Determination of indigestible neutral detergent fibre contents of grasses and its prediction from chemical composition

F. Jančík[1,2], P. Homolka[1], B. Čermák[2], F. Lád[2]

[1]Institute of Animal Science, Prague-Uhříněves, Czech Republic
[2]Faculty of Agriculture, University of South Bohemia in České Budějovice, Czech Republic

ABSTRACT: Five grass species (*Dactylis glomerata* L., *Phleum pratense* L., *Lolium perenne* L., *Festuca arundinacea* L. and the hybrid Felina (*Lolium multiflorum* L. × *Festuca arundinacea* L.)), commonly used in roughages for ruminants, were harvested at different maturities of primary growth (n = 60) and evaluated for contents of dry matter (DM), crude protein (CP), ash, crude fat, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), indigestible neutral detergent fibre (INDF) and digestible neutral detergent fibre (DNDF). INDF and DNDF contents were determined by *in sacco* rumen degradation of grasses for 12 days in non-lactating cows. ADL content was found to be highly correlated ($P < 0.05$) with DNDF ($r = -0.87$) and presented a reliable ($R^2 = 0.78$; residual mean square error of 17.65 g/kg DM; $P < 0.0001$) parameter to predict INDF contents. Over a six-week period of maturation INDF contents increased ($P < 0.0001$) in all studied grasses. It was confirmed by this study that the INDF contents of grasses, which markedly increased during maturation, could be effectively predicted from ADL contents.

Keywords: grass; indigestible neutral detergent fibre; *in sacco* method

Grasses, one of the most important sources of roughage used in ruminant nutrition, contain substantial amounts of cell wall carbohydrates. Cell wall carbohydrates can be quantified by determination of neutral detergent fibre (NDF), which includes cellulose, hemicellulose and lignin as the major components (Van Soest et al., 1991). Due to the variability of NDF in rumen degradation and its influence on animal performance, the knowledge of NDF digestibility in forage is critical for effective ruminant feeding (Oba and Allen, 1999).

The *in sacco* nylon bag technique, often applied to determine rumen degradation of protein (Tománková and Kopečný, 1995; Homolka, 2000), organic matter (Van Vuuren et al., 1991) or starch (Herrera-Saldana et al., 1990; Tománková and Homolka, 2004), is the most common method used to estimate NDF digestion (Huhtanen et al., 2006a). The content of indigestible NDF (INDF), which needs long *in sacco* incubation periods (Fonseca et al., 1998), presents an important indicator of the quality of grass cell wall carbohydrates and can be a good predictor of *in vivo* digestibility of roughages (Nousiainen et al., 2003). Forage digestibility in ruminants is constrained by the extent of cell wall (NDF) digestion (Van Soest, 1994). INDF is the most important factor affecting the total diet organic matter digestibility (Nousiainen et al., 2004). A part of the forage cell wall, i.e. INDF, is unavailable to microbial digestion in ruminants,

Supported by the Ministry of Agriculture of the Czech Republic (Project MZe 0002701403) and the Ministry of Education, Youth and Sports of the Czech Republic (Project MSM 6007665806).

even if the total tract residence time of fibre could be extended to an infinite time (Huhtanen et al., 2006b). INDF represents the actually indigestible part of NDF.

Methods for INDF determination are time consuming and expensive. Prediction equations, based on basic parameters of chemical analysis, are cheaper and faster for institutions without availability of experimental animals.

The influence of maturity at harvest on the chemical composition and digestibility of grasses is more pronounced than other management factors such as particle size, dry matter (DM) at harvest or harvesting system (Harrison et al., 2003). Incomplete degradation of cell walls is a major factor limiting the value of forages and straws for animals (Ahmad and Wilman, 2001). Grenet and Besle (1991) and Nagadi et al. (2000) postulated that the cell wall carbohydrates are little degraded in the rumen due to a high extent of lignification. Lignin is generally accepted as the primary component responsible for limiting the digestion of forages (Van Soest, 1994; Traxler et al., 1998; Agbagla-Dohnani et al., 2001).

