Ruminal Proteolysis in Forages with Distinct Endopeptidases Activities

G. Pichard
Pontificia Universidad Católica de Chile

C. Tapia
Pontificia Universidad Católica de Chile

R. Larrain
Pontificia Universidad Católica de Chile

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005. The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

Ruminal proteolysis in forages with distinct endopeptidases activities

G. Pichard, C. Tapia and R. Larraín
Department of Animal Sciences, School of Agriculture, Pontificia Universidad Católica de Chile Email: gpichard@puc.cl

Keywords: forages, proteolysis, rumen, plant peptidases

Introduction Improving livestock efficiency in utilisation of nitrogen resources continues to be a major environmental and economic objective. Zhu *et al.* (1999) have shown that plant endopeptidases are activated as a response to cutting stress. Previous work in our laboratory explored over 300 entries of forage genotypes and found a broad diversity in enzymatic activity by means of hydrolysis in gelatine and direct autolysis assays in forage tissues. The objective of this work was to assess if the species previously identified as having high or low endopeptidase activity, would behave consistently when exposed to ruminal microbial proteolysis.

Materials and methods Two groups of forages were selected according with their level of peptidase activity (Table 1). They were grown in a greenhouse, fresh leaf samples were collected in vegetative stages, submitted to a molar-like pressing device and further chopped to 1 cm. The rumen fluid inocula was subjected to 3 hours pre-incubation with sugar for depletion of free N and further 2 hours with hydrazine-cloramphenicol inhibitors (Broderick, 1987). Fresh forage samples (2 g) were incubated *in vitro* during 6 hours (T_0 to T_6) with inhibited rumen fluid (IRF). The residue of neutral detergent insoluble nitrogen (NDIN) (Licitra *et al.,* 1996) and non protein soluble nitrogen (NPSN) were determined.

Table 1 Extent and rates of solubility of N compounds incubated in inhibited rumen fluid

Species	EPA*	Total N	NDIN (% of TN)			NPSN (% of TN)		
		(g/kg)	T_0	T_6	k_{0-6}	T_0	T_6	k_{0-6}
Avena strigosa cv. Negra	High	32	46	20	4.4	19	32	2.
Festuca arundinacea cv. Conway	High	23	62	17	7.5	22	31	1.5
Medicago sativa cv. Innovator	High	35	56	18	6.3	21	35	2.4
Trifolium repens cv. Blanca	High	37	51	19	5.4	22	34	1.9
Mean			54	19	5.9	22	33	1.9
Bromus unioloides cv. M. Fierro	Low	25	62	31	5.3	20	27	1.2
Lolium hybridum cv. Galaxy	Low	25	54	29	4.3	20	28	1.4
Trifolium pratense cv. Resistenta	Low	37	48	32	2.7	19	27	1.3
Trifolium repens cv. Kopu	Low	33	50	38	1.9	17	25	1.2
Mean			53	32	3.6	19	27	1.3
Effect of endopeptidases (*P* value)			NS	0.003	0.06	NS	0.012	0.03

* Endopeptidases activity

Results and discussion The extents and rates of NDIN disappearance and NPSN accumulation were statistically different between the groups of high and low enzymatic activity (Table 1). The extent of nitrogen solubilised from the ND residue was not accounted for by the fraction of NPSN, thus suggesting that it remains as a soluble true protein. Also, within the group with high endopeptidase activity, no major differences were observed between legumes and grasses.

Conclusion The activity of plant endopeptidases varies among different germplasms and affects ruminal proteolysis. Such plant diversity supports the idea that one way of improving nitrogen utilisation in pasture-based animal production systems is the genetic improvement of germplasms that have the potential for delayed protein hydrolysis. The laboratory enzymatic assays with gelatine showed to be consistent with the *in vitro* rumen microbial proteases. The large fraction of soluble proteins released in rumen soon after eating suggests that the potential for rumen by pass with the liquid phase may be high and should be reassessed.

Acknowledgements Funded by Fondecyt Grant 1030918

References

Broderick, G.A. (1987). Determination of protein degradation rates using a rumen *in vitro* system containing inhibitors of microbial nitrogen metabolism. *British Journal of Nutrition*, 58, 463-475.

Licitra, G., T.M. Hernández & P.J. Van Soest (1996). Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology*, 57, 347-358.

Zhu W-Y., A.H. Kingston-Smith, D. Troncoso, R.J. Merry, D.R. Davies, G. Pichard, H. Thomas & M.K. Theodorou (1999). Evidence of a role for plant proteases in the degradation of herbage proteins in the rumen of grazing cattle. *Journal of Dairy Science*, 82, 2651-2658.