Comparison of analyses to predict ruminal fibre degradability and indigestible fibre in temperate grass silages

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Abstract

The objective of this study was to compare the ruminal degradability of neutral detergent fibre (NDF) and indigestible NDF (INDF) between silages (n = 24) that originated from three different temperate grass species, i.e. *Dactylis glomerata* L., *Festuca arundinacea* L. and hybrid, Felina – *Lolium multiflorum* L. × *Festuca arundinacea* S. The data is used to create prediction equations for the effective degradability of NDF (ED_{NDF}), assuming ruminal solid outflow rates of 0.02 (low), 0.05 (medium) and 0.08/h (high), and INDF. The highest values for the potentially degradable fraction of NDF (*b*), ED_{NDF2}, ED_{NDF5} and ED_{NDF8} (874, 560, 366 and 272 g/kg NDF, respectively) and the lowest for INDF (73.1 g/kg DM) were found for *F. arundinacea.* These parameters were significantly different from the hybrid Felina, which presented the lowest rate of NDF degradation (*c*), ED_{NDF5} and ED_{NDF8} (0.033/h, 341 and 250 g/kg NDF, respectively). The highest for *c* (0.038/h) and INDF (86.9 g/kg DM) and the lowest for *b* (847 g/kg NDF) were detected for *D. glomerata*. The combination of acid detergent fibre (ADF) and acid detergent lignin (ADL) (R^2 = 0.844) were identified by a backward, stepwise, multiple regression analysis as the most accurate to predict INDF. Crude protein, ADF and NDF were found as the most suitable combination for predicting ED_{NDF2} (R^2 = 0.715), ED_{NDF5} (R^2 = 0.669) and ED_{NDF8} (R^2 = 0.648). Calculated equations found practical application in laboratory analyses to evaluate the nutritional quality of feeds for ruminants.

Keywords: Grass silage, neutral detergent fibre, rumen degradation, prediction equations
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Introduction

Grass is one of the most important sources of nutrients for domesticated ruminants during a large part of the year (Taweel *et al.*, 2005). Yan & Agnew (2004) noted that despite being a major feed for ruminants across the world, the nutritive value of grass silage is extremely variable. The digestibility of the different grass species could be distinctly different, and is also influenced by area of origin, including temperature, light intensity, total rainfall, soil type, fertilization level, and by stage of maturity and preservation method (Huhtanen *et al.*, 2006; Jančík *et al.*, 2009). This is especially evident in the rate and extent of ruminal degradation of neutral detergent fibre (NDF) (Hoffman *et al.*, 1993). Several authors have compared the chemical composition and NDF degradability of different grass species (Cherney *et al.*, 1993; Hoffman *et al.*, 1993; Jensen *et al.*, 2003). However, comparisons of NDF degradability and indigestible neutral detergent fibre (INDF) of grass silages, made from *Dactylis glomerata*, *Festuca arundinacea* and grass hybrids (*Lolium multiflorum* L. × *Festuca arundinacea* S.*)* grown under the same conditions, are lacking.

The extent and rate of digestion of structural carbohydrates of the cell wall, which is mainly degraded in the rumen with the aid of ruminal microorganisms (Zhang *et al.*, 2007), are highly related to the nutritive value of ruminant feeds (Andrés *et al.*, 2005). The NDF concentration, which represents cell walls of feeds, could be affected by several factors such as temperature, light intensity, water availability, latitude, maturity, and harvesting and storage methods (Van Soest, 1994). Furthermore, the intrinsic characteristics of the carbohydrate fraction, such as the proportion of starch or cellulose and lignification of the cell wall, could

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have an influence on the rate of fermentation of feeds (Nagadi *et al.*, 2000). Indigestible neutral detergent fibre is a part of the cell wall that is unavailable to microbial digestion in ruminants, even when the total tract residence time of the fibre could be extended to an infinite time period (Van Soest, 1994). Lund *et al.* (2004) showed that the NDF concentration and potential digestibility of NDF are important factors to be included in new feed evaluation systems. If the incorporation of degradation characteristics into a forage quality prediction system seems promising, methods to replace laborious conventional *in situ* or *in vitro* procedures to estimate these characteristics on a routine basis, should be evaluated (Hackmann *et al.*, 2008).

The objectives of the present study were to (1) compare ruminal degradability parameters of NDF and indigestible NDF (INDF) among silages made from three different grasses, and (2) to develop prediction equations for ruminal degradability parameters of NDF and INDF from their chemical composition.

Materials and Methods

Twenty four silage samples made from three different temperate grasses, i.e. orchardgrass - *Dactylis glomerata* L. cv. Dana; fescue - *Festuca arundinacea* L. cv. Prolate and the hybrid Felina – *Lolium multiflorum* L. × *Festuca arundinacea* S. cv. Felina were evaluated in the present study. The grass was harvested from the primary growth of monocultured grasses on 19 and 26 May of 2004 and 27 May and 10 June of 2005 at the Větrov Breeding Station, Tábor region, Czech Republic (49° 31' 2.04" N lat, 14° 28' 4.9" E long; 620 m altitude). The growth stages of the grass used for silage making are presented in Table 1. The annual mean temperatures in 2004 and 2005 were 8.4 and 8.3 °C, and the annual rainfall 1020 and 1047 mm, respectively. The grass was wilted for up to 4 h on a table drier with cool air ventilation for 300 to 400 g/kg dry matter (DM), whereafter it was cut with a cutting machine (RP03, OPP Polička a.s., Czech Republic) to a theoretical length of 1.0 to 1.5 cm and ensiled, without additives, in hermetic glass vessels (3 L capacity). For each grass species and harvest time, two vessels were stored in a dark room at a temperature of 15 °C for storage periods of 10 and 20 weeks, respectively. At the opening the silages were analyzed for fermentation quality, i.e. pH (measured with a pH meter, WTW Level 1) and concentration of lactic, acetic and butyric acids, determined by isotachophoresis (Kvasnička, 2000) using an Ionosep 2001 apparatus (RECMAN - Laboratory Systems, Ostrava, Czech Republic). Isotachophoresis is one of the basic electrophoretic techniques used based on injecting a sample solution between leading and terminating solutions present in the separation compartment. The leading electrolyte solution contains an anion or cation which has a higher effective mobility than those of the sample constituents to be analyzed. The terminating electrolyte solution contains an anion or cation of a lower effective mobility. The electric field was applied to separate the sample constituents (Kvasnička, 2000). The silage samples were subsequently oven-dried at 50 °C for 48 h and milled to pass a 1 mm screen.

