Timothy and Red Clover as Forage for Dairy Production

In vitro Degradation Characteristics and Chemical Composition

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Abstract

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The aim of this thesis was to describe and compare timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.) as forage for dairy cattle on the basis of the chemical composition and the in vitro degradation characteristics. Initially the degradation characteristics determined with an in vitro gas production technique and an in vitro filter bag technique were compared. Both techniques were found to be interesting tools for providing relevant degradation parameters for feed evaluation models. Thereafter the degradation characteristics and nutritive value of forages of timothy and red clover were described with recordings of in vitro gas production and chemical analysis.

Analysis of the herbage of red clover and timothy showed that red clover contained lower concentrations of neutral detergent fibre (NDF) and had lower degradability of neutral detergent fibre (NDFD) on all harvest occasions. Red clover had on average a higher maximal fractional degradation rate (FDR_{max}) of the whole forage compared to timothy. The FDR_{max} for the NDF in red clover was not different from that in timothy. In timothy the components soluble in neutral detergent solution had a higher FDR_{max} compared to those in red clover.

Timothy and red clover were conserved as direct cut silages with the addition of conservation additives. It was found that timothy and red clover herbage responded differently to the silage additives. Silages treated with additives had a shorter time until half of the gas was produced, as well as a shorter time until the maximal fractional degradation rate was reached, indicating a faster in vitro degradation compared to that of untreated silages. The use of additives improved the NDFD in silage with red clover but not that in the silage of pure timothy.

The relationships between the feed characteristics and the relative silage intake in dairy cows were evaluated. It was found that the preparation technique for silage samples was important and that wet silage samples had a higher gas production and different kinetics compared to dried samples. It was concluded that chemical analysis in combination with the in vitro gas production technique can be a cost-efficient tool for relative prediction of silage intake based on feed characteristics. The predictions can be used to compose feed rations that will optimize silage intake in relation to milk production and to improve conservation management.

Key words: timothy, red clover, in vitro gas production, artificial filter bag technique, degradation characteristics, fermentation products, silage intake

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“Befria flugan från sirapen och du skall få se den flyga”
En författares dagbok, Fjodor Dostojevskij, 1821–1881

Contents

Appendix

Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Hetta, M., Nordheim, H., Gustavsson, A-M. & Martinsson, K. 2004. Comparison of in vitro degradation of temperate forages described with the gas production and filter bag techniques. *Submitted for publication.*

II. Hetta, M., Gustavsson, A.-M., Cone, J.W. & Martinsson, K. 2004. In vitro degradation characteristics of timothy and red clover at different harvest times. *Acta Agriculturae Scandinavica, Sect. A,* 54, 20-29.

III. Hetta, M., Cone, J.W., Gustavsson, A.-M. & Martinsson, K. 2003. The effect of additives in silages of pure timothy and timothy mixed with red clover on chemical composition and in vitro rumen fermentation characteristics. *Grass and Forage Science* 58, 249-257.

IV. Hetta, M., Cone, J.W., Bernes, G., Gustavsson, A.-M. & Martinsson, K. 2004. Voluntary intake of silages depending on chemical composition and in vitro degradation characteristics. *Submitted for publication.*

List of abbreviations

ADF	Acid detergent fibre
ADF-N	Acid detergent fibre bound nitrogen
CFU	Colony forming units
CP	Crude protein
DM	Dry matter
FB	Filter bag
FDR_{max}	Maximal fractional degradation rate
GP	Gas production
ME	Metabolisable energy
NDF	Neutral detergent fibre
NDFD	Neutral detergent fibre degradability
NDS	Neutral detergent solution
NIRS	Near infrared reflectance spectroscopy
OM	Organic matter
OMD	Organic matter degradation
t_{50}	Time when half of the gas was produced
t_{max}	Time of maximal fractional degradation rate
t_{90}	Time when 90 percent of the gas was produced
WSC	Water-soluble carbohydrates

Introduction

Background

There is an increased interest from the dairy industry in feed sources, which are produced on farms and reinforce the consumer's confidence in dairy products in terms of animal welfare and product quality (Cunningham, 2003). Forages from leys (short-term grassland) are the most important crops in Northern Scandinavia. The Department for Agricultural Research in Northern Sweden has for a long time been carrying out research on red clover (*Phleum pratense* L.) and timothy (*Trifolium pratense* L.), with a focus on the nutritive value for dairy production. The research has been a collaborative effort between the Sections of Crop Science and Animal Science and has been initiated to contribute to a more sustainable and competitive dairy production in Northern Scandinavia.

Timothy and red clover are traditional forages for dairy cows in northern areas. The high nutritive value of the two species for dairy cows was established as early as during the nineteenth century (http://linnaeus.nrm.se/flora/welcome.html; 13-Feb-2002). Both species are found as native plants all over Scandinavia. Olof Rudbeck the elder became the first to describe red clover scientifically in 1658**.** Timothy is named after Timothy Hanson, an 18th century American farmer who promoted it for hay. Originally in Sweden it was known under the name "Ängskampe" and was first scientifically described by Anders Celsius in 1732. Both species were, however, known in older literature dating back to the Middle Ages. In modern agriculture the two species are subjected to plant breeding and are available to the farming community as different commercial varieties selected for most temperate regions.

Forages cultivated on high latitudes are known to be highly digestible to ruminants (Van Soest, Mertens & Deinum, 1978), which could be a competitive advantage for the dairy farmers in Northern Scandinavia. Timothy grown in Tromsø, Norway (latitude 69 ºN) has been found by Deinum *et al*. (1981) to be more digestible compared to timothy grown in Wageningen, the Netherlands (latitude 52 ºN) at the same stage of maturity. Red clover and other forage legumes are known to have a high nutritional value for ruminants (Sheldrick, Newman & Roberts*,* 1995) and to be able to fixate nitrogen from the atmosphere, up to 400 kg N ha^{-1} per year (Carlsson & Huss-Danell, 2003). This makes red clover a valuable crop in organic farming, where the use of fertilizers and concentrates is limited.

Timothy is at present the most important forage grass in Northern Scandinavia (Thorvaldsson & Andersson, 1986; Nissinen & Hakkola, 1995). It is well known for its winter hardiness and high palatability (Balasko & Nelson, 2003). Other forage grasses grown in the region are meadow fescue (*Festuca pratensis* L.) and annual ryegrass (*Lolium multiflorum* L.). Within the legumes red clover is the most important forage species in northern areas. In comparison to alfalfa (*Medicago sativa* L.), red clover can grow in less drained and more acid soils and will persist in short-time leys, for up to three years in a tough winter climate (McGraw & Nelson, 2003). Other forage legumes suitable for northern areas are white clover (*Trifolium repens* L.) and common vetch (*Vicia sativa* L.).

Dairy production has traditionally been based on forages as the primary feed, either as grazed grass or as conserved silage or hay. The forage quality is important for animal health, milk production and milk quality (Van Soest, 1994). Producers can manage the quality of forages mainly by the alteration of the botanical composition, the harvest time and the conservation methods. In modern feed evaluation systems such as the Cornell net carbohydrate and protein system (Fox *et al*., 2004), the degradation kinetics of feeds is of importance. Very little is known about the effects of forage management on the degradation kinetics and intake of forages from Northern Scandinavia. The relatively low prices of agricultural commodities such as grain and the intensive livestock management systems have reduced the amount of forages in the diets of dairy cows in Scandinavia. In order to achieve higher proportions of forages in the diets of high-producing dairy cows, it is necessary to improve the knowledge of the nutritive value of the forages on different harvest occasions and using different conservation methods.

Aims of this thesis

The overall aim of this thesis was to describe and compare timothy and red clover as forage for dairy cattle on the basis of the chemical composition and the in vitro degradation characteristics.

The specific objectives were to investigate the following:

- The in vitro degradation of temperate forages described with the gas production and the filter bag techniques.
- The in vitro degradation characteristics and chemical composition of timothy and red clover at different harvest times.
- The effect of additives on the chemical composition and rumen fermentation characteristics in silages of pure timothy and timothy mixed with red clover.
- The voluntary intake of silages depending on the chemical composition and in vitro degradation characteristics.

Materials and Methods

Comparison of in vitro degradation of temperate forages described with two techniques: Paper I

A set of 20 temperate forage samples were analysed for their chemical composition and in vitro dry matter (DM) digestibility. Degradation in the rumen was described with the in vitro gas production and in vitro filter bag techniques. The degradation data were fitted to Michaelis-Menten equations. The effective fermentation of organic matter (OM) was calculated with a dynamic model.

In vitro degradation characteristics of timothy and red clover: Paper II

The nutritional value of red clover and timothy was studied over two consecutive growing seasons (1995 and 1996). The crops were cut four times during the spring and summer growth and were analysed with an in vitro gas production technique and chemical analysis. The degradation data were fitted to Michaelis-Menten equations.

The effects of additives in silages of pure timothy and timothy mixed with red clover: Paper III

First and second growth cuts of pure timothy and timothy mixed with red clover were conserved during three consecutive years, 1994, 1995 and 1996, either without any additive or with the addition of formic acid or lactic acid bacteria in combination with molasses. The degradation characteristics of the herbage and the silages were analysed using an in vitro gas production technique and chemical analysis.

Predictions of voluntary intake of highly degradable silages: Paper IV

The relationships between the intake of 15 silages and their chemical composition and degradation parameters measured with an in vitro gas production technique were evaluated. The silages were incubated as dry or wet samples and in combinations with concentrate. The gas production profiles were fitted to a Michaelis-Menten equation. The silage DM intake in relation to the body weight (BW) was recorded in five experiments with lactating dairy cows.