The aim of the present study was to determine the content of indigestible neutral detergent fibre (INDF) in grasses by an *in sacco* nylon bag technique, to compare the effect of consecutive harvest dates on INDF content in grasses, and to predict INDF content from chemical composition.

MATERIAL AND METHODS

Samples

Five of the most widely used grass species in ruminant nutrition, *Dactylis glomerata* L. – Dana variety, *Phleum pratense* L. – Sobol variety, *Lolium perenne* L. – Jaspis variety, *Festuca arundinacea* L. – Prolate variety and the hybrid Felina (hybridization of *Lolium multiflorum* L. and *Festuca arundinacea* L.) were grown as monocultures at the Breeding Station in Větrov, Tábor district, Czech Republic (49° 31' 2.04" N lat, 14° 28' 4.9" E long; 620 m altitude). Grasses were harvested from primary growth at six dates in 2004 and 2005 (Table 1.) After drying (at 50°C for 48 h), grass samples were milled through a 1-mm sieve for chemical analysis and *in sacco* incubation.

Chemical analysis

Samples were analyzed for contents of DM, crude protein (CP), ash, crude fat, NDF, acid detergent fibre (ADF) and acid detergent lignin (ADL). DM was determined after drying at 105°C, and ash after combustion at 550°C (Regulation No. 497/2004, 2004). Crude fat was extracted for 6 h with petroleum ether, whereas the Kjeldahl method

Table 1. Maturity stages[1] of grasses at harvest dates

Harvest	Date	Grass				
		Dactylis glomerata	*Phleum pratense*	*Lolium perenne*	*Festuca arundinacea*	Felina hybrid
1	13.5. 04	32	30	30	30	31
	13.5. 05	31	30	30	30	31
2	19.5. 04	35	31	31	31	38
	20.5. 05	34	31	30	31	37
3	26.5. 04	51	32	32	32	50
	27.5. 05	51	32	32	37	51
4	2.6. 04	57	32	32	32	55
	3.6. 05	60	37	37	51	59
5	9.6. 04	59	51	51	51	57
	10.6. 05	61	51	51	55	59
6	16.6. 04	65	53	51	55	61
	17.6. 05	65	55	54	58	61

[1]based on the decimal code described by Zadoks et al. (1974) in which 30 to 39 refers to stem elongation, 50 to 59 to inflorescence emergence, and 60 to 69 to anthesis

Table 2. Chemical composition and NDF digestibility parameters of grasses (n = 60)

	Mean	SD	Minimum	Maximum
Dry matter (g/kg)	234.9	44.3	159.1	341.5
Chemical composition (g/kg DM)				
CP	130.3	37.9	64.5	211.3
Ash	76.6	14.5	49.0	105.3
Fat	23.5	8.0	5.4	42.6
CF	281.2	49.7	166.5	373.8
NDF	544.5	75.1	337.0	691.2
ADF	301.9	48.7	183.0	382.8
ADL	22.7	8.0	8.2	42.2
Hemicellulose	242.5	33.4	144.5	308.4
Cellulose	279.2	42.7	168.8	350.7
Parameters of NDF digestibility				
INDF (g/kg DM)	68.9	37.6	18.0	175.7
DNDF (g/kg NDF)	878.5	55.1	724.8	959.5

CP = crude protein; CF = crude fibre; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; INDF = indigestible neutral detergent fibre; DNDF = digestible neutral detergent fibre; SD = standard deviation

was used to determine nitrogen (N) (AOAC, 1990). CP was calculated as N × 6.25. NDF, ADF and ADL were determined according to the methods of Van Soest et al. (1991) using an ANKOM 220 Fibre Analyzer (ANKOM Technology Corporation, NY, USA). Hemicellulose was calculated as NDF – ADF and cellulose as ADF – ADL (Rinne et al., 1997a).

