

## Protein fractions and ruminal undegradable proteins in alfalfa

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Tremblay, G. F., Michaud, R. and Bélanger, G. 2003. **Protein fractions and ruminal undegradable proteins in alfalfa.** *Can. J. Plant Sci.* **83**: 555–559. The relationship between protein fractions of the Cornell Net Carbohydrate and Protein System (CNCPS) and in vitro ruminal undegradable protein (RUP) concentration was studied using variability among 14 genotypes and 27 cultivars of alfalfa harvested at early bloom in the spring growth. Significant differences in soluble N concentration (fraction A + B1), degradable true protein fractions (fractions B2 and B3), and in vitro RUP concentration were found among genotypes but not among cultivars. Correlations between in vitro RUP values and fractions A + B1 and B2 were significant for genotypes ( $r = -0.77$  and  $r = 0.78$ ) and cultivars ( $r = -0.72$  and  $r = 0.64$ ). Protein fractions of the CNCPS should be considered as an alternative laboratory method for in vitro RUP to screen alfalfa genotypes for breeding purposes.

**Key words:** Protein fractions, ruminal undegradable protein, alfalfa

Tremblay, G. F., Michaud, R. et Bélanger, G. 2003. **Protéines non dégradables au niveau du rumen et fractions protéiques de la luzerne.** *Can. J. Plant Sci.* **83**: 555–559. La relation entre les fractions protéiques du “Cornell Net Carbohydrate and Protein System” (CNCPS) et la concentration en protéines non dégradables au niveau du rumen mesurée in vitro (RUP) a été étudiée en utilisant la variabilité observée entre 14 génotypes et 27 cultivars de luzerne récoltés au stade début floraison d’une croissance printanière. Des variations significatives des concentrations en N soluble (fraction A + B1), en protéines vraies dégradables (fractions B2 et B3) et en RUP mesurée in vitro ont été observées entre les génotypes mais pas entre les cultivars. Les corrélations entre les valeurs de RUP mesurées in vitro et les fractions A + B1 et B2 étaient significatives chez les génotypes ( $r = -0,77$  et  $r = 0,78$ ) et les cultivars ( $r = 0,72$  et  $r = 0,64$ ). Les fractions protéiques du CNCPS pourraient être utilisées comme alternative à la mesure in vitro de la concentration en RUP lors de la sélection de génotypes de luzerne.

**Mots clés:** Fractions protéiques, protéine non dégradable, luzerne

Alfalfa is an important source of protein for ruminants, but its protein is often poorly used because it is extensively degraded during ruminal fermentation. This degradation may be the most limiting factor of high-quality forage legumes. The nutritional quality of alfalfa could be greatly enhanced by increasing the amount of protein that escapes microbial degradation in the rumen. Significant genetic variation has been reported in alfalfa for ruminal in vitro protein degradability (Broderick and Buxton 1991; Skinner et al. 1994; Griffin et al. 1994; Guines et al. 2000; Tremblay et al. 2000).

Several in situ or in vitro techniques that require ruminal experimentation have been used to estimate protein degradation. These procedures are limited for use in plant breeding programs; they require fistulated animals that are expensive to maintain, and they are difficult to standardize due to the variability associated with diet and cows.

In vitro procedures that utilize enzymes or chemicals have shown promise as routine laboratory techniques to estimate RUP; the Cornell Net Carbohydrate and Protein System (Sniffen et al. 1992) has been used extensively to characterize protein degradation in forages. The characterization of the crude protein (CP) fractions according to the

CNCPS is as follows: fraction A is non-protein N, B is true protein, and C is unavailable true protein. Fraction B is further divided into three fractions (B1, B2, and B3) that have different rates of ruminal degradation. Fractions A and B1 are soluble in borate-phosphate buffer and are rapidly degraded in the rumen. Fraction B1 is soluble in this buffer but insoluble in trichloroacetic or tungstic acid solution. Fraction B2 is insoluble in this buffer but soluble in neutral detergent, and fraction B3 is insoluble in this buffer and neutral detergent but is soluble in acid detergent. Fraction C is insoluble in acid detergent and is assumed to be unavailable to the animal. Fraction B3 is believed to be degraded more slowly in the rumen than fractions B1 and B2, and a large portion of fraction B3 is believed to escape the rumen.

