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Prediction of silage composition and organic matter digestibility from herbage composition and pepsin-cellulase solubility

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A dataset of grasses and respective silages was collected by systematically varying the harvesting time in primary growth (n = 27) and in regrowth (n = 25). The swards were mixtures of timothy and meadow fescue. The grasses were ensiled unwilted with formic acid. Fixed or mixed regression procedure of SAS was used to investigate the relationships between composition of grasses and respective silages and to develop regression equations for predicting silage *in vivo* organic matter digestibility (OMD) from herbage pepsin-cellulase organic matter solubility (OMS). The silages were well preserved showing only limited amounts of secondary fermentation products. The silage dry matter (DM), crude protein and neutral detergent fibre contents could be estimated relatively accurately from grass variables as judged by relatively small prediction errors ($\mathrm{RMSE_{mixed}}$ = 3.6, 8.1 and 18 g (kg DM)$^{-1}$, respectively). The average OMS of grasses was significantly higher than that of respective silages (779 vs. 756 g (kg DM)$^{-1}$, $P < 0.001$). However, silage OMD was equally accurately predicted from grass and silage OMS ($\mathrm{RMSE_{mixed}}$ = 15.1 and 15.8 g (kg DM)$^{-1}$, respectively). When predicting silage OMD from OMS, specific equations should be used for primary growth and regrowth silages, because the slopes and intercepts of correction equations were numerically though not statistically significantly different. It is concluded that silage composition and digestibility can be reliably predicted from herbage characteristics provided that silages are well preserved with moderate ensiling losses.

Key words: digestibility, ensiling, ensilage, grasses, herbage, pepsin-cellulase solubility, silage

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Introduction

Grass silage has remained the most important feed component of dairy cow diets in Finland despite reduced concentrate prices and increased production levels. The performance of dairy cows depends strongly on silage quality, both in terms of digestibility and fermentation characteristics. In recent studies one kilogram of concentrate supplements was required to compensate for a decrease of 10 g kg^{-1} in silage D-value [concentration of digestible organic matter (OM) in dry matter (DM)] of primary growth silages (Rinne et al. 1999, Kuoppala et al. 2004). Production responses to improved silage digestibility are derived both from higher energy concentration and increased silage DM intake (Rinne 2000). Based on a literature analysis of published data, daily silage DM intake increased by 0.16 kg per 10 g kg^{-1} increment in silage D-value (Huhtanen et al. 2002).

Under Finnish climatic conditions, organic matter digestibility (OMD) of the primary growth grass decreases and DM yield increases rapidly with advancing maturity in early summer (Rinne 2000). Accurate and precise predictions of digestibility at harvesting time are essential in management of milk production systems based on a large proportion of grass silage in the diet. Therefore, a meteorological model using cumulative temperature and geographical location has been developed in Finland to predict digestibility of swards in order to correctly time the harvest (Rinne et al. 2001, Artturi 2004). The OMD of herbage samples used in the model data was determined by *in vitro* organic matter pepsin-cellulase solubility (OMS) as described by Nousiainen et al. (2003a). This approach assumes that OMD of ensiled grass does not substantially change during the in-silo fermentation. However, theoretically silage digestibility should be lower than that of the ensiled herbage due to OM losses in effluent, respiration and fermentation, which all reduce the completely digestible fraction of grass.

Sampling of herbage during silage harvesting allows obtaining more representative samples and provides a more detailed illustration of the variation in silage digestibility than samples taken from the silos, especially those drilled from the top layer of large tower silos. Advance information of silage digestibility would also be useful in the ration planning for the next indoor feeding period, provided that silage OMD could accurately be predicted from herbage samples.

Because the OMD predictions of the herbage D-value model (Rinne et al. 2001) need to be confirmed by *in vivo* measurements and reliable predictions of silage digestibility from herbage instead of silage samples would provide several advantages in ration formulation, we investigated relationships between OMS of herbage samples and *in vivo* OMD of the respective silages. The data are derived from digestibility experiments conducted in MTT Agrifood Research Finland in order to develop meteorological D-value model and to study laboratory techniques in predicting feeding value of grass silages.

Material and methods

Herbages and respective silages, chemical analyses and digestibility determination

The silages were harvested from primary growth (n = 27) and regrowth (n = 25) of mixed timothy (*Phleum pratense)* meadow fescue (*Festuca pratensis*) swards in 1994–2002 in Jokioinen, Finland (for details, see Nousiainen et al. 2004). Representative samples of herbages were collected during ensiling for analyses.