Results and discussion

Comparisons of the degradation of temperate forages described with two in vitro techniques

In vitro methods

The utilisation of forages is primarily determined on the basis of the degradation in the rumen (Van Soest, 1994). Consequently, there has been a long scientific development of methods for rumen studies of forage degradation. Early studies of rumen degradation kinetics were performed by Quin, Van der Wath & Myburg (1938), who used measurements of rumen gas production in vivo, as well as incubated feeds in silk bags in rumen-fistulated sheep, to measure rumen kinetics. The feed value of forages in Sweden for dairy cows is at present expressed as metabolisable energy (ME) determined with a simple in vitro technique (Lindgren, 1979) similar to the method of Tilley & Terry (1963). The concentration of ME is estimated in vivo from experiments with sheep (wethers) fed at maintenance. The Swedish feed evaluation gives no prediction of silage intake and has not been developed for the analysis of the nutritive value of silages. The system is at present under reconsideration by the Swedish Dairy Association.

Many feed evaluations systems such as the Nordic protein evaluation system (Madsen, 1985) used in Sweden are based on measurements with the in sacco technique (Mehrez & Ørskov, 1977). The technique is laborious, requires rumen-fistulated animals and is less suited to soluble components (Ørskov, 2000). The low analytical capacity of the in sacco technique (Wilman, Foulkes & Givens, 1996) and the high costs for keeping fistulated animals have resulted in that the technique mainly being used for the development of feed tables, which are rarely updated. There is therefore a need for new technologies, which can provide cost-efficient diet-specific feed parameters (Bannink *et al*., 1997). Two of the most promising alternative techniques are the in vitro gas production (Blummel & Ørskov, 1993) and the in vitro filter bag (Vogel *et al.,* 1999) techniques, presented in Paper I. They can be performed without the use of surgically modified animals and the equipment is not too expensive. Examples of results from the two techniques are presented in Fig.1.

The in vitro filter bag and the gas production techniques

The in vitro filter bag technique (Vogel *et al.,* 1999) is similar to the in sacco technique (Ørskov, 2000), but instead of incubating the feed samples in nylon bags in a rumen-fistulated animal, the feeds are incubated in filter bags which are kept soaked in buffered rumen fluid in an incubator at 39º C. The technique enables incubation of larger numbers of samples (100 samples per incubator) compared to the in sacco technique. The in vitro gas production technique (Blummel & Ørskov, 1993) records the gas produced when rumen microbes ferment a feed sample (Getachew *et al*., 1998). The production of gas in relation to the degradation is dependent on the chemical composition of the feeds (Cone & van Gelder, 1999). The technique is advantageous compared to the filter bag technique, as it can be used to parameterize the degradation of soluble and non-soluble components (Pell & Schofield, 1993).

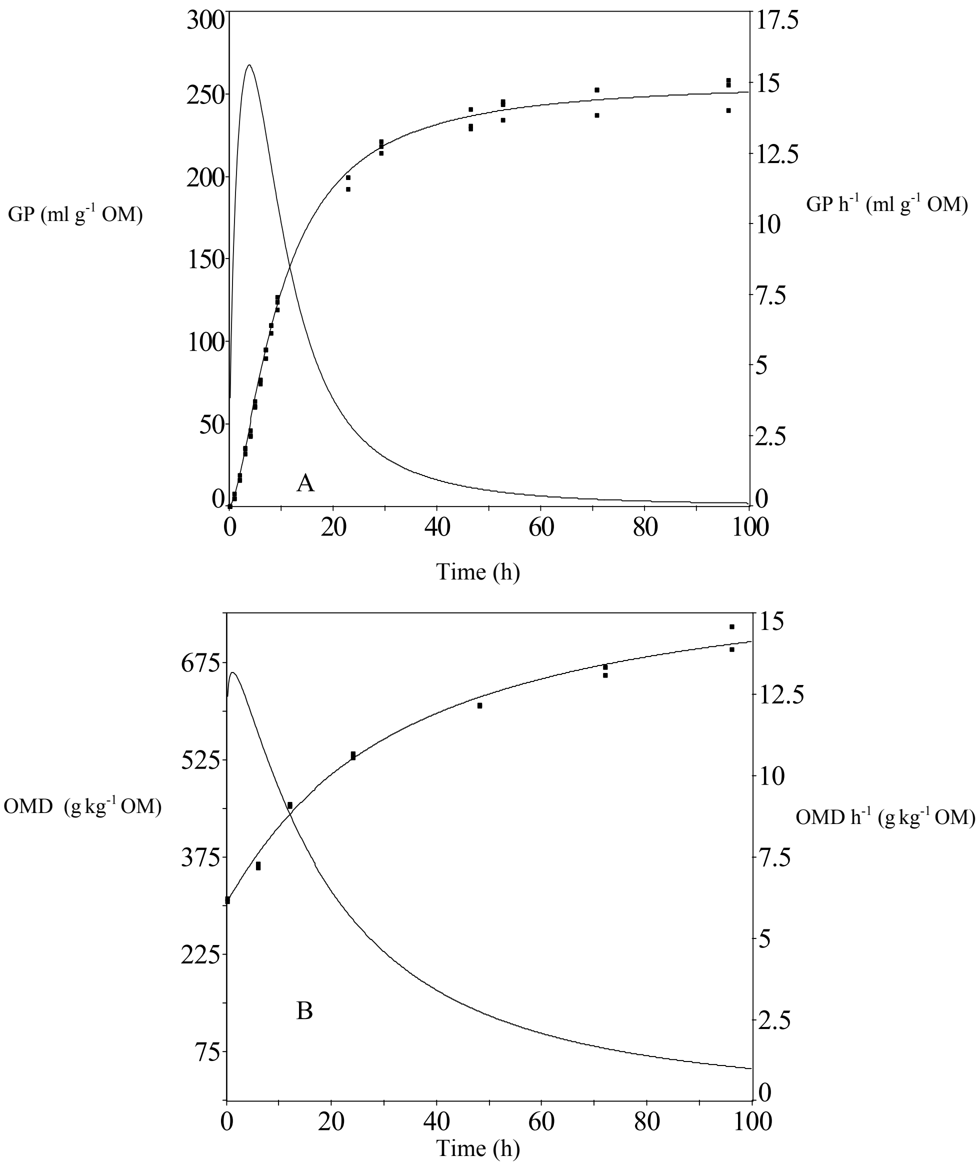


Fig. 1. Data describing organic matter degradation (OMD) (left y-axis) of red clover during 96 h from the in vitro gas production (GP) technique (panel A) and the in vitro filter bag technique (panel B), fitted to the Michaelis-Menten model described in Paper I. The first derivative of the model during the incubation is presented on the right y-axis.

Gas production and disappearance as measurements of rumen degradation

The results of the different techniques in Paper I are difficult to compare, as they are based on different assumptions. The underlying assumption in the in vitro gas production technique is that the gas produced is linearly correlated to the degradation. For the filter bag technique it is assumed that the disappearance from the bags represents the degradation. These assumptions have unfortunately some weak points, which will affect the validity of the comparisons of the methods in Paper I.

During the initial incubation small feed particles and soluble components may leave the filter bags without having been fermented (Dewhurst, Hepper & Webster, 1995). This problem is of great importance for the forages analysed in Paper I, which show a large variance in the concentration of soluble components, as well as a large variance in the concentration of crude protein (CP). The differences in the concentration of CP are problematic for the assumptions behind the filter bag and the gas production technique. As a result, the degradation of high nitrogenous forages such as legumes may be underestimated by the gas production technique, because less gas is produced when protein is degraded compared to when carbohydrates are degraded (Cone & van Gelder, 1999). The filter bag technique may overestimate the initial degradation, as forage proteins are highly soluble in rumen fluid and may therefore degrade at a different rate compared to the disappearance from the bags (Broderick, 1994).

To compensate for the shortcomings of the relationship between gas production and degradation, Blummel & Bullerdieck (1997) suggested that gas production profiles should be complemented with in sacco degradabilities at 24 and 48 h to improve the prediction of voluntary intake. Cone & van Gelder (1999) have shown that gas production profiles of grasses with increasing maturities are more comparable to the results from the in sacco technique if the cumulative gas production is corrected for the influence of the concentration of CP.

In Paper I, a different approach was used to improve the correlation between the two methods. The relationship between the amount of gas and the amount of OM degraded at 96 h was used to transform the gas production profiles to organic matter degradation (OMD). The transformation improved the relationship between the two methods at all intervals from 6 to 96 h. The procedure also enabled a robust curve fitting procedure with a biphasic model describing the degradation of the whole forage. The advantage of the approach in Paper I compared to the method of Cone & van Gelder (1999) is that the degradation described with the two techniques is given the same units. The approach is also preferable to the suggestion of Blummel & Bullerdieck (1997), as there is no need for expensive in sacco measurements to complement the gas production recordings.

Models to describe in vitro degradation

The kinetic degradation of forages can be described by data for different intervals or by mathematical parameters (e.g. Dewhurst, Hepper & Webster, 1995). The latter method is often used in feed tables, as it is more feasible to apply mathematical parameters in feed evaluation systems. Many feed evaluation systems, such as the Nordic protein evaluation system (Madsen, 1985), are based on the monomolecular equation (first order kinetics), applied to in sacco data by Ørskov & McDonald (1979). The model is, however, biologically incorrect, as it assumes that the degradation is only limited by the substrate, which is unlikely for microbial degradation (Lopez, Dijkstra & France, 2000).