**Abbreviations:** ADF, acid detergent fiber; CNCPS, Cornell net carbohydrate and protein system; CP, crude protein; DM, dry matter; LWR, leaf to weight ratio; NDF, neutral detergent fiber; PC, principal component; PCA, principal component analyses; RUP, ruminal undegradable proteins; TN, total nitrogen

**Table 1. Statistics for variates measured on alfalfa genotypes and cultivars**

	A+B1	B2	B3	C	in vitro RUP	TN	ADF	NDF	DM yield (Mg ha <sup>-1</sup> )
	(mg g <sup>-1</sup> of TN)					(mg g <sup>-1</sup> of DM)			
<i>Genotypes</i>									
Minimum	329	462	30	28	266	30.5	264	314	— <sup>z</sup>
Maximum	468	596	49	40	360	36.2	325	380	—
Mean	406	520	41	34	308	33.6	293	353	—
SEM	14.4	14.9	3.7	2.4	17.6	1.40	15.1	16.7	—
LSD (5%) lower limit <sup>y</sup>	385	499	35	30	283	31.6	271	329	—
LSD (5%) upper limit <sup>x</sup>	426	541	46	37	333	35.6	314	377	—
Significance probability <sup>w</sup>	<0.01	<0.01	0.04	0.09	0.003	0.07	0.20	0.26	—
<i>Cultivars</i>									
Minimum	425	469	18	34	225	29.5	282	347	1.53
Maximum	472	518	27	48	263	34.9	339	413	2.79
Mean	443	494	22	41	243	31.6	306	375	2.39
SEM	13.1	12.7	2.6	2.7	8.5	1.13	10.6	12.0	0.126
LSD (5%) lower limit <sup>y</sup>	425	476	19	37	231	30.0	291	358	2.21
LSD (5%) upper limit <sup>x</sup>	462	512	26	45	256	33.2	321	392	2.57
Significance probability <sup>w</sup>	0.59	0.59	0.78	0.11	0.16	0.20	0.05	0.05	<0.01

<sup>z</sup>DM yield of the genotypes was not measured.

<sup>y</sup>Lower limit centered on overall mean.

<sup>x</sup>Upper limit centered on overall mean.

<sup>w</sup>Significance probability of genotype and cultivar effects based on the analysis of variance.

This study was undertaken to determine the relationship between CNCPS protein fractions and in vitro RUP concentration, and the variability of protein fractions among alfalfa genotypes and cultivars. In the first experiment, 14 alfalfa genotypes were grown as spaced plants in a field at Saint-David-de-l'Auberivière, QC, Canada, (Lat. 46°46'N, Long. 71°11'W) under a randomized complete block design with four replications. Nine genotypes were from the cultivar DK 125 (DK series) and five were from Ultra (UL series). The genotypes used in this experiment had been selected for vigour and good appearance but DM yield was not measured. In the first production year, the 14 genotypes were all harvested on 23 June 1997 at the early bloom stage of development. The samples were dried at 55°C and ground in a Wiley mill through a 1-mm screen.

In the second experiment, 27 alfalfa cultivars, chosen to represent a wide variability in genetic background, were seeded in a field at the Normandin Research Farm (QC, Canada) of Agriculture and Agri-Food Canada (Lat. 48°51'N, Long. 72°32'W) in the spring of 1995 under a randomized complete block design with three replications. On 27 June 1997, when cultivars reached the early bloom stage of development, a 3-m<sup>2</sup> strip was harvested from each 9-m<sup>2</sup> plot to determine DM yield. Whole plant samples of approximately 400 g were taken from each plot, dried at 55°C, and ground in a Wiley mill through a 1-mm screen. Plot management and details on the conduct of this field experiment are described in Tremblay et al. (2000).

