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Original article

Variation in protein degradability in dried forage legumes

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Abstract — Proteins in forage legumes are rapidly degraded in the rumen, inducing a poor dietary protein efficiency, risk of bloat, and nitrogen loss detrimental to the environment. The aim of this study was to investigate the variation in ruminal protein degradability among legume species and cultivars. Four species, each represented by 1 to 16 cultivars, were studied: lucerne (*Medicago sativa*), white clover (*Trifolium repens*), birdsfoot trefoil (*Lotus* sp.) and crownvetch (*Coronilla varia*). In a first experiment, forage samples of 16 cultivars of lucerne harvested in the autumn of 1998 were incubated in nylon bags in 3 fistulated cows in order to obtain the kinetics of ruminal degradation of crude protein and dry matter. The phenotypic variation in crude protein degradation was not significant, and was partly related to the dry matter degradation. In a second experiment, lucerne (5), birdsfoot trefoil (5), white clover (4) and crownvetch (1) cultivars were harvested in two cuts in 2000, and dry matter and crude protein degradation were analysed at 3 incubation times (2, 8 and 48 h) in the rumen of the fistulated cows. Crude protein degradation was higher for lucerne than for white clover, and these two tannin-free species exhibited greater crude protein degradation than crownvetch and birdsfoot trefoil. In birdsfoot trefoil, crude protein degradation was negatively correlated to condensed tannin content but positively correlated to dry matter degradation. Except for birdsfoot trefoil, the range of genetic variation within species for in situ crude protein degradation was low.

protein degradation / dry matter degradation / *Medicago sativa* / *Lotus* sp. / *Trifolium repens* / *Coronilla varia*

Résumé — Variabilité pour la dégradabilité des protéines de légumineuses fourragères. Les protéines des légumineuses fourragères sont rapidement dégradées dans le rumen, conduisant à une faible efficacité des protéines de la ration, des risques de météorisation et des pertes d'azote polluant l'environnement. L'objectif de cette étude était d'évaluer la variabilité de la dégradabilité ruminale des protéines entre espèces et cultivars de légumineuses fourragères. Quatre espèces, chacune représentée par 1 à 16 cultivars, ont été étudiées : la luzerne (*Medicago sativa*), le trèfle blanc (*Trifolium repens*), le lotier corniculé (*Lotus* sp.) et la coronille (*Coronilla varia*). Dans une première expérience,

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des échantillons de 16 variétés de luzerne récoltés durant l'automne 1998 ont été introduits dans des sachets de Nylon et placés dans le rumen de trois vaches fistulées pour obtenir la cinétique de dégradation ruminale des protéines et de la matière sèche. La variabilité phénotypique pour la dégradabilité des protéines n'était pas significative, et elle était en partie reliée à la dégradation de la matière sèche. Dans une seconde expérience, des variétés de luzerne (5), de lotier corniculé (5), de trèfle blanc (4) et de coronille (1) ont été récoltés en deux coupes en 2000, et les dégradations des protéines et de la matière sèche ont été analysées à trois temps d'incubation (2, 8 et 48 h) dans le rumen de vaches fistulées. La dégradation des protéines était plus élevée pour la luzerne que pour le trèfle blanc, et ces deux espèces sans tannin avaient une plus forte dégradation des protéines que le lotier et la coronille. Pour le lotier, la dégradation des protéines était corrélée négativement à la teneur en tannins condensés, mais corrélée positivement à la dégradation de la matière sèche. A l'exception du lotier, la variabilité à l'intérieur d'une espèce pour la dégradabilité des protéines in situ était faible.

dégradation des protéines / dégradation de la matière sèche / *Medicago sativa* / *Lotus* sp. / *Trifolium repens* / *Coronilla varia*

1. INTRODUCTION

Forage legumes produce protein-rich forage, but the proteins are extensively degraded into amino acids and ammonia in the rumen. Protein degradation contributes to microorganism growth, but proteins may be lost through ammonia when the energy of the diet is not sufficient. The nitrogen lost into the environment can cause pollution [36]. Protein degradation may induce the formation of foaming agents causing bloat [17]. Rapid and extensive ruminal degradation of proteins generally leads to the inefficient use of dietary protein. Excessive ruminal protein degradation may be the most limiting nutritional factor in high-quality temperate legume forages [8, 12]. The most profitable situation for the animal is when a large proportion of the proteins go directly into the small intestine where they are degraded into amino acids and absorbed. Proteins escaping rumen degradation are called escape proteins in the American system [28] and PDI (Digestible Proteins in the Intestine) in the French system [41].