In vivo digestibility of the silages was determined in sheep fed at maintenance level (35 g dry matter DM per kg LW$^{0.75}$) by the total faecal collection method (7 d collection period) in complete or incomplete Latin Square designs. The digestibility data are based on results from four (46 silages) or three sheep (6 silages). The digestibility determinations were conducted under supervision of the local ethical animal experiment committee and are described in detail by Nousiainen et al. (2004).

DM content of both herbage and silage samples was determined by oven drying overnight at 103°C. The DM content of silage samples was corrected for loss of volatiles according to Huida et al. (1986). Nitrogen (N) content was determined either by the Kjeldahl method or by the Dumas method (Leco FP-428 N analyzer). Crude protein (CP) content was calculated as 6.25 × N. Cell wall composition was determined by analysing neutral detergent fibre (NDF) content according to Van Soest et al. (1991) in the presence of sodium sulphite and acid detergent fibre (ADF) and lignin content as described by Robertson and Van Soest (1981). Indigestible NDF (INDF) was determined from silage samples by prolonged (12 d) *in situ* incubation with dairy cows fed forage-based diets using nylon bags of small pore size (6 or 17 µm) as described by Huhtanen et al. (1994). Silage fermentation characteristics [pH, ammonia N [g (kg total N) $^{-1}$], lactic acid [g (kg DM)$^{-1}$] and volatile fatty acids [VFA, g (kg DM)$^{-1}$)] were analysed as described by Shingfield et al. (2001). Silage total acids were calculated as lactic acid + VFA [TA, g (kg DM)$^{-1}$)]. *In vitro* OM pepsin-cellulase solubility of herbage and silage samples was determined as described by Nousiainen et al. (2003a).

Statistical methods

Relationships between laboratory measurements of herbage and silage samples were analysed by a fixed or mixed regression model (Littel et al. 1996), using year of harvest as a random factor. Using random year effect in the model can be justified e.g. by annual variation in the climatic conditions affecting the biological growth processes of herbage, in the activity of enzymes used in OMS determination and in analytical and digestibility results (typically the samples from the same year were analysed in the same batch).

Residual mean square error (RMSE) and coefficient of determination adjusted for degrees of freedom (Adj. R^2) were calculated for both fixed and mixed models. Because of different relationships between OMS and *in vivo* OMD for the primary and regrowth silages (Nousiainen et al. 2003b), cut (primary growth vs. regrowth) was used as a fixed factor in the model estimating relationships between herbage OMS and silage OMD. Bi- or trivariate regression models were used to investigate the effects of in-silo fermentation and changes in silage composition on the relationship between herbage OMS and silage *in vivo* OMD.

Results

Composition of grasses and silages

The characteristics of herbages and respective silages are presented in Table 1. All the grass and silage variables showed large variation and were normally distributed. The fermentation quality of silages was typical for well-preserved silages ensiled with acid-based additives. In general, the composition of silages closely reflected the corresponding characteristics of grasses. However, CP content of silages was slightly lower [151 vs. 154 g (kg DM)$^{-1}$; $P < 0.05$] than that of grasses. The change in CP content (ΔCP) during ensilage ($CP_{herbage} - CP_{silage}$) was associated with silage pH: ΔCP [g (kg DM)$^{-1}$] = 61.1(±14.4) – 14.4(±5.1) × Silage pH (RSME = 5.5; $P < 0.01$). Silage ammonia N or DM content of the grass ensiled had no influence on the change in CP content during ensilage.

Some degradation of NDF occurred during in silo fermentation, which was reflected as a significantly ($P < 0.001$) lower NDF content in silages than in original herbages [554 vs. 582 g (kg DM)$^{-1}$]. The decrease in NDF content ($NDF_{herbage} - NDF_{silage}$) was positively related to herbage OMS ($P < 0.001$) and silage pH ($P = 0.05$) and negatively related to herbage NDF ($P < 0.001$) and silage INDF content ($P < 0.001$), when analysed with a mixed model and using year of harvest as a random effect. Using herbage NDF content and silage pH as independent variables in a mixed analysis, the following relationship was estimated: ΔNDF [g (kg DM)$^{-1}$] = 59(±76) – 0.35(±0.06) × herbage NDF + 42(±16) × silage pH (RSME = 16.9, Adj. $R^2 = 0.55$).

