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Prediction of *in situ* rumen protein degradability of grass and lucerne by chemical composition or by NIRS*

J.L. De Boever[1], Anna M. Antoniewicz[2] and Ch.V. Boucqué[1]

[1]Department Animal Nutrition and Husbandry – Agricultural Research Centre – Ghent
Scheldeweg 68, B-9090 Melle-Gontrode, Belgium
[2]Institute of Animal Production, Department of Animal Nutrition
Sarego 2, 31-047 Kraków, Poland

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ABSTRACT

Sixty one samples of three grass species and seventy three lucerne samples collected from different growth stages and cuts during three seasons were used to derive regression equations based on crude protein (CP), crude fibre (CF) or harvest date (D) as well as near infrared reflectance spectroscopy (NIRS) calibrations to predict potential (a+b) and effective (ED) CP degradability.

Best regression equations to predict a+b and ED of grass were based on a combination of CP and CF, resulting in an equal residual standard deviation (RSD) of 3.7%-units. For lucerne, two-term regressions with CF and D resulted in the lowest RSD, being 2.3%-units for a+b and 2.5%-units for ED. For both grass and lucerne, a still higher prediction accuracy was obtained with NIRS. In the case of grass, calibrations based on 4 raw absorbances gave the lowest standard error of cross-validation (SEC) for a+b (2.7%-units) and for ED (2.5%-units). For lucerne, calibrations with 4 second derivatives performed best with SEC-values of 1.9 and 1.4%-units for a+b and ED, respectively. Validation on an independent set of UK grasses however showed that the performance of NIRS-calibrations can be heavily disturbed by the way of sample preparation.

KEY WORDS: grass, lucerne, protein degradability, NIRS

INTRODUCTION

In the current protein evaluation systems, degradability of feed protein in the rumen is an important factor. The *in situ* procedure, by which feed samples, in-

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cluded in nylon bags, are incubated during different time intervals in the rumen of cows or sheep, is accepted as the reference method. Besides the need for fistulated animals, the method is too cumbrous, time-consuming and expensive for routine evaluation. Instead, tabular values are used for most feedstuffs. De Boever et al. (1995) showed that the calculation of the protein value of compound feeds based on the tabular values for the ingredients corresponded reasonably well with that obtained *in situ*. Compared with concentrates however, the composition of some forages may vary much more, depending on species, maturity, fertilisation level, season, soil type and weather conditions (Van Straalen and Tamminga, 1990). Therefore, regression equations based on chemical parameters (dry matter, crude protein, crude fibre) and/or day of harvest were developed. Because the near infrared reflectance spectroscopy (NIRS) technique is nowadays currently used by feed laboratories to predict chemical composition and digestibility, its potential to predict protein degradation characteristics is interesting to know. The results from limited studies with grass (Waters and Givens, 1992; Antoniewicz et al., 1995; Halgerson et al., 1995) and lucerne (Atanassova et al., 1994; Antoniewicz et al., 1995; Halgerson et al., 1995) seem very promising.

The aim of this study was to compare the accuracy of regression equations based on chemical parameters and harvest date with NIRS-calibrations to predict protein degradability of grass and lucerne samples from three harvest years.

MATERIAL AND METHODS

Forages and sampling

The studied forages originated from Poland grown in the seasons 1991, 1993 and 1994 for grass and 1992, 1993 and 1994 for lucerne. Grass of 1991 originated from one site (A) and that from 1993 and 1994 from two sites: A and B and A and C, respectively. Grass from A comprised ± 70% *Dactylis glomerata*, ±20% *Poa pratensis* and ± 10% *Festuca pratensis* and others. Grass from B consisted mainly of *Phleum pratense* (timothy), whereas that of C was sown with *Lolium multiflorum* (Italian ryegrass cv. Polus, tetraploid). The grasses were fertilized with 50 kg N in spring, followed by 30 kg N after cutting. Lucerne, a monoculture of *Medicago sativa* grown at one site, received 25 kg P_2O_5 and 50 kg K_2O in early spring. By sampling primary growths and regrowths weekly or biweekly, in total 61 grass and 73 lucerne samples were collected over the three years. Samples were dried at 35°C and ground to pass a 1 mm screen using a Wiley mill.