In sacco analysis

Digestible neutral detergent fibre (DNDF) and INDF contents were determined after a 288-h rumen incubation period (Rinne et al., 1997b; Nousiainen et al., 2004) of grass samples in nylon bags with two non-lactating cows fitted with rumen cannulas. Animals had *ad libitum* access to meadow hay and were fed 1 kg of barley meal per day. The small pore size of 17 μm was used for nylon bags (Swiss Silk Bolting Cloth Ltd., Zurich, Switzerland; external dimensions 60 × 120 mm) to minimize particle inflow and outflow, but still ensuring sufficient microbial activities inside the bags (Huhtanen et al., 1998; Nousiainen et al., 2003). Each sample was weighed in an amount of 3 g into nylon bags and incubated in 3 repetitions in each cow. After incubation the bags were rinsed by hand with cold water for 30 min and dried at 50°C for 48 h.

Statistical analysis

Simple linear correlation coefficients were calculated to evaluate the linear relationships between analysed components. Linear, multiple, and stepwise multiple regression analyses were used to develop prediction equations for INDF content using nutrient concentrations in grasses. These statistics were calculated using Statistica 6 (2001).

The MIXED procedure of SAS (SAS, 2002–2003) was used for comparison of differences between INDF contents as influenced by harvest dates and grasses. The effects of year and grass were considered as fixed effects and harvest date was nested in each level of the factor grass as a covariate. Because of heterogeneity of the variance the different variances for harvest dates were taken into account in the variance-covariance structure of the model. The estimates of the parameters for a change in INDF contents at consecutive harvest dates were compared by pairwise comparison with the Bonferroni correction to control the overall type I error rate (Rasch et al., 1994; Rasch et al., 1999).

RESULTS AND DISCUSSION

The chemical composition and NDF digestibility parameters of studied grasses are presented in

Table 3. Coefficients of linear correlations between chemical components and NDF digestibility

Parameter	CP	CF	NDF	ADF	ADL	Hemi-cellulose	Cellulose
CF	–0.59*						
NDF	–0.59*	0.89*					
ADF	–0.65*	0.86*	0.94*				
ADL	–0.61*	0.61*	0.68*	0.79*			
Hemicellulose	–0.36*	0.75*	0.87*	0.66*	0.37*		
Cellulose	–0.63*	0.87*	0.95*	0.99*	0.71*	0.68*	
INDF	–0.74*	0.72*	0.77*	0.83*	0.88*	0.51*	0.78*
DNDF	0.74*	–0.64*	–0.68*	–0.77*	–0.87*	–0.41*	–0.71*

*$P < 0.05$

Table 2. Similar results were reported by Cherney et al. (1993) for *Phleum pratense* and *Festuca arundinacea*, López et al. (1998) for grass hay, Jensen et al. (2003) for *Dactylis glomerata* and *Lolium perenne* and Sommer et al. (2005) for meadow hay.

Correlation coefficients (r) of the relationships between the chemical composition and parameters of NDF digestibility are given in Table 3. INDF and DNDF were highly correlated with ADL ($P < 0.05$) and less with ADF and NDF ($P < 0.05$). Similar correlation coefficients like those found in the present study were reported by Traxler et al. (1998) between INDF and ADL ($r = 0.81$), ADF ($r = 0.71$) and NDF ($r = 0.62$) contents for a group of forages (C_3 grasses, C_4 grasses and legumes). Koukolová et al. (2004) calculated a correlation coefficient of – 0.82 between DNDF and ADL contents for a set of samples including fresh grasses and grass silages. In silage made of legumes Rinne et al. (2006) reported that INDF was highly related ($r = 0.85$) to lignin content as well. In the present study (Table 3) CP was highly correlated ($P < 0.05$) with ADF, NDF with cellulose, ADF with cellulose and ADL with ADF.

Table 4. Prediction of indigestible neutral detergent fibre (y) of grasses from chemical components