All ensiled samples (n = 24) were analyzed for DM, crude protein (CP), ash, ether extract (EE), NDF, acid detergent fibre (ADF) and acid detergent lignin (ADL). The residual moisture of the samples was determined at 105 °C for 12 h of oven-drying, and ash content was determined after combustion at 550 °C for 4.5 h (Regulation No. 497/2004, 2004). The EE level was determined, using the Soxtec extraction method with petroleum-ether (AOAC Official Method 920.39; AOAC, 2005). The Kjeldahl method was used for determining of nitrogen (N) (AOAC Official Method 976.05; AOAC, 2005), with the CP content

Table 1 Maturity stages[1] of used grass for silage-making by harvest date

Year	Date	Grass species		
		Dactylis glomerata	*Festuca arundinacea*	Hybrid Felina
2004	19 May	35	31	38
	26 May	51	32	50
2005	27 May	51	37	51
	10 June	61	55	59

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

calculated as N x 6.25. Ash-free ADF and ADL were analyzed according to the AOAC Official Method 973.18 (AOAC, 2005). The NDF concentration (Van Soest *et al.*, 1991) of samples and digested residues were analysed in the presence of sodium sulphite, but without α-amylase treatment, and is presented as ash-free. All fibre analyses were performed in an ANKOM 220 Fiber Analyzer (ANKOM Technology Corporation, NY. USA).

In situ experiments complied with the guidelines of the Ministry of Agriculture of the Czech Republic (Act No 246/1992 Coll.) in respect of animal experimentation and the care of animals under study. For *in situ* analyses, two Holstein steers (live weight 800 kg) fitted with rumen cannulas (120 mm in inside diameter), were used. The animals had *ad libitum* access to meadow hay, and were fed twice a day with 1 kg of barley meal per animal. The meadow hay was made from growth, containing orchardgrass (*Dactylis glomerata* L.; 30%), timothy (*Phleum pratense* L.; 20 %), meadow fescue (*Festuca pratensis* H.; 25%), ryegrass (*Lolium perenne* L.; 10%), white clover (*Trifolium repens* L.; 10%) and herbs (5%). For the *in situ* determination, the silage samples were oven-dried at 50 °C for 48 h and milled to pass a 1 mm screen. The rumen degradability of the NDF, in the experimental silages, was determined by incubating 1.5 g of each silage (in triplicate) in nylon bags (pore size 42 µm, internal dimensions 50 × 120 mm, Uhelon 130 T, Silk and Progress Moravská Chrastová, Czech Republic) for 6, 12, 24, 48, 72 and 96 h in the rumen of the steers.

Indigestible NDF contents of the silage samples were determined by using a 288 h rumen incubation period (Rinne *et al.*, 1997; Nousiainen *et al.*, 2004). Nylon bags (Swiss Silk Bolting Cloth Ltd., Zurich, Switzerland; external dimensions 60 x 120 mm) with a small pore size (17 µm), which minimize particle inflow and outflow but still ensure sufficient microbial activities inside the bags (Huhtanen *et al.*, 1998; Nousiainen *et al.*, 2003b), were used. Three grams of each sample were weighed into a bag and incubated in three replicates per animal. After incubation, the bags were rinsed by hand with cold water for 30 minutes and dried at 50 °C for 48 h.

Parameters of NDF ruminal degradability ($Deg_{(t)}$) and effective degradability of NDF (ED_{NDF}) were calculated according to equations adapted from Ørskov & McDonald (1979):

$$Deg_{(t)} = b \times (1 - \exp^{-ct}); \quad ED_{NDF} = b \times [c / (c + k)]$$

where b = fraction of NDF potentially degradable in the rumen, c = rate constant of degradation of fraction b, t = time of incubation and k = ruminal outflow rate. ED_{NDF} was estimated for each sample assuming ruminal solid outflow rates of 0.02, 0.05 and 0.08/h (ED_{NDF2}, ED_{NDF5} and ED_{NDF8}, respectively), which represent low, medium and high feed intakes, respectively (Petit & Tremblay, 1992). The soluble fraction was omitted from the equations, as NDF is by definition insoluble.

The results for chemical composition (ash, EE, CP, NDF, ADF and ADL), rumen degradation parameters (c, b, ED_{NDF2}, ED_{NDF5} and ED_{NDF8}) and INDF were analyzed using the MIXED procedure of SAS (SAS, 2002-2003). For the evaluation of rumen degradation parameters and INDF, grass species, year, storage period and animal were used as fixed effects, and harvest time nested within year used as a random effect. The grass species × year, grass species × storage period, year × storage period, grass species × animal, year × animal and storage period × animal interactions were included in the model. The data for chemical composition was analyzed with a similar model as described above, but with the animal effect excluded. Differences in mean values were evaluated by Scheffe's tests (Scheffé, 1953).