To validate the grass regressions and calibrations, a collection of 17 grass samples was provided by the ADAS-Institute for Feed Evaluation and Nutritional

Sciences (Drayton, UK), used in the study of Waters and Givens (1992). These samples originated from primary growths and regrowths of two ryegrass-dominant commercial swards in England, cut in 1987. Samples were frozen.

In situ protein degradability

Three rumen fistulated dry cows, fed meadow hay *ad libitum* and 0.5 kg ground barley twice daily at 7 h am and 14 h pm, were used the *in situ* experiments. Samples of about 3 g were weighed in nylon bags with a pore size of 40 µm. The bags were incubated for 2, 4, 8, 16, 24 and 48 h (three replicates per sample and per incubation time). After incubation the bags were rinsed in an automatic washing machine (three cycles of cold water rinsing and spinning, total time: 18 min.). Zero-h bags were not incubated, only subjected to the same rinsing procedure. The bags + residues were dried in a ventilated oven at 60°C overnight, weighed and total N content in the residue was determined.

Protein degradation characteristics were calculated using the model of McDonald (1981), including a lag time. These are 'a': the soluble fraction, 'b': the potentially degradable fraction and 'c': the degradation rate of b.

In situ degradation of the 17 grass samples from the UK was determined with three mature wether sheep fed a maintenance diet of grass hay and a compound feed (60:40 on DM-basis). Samples of frozen herbage were thawed and chopped to 1 cm length. About 20 g of chopped material was weighed in synthetic fibre bags with a pore size of 43 µm. Two samples of each herbage were incubated in each sheep for 3, 8, 16, 24, 45 and 72 h. After incubation, bags were washed in a machine set to a cold rinse cycle, then stored at -20°C. Zero-h bags were only rinsed. On completion of all incubations, the bags were thawed and dried to a constant weight at 60°C. The residues were ground to 1 mm and analysed for total N. Protein degradation characteristics a, b and c were calculated according to the model of Ørskov and Mc Donald (1979) without a lag time.

The potential degradability was considered as a+b. The effective degradability (ED) was calculated with the equation $a + b \times (c/(c + k_p))$ and assuming a passage rate (k_p) of 0.04 h^{-1}. The residues in the bags after rumen incubation were not corrected for contamination with microbial matter.

Chemical analyses

Dry matter (DM) was determined by oven drying at 105° C to a constant weight. Nitrogen was determined following Kjehldahl and crude protein (CP) was calculated as N x 6.25. The crude fibre (CF) content of the Polish samples was determined using a Fibertec System M6 (Tecator, Sweden).

Regression equations

Single and two-term linear regressions were calculated between the chemical parameters CP and CF and the days of harvesting after 1 April (D) at one side and the potential (a+b) or the effective protein degradability (ED) at the other. Regression analysis was carried out separately for grass and lucerne and separately for first cut, regrowth and all samples. For harvests later than 13 September a constant D of 165 was assumed (CVB, 1992). Only regression equations with significantly contributing ($P<0.05$) independent variables were withheld. The grass regressions based on CP and/or harvest date were tested on the 17 UK samples.

NIRS-calibrations

NIRS-analysis was carried out with an InfraAlyser 500 scanning monochromator (Bran and Luebbe, Norderstedt, Germany), taking the spectrum from 1100 to 2500 nm in steps of 4 nm. The dried samples, ground in a cyclone mill fitted with a 1 mm screen (1093 Cyclotec, Tecator, Sweden), were scanned twice on two days and the spectra were averaged. Calibrations were developed for CP, CF, a+b and ED using multiple linear regression analysis (MLR) by IDAS (Bran and Luebbe, Norderstedt, Germany) and partial least squares regression (PLSR) by Unscrambler (version 5.01, Camo, Trondheim, Norway). For MLR, raw absorbance (log 1/R), first and second derivative values were used as independent variables, whereas only log 1/R values for PLSR. The best way to evaluate the accuracy of NIRS-calibrations is to test them on samples not belonging to the calibration set. However, a complete independent validation could only be carried out on the rather limited collection of UK grass samples, resulting in the standard error of prediction (SEP). As an alternative, the calibration set was divided in nine subsets. Then, for each parameter, number and nature of the independent variables, nine calibrations were calculated, leaving out one subset, which was subsequently used for validation. The mean of the nine prediction errors is called the standard error of cross-validation (SEC). In the case of PLS, the more convenient software allowed to consider 20 sub-sets.