Equation	RMSE[1]	R^2	Probability
Simple linear regression			
y = 164.2 – 7.308 CP	25.60	0.544	<0.0001
y = –83.16 + 5.408 CF	26.48	0.512	<0.0001
y = –139.8 + 3.834 NDF	24.39	0.586	<0.0001
y = –124.3 + 6.399 ADF	21.20	0.687	<0.0001
y = –25.17 + 41.36 ADL	17.65	0.783	<0.0001
y = –75.5 + 5.749 hemicellulose	32.57	0.262	<0.0001
y = –122.8 + 6.867 cellulose	23.75	0.607	<0.0001
Multiple regression			
y = –86.98 + 1.542 NDF + 31.63 ADL	15.55	0.835	<0.0001
y = –77.30 + 2.692 ADF + 28.55 ADL	15.78	0.830	<0.0001
y = –65.84 + 2.088 CF + 33.43 ADL	15.73	0.831	<0.0001
y = 36.28 + 32.36 ADL – 3.144 CP	14.97	0.847	<0.0001
Stepwise multiple regression			
y = –21.15 + 27 ADL – 2.524 CP + 1.13 NDF	13.80	0.872	<0.0001

[1]residual mean square error

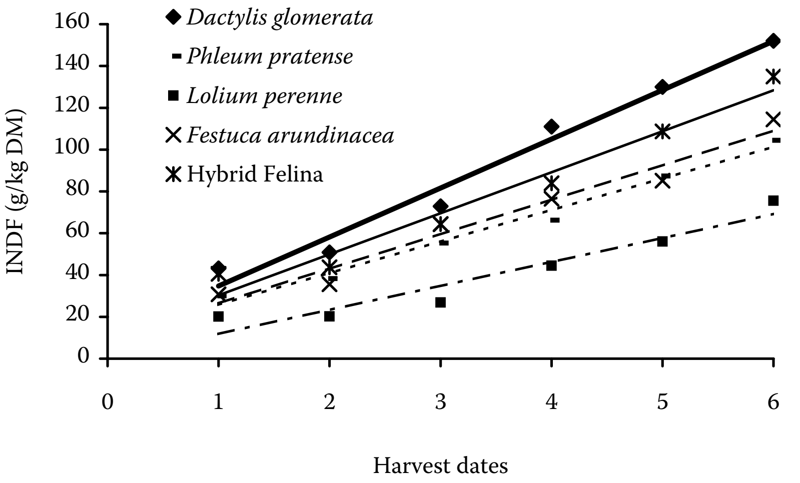

Figure 1. Accumulation of INDF contents of grasses at consecutive harvest dates (mean values of two years)

Table 4 shows the regression equations describing the relationships between INDF and the chemical composition of grasses. According to R^2-values and residual mean square errors ADL represented the best single predictor of INDF content and hemicellulose the worst. Traxler et al. (1998) predicted INDF from ADL contents with R^2-values 0.63, 0.69, 0.66 and 0.79 for C_3 grasses, C_4 grasses, legumes and combined forages, respectively. According to multiple regression including ADL and CP contents INDF could be predicted with the R^2-value almost 0.85, whereas stepwise multiple regression included NDF contents in the above equation to increase the R^2-value to 0.87. In accordance with the present study Nousiainen et al. (2003) and Huhtanen et al. (2006b) found ADL as the best single predictor of *in vivo* organic matter digestibility for grass silages (R^2 = 0.62) and for a set of forage samples (grasses, legumes and whole crops) (R^2 = 0.43), respectively.

Increasing INDF contents were observed in all grass species as the harvest time progressed (Figure 1). This indicates an increase in cell wall fractions with harvest dates that was also found by Coblentz et al. (1998) and Cone et al. (1999). The highest accumulation of INDF was detected for *Dactylis glomerata* and the lowest for *Lolium perenne*, being however statistically significant (P < 0.0001) in all grasses (Table 5). Pozdíšek et al. (2003) reported higher digestibility of NDF for *Festuca arundinacea* compared to the Hykor hybrid. In the present study ADL increased from 15 to 38, 16 to 32, 14 to 26, 19 to 29 and 10 to 31 g/kg DM with harvest dates for *Dactylis glomerata*, *Phleum pratense*, *Lolium perenne*, *Festuca arundinacea* and Felina hybrid, respectively. According to Beck et al. (2007) NDF contents of grasses increased from 587 to 722 g per g DM, ADF contents from 334 to 433 g/kg DM within one month. The maturity of grass caused decreased degradability and digestibility, both in magnitude and rate (Cone et al., 1999; Dawson et al., 2002). For example Rinne et al. (2002) reported that the INDF content of grass silage made from grasses (mixed growth of *Phleum pratense* and *Festuca pratensis*) harvested from June 13 to July 4 increased from 48 to 124 g/kg DM. Both Di Marco et al. (2002) and Harrison et al. (2003) presented evidence that the harvest of grass and maize at younger

