Effects of particle size in forage samples for protein breakdown studies

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Introduction Coupling ruminal processes of hydrolysis and synthesis continues to be a research issue where more progress is needed. This requires the development of good protein assessment methods, particularly when representing the breakdown processes that occur in fresh pastures eaten by herbivores. Laboratory analyses need to deal with small and homogeneous samples, but the mechanical reduction of particle size may not reflect the actual digestion kinetics occurring when the original fresh forage is consumed. Such physical traits may alter the release of non-structural compounds and the penetration of microbial enzymes (Boudon *et al.,* 2002). The objective of this work was to assess in fresh samples the effect of reducing particle size upon the *in vitro* breakdown of proteins during the early rumen fermentation period.

Materials and methods Eight fresh forage samples with contrasting endopeptidase activities were subjected to different strategies for particle size reduction. Protein hydrolysis was assessed by measuring the residual neutral detergent insoluble nitrogen (NDIN) (Licitra *et al*., 1996) and the accumulation of non-protein soluble nitrogen (NPSN) after 6 h *in vitro* rumen fermentation (IIV, Broderick, 1987). In fresh samples mastication-like damage was obtained with a device in which forage samples were pressed between two stony surfaces that simulated the animal molar surfaces. During the development of this method, microscopic observation was used in order to obtain a similar damage to that observed in samples obtained from the cardias of a fistulated adult cow fed the same type of fresh long forage. Three chopping sizes and two macerations were tested. Chopping was preceded by laboratory-mastication and further cutting to 3 cm, 1 cm or 0.25 cm; maceration was thoroughly done in a mortar with dry ice (CO_2) or liquid nitrogen. Treatments means were compared by Tukey-Kramer test at $P<0.05$.

Results and discussion Sample size significantly affected ($P<0.05$) the fractions of NDIN and NPSN (Figure 1), but the two macerates were essentially identical. As expected, the smaller the chopping the greater the solubility, with this effect being more pronounced in cultivars with lower endopeptidase activity. Mechanical particle comminution may facilitate access of external enzymes and activate the endogenous enzymatic system.

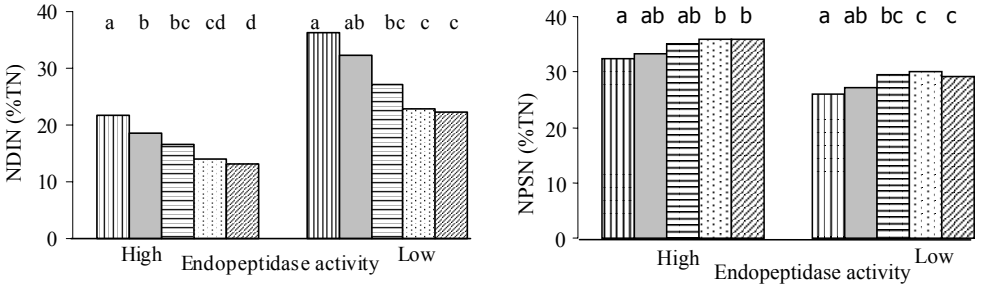

Figure 1 Effect of forage particle size on protein solubilisation during 6 h *in vitro* rumen fermentation
▥ : 3cm.; ▩ : 1cm.; ▤ : 0.25cm.; ⬚ : CO_2; ▨ : N_{Liq}

Conclusion Our results show that particle size is a major source of variation when studying kinetics of protein breakdown. The mechanical damage affects the release of fermentable substrates as well as the accessibility of bacteria or their enzymes into the plant cells and the activity of plant endopeptidases. Larger particles may be more representative of actual animal behaviour, but they present practical problems for analytical purposes.

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