The in situ degradation technique, based on the incubation of feed samples in nylon bags in the rumen of fistulated animals for various time intervals, is widely used and has been standardised to characterise the ruminal degradability of dietary nitrogen [19, 21, 26]. In vitro methods, based on the incubation in a buffer [42], in proteolytic enzymes [29] or in a system with rumen fluid and inhibitors [7], have also been developed to predict the proportion of protein escaping ruminal fermentation.

In lucerne, Broderick and Buxton [10] found variation in protein degradability among 19 *M. sativa* accessions and 3 *M. falcata* accessions. Skinner et al. [33] found differences in the degradation rates of proteins among nine lucerne accessions. Griffin et al. [13] found differences among cultivars for escape proteins, but the differences were inconsistent across years and cuttings. Tremblay et al. [38] analysed genetic differences among 27 lucerne cultivars for the ruminal undegradable protein concentration, the protein degradation rate, and dry matter yield. They found low genetic variation among the cultivars for these traits, but concluded it is feasible to combine a high dry matter yield with a low protein degradation rate. Gutek et al. [14] reported low inheritance for soluble protein in lucerne, and Rooney et al. [30] found that the heredity of protein degradability traits is mainly additive.

In white clover, no description of genetic variation for protein degradation is available in the literature, but Ayres et al. [5] indicated that protein degradability decreases with advancing maturity.

In birdsfoot trefoil (*Lotus corniculatus*), genetic variation in protein degradation is attributed to the condensed tannin content [6, 15, 23], although Broderick and Albrecht [9] stated that the tannin content explains only part of the variation in protein degradation. Rioux et al. [31], comparing two cultivars, found differences in crude protein content, in situ crude protein degradation and tannin content.

Griffin et al. [13] indicated that increases in in situ escape protein in lucerne are concommitant to a decrease in crude protein content and in situ dry matter degradation and to an increase in cell-wall concentration. Indeed, Coulman et al. [11] developed a bloat-reduced lucerne cultivar through 4 cycles of selection based on a reduction of the initial rate of degradation of dry matter. These two studies indicate a conflict between escape protein content and other parameters of forage quality.

The objective of this present study was to determine, among and within temperate legume species, the variation in ruminal protein and dry matter degradation using the in situ nylon bag technique.

2. MATERIALS AND METHODS

2.1. Experiment 1

One hundred lucerne cultivars were sown at Lusignan (France) in 1996 in a randomised three-block design. The samples were harvested on the fourth cut on 15 October 1998, at the early bud stage (all the cultivars belonged to the same dormancy group so no significant variation was noticed). This cut was chosen because autumn cuts always present high crude protein contents that could increase the chance of detecting genetic variation in crude protein degradation. Crude protein and NDF contents were predicted by NIRS for each cultivar in each block, and 16 cultivars (Tab. I) were chosen to cover the range of crude protein content and NDF content observed for the 100 cultivars (6 units of percentage for both traits). Samples of the 3 blocks were bulked, dried at 60 ºC for 72 h and ground to pass through a 2-mm sieve. The bulking of blocks was done to adapt the number of individual samples to the technical constraints of the in situ experiment.

The in situ experiment was conducted with three fistulated cows fed twice daily in equal portions (70% lucerne and 30% concentrate; 1.3% body weight per day on a dry matter basis at 08.00 and 16.00 h; continuous access to water). The concentrate composition was as described by Michalet-Doreau et al. [24]. The lucerne was harvested at the bud stage and well stored. Nylon bags (size 10×7 cm, pore size 46 µm) containing 2.5 g of a dried sample were prepared. The residuals were obtained after 2, 4, 8, 14, 24, 48 or 72 h of incubation for the 16 cultivars, each in three cows with 2 replicates (i.e. 672 bags). A control sample, composed of maize stem was included and removed after 8 h of incubation. Each animal received 8 series over a 3 week period. In each series, the bags corresponding to 4 cultivars in one replicate were placed in the rumen of fistulated cows before the morning feed and were removed after the corresponding time. The bags were frozen and washed three times in cold water (using a washing machine with a washing program of 5 min) to remove a large part of bacterial contamination, and dried at 60 ºC for 72 h. The residues of the two replicates were pooled and ground to pass a 1 mm-sieve. The effect of the series was not taken into account because the control sample gave stable results. The residues and the initial forage samples were analysed in triplicate for nitrogen content by the Kjeldahl procedure [1]. Crude protein degradation was calculated for each cow from the dry matter degradation and the crude protein content of the residue.

