In vitro rumen simulated metabolism (RUSITEC) of freshly cut or wilted grasses with contrasting polyphenol oxidase activities: the effect on rumen parameters, lipolysis and biohydrogenation

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Introduction Polyphenol oxidase (PPO) in red clover has been shown to reduce both proteolysis and lipolysis in the silo and rumen. Lee *et al.* (2006) showed *in vitro* that grass PPO resulted in a reduction in plant mediated proteolysis and lipolysis. However, it is yet to be determined whether grass PPO has any effect on proteolysis and lipolysis in the presence of rumen micro-organisms. For PPO activity to occur, cell damage (mixing of enzyme and substrate) and aeration (oxidation) are required. These two criteria are easily met during silage making but during grazing the anaerobic nature of the rumen provides only a small window of opportunity during mastication for PPO activation. Therefore this study investigated the need to mix enzyme and substrate in the field by wilting and chopping versus cut grass in two species cocksfoot (*Dactylis glomerata*; high PPO) and tall fescue (*Festuca arundinacea*; low PPO).

Materials and methods A 16 vessel RUSITEC as described by Czerkawski and Breckenridge (1977) was used with four treatment combinations: cocksfoot wilted (C_w); cocksfoot fresh (C_f); tall fescue wilted (TF_w) and tall fescue fresh (TF_f). Rumen liquor was collected from 4 fistulated dairy cows maintained on permanent pasture. The wilted treatments (C_w and TF_w) were cut the previous day and left on the laboratory bench for 24 h. The fresh treatments (C_f and TF_f) were collected daily on ice. Once transferred to the laboratory all treatments were passed through a garden shredder, a sample taken for chemical analysis and ca. 10 g DM of each grass weighed into 4 Dacron bags for the relevant vessels. This was repeated daily with the bags remaining in the vessels for 48 h. The experiment ran for 12 days with sampling of effluent for rumen parameters on days 10 and 11. At the end of d 12 the grass residue and effluent from the vessels were collected and analysed for N and fatty acids. Lipolysis was calculated as the proportional loss of membrane glycerol-based lipid between the forage and 24 h residue. Biohydrogenation was calculated as the retention of C18 polyunsaturated fatty acids (PUFA) during incubation per unit PUFA supply. All analysis was performed as a general analysis of variance with species x wilt as the fixed effects (Genstat, Release 11.1).

Results PPO was significantly higher in C_f than the other treatments; C_w was higher than both TF treatments, with no difference between TF_w and TF_f. The level of bound phenol (product of oxidation reaction) was higher for C_w and TF_w than C_f and TF_f, and for C than TF. As an average across the day ammonia-N was lower in C than TF, despite the lower N concentration of the respective grasses, and in fresh as opposed to wilted grass. There was a trend for lipolysis to be lower in C than TF, and for both wilted treatments to be lower than the fresh. There was no difference in lipolysis between the species during wilting 0.14 and 0.11 for C and TF, respectively. Biohydrogenation and total VFA were not different between treatments.

Table 1 Chemical composition of grasses and the rumen parameters, lipolysis and biohydrogenation in RUSITEC

	Cocksfoot		Tall Fescue		S.e.d	P		
	Fresh	Wilted	Fresh	Wilted		Sp	F/W	Sp x F/W
PPO (µkatal (µmol/s))	15.7	8.06	1.26	1.57	0.534	***	*	***
Bound Phenol (mg/g DM)	1.81	2.11	0.21	0.52	0.279	***	*	NS
Grass N (g/kg DM)	27.1	27.4	25.3	25.5	0.71	**	NS	NS
Total VFA (mmol/l)	36.1	38.6	36.8	37.9	4.84	NS	NS	NS
Ammonia-N (µg/ml)	77.6	82.3	82.9	94.1	6.21	**	**	NS
Lipolysis (g/g membrane lipid)	0.83	0.80	0.87	0.82	0.023	†	*	NS
C18:2 Biohydrogenation (mg/g C18:2 input)	33.7	27.1	25.2	27.6	3.58	NS	NS	NS
C18:3 Biohydrogenation (mg/g C18:3 input)	7.70	7.25	6.05	9.16	2.505	NS	NS	NS

Sp, species effect; F/W, wilting effect; Sp x F/W, interaction; †P<0.1; *P<0.05; **P<0.01; ***P<0.001; NS P>0.1.

Conclusions As expected C had higher PPO activity and subsequent bound phenol concentrations than TF which may have resulted in a lower rumen ammonia N in C despite its greater N content. The greater level of ammonia N in wilted compared to fresh was possibly due to levels of plant mediated proteolysis during wilting. The effect of PPO in reducing proteolysis could also help explain the smaller effect on C as opposed to TF when the grasses were wilted. There was a trend for lower lipolysis in C than TF during incubation. Wilting resulted in a lower lipolysis than the fresh grasses during incubation which may have been due to the lower initial level of membrane lipid due to lipolysis during wilting. There was no effect on the flow of C18 PUFA in a simulated rumen environment. Differences in grasses other than just PPO activity such as lipase activity and digestibility may have confounding effects between grasses thus diminishing the protective effect of PPO.

References

Czerkawski, J.W. and Breckenridge, G. 1977. British Journal of Nutrition 38, 371-380.
Lee, M.R.F., Olmos Colmenero, J. J., Winters, A.L., Scollan, N.D. and Minchin, F.R. 2006. Journal of the Science of Food and Agruculture 86, 1503-1511

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