Letters to the Editors

Is proteolysis in the rumen of grazing animals mediated by plant enzymes?

Proteolytic enzymes in plants are extremely robust with broad spectrum pH and temperature optima and wide substrate specificities. They reside predominantly in the plant cell vacuole and are intimately associated with controlled cell death (apoptosis) during senescence. Vacuolar proteases are also involved in plant cell death in the silo, following the cutting of grass for silage. This phenomenon of proteolysis mediated by plant enzymes during ensilage is well documented, resulting in the conversion of plant proteins to lower molecular weight peptides and amino acids which are then degraded further by the developing silage microflora (Wetherall *et al.* 1995).

According to current dogma, protein breakdown in the rumen is generally regarded as a two-stage process whereby extra-cellular proteases produced by rumen micro-organisms convert plant proteins to lower molecular weight peptides and these are then degraded to ammonia by deaminases. However, the proteolytic activity of the rumen microbial population is only moderate when compared with other proteolytic micro-organisms and the animal's own gastric and pancreatic secretions (Wallace, 1995).

To our knowledge, no one has considered the possibility that proteolysis in grazing animals may occur under the influence of proteases of plant origin. Ruminants generally ingest intact fragments of plant biomass. These are abraded by initial chewing, coated in saliva, swallowed and incubated for many hours before particle size reduction during rumination. It is during the initial period of incubation of herbage in the rumen that we propose a role for plant proteases. Factors in favour of our hypothesis include (*a*) the elevated temperature (39°) of ingested herbage in the rumen, (*b*) a neutral to slightly acidic but reasonably constant ruminal pH, and (*c*) the anaerobic conditions in the rumen, all of which will contribute to plant cell death and hasten plant protease activity.

Plant proteases have not been considered in ruminal proteolysis in the past and this may be related to the fact that *in vitro* and *in sacco* digestion studies are usually conducted with finely ground, oven-dried plant particles and not with freshly harvested (living) plant biomass. Clearly, under these conditions plant protease activity would be inactivated before contact with rumen micro-organisms. Moreover, even where plant proteases do survive, in air- or freeze-dried plant biomass, the finely ground nature of the substrate with its increased surface area will promote bacterial attachment and activity, thus masking the effect of any plant-enzyme-mediated processes.

With regard to the apparently responsible proteolytic rumen bacteria, we would add that the major cellulolytic species, i.e. those most closely associated with (attached to) plant biomass, are not in fact proteolytic. Even with the anaerobic fungi, which are both cellulolytic and proteolytic, it was recently commented upon that their proteolytic activity was only sufficient to account for internal protein turnover, and thus they are not likely to have a significant role in the breakdown of plant proteins in the rumen (Asoa *et al.* 1993). If our hypothesis is correct, it will impact on the way we view proteolysis in ruminants and present us with alternative strategies to align protein and carbohydrate breakdown in the rumen.

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Multi-frequency impedance technique

We recently read with interest the article by Deurenberg *et al.* (1995) entitled 'Multi-frequency impedance for the prediction of extracellular water and total body water' published in the *British Journal of Nutrition*.

This article reports a well conducted study in which multi-frequency bioelectrical impedance (MFBIA) measures at low (1 and 5 kHz) and high (100 kHz) frequencies correlated with independent estimates of extracellular water (ECW) and total body water (TBW) respectively. These observations accord with theoretical predictions (Cornish *et al.* 1993). Ideally, however, MFBIA-derived prediction algorithms for these body-water compartments would be based upon impedances measured at zero frequency for ECW and much higher frequencies or infinite frequency (van Marken Lichtenbelt *et al.* 1994) or at the characteristic frequency (Cornish *et al.* 1993) for TBW. Whilst this point is made by Deurenberg *et al.* (1995), the authors then state 'Unfortunately, however, due to technical limitations and to interactions between electrodes and skin (Schwan, 1963), the *instruments available today are not able to measure impedances reliably at frequencies higher than 100 to 200 kHz (van Marken Lichtenbelt et al. 1994)*' [our italics]. It is with this statement that we take issue.

Deurenberg *et al.* (1995) did not measure impedance at any frequency higher than 100 kHz. They do not present evidence that the impedance analyser used in their study (Dietosystems, Milan, Italy) is indeed unreliable at frequencies above this value. We also supply multiple-frequency impedance analysers for research purposes which have been clearly demonstrated to perform reliably at much higher frequencies (at least up to 800 kHz) in both man (Stroud *et al.* 1995) and animals (Cornish *et al.* 1994). The availability of impedance data for this extended range of frequencies allows an alternative method of analysis to that used by Deurenberg *et al.* (1995), the so-called Cole–Cole plot (Thomas *et al.* 1992; van Marken Lichtenbelt *et al.* 1994), which may improve the predictive power of MFBIA (Cornish *et al.* 1993). The statement that impedance analysers are unreliable at high frequencies, precluding the Cole–Cole analytical approach, does MFBIA a disservice and may inhibit further research in this area.

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Reply from Deurenberg

In responding to the letter of Janet Caffin, we would like to make the following comments.

In the study published in the *British Journal of Nutrition* (Deurenberg *et al.* 1995*a*) we indeed did not measure impedance at higher frequencies than 100 kHz and not at lower frequencies than 1 kHz as the impedance analyser used (HUMAN-IM SCAN, Dietosystem, Milan, Italy) did not allow that. Of course we are aware of the fact that, theoretically, impedance at zero frequency and at infinite frequency is the best measure of extracellular water and total body water respectively.

However, as we could show in two studies, extrapolating impedance curves, both as impedance *v.* frequency (Deurenberg, 1995) as well as impedance *v.* reactance (Deurenberg *et al.* 1995*b*), has no clear advantages over measuring impedance at fixed low or high frequency. This is in accordance with other studies, both published (Gudivaka *et al.* 1994) as well as in the press (Hannan, personal communication). Possibly the errors in the estimated impedance values at zero frequency and infinite frequency are responsible for that phenomenon. Thus we doubt the possibility of obtaining better predictive equations with lower standard errors of estimates. All papers on multi-frequency impedance, however, have one thing in common. They all show that the specific resistivity of intracellular fluid is higher than of extracellular fluid. This is recognized in a recent consensus statement on bioelectrical impedance (National Institutes of Health, 1995). This means in our view that the prediction of total body water, using either the Cole–Cole plot or the simple impedance index approach, will always be dependent on the distribution of the two main body-water compartments. This is in fact the limitation the impedance method has for the quantitative prediction of body-water compartments. That impedance can, nevertheless, be a useful tool in assessing body hydration and changes in body hydration we showed in a short overview, recently published (Deurenberg *et al.* 1995*c*).

With our paper in the *British Journal of Nutrition* we did not want to discourage or inhibit research on (multi-frequency) impedance. In our view, research is best served in presenting findings as they are and discussing not only advantages but also limitations.

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