Röbäcksdalen meddelar

Improved analytical capacity for determination of forage quality, utilising the gas production technique

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List of abbreviations

AA	Acetic acid
ADF	Acid detergent fibre
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
GP	Gas production
LA	Lactic acid
LWG	Live weight gain
OM	Organic matter
OMD	Organic matter degradation
ME	Metabolizable energy
NIRS	Near infrared reflectance spectroscopy
NDF	Neutral detergent fibre
NDFD	Neutral detergent fibre degradability
NDS	Neutral detergent solution
SDMI	Silage dry matter intake
VFA	Volatile fatty acids
WSC	Water-soluble carbohydrates

Abstract

Voluntary intake and digestibility of forages are characteristics that affect the animal's performance (Mertens, 1994). Today there are several techniques that can be used for studying forage quality and from where the results can be correlated to feed intake and animal performance. The main factor that affects dry matter intake (DMI) is the energy requirement, which on the other hand is affected by environmental temperature, physical activity of the animal and the production level (Faverdin et al., 1995). Forage intake is mainly restricted by low digestibility, where the content of the cell wall constituents have the greatest impact on digestibility (e.g. Blummel & Becker, 1997; Mould, 2003).

The gas production (GP) technique is an *in vitro* method that is a simple, relatively quick and inexpensive. In this method feed samples are incubated in buffered rumen fluid and the gas that is produced due to fermentation is recorded. The GP gives information on forage digestibility and rumen fermentation pattern (Getachew et al, 1997). Several authors have found high correlations between *in vitro* GP studies and DMI of forages (e.g. Blummel & Becker, 1997; Hetta et al., 2007). In the original method, the incubation time for GP analyses is 24 h (Menke et al. 1979), but today it is more common with incubation times between 24 h up to 96 h. The analytical capacity of the technique is affected by incubation time, as a smaller number of experiments can be conducted per time unit (week) in the laboratory. Studies on high quality forages have shown that 90 % of the gas that is produced during the incubation is produced within the first 24 hours (Hetta et al., 2003) and also that after 15 to 20 h of incubation, the GP decreases although it doesn't stop (Cone et al., 1998).

Fifteen ensilage samples with known silage dry matter intake (SDMI) and chemical composition were analyzed with the gas production technique (Cone et al., 1996). Feed samples that have been analysed in this study are the same as the ones that were studied by Hetta et al. (2007). The aim of this study was to evaluate the consequences of reducing the length of incubation time from 72 h to 24 h for studies of high quality forages, utilizing the GP technique. Results from this study, with 24 h incubation, were compared to the results from the research by Hetta et al. (2007) where the incubation time was 72 h. A special focus in this study was put on the effects on reproducibility of the measurements, correlations with other feed parameters and silage intake of the forages. The results show that there is a great potential for improving the analytical capacity of the technique, by reducing the length of incubation from 72 to 24 h for studies on high quality forages. This study has shown that 24 h incubation gives informative results with high reproducibility of the measurements, clear relationships and high correlations between different parameters and feed intake, and also reliable models for prediction of silage intake with high values of the coefficient of determination.

Sammanfattning

Frivillig foderkonsumtion och smältbarhet av grovfoder är egenskaper påverkar djurets produktion (Mertens, 1994). Idag finns det flera olika tekniker som kan användas för att studera grovfoderkvalitet och från vilka resultaten korreleras till foderkonsumtion och produktion. Den faktor som har störst påverkan för djurets konsumtion av torrsubstans foder är energibehovet, vilken å andra sidan påverkas av omgivningens temperatur, fysisk aktivitet samt produktionsnivå hos djuret (Faverdin et al., 1995). Grovfoderkonsumtionen påverkas i huvudsak av låg smältbarhet, där cellväggsdelarna har störst påverkan på smältbarheten (t.ex. Blummel & Becker, 1997; Mould, 2003).

Gasproduktionstekniken är en *in vitro* metod som är enkel, relativt snabb och billig. I denna metod inkuberas foderprover i buffrad våmvätska och gasen som produceras till följe av fermentation registreras. Gasproduktionen ger information om grovfodrets smältbarhet och dess nedbrytningsmönster (Getachew et al, 1997). Ett flertal forskare har funnit starka samband mellan *in vitro* gasproduktion och konsumtion av grovfoder (e.g. Blummel & Becker, 1997; Hetta et al., 2007). I orginalmetoden från 1979, är inkubationstiden för gasproduktionanalyser 24 timmar (Menke et al. 1979), men idag är det mer vanligt med inkubationstider mellan 24-96 timmar. Den analytiska kapaciteten för tekniken påverkas till stor del av hur lång inkubationstiden är, eftersom detta påverkar antalet analyser som kan göras per vecka. Studier på högkvalitativa grovfoder har visat att 90 % av gasen som produceras under en inkubation produceras under de första 24 timmarna (Hetta et al. 2003), samt även att efter 15 till 20 timmars inkubation så kommer gasproduktionen att sjunka, även om den inte stannar helt (Cone et al., 1998).

Femton ensilageprov med känd ensilagekonsumtion och kemisk sammansättning analyserades med gasproduktionstekniken (Cone et al., 1996). De analyserade foderproven är desamma som analyserades av Hetta et al. (2007). Syftet med denna studie var att utvärdera konsekvenserna med att korta ner inkubationstiden från 72 timmar till 24 timmar, för studier på högkvalitativa grovfoder med gasproduktionstekniken. Resultaten från denna studie med en inkubationstid på 24 timmar, har jämförts med studien som gjordes av Hetta et al (2007) där inkubationstiden var 72 timmar. Speciellt fokus i denna studie sattes på effekterna på reproducerbarheten mellan mätningar, korrelationer mellan foderparametrar och konsumtion av ensilage. Resultaten visar att det finns stor potential med att sänka inkubationstiden från 72 timmar till 24 timmar för studier på högkvalitativa grovfoder. Denna studie har också visat att 24 timmars inkubation ger resultat med hög reproducerbarhet mellan mätningar, tydliga samband och klara korrelationer mellan olika parametrar och foderkonsumtion, samt även tillförlitliga modeller som kan användas för att förutse konsumtionen av ensilage.

Introduction

Good quality forage is of importance for high producing animals, especially in northern Scandinavia where the climate limits the cultivation of cereal grains. The conditions for growing forages with high nutritional values and high digestibility are good in Scandinavia due to the climate with long days during the summer. Cultivation of forage in northern Scandinavia is also positive from the aspect that the agricultural land resources are used, which contributes to biodiversity and keeps the landscape open.

It is generally accepted that feed intake has the biggest impact on the animal's performance and production (Huhtanen et al., 2007). Both milk yield and growth of the ruminant animals are largely limited by forage quality (Minson, 1990). Therefore recording of the performance in animals fed the particular forages does the best evaluation of forage quality. Voluntary intake and digestibility of forages are characteristics of forages that affect the animal's performance (Mertens, 1994). With this knowledge, it is of interest to develop reliable models that can predict animals' feed intake. Today there are several techniques that can be used for studying forage quality and from where the results can be correlated to feed intake and animal performance. The negative aspects with both *in vivo* and *in sacco* method, like that they are laborious, time consuming and expensive, has made alternative laboratory techniques for estimating feed values interesting. The GP technique as an *in vitro* method is a simple, relative quick and an inexpensive method. The GP gives information on forage digestibility and rumen fermentation pattern (Getachew et al., 1997). The analytical capacity of the different methods is limited by incubation time. With long incubation time, like for the *in vivo* method (2 weeks) and the *in sacco* method (1 week), the use of the methods is limited. Gas *in vitro* studies have high analytical capacity, as the incubation time is much shorter and as the number of samples that can be analysed per run is higher. In the original method, the incubation time for GP analyses is 24 h (Menke et al., 1979). Today it is more common with incubation between 24 h up to 96 h, even though studies have shown that after 15 h to 20 h of incubation, the GP decreases although it doesn't stop (Cone et al., 1998). Results from the research by Hetta et al. (2003), where high quality forages were incubated for 72 h, showed that 90 % of the gas that were produced during the incubation was produced within the first 24 h.

The GP technique has proved to be an alternative to the *in sacco* technique (Cone et al., 1998) and several authors have found high correlations between *in vitro* GP studies and DMI of forages (e.g. Blummel & Becker, 1997; Hetta et al., 2007). The aim of this study was to evaluate the consequences of reducing the length of incubation time from 72 h to 24 h for studies of high quality forages, utilizing the GP technique. Special focus was put on the effects on reproducibility of the measurements, correlations with other feed parameters and silage intake of the forages.