Lopez *et al.* (1999) have shown that more biologically correct models, such as the model presented in Paper I, can be fitted to in sacco data with similar intervals of recordings as Paper I. The model (Equation 1) is a modified version of the equation developed by Leonor Michaelis and Maud Menten in 1913 (http://www.cdnmedhall.org/Inductees/menten_98.htm; 04-Apr-2004) where enzymatic limitations are considered. Groot *et al.* (1996) have described the mathematical function of the parameters in the model when applied to gas production kinetics. Parameter Ax represents the asymptotic OMD (g kg-1 OM) or gas production (ml g-1 OM), and parameter Bx (h) is the time when half of Ax is reached. Parameter Cx is dimensionless and determines the sharpness of the switching characteristics of the curve. In the model x represents the number of the subcurve. An example of the model fitted to filter bag and gas production data is presented in Fig. 1.

$$Y = \sum \left(\frac{A_x}{1 + (B_x / t)^{C_x}} \right) \qquad \text{(Equation 1)}$$

The chosen model is highly flexible and can be fitted to sigmoidal and non-sigmoidal degradation profiles (Dhanoa *et al.,* 2000). The biological meaning of the parameters has been described to a lesser degree. However, parameter A_x could represent the potential availability of the substrate to the microbes. The enzymatic activity of the rumen bacteria is determined by the interplay between parameter B_x and C_x, reflecting the barriers that the microbes need to overcome before the substrate is completely degraded. As the model has a time-dependent degradation rate, there is less need to divide the forage fraction into fast and slow sub-fractions as in the model of Mertens & Eley (1979).

There are two main reasons for applying multiphasic models, namely that they are more flexible compared to monophasic models and can give a better mechanistic description of the degradation of different feed components (Groot *et al*., 1996). Multiphasic curve fitting can be achieved by inspection of the first and second derivatives of the data (Groot *et al.,* 1996), or by incubating different feed fractions and subtracting the gas production curves of the subcomponents from the gas production curve of the whole forage (Schofield & Pell, 1995). Since the data in Paper I were obtained from manual recordings, the possibility of identifying different phases as described by Groot *et al.* (1996) was limited. The curve subtraction procedure of Schofield & Pell (1995) is laborious and assumes that the separation of the fractions does not alter the properties of the forage, which can be questioned. Therefore, a different approach from multiphasic curve fitting was used in Paper I.

The sizes of the two parameters for degradation of soluble components (cell contents) and non-soluble components (cell walls) were identified from the amount of degraded OM and NDF (neutral detergent fibre) at 96 h. The shape of the first subcurve, the degradation of cell contents, was determined from the initial 8 h of gas production utilising the results of Schofield & Pell (1995) and Calabro *et al.* (2001) showing that the initial gas production reflects the degradation of the components soluble in neutral detergent solution (NDS). The second subcurve was then determined by subtracting the curve describing the degradation of the cell contents from the model for the whole forage. For the in vitro filter bag technique the fractions were defined as the buffer-soluble fraction at time zero and the non-soluble degradable fraction at 96 h.

Comparison between the two techniques

Several studies have been conducted to correlate results from gas production techniques with those from filter bag techniques. Most of these studies have shown lower correlations (Valentin *et al.,* 1999) compared to the comparisons between the techniques in Paper I. The low correlation between in sacco methods and in vitro gas production parameters in many previous studies may be explained by the fact that inappropriate mathematical equations were applied and that the gas production curves were not corrected for endpoint degradation or CP concentration. The relatively good correlation between the results of the two techniques in Paper I was most likely achieved by the transformation of gas to OMD, the application of an appropriate mathematical equation, and the fact that both methods were conducted in vitro. The conclusion in Paper I that a dynamic rumen model (Fig. 2) is a powerful tool to evaluate degradation curves determined with different techniques is comparable to the results of Sileshi *et al.* (1996). They found that the calculated effective in vitro gas production was well correlated with the calculated effective degradation of DM determined with the in sacco technique. Forage evaluation in Sweden is based on parameters from a feed table (Spörndly, 1999). The value of such an approach in modern feed evaluation has been questioned by Givens (2000), due to the large heterogeneity of forages and because feed tables soon become irrelevant. Paper I shows that both techniques described are interesting alternatives to the use of feed tables for providing relevant degradation parameters for dairy producers.

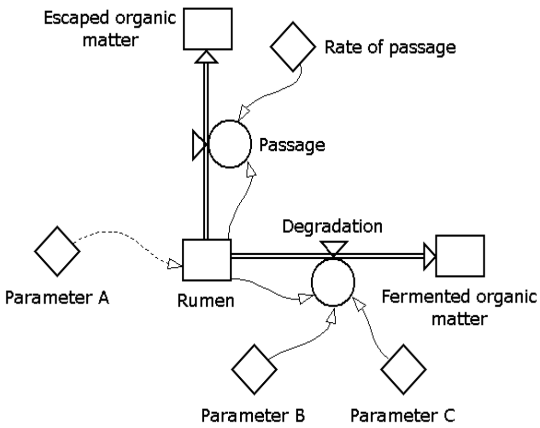


Fig. 2. Flow chart of the simple dynamic rumen model used in Paper I.

In vitro rumen degradation of timothy and red clover at different harvest occasions

All living systems are in a continuous stage of development, and so too are forage plants. The irreversible process of senescence causes the plants to grow, flower and fall into dormancy (Mohr & Shopfer, 1994). The phenological development predestined by the genotype will be heavily affected by external factors such as weather, day length, soil nutrients and living organisms in the ambience (e.g. Van Soest, 1994). The nutritive value of forages declines with the maturation of the plants (Collins & Fritz, 2003). Therefore, forages are cut several times per season to optimize the nutritive quality in relation to the forage yield. In Northern Scandinavia forage leys are cut two to three times per season (Rinne & Nykänen, 2000).

Red clover and timothy differ in their morphology and phenological development from seedling to mature plant (Fagerberg, 1988). Due to the different pace of phenological development between forage species and varieties, comparisons between forages based on defined stages of maturity such as "ear emergence" can be misleading (Minson *et al.,* 1964). Stefanon, Pell & Schofield (1996) compared alfalfa and bromegrass (*Bromus inermis,* Leyss) at different stages of maturity using a similar in vitro technique to that used in Paper II. Due to the different pattern of maturation of the two forages, the bromegrass was cut one to two weeks before the alfalfa, and hence at the same stage of maturity. In Scandinavia red clover is in general grown in mixes with timothy and will in practical farming be cut on the same day. Therefore, in mixed forages it is preferable to compare the forages on the same harvest occasions, as in Paper II.

Potential degradability of OM and NDF on different harvest occasions

The most well known effect of delayed harvest on the nutritive value of forages is the reduction in the potential degradability of OM and NDF, which is also shown in Paper II. The reduction in degradability is an effect of the change in the leaf to stem ratio in the plant (Troelsen & Campell, 1969), as well as the maturation of the cell walls with increasing resistance to microbial degradation as a result. It has been assumed that rumen microbes degrade forage tissues from the lumen of the plant cells into the cell walls. Therefore, the soluble components are degraded prior to the cell walls (Cheng *et al.,* 1983). This is also an assumption behind the degradation models applied in Paper I and II.

The cell wall is a product of secretions from the protoplast. It works as a rigid framework, which keeps the cell from bursting due to the osmotic pressure. The cell wall is divided into a primary and a secondary structure, which consist mainly of polymeric carbohydrates and lignin reinforcements (Mohr & Shopfer, 1994). As the cell wall matures, it gets thicker and degradation enzymes from the rumen bacteria will be subjected to increasing hindrances caused by the cellulose-lignin complexes (Jung & Deetz, 1993; Gustavsson & Martinsson, 2004). An important factor in the maturation of the plants is the lignification of the plants, which has been correlated to the degradability of cell walls, even though the mechanism is still unclear (Hatfield, 1993). Not all species have a good correlation between the concentration of lignin and the cell wall degradability. In a study of Smith, Goering & Gordon (1972) using different forage species, red clover showed the poorest correlation between lignification and in vitro digestibility compared to other forage species. The structure of the cell walls deviates greatly between grasses and legumes (Mohr & Shopfer, 1994). The primary cell wall of grasses is known to have a more compact structure compared to that of legumes, and it has been suggested that legume cell walls are more accessible for microbial degradation compared to the corresponding structure in grasses (Hatfield, 1993). However, theoretical models of cell wall degradation are awkward to apply in larger studies such as Paper II and III, as the models are based on studies of limited plant material on a tissue level.

The results in Paper II showing lower neutral detergent fibre degradability (NDFD) in red clover compared to that in timothy are in agreement with the results of other comparisons of grasses and legumes (e.g. Smith, Goering & Gordon, 1972; Bertilsson & Murphy, 2003). In red clover the concentration of peptic substances can be as high as 100 g kg^{-1} DM (Åman & Nordkvist, 1983). In Paper II it is therefore suggested that the lower degradability of cell walls (NDF) in red clover could be a result of the fact that the NDS removes the degradable pectins from the cell wall in red clover. Besides the change in the degradability of the cell walls, the results in Paper II show that, as the plants mature, the soluble fraction (cell contents) decreases in both species. The degradable cell wall fraction follows a more curve linear development during maturation due to the enlargement of the NDF fraction in relation to decreased degradability of the NDF. Initially the degradable NDF fraction increases until the maturity decreases the degradability of the fibre so that the fraction starts to decrease.

Determinations of the rate of degradation of forage fractions

In more dynamic feed evaluation models, correct determinations of the rates of degradation of the feeds are crucial (Ruiz *et al.,* 2002). As discussed in Paper I, several techniques have been developed to estimate the rate of degradation in forages. The estimations of degradation rates are dependent on the applied techniques as well as the inocula (Cone *et al.,* 1996). Wallace *et al.* (2001) have concluded that the degradation of cell walls is limited more by the enzymatic activity in the rumen fluid than by the properties of the cell walls. In Table 1 the range of the estimated maximum fractional degradation rates in Paper II is compared with results from previous studies.