Table 1. Chemical composition and digestibility of the herbages and the respective silages.

	Grass				Silage			
	Mean	Std.Dev.	Minimum	Maximum	Mean	Std.Dev.	Minimum	Maximum
Primary growth (n = 27)								
Dry matter, g kg^{-1}	205	37.1	136	285	218	29.7	171	285
pH					4.09	0.144	3.79	4.36
Ammonia N, g kg^{-1} total N					52	19.9	28	116
In dry matter, g kg^{-1}								
Ash	74	7.9	62	93	72	7.3	60	87
Crude protein	156	36.5	109	246	155	33.1	112	239
Neutral detergent fibre (NDF)	600	58.6	500	687	576	74.0	402	669
Indigestible NDF					79	40.9	17	158
Water soluble carbohydrates	112	32.4	48	197	40	17.1	17	78
Lactic acid					52	14.3	32	93
Acetic acid					20	8.3	9	49
Propionic acid					0.1	0.21	0.0	0.8
Butyric acid					0.5	0.71	0.0	2.2
Total acids [a]					73	16.8	48	118
OMS, g kg^{-1} [b]	781	67.7	655	897	757	73.8	634	878
OMD, g kg^{-1} [c]					734	64.8	613	840
Regrowth (n = 25)								
Dry matter, g kg^{-1}	216	54.9	128	319	225	44.8	151	321
pH					4.04	0.255	3.72	4.60
Ammonia N, g kg^{-1} total N					61	12.4	31	81
In dry matter, g kg^{-1}								
Ash	92	6.6	83	103	93	8.7	79	108
Crude protein	151	26.7	115	211	146	24.5	111	207
NDF	561	20.7	506	608	531	33.6	465	587
Indigestible NDF					108	28.0	60	167
Water soluble carbohydrates	99	18.5	61	144	83	23.6	34	127
Lactic acid					42	12.8	11	68
Acetic acid					12	2.6	8	17
Propionic acid					0.1	0.13	0.0	0.6
Butyric acid					0.3	0.62	0.0	2.7
Total acids [a]					55	14.5	23	88
OMS, g kg^{-1} [b]	777	32.6	709	843	756	28.5	700	811
OMD, g kg^{-1} [c]					693	35.2	610	766

[a] Calculated as VFA + Lactic acid

[b] Pepsin-cellulase solubility of organic matter

[c] *In vivo* digestibility of organic matter determined with sheep by total faecal collection

The *in vitro* OMS was on average 22.3 g kg^{-1} higher ($P < 0.001$) in grasses than in silages. The difference increased ($P < 0.01$) with increased NDF content of herbage ensiled. Pepsin-cellulase organic matter solubility, DM content of grass ensiled or fermentation characteristics of silage had no influence on the decrease in OMS during ensilage. The *in vivo* OMD of silages was numerically slightly lower than OMS in silages (734 vs. 757 g kg^{-1}) and in respective grasses (781 g kg^{-1}) in primary growth. However, in regrowth silages, OMD was markedly lower than OMS in silages (693 vs. 756 g kg^{-1}) and in grasses (777 g kg^{-1}).

Predictions of silage composition from herbage composition

The predictions of silage ash, CP, NDF and OMS from grass composition are shown in Figure 1. In general, the silage composition could be predicted