Literature review

In vivo methods

From a historical point of view the *in vivo* method has generated a lot of important knowledge about feed fermentation for different types of feeds (Kitessa et al., 1999). This method will also be important in the future, because *in vivo* trials will be needed as it represents the actual animal response to a dietary treatment. Even though this method gives the most realistic and accurate values about the digestibility of a feedstuff, it has several negative aspects that should be considered (López et al., 2000). It is laborious, expensive, time consuming, requires large quantities of feed and is unsuitable for large-scale feed evaluation (Coelho et al., 1988). These factors are the reasons to why laboratory methods have been developed to predict intake and the nutritive value of ruminant feed (Getachew et al., 1997).

The *in sacco* method

The *in sacco* method, where feed samples in nylon mesh bags are incubated in the rumen, gives information about the rate and extent of disappearance of feed constituents (Mehrez & Ørskov, 1977). Time course disappearance curves for each substrate are used to evaluate the kinetics of degradation of feeds in the rumen, by assuming that disappearance from the bag equals degradation in the rumen (Dewhurst et al., 1995). The number of time point during the incubation should be adequate to detect an observable lag time, to detect multiple rate components and to detect an end point of digestion. For forages, an incubation time on 72 h up to 96 h is required (Nocek, 1988). The exponential model as described by Ørskov & McDonald (1979) has been widely used in ruminant feed evaluation to describe degradation kinetics as measured with the *in sacco* method.

The *in sacco* method forms the basis for many of todays feed evaluation systems (Tamminga et al., 1994) and has generally been considered as a standard method to which other techniques accuracy should be tested (Kitessa et al., 1999). In this method feed samples are allowed to come in near contact with the ruminal environment, although it is not exposed to chewing, rumination and passage (Getachew et al., 1997). This is the most accurate method, both in theory and practice, of the non *in vivo* methods that predicts digestibility (Blummel & Bullerdieck, 1997; Kitessa et al., 1999). Disabilities with this method are that it requires at least three fistulated animals to reduce variations between measurements and the analytical capacity in this method is low as only a small number of feed samples can be analyzed per time (Mehrez & Ørskov, 1977). This method is also laborious and requires large amounts of feed samples (Tamminga et al., 1994). Feed samples that are soluble, feed with high proportion of starch and lipid and feed with other small particles are not appropriate for the nylon bags because they disappear undegraded from the nylon bags (Cone et al., 1994; Tamminga et al., 1994). This gives a lack of correlation between silage dry matter intake (SDMI) and degradation kinetics (Cone et al., 1999). Compared to

laboratory techniques, these factors limit the use of the *in sacco* method (Getachew et al., 1997).

The near infra red spectroscopy method

Near infra red spectroscopy (NIRS) is routinely used in the agricultural sector to measure organic constituents in food and other products. The interest of using NIRS for functional properties, like digestibility, has increased (Kitessa et al., 1999). This is a rapid physical method that makes non-destructive measurements and it has shown to have potential to predict *in vivo* organic matter degradability (OMD) (Barber et al., 1990) and feed intake (Steen et al., 1998). By calibrating NIR to feed samples with known *in vivo* digestibility, it can be used to predict digestibility of feeds. The results with NIRS are comparable with *in vitro* methods, with equal or even better accuracy. The lack of adequate numbers of feed samples with known *in vivo* values for calibration limits the use of NIRS for predicting digestibility. Otherwise this method has potential for standardized analysis between laboratories (Kitessa et al., 1999).

The *in vitro* fermentation technique

This is a laboratory method that estimates digestion of feed samples. There are several types of *in vitro* methods that simulate the digestion process and by that determine the nutritive value of ruminant feed (Faithfull, 2002). The *in vitro* method has several advantages compared to *in vivo* trials; it is low costing, less time consuming and it allows the experimental conditions to maintain more accurate (Getachew et al., 1997). *In vitro* digestibility values are corrected by using regression equations where *in vitro* and *in vivo* digestibilities from a large sample set are related. Another way to relate *in vitro* values with *in vivo* values is to include standard samples of known *in vivo* digestibility in each run (Kitessa et al., 1999). There are strong correlations between *in vitro* rumen digestion and *in vivo* digestibility and intake, see figure 1. It is also clear that the times at which *in vitro* results relate to intake and digestibility differ clearly. Maximum correlation between *in vitro* fermentation and intake is seen already at 6 h, while the strongest correlation between *in vitro* fermentation and digestibility is seen at 36 h (Van Soest et al., 1987).

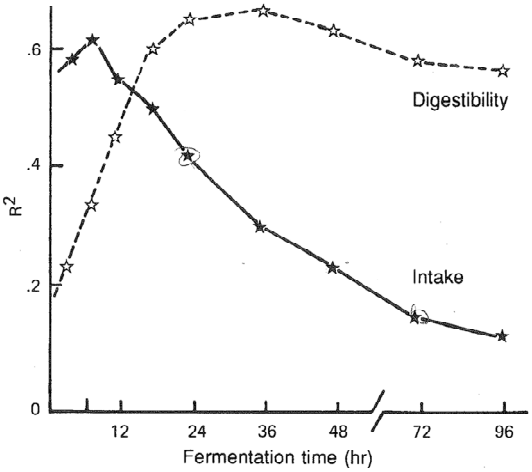

Figure 1. The relationship between *in vitro* rumen digestion and *in vivo* digestibility and intake (Van Soest et al., 1987).

The method by Tilley & Terry (1963)

The method by Tilley & Terry (1963) has been widely tested and used, and has also been an important source for the development of other *in vitro* methods (Faithfull, 2002). This method simulates ruminal and postruminal digestion and has shown good correlations with *in vivo* digestibility (Omed et al., 2000). The original *in vitro* technique of Tilley & Terry (1963) contains two stages where the feed sample initially is fermented in a buffer solution (McDougall's artificial salvia solution) together with rumen fluid for 48 h. After incubation, pepsin in an acid solution is added to the samples. The samples are further incubated in 48 h and then filtered through a porous alumina crucible before oven drying, weighing and ashing. This method characterizes OMD in the rumen and abomasum (Getachew et al., 1997) and it has been verified by *in vivo* results (Faithfull, 2002). The equipment that is used is simple to handle and many samples can be examined at one time. The method gives one observation per feed sample, it is a so-called endpoint measurement, and it doesn't give information about the kinetics of forage digestion (Getachew et al., 1997).

One-stage in vitro method

This is also a laboratory method, which also aims to imitate naturally occurring processes in the rumen. This method is a version of the method of Tilley & Terry (1963), with one major difference. In the one-stage *in vitro* method, feed samples are incubated in rumen fluid and buffer four days (96 h) and no incubation in pepsin occurs. This is the official method for measuring metabolizable energy in Sweden. The analyses on OMD give a coefficient of the digestibility, which can be transferred to a regression equation that calculates the energy content of the forage. The equation is based on *in vivo* feeding trials with sheep's (Lindgren, 1979).

Alternative sources of inoculum

The most predominant reasons for the development of alternative inoculum sources have been to reduce the use of fistulated animals and to provide a more standardized inoculum source. Using faeces as an inoculum is one possible way to reduce the use of rumen fluid, but the results with faeces compared to rumen fluid differ due to different microbial population in the inoculum sources. In theory a bacteria culture could be used, but it requires a lot of work and research to obtain a well functioning mixture (Rymer et al., 2005).

The enzymatic method, where enzymes instead of rumen microorganisms are used, is an end-point measurement. This method was developed by Jones & Hayward (1975) and was based on the two-stage Tilley & Terry (1963) method, but today there are different variations of this method. In the original method feed samples were incubated in pepsin for 24 h, followed by incubation in cellulose preparations for 48 h. Improvements have been made in several steps since then, with addition of amylase (Dowman & Collins, 1982), higher temperature digestion (De Boever et al., 1986) and pre-treatment with neutral detergent solution (NDS) (Roughan & Holland, 1977). De Boever et al. (1986) also made the procedure time shorter, by decreasing the incubation times to 24 h each. Comparing the enzymatic method and *in sacco* method, Nocek & Hall (1984) found that enzymatic combinations got lower values on digestibility compared to values obtained from the *in sacco* method. This method has not been certified by *in vivo* measurements (Getachew et al., 1997), which might make this technique more useable for measuring differences between different feeds, than used to provide absolute digestibility values (Nocek & Hall, 1984).