Table 5. Evaluation of INDF contents of grasses in relation to harvest date

Grass	Estimate[1]	SE	Probability
Dactylis glomerata	23.61	0.999	<0.0001
Phleum pratense	15.26	0.427	<0.0001
Lolium perenne	11.46	0.466	<0.0001
Festuca arundinacea	16.34	1.200	<0.0001
Felina hybrid	19.74	0.624	<0.0001

[1]estimate values indicate an increase in INDF content (g/kg DM) at consecutive dates of harvest

Table 6. Comparison of grasses according to changes in INDF contents (g/kg DM) at harvest dates

Grasses[1]	Estimate	SE	Probability
1 vs. 2	8.353	1.086	<0.001
1 vs. 3	12.14	1.102	<0.001
1 vs. 4	7.273	1.561	<0.001
1 vs. 5	3.870	1.177	0.013
2 vs. 3	3.796	0.632	<0.001
2 vs. 4	−1.080	1.274	1.000
2 vs. 5	−4.483	0.756	<0.001
3 vs. 4	−4.875	1.287	0.002
3 vs. 5	−8.279	0.786	<0.001
4 vs. 5	−3.403	1.353	0.130

[1]1 = *Dactylis glomerata*; 2 = *Phleum pratense*; 3 = *Lolium perenne*; 4 = *Festuca arundinacea*; 5 = Felina hybrid

stages improved NDF digestibility. In contrast, in the present study the contents of CP decreased from 188 to 98, 176 to 83, 182 to 100, 201 to 95 and 154 to 68 g per g DM with harvest dates for *Dactylis glomerata*, *Phleum pratense*, *Lolium perenne*, *Festuca arundinacea* and Felina hybrid, respectively. Hoffman et al. (1993) and Rinne and Nykänen (2000) found a similar decrease in CP content during the maturation of grasses. Differences between grass species in changes in INDF content at harvest dates are shown in Table 6. There were no differences between *Phleum pratense* and *Festuca arundinacea* and between *Festuca arundinacea* and Felina hybrid. Other differences between grasses were statistically significant ($P < 0.05$). Harrison et al. (2003) also described significant differences in NDF digestibility between perennial ryegrass cultivars.

CONCLUSION

It was confirmed by this study that the INDF contents of grasses could be effectively predicted from ADL contents. INDF contents markedly increased during the maturation of grasses, which has practical implications for the time of harvest.

REFERENCES

Agbagla-Dohnani A., Nozière P., Clément G., Doreau M. (2001): *In sacco* degradability, chemical and morphological composition of 15 varieties of European rice straw. Anim. Feed Sci. Technol., 94, 15–27.

Ahmad N., Wilman D. (2001): The degradation of the cell walls of lucerne, Italian ryegrass and wheat straw when fed to cattle, sheep and rabbits. J. Agric. Sci., 137, 337–349.

AOAC (1990): Official Methods of Analysis, Association of Official Analytical Chemists. 15th ed. Washington, USA.

Beck P.A., Hutchison S., Gunter S.A., Losi T.C., Stewart C.B., Capps P.K., Phillips J.M. (2007): Chemical composition and *in situ* dry matter and fiber disappearance of sorghum × Sudangrass hybrids. J. Anim. Sci., 85, 545–555.

Coblentz W.K., Fritz J.O., Fick W.H., Cochran R.C., Shirley J.E. (1998): *In situ* dry matter, nitrogen, and fibre degradation of alfalfa, red clover, and Eastern Gamagrass at four maturities. J. Dairy Sci., 81, 150–161.

Cone J.W., Van Gelder A.H., Soliman I.A., De Visser H., Van Vuuren A.M. (1999): Different techniques to study rumen fermentation characteristics of maturing grass and grass silage. J. Dairy Sci., 82, 957–966.

