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Variation for protein degradation in three forage legume species

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SUMMARY – The efficiency of proteins of legume forages in ruminant nutrition is poor because of their extensive degradation in the rumen. The objective of this study was to evaluate the variation available for protein degradation in tannin-free (alfalfa and white clover) and tannin-rich (*Lotus* sp.) legume species. Protein and dry matter degradation were measured by *in sacco* incubation of forage samples in the rumen of fistulated cows, for 2, 8 or 48 h. Condensed tannin content was measured on *Lotus* samples. Variation in protein degradation was low among alfalfa and white clover cultivars, but the degradation was more important for alfalfa than for clover cultivars. Protein degradation was less important in *Lotus* sp. than in alfalfa or clover, and the variation among *Lotus* cultivars was mainly related to condensed tannin content.

Key words: Alfalfa, white clover, *Lotus,* tannin.

RESUME – *"Variabilité pour la dégradation des protéines chez trois espèces de légumineuses fourragères". La valorisation des protéines issues des légumineuses fourragères dans l'alimentation des ruminants est médiocre à cause de leur importante dégradabilité ruminale. L'objectif de l'étude était d'évaluer la variabilité disponible pour la dégradabilité des protéines chez des espèces sans tannins (luzerne et trèfle blanc) et chez des espèces riches en tannins (lotiers,* Lotus *sp.). La dégradation des protéines et de la matière sèche a été mesurée par l'incubation* in sacco *dans le rumen de vaches fistulées d'échantillons de fourrage pendant 2, 8 ou 48 h. La teneur en tannins condensés a été mesurée pour les échantillons de lotiers. La variabilité entre cultivars de luzerne ou de trèfle pour la dégradation des protéines est faible, mais la dégradation de la luzerne est plus importante que celle du trèfle. La dégradation des protéines des lotiers est moins importante que celle de la luzerne et du trèfle, et la variation entre cultivars de lotier est principalement liée à la teneur en tannins.*

Mots-clés : *Luzerne, trèfle blanc, lotier, tannin.*

Introduction

Legume forage species produce forage with high protein content. However, a large part of these proteins are degraded in the rumen, inducing a low efficiency of the proteins of the diet, nitrogen losses detrimental to the environment, and risks of bloat when the forages are grazed. Some legume species produce secondary metabolites, the condensed tannins, known to reduce the ruminal degradation of the proteins (McMahon *et al*., 2000). But the tannins are also known to reduce the dry matter digestibility and the palatability. Condensed tannins are present in the birdsfoot trefoil (*Lotus corniculatus*) and other *Lotus* species, in various concentrations depending on the species, the cultivar and the growing conditions (McMahon *et al*., 2000). The most cultivated forage legumes in Europe, alfalfa (*Medicago sativa*) and white clover (*Trifolium repens*), have leaves and stems free of tannins (Robbins and Morris, 1999).

The objective of this study was to assess the genetic variation for protein degradation in two tannin-free legume species (alfalfa and white clover) and tannin-rich species of the Lotus genus. On *Lotus* sp., an experiment was run to analyse the relationship between protein degradation and condensed tannin concentration.

Materials and methods

Experiment 1

A trial with five alfalfa cultivars (Luisante, Milfeuil, Kayserie, 5715, 4G73), four white clover cultivars (Grassland Huia, Aberherald, Aberdai, Aran) and five lotus cultivars (three *L. corniculatus*: Exact, Wellington, Leo; one *L. pedunculatus:* Maku; and one *L. tenuis*) was sown in spring 1999 at INRA Lusignan (France). The first cut of spring 2000 was harvested, and the samples were dried at 60ºC and ground to pass a 2 mm-grid. Protein degradation was measured by the incubation of Nylon bags containing 2.5 g forage samples placed in the rumen of fistulated cows, during 2, 8 and 48 h, in triplicate. After the incubation, the bags were rinsed, dried and weighed. Protein content of the initial samples and of the residues was determined. Tannin content was determined in *Lotus* samples (Terrill *et al*., 1992). Analyses of variance were made to test the significance of the effects of species and cultivar among species for the protein and dry matter degradation after each time of incubation.

