Effect of forage species, ryegrass water soluble carbohydrate concentration, and red clover polyphenol oxidase activity on *in vitro* rumen efficiency of nitrogen utilization

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Introduction A relatively small proportion of the nitrogen (N) consumed by the ruminant is transferred into meat (5-15%) or milk (15-30%), and most of the rest is excreted, with potential impacts on water, soil and air quality. Many fresh forage diets used for dairy cows present an imbalance between the rapidly available N and the slowly available energy in the rumen, which can limit microbial protein synthesis and increase excretion losses. In this experiment two strategies to improve the dietary energy/protein balance and their effects on microbial synthesis were studied. The first was to use perennial ryegrass (*Lolium perenne*) bred to express high water soluble carbohydrate (HWSC) concentrations. The second was to reduce and/or delay protein degradation in the rumen, to improve the energy/N synchronisation, by employing the activity of the enzyme polyphenol oxidase (PPO) in red clover (*Trifolium pratense;* RC). The latter promotes the formation of protease resistant cross-linked protein complexes (Lee *et al*., 2004).

Material and methods Two ryegrass varieties with high (AberMagic®; HWSC) or control (Premium; LWSC) WSC concentrations were used. Two types of RC were used: wild type (PPO+) or knockout (PPO-) strains, with normal and undetectable PPO activities respectively. The experiment was carried out in sixteen continuous culture rumen simulation technique (Rusitec) systems inoculated with fresh rumen contents from cows, using 4 vessels (700 ml nominal volume) per treatment. Grass was harvested daily during May 2009 and used fresh to avoid WSC degradation. Red clover material was grown in pots and was frozen to collect sufficient material. It was defrosted before use, which also helped to activate the PPO enzyme. All plants were chopped into 5 cm lengths, placed in nylon bags (50 g fresh material per bag) and remained in the fermenter vessels for 48h. The liquid dilution rate was maintained at 3.65%/h by continuous infusion of artificial saliva. After 9 days adaptation 2.3 mg ^{15}N/d was continuously infused to label the microbial protein. Days 10 to 12 of the experiment were used to determine the diet degradability, gas production and rumen fermentation, while effluent was collected during days 13 and 14 and mixed with their correspondent bag residue to reconstitute the digesta flow. Finally, on day 15 total (TB), liquid- (LAB) and solid-associated bacteria (SAB) were isolated (Carro and Miller, 1999) to determine microbial synthesis. Data were analysed by ANOVA blocking by machine and using three orthogonal contrasts to separately determine the effect of forage, WSC and PPO: C1 (grass *vs*. red clover), C2 (HWSC *vs*. LWSC) and C3 (PPO+ *vs*. PPO-).

Table 1 Effect of WSC and PPO content on rumen fermentation and microbial synthesis

	Ryegrass		Red clover		SED	Significance		
	HWSC	LWSC	PPO+	PPO-	*n=4*	C1	C2	C3
Rumen fermentation								
pH	6.76	6.75	6.88	6.87	0.035	***	NS	NS
VFA (mM)	48	51	42	41	4.2	*	NS	NS
N-NH_3 (mg/dl)	6.6	5.8	9.3	9.6	0.33	***	*	NS
Gas production (ml/h)								
Total	84	70	67	65	8.6	†	NS	NS
Methane	0.74	0.71	0.63	0.57	0.371	NS	NS	NS
Degradability (%)								
OM	63	59	73	73	2.1	***	†	NS
N	75	72	85	83	2.0	***	NS	NS
NDF	38	29	36	16	4.2	NS	*	*
ADF	28	17	21	22	4.2	NS	*	NS
Post-ruminal flow (g/d)								
OM	9.2	9.9	9.5	10.2	0.99	NS	NS	*
N	0.16	0.15	0.29	0.32	0.015	***	NS	NS
NAN	0.11	0.10	0.20	0.21	0.008	***	NS	NS
NAN/N intaked	0.56	0.52	0.58	0.62	0.027	*	NS	†
Microbial N flow (g/d)								
TB	0.06	0.06	0.10	0.10	0.005	***	NS	NS
LAB	0.08	0.07	0.09	0.10	0.005	***	NS	†
SAB	0.13	0.12	0.18	0.19	0.010	***	NS	NS
Synthesis efficiency (g MN/kg OMADR)								
TB	10	10	19	20	0.9	***	NS	NS
LAB	12	12	18	19	1.1	***	NS	NS
SAB	20	20	35	38	2.2	***	NS	NS

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

Results Ryegrass led to higher VFA concentrations, while RC increased pH, ammonia concentrations and degradabilities of OM and N. Red clover also increased the flow of total N and non-ammonia-N (NAN) compared with ryegrass, even when normalized for N supply. In agreement with that, RC also increased microbial N flow and synthesis efficiency in comparison with grass, regardless of the microbial extraction used. Estimates of SAB synthesis were higher than those estimated using TB and LAB. No differences between treatments were observed in either gas production or methane emissions. Within ryegrass treatments, WSC promoted an increase in ammonia and fibre degradability but did not affect microbial synthesis. PPO activity neither decreased the N degradability nor improved the N utilization by this system.

Conclusion RC promoted a more efficient use of dietary energy and N than ryegrass; however no significant effects of WSC content or PPO activity were observed under our conditions.

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References Carro, M.D. and Miller, E.L., 2002. Animal Science 75, 315-321
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