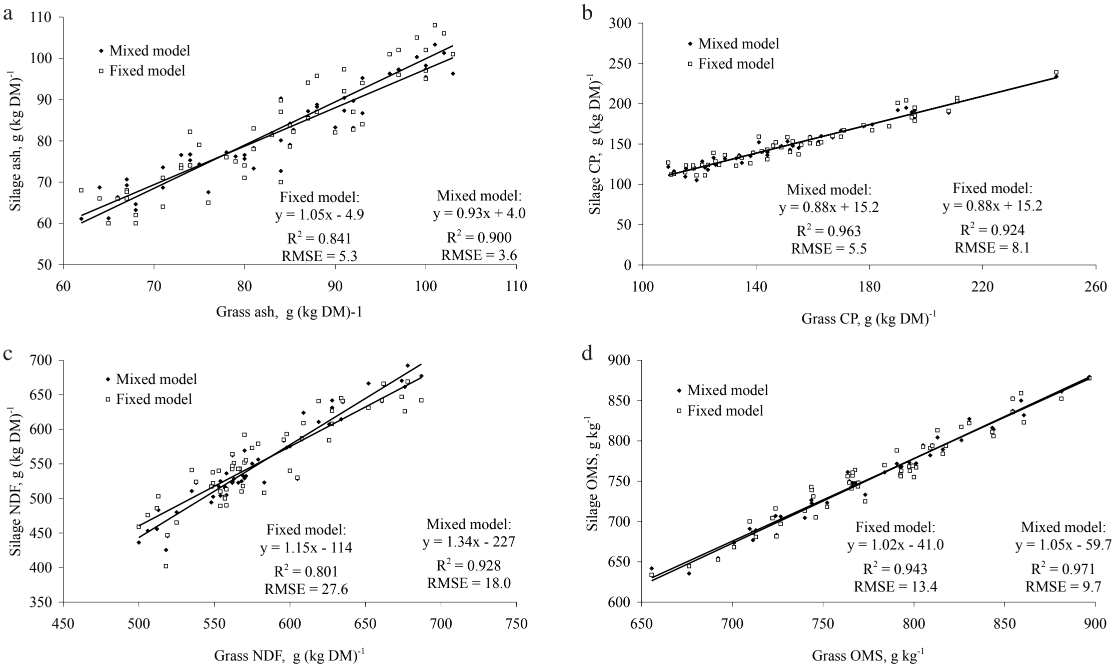

Fig. 1. The relationships between grass and respective silage characteristics; ash (a), crude protein (CP; b), neutral detergent fibre (NDF, c) and in vitro pepsin-cellulase solubility (OMS, d). The silage characteristics were estimated from grass composition using either a fixed (Y = a + bX) or a mixed (Y = Year + a + bX) regression model, where Y = silage variable, X = grass variable and Year is a random effect of the year of harvest (n = 52).

rather accurately from grass variables with relatively high coefficient of determination ($R^2 > 0.8$) and low RMSE values. The outcome of fixed and mixed regressions was very similar for CP and OMS content (Figures 1b and 1d). The year effects in the predictions of silage ash and NDF content were significant, resulting in slightly different equations between fixed and mixed regression models (Figures 1a and 1c).

Prediction of silage OMD from herbage OMS

The predictions of silage OMD from grass OMS in primary growth and regrowth samples are presented in Figures 2a and 2b, respectively. The accuracy of the regression equation in primary growth was very good (mixed model $R^2 = 0.971$ and RMSE = 11.1 g kg^{-1}), with practically no difference between fixed and mixed models. However, the performance of fixed regression model in regrowth was much poorer than that of mixed model. Also the model parameters differed between fixed and mixed regression methods. This is most probably due to marked year effects leading to variable *in vivo* OMD at certain OMS between harvesting years. The intercepts in the mixed regression equations between primary growth and regrowth were markedly different (–1.3 vs. –136 g kg^{-1}) showing that at a constant *in vivo* OMD *in vitro* pepsin-cellulase treatment solubilised more OM from regrowth silages.

The bi- and trivariate fixed and mixed regression models in predicting silage OMD are presented in Table 2. In general, only slight improvements in the performance of models were detected when

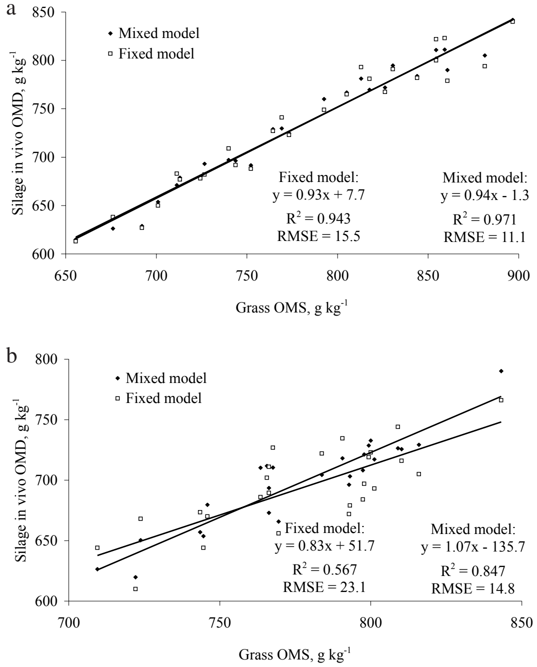

Fig. 2. The relationships between primary growth (a, n = 27) and regrowth (b, n = 25) grass organic matter pepsin-cellulase solubility (OMS) and in vivo digestibility (OMD) estimated either by a fixed (Y = a + bX) or a mixed (Y = Year + a + bX) regression model, where Y = silage OMD, X = grass OMS and Year is a random effect of harvesting year.