Experiment 2

Several accessions of *Lotus* sp. were grown in various experimental conditions in glasshouse at IGER, Aberystwyth (UK). For 5 samples, aerial parts were harvested, and leaves and stems were separated, and freeze-dried. After grinding, leaves were analysed for protein degradation in the rumen of fistulated cows as previously described. Condensed tannin content was determined. Analysis of variance was performed to test the effect of the sample on the protein and the dry matter degradation. Correlation between condensed tannin content and degradation was calculated.

Results and discussion

Experiment 1

On average, the *Lotus* and clover samples had higher protein content than alfalfa (Table 1). For dry matter degradation, clover samples were more degraded at each time of incubation than *Lotus* and alfalfa. For protein degradation, alfalfa was the most degraded after 2 and 8 h of incubation, followed by clover, and *Lotus* was the least degraded. Over the 14 samples, there was significant variation among species and among cultivars within species (Table 2). Comparing alfalfa and red clover (*Trifolium pratense*), Owens *et al*. (1999) also found a higher protein degradation in alfalfa than in clover.

Among species, there was variation in dry matter degradation in *Lotus* and in alfalfa, but not in clover. For protein degradation, there was no variation in alfalfa and clover, but there was significant variation among *Lotus* samples (Table 3). Similarly, on alfalfa, Tremblay *et al*. (2000) found a limited genetic variation among cultivars for protein degradability. Coulman *et al*. (2000) developed a bloat-reduced alfalfa cultivar, but the bloat safety is associated with a reduction in the initial rate of dry matter degradation. On clover species, Cohen (2001) established that most variations for protein degradability were associated to variation in protein content.

Among the 5 *Lotus* cultivars, the correlations between condensed tannin content and dry matter or protein degradation were higher than 0.9. Hedqvist *et al.* (2000) published that protein degradability was correlated to tannin concentration, despite the fact that their concentrations were low.

Experiment 2

Tannin content in *Lotus* leaf samples varied from 0.65 to 5.57%, but protein content was rather similar (Table 4). Dry matter and protein degradation varied a lot after 2 or 8 hours of incubation (Table 5), and the variations were highly correlated to tannin content. After 48 h of incubation, variation in dry matter degradation, and even more in protein was very limited. Tannins seemed to delay protein degradation rather than to reduce the potential degradation after a long time of incubation in the rumen. Sample H, with a high tannin content had an extensive protein and dry matter degradation. This result suggests that the analysis of tannin structure (monomeric composition) could help in explaining the variation in protein degradation not entirely linked to condensed tannin content. Aerts *et al*. (1999) showed that the

condensed tannins from *L. corniculatus* and *L. pedunculatus* had different effectiveness in preventing protein degradation.

Table 1. Protein content, residual dry matter (DM) and protein after 2, 8 and 48 hours of incubation of the rumen of fistulated cows, and condensed tannin (CT) content for 14 cultivars of 3 legume species, harvested in spring 2000

Species	Cultivar	Protein content	% residual DM after			% residual protein after			CT content
			2 h	8 h	48 h	2 h	8 h	48 h	
Lotus	6 - Exact	20.9	62.4	51.2	25.1	55.9	44.4	9.3	1.17
Lotus	7 - Wellington	21.3	62.1	53.4	23.7	56.7	48.8	8.8	1.23
Lotus	8 - Leo	22.8	62.0	51.2	23.6	55.3	42.3	8.5	0.83
Lotus	9 - *L. ped.* Maku	21.7	68.3	64.4	30.5	72.4	66.9	19.9	2.33
Lotus	10 - *L. tenuis*	20.6	61.3	50.9	23.5	54.1	45.4	7.7	0.67
Alfalfa	1 - Luisante	19.8	61.6	50.3	24.1	43.5	36.4	8.2	–
Alfalfa	2 - Kayserie	18.9	66.0	51.6	27.6	46.5	33.0	10.0	–
Alfalfa	3 - 5715	16.8	65.2	51.2	27.7	49.6	35.4	10.1	–
Alfalfa	4 - 4G73	19.9	65.0	49.9	25.3	48.2	32.6	8.7	–
Alfalfa	5 - Milfeuil	19.5	64.4	51.1	26.5	46.0	30.3	9.0	–
Clover	11 - Grassland Huia	21.3	58.5	48.0	10.3	50.7	42.6	4.8	–
Clover	12 - Aberherald	21.1	58.5	48.3	11.1	50.7	43.4	6.0	–
Clover	13 - Aberdai	21.9	57.0	46.0	10.1	49.0	40.6	5.6	–
Clover	14 - Aran	20.4	56.8	44.6	9.5	49.0	39.4	4.2	–
Means									
Overall		20.8	62.1	51.1	21.6	52.4	42.2	8.8	
Lotus		21.5	63.2	54.2	25.3	58.9	49.6	10.8	1.25
Alfalfa		19.0	64.5	50.5	26.2	47.3	33.2	9.3	–
Clover		21.2	57.7	46.7	10.3	49.8	41.5	5.1	–

