# Invited commentary

# The need for nitrogen

That microbial fermentation in the rumen can progress without added amino acids, peptides or proteins was elegantly demonstrated by Virtanen (1966) who maintained lactating dairy cows for prolonged periods on purified diets with urea as the only N source. However, there have also been numerous studies which have demonstrated benefits of feeding pre-formed amino acids, either as free amino acids, peptides or proteins, in terms of increased microbial growth and/or fibre breakdown in the rumen (Hume, 1970; Maeng & Baldwin, 1976; Maeng *et al*. 1976; Cotta & Russell, 1982; Rooke & Armstrong, 1989; Merry *et al*. 1990; McAllan, 1991; Chikunya *et al*. 1996). Pre-formed amino acids will become available to the rumen during the degradation of dietary protein and may also be added as dietary amino acid or peptide supplements, and a clear understanding of the optimum balance of $NH_3$ N to pre-formed amino acid N is required if rations are to be formulated for optimal rumen fermentation, and hence animal production. Together with the obvious economic significance of getting this balance correct, urea N is almost always cheaper than dietary protein N. Unbalanced N metabolism in the rumen may also have wider significance. Excess $NH_3$ in the rumen is absorbed into the bloodstream and converted to urea in the liver, before at least a fraction being excreted in the urine. Excessive urea formation will have deleterious consequences; for the environment, in terms of N-scorching of pasture and N leaching into watercourses (Tamminga, 1996), and also potentially animal health, in particular fertility (Butler, 1998).

With such concerns in mind, modern ruminant rationing programs have moved from an empirical approach to estimating animal production to a more mechanistic approach based on modelling of reactions in both the rumen and the animal's tissues. Prominent among such models are the various implementations based on the Cornell net carbohydrate and protein system (Russell *et al*. 1992; Pitt *et al*. 1996). The Cornell net carbohydrate and protein system recognizes the importance of peptides and amino acids in rumen fermentation. However, the requirement for such N sources is assumed to be limited to bacteria fermenting non-structural carbohydrates, with bacteria that utilize structural carbohydrates assumed to utilize only $NH_3$; in addition the requirement of the non-structural-carbohydrate-fermenting bacteria for pre-formed amino acids is assumed to be fixed. Recently several authors have questioned these assumptions. It is now clear that in non-structural-carbohydrate-fermenting bacteria the proportion of cellular N derived from $NH_3$ is not fixed and varies in accordance with the N source available (Atasoglu *et al*. 1998, 1999). Furthermore, pure culture studies have shown that structural-carbohydrate-fermenting bacteria can derive

a significant amount of their cellular N from sources other than $NH_3$, although the extent to which this proportion varies with different N sources is less clear (Ling & Armstead, 1995; Wallace *et al*. 1999). Indeed the division of rumen bacteria into those that ferment structural and non-structural carbohydrates may be simplistic. In this issue, Carro & Miller (1999) report the responses of rumen bacteria to added amino acid sources with structural carbohydrate (neutral-detergent fibre) as the only available energy source and note that there are distinct differences in the responses of solid-attached and liquid-associated bacteria. Solids-attached bacteria failed to show any consistent response to added amino acids, peptides or protein while the yield of liquid-associated bacteria increased and the proportion of their cellular N derived from $NH_3$ decreased when pre-formed amino acids were added. This may reflect differences in the microbes present in the two pools even with relatively homogeneous substrates such as neutral-detergent fibre. It may also reflect differences in the fermentation of essentially the same bacteria on different energy sources. Cruz Soto *et al*. (1994) found that the growth of the cellulolytic bacteria *Fibrobacter succinogenes*, *Ruminococcus albus* and *R. flavefaciens* was stimulated by amino acids and peptides when the bacteria were grown with cellobiose but not when they were grown on cellulose. They speculated that the stimulatory effect of amino acids was dependent on the energy source, with pre-formed amino acids being stimulatory only when the energy source in the diet was capable of sustaining high rates of microbial growth in the rumen. This hypothesis was apparently confirmed by Chikunya *et al*. (1996) who found that added pre-formed amino acids stimulated DM digestion and microbial protein synthesis in the rumen of sheep fed on a diet of readily degradable sugarbeet pulp but not when more recalcitrant hay was given. However, in agreement with the lack of an effect of pre-formed amino acids on solids-attached bacteria in the study of Carro & Miller (1999), the number of cellulolytic bacteria in the rumen did not increase (Chikunya *et al*. 1996). Fibre digestion in the rumen is carried out by a consortia of microbes, with non-cellulolytic organisms important in the removal of the products formed by the cellulolytic organisms, thus preventing feedback inhibition of cellulolysis (Cheng & McAllister, 1997). Possibly the stimulation in fibre breakdown when pre-formed amino acids are fed results from a stimulation of the non-cellulolytic partners of the microbial consortia associated with the fibre rather than a direct effect on the cellulolytic organisms *per se*. Thus, while a clear argument can be made for adequate provision of pre-formed amino acids to the rumen to maintain maximal growth and fibre breakdown, more work is required to define both the

relationship between the degradability of fibre sources and their response to the presence of pre-formed amino acids, and also to reconcile the observed stimulation in degradability to the lack of a detectable effect on the numbers of cellulolytic bacteria.

On a methodological level it is interesting that the authors carried out their experiments in the Rusitec, rumen-simulating fermenter. Czerkawski (1986) himself noted the large number of rumen-simulating fermenters that have been described but rarely used for experimental work. He left it to the reader to speculate on the relationship between the operational complexity of such devices and their usefulness as experimental tools. It is a complement to the elegant simplicity of his design for a compartmentalized long-term rumen fermenter that Rusitec is still the tool of choice almost a quarter of a century after its initial design.

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