grass or silage parameters in addition to grass OMS were included as regression variables. Irrespective of the model used, the effect of cut (primary growth vs. regrowth) was highly significant ($P < 0.001$). Numerically the effect of cut was 34–41 g kg^{-1} meaning that at a constant OMS of grass, *in vivo* OMD of primary growth silages was higher than that of regrowth silages.

The fixed regression models suggest that higher grass DM and greater difference between grass and silage DM lead to lower silage OMD. However, this effect was less pronounced in mixed models in which the random effect of harvesting year was taken into account. The effects of changes in the DM and ash content during ensiling and silage fermentation characteristics (TA and ammonia N) on accuracy of OMD prediction were not significant ($P > 0.1$) in the trivariate mixed models (Table 2).

Discussion

Herbage and respective silage composition

Although the mean decline in CP content during ensiling was small (Table 1), the slope between herbage and silage CP was significantly different from one indicating that N losses were elevated with increasing herbage CP concentaration (Figure 1b). Because the change in CP content was almost similarly related to the CP content both in grasses and silages, the relationship between herbage CP content and the change in CP content evidently cannot be attributed to analytical bias. The possible forms of N losses during ensilage are effluent production and gaseous losses as ammonia or N oxides. Because the change in DM content during ensilage and silage ammonia content (analysed from fresh silage) were not associated with CP decline, it is unlikely that effluent losses or ammonia volatilization were responsible for the CP decline. It could, however, be associated with gaseous N oxide losses from feeds with low pH and high CP content (McDonald et al. 1991), which probably also had high nitrate concentrations.

The decrease in NDF content during ensiling [28 g (kg DM)$^{-1}$] corresponds well to the increase in the total amount of fermentation products (lactic acid, VFA and ethanol) and amount of residual WSC in silage compared with that in grass ensiled [26 g (kg DM)$^{-1}$]. This is in agreement with several earlier studies (see McDonald et al. 1991). The extent of hydrolysis was significantly related to the cell wall characteristics of grass ensiled. The more digestible the grass ensiled was, the more NDF was degraded in the silo as indicated by the negative relationship between grass NDF or silage INDF content and the extent of NDF hydrolysis. Also Rinne et al. (1997) and Keady et al. (2000)

Table 2. Predictions of silage organic matter digestibility (g kg^{-1}) from grass pepsin-cellulase organic matter solubility and other herbage characteristics by fixed or mixed (harvest year as a random effect) regression models ($^{a}Y = A + BX_1 + CX_2 + DX_3$, n = 52).

Variable[b] (X_1, X_2, X_3)	Model	A	SE	P-value	B	SE	P-value	C	SE	P-value	D	SE	P-value	RMSE	Adjusted R^2
OMS, Cut	Fixed	59	41.0	0.156	0.91	0.051	<0.001	37	5.4	<0.001				19.4	0.880
OMS, Cut	Mixed	–51	34.4	0.190	0.96	0.044	<0.001	38	5.2	<0.001				15.1	0.930
OMS, Cut, DM	Fixed	23	41.8	0.584	0.90	0.049	<0.001	36	5.2	<0.001	–0.13	0.06	0.028	18.7	0.895
OMS, Cut, DM	Mixed	–8	40.3	0.857	0.94	0.044	<0.001	38	5.1	<0.001	–0.13	0.07	0.061	14.7	0.933
OMS, Cut, DMDiff	Fixed	–12	39.1	0.752	0.90	0.050	<0.001	36	5.3	0.079	–0.37	0.20	<0.001	19.0	0.892
OMS, Cut, DMDiff	Mixed	–48	34.1	0.211	0.95	0.044	<0.001	38	5.2	<0.001	–0.27	0.20	0.182	15.0	0.931
OMS, Cut, Ashdiff	Fixed	–5	40.8	0.895	0.90	0.052	<0.001	35	5.6	<0.001	0.60	0.550	0.282	19.4	0.887
OMS, Cut, Ashdiff	Mixed	–41	35.4	0.293	0.94	0.045	<0.001	37	5.2	<0.001	0.66	0.572	0.254	15.0	0.932
OMS, Cut, NH_3	Fixed	23	45.9	0.622	0.88	0.053	<0.001	35	5.5	<0.001	–0.27	0.171	0.118	19.1	0.890
OMS, Cut, NH_3	Mixed	–19	41.2	0.667	0.93	0.047	<0.001	38	5.2	<0.001	–0.23	0.162	0.169	14.9	0.932
OMS, Cut, TA	Fixed	–14	40.4	0.739	0.90	0.055	<0.001	36	6.3	<0.001	0.08	0.187	0.685	19.6	0.885
OMS, Cut, TA	Mixed	–52	34.6	0.181	0.97	0.046	<0.001	39	5.9	<0.001	–0.10	0.174	0.565	15.0	0.930

