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Crude protein degradation in leaves and stems of alfalfa (*Medicago sativa*)

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SUMMARY – Leaves, stems, and whole-plants of 27 cultivars were assessed for ruminal undegradable proteins (RUP) using an inhibitor *in vitro* procedure and for protein fractions using the Cornell Net Carbohydrate and Protein System (CNCPS). The CNCPS divides the protein into soluble non protein nitrogen (A), soluble true protein (B1), rapidly degradable true protein (B2), slowly degradable protein (B3), and undegradable protein (C). Whole-plant crude protein (CP) (19.7% of DM) was intermediate between leaf CP (28.4%) and stem CP (10.7%), and differences among cultivars were significant for leaf CP. The cultivars differed significantly for leaf and stem RUP but not for whole-plant RUP. In whole-plants, soluble protein fractions (A and B1) accounted for 44.3% of the CP whereas fractions B2, B3, and C accounted for 49.4, 2.2, and 4.1%, respectively. The cultivars did not differ significantly for any of these protein fractions in whole-plants. The soluble fractions (A and B1) accounted for 32.2% of CP in leaves and for 42.6% in stems whereas the fraction B2 was 62.5 and 40.5% of the CP in leaves and in stems, respectively. Significant differences were observed among cultivars for A and B1 fractions in leaves and stems and for the B2 fraction in leaves. No difference was observed among cultivars for B3 and C fractions in leaves and stems. Maturity affected all protein fractions of whole-plant CP and the B3 fraction in leaves and the C fraction in stems. In both leaves and stems, RUP values were negatively correlated with the soluble fractions (A and B1) but positively correlated with degradable true protein fractions (B2 and B3).

Key words: Protein degradability, protein fractions, ruminal undegradable protein.

RESUME – *"Dégradation des protéines brutes des feuilles et des tiges de luzerne* (Medicago sativa)*". Les feuilles, les tiges et les plantes entières de 27 cultivars ont été évaluées pour leur teneur en protéine non dégradable dans le rumen (RUP) selon une méthode* in vitro *et pour les fractions protéiques du Cornell Net Carbohydrate Protein System (CNCPS). Selon le CNCPS, la protéine brute (PB) est divisée en azote soluble non protéique (A) et en azote protéique soluble (B1), rapidement dégradable (B2), lentement dégradable (B3) et non dégradable (C). La PB des plantes entières (19,7% de la MS) était intermédiaire entre celle des feuilles (28,4%) et des tiges (10,7%), et les différences entre les cultivars étaient significatives pour la PB des feuilles. Les cultivars différaient significativement pour la RUP des feuilles et des tiges mais non pour celle des plantes entières. Chez les plantes entières, les fractions protéiques solubles (A et B1) totalisaient 44,3% de la PB tandis que les fractions B2, B3 et C représentaient respectivement 49,4, 2,2 et 4,1% ; les cultivars ne différant significativement pas, pour aucune de ces fractions protéiques. Les fractions solubles (A et B1) représentaient 32,2% de la PB des feuilles et 42,6% de celle des tiges tandis que la fraction B2 représentait 62,5 et 40,5% de la PB des feuilles et des tiges, respectivement. Des différences significatives entre les cultivars ont été observées pour les fractions A et B1 des feuilles et des tiges et pour la fraction B2 des feuilles. Aucune différence n'a été observée entre les cultivars pour les fractions B3 et C des feuilles et des tiges. La maturité a affecté toutes les fractions protéiques des plantes entières, la fraction B3 des feuilles et la fraction C des tiges. Chez les feuilles et les tiges, les valeurs RUP étaient corrélées négativement avec les fractions solubles (A et B1) mais corrélées positivement avec les fractions protéiques dégradables (B2 et B3).*

Mots-clés : *Protéine non dégradable, fraction protéique, dégradabilité de la protéine.*

Introduction

Alfalfa is a very important source of protein for ruminants, but its protein is often poorly used because of extensive ruminal degradation. Thus, the nutritional quality of alfalfa would be greatly enhanced if the amount of protein escaping microbial degradation in the rumen was increased. Significant genetic variation was reported in alfalfa for ruminal *in vitro* protein degradability (Broderick and Buxton, 1991; Griffin *et al.,*1994; Skinner *et al.,* 1994; Guines *et al.,* 2000; Tremblay *et al.,* 2000). Aufrere *et al*. (1994) found that rumen undegraded protein (RUP) concentration, estimated by *in situ* procedure, was lower in leaves than in stems. However, Broderick *et al*. (1993) reported that RUP concentration, measured with an inhibitor *in vitro* method, was higher in leaves than in stems. Hoffman *et al*. (1993) and Griffin *et al.*