RESULTS

Chemical composition and protein degradability

In Table 1 the mean and range in CP, CF and protein degradability of the grass and lucerne samples are given per season and site. The variation in chemical composition was mainly caused by the growth stage, but also by the season and the

TABLE I

Mean and range in chemical composition and protein degradability of grass and lucerne

Season/ place	n	D[1] day	CP g/kg DM	CF g/kg DM	a+b[2] [20]%	ED[2] [30]%
			Grass			
1991 A	16	37-165	127	306	83.1	70.5
			53 -200	218 -394	56.1-95.6	51.0-81.8
1993 A	11	33 -132	156	304	89.3	72.8
			104-249	239 - 353	80.3-97.9	67.2-81.2
1993 B	8	62-154	101	338	78.7	64.7
			61-137	287- 375	67.3-85.3	58.0-71.2
1994 A	12	33-165	177	275	94.4	78.9
			120-254	214 -341	87.5-99.5	74.4-86.0
1994 C	14	21-122	1125	268	82.9	74.8
			4 -253	147 -368	63.0-98.8	57.6-91.2
Total	61	21-165	135	295	85.8	72.8
			53-254	147-394	56.1-99.5	51.0-91.2
1987UK	17	40-165	171	–	91.9	72.6
			74-256		72.9-96.0	56.2-78.7
			Lucerne			
1992	38	27-165	186	294	89.4	82.6
			88-306	160-472	69.3-97.7	64.7-90.8
1993	13	19-123	205	291	91.8	83.4
			132-277	198-386	83.0-98.5	75.5-89.0
1994	22	26-165	204	279	90.5	80.7
			114-357	114-457	68.6-99.9	61.8-88.6
Total	73	19-165	195	289	90.1	82.1
			88-357	114-472	68.6-99.9	61.8-90.8

[1] harvest date, expressed as days since 1 April

[2] potential degradability

[3] effective degradability calculated for a rumen passage rate of 0.04 h^{-1}

grass species. Although the frequency of sampling differed among seasons, some general tendencies could be found. On site A with *Dactylis glomerata* as dominating grass species, CP-content was on average higher during 1993 and 1994 than in 1991. Compared with A, the chemical composition of grass from site B, mainly consisting of timothy, varied less during the growth season and was characterized by a lower mean CP- and a higher CF-content. On the contrary, the CP- and CF-contents of grass from C, sown with Italian ryegrass, were on average lower and showed a larger range than grass from A. The UK-samples from predominant ryegrass were most comparable with those from site A concerning content and range in CP.

For lucerne, the mean CP- and CF-content were similar among seasons, whereas the variation in chemical composition was lower in 1993 than in the other two seasons. The range in potential and effective degradability was mainly determined by the chemical composition, as will be discussed later. For the 61 grass samples, a+b varied from 56.1 to 99.5% and ED from 51.0 to 91.2%. Despite the similar range in CP-content for the Polish and the UK samples, the protein degradability values of the latter varied less. For the 73 lucerne samples, a+b varied from 68.6 to 99.9% and ED from 61.8 to 90.8%.

Prediction of protein degradability by chemical composition and harvest date

The single and two-term regression equations to predict the potential or effective protein degradability of the Polish grass samples based on CP, CF and harvest date are given in Tables 2 and 3, respectively. For first cut as well as for regrowth samples, the potential and effective protein degradability of grass increased with higher CP-content or lower CF-content. For a+b, CP showed a higher determination coefficient than CF, whereas for ED, CF was more determinant. The regression coefficients or slopes for CP and CF were respectively lower and higher for regrowth grass than for first cut grass. Compared with the single regressions, the two parameters together could explain somewhat more of the variation in a+b for first cut samples (82.9%) and in ED for first cut (85.5%) as well as for regrowth samples (73.6%). For first cut samples, a+b and ED clearly decreased with the