Correlation and simple linear regression were used for the evaluation of relationships within the parameters of chemical composition and utilization of NDF of the grass silages. Backward, stepwise, multiple regression analysis was applied to determine the best combination of chemical components for the prediction of ED_{NDF} and INDF.

Results and Discussion

The chemical composition, fermentation quality, NDF ruminal degradation parameters and INDF of grass silages evaluated are presented in Table 2. The values found in the present study showed a high variability in the chemical composition and degradability parameters, reflecting a wide range in NDF quality in the experimental silages. A wide variability is desired for developing prediction equations. The values of chemical composition are comparable to the results obtained by Huhtanen *et al.* (2002), Koukolová *et al.* (2004) and Nousiainen *et al.* (2004). Wilman *et al.* (2000) reported mean b and c values of 741 g/kg NDF and 0.048/h, respectively, for NDF degradability of grass silages that contained 68 g/kg DM of lignin, whereas Spanghero *et al.* (2007) found ED_{NDF} values of grass hay to vary from 239 to 483 g/kg NDF (k = 0.03/h). A mean value of 860 g/kg NDF for the potential digestibility of NDF, and 71.8 g/kg DM for INDF, was obtained for grass silage by Oba & Allen (1999). According to Rinne *et al.* (2002), the INDF contents of grass silages harvested from 13 June to 4 July in Finland ranged from 48 to 124 g/kg DM, and Lund *et al.* (2007) reported values of 49 and 137 g/kg DM INDF for early and late cut grass silages, respectively.

The differences in chemical composition of grass species are indicated in Table 3. The highest CP content was found in *F. arundinacea* and the lowest (P <0.05) in the hybrid Felina. The highest NDF content was detected in hybrid Felina (P <0.05). A significantly higher mean value of ADF was detected in hybrid, Felina compared to *F. arundinacea*. No significant differences in content of ADL and EE were found in the different grass species. *D. glomerata* had the lowest (P <0.05) level of ash. Similar to current results, Skládanka *et al.* (2008) detected a higher NDF content in the hybrid Felina, compared to *D. glomerata*. The same authors, however, stated a better quality (according to chemical composition) for the hybrid Felina, than for *F. arundinacea*. With the interactions between grass species × storage period and year × storage period being non-significant, both year and storage period, as main factors, did not present any differences (P >0.05). The interactions between grass species × year were different for ash, CP, NDF and ADF (P <0.05) (Table 6).

Table 2 Chemical composition, silage fermentation quality and NDF utilization of grass silages (n = 24)

	Mean	SD	Minimum	Maximum
Dry matter (DM, g/kg)	321	65.8	191	470
Silage chemical composition (g/kg DM)				
Ash	83.5	12.98	64.6	110
Ether extract (EE)	29.2	4.43	20.0	38.6
Crude protein (CP)	149	35.9	93.7	214
Acid detergent fibre (ADF)	331	35.3	253	386
Neutral detergent fibre (NDF)	549	60.5	440	638
Acid detergent lignin (ADL)	27.6	8.56	12.6	45.9
Parameters of fermentation process				
pH	4.65	0.378	4.04	5.33
Lactic acid (g/kg DM)	24.7	11.19	8.11	64.4
Acetic acid (g/kg DM)	6.66	3.819	3.00	18.3
Butyric acid (g/kg DM)	1.41	3.012	0.00	11.4
Ammonia N (g/kg DM)	0.36	0.120	0.20	0.62
Utilization of silages NDF				
b (g/kg NDF)	864	44.5	783	923
c (/h)	0.036	0.0070	0.027	0.049
ED_{NDF2} (g/kg NDF)	549	52.6	463	643
ED_{NDF5} (g/kg NDF)	357	48.1	284	449
ED_{NDF8} (g/kg NDF)	264	41.0	204	345
INDF (g/kg DM)	81.5	31.28	39.9	144

b - fraction of NDF potentially degradable in the rumen; *c* - rate constant of disappearance of fraction *b*.
ED_{NDF2}, ED_{NDF5} and ED_{NDF8} - effective degradability of NDF at rumen solid outflow rates of 0.02, 0.05 and 0.08/h.
INDF - indigestible neutral detergent fibre.

No significant interactions in grass species × storage period, grass species × animal, year × animal, storage period × animal and the animal effect were found for any of the parameters. Significant differences have been found in grass species regarding NDF degradation parameters (*b*, *c*, ED_{NDF}) and INDF content (Table 4). *Dactylis glomerata* presented the lowest (P < 0.05) value for *b* in comparison with *F. arundinacea* and hybrid Felina. The lowest *c* value (P <0.05) was found for hybrid Felina. The rate of NDF degradation (*c*) presented differences between year (*P* <0.0001) (Table 5) and storage periods (*P* = 0.019) (Table 5). The ED_{NDF2}, ED_{NDF5} and ED_{NDF8} were significantly higher after 20 weeks compared to 10 weeks of storage (*P* <0.001) (Table 5). At outflow rates of 0.05 and 0.08/h, hybrid Felina presented the lowest (*P* <0.05) ED_{NDF}. The lowest (*P* <0.05) content of INDF was found in *F. arundinacea*, whereas *D. glomerata* showed the highest numerical INDF content. However, it only differed significantly from *F. arundinacea* (Table 4).