Protein fractions of the CNCPS were estimated in duplicate according to the procedure described by Roe et al. (1990); the TN concentration was determined in whole samples and in a borate-phosphate buffer (pH 6.7–6.8), neutral detergent, and acid detergent insoluble fractions using the Kjeldahl procedure (Association of Official Analytical Chemists 1990). The A + B1 fraction was estimated by subtracting TN in the borate-phosphate buffer insoluble fraction from the whole sample TN. Ruminal protein degradation

was assessed in triplicate using an inhibitor in vitro procedure (Broderick 1987) as described by Tremblay et al. (2000). Net release of NH<sub>3</sub> and total amino acids after incubating for 2 h in rumen fluid were used to estimate the ruminal protein degradation rate. In vitro RUP concentration was calculated from the estimated protein degradation rate, based on a passage rate from the rumen of 0.06 h<sup>-1</sup>.

Data of both experiments were analyzed by ANOVA (SAS Institute, Inc. 1999). Genotype and cultivar means, SEM, and LSD (5%) range were calculated. Correlations between variates were computed on the genotype and cultivar means from the ANOVA. Principal component analyses (PCA) were performed on these means using the PROC PRINCOMP procedure (SAS Institute, Inc. 1999). The correlation matrix between variates was calculated for each experiment, and the correlations with the principal component (PC) scores were augmented to the correlation matrix to assist in identifying the contribution of each variate to a PCA axis. Only significant ( $P < 0.05$ ,  $n = 14$  for genotypes and  $n = 27$  for cultivars) correlation coefficients are presented. Genotypes and cultivars with PC scores higher or lower than the LSD (5%) limits centred on the overall mean were grouped.

Soluble N fractions (A + B1) averaged across genotypes accounted for 406 mg g<sup>-1</sup> of TN, whereas fractions B2, B3, and C accounted for 520, 41, and 34 mg g<sup>-1</sup> of TN, respectively (Table 1). These values are similar to those obtained by Elizalde et al. (1999) and confirm that alfalfa CP is highly soluble and would be readily degraded in the rumen. Significant differences ( $P < 0.05$ ) among genotypes were observed for soluble N fractions (A + B1), degradable true protein fractions (B2 and B3), and in vitro RUP concentration (Table 1). Genotypes did not differ for concentrations of TN ( $P = 0.07$ ), fraction C ( $P = 0.09$ ), ADF ( $P = 0.20$ ), and NDF ( $P = 0.26$ ).

The PC scores for the first axis (50% of the total covariation) defined a contrast; in vitro RUP, B2, TN, and B3 ver-