TABLE 2

Regression equations to predict the potential protein degradability (a+b = Y) of grass based on CP, CF and harvest date (D**)

Regression equation	R^2, %	RSD, %
First cut (n = 35; a+b: 84.7 ± 10.9%, 56.1 – 99.5%)		
Y = 61.1 + 0.174 CP*	76.0	5.4
= 129.8 – 0.150 CF*	71.1	5.9
= 115.9 – 0.519 D**	89.4	3.6
= 92.6 +0.109 CP – 0.075 CF	82.9	4.5
Regrowths (n = 26; a+b: 87.4 ± 9.5%, 63.0 – 98.6%)		
Y = 65.6 + 0.161 CP	69.8	5.2
= 151.3 – 0.222 CF	47.9	6.9
All (n = 61; a+b: 85.8 ± 10.3%, 56.1 – 99.5%)		
Y = 62.9 + 0.169 CP	72.9	5.4
= 133.6 – 0.162 CF	62.5	6.3
= 92.8 + 0.058 CP – 0.095 CF	79.5	3.7

* CP and CF in g per kg DM

** D = harvest date in days since 1 April

TABLE 3

Regression equations to predict effective protein degradability (ED = Y) of grass based on CP, CF and harvest date (D**)

Regression equation	R^2, %	RSD, %
First cut (n = 35; ED: 73.4 ± 9.1%, 51.0 91.2%)		
Y = 55.1 + 0.138 CP*	66.2	5.3
= 113.9 – 0.135 CF*	82.7	3.8
= 99.5 – 0.433 D**	89.4	3.0
= 97.9 + 0.047 CP – 0.102 CF	85.5	3.5
Regrowths (n = 26; ED: 71.9 ± 6.7%, 57.6 – 82.7%)		
Y = 57.8 + 0.104 CP	57.0	4.4
= 123.8 – 0.180 CF	64.5	4.0
= 99.0 + 0.056 CP – 0.121 CF	73.6	3.5
= 68.4 + 0.115 CP – 0.096 D	74.5	3.4
All (n = 61; ED: 72.8 ± 8.1%, 51.0 – 91.2%)		
Y = 56.0 +0.124 CP	62.6	5.0
= 113.4 – 0.138 CF	72.9	4.2
= 80.4 – 0.087 D	17.7	7.4
= 92.8 +0.058 CP – 0.095 CF	79.5	3.7
= 62.4 +0.115 CP – 0.058 D	70.4	4.4
= 115.0 – 0.128 CF – 0.050 D	78.6	3.8

* CP and CF in g per kg DM

** D = harvest date in days since 1 April

number of days since 1 April; harvest date explained more of the variance in potential and effective protein degradability than the combination of the two chemical parameters, almost 90%. On the contrary, harvest date had no effect on protein degradability for regrowth samples. When first cut and regrowth grasses were pooled, the combination of CP and CF explained about 80% of the variance, resulting in a residual standard deviation (RSD) of 3.7%-units for both the potential and effective degradability. The regression equations derived from all grass samples and based on CP and/or D were validated on the 17 UK samples with a potential degradability (mean ± SD) of 91.9 ± 5.5% and an effective degradability of 72.6 ± 6.4%. Prediction of a+b with the CP-equation resulted in a mean difference between the predicted and the determined values of -0.1 ± 7.3%-units and a prediction error of 7.6%-units. For ED, application of the equations based on CP or CP and D gave mean differences of 4.6 ± 5.8%-units and 2.5 ± 5.4%-units, respectively and prediction errors of 7.8 and 6.2%-units.

The single and two-term regression equations to predict a+b and ED of the Polish lucerne samples are given in Tables 4 and 5, respectively. Similarly to grass, the potential and effective protein degradability of lucerne increased with higher CP- and lower CF-content and this for first cut as well as for regrowth samples.