Table 1. *Range of rates of degradation of fractions in timothy and red clover in different studies determined with the in vitro, in vitro gas production (GP) and the in sacco methods*

Crop	Method	Fraction	Range of rates		Reference
			Min	Max	
Timothy	In sacco[1]	OM	0.05	0.11	Lindberg & Lindgren, 1988
Timothy	GP[2]	DM	-	0.09	Schofield & Pell, 1995
Timothy	GP [1]	DM	0.04	0.05	Rinne & Nykänen, 2000
Timothy	GP[2]	OM	0.08	0.11	Paper II, 2004
Timothy	In sacco[1]	NDF	0.05	0.09	Lindberg & Lindgren, 1988
Timothy	GP [2]	NDF	-	0.14	Schofield & Pell, 1995
Timothy	GP[2]	NDF	0.05	0.09	Paper II, 2004
Timothy	GP[2]	NDS	-	0.19	Schofield & Pell, 1995
Timothy	GP[2]	NDS	0.15	0.33	Paper II, 2004
Red clover	GP[2]	DM	-	0.15	Schofield & Pell, 1995
Red clover	GP[1]	DM	0.07	0.07	Rinne & Nykänen, 2000
Red clover	GP[1]	OM	0.09	0.12	Paper II, 2004
Red clover	In vitro[1]	NDF	0.06	0.09	Smith, Goering & Gordon, 1972
Red clover	GP[2]	NDF	-	0.21	Schofield & Pell, 1995
Red clover	In sacco[1]	NDF	0.09	0.15	Bertilsson & Murphy, 2003
Red clover	GP[2]	NDF	0.05	0.13	Paper II, 2004
Red clover	GP[2]	NDS	-	0.15	Schofield & Pell 1995
Red clover	GP[2]	NDS	0.12	0.17	Paper II, 2004

[1]*First order kinetics,* [2]*Maximal fractional degradation rate in time dependent models OM=organic matter, DM=dry matter, NDF=neutral detergent fibre, NDS=neutral detergent soluble components*

The comparison in Table 1 indicates that the measurements and the applied degradation model in Paper II gave a determination of the rates of degradation for all the fractions that was comparable to that of other studies, even though different degradation models have been applied in the studies. A gas production analysis as performed in Paper II costs less than 25 percent of an in sacco analysis. It is therefore concluded that the in vitro gas production technique described in Paper II is a cost-efficient tool for parameterizing the degradation of soluble and non-soluble fractions as well as the whole forage of timothy and red clover.

Statistical challenges in comparisons of degradation kinetics

The estimated degradation parameters in Papers I to IV are based on non-linear regression. For correct estimates of the variance of the estimated parameters, normal distribution of the residuals with equal variance during the whole incubations is necessary (Nathanaelsson & Sandström, 2004). Fig. 3 is an example of degradation data and the fitted degradation curve from the study in Paper II. The pattern of the plotted residuals shows that these requirements are not fulfilled. Therefore, the estimated variance for the degradation parameters given by the software used in Paper I-IV is not valid. Due to this anomaly, the estimated degradation parameters are presented without subjection to analysis of variance in Paper II. Further studies are needed to determine robust procedures for correct statistical evaluations of gas production kinetics.

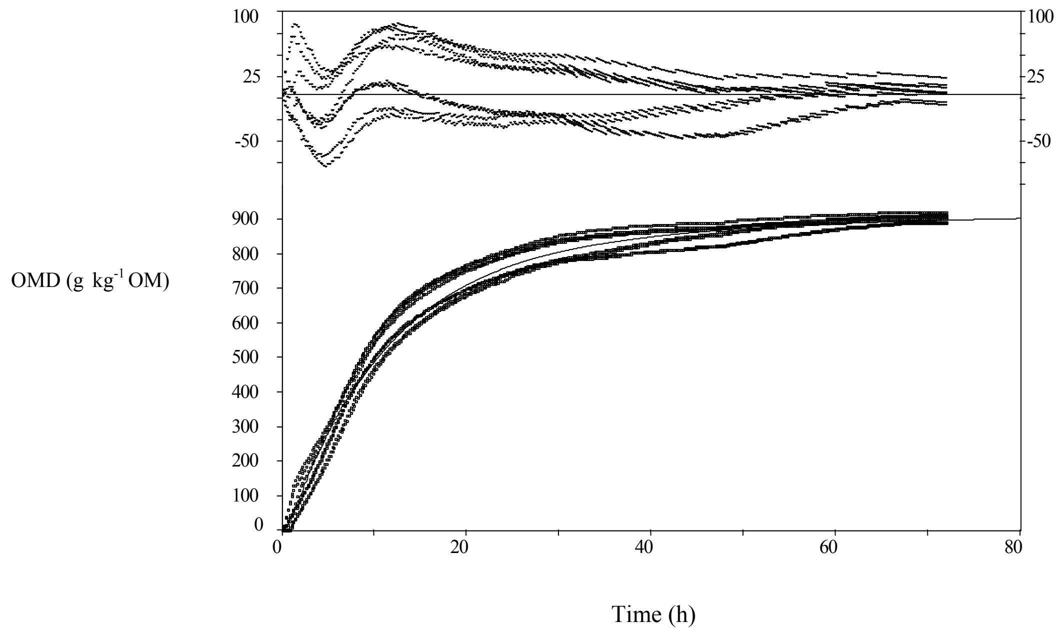


Fig. 3. Calculated degradation of OM (g kg^{-1} OM) during 72 h and fitted kinetic model for timothy cut on 27 June 1996. The figure represents data from two field plots analysed in duplicate two times in different runs. The panel above displays the residuals from the fitted line OMD = 338 $/(1+(3.8/t))^{1.7}$+586 $/(1+(13.9/t))^{1.9}$ with residual fitted standard error FE= 31 g kg^{-1}OM and coefficient of determination, R^2 =0.98.

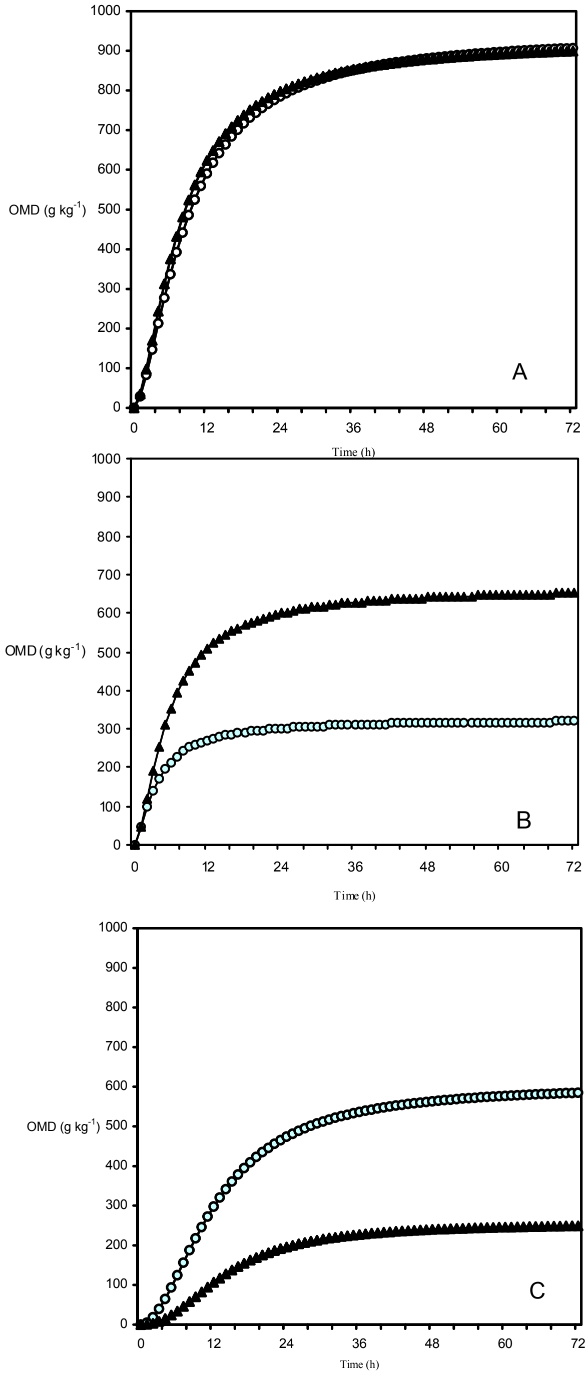


Fig. 4. The predicted rumen degradation of the fractions, the whole forage (A), the neutral detergent soluble components (B) and the neutral detergent fibre (C) of timothy (circles) and red clover (triangles) cut on 24 June in 1996.

Differences in degradation kinetics between timothy and red clover

In Paper II it was concluded that, despite the different chemical composition and degradabilities, the degradation pattern of the whole forage of timothy and that of red clover are similar. An example of that phenomenon is presented in Fig. 4 (Panel A). Further, it was concluded that the NDS fraction in red clover was larger and had a lower maximal fractional degradation rate (FDRmax) compared to that in timothy, which is exemplified in Fig. 4 (Panel B). The higher FDRmax of the NDS fraction in timothy compared to that in red clover in Paper II is consistent with the results of Schofield & Pell (1995) in a similar study. The difference in degradation rates between the species could be a result of the different chemical composition, which is suggested in Paper II. This difference could also depend on a different relationship between the substrate and the inocula. Rymer, Huntington & Givens (1999) have shown that the rate of degradation of the soluble fraction in grass is dependent on the relationship between the concentration of substrate and inocula, resulting in that small amounts of the soluble fractions degrade faster compared to larger amounts soluble fractions.

In Paper II it was found that the rate of degradation of NDF in red clover and that in timothy were not different, even though there was a larger range for the degradation rate of NDF in red clover. An example of the degradation of NDF in red clover and timothy is presented in Fig. 4 (Panel C). The results in Paper II indicate that the conclusion of Smith, Goering & Gordon (1972) that legumes in general have a faster rate of degradation of NDF compared to grasses is not valid for the timothy and red clover studied in Paper II. This conclusion, in combination with the finding in Paper II that red clover has higher concentrations of NDS soluble components compared to timothy on all harvest occasions, may explain the slightly higher maximal fractional degradation rate of the whole forage of red clover compared with that of timothy. This finding is in line with the suggestion of Mertens (1993) that the degradation of forages is primarily determined by the concentration of cell contents and secondly by the properties of the cell wall.