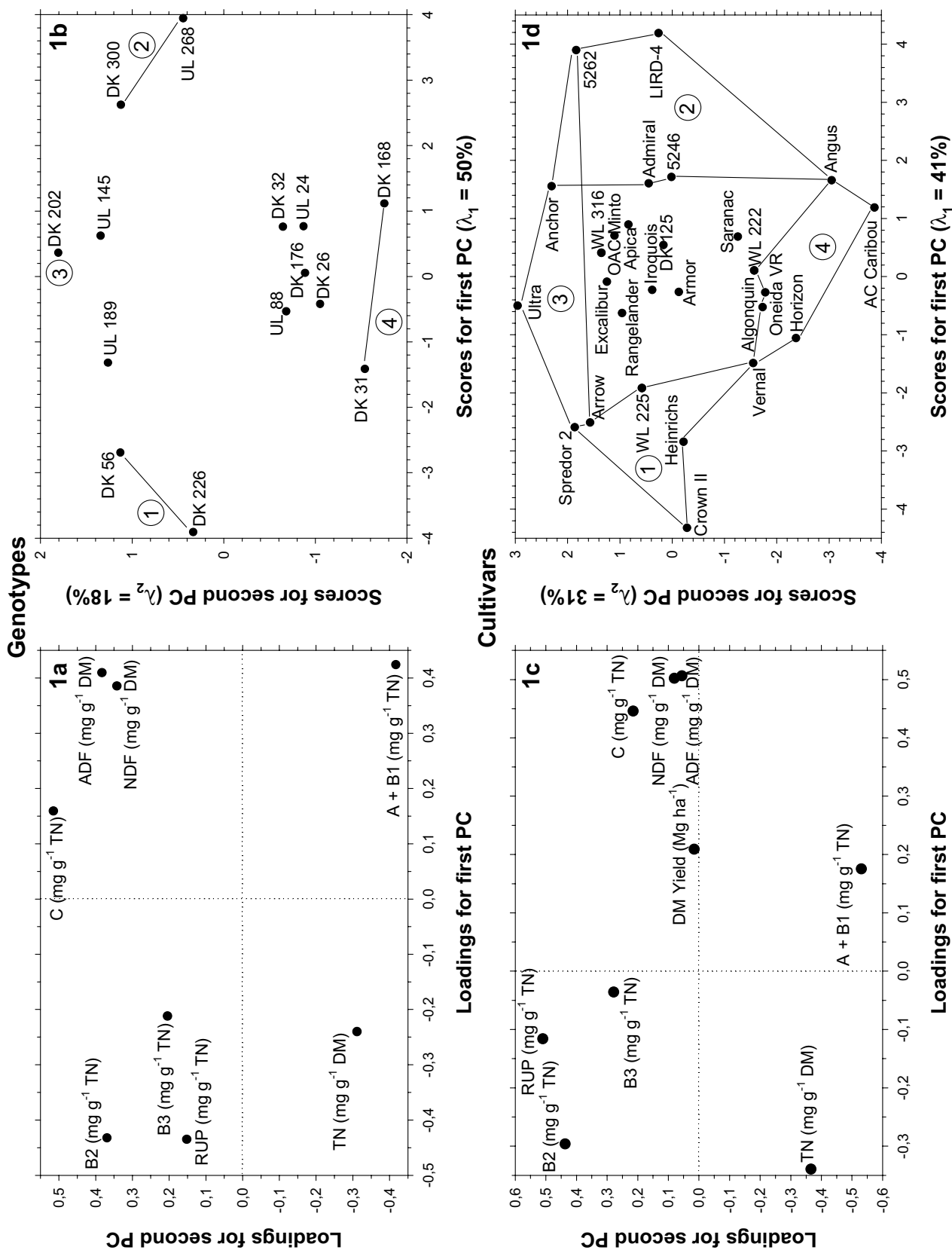


Fig. 1. Diagrams of the loadings and scores of the first two principal components for genotypes (1a and 1b) and cultivars (1c and 1d), respectively.

sus A + B1, ADF, and NDF (Fig. 1a). Fraction B2 and in vitro RUP concentrations were negatively correlated with A + B1, ADF, and NDF concentrations, and positively correlated with TN and B3 concentrations (Fig. 1a). The contribution of a variate to a PC axis can be seen from its loading (Fig. 1a) or its correlation coefficient. The first PC scores were largely determined by in vitro RUP ( $r = -0.87$ ) and B2 ( $r = -0.86$ ), and positively associated with A + B1 ( $r = 0.85$ ), ADF ( $r = 0.82$ ), and NDF ( $r = 0.77$ ).

The two genotypes DK 226 and DK 56 (group 1, Fig. 1b) had significantly lower scores than those of UL 268 and DK 300 on the first axis (group 2, Fig. 1b). Genotypes DK 226 and DK 56 also showed high TN, B2, B3, and in vitro RUP concentrations and low soluble N (A + B1) concentrations (data not shown). The genotype DK 226 also had low ADF and NDF concentrations.

On the second axis (18% of the total covariation), there was also a contrast; A + B1 and TN versus C, ADF, B2, NDF, and B3 (Fig. 1a). In this axis, there were some unique genotypes with extreme PC scores; DK 168 and DK 31 (group 4, Fig. 1b) had high negative scores while DK 202 had a high positive score (group 3, Fig. 1b). The genotype DK 202 had simultaneously higher C, ADF, B2, NDF, and B3 concentrations, and lower A + B1 and TN concentrations than the average of all genotypes; genotypes from the group 4 were just the opposite.