TABLE 4

Regression equations to predict potential protein degradability (a+b = Y) of lucerne based on CP, CF and harvest date (D**)

Regression equation	R^2, %	RSD, %
First cut (n = 33; a+b: 88.8 ± 7.9%, 68.6 – 99.9%)		
Y = 69.7 + 0.101 CP*	78.5	3.7
= 112.1 – 0.075 CF*	87.5	2.8
= 102.1 – 0.168 D**	83.2	3.2
= 140.1 – 0.070 CP – 0.123 CF	88.9	2.6
= 88.3 + 0.047 CP – 0.105 D	88.0	2.7
= 109.1 – 0.048 CF – 0.068 D	89.8	2.5
Regrowths (n = 40; a+b: 91.2 ± 4.8%, 81.7 – 98.0%)		
Y = 74.6 + 0.083 CP	78.5	2.2
= 108.1 – 0.062 CF	77.6	2.3
= 97.9 – 0.057 D	13.2	4.4
= 90.5 + 0.046 CP – 0.031 CF	81.9	2.0
= 110.7 – 0.059 CF – 0.030 D	81.1	2.1
All (n = 73; a+b: 90.1 ± 6.4%, 68.6 – 99.9%)		
Y = 71.6 + 0.095 CP	77.7	3.0
= 110.3 – 0.070 CF	84.6	2.5
= 98.6 – 0.085 D	29.4	5.4
= 111.2 – 0.064 CF – 0.026 D	86.6	2.3

* CP and CF in g per kg DM

** D = harvest date in days since 1 April

Compared with CP, CF explained more of the variation in a+b of first cuts and all samples and in ED of all samples. The combination of the two chemical parameters was only marginally better than the best single parameter. The negative effect of harvest date on a+b and ED was again strongly pronounced for first cuts, but almost insignificant for regrowths. Considering all samples, most of the variation in a+b and ED could be explained by CF, resulting in a RSD of 2.5 and 2.8%-units, respectively. By taking D into account as supplemental variable, the prediction could only marginally be improved.

NIRS-calibrations

In Table 6 the accuracy of the NIRS calibrations to predict CP, CF and protein degradability of grass is presented. The calibrations for CP and CF showed very high determination coefficients with all but one higher than 95%. From the cross-validation test appeared that a multiple regression with four log 1/R values gave the best results for both CP and CF. The two most determining wavelengths were 2152 and 2160 nm for CP and 2300 and 2364 nm for CF. Validation of the CP-

TABLE 5
Regression equations to predict effective protein degradability (ED = Y) of lucerne based on CP, CF and harvest date (D**)

Regression equation	R, %	RSD, %
First cut (n = 33; ED: 81.5 ± 7.0%, 61.8 – 90.8%)		
Y = 65.9 + 0.082 CP*	64.5	4.2
= 101.0 – 0.063 CF*	77.5	3.3
= 93.4 – 0.151 D**	84.0	2.8
= 151.6 – 0.126 CP – 0.150 CF	84.0	2.8
Regrowths (n = 40; ED: 82.7 ± 4.7%, 71.0 – 89.9%)		
Y = 67.4 +0.077 CP	68.2	2.7
= 99.6 – 0.062 CF	78.5	2.2
= 89.9 – 0.062 D	16.2	4.3
= 102.6 – 0.058 CF – 0.034 D	83.6	1.9
All (n = 73; ED: 82.1 ± 5.9%, 61.8 – 90.8%)		
= 66.5 + 0.080 CP	66.5	3.4
= 99.8 – 0.061 CF	77.5	2.8
= 90.5 – 0.084 D	35.0	4.7
= 71.4 + 0.069 CP – 0.028 D	68.8	3.3
= 101.1 – 0.053 CF – 0.035 D	82.3	2.5

* CP and CF in g per kg DM
** D = harvest date in days since 1 April

calibrations on the independent set of UK-samples with a mean content of 171±51 g/kg DM revealed that a three-term regression based on first derivatives resulted in the lowest prediction error (20 g/kg DM). All examined NIRS-calibrations for the potential and effective protein degradability showed higher determination coefficients than the regression equations based on chemical composition (Tables 2 and 3). Although not completely comparable, the standard errors of cross-validation of the calibrations were also lower than the the residual standard errors of the regressions. A calibration based on four log 1/R values performed best for the prediction of both the potential and effective degradability of the Polish grass samples. The two main determining wavelengths were 2208 and 2264 nm for a+b and 1468 and 1820 nm for ED. The protein degradability of the UK samples was best predicted by a partial least square regression with 8 factors for a+b and and 3 factors for ED, resulting in a prediction error of 5.6 and 5.1%-units, respectively.