Table 3 Chemical composition (g/kg DM) of silages as influenced by grass species (n = 8 per grass species)

Grass species	Ash	EE	CP	NDF	ADF	ADL
Dactylis glomerata	76.6^{b}	30.8	149^{b}	541^{b}	333^{ab}	31.2
Festuca arundinacea	85.9^{a}	27.6	178^{a}	512^{b}	311^{b}	26.6
Hybrid Felina	87.8^{a}	29.2	119^{c}	595^{a}	349^{a}	25.1
SE	3.69	1.59	14.2	28.5	15.3	3.82

$^{a-c}$ Within a column, means without a common superscript differ (*P* <0.05).
DM - dry matter; EE - ether extract; CP - crude protein; ADF - acid detergent fibre; NDF - neutral detergent fibre; ADL - acid detergent lignin; SE - standard error.

Table 4 Rumen neutral detergent fibre (NDF) degradability and indigestible neutral detergent fibre content of silages as influenced by grass species (*n* = 8 per grass species)

Grass species	*b*	*c*	ED_{NDF2}	ED_{NDF5}	ED_{NDF8}	INDF
Dactylis glomerata	847^{b}	0.038^{a}	550^{ab}	363^{a}	271^{a}	86.9^{a}
Festuca arundinacea	874^{a}	0.037^{a}	560^{a}	366^{a}	272^{a}	73.1^{b}
Hybrid Felina	871^{a}	0.033^{b}	537^{b}	341^{b}	250^{b}	84.5^{a}
SE	24.1	0.0007	16.7	11.5	8.82	15.26

^{a,b} Within a column, means without a common superscript differ (*P* <0.05).
b - fraction of NDF potentially degradable in the rumen (g/kg NDF); *c* - rate constant of disappearance of fraction *b* (/h); ED_{NDF2}, ED_{NDF5} and ED_{NDF8} - effective degradability of NDF (g/kg NDF) assuming rumen solid outflow rates of 0.02, 0.05 and 0.08/h; INDF - indigestible neutral detergent fibre (g/kg DM); SE - standard error.

Table 5 Differences of parameters describing utilization of silages' neutral detergent fibre (NDF) evaluated between years and between storage periods

	Year		SE	Storage period (weeks)		SE
	2004	2005		10	20	
b (g/kg NDF)	881	847	33.8	863	865	24.0
c (h^{-1})	0.041^{b}	0.030^{a}	0.0006	0.034^{a}	0.037^{b}	0.0006
ED_{NDF2} (g/kg NDF)	589	509	23.3	542^{a}	556^{b}	16.6
ED_{NDF5} (g/kg NDF)	394	319	15.9	349^{a}	364^{b}	11.4
ED_{NDF8} (g/kg NDF)	297	232	12.0	258^{a}	271^{b}	8.65
INDF (g/kg DM)	62.7	100	21.5	82.2	80.8	15.2

^{a,b} Within a row, means without a common superscript differ (*P* <0.05).
b - fraction of NDF potentially degradable in the rumen (g/kg NDF); *c* - rate constant of disappearance of fraction *b* (/h); ED_{NDF2}, ED_{NDF5} and ED_{NDF8} - effective degradability of NDF (g/kg NDF) assuming rumen solid outflow rates of 0.02, 0.05 and 0.08/h; INDF - indigestible neutral detergent fibre (g/kg DM); SE - standard error.

Table 6 Differences in chemical composition and parameters describing utilization of silages' neutral detergent fibre (NDF) evaluated for grass species × year interaction

Year	2004			2005			SE
Grass	D	F	H	D	F	H	
Silage chemical composition (g/kg dry matter, DM)							
Ash	71.7[ab]	71.4[ab]	79.3[ab]	81.6[a]	100[b]	96.4[b]	5.22
Ether extract	31.5	26.2	26.3	30.1	29.0	32.0	2.25
Crude protein	166[bde]	198[cde]	117[ade]	132[abcd]	158[abce]	120[abcd]	20.1
NDF	527[a]	479[a]	608[b]	556[ab]	545[ab]	582[ab]	40.3
ADF	323[b]	278[a]	356[b]	343[ab]	344[ab]	342[ab]	21.6
ADL	29.1	21.1	20.0	33.2	32.1	30.2	5.40
Utilization of silages NDF							
b (g/kg NDF)	850[a]	907[b]	888[b]	843[ab]	842[ab]	855[ab]	34.1
c (/h)	0.044[c]	0.044[c]	0.035[b]	0.032[b]	0.030[a]	0.030[a]	0.001
ED_{NDF2} (g/kg NDF)	585[a]	620[b]	562[b]	515[ab]	500[a]	512[a]	23.6
ED_{NDF5} (g/kg NDF)	399[b]	421[b]	363[a]	326[a]	311[a]	319[a]	16.3
ED_{NDF8} (g/kg NDF)	303[b]	319[b]	268[a]	239[a]	226[a]	232[a]	12.5
INDF (g/kg DM)	71.8[b]	44.5[a]	72.0[b]	102[ab]	102[ab]	97.0[ab]	21.6

[a-e] Within a row means without a common superscript differ (P <0.05).
D – *Dactylis glomerata* L.; F – *Festuca arundinacea* L.; H – hybrid Felina; ADF - acid detergent fibre; NDF - neutral detergent fibre; ADL - acid detergent lignin; *b* - fraction of NDF potentially degradable in the rumen (g/kg NDF); *c* - rate constant of disappearance of fraction *b* (/h); ED_{NDF2}, ED_{NDF5} and ED_{NDF8} - effective degradability of NDF (g/kg NDF) assuming rumen solid outflow rates of 0.02, 0.05 and 0.08/h; INDF - indigestible neutral detergent fibre (g/kg DM); SE - standard error.