The neutral detergent fibre method

As fibre composition in forage is a major determinant of forage quality, laboratory methods for analyzing fibre in forage has been developed (Givens et al., 2000). Cell wall contents are highly related to OMD, which on the other hand is positively correlated to feed intake (Van Soest, 1994; Steen et al., 1998). The methods for estimating cell wall content of feed can be grouped in three main categories: the direct or gravimetric method (the neutral detergent fibre method), fractionation method into separate constituents and the physico-chemical methods. The neutral detergent fibre (NDF) method is robust, easy to handle and seems to be the most adequate method for routine analysis (Giger-Reverdin, 1995). This method is well documented and widely used. Treatment with NDS removes cell contents and distinguishes the majority of carbohydrates, including lignin, cellulose and hemicellulosa, which are not digested by endogenous enzymes (Van Soest et al., 1991). The original method was described by Van Soest & Wine (1967), but it has later been modified in several steps (e.g.Giger-Reverdin, 1995; Van Soest et al., 1991). Chai & Udén (1998) have studied and developed this method to a well functioning 16 h oven method at 90°C, which gives several advantages compared to the original method. The laboratory work and the detergent strength are reduced, which results in easier filtering, lower cost of both equipment and chemical and it also decrease the risk for wastewater pollution.

The gas production technique

Although chemical analyses give good information about the forage quality, it doesn't give sufficient information to determine the feeds true nutritive value (Cherney, 2000). As utilisation of forage is largely dependent on microbial degradation within the rumen, description of forages in term of their degradation characteristics is interesting. Mathematical descriptions of GP profiles allow analysis of data and various types of models have been used to describe GP profiles. An exponential model can be used to describe kinetics of GP data, but as it assumes a constant fractional fermentation rate which is unlikely for microbial degradation, these models are not generally valid (Getachew et al., 1998). Menke et al. (1979) found a high precision in prediction of *in vivo* OMD, by using *in vitro* gas measurement and chemical composition in multiple regression equation. Groot et al (1996) introduced a three-phasic model to GP kinetics, which differentiates soluble, insoluble but fermentable, and microbial turnover. This model provides useful data on GP kinetics and the model has shown to be an alternative to the Tilley & Terry (1963) and the nylon bag technique (Cone et al., 1998). Multiphasic models are more flexible compared to monophasic models and can give better description of the degradation of different feed components (Groot et al., 1996). This model also makes it possible to describe the cumulative GP profiles very accurately, also during the first hours of GP which is not able with many other models (Cone et al., 1996).

The GP technique can be a powerful tool for studies on degradability of complex substrates such as grasses, legumes and other whole crop forages. It is used to get additional information about nutrient utilization and the efficiency by which an animal utilizes feed nutrients (Getachew et al., 1997). This method is not only useful for measuring degradability of a feedstuff, it can also detect differences in fermentability between different chemical and physical pretreatments of straw, fermentability of crops grown at different environmental conditions (Williams, 2000) and how different ensiling treatments can affect forage and the intake of forages (Beuvink & Spoelstra, 1994).

There are several methods for measuring gas GP; Menke's method with glass syringes (Menke et al., 1979), manometric method (Waghorn & Stafford, 1993), pressure transducer systems: manual (Theodorou et al., 1994); computerised (Pell & Schofield, 1993), combination of pressure transducer and gas release system (Devies et al., 1995; Cone et al., 1996) or by an automated liquid displacements system (Beuvink et al., 1992). This review will focus on Menke's method, also called Hohenheim gas test, which is widely used in many parts of the world (Blummel & Becker, 1997; Schofield, 2000). This is also the official technique in Germany for estimation of *in vivo* digestibility and ME for ruminants (Menke & Steingass, 1988).

The principles for the GP technique, where feed samples are incubated in rumen fluid, were developed by Menke et al. (1979). A lot of trials have been made after this to measure the accurate kinetics of GP. The method by Menke et al. (1979) has been modified by Blummel & Ørskov (1993), Makkar et al. (1995) and Cone et al.

(1996). Automated GP techniques as an *in vitro* technique, is a simple method, but interactions between fermentation end products, buffering system and amount of gas produced are very complex (Beuvink & Spoelstra, 1992). This method detects and provides useful data on fermentation kinetics of both soluble cell contents and non-soluble fractions of feedstuffs (Getachew et al., 1997). Another advantage with this method is that it can analyse a large number of samples at one time, making the analytical capacity high (Cone et al., 1997). The most negative aspect with this method is that it requires donor animals to supply rumen fluid, which is an economic question where the costs for the animals are high (Kitessa et al., 1999). Another limitation with the GP technique is that it has a lack of standardization in the methodology, which makes it difficult to make comparisons on results between different laboratories (Williams, 2000).

Incubation in rumen fluid

By incubating feed samples with buffered rumen fluid, an estimation of the feed degradability can be made (Rymer et al., 2005). Rumen provides an optimal environment for its anaerobic micro-organisms in terms of pH, temperature, buffering capacity and nitrogen (Williams, 2000). Conditions within the rumen must be kept within limits to maintain normal microbial growth and metabolism and also for the well being of the ruminant. The rumen has an optimal pH range between 6.2-7.2 (Van Soest et al., 1994). The buffering system in the rumen with a copious flow of saliva, containing the main buffering components bicarbonate, phosphate, VFA produced by fermentation and buffering components from the feed, buffers the rumen fluid (Beuvink, 1993). Buffering of rumen fluid is very important, as some bacteria in the rumen fluid cannot grow at pH values below 6.0 (Omed et al., 2000). Also the rate of digestion is influenced negatively if pH decreases to below 6.2 (Van Soest et al., 1994).

The microbial activity of the rumen fluid is influenced by the diet and time of collection after feeding, as the diet strongly affects the rumen microbial flora (Kitessa et al., 1999). Feed samples that are examined should be incubated in rumen fluid from an animal eating comparable feed, to come closer to the *in vivo* situation (Cone et al., 1996; Cone et al., 2002). By sampling rumen fluid from several individuals and mixing it together, the risk of variation in rumen fluid decreases (Williams, 2000).

Gas measurements

The methods for measuring GP were developed around the 1940s, but the knowledge about the close association between fermentation and gas production has been known for over a century (Getachew et al., 1997). Gas measurement is a direct measure of microbial activity, where the gas is an indirect indicator of fermentation kinetics and can be used to predict the extent and rate of feed digestion (Rymer et al., 2005). The GP gives a good picture of the fermentation kinetics, as it allows recording of GP at several times in the incubation period (Cone et al., 1996). Gas volumes can be measured either by collecting gas at atmospheric pressure where its volume is determined directly, or by measuring gas

in a fixed volume container where the volume is calculated from pressure changes (Getachew et al., 1997).

Gas measurements reflect how much of the substrate is converted into VFAs and gases and should be combined with residue determination, where the residues reveals how much of the substrate is fermented (Getachew et al., 1997). After a fixed incubation period, degradability of organic matter (OMD) and NDF (NDFD), can be derived by looking at the disappearance of DM and amount of gas produced (Tilley & Terry, 1963; Menke et al., 1979). In general it is assumed that there is a constant relationship between GP and OMD (López et al., 2007). Menke et al. (1979) found a high correlation between cumulative GP *in vitro* and digestibility *in vivo*.

Direct and indirect gas production

Anaerobic micro-organisms (bacteria, fungi and protozoa) in the rumen ferment carbohydrates to volatile fatty acids (VFA) (acetate, propionate and butyrate), gases (mainly CO_2 and CH_4) and microbial cells (biomass) (Wolin, 1960; Beuvink & Spoelstra, 1992). Volatile fatty acids are the major sources of energy to the ruminant. Fermentation of protein generates in relatively small GP (130 ml gas/g substrate), compared to the fermentation of carbohydrate (340-370 ml gas/g substrate) (Cone & van Gelder, 1999). The GP from fat fermentation is insignificant (1-2 ml gas/g substrate) (Menke & Steingass, 1988).

The gas that is produced in the GP technique comes from two different sources; direct and indirect GP. The direct gas is a result of fermentation that yields in CO_2 and CH_4. The indirect gas is produced from reactions with fermentation products, e.g. buffering of VFAs. The buffering neutralizes the liquid, yielding in CO_2 that is released from the buffer (Beuvink & Spoelstra, 1992). When bicarbonate buffer is used in incubations with forage samples, 50 % of the gas production (GP) is from indirect GP and the remaining 50 % from direct GP (Blummel & Ørskov, 1993).