The accuracy of the NIRS-calibrations to predict CP, CF and protein degradability of lucerne is given in Table 7. The calibrations for CP and CF had very high determination coefficients, varying between 94 and 99%. The lowest SEC was obtained with three or four log 1/R values for CP and with three or four first derivatives for CF. The pair of determining wavelengths was 2156 and 2184 nm for CP and 1928 and 2308 nm for CF. For the potential and effective protein degradabilty,

TABLE 6

Accuracy of NIRS-calibrations to predict CP, CF, potential (a+b) and effective (ED) protein degradability of grass (n = 61)

X-variable	n[1]	CP			CF		a+b			ED		
		R^2	SEC[3]	SEP[4]	R^2	SEC	R^2	SEC	SEP	R^2	SEC	SEP
		%	g/kg DM		%	g/kg DM	%	%-units		%	%-units	
log 1/R	2	96.2	10	42	91.0	16	90.4	3.3	9.4	85.0	3.3	19.6
log 1/R	3	96.8	9	24	95.5	15	93.7	3.0	6.1	89.7	2.9	19.2
log 1/R	4	98.4	7	48	97.2	10	95.5	2.7	17.7	93.1	2.5	8.2
1st der.	2	97.2	11	64	94.9	15	92.9	3.1	13.6	90.1	2.9	14.1
1st der.	3	98.0	10	20	96.2	11	93.7	3.4	18.6	91.2	2.7	10.7
1st der.	4	98.6	9	30	97.0	10	94.7	3.0	8.8	92.9	2.7	14.3
2nd der.	2	97.4	12	34	95.4	13	93.7	3.2	7.0	87.2	3.2	11.8
2nd der.	3	97.6	11	47	97.0	11	95.3	3.0	6.4	90.4	2.9	6.4
2nd der.	4	98.6	10	42	97.2	10	96.0	3.1	7.2	92.4	2.8	6.3
PLS	*	97.4	10	66	96.2	12	95.1	2.9	5.6	85.2	3.4	5.1

[1] number of wavelengths
[2] determination coefficient
[3] standard error of cross-validation
[4] standard error of prediction, using the UK grass samples as validation set
* optimal number of PLS-factors: CP (6), CF (7), a+b (8) and ED (3)

TABLE 7

Accuracy of NIRS-calibrations to predict CP, CF, potential (a+b) and effective (ED) protein degradability of lucerne (n = 73)

X-variable	n[1]	CP		CF		a+b		ED	
		R^2 %	SEC[3] g/kg DM	R^2 %	SEC g/kg DM	R^2 %	SEC %-units	R^2 %	SEC %-units
log 1/R	2	97.8	9	96.2	18	88.9	2.1	91.6	1.6
log 1/R	3	98.4	8	96.6	17	89.7	2.2	92.7	1.5
log 1/R	4	98.6	8	98.0	15	91.8	2.0	93.9	1.6
1st der.	2	93.9	11	96.2	15	89.7	2.0	89.7	1.9
1st der.	3	94.5	11	96.6	14	90.3	2.1	90.4	1.7
1st der.	4	94.9	9	96.8	14	91.8	2.1	91.4	1.6
2nd der.	2	94.3	11	95.8	19	88.5	2.1	91.2	1.6
2nd der.	3	94.9	10	96.4	16	89.7	2.1	92.5	1.5
2nd der.	4	95.1	9	96.8	15	92.5	1.9	93.1	1.4
PLS	*	96.4	12	97.2	17	87.2	2.5	93.3	1.8

[1] number of wavelengths or PLS-factors
[2] determination coefficient
[3] standard error of cross-validation
* optimal number of PLS-factors: CP (3), CF (6), a+b (3) and ED (6)

all examined NIRS-calibrations showed higher determination coefficients and lower errors than the regression equations based on chemical composition and harvest date (Table 4 and 5). A calibration based on four second derivative values gave the best performance for a+b as well as for ED. The determining wavelengths were 1228 and 1708 nm for a+b and 1280 and 1900 nm for ED.