Crude protein and dry matter degradation curves were fitted to the Ørskov and

Table I. Origin, crude protein content, neutral detergent fibre (NDF) content and parameters of the crude protein degradation kinetics and asymptotic standard errors (in parentheses) for 16 lucerne cultivars (Experiment 1). The extreme values are in bold.

Cultivars	Origin	Crude protein[1] (% of DM[2])	NDF (% of DM)	a[3] (%)	b[4] (%)	c[5] (h^{-1})	U[6] (%)	ED[7] (%)
Opal	US	21.5	32.0	37.1 (2.4)	53.3 (2.4)	0.073 (0.009)	9.6 (4.3)	66.4 (4.3)
4G73	US	**25.0**	30.5	38.6 (1.8)	52.5 (1.9)	0.080 (0.007)	8.9 (3.4)	68.6 (3.3)
Kara	France	23.5	31.5	39.6 (2.0)	51.5 (1.9)	**0.088 (0.008)**	8.9 (3.6)	**70.3 (3.5)**
Vermont	France	21.4	**34.8**	38.4 (2.7)	51.5 (2.8)	0.077 (0.011)	10.1 (5.0)	67.3 (4.9)
Europe	France	22.4	31.0	37.8 (2.9)	**54.1 (3.0)**	0.074 (0.011)	8.0 (5.3)	67.8 (5.3)
8920MF	US	23.6	30.7	**41.6 (3.2)**	51.6 (3.4)	**0.053 (0.011)**	**6.8 (5.5)**	65.8 (5.7)
Esterel	France	21.2	31.1	40.3 (2.6)	50.2 (2.7)	0.070 (0.010)	9.5 (4.7)	67.4 (4.7)
Cherokee	US	20.4	33.6	39.3 (2.6)	49.0 (2.7)	0.072 (0.011)	11.7 (4.8)	66.1 (4.7)
5715	US	**19.3**	33.2	39.8 (2.10)	**46.0 (2.2)**	0.063 (0.009)	**14.3 (3.7)**	**63.4 (3.7)**
Milfeuil	France	22.2	31.3	40.6 (2.8)	51.0 (3.0)	0.064 (0.011)	8.4 (4.9)	66.9 (5.0)
5151	US	22.0	30.4	**34.7 (2.4)**	57.1 (2.4)	0.072 (0.008)	8.2 (4.3)	66.0 (4.2)
Yliki	Greece	19.4	34.2	40.2 (3.0)	47.9 (3.1)	0.063 (0.012)	11.9 (5.2)	64.8 (5.3)
Luisante × Rival	France	21.5	30.9	38.0 (2.5)	**54.1 (2.6)**	0.071 (0.009)	7.9 (4.5)	67.5 (4.5)
Verko	Hungary	22.5	**29.1**	40.7 (2.5)	51.4 (2.6)	0.069 (0.010)	7.9 (4.6)	68.3 (4.5)
Topaz	Romania	22.4	30.6	38.7 (2.7)	53.0 (2.9)	0.065 (0.010)	8.3 (4.8)	66.2 (4.9)
4G68	US	23.5	31.4	35.5 (2.2)	55.4 (2.3)	0.071 (0.008)	9.1 (3.9)	65.6 (3.9)
Mean		22.0	31.6	38.8	51.8	0.070	9.3	66.8
Statistical test[8]		0.3	0.5	8.6 (ns)	19.2 (ns)	10.9 (ns)	2.9 (ns)	2.8 (ns)

[1] crude protein = total nitrogen × 6.25; [2] dry matter; [3] soluble fraction; [4] potentially degradable fraction; [5] rate of degradation of b fraction; [6] undegradable fraction; [7] effective degradability fixing the rate of passage in the rumen at $0.06 \cdot h^{-1}$; [8] the statistics used to test the differences among the 16 cultivars was a residual standard error for crude protein and NDF content in an analysis of variance with the effect of cultivar and block; it was a Wald test (Sw) for degradation parameters. Wald statistics is to be compared to a χ^2 with 15 d.f (25.0). A statistics lower than 25.0 indicates no differences among cultivars (ns).