Depending on the substrate that is fermented, there are differences in VFA production, total GP and fermentation pattern. The type of substrate that is fermented also determines the proportion of acetate, propionate and butyrate that is produced (Beuvink & Spoelstra, 1992). Easily digestible carbohydrates, like starch, generates in a higher proportion of propionate, while slowly fermentable carbohydrates generates in more acetate (Beuvink & Spoelstra 1992; Blummel & Ørskov, 1993). Beuvink & Spoelstra (1992) showed that the total GP depends on the composition of the end products of the fermentation. Comparing for example glucose, rice starch and cellulose, rice starch has the highest total GP; glucose is fermented fastest, while cellulose has the lowest fermentation rate but the highest VFA production.

When microbes ferment carbohydrates to acetate and butyrate, it generates in more GP compared to GP from propionate. The GP from propionate generates only in indirect GP (see table 1), that is the GP from buffering of the acid (Wolin, 1960). This means that a higher proportion of acetate will increase the total GP.

Table 1. *Direct and indirect gas production (GP) in mol, from 1 mol glucose fermented to different acidic end products (Beuvink & Spoelstra, 1992).*

Acidic end prod.	Direct GP	Indirect GP	Total GP
2 acetic acid	2 CO_2	2 CO_2	4 CO_2
1 butyric acid	2 CO_2	1 CO_2	3 CO_2
2 propionic acid		2 CO_2	2 CO_2
2 lactic acid		2 CO_2	2 CO_2

[a] Assuming that 1 mol organic acid releases 1 mol gas from the buffer

Feed samples

Sample preparations are important in GP studies and needs considerable care, especially if the results are to be meaningfully extrapolated to *in vivo* situations. Drying, in an oven or by a freeze drier, and grounding makes it possible to get homogenous samples that can be stored for a longer period and used when required (Lowman et al., 2002). By grounding samples to a standard mesh size, variations within and between laboratories are minimized (Kitessa et al., 1999). A negative aspect with dried samples is that the feed commonly is digested fresh, so from one aspect it can be misleading to incubate dried samples (Lowman et al., 2002). Another negative aspect with drying samples is that the content of OM can decrease (Acosta & Kothmann, 1978). Fresh samples on the other hand, are more inconvenient to handle and more difficult to grind (Lowman et al., 2002). Measurements with fresh samples also give bigger variations in studies (Lopez et al., 1995). The GP profiles are different between fresh silage samples and dried silage, but the total gas volume that is produced is the same (Cone, 1998; Menke & Steingass, 1988). The size of the sample results in a linear increase in total gas volume, but the GP rate is not affected (e.g. Schöner, 1981 & Theodorou et al., 1994). It should also be considered that it is easier to get errors in weighting small samples, while with large samples it is important that the system is capable of buffering the produced acid and that the stored pressure doesn't increase in such a way that it affects the GP profile (e.g. Pell & Schofield, 1993). There are no practical problems in enlarging the sample size in GP experiments, as long as the quantities of feed that is incubated are set in proper relation to the volume of buffered rumen fluid medium (Beuvink & Spoelsta, 1992).

Blank samples are used routinely during GP measurements. The blank vessels contain the same components as the other vessels, but with exclusion of substrate (Rymer et al., 2005). The blank series can correct for the residual of fermentable OM that is included with the inoculum and also for changes in atmospheric pressure (Cone et al., 1996), but as the GP from the blanks may not be relevant to what occurs in presence of substrate, the GP from the blank samples are not always subtracted from the substrate profiles prior to curve fitting. In order to overcome the differences between inoculum, standard samples of forages can be included in the incubations. They can be used as references, which make it possible to make adjustments, if needed, in variations between groups and also between runs (Williams, 2000).

Variations between incubations

Type of feed sample, donor animals, management routines of donor animals, adaptation of the micro-organisms in the rumen to the feed samples and the time of sampling of rumen fluid after feeding has an impact on the GP curve. Also factors like anaerobiosis, proper temperature, suitable pH, right buffering, sample size (Getachew et al., 1997), sample preparation (Lowman et al., 2002) and the condition of rumen fluid influences the GP curve (Cone et al., 1995). The construction of the GP equipment is another source for variation. There are big variations regarding agitation of bottles during incubation, where both automated systems with shaking water baths and manual pressure transducer techniques appears. Rymer et al. (2005) compared Cone et al. (1996) automated and agitating system with Davies et al. (2000) automated system without agitation, and did not found any difference of importance between apparatus, indicating that shaking the medium has relatively little effect on the results within and between laboratories. Even though there are several factors between systems that can have an impact on the GP, the biggest cause for variation seem to be the inoculum (Rymer et al., 2005). Even within donor animals, the rumen fluid can vary with time. A part of this variation can be minimized by collecting rumen fluid from more than one donor animal and at a fixed time relative to feeding (Williams, 2000).

Incubation time

From a historical point of view it has been more interesting to measure digestibility of forages, rather than getting information regarding how GP profiles can provide information about feed intake. This has also affected the length of incubation, as detecting digestibility requires longer incubations compared to detection of feed intake (Van Soest, 1994). In the original *in vitro* method, the incubation time for GP analyses is 24 h (Menke et al. 1979), but today it is more common with incubation times between 24 h up to 96 h.

The GP studies show that young samples have fast degradation rates and that the degradation rate decreases as grass and silage matures (Cone et al., 1998), meaning that studies on high quality forages require shorter incubation time, compared to forage with low degradability. For feeds with low degradability it is of importance that the incubation time is long enough (e.g. 72 h or even up to 96 h), so that the switching character of the GP curve (the asymptote) can be defined, otherwise fitting of the model may not be possible, see figure 2 (Dhanoa, 1988). For studies on high quality forages, an incubation time on 72-96 h will reduce the analytical capacity of the technique, as only 1-2 experiments can be conducted per week. The fact that high quality forages doesn't need that long incubation time can be supported by the research by Hetta et al. (2003) where high quality forages were incubated for 72 h. The results show that 90 % of the gas that is produced during the incubation is produced within the first 24 h. Also a study by Cone et al. (1998) shows that after 15 h to 20 h of incubation, the GP decreases although it doesn't stop (Cone et al., 1998).

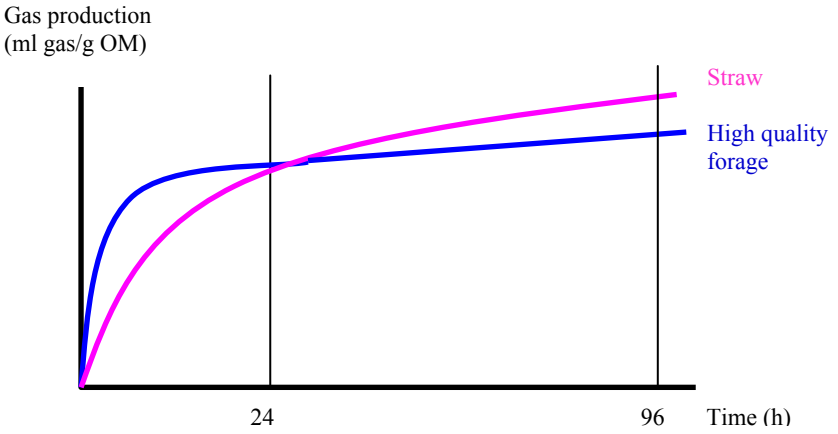

Figure 2. Forage with low degradability (straw) needs longer incubation time compared to forage with high degradability before the asymptote is defined.

The relationship between in vitro fermentation and feed intake

Van Soest et al. (1978) found maximum relationship between *in vitro* degradability and DMI at 6 h incubation. After 6 h of incubation the relationship between degradability and intake decreases. This research suggested incubation times between 6 h and 12 h for best prediction of DMI, which corresponds with Rodriguez et al. (2002) that found that the first 12 h of fermentation are the most important hours for prediction of forage intake. Also in a study by Blummel & Becker (1997), the highest correlation between *in vitro* degradability and DMI was found at 8 h incubation. In the same research, but with a shortened incubation time, from 96 h to 24 h, the results show a maximum correlation at 6 h incubation. According to Blummel et al. (1997) it seems that a combination of gas volume measurements (4-8 h) and the amount of substrate degraded (>24 h) is superior to the models based on only GP kinetics.

Factors affecting silage intake

The factors related to voluntary feed intake can be divided into three distinct groups; animal factors, dietary factors and environmental factors. It is not possible to measure or obtain an exact value for each factor, so it is important to know how these factors interact and if they are correlated The energy requirement of the animal, which depends on the environmental temperature, physical activity of the animal and the production level, is the main factor that affects DMI (Faverdin et al., 1995). Both long- and short-term mechanisms regulate the feed intake. Factors like stretch- and mechanical receptors of the rumen wall and hormonal signalling are short-term mechanisms, while nutritional requirements and body reserves are long-term regulating factors. Long-term mechanisms are often found among animal factors and short-term factors among dietary factors (Faverdin et al., 1995). Other animal factors that affect the consumption are type of breed, age, physiological stage and production level. Environmental factors are climate, temperature, housing conditions, photoperiod and feeding system. Among dietary

factors, the quality of forage is one of the most important factors that affect voluntary feed intake (Huhtanen et al., 2002).