Table 2. Analysis of variance for dry matter and protein degradation, with the effect of species, cultivar within species and cow. The mean squares are indicated

	% residual DM after			% residual protein after		
	2 h	8 h	48 h	2 h	8 h	48 h
Species	112.9***	156.7***	699.4***	373.5***	788.3***	80.8***
Cultivar (species)	12.2***	40.5***	13.0***	66.6***	115.0***	29.4***
Cow	3.1**	24.0**	0.33 NS	37.1**	17.4 NS	1.2 NS
Residual	0.5	3.6	0.3	6.5	7.5	0.7

Table 3. Analysis of variance for dry matter and protein degradation, for each species, with the effect of cultivar and cow. The mean squares are indicated

Species	Effect	% residual DM after			% residual protein after		
		2 h	8 h	48 h	2 h	8 h	48 h
Lotus	Cultivar	24.6***	100.1***	27.2***	174.2***	297.1***	78.0***
	Cow	1.2*	18.6**	0.4 NS	5.0 NS	23.4 NS	0.8 NS
	Residual	0.3	2.4	0.2	5.8	7.1	1.8
Alfalfa	Cultivar	6.9**	4.2*	7.8**	6.8 NS	11.8 NS	1.5*
	Cow	1.6 NS	14.0**	0.3 NS	46.5*	8.1 NS	1.0 NS
	Residual	0.5	1.0	0.5	8.7	8.4 NS	0.4
Clover	Cultivar	2.7 NS	9.4 NS	1.2*	2.8 NS	9.7 NS	1.9**
	Cow	0.7 NS	3.0 NS	0.0 NS	6.2 NS	8.3 NS	0.0 NS
	Residual	0.9	5.6	0.2	1.4	4.4	0.1

Table 4. Protein content, residual dry matter (DM) and protein after 2, 8 and 48 hours of incubation of the rumen of fistulated cows, and condensed tannin (CT) content (%) for leaves of *Lotus* sp.

Sample	Protein content	% residual DM after			% residual protein after			CT content
		2 h	8 h	48 h	2 h	8 h	48 h	
A	26.4	47.0	18.8	4.8	48.1	19.4	2.6	0.65
D	26.3	50.0	27.2	6.8	52.1	28.9	2.8	1.92
F	25.8	53.2	34.4	8.8	61.9	41.1	5.5	3.00
H	27.9	52.6	17.4	5.2	60.5	20.3	2.9	2.59
J	24.7	52.9	43.1	9.7	64.2	57.2	5.4	5.57
Mean	26.2	51.1	28.2	7.1	57.4	33.4	3.9	2.75

Table 5. Analysis of variance for dry matter and protein degradation, with the effect of *Lotus* sample and cow. The mean squares are indicated

Effect	% residual DM after			% residual protein after		
	2h	8h	48h	2h	8h	48h
Sample	20.6***	349.0***	14.0**	142.4***	763.2***	6.6**
Cow	0.3 NS	42.6**	8.8*	2.2 NS	63.1*	5.3*
Residual	1.3	5.5	1.9	2.4	12.2	0.7

Conclusions

Variation for protein degradation in tannin-free forage species (alfalfa, white clover) seems to be very limited. On the contrary, protein degradation in *Lotus* species is more variable, and is partly related to condensed tannin concentration. Barry and McNabb (1999) defined that a condensed tannin content below 5% reduces the rate of degradation of proteins in the rumen and increased animal production. Above 5%, the tannins are detrimental to animal growth and performance. Prospects in reducing protein degradation in alfalfa and clover could be: (i) to mix, in the diet or in the stands, these species with tannin-rich species as *Lotus*; and (ii) to introduce genes of condensed tannin pathway by transformation. Both alfalfa and white clover produce tannins in specialised organs or tissues within the organs, seed coats and petals (Morris and Robbins, 1997), indicating that the genes of the pathway are present but not expressed in the stems and the leaves of these species.

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