Our results confirm that there is significant variability in protein fractions and protein degradability among alfalfa genotypes, corroborating previous findings on other genotypes (Broderick and Buxton 1991; Skinner et al. 1994). Broderick and Buxton (1991) also reported significant difference among 22 alfalfa entries for fraction A (degraded N at 0 h of incubation with rumen fluid), fraction B [potentially degradable N =  $1000 - (A + C)$ ], and in vitro RUP concentration, which were all determined using the ruminal inhibitor in vitro system (Broderick 1987). From a nutritional and breeding point of view, genotypes such as DK 56 and DK 226 are desirable because they combine high CP values with low protein degradability. Selection of such genotypes should aid in the development of populations with higher protein of better quality for ruminant nutrition.

Tremblay et al. (2000) reported differences among 27 alfalfa cultivars for whole plant in vitro RUP but protein fractions were not measured. In the present study, only the first harvest of the second production year of one seeding from the experiment of Tremblay et al. (2000) was evaluated for both protein fractions and in vitro RUP. In the whole plants of that specific harvest, soluble N fractions (A + B1) accounted for  $443 \text{ mg g}^{-1}$  of the TN concentration, whereas fractions B2, B3, and C accounted for 494, 22, and  $41 \text{ mg g}^{-1}$  of TN, respectively (Table 1). The cultivars did not differ significantly ( $P > 0.05$ ) for any of these protein fractions neither for in vitro RUP nor TN concentrations (Table 1). Once again, these 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.

The first PC axis explained 41% of the total covariation with the scores being largely determined by ADF ( $r = 0.97$ )

and NDF ( $r = 0.96$ ), and positively associated with C ( $r = 0.85$ ) and DM yield ( $r = 0.40$ , Fig. 1c), and negatively associated with TN ( $r = -0.65$ ) and B2 ( $r = -0.57$ ). On this axis, cultivars LIRD-4, 5262, 5246, Angus, Admiral, and Anchor had high scores (group 2, Fig. 1d) that were significantly higher than the other extreme cultivars (Crown II, Heinrichs, Spredor 2, Arrow, WL 225, and Vernal: group 1, Fig. 1d) for one or more of the following variates: ADF, NDF, C, and DM yield. Conversely, cultivars of group 1 (Fig. 1d) had higher TN and B2 concentrations than those of group 2; the two cultivars with a small degree of expression of the multifoliate trait (Crown II and WL 225) were in group 1.

In the second axis ( $\lambda_2 = 31\%$ ), there were some unique cultivars with extreme PC scores; Ultra, Anchor, Spredor 2, 5262, and Arrow (group 3, Fig. 1d) had large positive scores while AC Caribou, Angus, Horizon, Oneida VR, Algonquin, Vernal, and WL 222 (group 4, Fig. 1d) had negative scores. Cultivars of group 3 were higher than their neighbours on the second axis in in vitro RUP, B2, and B3, and lower in A + B1 and TN; cultivars of group 4 were just the opposite. Tremblay et al. (2002) reported that the leaf to weight ratio (LWR) varied among cultivars and it was positively related to plant RUP in spring growth. Consequently, it is also possible that the LWR accounts for some of the genotype and cultivar differences observed in the present experiments.

For both genotypes and cultivars, the PCA indicated a close relationship between the in vitro RUP and fraction B2 (Fig. 1a and 1c). This is confirmed by the high positive and significant correlations between in vitro RUP and B2 concentrations ( $\text{mg g}^{-1}$  of TN) for both genotypes ( $r = 0.78$ ) and cultivars ( $r = 0.64$ ). Furthermore, in vitro RUP values were negatively correlated with the A + B1 fraction for both genotypes ( $r = -0.77$ ) and cultivars ( $r = -0.72$ ). When in vitro RUP and protein fractions were expressed on a DM basis, the relationship is even stronger; correlations between in vitro RUP and B2 ( $\text{mg g}^{-1}$  DM) were 0.89 for genotypes and 0.77 for cultivars. The experiments with genotypes and cultivars were conducted independently at different locations and years. However, further research is required to establish the stability of the relationship between protein fractions and in vitro RUP across different environments. Our results strongly suggest that protein fractions of the CNCPS should be considered as a reliable alternative laboratory method for in vitro RUP to screen genotypes for breeding purposes.

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