DISCUSSION

The grass and lucerne samples in this study covered a wide range in chemical composition, mainly caused by the frequent sampling of primary growths and re-growths at different maturity stages and over three seasons. It is well-known that during maturation of herbage, the CP-content declines and the fibre content increases. The decrease in CP-content may be due to an increase in the proportion of stems (lower CP than leaves) and/or a decrease of CP in leaf and stem fractions (Lyttleton, 1973). Moreover, for grass three species were involved, of wich *Dactylis glomerata* and *Phleum pratense* are typical for a cold, dry land climate and *Lolium multiflorum* predominates in a moist sea climate (Lyttleton, 1973). The range in CP-content of the Polish grass samples was similar to that of the UK ryegrass samples. The reason for the smaller variation in protein degradability of the UK grass samples than for the Polish samples could be explained by differences in the *in situ* method. The Polish samples were dried and ground for incubation, whereas the UK samples were unthawed and chopped. As a consequence, the Polish samples had a much higher soluble fraction, on average 45.4 vs 18.7% for the UK samples; on the other hand the potentially degradable fraction was lower for the former on average 40.4 vs 73.2%. The reason for these differences may be that grinding ruptures more cell walls than chopping, so that more free protein and chloroplasts are washed out of the bags (Van Vuuren, 1993). Besides, some proteolysis might have taken place during low temperature drying. The different way of sample preparation had probably most effect on ED. Another difference was the longest incubation period, being 48 h for the Polish and 72 h for the UK samples, which could have affected potential degradability. The effect of the fitted model, with lag time for the Polish and without for the UK samples, on the protein degradability on these relatively good quality forages is to be expected small.

The higher CP-content of lucerne than of grass throughout the season was also observed by Sanderson and Wedin (1989). These authors also found that more than 80% of the total N in herbage was present in the cell solubles, originating from chloroplasts and cytoplasm, whereas the remaining part is either bound to the cell walls or denatured protein. The potential and effective protein degradability of lucerne were on average higher than those of grass. This agrees with Amrane and Michalet-Doreau (1993), who compared lucerne with Italian ryegrass and Balde

et al. (1993), comparing lucerne with *Dactylis glomerata*. Similarly, Sanderson and Wedin (1989) observed that the proportion of ADF-N or unavailable N in total N was higher in grasses than in lucerne.

The positive effect of CP-content and the negative effect of fibre – content or harvest date on protein degradability was also observed by Waters and Givens (1992), Van Vuuren (1993) and Antoniewicz et al. (1995) for grass and by Atanassova et al. (1994) and Antoniewicz et al. (1995) for lucerne. In our study, these effects are mainly due to the process of maturation. During maturing cell walls become more resistant to microbial breakdown, so that cell contents are slower released, and a greater proportion of N is associated with the cell walls (Van Vuuren et al., 1991). The effects of CP and CF on protein degradability were similar for first cut and regrowth samples of both grass and lucerne. For these parameters, samples could be pooled without much loss in the accuracy of the regression equations. On the other hand, harvest date was a highly significant predictor of protein degradability for first cut samples of grass and lucerne but almost insignificant for regrowths. The best combination of two parameters for predicting the potential as well as the effective degradability of grass was with CP and CF and resulted in an equal RSD of 3.7%-units. For lucerne, CF and harvest date gave the most accurate two-term regression to predict the potential and effective protein degradability, resulting in a RSD of 2.3 and 2.5%-units, respectively. The lower RSD for lucerne than for grass is probably due to the smaller variability in the data of the former, because it concerned only one species and one site.