(1994) demonstrated that *in situ* protein degradability decreased with maturity in spring growth whereas Broderick *et al.* (1992) found no effect of maturity on protein degradability when an *in vitro* method was used. Our objectives were: (i) to determine the extent of genetic variation among 27 alfalfa cultivars for whole-plant, leaf and stem *in vitro* RUP concentrations measured using an inhibitor *in vitro* system (Broderick, 1987), and protein fractions measured according to the Cornell Net Carbohydrate and Protein System (CNCPS) (Licitra *et al.,* 1996); and (ii) to evaluate the effect of maturity at harvest on RUP and protein fractions.

Materials and methods

Twenty-seven alfalfa cultivars were seeded in a field at the Normandin Research Farm of Agriculture and Agri-Food Canada (Lat. 48º51'N, Long. 72º32'W) in the spring of 1995 under a randomized complete block design with three replications. In the spring of 1997, all cultivars were harvested on the same date when the majority of them reached 10% bloom. A second sampling was made one week later on three cultivars to determine the effect of maturity on protein fractions and RUP. Two whole-plant samples of approximately 400 g were taken from each plot and dried at 55ºC. Leaves and stems were separated on one sample. All samples were ground in a Wiley mill through a 1-mm screen.

Ruminal protein degradation was assessed using an inhibitor *in vitro* procedure (Broderick, 1987). The net release of NH_3 and total amino acids after incubating for 2 h in rumen fluid was used to estimate the ruminal protein degradation rate (PDR). *In vitro* RUP concentration was calculated from the PDR estimate, assuming a passage rate from the rumen of 0.06/h. Each sample was analysed in triplicate and RUP was expressed on a crude protein basis.

Protein fractions were estimated using a borate-phosphate buffer under a pH of 6.7-6.8, and NDF and ADF solutions as indicated in the CNCPS procedure (Licitra *et al*., 1996; Michaud and Tremblay, 1999). The total crude protein (CP) and the CP concentration of ADF and NDF were determined by Kjeldahl analysis (AOAC, 1990). All samples were analysed in duplicate.

Results and discussion

Whole-plant CP was intermediate between leaf and stem CP (Table 1). Differences among cultivars were significant for leaf CP. The cultivars differed significantly for leaf and stem RUP but not for whole-plant RUP. Tremblay *et al*. (2000) reported significant differences among alfalfa cultivars for whole-plant RUP at the significance level of P = 0.10. In whole-plants, soluble protein fractions (A and B1) accounted for 44.3% of the CP concentration whereas fractions B2, B3, and C accounted for 49.4, 2.2, and 4.1%, respectively. The cultivars did not differ significantly for any of these protein fractions in whole-plants (Table 1). Broderick and Buxton (1991) also reported no significant difference in total N or acid detergent insoluble nitrogen (fraction C) among 22 alfalfa entries. However, they detected significant effects of the germplasm source on fractions A and B, and *in vitro* RUP concentration which were all determined with the ruminal inhibitor *in vitro* system described by Broderick (1987).

The soluble fractions (A and B1) accounted for 32.2% of the CP in leaves and for 42.6% in stems whereas the fraction B2 was 62.5 and 40.5% of the CP in leaves and stems, respectively. Significant differences were observed among cultivars for A and B1 fractions in leaves and stems and for the B2 fraction in leaves. Our data are very similar to those obtained by Elizalde *et al.* (1999) and confirm that alfalfa CP is highly soluble and readily degraded in the rumen. Our results are similar to those of Aufrere *et al*. (1994) who reported that there was more soluble protein in stems (35.4% of CP) than in leaves (27.4% of CP). No difference in leaves and stems was observed among cultivars for fractions B3 and C. Michaud and Tremblay (1999) also reported no difference among genotypes in whole-plants for fraction C.

In both leaves and stems, RUP values were negatively correlated with the soluble fractions (A and B1) but positively correlated with degradable true protein fractions (B2 and B3) (Table 2). Lower values estimated for RUP than for fractions B2 and B3 (Table 1) were expected because a certain proportion of the fraction B2 is readily degraded in the rumen resulting in lower RUP values. However, the very high correlations between RUP values and fractions B2 and B3 indicate a very close relationship between the values estimated using a rumen *in vitro* system and the CNPCS. The significant negative correlations

obtained between soluble protein fractions (A and B1) and insoluble fractions (B2 and B3) differ from the lack of correlation previously reported on individual genotypes (Michaud and Tremblay, 1999).