[a] Regression equation: A, Intercept; B, coefficient of the first variable; C, coefficient of the second variable; D, coefficient of the third variable

[b] OMS, organic matter pepsin-cellulase solubility (g kg^{-1}); Cut, difference between primary growth and regrowth; DM, grass dry matter content (g kg^{-1}); DMDiff, difference between grass and silage DM content (g kg^{-1}); Ashdiff difference between grass and silage ash content [g (kg DM)$^{-1}$]; NH_3, silage ammonia N [g (kg N)$^{-1}$]; TA, total silage fermentation acids [g (kg DM)$^{-1}$]

found a positive relationship between NDF breakdown and silage digestibility.

McDonald et al. (1991) suggested three possible reasons for the breakdown of cell wall carbohydrates during ensiling: hemicellulases present in the original herbage (1), microbial hemicellulases (2) or acid hydrolysis (3). In addition to these factors, silage additives such as formic acid could contribute to breakdown of hemicellulose. Both simple and mixed model (adjusted for random year effect) regression analyses suggested, that organic acids produced during fermentation were not responsible for the NDF breakdown. The effect of silage TA content on NDF hydrolysis was not significant, either when used as a single regression factor or together with grass NDF content in a bivariate model. In contrast, the extent of NDF hydrolysis was significantly and positively associated with silage pH, when pH was used as a single factor or together with grass NDF in a bivariate model. This agrees with Dewar et al. (1963), who observed that the extent of hydrolysis of grass hemicellulose was positively related to pH. This may indicate that plant enzymes were responsible of the NDF hydrolysis.

One possible reason for the decrease in NDF content is the considerable hydrolysis of cell wall-bound N during ensilage (Jones et al. 1992, Rinne et al. 1997, Keady et al. 2000), especially when NDF is analysed without sodium sulphite. The decline seems to be the greater, the higher the digestibility of the silage (Rinne et al. 1997, Keady et al. 2000). The higher recovery (0.82 vs. 0.09) of hydrolysed NDF as non-structural carbohydrates (OM-CP-NDF-fat) compared with fermentation products and WSC supports the view that the hydrolysis of cell wall-bound N was the main contributor to the decline in NDF content during ensilage, even when sodium sulphite was used in NDF analysis.

Pepsin-cellulase solubility of grass ensiled was significantly higher than that of the resultant silages. The difference in OMS between the original grasses and the respective silages corresponds to a DM loss of 92 (s.e. 8) g (kg DM)$^{-1}$, which is slightly higher than the minimum loss of 70 (kg DM)$^{-1}$ suggested by Zimmer (1980). Theoretically the digestibility of original grass should be slightly higher than that of the respective silage, since losses due to plant respiration, in-silo fermentation and effluent production reduce those OM fractions, which are completely digestible. This is in agreement with results of Rogers et al. (1979), who found decreased digestibility associated with ensiling wet herbage, and those from Zimmer and Wilkins (1984), who reported decreased digestibility with prolonged wilting of grass. On contrary, McDonald and Edwards (1976) found no differences in the *in vivo* digestibility between 36 grasses and respective silages.