Dawson, L.E.R., Kirkland R.M., Ferris C.P., Steen R.W.J., Kilpatrick D.J., Gordon F.J. (2002): The effect of stage of perennial ryegrass maturity at harvesting, fermentation characteristics and concentrate supplementation, on the quality and intake of grass silage by beef cattle. Grass Forage Sci., 57, 255–267.

Di Marco O.N., Aello M.S., Nomdedeu M., Van Houtte S. (2002): Effect of maize crop maturity on silage chemical composition and digestibility (*in vivo*, *in situ* and *in vitro*). Anim. Feed Sci. Technol., 99, 37–43.

Fonseca A.J.M., Dias-da-Silva A.A., Orskov E.R. (1998): *In sacco* degradation characteristics as predictors of digestibility and voluntary intake of roughages by mature ewes. Anim. Feed Sci. Tech., 72, 205–219.

Grenet E., Besle J.M. (1991): Microbes and fibre degradation. In: Jouany J.P. (ed.): Rumen Microbial Metabolism and Ruminant Digestion, Institut National de la Recherche Agronomique, Paris, France, 107–129.

Harrison J., Huhtanen P., Collins M. (2003): Perennial grasses. In: Silage Science and Technology. Agron. Monogr., 42, 665–747.

Herrera-Saldana R.E., Huber J.T., Poore M.H. (1990): Dry matter, crude protein, and starch degradability of five cereal grains. J. Dairy Sci., 73, 2386–2393.

Hoffman P.C., Sievert S.J., Shaver R.D., Welch D.A., Combs D.K. (1993): *In situ* dry matter, protein, and fibre degradation of perennial forages. J. Dairy Sci., 76, 2632–2643.

Homolka P. (2000): Digestibility of nutrients, nitrogen degradability and intestinal digestibility of rumen undegraded protein of alkali-treated barley. Czech J. Anim. Sci., 45, 447–450.

Huhtanen P., Vanhatalo A., Varvikko T. (1998): Enzyme activities of rumen particles and feed samples incubated *in situ* with differing types of cloth. Brit. J. Nutr., 79, 161–168.

Huhtanen P., Ahvenjärvi S., Weisbjerg M.R., Norgaard P. (2006a): Digestion and passage of fibre in ruminants. In: Sjersen K., Hvelplund T., Nielsen M.O. (eds.): Ruminant Physiology, Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress. Wageningen Academic Publishers, Netherlands, 87–135.

Huhtanen P., Nousiainen J., Rinne M. (2006b): Recent developments in forage evaluation with special reference to practical applications. Agric. Food Sci., 15, 293–323.

Cherney D.J.R., Cherney J.H., Lucey R.F. (1993): *In vitro* digestion kinetics and quality of perennial grasses as influenced by forage maturity. J. Dairy Sci., 76, 790–797.

Jensen K.B., Waldron B.L., Asay K.H., Johnson D.A., Monaco T.A. (2003): Forage nutritional characteristics of orchardgrass and perennial ryegrass at five irrigation levels. Agron. J., 95, 668–675.

Koukolová V., Weisbjerg M.R., Hvelplund T., Lund P., Čermák B. (2004): Prediction of NDF degradation characteristics of grass and grass/clover forages based on laboratory methods. J. Anim. Feed Sci., 13, 691–708.

López S., Carro M.D., González J.S., Ovejero F.J. (1998): Comparison of different *in vitro* and *in situ* methods to estimate the extent and rate of degradation of hays in the rumen. Anim. Feed Sci. Technol., 73, 99–113.

Nagadi S., Herrero M., Jessop N.S. (2000): The effect of fermentable nitrogen availability on *in vitro* gas production and degradability of NDF. Anim. Feed Sci. Technol., 87, 241–251.

Nousiainen J., Rinne M., Hellämäki M., Huhtanen P. (2003): Prediction of the digestibility of the primary growth and regrowth grass silages from chemical composition, pepsin-cellulase solubility and indigestible cell wall content. Anim. Feed Sci. Technol., 110, 61–74.