Digestibility of forages

The digestibility of a feedstuff and the fermentation pattern influences the daily DMI, this is of importance to today's high yielding dairy cows. Feed fractions that have the greatest impact on the digestibility are the cell wall constituents NDF, acid detergent fiber (ADF) and lignin (e.g. Blummel & Becker, 1997; Mould, 2003). As the grass and silage mature, the cell wall content increases which leads to a decreased digestibility (Cone et al., 1998). Forage cell walls have considerable influence on voluntary feed intake through rumen fill mechanism (Van Soest, 1994). It is the rate of passage from the digestive tract and the amount of undigested materials in the digestive tract that determines feed intake (Blaxter et al., 1961). This means that highly digestible feed can be eaten in greater quantities before physical limitations apply, due to faster passage in rumen (Blummel & Becker, 1997). Several studies show an evident and positive relationship between feed intake and digestibility (e.g. Blaxter et al., 1961; Steen et al., 1998), meaning that SDMI increases with improved digestibility (Huhtanen et al., 2002). Bad forage quality results in low voluntary intake and digestibility (Blaxter et al., 1961; Minson, 1990).

Other factors

Maturity of grass does not only cause a decrease in OMD and increase of NDF, ADF and lignin content, it also causes a decrease in crude protein (CP) content (Cone et al., 1998). It is known that there is a positive relationship between CP content in silage and intake. This can be associated with the fact that as the grass matures, the protein concentration decreases while fibre concentration increases, which results in silages with low protein content and low digestibility (Steen et al., 1998). Water-soluble carbohydrates (WSC) content in the silage is also positively correlated with SDMI (Huhtanen et al., 2002). Also DM content, which is affected by the stage of grass maturity at harvest, of the silage can have a significant impact on intake. The SDMI increases with increasing DM in the silage, especially at low DM concentrations (Steen et al., 1998; Huhtanen et al., 2007). The water dilutes the energy content and also restricts the intake by its physical presence (Mould, 2003). Steen et al. (1998) found a maximum intake of silage at a DM concentration of 320 g/kg. The relationship between pH and SDMI is weak (e.g. Steen et al., 1998; Huhtanen et al., 2002), but according to McDonald et al. (2002), silage of good quality has a pH value between 4.0 and 4.5.

Characteristics of the silage fermentation products also have an impact on SDMI (Huhtanen et al., 2002). Organic acids and nitrogenous substances, such as amines, that are produced in the fermentation process of silage can have a negative impact on silage intake (Steen et al., 1998). Huhtanen et al. (2002) suggested that it is more likely that SDMI is more related to extent, meaning the additive effects of a number of substances, than type of in-silo fermentation. Also additives, which are

used for improving the silage, have shown to have a negative impact on silage intake (Romney & Gill, 2000).

Type of grass can also have an impact on the DMI and it is well known that silages that contain legumes affect SDMI positively (Martinsson, 1992). It is also known that amount and type of concentrate and supplementary protein feeding have influence on SDMI (Huhtanen et al., 1998) and that an increase in the amount of concentrate decreases the silage intake (Forbes, 1995). Protein as a supplementary feed gives an increase in SDMI, especially if the silage has low protein content. Silage DM intake is also positively related to shorter chop length, suggesting faster intake and passage in the rumen due to this (Romney & Gill, 2000). Palatability is another factor that can have an impact on SDMI, but it is not a major determinant of intake (McDonald et al., 2002). It seems like this is a factor that is more connected to adaptation of a feedstuff rather than to palatability itself (Huhtanen et al., 2002).

Prediction of silage intake

It is generally accepted that feed intake has the biggest impact on the animals' performance and production (Huhtanen et al., 2007). With this knowledge, it is of interest to develop reliable models that can predict animals' feed intake. The role of feed intake models is to optimize the economic performance of the agricultural system, consequently have an optimal feeding plan where the feed is utilized to a maximum, both from a nutritional and an environmental aspect (Yearsley et al., 2001). Models for predicting SDMI also give a better understanding of the factors that controls silage intake (Steen et el., 1998). Several researchers have used simple or multiple regression analyses to examine relationships between individual parameters, or groups of parameters, to predict SDMI. Statistical regression models are the most common models for predicting feed intake, with the negative aspect that these models normally lack generality and can't be applied outside its original parameters (Yearsley et al., 2001).

Factors like chemical composition, digestibility and fermentation quality of the silage are important for predicting voluntary silage intake (Huhtanen et al., 2002). Prediction of silage intake is difficult, as there are often many non-forage factors that may have influence on the intake. Even though there are correlations between chemical composition and SDMI, it is difficult to predict intake only from silage quality. *In vitro* gas measurements can be used to predict DMI and several researchers show significant correlation between *in vitro* GP and DMI (e.g. Blummel & Becker, 1997; Hetta et al., 2007). The kinetics of fermentation is one of several factors that influences voluntary feed intake by ruminants (Getachew et al., 1997). Models for prediction of intake often include parameters related to feedstuffs characteristics, especially to degradability of carbohydrates. As the cumulative GP can provide information about fermentation kinetics, it makes the GP technique a useful tool in making models for prediction of silage intake.

Material and methods

Feed samples

In this study 15 different grass samples, consisting of timothy *(Phleum pretense* L.), and a standard forage hay were analyzed. The grass was harvested during primary growth and regrowth in 1999 and 2000 from fields at the Forage Research Centre, Röbäcksdalen, Umeå, Sweden. After harvesting, wilting and chopping the grass was ensiled in bunker silos. Representative feed samples were taken from the silos 4 to 5 months after ensiling. The samples were freeze dried and grinded. For all samples pH, DM, CP, NDF, lignin, WSC, lactic acid (LA), acetic acid (AA), ethanol and ammonia-N was known, see table 2. The SDMI of the different silages were determined by Hetta et al. (2007) and is also presented in table 2.

All the feed samples, the standard forage hay and the blanks were analysed for *in vitro* fermentation kinetics with a fully automated *in vitro* gas recording system (Cone et al., 1996). The DM content of the 15 feed samples and the standard hay were determined before the experimental work started. After 24 hours in the oven at 105°C, the DM content was calculated. Every sample was examined in duplicates.

Donor animals

Three heifers of the Swedish red breed from the research barn at Röbäcksdalen, Umeå, Sweden, were used as donors of rumen fluid. The live weight of the heifers was about 350 kg at the start of the trial. The animals were fed according to an optimal feeding plan, to obtain a live weight gain (LWG) of 700 g/day. The heifers were kept on a daily ration of 26 kg (6,5 kg DM) grass silage and 300 g (250 g DM) pelleted soya meal. Straw was also given to the heifers, to stimulate saliva production. The daily ration was provided in two meals of equal size, at 07.00 and 15.00.. The feeding plan was introduced two weeks before rumen fluid sampling, so that the rumen micro-organisms were well adapted to the feeds to be investigated. The health of the heifers as well as body condition and live weight, were checked routinely.