The decrease in OM solubility between grasses and silages was not related to the DM content of the grass ensiled, suggesting that variation in effluent production did not have a significant contribution to the decrease in OM solubility. Further, total acid or VFA contents did not explain the difference in OMS between the grass and silage samples. In the present study, all silages were ensiled using a high application rate of formic acid (4 litres per ton) resulting in a high fermentation quality. In contrast, when grass was ensiled with varying additive treatments and a wider range of fermentation quality was observed, the decrease in silage digestibility compared to the original grass was associated with increased concentrations of ammonia N and volatile fatty acids (Demarquilly 1973).

The difference in OM solubility between grass and silage samples increased when herbage NDF content increased. The smaller decline in OMS for low NDF silages ($P < 0.01$) could be related to a greater extent of NDF hydrolysis during ensilage, which could facilitate the access of enzymes to substrates during *in vitro* digestion.

Prediction of silage OMD from herbage OMS

The present data indicate that OMD in primary growth grass silages can be predicted accurately from the herbage OMS (Figure 2a: $R^2_{mixed} = 0.971$ and RMSE 11.1 g kg^{-1}). The outcome of the fixed and mixed (adjusted for random effects of the year of harvest) regression models was similar, suggesting that a general relationship existed between grass OMS and silage OMD. The slope for the correction equation was below one, and the intercept close to zero, which is in agreement with our earlier results from grass silages (Nousiainen et al. 2003a, b). However, if OMS would correspond directly to *in vivo* OMD, the slope should be one and the intercept significantly below zero due to metabolic faecal material that is essentially not produced during the *in vitro* incubation. Nousiainen (2004) calculated with the Lucas equation, that the excretion of metabolic faecal OM is between 90 to 100 g (kg digested OM)$^{-1}$ in grass silages, which is in good agreement with the results of Van Soest (1994) and Weisbjerg et al. (2004), who included both forages and concentrates in the test.

Several *in vitro* procedures based on cell wall degrading cellulases have successfully been developed for the prediction of OMD in forages (Jones and Theodorou 2000, Nousiainen et al. 2003a, b). As demonstrated by Nousiainen (2004), the *in vitro* cellulase method based on pre-treatment with an acid pepsin solution solubilizes proportionally 0.60 to 0.75 of the NDF in grass silages compared to the potential degradation of cell walls during a prolonged *in situ* ruminal incubation. Although it is assumed that cell solubles (OM–NDF) behave uniformly in the *in vitro* system, specific statistical correction equations are required to convert OMS into *in vivo* OMD of different forage species, corresponding to values determined by the reference method (typically *in vivo* digestibility with sheep).

In agreement with earlier results for silage (Nousiainen 2003b), the prediction of silage OMD from regrowth herbage OMS was somewhat less accurate compared to that from primary growth (RMSE 14.8 vs. 11.1 g kg^{-1}, respectively). If both cuts were analysed together, the RMSE increased to 22.0 g kg^{-1}. The mixed regression equation for predicting silage OMD of regrowth herbages differed also numerically from that of primary growth (Figure 2) the slope being higher (1.07 vs. 0.94 g kg^{-1}, P = 0.6) and the intercept being lower (–1.3 vs. 135.7 g kg^{-1}, P = 0.4). Although the differences in equation parameters were non-significant, a similar tendency was detected for primary growth and regrowth grass silages (Nousiainen et al. 2003b), suggesting that specific correction equations should be used for different cuts of grass. Accordingly, Givens et al. (1993) reported markedly different slopes for OMS in spring and autumn herbages in predicting *in vivo* D-value of the respective forages.

These results suggest a maturity × cut interaction between OMS and *in vivo* OMD, indicating that at a constant OMS the *in vivo* OMD is lower in regrowth than in primary growth grasses. This is probably due to differences in cell wall structure between the primary growth and regrowth grasses and that the commercial fungal (*Trichoderma viride*) cellulase reacts differently to cell walls of these forage types with advancing maturity. This may be justified by the results of Nousiainen (2004), which showed that the relative potential NDF digestion (enzyme solubility/potential *in situ* NDF digestion) was significantly dependent on the maturity in primary growth silages but not in regrowth. The difference in cell wall structure between primary growth and regrowth silage was also demonstrated by the fact that in spite of lower mean NDF content in regrowth silages, INFD content was higher than in primary growth (Nousiainen 2004).