McDonald [27] exponential model, for each cultivar, using the values of the 3 cows:

$$p = a + b\,(1 - \exp^{-ct})$$

where p is the percentage of crude protein or dry matter degradation, a is the soluble (or rapidly degraded) fraction, b is the potentially degradable fraction, and c is the rate of degradation of fraction b (h^{-1}) in the rumen. The parameters a, b and c and their asymptotic standard errors were estimated with the non-linear regression procedure NLIN of SAS [32], using the Marquardt option. The effective degradability of crude protein or dry matter was calculated with the Ørskov and McDonald [27] equation:

$$ED = a + bc/(c + k)$$

assuming a constant ruminal passage rate ($k = 0.06 \cdot h^{-1}$, [41]). The crude protein effective degradability was not corrected for microbial contamination [26]. The undegradable fraction U was calculated as:

$$U = 100 - (a + b).$$

The statistical test of difference for each parameter among cultivars was performed through a Wald test based on the asymptotic standard errors of the parameters and the assymtotic correlations among parameters [18], using S-Plus software [35]. Correlations between in situ crude protein degradation and chemical measurements were calculated with the CORR procedure of SAS [32].

2.2. Experiment 2

A trial with 5 lucerne cultivars (Luisante, Milfeuil, Kayserie, 5715, 4G73), 4 white clover cultivars (Grassland Huia, Aberherald, Aberdai, Aran), 5 birdsfoot trefoil cultivars (three *L. corniculatus*: Exact, Wellington, Leo, 1 *L. pedunculatus* Maku, and 1 *L. tenuis* accession 3403) and one crownvetch (*Coronilla varia*) cultivar (Lucor) was planted in the spring of 1999 at INRA Lusignan (France) in a randomised block design. In 2000, at the beginning of flowering for each species, the first two cuts for clover (17 May and 15 June) and birdsfoot trefoil (17 May and 20 June) and the first and the third cuts for lucerne and crownvetch (19 May and 09 August) were harvested with a forage harvester, and a sample of about 400 g of fresh forage per plot was taken by hand. The samples were dried at 60 °C, and ground to pass through a 2 mm-sieve. Dry matter and crude protein degradation were measured as in experiment 1 but at only 2, 8 and 48 h of ruminal incubation (i.e. 270 bags) as described by Mathis et al. [22]. Condensed tannin content was determined in birdsfoot trefoil and crownvetch samples [37].

On the initial forage samples, ground to pass through a 1 mm sieve, crude protein content was determined with the Dumas method [1], and the percentage of residual crude protein was calculated for each cultivar after each time of incubation.

For each species, the design was analysed as a split-plot. For each incubation time, the effects of the cut and cow were tested against the cut × cow interaction, and the effects of cultivar and cultivar × cut interaction were tested against the residual error, using the GLM procedure of SAS [32]. For the four species, the analysis of variance was restricted to the first cut, and the effects of species, cultivar among species and cow were tested for the crude protein and dry matter degradation after each time of incubation. The data of birdsfoot trefoil and crownvetch were assembled to represent the crude protein residue and dry matter residue as a function of condensed tannin content.

3. RESULTS

3.1. Experiment 1

The 16 lucerne cultivars showed a variation in crude protein and NDF contents, with 5.7 percentage units of difference for

crude protein content between the two extreme cultivars (Tab. I), and 5.7 percentage units for NDF content (Tab. II).

Table I shows the parameters of the crude protein degradation kinetics of the 16 cultivars. Effective degradability ranged from 63.4 to 70.3%. Kara had the highest effective degradability with the highest rate of degradation of the potentially degradable fraction. Cv. 5715 had the lowest effective degradability and the highest undegradable fraction. Cultivars with high effective crude protein degradability also exhibited high rates of degradation of the potentially degradable fraction, and a low undegradable fraction. Even if the parameters of crude protein degradation kinetics varied among cultivars, these variations were not significant according to the Wald test (Tab. I).

Cultivars with high effective degradability of dry matter (Tab. II) also had a low undegradable fraction (Europe), and a large rate of degradation, while cultivars with the lowest effective degradability had the highest undegradable fraction (Yliki) and a low rate of degradation. The cultivar with the highest effective degradability of crude protein (Kara) had an intermediate dry matter effective degradability, but in general, cultivars with a high effective degradability

Table II. Parameters of the dry matter degradation kinetics of 16 lucerne cultivars (Experiment 1). The extreme values are in bold.