Impact of degradation characteristics on animal production

Numerous experiments and practical farming have shown that dairy cows have a higher forage intake and milk production when fed legumes compared to when fed grasses (Sheldrick, Newman & Roberts, 1995). The higher intake of legumes in dairy cows has been related to the higher rate of rumen degradation in legumes compared to that in grasses (e.g. Beever *et al.,* 1986). In Paper II, it is questioned if this difference in degradation rate between grasses and legumes, is large enough to be the only explanation for the higher voluntary intake of legumes versus grasses. Therefore, additional explanations have been suggested in Paper II. In a study of Bertilsson & Murphy (2003) it was suggested that the less digestible NDF should have a higher density and therefore have a higher passage from the rumen. The lower digestibility of NDF in red clover compared to timothy could therefore have a positive effect on intake. Dewhurst *et al.* (2003) found that cows fed silage of red clover ate larger meals and ate more frequently, compared to cows fed silage of perennial ryegrass. One explanation for this difference could be the suggestion in Paper II that cows fed grass spend more time ruminating compared to cows fed legumes (Penning *et al.,* 1991), which limits the voluntary intake and is more energy spilling. This can be further visualised by using a metaphor and comparing dairy cows with herbivorous insects. When cows are fed red clover, they become like the idle aphids, which feed on plant juices, and when fed grass like timothy, they behave more like diligent termites that rely on digestion of cellulose.

The use of additives in direct cut silages

Silage is the most important forage for dairy cows in Scandinavia. It is also the most variable and unstable feedstuff used in rations for dairy cows. In order to improve the nutritive value and hygienic quality of forages, several silage additives have been developed (Muck & Kung, 1997). Major advances in the use of acids as additives in the conservation of silages were achieved by the Finnish scientist Artturi Virtanen, who received the Nobel Prize in Chemistry in 1945 for his work. Besides additives, the quality of silage is also affected by other treatments, such as chopping and wilting (Pauly & Lingvall, 1999). The dairy industry is well aware of the importance of silage quality for the possibility of producing dairy products with a high added value (e.g. high-quality hard cheese). Standards for good silage quality in Sweden are presented in Table 2.

Table 2. *Guidelines for good silage*

Parameter	Criteria	Comment
Metabolisable energy[1]	Min. 11.0 MJ kg^{-1} DM	11.0 MJ kg^{-1} DM = 850 g kg^{-1} OMD[2] for grasses and 890 g kg^{-1} OMD[2] for legumes
Crude protein	120-150 g kg^{-1}DM	
ADF-N	Max. 50 g kg^{-1} N	
Ammonium-N	Max. 80 g kg^{-1} N	
NDF	450-550 g kg^{-1} DM	NDF according to Chai & Udén (1998).
pH	Min. 4.2	(For direct cut silages)
Butyric acid	Max. 10 g kg^{-1} DM	
Lactic acid	30-100 g kg^{-1} DM	
WSC	Min. 50 g kg^{-1} DM	
Clostridia spores	< 10^3 CFU kg^{-1} feed	

[1]*Spörndly, 1999* [2]*Lindgren, 1979, ADF-N=Acid detergent fibre bound nitrogen, NDF=Neutral detergent fibre, WSC=Water soluble carbohydrates, CFU=Colony forming units*

Preserving the crops as direct cut silage as described in Paper III may seem rather old fashioned, as most farmers today wilt their silage on the field as was done in Paper IV. Wilting reduces the need for transport of the fresh matter from the field to the silo. It also reduces the losses through effluents and the requirement for a low pH in order to prevent the growth of clostridia (Collins & Owens, 2003). However, there are advantages to be gained by using direct cut silages. According to Rotz & Muck (1994) a minimum of DM losses in the chain from the field to the feed bunk is achieved by direct cut silage with formic acid as an additive, compared to hay making and wilting silage.

Conservation properties of the crops

The fermentation processes in the silo are different from the fermentation processes in rumen. Therefore the properties of forage for silage conservation are different from the nutritional properties. Respiration, proteolysis, fermentation and other enzymatic reactions during ensiling mainly affect the herbage components soluble in NDS (Van Soest, 1994). The high concentration of NDS soluble components in the mixed crop of timothy and red clover in Paper III makes it more sensitive to the effects of additives. Another important factor for the effect of additives in silages is the DM content of the herbage (Muck & Kung, 1997). The mixed crop in Paper III had a significantly lower DM content compared to the grass. The low DM contents, the thick fleshy stems and the leaves that easily shatter make red clover a difficult crop to wilt (Sheldrick, Roberts & Newman, 1995). Timothy is, in contrast to red clover, well known for being easy to wilt and for resulting in good silage quality in most conditions.

Red clover and other legumes have a higher buffering capacity compared to grasses such as timothy, mainly due to higher concentrations of anions and organic acids (Playne & McDonald, 1966). This is the reason for the higher buffering capacity in the mixed crop in Paper III. For good conservation of the crop, soluble carbohydrates available for the fermentation in the silo are important. The content of water-soluble carbohydrates (WSC) in the herbage of the mixed crop in Paper III is comparable to that in other studies with red clover silages (e.g. Lättemäe, Ohlsson & Lingvall, 1996). The concentration of WSC in the timothy crop in Paper III was not significantly higher compared to the concentration of WSC in red clover, which was unexpected.

Chemical composition of direct cut silages

The low DM content in direct cut silages results in high concentrations of fermentation products and low concentrations of WSC, if no additives are used to control the in silo fermentation. This effect is obvious in Paper III, where the concentrations of WSC in most silages were lower compared to the recommendation of good silage (Table 2). Conditioning and wilting had probably improved the effect of the addition of molasses in both crops, which resulted in undesirably high concentrations of lactic acid compared to the standard of good silage. Lactic acid is the strongest acid produced during fermentation (McDonald, Henderson & Heron, 1991) and is also produced during the fast degradation of starch in the rumen. High production of lactic acid in the rumen can cause acidosis (Van Soest, 1994).

One of the more overlooked fermentation products in silages is ethanol. High concentrations of ethanol in silages could result in an off-flavour in the milk, but are not a major problem for the metabolism of the dairy cow (Randby *et al.*, 1999). Ethanol is not a significant product for badly fermented silages, as high concentrations of ethanol are more frequent in well-fermented silages (Randby *et al.*, 1999; Driehuis & Wikselaar, 2000). The concentrations of ethanol in the silages treated with molasses in Paper III were much higher compared to the results of Lättemäe, Ohlsson & Lingvall (1996), but comparable to the concentrations reported by Driehuis & Wikselaar (2000). Lättemäe, Ohlsson & Lingvall (1996) suggested that ethanol in silages is a result of air leaking into the silage and fermentation of WSC by yeasts. Therefore, they concluded that molasses improved the packing of the silages and excluded air from the silage. This conclusion is different from the results of Driehuis & Wikselaar (2000), who suggested that ethanol is produced in silages with high concentration of WSC and high water activity. Driehuis & Wikselaar (2000) were able to restrict the production of ethanol with the addition of inoculants, an effect that was not achieved by the inoculant used in Paper III.

Rumen microbes are more efficient if peptides and amino acids are used as a substrate for microbial synthesis compared to the use of ammonia (Firkins, 1996). The high concentration of NH_4 –N in the untreated silages in Paper III could therefore be a limiting factor for the microbial synthesis of protein in the rumen. This implies that feeding the untreated silages in Paper III could result in lower animal production compared to feeding the treated silages.

Effects of additives on fibre quality

The untreated mixed crop in Paper III showed a lower NDFD compared to the treated silages. Maillard reactions reduce the degradability of silages. However, Maillard reactions occur most frequently in low moisture silage (Rotz & Muck, 1994). Due to the low DM content in the silages in Paper III, the reduction in NDFD in the untreated red clover silages is most likely an effect of partial fermentation of cell wall carbohydrates, and not the effect of Maillard reactions. Lättemäe, Ohlsson & Lingvall (1996) draw similar conclusions in a study of the effect of additives in direct cut red clover silage. The response to additives of the NDFD did not appear in the pure timothy crop. The different responses of the NDFD to additives in the two crops indicate that the NDF fraction in timothy is less fermentable in the silo, compared to that in red clover.

Cell wall degrading enzymes are in general added to the herbage during ensiling to enlarge the fraction of fermentable carbohydrates available for the lactic acid producing bacteria (Rodrigues *et al.*, 2001). The effect of enzymes in Paper III is very difficult to evaluate, as the treatment with enzymes also included inoculation with lactic acid bacteria and molasses. Most likely the complex treatment mainly diluted the NDF concentration, which is also concluded in Paper III.

