

## Ruminal proteolysis in forages with distinct endopeptidases activities

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**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-chloramphenicol inhibitors (Broderick, 1987). Fresh forage samples (2 g) were incubated *in vitro* during 6 hours (T<sub>0</sub> to T<sub>6</sub>) 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 (g/kg)	NDIN (% of TN)			NPSN (% of TN)		
			T <sub>0</sub>	T <sub>6</sub>	k <sub>0-6</sub>	T <sub>0</sub>	T <sub>6</sub>	k <sub>0-6</sub>
<i>Avena strigosa</i> cv. Negra	High	32	46	20	4.4	19	32	2.
<i>Festuca arundinacea</i> cv. Conway	High	23	62	17	7.5	22	31	1.5
<i>Medicago sativa</i> cv. Innovator	High	35	56	18	6.3	21	35	2.4
<i>Trifolium repens</i> cv. Blanca	High	37	51	19	5.4	22	34	1.9
	Mean		54	19	5.9	22	33	1.9
<i>Bromus unioloides</i> cv. M. Fierro	Low	25	62	31	5.3	20	27	1.2
<i>Lolium hybridum</i> cv. Galaxy	Low	25	54	29	4.3	20	28	1.4
<i>Trifolium pratense</i> cv. Resistenta	Low	37	48	32	2.7	19	27	1.3
<i>Trifolium repens</i> cv. Kopu	Low	33	50	38	1.9	17	25	1.2
	Mean		53	32	3.6	19	27	1.3
Effect of endopeptidases ( <i>P</i> 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 germplasm 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 germplasm 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.

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### References

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