Cultivars	*a* (%)	*b* (%)	*c* (h^{-1})	*U* (%)	*ED* (%)
Opal	34.0 (2.6)	45.5 (2.6)	0.090 (0.012)	20.5 (4.7)	61.4 (4.7)
4G73	32.0 (2.4)	**49.8 (2.3)**	**0.111 (0.012)**	18.2 (4.5)	64.5 (4.4)
Kara	31.5 (1.8)	48.3 (1.8)	0.108 (0.009)	20.2 (3.4)	62.6 (3.4)
Vermont	**29.5 (2.3)**	47.8 (2.7)	**0.111 (0.012)**	22.7 (4.4)	60.6 (4.3)
Europe	**35.3 (4.0)**	48.2 (3.9)	0.095 (0.019)	16.6 (7.4)	**64.9 (7.3)**
8920MF	34.4 (2.9)	48.0 (2.9)	**0.079 (0.013)**	17.5 (5.3)	61.8 (5.2)
Esterel	32.3 (2.9)	47.5 (2.8)	0.093 (0.014)	20.2 (5.3)	61.3 (5.2)
Cherokee	32.9 (2.9)	45.6 (2.8)	0.095 (0.014)	21.5 (5.3)	60.8 (5.3)
5715	33.0 (2.1)	**43.9 (2.1)**	0.089 (0.011)	23.1 (3.9)	59.3 (3.9)
Milfeuil	32.6 (3.0)	49.0 (3.0)	0.085 (0.013)	18.4 (5.5)	61.4 (5.4)
5151	34.0 (2.1)	**49.8 (2.1)**	0.090 (0.009)	**16.2 (3.9)**	64.0 (3.8)
Yliki	29.6 (2.7)	46.9 (2.7)	0.086 (0.013)	**23.4 (5.0)**	**57.3 (5.0)**
Luisante × Rival	33.5 (2.6)	48.7 (2.6)	0.090 (0.012)	17.8 (4.7)	62.8 (4.7)
Verko	34.2 (2.5)	48.4 (2.4)	0.096 (0.012)	17.4 (4.6)	64.1 (4.5)
Topaz	32.0 (2.2)	48.7 (2.2)	0.092 (0.010)	19.3 (4.0)	61.5 (3.9)
4G68	34.0 (2.6)	48.6 (2.6)	0.096 (0.012)	17.4 (4.8)	64.0 (4.8)
Mean	32.8	47.8	0.094	19.4	62.0
Statistical test	5.9	7.3	9.6 (ns)	3.9 (ns)	2.8 (ns)

See legend in Table I.

of dry matter also had a high effective degradability of crude proteins (r = 0.840, $P < 0.001$, 14 d.f.).

The effective degradability of crude protein was correlated to crude protein content ($r = 0.619$, $P = 0.01$, 14 d.f.) but weakly correlated to NDF content ($r = –0.464$, $P = 0.07$, 14 d.f.).

3.2. Experiment 2

On average, birdsfoot trefoil, white clover and crownvetch samples had significantly higher crude protein content than lucerne (Tab. III). In the first cut, there was greater dry matter degradation for clover samples than for birdsfoot trefoil, lucerne

Table III. Crude protein content, condensed tannin (CT) content, residual dry matter and crude protein after 2, 8 and 48 hours of incubation of the rumen of fistulated cows, for 15 cultivars of 4 legume species, harvested in the spring of 2000 (Experiment 2).