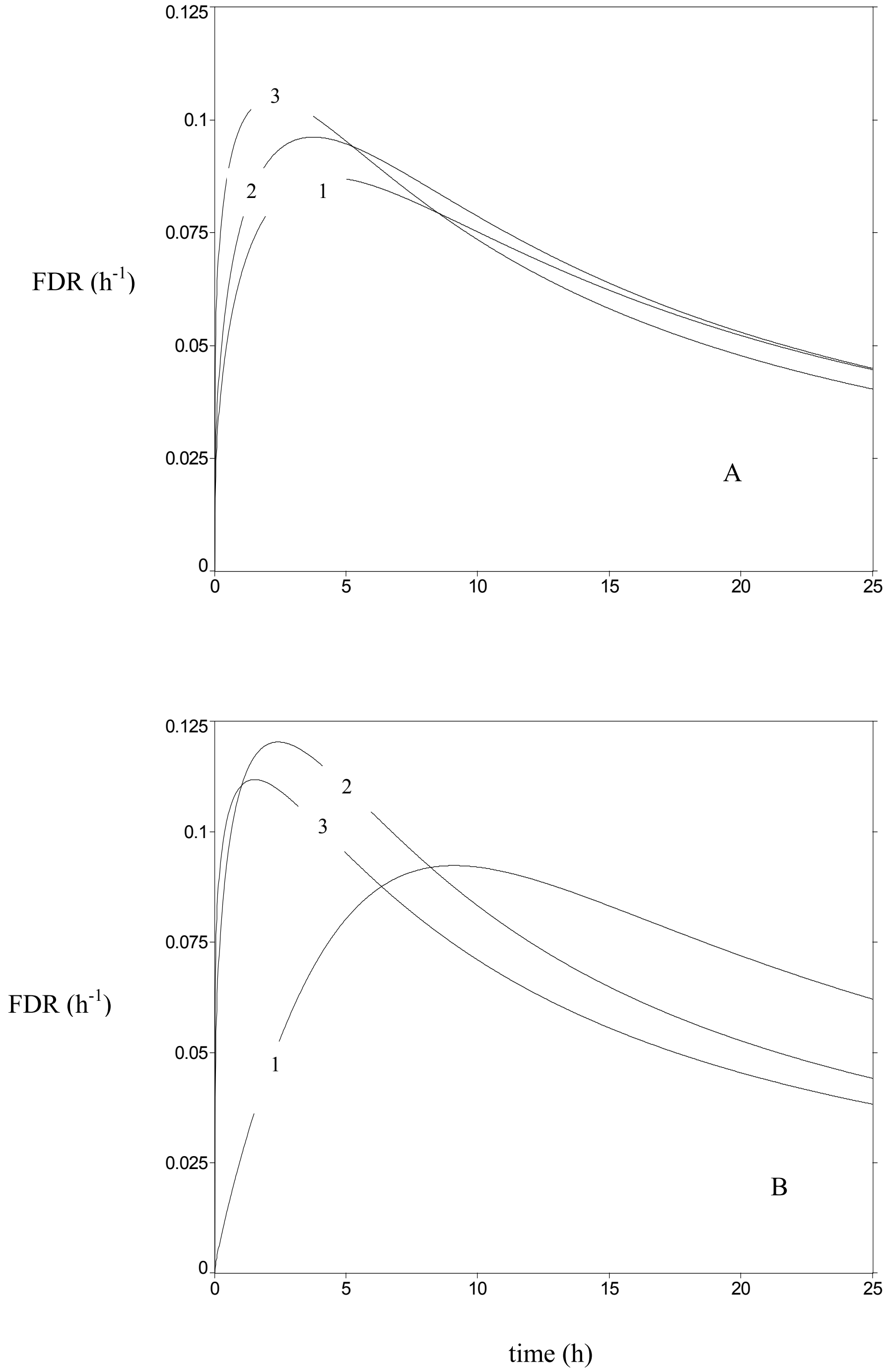
Effects of the additives on in vitro gas production profiles and OM degradability

Silage additives can affect the total in vitro gas production and OMD (Paper III), which will affect the potential feed value of the silages. The response of a reduction in the OMD was detected in both crops in Paper III, but the response of a reduction in the gas production was only detected in the mixed crop. The reduced gas production in the untreated mixed crop indicates that rumen-fermentable OM had been lost during the in silo fermentation, which may result in a lower feeding value.

Besides the effects on the potential degradation, the effects of the additives on the degradation kinetics in silages are of importance (e.g. Lättemäe, Ohlsson & Lingvall*,* 1996; Rodrigues *et al.*, 2001). It is difficult to correlate the gas production of the NDS soluble fraction to the OM degradation due to the fermentation of the fraction in the silo. To give the gas production profiles of the silages in Paper III, a robust and simple description, a monophasic model was applied. Deaville & Givens (2001) draw the same conclusion for the modelling of gas production profiles of corn silage. To improve the understanding of the results, the gas production profiles in Paper III were described with simplistic parameters, the time (h) when half of the gas was produced (t_{50}), the time (h) of the maximal fractional gas production rate (t_{max}), the time when 0.90 of the gas was produced (t_{90}) and the maximal fractional gas production rate ($FGPR_{max}$). These parameters can be derived directly from data or from any equation describing the gas production.

The length (h) of the parameters t_{max} and t_{90} in Paper III is comparable to that of the corresponding parameters for silages of perennial ryegrass treated with cell wall degrading enzymes (Beuvink & Spoelstra, 1994), where t_{max} occurred between 4 to 6.5 h and the time when 0.95 of the gas was produced occurred after 19 to 29 h. This comparison strengthens the conclusions in Paper III that it is the initial 24 h of degradation that are of importance in studies of the effects of additives in silages and that most effects occur within the first 8 h. To highlight the effects of the additives on the gas production kinetics in Paper III, the fractional degradation rates of the gas production profiles are presented in Fig. 5. By comparing the fractional degradation rates, it is evident that the mixed crop was more affected by the treatments compared to the pure timothy and that the effects were greatest during the start of the degradation. The reduction and the delay of the peak gas production rate in the untreated silages could, as suggested in Paper III, have had a negative effect on the intake, as high initial gas production is correlated to high intake (Rodrigues *et al*., 2002).

By using the in sacco technique it has been difficult to detect effects of the addition of molasses (Lättemäe, Ohlsson & Lingvall, 1996) and formic acid (Salawu, Warren & Adesogan, 2001) on the degradation profiles of the silages. The in vitro gas production technique has proved to be a powerful tool to describe the effect of wilting and the addition of cell wall degrading enzymes on in vitro degradation kinetics in grass (Beuvink & Spoelstra, 1994) and corn silages (Colombatto *et al.,* 2004). The results in Paper III show that the corresponding effects of formic acid and molasses in grass and legume silages could be detected by using the in vitro gas production technique. Silage additives are commonly used in Scandinavia, and the possibilities of detecting the effects of different additives on rumen fermentation as presented in Paper III make the gas production technique a useful tool for improving the profitability of dairy producers and the dairy industry.


0.125
0.1
0.075
0.05
0.025
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5
10
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FDR (h-1)
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2
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A
0.125
0.1
0.075
0.05
0.025
0
0
5
10
15
20
25
FDR (h-1)
2
3
1
B
time (h)

Fig. 5. The effect of no additive (1), formic acid (2) and molasses and lactobacillus (3) on the fractional degradation rates (FDR h^{-1}) of pure timothy, panel (A) and the mixed crop, panel (B) during the first 25 h at second cut 1996.

Predictions of voluntary intake of silages in dairy cattle based on feed characteristics

The primary nutritional factor controlling animal production is intake (Minson & Wilson, 1994). Intake is controlled by the nervous system by a complex interplay between dietary and animal factors (Forbes, 1995). When herbage is preserved as silage, the conservation process changes the chemical composition and the nutritional value of the forage (Van Soest, 1994). Therefore, the intake of silages is in general lower in relation to the fresh crop or hay (Minson, 1990; Forbes, 1995). However, reports dealing with the intake of silages in dairy cows are ambiguous, and silage intake in relation to the intake of hay from the same herbage has sometimes been shown to be similar (Bertilsson & Burstedt, 1983).

In Scandinavia silages are in general fed separately from the concentrates, even though feeding systems with total mixed rations occur. If the farmer has access to cheap forages, underestimation of the potential silage intake can be very costly, as it will increase the amount of concentrates in the diets. An increase in the amount of concentrate will further decrease the silage intake (Forbes, 1995) and can thereby affect the competitiveness of the milk production. Some of the current feed evaluation systems have predictions of intake, such as the French fill unit system developed by Jarrige *et al*. (1986), which is based on a large database of recorded feed intakes, in relation to a standard feed. In Finland Huhtanen *et al.* (2002) have developed an empirical index model for predicting silage intake based on in vitro digestibility and fermentation parameters.

Dietary factors correlated to the voluntary intake of silage

For dry feeds the relation between intake and digestibility is stronger compared to that for wet and fermented feeds (Huhtanen *et al.,* 2002). To improve the relationships between the feed characteristics and silage intake, several models with other nutritional parameters have been developed. In a study of silage intake in beef cattle (Rook & Gill, 1990) and in a study of intake in dairy cows (Huhtanen *et al.,* 2002), the concentrations of volatile fatty acids (VFA) in silages had a negative effect on the intake. The negative effect of VFA on intake has also been established by infusions of the acids into the rumen (Forbes, 1995). No effects on silage intake from the concentrations of fermentation acids were found in Paper IV. The lack of response to the concentration of fermentation acids concerning the intake in Paper IV may be explained by the higher levels of milk production and concentrate supplementation compared to the levels in the study of Huhtanen *et al.* (2002).

The concentration of NH_4-N in relation to the total N in silage is an important feed characteristic with a negative effect on silage intake (Forbes, 1995). This negative effect on the intake was also found in Paper IV. A more unexpected relationship between the intake and fermentation products in Paper IV was the positive correlations between the ethanol concentration and the silage intake. In Paper IV it is suggested that the effect of the ethanol concentration on the intake is an associative effect. One explanation for the relationship could be that the concentration of NH_4-N was negatively correlated (r=-0.7, p=0.006) to the concentration of ethanol. The response to ethanol could therefore be an image effect of the concentration of NH_4-N. The negative correlation between the concentrations of ethanol and the concentration of NH_4-N could depend on the fact that the rapid fermentation of sugar into ethanol reduces the proteolysis in the silage (Driehuis & Wikselaar, 2000).

Two of the cornerstones in the theories on the regulation of intake are the physical limits of gut fill and the metabolic demand for energy. The regulation of intake from dietary factors, expressed as fill effects and energy concentration in the diets, is highlighted in the net energy-NDF theory of Mertens (1994). In general, the relationships between intake and the concentration of NDF in forages have been found negative (Minson, 1990). This negative correlation is, according to the theories of Mertens (1994), only valid for NDF concentrations in the diet larger than 500 g NDF kg $^{-1}$ DM and when the intake is restricted by rumen fill. At lower concentrations of NDF and when the energy requirement of the animal is regulating intake, a positive relationship between the concentration of NDF in the diet and intake is suggested. The mean concentrations of NDF in the diets in Paper IV ranged from 400 to 500 g NDF kg $^{-1}$ DM, indicating that the diets were below the upper NDF limit for a positive response to the NDF concentration. This supports the suggestion in Paper IV that the cows were not limited by fill and therefore could regulate their silage intake to meet the demand for energy.