Table 1. Comparison between 27 alfalfa cultivars for whole-plant, leaf and stem protein fractions and *in vitro* rumen undegradable proteins (RUP)

	CP (% of DM)	Protein fractions (% of CP)				*In vitro* RUP (% of CP)
		A and B1	B2	B3	C	
Whole plant						
Mean	19.7	44.3	49.4	2.2	4.1	24.3
Range	18.4-21.8^{NS}	42.5-47.2^{NS}	46.9-51.8^{NS}	1.8-2.7^{NS}	3.4-4.8^{NS}	22.5-26.3^{NS}
SEM†	0.705	1.309	1.265	0.263	0.269	0.853
Leaves						
Mean	28.4	32.2	62.5	3.7	1.6	27.6
Range	25.4-30.3**	26.3-40.2**	54.9-68.7**	2.8-4.3^{NS}	1.5-1.9^{NS}	23.7-33.2*
SEM	0.786	2.580	2.413	0.407	0.085	1.517
Stems						
Mean	10.7	42.6	40.5	7.5	9.4	27.0
Range	9.5-12.0^{NS}	35.9-50.5*	34.7-44.1^{NS}	5.0-10.7^{NS}	8.5-10.6^{NS}	20.3-36.5**
SEM	0.500	2.584	1.710	1.147	0.597	1.895

†SEM = Standard error of the mean.

*Significant at P < 0.05, **Significant at P < 0.01, NS = non-significant.

Table 2. Pearson correlation coefficients between protein quality traits measured on 27 alfalfa cultivars at 10% bloom stage and expressed on a DM basis. Values for stems and leaves are shown in the upper and lower diagonals, respectively

Traits (% of DM)	Crude protein	*In vitro* RUP	A and B1	B2	B3	C
Crude protein		-0.50*	0.88*	0.04	-0.55*	0.24*
In vitro RUP	-0.23*		-0.78*	0.65*	0.82*	-0.33*
Fractions A and B1	0.54*	-0.71*		-0.42*	-0.81*	0.32*
Fraction B2	0.19	0.61*	-0.72*		0.54*	-0.23*
Fraction B3	-0.13	0.66*	-0.51*	0.40*		-0.50*
Fraction C	0.13	0.16	-0.11	0.21	0.18	

*Significant at P < 0.05.

Maturity had a highly significant effect on all protein fractions of whole-plant CP (Table 3) and on the B3 fraction in leaves and the C fraction in stems (data not shown). In whole-plants, the soluble protein fractions (A and B1) decreased whereas fractions B2, B3 and C, and *in vitro* RUP increased with maturity. Our results based on three cultivars and one week difference in maturity seem to confirm the findings of Hoffman *et al*. (1993) and Griffin *et al.* (1994) who reported that protein degradability decreased with maturity in spring growth of alfalfa. However, our results and those of Griffin *et al*. (1994) indicate that a decrease in protein degradability with maturity was concomitant with a decrease in CP and possibly other forage quality parameters.

Conclusions

Our results indicate that leaves and stems differ in protein fractions and RUP. Furthermore, differences exist among cultivars in fractions A and B1, and RUP in leaves and stems, and in fraction B2 in leaves. Our results indicate that it is unlikely that improved whole-plant RUP would be achieved by

simply selecting for high RUP in leaves although approximately two-thirds of the protein in alfalfa herbage is in leaves. We previously reported that high RUP concentration is often associated with low yielding cultivars (Tremblay *et al*., 2000). Therefore, considerable attention should be paid to yield when selecting for RUP. Maturity affected all protein fractions of whole-plant CP, the B3 fraction in leaves and the C fraction in stems. In both leaves and stems, RUP values were negatively correlated with the soluble fractions (A and B1) but positively correlated with degradable true protein fractions (B2 and B3).

Table 3. Effects of maturity on whole-plant protein quality traits measured on three alfalfa cultivars

	10% bloom	1 week later	Significance	SEM†
Crude protein (% of DM)	20.0	18.4	**	0.398
Protein fractions (% of CP)				
A and B1	43.5	35.7	***	0.774
B2	50.2	54.1	**	0.915
B3	2.3	5.2	***	0.268
C	4.0	4.9	**	0.202
In vitro RUP (% of CP)	25.2	30.4	***	0.400

†SEM = Standard error of the mean.

**Significant at P< 0.01, ***Significant at P < 0.001.

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