Cut	Species	Cultivar	Crude protein	CT[1]	% residual DM after			% residual crude protein after		
			(% of DM)	(% of DM)	2 h	8 h	48 h	2 h	8 h	48 h
1	Crownvetch	Lucor	24.4	1.13	62.1	56.6	25.1	55.8	52.7	11.2
1	B. trefoil[2]	Exact	20.9	1.59	62.4	51.2	25.1	55.9	44.4	9.3
1	B. trefoil	Wellington	21.3	1.44	62.1	53.4	23.7	56.7	48.8	8.8
1	B. trefoil	Leo	22.8	1.35	62.0	51.2	23.6	55.3	42.3	8.5
1	B. trefoil	Maku	21.7	4.43	68.3	64.4	30.5	72.4	66.9	19.9
1	B. trefoil	3403	20.6	0.67	61.3	50.9	23.5	54.1	45.4	7.7
1	Lucerne	Luisante	19.8	–	61.6	50.3	24.1	43.5	36.4	8.2
1	Lucerne	Kayserie	18.9	–	66.0	51.6	27.6	46.5	33.0	10.0
1	Lucerne	5715	16.8	–	65.2	51.2	27.7	49.6	35.4	10.1
1	Lucerne	4G73	19.9	–	65.0	49.9	25.3	48.2	32.6	8.7
1	Lucerne	Milfeuil	19.5	–	64.4	51.1	26.5	46.0	30.3	9.0
1	W. clover	Grassland Huia	21.3	–	58.5	48.0	10.3	50.7	42.6	4.8
1	W. clover	Aberherald	21.1	–	58.5	48.3	11.1	50.7	43.4	6.0
1	W. clover	Aberdai	21.9	–	57.0	46.0	10.1	49.0	40.6	5.6
1	W. clover	Aran	20.4	–	56.8	44.6	9.5	49.0	39.4	4.2
3	Crownvetch	Lucor	25.6	0.43	50.9	43.3	12.5	58.3	47.9	6.4
2	B. trefoil	Exact	21.2	1.17	55.7	41.3	14.9	58.4	41.5	5.2
2	B. trefoil	Wellington	23.0	1.23	55.1	41.8	13.6	56.6	42.5	5.3
2	B. trefoil	Leo	23.3	0.83	57.4	43.0	15.5	56.2	40.9	5.6
2	B. trefoil	Maku	23.1	2.33	65.4	60.4	15.1	80.8	77.2	9.9
2	B. trefoil	3403	20.4	0.67	54.9	44.2	13.4	61.5	49.0	5.6
3	Lucerne	Luisante	20.6	–	55.9	43.7	21.7	40.0	26.2	5.8
3	Lucerne	Kayserie	18.7	–	57.1	44.9	23.6	38.3	25.5	7.0
3	Lucerne	5715	16.3	–	60.1	50.1	26.6	41.7	30.8	8.6
3	Lucerne	4G73	21.4	–	57.4	45.5	22.4	39.1	24.2	5.6
3	Lucerne	Milfeuil	19.9	–	56.2	44.2	22.3	43.1	27.8	6.1
2	W. clover	Grassland Huia	24.9	–	61.1	40.3	7.3	63.0	44.7	4.8
2	W. clover	Aberherald	22.7	–	61.0	41.7	7.3	65.5	49.3	4.8
2	W. clover	Aberdai	23.4	–	60.5	45.5	8.8	63.2	48.6	6.0
2	W. clover	Aran	22.5	–	58.0	43.7	7.7	60.8	45.3	5.9
Means										
1st cut			20.8	1.89	62.1	51.1	21.6	52.4	42.2	8.8
2nd cut			21.8	1.11	57.8	44.9	15.5	55.1	41.4	6.2
	Crownvetch		25.0	0.78	56.5	49.9	18.8	57.0	50.3	8.8
	B. trefoil		21.8	1.57	60.5	50.2	19.9	60.8	49.9	8.6
	Lucerne		19.2	–	60.9	48.1	24.7	43.9	30.0	8.0
	Clover		22.3	–	58.9	44.8	9.0	56.5	44.2	5.3

[1] Crownvetch and birdsfoot trefoil only; [2] Birdsfoot trefoil.

Table IV. Analysis of variance for crude protein and dry matter residues after 2, 8 and 48 hours of incubation of the rumen of fistulated cows, for each species, with the effects of cut, cow and cultivar in a split-plot design (Experiment 2). The mean squares are shown.