Oba & Allen (1999) found that increased NDFD increased milk production and intake. The positive effect of increased NDFD on intake is in line with the results in Paper IV. The net energy-NDF theory (Mertens, 1994) for the prediction of intake does not include quality parameters for the NDF such as the degradability and rate of degradation. Allen (1996) stated that the NDF concentration alone is insufficient as a predictor of forage intake due to the variable rumen availability of NDF in forages. Introducing NDFD as a parameter to predict intake may improve predictions for types of forages similar to the grass silages in Paper IV. However, NDFD cannot explain the general higher intake of legumes compared to that of grasses. Due to winterkill of red clover in 1999 and 2000, none of the silages in Paper IV contained red clover. As shown in Paper II, red clover has a lower NDF concentration and degradability on all harvest occasions compared to timothy. If the study in Paper IV had included red clover silages, the results of the regression analysis would therefore have been different.

The linear relationships between the individual kinetic feed parameters describing the gas production profiles in Paper IV and the forage intake were stronger compared to the results of Steen *et al.* (1998), who used the in sacco technique to predict silage intake in steers. In all the types of incubations used to determine the degradation characteristics in Paper IV, the parameters describing the shape of the gas production curves (B and C in Equation 1) had a stronger correlation to the silage intake compared to the asymptotic gas production (Parameter A). The lack of correlation between parameter A (Equation 1) and the voluntary intake in Paper IV is consistent with the work of Carro *et al.* (2002), who concluded that the voluntary intake is primarily regulated by the parameters describing the rate of degradation. The mechanistic effects of the changes in the gas production curvature on the intake are not yet known, even though such changes could represent a change of the initial release of energy from the silages. The samples incubated wet resulted in different correlations compared to the dried samples (Paper IV). In Paper IV it is suggested that the reason for this difference is that the different sample preparation techniques affects the chemical composition of the silage samples.

The strongest correlation between intake and in vitro degradation occurs at 6 h of incubation (Van Soest, 1994). As the incubation proceeds beyond 6 h, the relationship between degradability and intake reduces. This conclusion is in line with the lack of a relation between the OMD of the silages at 72 h and the intake in Paper IV. Blummel & Becker (1997) found that in vitro gas production at 8 h gave the best correlation to intake compared to other parameters describing the gas production profile. The value of short-time incubations in forage evaluation has also been suggested by Akin (1979), who found that the interval between 4 and 8 h is the most useful interval for the incubation of plant tissues when using the scanning electron microscope in a plant breeding programme for enhanced forage quality.

Sample preparation for silages

Lowman, Theodorou & Cuddeford (2002) have highlighted the importance of sample preparation techniques in gas production experiments. The silages in Paper III were freeze-dried to reduce the losses of volatile components. This is an interesting alternative technique for research purposes (Cone, van Gelder & Marvin, 1996), but is too laborious for routine analysis. One of the largest obstacles to analysing fresh silages is finding methods to obtain representative samples (Paper IV). Porter (1992) determined the gross energy content in fresh silages and found that grinding the silages with an addition of liquid nitrogen was the method that gave the highest precision. In Paper IV the wet (thawed) samples produced more gas and had a higher initial gas production compared to the dried samples of the same silages. An example of the differences in degradation kinetics between the different sample preparation techniques in Paper IV is presented in Fig. 6. The degradation parameters of the wet samples gave poorer predictions of intake compared to the parameters of the dried samples (Paper IV). The poorer predictions may be due to the fact that the wet samples were less representative of the silage consumed by the cows and that wet samples need a more flexible gas production model compared to the dried samples (Paper IV). Porter (1992) improved the precision in his experiments with fresh grass samples by increasing the sample size from 2 to 4 g. There are no practical problems in enlarging the sample size in gas production experiments, as long as the volume of the buffering inocula is increased in parallel with the weight of the sample (Beuvink & Spoelstra, 1992).

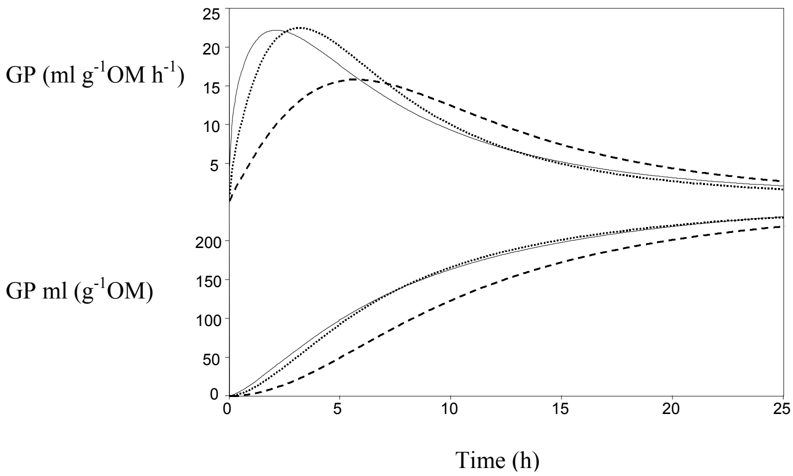

Fig. 6. Cumulative in vitro gas production (GP) during 25 h for a silage sample, incubated wet (solid line), incubated dried (dashed), and the supplementing concentrate feed (dotted line) in Paper IV. The upper panel displays the first derivatives of the gas production curves below.

Techniques to determine degradation characteristics as intake predictors

Intuitively one might suggest that the best techniques for predicting silage intake should involve the use of parameters from in vivo measurements. However, Nsahlai & Umunna (1996) have found that in vivo digestibility might give less accurate predictions of intake compared to the in sacco method. As discussed in Paper I, parameters based on in sacco degradabilities are only valid for research purposes and to build up feed tables, as they for practical reasons cannot be applied on the farm level. Wilman, Foulkes & Givens (1996) concluded that a simple in vitro method for measurements of NDF degradation was superior to the in sacco method in terms of accuracy and cost-efficiency. Determination of the in vitro NDFD is not only a less expensive alternative, but it can also lead to more valuable results. Oba and Allen (1999) concluded that in vitro measurements of the NDFD give more accurate predictions of intake compared to in vivo measurements, where measurements of potential degradability are often confounded with the retention time. Intake has also been related to the nature of the gas production curve (Khazaal *et al.*, 1995; Rodrigues *et al.*, 2002). Moreover, intake is predicted with near infrared spectrometry (NIRS) (Deaville & Flinn, 2000). This technique uses NIRS reference spectra of silages with known intakes to predict silage intake.

Effect of particle size reduction on intake

Forages need to be broken down into small particles before they can escape the rumen (Allen, 1996). Particle size reduction depends on silage animal interactions and not a specific feed parameter. Chewing and rumination take up a large part of a dairy cow's day and the time available for eating can be limiting for intake (Martinsson, 1992). The limiting effect of access on intake may be different in timothy compared to that in red clover, as the different forages have different feeding patterns (Dewhurst *et al*., 2003). Few feed evaluation systems for dairy cows, except for the Danish (Nørgaard, 2003), have estimates of chewing time as an intake parameter. Other factors affecting the particle size reduction are differences in fragility and buoyancy between forages, which may affect the retention-time in the rumen and thereby the voluntary intake of forages (Allen, 1996). The particle size distribution and reduction of silages in the rumen are not studied in this thesis and need further research to explore their importance for the voluntary intake of red clover and timothy silages.

Challenges in modelling forage intake

One type of model for predicting intake is based on non-dietary factors such as milk production and the level of supplementation (e.g. Heather *et al*., 1984). This type of model cannot rank feeds and its usefulness in practical farming can be discussed, as milk production is partly a function of intake. To improve silage management, it is therefore preferable to predict the relative intake of the forages on the basis of the feed characteristics, as accomplished by Huhtanen *et al*. (2002) and in Paper IV.

Mertens (1994) has suggested that, when evaluating the intake potential of high quality forages, the studies must be conducted with animals with high-energy demands so that the genetic and physiological potential does not limit the intake. Therefore, sheep fed at maintenance do not constitute a good model animal for predicting the feed intake in dairy cows. Most studies undertaken to correlate the degradation characteristics of forages have, however, been conducted with animals, which have had relatively low energy, requirements and which have been fed straw and hay (e.g. Blummel & Ørskov, 1993; Rodrigues *et al.,* 2002). Few studies have been conducted to predict silage intake on the basis of the feed characteristics of highly digestible silages fed to high yielding dairy cows, as in Paper IV. The cows in Paper IV had an average milk production of almost 30 kg of milk per day and were in mid-lactation. This implies that the physical constraints of early lactation were overcome and that the animals were in a positive energy balance. Another important criterion for correct intake studies is that the energy concentrations of the diets must be high enough for the animals to be able to consume enough feeds to meet their requirements (Mertens, 1994). To meet the criterion of energy concentration, the animals studied in Paper IV were allocated to blocks depending on their parity, stage of lactation and milk production, and supplemented according to their pre-treatment milk production. High-quality forages have a much higher variation in intake between individual animals compared to low quality forages (Van Soest, 1994). Therefore, a large number of observations are needed to develop robust predictions of highly digestible forages. Further research on the intake of highly digestible silages should take this aspect into consideration.

Incubations of complete diets

Incubations of complete diets are an interesting alternative, especially in feeding systems with total mixed rations. One of the initial applications of the in vitro gas production technique (Menke *et al.,* 1979) was developed to predict the concentration of ME in various feedstuffs, including compound feeds. The rumen ecosystem is far more complex than the batch culture used in the in vitro gas production technique. Therefore, progressing from determination of the ME concentration in compounds to determination of the course of degradation in complete diets requires taking a large step. Sandoval-Castro *et al.* (2002) investigated the associative effects of feed mixes on gas production parameters by incubating tanniferous feeds with grass and soybean meal and found the technique useful. However, in their study there were no in vivo trials, as there were in Paper IV. The usefulness of the results of the incubations of the mixes in Paper IV is difficult to interpret, as all the silages were incubated with the same ratio to the concentrate, even though there were specific relationships for each animal.

