in their rumen on the same diet (Teather *et al.* 1984). Application of results to individual animals may therefore require correction using indicators of rumen microbial activity to be derived, e.g. from milk constituents (Vlaeminck *et al*. 2003).

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