	% residual crude protein after			% residual dry matter after		
	2 h	8 h	48 h	2 h	8 h	48 h
Birdsfoot trefoil						
Cut	110.5 *	3.3 ns	150.1 ns	227.5 **	489.0 *	874.9 ***
Cow	1.9 ns	14.9 ns	7.9 ns	0.8 ns	19.9 ns	1.7 ns
Cut × cow	6.4 ns	66.0 *	13.5 **	0.9 ns	7.6 ns	1.1 ns
Cultivar	472.0 ***	946.5 ***	75.1 ***	78.6 ***	282.0 ***	18.6 ***
Cultivar × cut	22.3 *	62.6 **	15.0 **	4.5 ***	12.7 **	11.3 ***
Residual	7.4	11.9	1.9	0.5	2.3	0.6
Lucerne						
Cut	358.2 ns	297.6 ns	53.3 **	386.0 *	174.8 ns	62.2 ***
Cow	4.3 ns	7.9 ns	1.6 *	15.4 ns	0.7 ns	0.6 ns
Cut × cow	81.3 ***	25.4 *	0.1 ns	13.1 ***	28.6 ***	1.7 ns
Cultivar	9.1 ns	20.8 *	5.3 ***	11.6 ***	15.6 *	16.7 ***
Cultivar × cut	9.0 ns	10.7 ns	0.6 ns	3.7 *	8.0 *	2.5 *
Residual	7.0	6.6	0.2	1.0	1.9	0.6
White clover						
Cut	1057.8 *	414.6 ns	0.3 ns	35.9 ns	0.0 ns	38.3 **
Cow	2.6 ns	22.9 ns	3.9 ns	0.28 ns	15.4 ns	0.4 ns
Cut × cow	24.7 **	16.7 *	3.3 ns	2.4 *	21.6 *	0.20 ns
Cultivar	10.8 *	11.1 ns	1.1 ns	7.9 ***	0.5 ns	0.8 *
Cultivar × cut	3.1 ns	4.9 ns	2.0 ns	1.2 ns	19.2 *	2.0 ***
Residual	6.2	3.3	1.5	0.8	4.3	0.2
Crownvetch						
Cut	9.0 ns	7.1 ns	35.0 ns	187.4 **	78.8 ns	237.2 ***
Cow	8.9 ns	16.2 ns	2.2 ns	0.8 ns	8.3 ns	0.3 ns
Residual	4.3	23.3	5.3	2.0	0.8	0.1

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant.

and crownvetch at each incubation time. In the second cut, there was less dry matter degradation after 2 h of incubation for clover than for the other three species, but more degradation after 8 or 48 h of incubation. The crude protein degradation after 2 and 8 h was greater in lucerne than in clover, birdsfoot trefoil and crownvetch, but crude protein degradation after 48 h of incubation was the highest in clover. In

Table V. Analysis of variance for crude protein and dry matter residues after 2, 8 and 48 hours of incubation of the rumen of fistulated cows, in the spring cut, with the effects of species, cultivar within species and cow (Experiment 2). The mean squares are shown.

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

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: not significant.

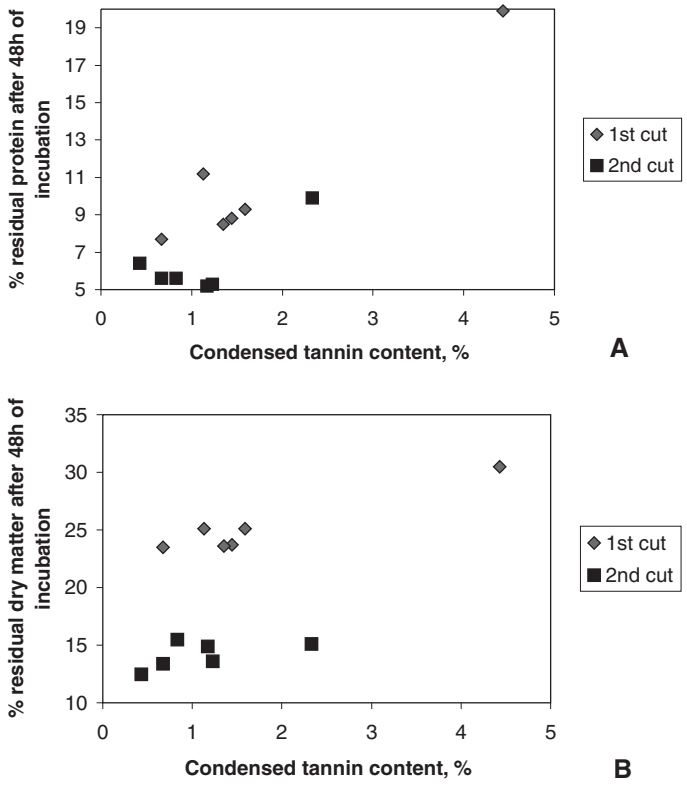

Figure 1. Relationship between condensed tannin content (% of DM) and crude protein residual after 48 h of incubation (A) and dry matter residual after 48 h of incubation (B).

analysis of variance for each species, the effect of the cut was often significant for dry matter degradation, but not for crude protein degradation (Tab. IV). Within birdsfoot trefoil species (Tab. IV), the cultivar effect was significant for all traits of degradation, and the interaction between the cultivar and the cut was also significant. In lucerne, a cultivar effect was observed for dry matter degradation, but it was unsignificant at 2 h for crude protein degradation and weakly significant at 8 h. The interaction between

the cultivar and the cut was only significant for dry matter degradation. In the white clover, the cultivar effect was only significant for crude protein degradation after a 2 h incubation and for dry matter degradation after 2 and 48 h. For the data of the first cut, the effects of the species and cultivar within species were significant for all traits (Tab. V).