Table 2. *Information about the silages concerning date of harvest and means of pH, dry matter (DM) (g/kg), and means (g/kg DM) of crude protein (CP), neutral detergent fibre (NDF), lignin, water-soluble carbohydrates (WSC), lactic acid (LA), acetic acid (AA), ethanol, ammonia-N (NH_4-N) (g/kg N) and silage DM intake (SDMI) (kg/100 kg live weight/day).*

Silage	Year	Cut	PH	DM	CP	NDF	Lignin	WSC	LA	AA	Ethanol	NH_4-N	SDMI
1	1999	1	4.32	341	114	678	54	47	24.3	7.0	10.4	70	2.01
2	1999	1	4.07	283	139	642	43	29	49.2	11.0	9.1	73	1.99
3	1999	1	4.09	289	150	606	50	30	48.5	10.4	7.6	61	2.00
4	1999	2	3.79	249	118	617	50	9	90.1	26.3	15.5	50	2.00
5	1999	2	3.75	253	131	611	66	3	92.3	21.3	7.1	62	1.97
6	1999	2	3.84	262	146	542	66	20	89.8	22.9	10.3	59	1.98
7	2000	1	3.87	201	148	611	57	1	109.8	26.2	9.8	96	1.76
8	2000	1	3.90	242	155	611	55	5	88.4	17.1	8.0	91	1.96
9	2000	1	3.82	227	135	604	52	0	103.4	20.0	4.3	100	1.89
10	2000	1	3.86	207	158	596	55	0	105.6	25.1	9.2	88	2.02
11	2000	1	4.09	274	161	594	46	7	75.7	18.4	3.5	101	1.79
12	2000	2	3.75	256	100	603	50	39	89.0	15.9	2.7	87	1.81
13	2000	2	3.86	274	153	533	44	30	77.4	17.4	2.5	80	1.66
14	2000	2	3.91	279	129	588	42	35	64.0	16.1	3.0	86	1.81
15	2000	2	3.96	273	123	573	45	35	69.9	17.4	1.8	113	1.69

Experimental procedure

Inoculum source

Rumen fluid was sampled from two heifers 2 h after morning feeding for each run. The rumen fluid was collected with a flexible probe, by using oesophageal sampling. The rumen fluid was collected into pre-warmed CO_2 filled thermoses. Before the collected rumen fluid was mixed together, pH from each individual's rumen fluid was measured and later also from the mixture. Rumen fluid was filtered through a sieve and mixed with an anaerobic buffered mineral solution according to Menke & Steingass, 1988. All handling of rumen fluid at the laboratory was carried out under continuous flushing with CO_2 under anaerobic conditions.

Figure 3. Flushing rumen fluid with CO_2 to keep the correct pH.

Buffered mineral solution

Buffer and nutrient solutions were prepared one day before the experiment started, as described by Menke & Steingass (1998). Preparation of buffered mineral solution is done with the purpose to imitate the natural environment in the animals' rumen. Distilled water (790.0 ml), micro minerals (0.20 ml), macro minerals (395.0 ml) and buffer (395 ml) were mixed together on the first experimental day. The solution was warmed up to 39°C, which was the desired temperature, under continuous flushing with CO_2 until it got the desired pH (7.0-7.3). Peptone (2 g), that is a pancreatic-digested casein, was added to the mixed solution, with the aim to be an extra protein source for the rumen microbes. The reducing solution, which has the function to create a reducing redox potential similar to the ruminal environment, was mixed into the buffer just before the incubations started. After it was added to the buffered mineral solution, the solution was bubbled with CO_2, to get right pH, until a colour change from pink to transparent was obtained.

In vitro gas incubations

Each feed sample was incubated in duplicates in two separate series performed on two different weeks. Two blanks, containing buffered rumen fluid without sample, and two samples of standard hay were included in each series. The standard forage hay was incubated to monitor variations among runs (Williams, 2000).

Rumen fluid and the anaerobic buffered mineral solution, with a ratio of 1:2, were mixed together. About 500 mg of OM was incubated in 60 ml of buffered rumen fluid for 24 h in 250 ml serum bottles. The bottles were incubated at 39°C in a warm room under continuous agitation. A computer with a data logger was connected to the climate room to record *in vitro* gas recordings every 12-minute, from where GP curves and fermentation pattern for the feed samples was obtained. The computer also recorded temperature and atmospheric pressure in the room. This information was used as a control and for correcting the gas volumes recorded to estimate volumes at normal atmospheric pressure (1013,5 h Pa) (Cone et al., 1996).

Figure 4. The gas *in vitro* equipment.

End point measurements

After incubation the samples were put on ice to stop the fermentation. The rest of the mixture, with fermentation residues and buffered mineral solution were transferred to crucibles with fritted discs (porosity 2) to be filtrated. These were put under a light vacuum to remove the liquid fraction and then rinsed carefully with distilled water to remove all the bacteria. Thereafter 60 ml NDS was added to the crucibles with the aim to treat the remaining residues by using the oven method, as described by Chai & Udén (1998). The samples were put in an oven, 90°C, for 16 h. After this procedure the samples were washed several times with hot distilled water and finally rinsed with acetone to eliminate all residues of the detergent. The samples were dried overnight at 105°C. After weighing, the samples were incinerated at 550°C and weighed again. As the last step, OMD and NDFD were determined from the ND residues of the samples.

Data treatment and statistical analyses

The cumulative GP data from each sample (four runs/sample) were fitted to a monophasic model (Groot et al. 1996), using the software Tablecurve 2 D (Ver. 5.0®; SPSS Inc., Chicago, IL, USA). The equation; $GP = A / ((1 + (B/t)^c)$ predicts GP (ml gas/g OM) at a given time t (h). Parameter A (ml gas/g OM) is the asymptotic GP, parameter B (h) is the time at which half of the (A) GP has been produced and parameter C determines the sharpness of the switching curve and is dimensionless, see figure 3.

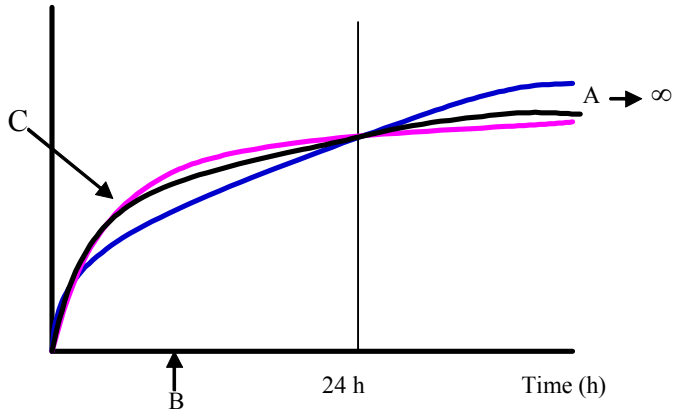

Figure 5. Parameters that describes the cumulative gas production (GP) data.

Pair wise comparisons of means were done by one-way analyses of variance utilising Tukey's test. Pearson's correlation was used to calculate correlations between feed characteristics between each pair of variables. Simple and multiple linear regressions evaluated the relationships between SDMI and other feed parameters. The Stepwise routine was used when the multiple relationships were selected (MINITAB TM ver. 13.2). Model evaluation was done by using the R^2 value, the fitted standard deviation (S.D.) and by examining residual plots for normality.

Results

Degradation characteristics and gas production data

Degradation characteristics, OMD and NDFD, and GP data are presented in table 3. Both OMD and NDFD were lower for silages incubated in 24 h, than for silages incubated in 72 h. Standard deviation (S.D.) of the means were higher for samples incubated for 24 h compared to 72 h and S.D. within samples was lower for samples incubated for 24 h compared to 72 h incubation.

The same pattern, with lower values for the 24 h incubation compared to the 72 h incubation, could be seen concerning the mean values for GP. Also the mean values for S.D. were higher for the 24 h incubation, both between and within samples.

The results for asymptotic GP (A) between 24 h and 72 h incubation were comparable, but the 24 h incubation had higher S.D. The time when half of the gas is produced (B) was lower in the 24 h incubation, compared to the 72 h incubation. Switching characters of the curves (C), meaning the shape of GP curve, between 24 h and 72 h incubation were comparable. Also the S.D. between incubations for both B and C were comparable. Coefficient of determination (R^2) between 24 and 72 h incubation were also comparable, both with high values and low S.D.

The relationship between NDFD and OMD estimated at different intervals of incubation, 24 h and 72 h are presented in figure 1. The results show that there were a good relationship between NDFD and OMD measured at 24 h and 72 h of incubation.