A faster rate of NDF degradation (*c*) detected after the longer storage period (20 weeks) (P = 0.001; SE = 0.0006) influenced the calculated ED_{NDF} values (P = 0.0005, 0.0003 and 0.0003; SE = 16.6, 11.4 and 8.7 for ED_{NDF2}, ED_{NDF5} and ED_{NDF8}, respectively) (Table 5). This could be attributed to the shortening of the cellulose chain length as a result of extra-cellular cellulase activity from silage microflora and hydrolysis of hemicellulose by organic acids produced during fermentation (Yahaya *et al.*, 2001). Cell walls, subjected to silage processing and microorganisms, may be more susceptible to enzymatic attack, mainly in early periods of fermentation in the rumen. Comparable to current results, NDF digestibility of *F. arundinacea* was found by Pozdíšek *et al.* (2003) to be higher than for the *festulolium* hybrid Hykor. Presented differences of ED_{NDF} in the different grass species are in agreement with our results on the *in situ* degradation of DM (Jančík *et al.*, 2009), where *F. arundinacea* had higher mean values of DM effective degradability than *D. glomerata* and hybrid Felina. Differences in grass species might be related to the differences in maturity of the observed grass (Table 1). *D. glomerata* and hybrid Felina are early maturing grass species, whereas *F. arundinacea* is a late-maturing grass. In the present study differences were found for interactions between grass species × year and year × storage period. The differences for interactions between grass species × year detected for all degradability parameters and INDF (P <0.0001) are presented in the Table 6. The interactions between year × storage period were different for parameter *c*, ED_{NDF2}, ED_{NDF5} and ED_{NDF8} (P <0.001) (Table 7).

The correlation coefficients (r) of the relationships between chemical composition and parameters of NDF degradation (*b*, *c*, ED_{NDF2}, ED_{NDF5} and ED_{NDF8}) and INDF are given in Table 8. Parameter *b* was the highest (P <0.05) correlated to ADL, and the lowest to ash. The lower ash content was found for silages made from younger grasses. It means that these silages had a higher OM content, and a higher OM content can lead to faster digestibility of feeds, especially with regard to the lower lignification of younger grasses. The correlation coefficient between *c* and ash (r = -0.73) shows that faster degraded silages had a lower ash content. In contrast, parameter *c* was the highest (P <0.05) correlated to ash, and the lowest to EE. The highest correlations of ED_{NDF} and INDF were found in ADF (P <0.05), whereas these measurements presented low correlations with EE.

Table 7 Differences of parameters describing utilization of silages' neutral detergent fibre (NDF) evaluated for year × storage period interaction

Year	2004		2005		SE
Storage period (weeks)	10	20	10	20	
b (g/kg NDF)	883	880	843	850	34.0
c (/h)	0.034^{b}	0.044^{c}	0.044^{d}	0.031^{a}	0.0008
ED_{NDF2} (g/kg NDF)	574^{a}	604^{b}	509^{ab}	509^{ab}	23.5
ED_{NDF5} (g/kg NDF)	378^{a}	411^{b}	320^{a}	318^{a}	16.1
ED_{NDF8} (g/kg NDF)	282^{a}	311^{b}	233^{a}	231^{a}	12.2
INDF (g/kg DM)	64.0	61.4	100	100	21.5

[a-d] Within a row, means without a common superscript differ (P < 0.05).
b - fraction of NDF potentially degradable in the rumen (g/kg NDF); *c* - rate constant of disappearance of fraction *b* (/h); ED_{NDF2}, ED_{NDF5} and ED_{NDF8} - effective degradability of NDF (g/kg NDF) assuming rumen solid outflow rates of 0.02, 0.05 and 0.08/h; INDF - indigestible neutral detergent fibre (g/kg DM); SE - standard error.

Table 8 Linear correlation coefficients between chemical components and fibre-related measurements (n = 24)

Parameter	Ash	Ether extract (EE)	Crude protein (CP)	Acid detergent fibre (ADF)	Neutral detergent fibre (NDF)	Acid detergent lignin (ADL)
EE	0.18					
CP	-0.11	-0.06				
ADF	0.18	0.10	-0.86^{*}			
NDF	0.05	0.02	-0.92^{*}	0.93^{*}		
ADL	0.08	0.33	-0.41^{*}	0.54^{*}	0.42^{*}	
b	0.04	-0.12	0.62^{*}	-0.68^{*}	-0.61^{*}	-0.80^{*}
c	-0.73^{*}	-0.01	0.56^{*}	-0.59^{*}	-0.48^{*}	-0.30
ED_{NDF2}	-0.51^{*}	-0.08	0.72^{*}	-0.78^{*}	-0.65^{*}	-0.65^{*}
ED_{NDF5}	-0.60^{*}	-0.07	0.69^{*}	-0.74^{*}	-0.62^{*}	-0.56^{*}
ED_{NDF8}	-0.63^{*}	-0.06	0.68^{*}	-0.72^{*}	-0.60^{*}	-0.52^{*}
INDF	0.21	0.10	-0.75^{*}	0.83^{*}	0.74^{*}	0.78^{*}

$^{*}P$ <0.05; *b* - fraction of NDF potentially degradable in the rumen (g/kg NDF); *c* - rate constant of disappearance of fraction *b* (/h); ED_{NDF2}, ED_{NDF5} and ED_{NDF8} - effective degradability of NDF (g/kg NDF) assuming rumen solid outflow rates of 0.02, 0.05 and 0.08/h; INDF - indigestible neutral detergent fibre (g/kg DM).