Nousiainen J., Ahvenjärvi S., Rinne M., Hellämäki M., Huhtanen P. (2004): Prediction of indigestible cell wall fraction of grass silage by near infrared reflectance spectroscopy. Anim. Feed Sci. Technol., 115, 295–311.

Oba M., Allen M.S. (1999): Evaluation of the importance of the digestibility of neutral detergent fibre from forage: effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci., 82, 589–596.

Pozdíšek J., Loučka R., Machačová E. (2003): Digestibility and nutrition value of grass silages. Czech J. Anim. Sci., 48, 359–364.

Rasch D., Tiku M.L., Sumpf D. (1994): Elsevier's dictionary of biometry. Elsevier Science B. V. chap. 221. Bonferroni Inequal., 64 pp.

Rasch D., Verdooren L.R., Gowers J.I. (1999): Fundamentals in the design and analysis of experiments and surveys. Oldenbourg Wissenschaftsverlag GmbH, chap. 4.3. Mult. Comp. Means, 126 pp.

Regulation No. 497/2004 (2004): Requirements of samples taking, methods for estimation of the feeds, supplements and premixes and way of samples storage. Collection of Law 2004. Czech Republic, part 172. (in Czech)

Rinne M., Nykänen A. (2000): Timing of primary growth harvest affects the yield and nutritive value of timothy-red clover mixtures. Agric. Food Sci. Finl., 9, 121–134.

Rinne M., Jaakkola S., Huhtanen P. (1997a): Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. Anim. Feed Sci. Tech., 67, 1–17.

Rinne M., Huhtanen P., Jaakkola S. (1997b): Grass maturity effects on cattle fed silage-based diets. 2. Cell wall digestibility, digestion and passage kinetics. Anim. Feed Sci. Technol., 67, 19–35.

Rinne M., Huhtanen P., Jaakkola S. (2002): Digestive processes of dairy cows fed silages harvested at four stages of grass maturity. J. Anim. Sci., 80, 1986–1998.

Rinne M., Olt A., Nousiainen J., Seppälä A., Tuori M., Paul C., Fraser D.M., Huhtanen P. (2006): Prediction of legume silage digestibility from various laboratory methods. Grass Forage Sci., 61, 354–362.

SAS (2002–2003): SAS System for Windows, Release 9.1 (TS1M3), SAS Inst., Inc., Cary, NC, USA

Sommer A., Vodňanský M., Petrikovič P., Požgaj R. (2005): Influence of lucerne and meadow hay quality on the digestibility of nutrients in the roe deer. Czech J. Anim. Sci., 50, 74–80.

Statistica (2001): Data analysis software system, Version 6, StatSoft, Inc., OK.

Tománková O., Kopečný J. (1995): Prediction of feed protein degradation in the rumen with bromelain. Anim. Feed Sci. Technol., 53, 71–80.

Tománková O., Homolka P. (2004): *In vitro* ruminal degradability of cereal grain starch. Czech J. Anim. Sci., 49, 151–155.

Traxler M.J., Fox D.G., Van Soest P.J., Pell A.N., Lascano C.E., Lanna D.P.D., Moore J.E., Lana R.P., Vélez M., Flores A. (1998): Predicting forage indigestible NDF from lignin concentration. J. Anim. Sci., 76, 1469–1480.

Van Soest P.J. (1994): Nutritional Ecology of the Ruminant. Cornell University, USA. 476 pp.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991): Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74, 3583–3597.

Van Vuuren A.M., Tamminga S., Ketelaar R.S. (1991): *In sacco* degradation of organic matter and crude protein of fresh grass (*Lolium perenne*) in the rumen of grazing dairy cows. J. Agric. Sci., 116, 429–436.

Zadoks J.C., Chang T.T., Konzak C.F. (1974): A decimal code for the growth stages of cereals. Weed Res., 14, 415–421.

Received: 2007–08–13
Accepted after corrections: 2007–11–26

Corresponding Author

Ing. Filip Jančík, Institute of Animal Science, Přátelství 815, Prague-Uhříněves, Czech Republic
Tel. +420 267 009 728, e-mail: jancik.filip@vuzv.cz