Table 3. *Comparison of in vitro degradation characteristics of silages with 72 h, respectively 24 h, incubation time.*

Silage	OMD				NDFD				GP				A		B		C		R^2	
	Mean 72 h	S.D. 72 h	Mean 24 h	S.D. 24 h	Mean 72 h	S.D. 72 h	Mean 24 h	S.D. 24 h	Mean 72 h	S.D. 72 h	Mean 24 h	S.D 24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h	24 h
1	873.9	5.02	829.6	7.01	829.9	6.77	770.2	9.45	248.15	14.41	185.12	10.19	255.9	206.5	10.58	8.66	1.78	2.10	0.97	0.99
2	857.3	4.13	808.5	3.28	796.1	5.90	726.4	4.68	229.50	4.12	176.80	8.53	245.5	213.0	10.98	8.68	1.74	2.01	0.98	0.99
3	884.4	8.17	827.8	2.63	827.4	12.20	742.9	3.92	234.19	7.71	190.67	20.68	255.7	222.6	10.99	8.92	2.00	1.98	0.96	0.96
4	861.9	11.58	831.2	6.14	798.5	5.65	753.8	8.96	240.03	17.82	194.83	18.54	258.5	226.3	10.71	8.66	1.52	1.84	0.94	0.96
5	864.2	11.31	802.0	2.93	799.5	16.70	707.7	4.33	234.07	25.53	183.99	16.25	253.2	215.3	10.52	8.94	1.75	1.84	0.99	0.97
6	873.8	3.04	845.2	3.08	789.9	5.06	742.1	5.12	228.21	19.43	155.74	24.07	237.1	212.3	9.90	7.65	1.56	1.87	0.94	0.95
7	863.1	1.74	800.2	1.38	772.5	2.61	696.8	2.09	237.17	5.95	192.60	34.39	241.8	231.0	9.93	9.86	2.25	1.84	0.99	0.90
8	908.9	4.15	848.2	2.69	841.9	6.27	769.7	4.08	237.75	14.90	189.40	36.48	242.0	207.9	9.87	8.12	2.35	2.26	0.97	0.91
9	861.8	3.93	796.2	3.04	770.1	6.01	690.9	4.62	213.35	16.00	180.67	36.62	218.3	209.8	9.71	8.43	2.13	1.90	0.97	0.89
10	919.4	5.44	866.9	3.63	854.1	8.52	792.8	5.65	234.88	15.37	227.38	29.77	248.2	261.3	9.76	8.89	2.18	1.98	0.97	0.94
11	894.0	5.19	827.7	2.77	819.1	7.72	739.3	4.19	212.27	23.80	203.07	19.86	216.6	242.3	10.00	9.13	2.28	1.86	0.92	097
12	868.4	5.93	791.3	1.12	778.8	10.30	681.0	1.71	240.62	17.0	208.40	46.49	246.8	253.5	9.45	8.63	2.08	1.56	0.96	0.84
13	891.8	4.11	823.5	1.62	789.7	6.50	694.5	2.80	221.62	19.3	207.36	22.83	225.0	262.3	9.90	9.74	2.31	1.58	0.95	0.95
14	877.0	2.59	812.5	2.94	784.4	16.63	702.9	4.66	234.04	34.4	213.34	36.02	259.0	271.4	10.00	9.92	2.12	1.59	0.99	0.91
15	846.0	3.20	767.3	3.71	730.6	12.92	625.4	5.98	251.43	11.8	193.47	25.38	260.7	247.4	10.51	9.81	1.92	1.51	0.98	0.93
Mean	876.4^{b}	5.30	818.5^{a}	3.20	798.8^{b}	8.65	722.4^{a}	4.82	233.15^{b}	16.51	193.52^{a}	25.7	244.3^{a}	232.2^{a}	10.19^{b}	8.94^{a}	2.00^{a}	1.85^{a}	0.96^{a}	0.94^{a}
S.D.	20.1		25.4		31.7		42.9		11.0		17.1		14.5	22.4	0.48	0.66	0.27	0.21	0.02	0.04

OMD = organic matter degradability (g/kg OM), NDFD = neutral detergent fibre degradability (g/kg NDF), GP = gas production (ml gas/g OM), A = asymptotic gas production (ml gas/g OM), B = time when half of the gas is produced (h), C = switching character of the curve (dimensionless), R^2 = coefficient of determination. Means with the same units and superscript do not differ significantly ($p>0.05$)

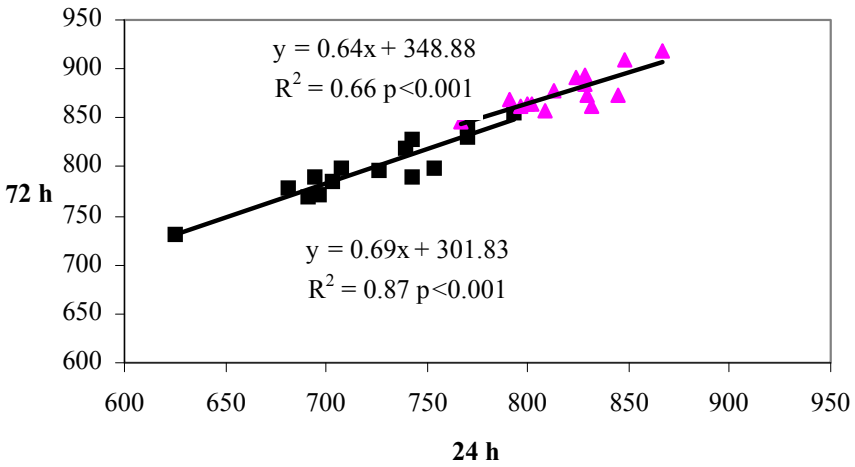

Figure 6. The relationship of neutral detergent fibre degradability (NDFD) (■) and organic matter degradability (OMD) (▲) determined at different intervals (24 h and 72 h).

Correlations and relationships between feed characteristics

There were significant correlations between SDMI, DM content, pH, crude protein, NDF, lignin, lactic acid, ethanol, ammonia-N, cut and degradation characteristics of the silages for both 24 h and 72 h incubation (see table 4). The number of significant correlations between different parameters was higher for the 24 h incubations compared to the 72 h.

Parameters correlated with silage intake

Both degradation characteristics and GP data were correlated to SDMI, with 5 and 2 parameters correlated to SDMI after the 24 h and 72 h incubation, respectively. The correlations were higher for the 24 h incubation compared to 72 h incubation. In both incubations SDMI was positively correlated to NDFD and positively respective negatively correlated to parameter B. In the 24 h incubation, SDMI also had positive correlations with OMD and parameter A, and negative correlations with parameter C.

Table 4. *Correlations between silage dry matter intake (SDMI), dry matter (DM) content, pH, crude protein (CP), neutral detergent fibre (NDF), lignin,, water soluble carbohydrates (WSC), lactic acid (LA), acetic acid (AA), ethanol, ammonia-N, harvest number and degradation characteristic of the silages.*

	72 h incubation					24 h incubation				
	OMD[a]	NDFD[b]	A[c]	B[d]	C[e]	OMD[a]	NDFD[b]	A[c]	B[d]	C[e]
SDMI	0.19	**0.65**	0.30	0.38	**−0.53**	**0.57**	**0.76**	**−0.60**	**−0.72**	**0.75**
DM	−0.17	0.07	0.25	**0.52**	−0.33	−0.06	0.03	−0.14	−0.01	0.01
pH	0.06	0.32	0.10	**0.50**	−0.03	0.13	0.29	−0.25	0.05	0.39
CP	**0.65**	0.43	**−0.51**	-0.11	0.48	**0.53**	0.37	0.01	0.02	0.38
NDF[f]	−0.16	0.34	0.34	0.43	−0.21	−0.01	0.36	−0.49	−0.17	**0.56**
Lignin	0.06	0.21	0.02	−0.15	−0.31	0.28	0.30	**−0.51**	**−0.53**	0.37
WSC[g]	−0.28	−0.21	0.39	0.26	−0.21	−0.28	−0.26	0.20	0.17	−0.35
Lactic acid	0.01	−0.15	−0.35	**−0.64**	0.26	0.04	−0.10	0.15	−0.06	−0.14
Acetic acid	0.06	−0.15	−0.17	−0.41	0.01	0.14	0.00	0.18	0.02	−0.17
Ethanol	0.01	0.42	0.31	0.40	**−0.57**	**0.52**	**0.66**	**−0.53**	−0.46	**0.60**
Ammonia-N	0.05	−0.35	−0.34	−0.49	**0.70**	−0.40	−0.48	0.37	0.43	−0.27
Cut[h]	−0.36	**−0.53**	0.29	−0.09	−0.37	−0.31	−0.48	0.39	0.17	**−0.75**
OMD[a]		**0.81**	−0.26	−0.37	**0.55**		0.92	−0.08	−0.46	**0.61**
NDFD[b]			−0.01	0.06	0.15			−0.30	**−0.52**	**0.79**
A[c]				**0.52**	−0.47				**0.67**	**−0.72**
B[d]					**−0.58**					**−0.60**

Numbers that are bold are statistically significant (P<0.05).