In agreement with the current study, Hoffman *et al.* (1993) presented r-values of -0.32, 0.60 and 0.50 between ADL and CP, ED_{NDF} and CP, and ED_{NDF} and ADL, respectively. Andrés *et al.* (2005) found a high correlation between *b* and CP (r = 0.65), and NDF degradability after a 96-h incubation period and CP (r = 0.80). Significant correlations of -0.82, -0.78, -0.78 and 0.88 were reported (Koukolová *et al.*, 2004; Jančík *et al.*, 2008) between *b* and ADL, *c* and NDF, *c* and ADF, and INDF and ADL, respectively. Several authors (Traxler *et al.*, 1998; Jančík *et al.*, 2008) used ADL content as the most reliable variable to predict the INDF content of grass. According to Grenet & Besle (1991) and Nagadi *et al.* (2000) degradability of cell wall carbohydrates is mainly limited by lignin content, accentuating its influence on feed utilization (Ahmad & Wilman, 2001). Bosch & Bruining (1995) confirmed that grass silages, with a high lignin content, have a lower digestibility compared to silages that contained low levels of lignin. Traxler *et al.* (1998) found an important relationship (r = 0.81) between INDF and the lignin content in the different type of grass. This was confirmed by a high negative r value (-0.80) between the NDF digestibility and ADL content of legumes and grasses by Jung *et al.* (1997).

The most reliable combinations of chemical components (CP, NDF, ADF, ADL) to predict ED_{NDF2}, ED_{NDF5}, ED_{NDF8} and INDF, calculated by backward, stepwise, multiple regression analysis, are presented in Table 9. A multiple regression analysis was used because of the low predictability of equations determined with simple linear regression and one predictor (R^2 = 0.272 to 0.689) (Table 10). For ED_{NDF5}, ED_{NDF8} and INDF the lowest residual mean square error (RMSE) was obtained with a combination of three or four predictors. The ED_{NDF2} had the lowest RMSE when a combination of four predictors was used. However, the RMSE value derived from the use of two predictors for INDF and three predictors for ED_{NDF2} did not differ substantially from the use of four predictors. The combination of CP, ADF and NDF was identified as the most suitable predictor for ED_{NDF2}, ED_{NDF5} and ED_{NDF8}, whereas ADF and NDF also predicted ED_{NDF2}, ED_{NDF5} and ED_{NDF8} with an acceptable degree of accuracy. The combination of ADF and ADL was found to predict INDF accurately. However, an R^2-value of 0.689 showed that ADF can be used as a single predictor for INDF. Jančík *et al.* (2010) reported that the combination of ADF and NDF was the most suitable predictor for ED_{NDF} of dried *F. arundinacea* (R^2 = 0.941, RMSE = 18.3).

Table 9 Best-fit combinations of chemical components to predict neutral detergent fibre effective degradability and indigestible neutral detergent fibre content when applying backward, stepwise, multiple regression (*n* = 24)

	Constant	CP	ADF	NDF	ADL	RMSE	R^2
	$461_{(193.4)}$	$0.97_{(0.412)}$	$-1.38_{(0.505)}$	$-0.81_{(0.346)}$	$-1.71_{(0.835)}$	27.9	0.767
ED_{NDF2}	$470_{(208.2)}$	$1.00_{(0.443)}$	$-1.83_{(0.488)}$	$-0.97_{(0.362)}$		30.1	0.715
	$922_{(65.0)}$		$-1.90_{(0.533)}$	$0.47_{(0.311)}$		32.9	0.642
	$241_{(205.4)}$	$0.95_{(0.437)}$	$-1.40_{(0.536)}$	$-0.85_{(0.367)}$	$-0.91_{(0.887)}$	29.7	0.686
ED_{NDF5}	$246_{(205.5)}$	$0.96_{(0.438)}$	$-1.64_{(0.482)}$	$-0.93_{(0.358)}$		29.7	0.669
	$680_{(63.8)}$		$-1.71_{(0.523)}$	$0.44_{(0.306)}$		32.3	0.588
	$157_{(182.4)}$	$0.82_{(0.389)}$	$-1.22_{(0.476)}$	$0.74_{(0.326)}$	$-0.59_{(0.788)}$	26.4	0.658
ED_{NDF8}	$160_{(180.3)}$	$0.83_{(0.384)}$	$-1.38_{(0.423)}$	$0.80_{(0.314)}$		26.1	0.648
	$534_{(55.8)}$		$-1.44_{(0.456)}$	$0.38_{(0.267)}$		28.3	0.566
	$21.5_{(86.18)}$	$-0.35_{(0.086)}$	$0.49_{(0.225)}$	$-0.17_{(0.154)}$	$1.68_{(0.372)}$	12.5	0.869
INDF	$-36.5_{(69.75)}$	$-0.22_{(0.146)}$	$0.31_{(0.161)}$		$1.78_{(0.365)}$	12.5	0.860
	$-136_{(26.0)}$		$0.51_{(0.090)}$		$1.70_{(0.373)}$	12.9	0.844

Values in subscript parentheses are SE; RMSE - residual mean square error; CP - crude protein; ADF - acid detergent fibre; NDF - neutral detergent fibre; ADL - acid detergent lignin; ED_{NDF2}, ED_{NDF5} and ED_{NDF8} - effective degradability of NDF (g/kg NDF) assuming rumen solid outflow rates of 0.02, 0.05 and 0.08/h; INDF - indigestible neutral detergent fibre (g/kg DM).

Chemical parameters as predictors of digestion become complicated when plant types such as legumes or gramineae are studied (Khazaal *et al.*, 1995). Although empirical equations have not been proven to be successful in determining DM digestibility (Khazaal *et al.*, 1995) or OM digestibility (Huhtanen *et al.*, 2006) from fibre components, the current study shows that prediction equations based on chemical components can produce equations with acceptable accuracy for NDF digestibility. However, it should be emphasized that equations have to be restricted to forage type and harvest stage.