In birdsfoot trefoil and crownvetch, the condensed tannin content varied from 0.43 to 4.43% of the dry matter (Tab. III). A relationship can be drawn between condensed tannin content and residual crude protein or dry matter after incubation in the rumen (Fig. 1), especially for the samples with a high condensed tannin content. After 8 h of incubation, the birdsfoot trefoil cultivar with the highest tannin content, Maku, had a residual dry matter of 64.4%, compared to 50.9% for the cultivar with the lowest tannin content, accession 3403; Maku had a residual crude protein of 66.9% instead of 45.4% for accession 3403.

4. DISCUSSION

On average, crude protein degradation was more rapid in lucerne than in white clover, and both were degraded more extensively than birdsfoot trefoil and crownvetch. This is in agreement with the studies of Broderick and Albrecht [9]. Similarly, comparisons between lucerne, birdsfoot trefoil and red clover (*T. pratense*) showed that clover proteins were less degraded than lucerne, but more degraded than proteins of birdsfoot trefoil cultivars with high tannin content [8]. Hoffman et al. [16] found differences among lucerne, birdsfoot trefoil and red clover for in situ protein and dry matter degradation; the rank of the species was modified according to the maturity stage, but the ruminally undegradable protein concentration was greater for lucerne than birdsfoot trefoil at all maturity stages.

Both experiments showed little variation in crude protein degradation in lucerne. In the evaluation of the 16 cultivars of the first experiment, the variation in the kinetics of dry matter degradation was limited too. Indeed the cut was performed in the autumn, with a high crude protein content and a low NDF content. In the second experiment, variations among lucerne cultivars were observed for dry matter degradation. Griffin et al. [13] and Tremblay et al. [38, 39] also found little variation for protein degradation among lucerne cultivars, with whole plant, leaf or stem samples. Broderick and Buxton [10] and Skinner et al. [33], who included *M. falcata* accessions in their studies, found differences between accessions. Julier et al. [20] described differences among lucerne cultivars for dry matter and fibre (NDF) degradation kinetics harvested in the spring. The rates of crude protein degradation presented in this study were lower than those obtained by Amrane and Michalet-Doreau [2], Antoniewicz et al. [3], or Aufrère et al. [4] with similar in situ experiments. The comparison of data from different groups are always difficult because the various conditions known to influence the results (sample preparation, grinding, animals, bags, washing…) are not standardised [26], even though recommendations that should increase the precision of in situ measurements have been recently presented [40].

Among white clover cultivars, small differences were observed after 2 h of incubation for crude protein degradation and after 2 and 48 h for dry matter degradation. To our knowledge, it is the first experiment that describes the genetic variation for in situ crude protein degradation of white clover.

Both crude protein and dry matter degradation differed markedly among birdsfoot trefoil cultivars. The condensed tannin content tended to correlate with dry matter and crude protein residuals after incubation in the rumen. The *L. pedunculatus* cultivar Maku had the highest tannin content and the least degraded crude protein and dry matter. The tannin had an effect on crude

protein and dry matter degradation even when the tannin content was low, as observed by Hedqvist et al. [15]. Condensed tannins would more delay the crude protein degradation than to reduce it, as proved by the crude protein residue after 48 h of incubation close to that of lucerne.

In lucerne and birdsfoot trefoil, a negative correlation was observed between degraded dry matter and non-degradable crude protein. This is in accordance with the fact that undegraded proteins after a long time of incubation are linked to the cell wall [34]. In birdsfoot trefoil, crude protein degradation is a combined result of cell wall degradation and condensed tannin content. Furthermore, tannins can have a negative impact on cell wall degradation [43].

Breeding for decreased crude protein degradability seems to offer limited prospects in lucerne and white clover as a consequence of low genetic variation among cultivars and good correlation with dry matter degradability. In birdsfoot trefoil, a variation of crude protein degradation can be achieved through condensed tannin content modification. Further progress probably implies to manipulate condensed tannin content using molecular techniques [6, 25]. In birdsfoot trefoil, the objective would be to reduce tannin content, or to modify its monomeric composition, without affecting protein degradability. In lucerne or white clover, for which the tannin pathway is effective in the seed coat, the activation of the pathway in leaves would be a major aim.

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