When analysing the whole data with mixed model and using the cut and OMS as regression variables, practically no difference was obtained in the prediction accuracy whether using either herbage or silage OMS as the regression variable (R^2_{mixed} = 0.930 or 0.920 and RMSE = 15.1 or 15.8 g kg^{-1}, respectively). The multiple regression equations showed that in addition to herbage OMS, the silage characteristics (i.e. DM content and fermentation quality) improved the prediction of OMD of the respective silages only marginally (Table 2). This suggests that when grass silages are well preserved with moderate conservation losses, as was the case in the present study, the equations in predicting silage OMD from herbage OMS are well applicable. However, if the ensiling losses due to effluent production, poor fermentation and/or wilting losses are marked, the present relationships should be applied with caution.

A completely similar approach has not been published earlier, but many other workers have demonstrated the potential of pepsin-cellulase OM solubility in predicting OMD of different types of forages (e.g. Givens et al. 1990, Steg et al. 1990, de Boever et al. 1999, Nousiainen 2004). In contrast to other workers, we compared the mixed and fixed regression methods in prediction equations (see Figure 2 and Table 2). Theoretically the mixed procedure should be followed, because it at least partly corrects for the annual differences in laboratory practises, purchased enzyme lots and growth and harvesting conditions of grass, and therefore results in less biased equations compared to the fixed regression method. However, to obtain a good accuracy in predicting OMD, each laboratory should estimate their own correction equation for each forage type due to species-specifity of cellulases, and evident problems in achieving comparable OMS results between laboratories (Weiss 1994, Nousiainen 2004).

Conclusions

Based on the systematically collected dataset comprising of grasses and respective silages, it is concluded that silage composition and digestibility can be reliably predicted from herbage characteristics provided that silages are well preserved with low or moderate ensiling losses. The pooled prediction error in estimating primary growth and regrowth silage OMD from pepsin-cellulase solubility of herbage OM was 15.1 g kg^{-1}, which is accurate enough for practical ration formulation. The results suggest that specific equations are needed for primary growth and regrowth samples in predicting OMD from pepsin-cellulase solubility.

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SELOSTUS

Säilörehun koostumuksen ja sulavuuden ennustaminen raaka-aineena käytetyn ruohon ominaisuuksien perusteella

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Tässä tutkimuksessa selvitettiin mahdollisuuksia ennustaa nurmisäilörehun koostumus ja sulavuus säilörehun raaka-aineena käytetyn ruohon koostumus- ja *in vitro* -sulavuusmääritysten perusteella. Aineisto koostui timotein ja nurminadan seoskasvustoista tehdyistä säilörehuista, joista 27 oli ensimmäisestä sadosta ja 25 jälkikasvusta. Säilörehujen ja raaka-aineiden koostumus määritettiin standardimenetelmin ja sulavuutta arvioitiin pepsiini-sellulaasiliukoisuudella. Säilörehujen *in vivo* sulavuus määritettiin pässeillä sonnan kokonaiskeruumenetelmällä. Raaka-aineen ja vastaavan säilörehun ominaisuuksien yhteyksiä tarkasteltiin regressioanalyysillä.

Kaikki säilörehut olivat hyvin säilyneitä. Säilörehun kuiva-aine-, raakavalkuais- ja solunseinäkuitupitoisuus pystyttiin arvioimaan suhteellisen tarkasti raaka-aineen perusteella, sillä ennustevirheet olivat melko pieniä. Ruohojen pepsiini-sellulaasiliukoisuus oli merkitsevästi korkeampi kuin säilörehujen [779 vs. 756 g (kg kuiva-ainetta)$^{-1}$], mikä johtuu pääasiassa säilönnän aikana tapahtuvan sulavan orgaanisen aineen hävikistä hengitys-, käymis- ja puristenestetappioiden takia. Säilörehun sulavuus pystyttiin kuitenkin arvioimaan yhtä hyvin ruohon ja säilörehun pepsiini-sellulaasiliukoisuuden perusteella. Ensi- ja jälkikasvusta tehdyille rehuille pitäisi käyttää eri korjausyhtälöitä pepsiini-sellulaasiliukoisuuden muuntamisessa sulavuudeksi, sillä regressioyhtälöiden vakiot ja kulmakertoimet poikkesivat lukuarvoiltaan selvästi toisistaan.

Säilörehun koostumus ja sulavuus voitiin arvioida luotettavasti raaka-aineena käytetyn ruohon perusteella, kun aineistona käytettiin hyvin säilyneitä säilörehuja.