The content of ADF was the best single predictor of ED_{NDF} and INDF (Table 10). However, a better prediction equation for INDF was found when using a combination of ADF and ADL (Table 9). This could be attributed to the fact that lignin is regarded as the principal factor limiting digestibility, although it does not affect all feed components. Non-cell wall components are not influenced by lignin, but they can often be highly correlated. Therefore, lignin concentration affects mainly the availability of cell wall polysaccharides (Van Soest, 1994). Cellulose, which along with lignin forms ADF, reduces the digestion rate and extent of digestion which are related to the lignin content. Hemicellulose (presenting NDF along with cellulose and lignin) is closely associated with lignin, and the digestibility of hemicellulose is directly related to that of cellulose and inversely related to lignification (Van Soest, 1994). In the present study, a combination of CP, ADF and NDF gave the best equations for ED_{NDF} based on three predictors. The higher effect of CP on ED_{NDF} was caused by the influence of parameter *c* in the calculation of ED_{NDF}. A high correlation between CP and parameter *c* most likely occurred because of a co-association when protein declines as grass matures and lignification proceeds (Van Soest, 1994; Nousiainen *et al.*, 2003a). Neutral detergent fibre represents the total insoluble matrix fibre, and it is better related to rumination and passage compared to other chemical components. Acid detergent fibre does not represent all insoluble fibre, but it is frequently better correlated with digestibility than NDF (Van Soest, 1994).

Table 10 Prediction of neutral detergent fibre effective degradability and indigestible neutral detergent fibre content using simple linear regression (n = 24)

	Constant	CP	ADF	NDF	ADL	RMSE	R^2
ED_{NDF2}	$393_{(33.2)}$	$1.05_{(0.218)}$				37.4	0.515
	$932_{(66.4)}$		$-1.16_{(0.200)}$			33.8	0.604
	$861_{(77.6)}$			$-0.57_{(0.140)}$		40.8	0.426
	$660_{(28.6)}$				$-4.00_{(0.993)}$	40.8	0.425
ED_{NDF5}	$219_{(31.7)}$	$0.92_{(0.207)}$				35.7	0.474
	$690_{(65.0)}$		$-1.01_{(0.195)}$			33.1	0.547
	$625_{(73.9)}$			$-0.49_{(0.134)}$		38.8	0.378
	$443_{(28.7)}$				$-3.12_{(0.996)}$	40.9	0.309
ED_{NDF8}	$150_{(27.4)}$	$0.77_{(0.179)}$				30.8	0.458
	$542_{(56.7)}$		$-0.84_{(0.170)}$			28.9	0.525
	$488_{(63.8)}$			$-0.41_{(0.115)}$		33.5	0.362
	$333_{(25.1)}$				$-2.50_{(0.870)}$	35.7	0.272
INDF	$179_{(18.6)}$	$-0.66_{(0.122)}$				21.0	0.569
	$-162_{(35.0)}$		$0.74_{(0.105)}$			17.8	0.689
	$-128_{(41.2)}$			$0.38_{(0.075)}$		21.6	0.543
	$-3.08_{(14.151)}$				$2.84_{(0.490)}$	20.1	0.604

Values in subscript parentheses are SE.; RMSE - residual mean square error; CP - crude protein; ADF - acid detergent fibre; NDF - neutral detergent fibre; ADL - acid detergent lignin; ED_{NDF2}, ED_{NDF5} and ED_{NDF8} - effective degradability of NDF (g/kg NDF) assuming rumen solid outflow rates of 0.02, 0.05 and 0.08/h; INDF - indigestible neutral detergent fibre (g/kg DM).

Conclusions

According to parameters describing NDF utilization, all tested types of grass are suitable forages to be ensiled for ruminant nutrition. However, *D. glomerata* and hybrid Felina, should be harvested earlier because of their rapid maturing rate. *F. arundinacea* matures more slowly and thus provides a high-quality forage for a longer vegetative period. This study confirmed that the ED_{NDF} and the INDF content of grass silages could be effectively predicted from a combination of two or three chemical components. Based on the current results, the use of a combination of CP, ADF and NDF for prediction of ED_{NDF}, and ADF and ADL for prediction of INDF, can be suggested. These equations could replace laborious and time-consuming *in situ* and *in vivo* techniques to evaluate the nutritional quality of feeds for ruminants. However, more research is needed on the relationships between NDF rumen degradation parameters (including INDF) and chemical components for silages made of legumes, maize, regrowth grass and whole grain cereals.

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References

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.

Andrés, S., Giráldez, F.J., González, J.S., Paláez, R., Prieto, N. & Calleja, A., 2005. Prediction of aspects of neutral detergent fibre digestion of forages by chemical composition and near infrared reflectance spectroscopy. Aust. J. Agric. Res. 56, 187-193.

AOAC, 2005. Official Methods of Analysis, AOAC International. 18th ed. Gaithersburg, USA, ISBN 0-935584-75-7.

Bosch, M.W. & Bruining, M., 1995. Passage rate and total clearance rate from the rumen of cows fed on grass silages differing in cell-wall content. Br. J. Nutr. 73, 41-49.

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.

Grenet, E. & Besle, J.M., 1991. Microbes and fibre degradation. In: Rumen Microbial Metabolism and Ruminant Digestion. Ed. Jouany, J.P., Institut national de la recherche agronomique, Paris, France, 107-129.

Hackmann, T.J., Sampson, J.D. & Spain, J.N., 2008. Comparing relative feed value with degradation parameters of grass and legume forages. J. Anim. Sci. 86, 2344-2356.