Multiple relationships between silage characteristics and intake

The complexity of the mechanisms regulating silage intake and the difficulties and costs involved in exploring them call for more global designs of prediction models. It is very expensive and laborious to develop new intake models for every feeding situation in each region. Global prediction models of forage intake are under development for NIRS analysis, and hence they need a large amount of reference material from various regions before they can be implemented.

The best multiple prediction (R^2=0.81) of silage intake in relation to BW based on feed characteristics in Paper IV came from the dry samples, and the in vitro NDFD of the silages at 72 h and the parameter C of the gas production curves. The equation suggested in Paper IV is similar to the equation for intake of hay and straw developed by Blummel & Becker (1997), which included the gas production at 8 h and the NDFD at 24 h. Blummel & Becker (1997) found no better prediction of intake using mathematical gas profile parameters as predictors compared to using the gas production directly. This could probably be explained by the fact that they applied a mathematical model with first order kinetics, which gives bad correlation to the initial gas production.

Compared to other studies (e.g. Rook & Gill, 1990; Huhtanen *et al.,* 2002) with multiple regressions of feed parameters to predict the relative silage intake, the predictions in Paper IV accounted relatively similarly for the variance of silage intake. The main difference from other studies is the relatively few predictors in the multiple regression models. This could probably be explained by the fact that only 15 silages were included in the study. The results in Paper IV show that the in vitro gas production technique can be a cost-efficient tool for developing relative predictions of silage intake based on feed characteristics. The predictions can be used to maximise the forage intake and to improve silage conservation management. More research is needed to understand the mechanism between gas production profiles and the intake in high yielding dairy cows fed temperate forages.

Conclusions, applications and suggested future research

Conclusions

- The in vitro gas production was correlated with the in vitro degradation of organic matter measured with the in vitro filter bag technique for temperate forages.
- The correlation between the results from the gas production and the filter bag techniques improved when the gas production was transformed to organic matter degradation.
- The second subcurve of the gas production and the filter bag techniques was found correlated for the degradation of the non-soluble fractions representing cell walls.
- The gas production and the filter bag techniques are interesting in vitro tools for describing the degradation of temperate forages.
- The results show that a simple dynamic rumen model is an interesting method to evaluate and compare the degradation of temperate forages determined with the different in vitro techniques.
- The decline in degradability with increasing maturity in timothy was faster than that in red clover during the spring growth, but similar to that in red clover during the summer growth. Red clover contained more readily degradable components at all harvest occasions.
- Timothy and red clover were found to have different intrinsic characteristics limiting the degradation rates of fermentation. Red clover had a slightly higher fractional degradation rate for the whole forage, but lower fractional degradation rates for neutral detergent soluble components compared to timothy.
- The use of NDF as the definition of the concentration of cell walls makes comparisons between the nutritional value of red clover and that of timothy difficult.
- Herbage from mixed crops of red clover and timothy had lower dry matter contents, higher crude protein concentrations and a higher buffering capacity compared to pure timothy during the spring and summer growth.

- Silages treated with additives had faster gas production compared to untreated silages. Mixed silages of red clover and timothy benefited more from additives compared to pure grass silages. The addition of molasses, in combination with a commercial inoculum, resulted in increased production of lactic acid and ethanol in the silages.

- The preparation technique for silage samples is of importance for the possibility of predicting the voluntary intake of silages. The wet silage samples had a higher gas production and different kinetics compared to dry samples. The best multiple linear relationships between the silage intake and the quality parameters were found for dry silage samples.

- When the silages were incubated as dry samples and dry samples were mixed with concentrate, the NDF degradability had a positive relationship to the silage intake. The switching characteristics of the gas curve (parameter C) had a negative relationship to the intake for dry samples and mixes of dry samples and concentrate.

Applications

- The in vitro gas production technique in combination with endpoint gravimetric measurements can be used for a routine analysis of temperate forages, to describe the course of degradation and determine the potential degradability of OM and NDF.

- The different response to maturation in spring and summer in red clover and timothy implies that in mixed leys, timothy might be the limiting forage species during the spring growth and red clover the limiting forage species during the summer growth, in terms of determining the optimal harvest time.

- Additives should be used for direct cut timothy and red clover in order to achieve good quality silages.

- Multiple correlations of chemical analysis, degradabilities and in vitro gas production parameters could be used in models to predict the relative intake of highly degradable silages.

Suggested future research

Feed evaluation for intake and milk quality

Grain and cereal products are relatively freely traded on a global market and offer no competitive advantage to farmers producing for more liberalised markets. Forages such as silages and pastures are local feed sources, which are essential for the welfare of the animals and the nutrient balance of the farms. With improved feed evaluation systems, the forages grown in Northern Scandinavia could improve the competitiveness of the farmers and add value to regional dairy products. Further research for a new feed evaluation system for dairy production should therefore emphasise the prediction of forage intake and milk quality.

Improved methodology of in vitro gas production procedures

The in vitro gas production procedures applied in most forage laboratories are built on recordings of the cumulative gas at regular intervals during the incubation. Different mathematical equations are then fitted to the data in order to parameterize the degradation. This approach is to a large extent a procedure, which has been developed for the in sacco technique, where only a few data points are obtained. The approach has led to long in vitro incubations, which reduces the analytical capacity of the gas recording equipment. With a more improved quality of the gas production recordings, the data itself can describe the degradation kinetics. Further research for new in vitro gas production procedures should therefore focus on the improvement of the quality of the data.

Development of new concentrates and feeding strategies

As the milk production of the dairy herd increases, the voluntary intake becomes more metabolically regulated. The increased use of the ensiling technique for the conservation of forages and farmers' endeavour to improve the nutritive value of forages have led to the main part of forages being cut early. This is resulting in an allocation of nutrients, which are readily available to the rumen microbes and is primarily affecting the metabolic regulation of intake. Further research is needed to develop concentrates and feeding strategies, which will stimulate forage intake through metabolic regulation.

Plant breeding for added value varieties of red clover and timothy

On the global market, seed companies are developing varieties of alfalfa, ryegrass and corn with improved characteristics for dairy production. These forages offer competitive advantages for the farmers utilizing them. In Northern Scandinavia the varieties of forages are selected for winter hardiness, pest-resistance and yield. To improve the competitiveness of dairy production in Northern Scandinavia, further research should focus on the development of varieties of grass and legumes with improved traits for dairy production.

Populärvetenskaplig sammanfattning

Institutionen för Norrländsk Jordbruksvetenskap bedriver grovfoderforskning med fokus på rödklöver (*Trifolium Pratense* L.) och timotej (*Phleum pratense* L.). Odling och utnyttjande av vallfoder studeras och utvecklas med avseende på kvalitet och näringsvärde. Forskningen genomförs i nära samarbete mellan avdelningarna för växtodling, växtskydd, och husdjursskötsel inom forskningsplattformen Grovfodercentrum, som syftar till att utveckla en mer lönsam och uthållig mjölk- och köttproduktion i norra Skandinavien.

Syftet med den här avhandlingen är att beskriva och jämföra timotej och rödklöver som foder till mjölkkor utifrån växternas kemiska sammansättning och in vitro nedbrytningskinetik i våmmen. Inledningsvis jämfördes två in vitro metoder, en filterpåsmetod och en gasproduktionsmetod. Båda metoderna kan vara användbara som alternativ till fodertabeller för att erbjuda relevanta nedbrytningsparametrar i fodervärderingssystem för mjölkkor. Därefter undersöktes nedbrytningskinetiken i våmmen med gasproduktion in vitro och kemiska analyser hos olika former av grovfoder av rödklöver och timotej.

Rödklöver och timotej skördades vid fyra tillfällen under första och andra skörd under två år, 1995 och 1996. Rödklöver innehöll högre koncentration av lösliga substanser och hade lägre smältbarhet hos fiber (NDF) vid alla skördetillfällen i förhållande till timotej. Rödklöver hade som foder högre nedbrytningshastighet i förhållande till timotej. Nedbrytningshastigheten för fraktionen NDF skiljde inte mellan rödklöver och timotej. De lösliga komponenterna hos timotej hade högre nedbrytningshastighet än motsvarande fraktion hos rödklöver.

Timotej och blandningar av timotej och rödklöver ensilerades som direktskördat ensilage med olika tillsatsmedel vid första och andra skörd under tre år 1994, 1995 och 1996. Ren timotej hade andra konserveringsegenskaper jämfört med blandningar av rödklöver och timotej. Timotej och blandningar av timotej och rödklöver gav därför olika respons på näringsvärdet beroende på tillsatsmedel. Smältbarheten hos fibern i ensilage av rödklöver förbättrades av tillsatsmedel, vilket inte var fallet hos timotej. Ensilage med tillsatsmedel hade högre nedbrytningshastighet i förhållande till ensilage utan tillsatsmedel.

För att beskriva sambandet mellan egenskaperna hos ensilage och foderintaget hos mjölkkor registrerades konsumtionen av 15 olika ensilage av timotej. I studien undersöktes olika metoder att behandla och analysera prover av ensilage. Sambanden mellan konsumtion och nedbrytningskinetik varierade beroende på behandlingen av ensilageproverna, vilket visade på betydelsen av korrekt provberedning. Resultaten visade att de genomförda analyserna kan vara kostnadseffektiva verktyg för att prediktera den relativa frivilliga konsumtionen av ensilage. Prediktioner av den relativa konsumtionen av ensilage kan användas för att optimera konsumtionen av ensilage i mjölkproduktionen och förbättra konserveringstekniken vid ensilering.

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