The suitability of NIRS to predict the CP- and CF-content of forages is well-known. However, one should be very careful when applying a calibration to samples which differ from those of the calibration set. In our study, calibrations were developed based on either MLR or PLSR. PLSR is the appropriate multivariate calibration technique to avoid the problem of the very high intercorrelation between absorbances, often occurring with MLR (Goedhart, 1990). Further within MLR, the optimal number and treatment of the absobance data were examined. Because the residual standard deviation of a NIRS-calibration underestimates the prediction error, either cross-validation or validation on a totally independent sample set is recommended. From the cross-validation of the CP-calibrations, an equation based on 4 raw absorbances appeared to be optimal. However, when testing the CP-calibrations on the UK-samples, a three-term regression with first derivatives appeared to perform best. An advantage of deriving spectra is that it removes baseline shifts, which can occur due to differences in particle size or moisture content between the calibration and validation samples (Williams and Norris, 1987). This was probably also the case here, considering the different sample preparation of the Polish and UK samples.

The best calibrations to predict a+b and ED of grass, as evaluated by cross-validation, contained 4 raw absorbance data. The standard error of cross-valida-

tion amounted to 2.7 and 2.5%-units, respectively, which is clearly lower than the best regession equations based on CP and CF. When applied to the UK samples, PLS-regressions performed best to predict protein degradability. Apparently, the physical nature of the samples was better modelled by one or more PLS-factors than by using raw or derived absorbances. The regressions for a+b and ED resulted in prediction errors of 5.6 and 5.1%-units respectively, thus also lower than with the regression equation based on CP. Better results with NIRS than with chemical parameters to predict protein degradability of grass were also obtained by Waters and Givens (1992) and Antoniewicz et al. (1995).

For lucerne, no independent sample set was available, so that the calibrations could only be evaluated by cross-validation. MLR-calibrations based on either 3 or 4 raw absorbances resulted in the lowest SEC for CP, whereas the best calibrations for CF contained 3 or 4 first derivatives. Because the risk of overfitting increases with the number of terms, the three-term calibrations are preferable. To predict a+b and ED, MLR-calibrations containing 4 second derivatives performed best, resulting in SEC-values of 1.9 and 1.4%-units, respectively, and thus again lower than the RSD-values of the best regression equations based on CF and harvest date. The better prediction of the protein degradability of lucerne with NIRS than with chemical parameters or harvest date was also found by Atanassova et al. (1994) and Antoniewicz et al. (1995).

The interpretation of NIR-spectra is not always straight forward because of the many overlapping bands (Williams and Norris, 1987). For CP and CF, the typical wavelengths for protein and cellulose were selected. For a+b and ED, which are in fact calculated values, the determining wavelengths are more difficult to identify but could mainly be attributed to cellulose rather than to protein .

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STRESZCZENIE

Określanie degradacji w żwaczu białka traw i lucerny na podstawie ich składu chemicznego lub metodą NIRS

Opracowano równania regresji na podstawie zawartości białka ogólnego (CP) i włókna surowego (CP) oraz daty (D) zbioru i na podstawie kalibracji metodą NIRS sześćdziesięciu jeden prób trzech gatunków traw i siedemdziesięciu trzech prób lucerny, zbieranych przez trzy lata w różnych fazach wzrostu, w celu określenia potencjalnej (a+b) i efektywnej (ED) ich degradacji.

Najlepsze równania regresji do określenia a+b i ED dla traw były oparte na kombinacji zawartości CP i CF, dające jednakowy współczynnik resztowego odchylenia standardowego (RSD) 3,7%. Najmniejszy współczynnik RSD dla lucerny otrzymano przy dwu-czynnikowej regresji CF i D, wynoszący 2,3 procentowych jednostek dla a+b i 2,5 dla ED. Przewidywana dokładność degradacji dla traw i lucerny była większa przy stosowaniu metody NIRS. W przypadku traw kalibracje oparte na czterech absorbancjach dały najmniejszy błąd standardowy walidacji (SEC) dla a+b (2,7 jednoste procentowych) i dla ED (2,5). Dla lucerny kalibracje z 4 drugich pochodnych miały najmniejsze wartości SEC 1,7 oraz 1,4 jednostek procentowych dla a+b i ED, odpowiednio. Jednakże walidacja wykonana na niezależnym zestawie traw wykazała, że kalibracje NIRS mogą być poważnie zakłócone przez sposób przygotowania prób.