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

Huhtanen, P., Vanhatalo, A. & Varvikko, T., 1998. Enzyme activities of rumen particles and feed samples incubated *in situ* with differing types of cloth. Br. J. Nutr. 79, 161-168.

Huhtanen, P., Khalili, H., Nousiainen, J.I., Rinne, M., Jaakkola, S., Heikkilä, T. & Nousiainen, J., 2002. Prediction of the relative intake potential of grass silage by dairy cows. Livest. Prod. Sci. 73, 111-130.

Huhtanen, P., Nousiainen, J. & Rinne, M., 2006. Recent developments in forage evaluation with special reference to practical applications. Agric. Food Sci. 15, 293-323.

Jančík, F., Homolka, P., Čermák, B. & Lád, F., 2008. Determination of indigestible neutral detergent fibre contents of grasses and its prediction from chemical composition. Czech J. Anim. Sci. 53, 128-135.

Jančík, F., Koukolová, V., Kubelková, P. & Čermák, B., 2009. Effects of grass species on ruminal degradability of silages and prediction of dry matter effective degradability. Czech J. Anim. Sci. 54, 315-323.

Jančík, F., Koukolová, V. & Homolka, P., 2010. Ruminal degradability of dry matter and neutral detergent fibre of grasses. Czech J. Anim. Sci. 55, 359-371.

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.

Jung, H.G., Mertens, D.R. & Payne, A.J., 1997. Correlation of acid detergent lignin and klason lignin with digestibility of forage dry matter and neutral detergent fiber. J. Dairy Sci. 80, 1622-1628.

Khazaal, K., Dentinho, M.T., Ribeiro, J.M. & Ørskov, E.R., 1995. Prediction of apparent digestibility and voluntary intake of hays fed to sheep: comparison between using fibre components, *in vitro* digestibility or characteristics of gas production or nylon bag degradation. Anim. Sci. 61, 527-538.

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.

Kvasnička, F., 2000. Application of isotachophoresis in food analysis. Electrophoresis 21, 2780-2787.

Lund, P., Weisbjerg, M.R., Ahvenjärvi, S., Huhtanen, P., Uden, P., Olafsson, B. & Volden, H., 2004. Nordic ringtest on INDF content and NDF degradation characteristics in three feeds. J. Anim. Feed Sci. 13, 139-142.

Lund, P., Weisbjerg, M.R., Hvelplund, T. & Knudsen, K.E.B., 2007. Determination of digestibility of different forages in dairy cows using indigestible NDF as marker. Acta Agric. Scand. 57, 16-29.

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., Helämäki, M. & Huhtanen, P., 2003a. Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsin-cellulase solubility. Anim. Feed Sci. Technol. 103, 97-111.

Nousiainen, J., Rinne, M., Helämäki, M. & Huhtanen, P., 2003b. 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 fiber from forage: Effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci. 82, 589-596.

Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J. Agric. Sci. 92, 499-503.

Petit, H.V. & Tremblay, G.F., 1992. *In situ* degradability of fresh grass and grass conserved under different harvesting methods. J. Dairy Sci. 75, 774-781.

Pozdíšek, J., Loučka, R. & Machačová, E., 2003. Digestibility and nutrition value of grass silages. Czech J. Anim. Sci. 48, 359-364.

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., Huhtanen, P. & Jaakkola, S., 1997. 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.

SAS, 2002-2003. SAS System for Windows, Release 9.1 (TS1M3), SAS Inst., Inc., Cary, N.C., USA.

Scheffé, H., 1953. A method for judging all contrasts in the analysis of variance. Biometrika 40, 87-104.

Skládanka, J., Dohnal, V. & Ježková, A., 2008. Fibre and ergosterol contents in forage of *Arrhenatherum elatius*, *Dactylis glomerata* and *Festulolium* at the end of the growing season. Czech J. Anim. Sci. 53, 320-329.

Spanghero, M., Gruber, L. & Zanfi, C., 2007. Precision and accuracy of the NDF rumen degradability of hays measured by the Daisy fermenter. Ital. J. Anim. Sci. 6, 363-365.

Taweel, H.Z., Tas, B.M., Smit, H.J., Elgersma, A., Dijkstra, J. & Tamminga, S., 2005. Improving the quality of perennial ryegrass (*Lolium perenne* L.) for dairy cows by selecting for fast clearing and/or degradable neutral detergent fiber. Livest. Prod. Sci. 96, 239-248.

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., Velez, 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. ISBN 0-8014-2772-X, 476 p.

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.

Wilman, D., Field, M., Lister, S.J. & Givens, D.I., 2000. The use of near infrared spectroscopy to investigate the composition of silages and the rate and extent of cell-wall degradation. Anim. Feed Sci. Technol. 88, 139-151.

Yahaya, M.S., Kimura, A., Harai, J., Nguyen, H.V., Kawai, M., Takahashi, J. & Matsuoka, S., 2001. Effect of length of ensiling on silo degradation and digestibility of structural carbohydrates of lucerne and orchardgrass. Anim. Feed Sci. Technol. 92, 141-148.

Yan, T. & Agnew, R.E., 2004. Prediction of nutritive values in grass silages: I. Nutrient digestibility and energy concentrations using nutrient compositions and fermentation characteristics. J. Anim. Sci. 82, 1367-1379.
Zadoks, J.C., Chang, T.T. & Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Res. 14, 415-421.
Zhang, Y.Q., Gao, W. & Meng, Q., 2007. Fermentation of plant cell walls by ruminal bacteria, protozoa and fungi and their interaction with fibre particle size. Arch. Anim. Nutr. 61, 114-125.