[a] Organic matter degradability

[b] Neutral detergent fibre degradability

[c] Asymptotic gas production

[d] Time when half of the gas is produced

[e] Switching character of the curve

[f] Neutral detergent fibre

[g] Water-soluble carbohydrates

[h] Primary or regrowth (1 or 2)

Parameters correlated with chemical composition and cut

CP was positively correlated with OMD in both incubations and higher correlation was seen in the 72 h incubation. NDF was positively and lignin was negatively correlated with parameter C and parameter B, respectively, in the 24 h incubation, but no significant correlations was found in the 72 h incubation. LA was negatively correlated with parameter B in the 72 h incubation. No correlation between LA and the other parameters was found in the 24 h incubation. Ethanol was correlated with parameter C in both incubations, negatively in the 72 h incubation and positively in the 24 h incubation. In the 24 h incubation, ethanol was also positively correlated with OMD and NDFD and negatively correlated with parameter A. Ammonia-N was positively correlated with parameter C in the 72 h incubation, but no significant correlation was found between ammonia-N and

parameter C in the 24 h incubation. Time of cutting, primary growth or re-growth, was negatively correlated to both NDFD in the 72 h incubation and to parameter C in the 24 h incubation.

Parameters correlated with degradation characteristics

OMD was positively correlated to NDFD and parameter C in both incubations, but with higher values in the 24 h incubation. NDFD was negatively correlated to parameter B and positively correlated to parameter C in the 24 h incubation. No significant correlations between these factors were found in the 72 h incubation.

Correlations between gas production parameters

Parameter A was positively correlated with parameter B and negatively correlated with parameter C in the 24 h incubation. No correlations between these factors were found in the 72 h incubation. Correlations between parameter B and parameter C had comparable correlations between incubations, with negative correlations in both incubations.

Relationship between silage intake and gas production data

The relationship between SDMI and gas volumes at different intervals during the incubation is presented in figure 7. Strongest connection between silage intake and gas volumes was found after 2 h incubation. The figure indicates a decrease in silage intake after 2 h of incubation.

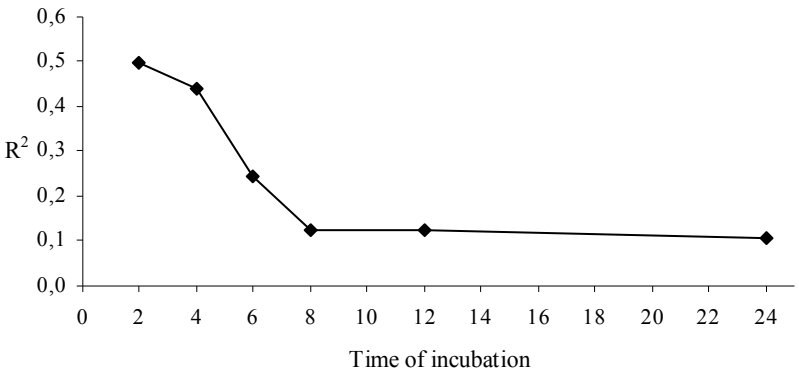

Figure 7. Coefficient of determination (R^2) between silage dry matter intake (SDMI) and gas volumes determined at different intervals (2, 4, 6, 6, 12, 24 h).

Regression models for prediction of silage intake

Two regression models for prediction of SDMI were obtained from the 24 h incubation, while the 72 h incubation yielded in one regression model, see table 5. For the 72 h incubation, a combination of NDFD and parameter C were related to silage intake (R^2=0.82). NDFD and parameter A as a combination (R^2=0.74), and parameter C and ammonia-N as a combination (R^2=0.79), were included in two

separate regression models for the 24 h incubation. The coefficient of determination, R^2, was comparable between models. Values for S.D. were low and comparable between models.

Table 5. *Multiple regression models for predicting silage intake from feed parameters (kg DM/100 kg live weight/day) from 72 h and 24 h incubation.*

Parameter	72 h			24 h			24 h		
	Coef.[a]	S.E.	P	Coef.[a]	S.E.	P	Coef.[a]	S.E.	P
NDFD[b]	0.0029	0.038	0.00	0.0019	0.0004	0.00			
A[c]				-0.0023	0.0019	0.0200			
C[d]	-0.266	0.055	0.00				0.360	0.080	0.00
NH_4-N[e]							-0.0034	0.001	0.00
Intercept	0.07	0.38	0.86	1.07	0.43	0.03	1.49	0.18	0.00
R^2	0.82			0.74			0.79		
S.D.[f]	0.06			0.07			0.06		

[a] Coefficient
[b] Neutral detergent fibre degradability (g/kg NDF)
[c] Asymptotic gas production (ml gas/g OM)
[d] Switching character of the curve
[e] Ammonia-N
[f] Fitted standard deviation

Discussion

Degradation characteristics and gas production data

Comparing the results from the 24 h incubation with the results from Hetta et al. (2007), the same pattern regarding both degradation characteristics and GP data was seen, see table 3 and figure 8, This indicates that a shorter incubation time can give a good picture of both degradation characteristics and GP. A shorter incubation time will also increase the analytical capacity of the GP technique as more analyses can be done per week.

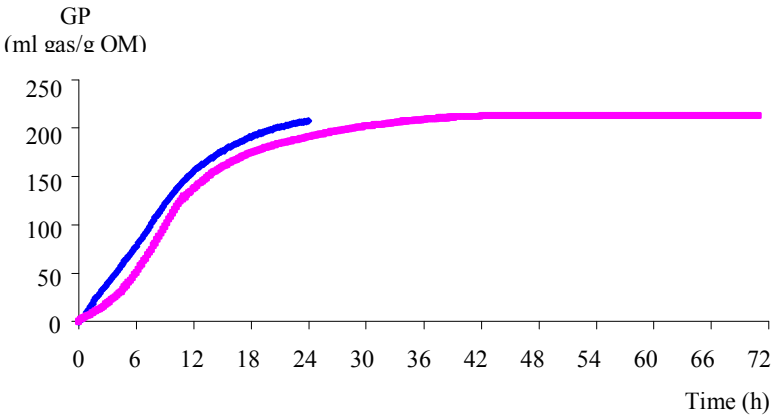

Figure 8. Gas production (GP) curves, 24 h (blue) and 72 h (pink) incubation on the mean GP values for one feed sample.

The GP curves also show that it is more likely to find differences between feed samples at early stages of incubation. With longer incubation times, the fermentation between the different feed samples tend to catch up on each other, which will lead to small differences between the results for the different feed samples. Several researchers support the fact that it is in the beginning of the incubation that most of the information is revealed (e.g. Blummel et al. 1997; Rodriguez et al. 2002). The GP technique can also be a useful tool in plant breeding, especially when different plant species are compared to each other. As there is an increased interest in developing plants with higher digestibility, it is important to know that this technique can be used to provide important information concerning the degradation characteristics of plants. With shorter incubation time, e.g. 24 h, clearer comparisons between different plants can be made and the differences between plants will be obvious.

High S.D values between feed samples is neither strange nor bad, it just indicates that the analyses have been conducted on different feed samples. S.D values within samples in the 24 h incubation were lower than in the 72 h incubation, indicating lower variation within the runs between all the feed samples. This can be an effect from shorter incubation time, but also the fact that the conditions for doing this

study were better than for the 72 h incubation. In the study by Hetta et al. (2007) the experiments were done on two different laboratories in two different countries (Sweden and the Netherlands), which can have had an impact on the variation of the results.

There was also a clear relationship between NDFD and OMD measured at 24 h and 72 h incubation, with high values on the coefficient of determination (R^2). Especially for NDFD there is a clear relationship, with R^2 being 0.87, between 24 h and 72 h incubation. These results also support the fact that a shorter incubation time will give results that are trustworthy, and it also strengthens the fact that shorter incubation time can provide results that are reliable and comparable with results from studies with longer incubation time.

Correlations between feed characteristics

More and clearer correlations were found in the 24 h incubation, which indicates that the 24 h incubation yields in more information compared to the 72 h incubation. Table 4 also shows that GP data can be used for detecting silage quality, as there were high correlations between several parameters connected to silage quality. Information about silage quality is interesting and of very big importance for high producing animals, as it will affect the intake of silages and thereby also the animal's production.

Correlations between feed characteristics that are seen between different parameters in table 4 can be explained from a biochemical point of view (McDonald et al., 1991), except for the parameters that were correlated to ethanol. As the feed samples that have been analysed in this experiment had low concentrations of ethanol, it is not likely that it will have an influence on both degradation characteristics and GP data, as these results suggests. The negative correlations between time of cutting (primary growth and re-growth) and NDFD (72 h incubation) and parameter C (24 h incubation), respectively, show that there is an effect of harvest number on the quality of fibre. This can be explained by the fact that timothy produces more leafy herbage, with less fibre during re-growth compared to primary growth (Fagerberg, 1988).

The relationship between gas production data and silage intake

As figure 1 showed, GP had the highest relationship with silage intake already at the 2nd hour of incubation, with a decrease in relation after this. GP measurements have shown to be a useful and informative tool for prediction of silage intake, and several researchers have also shown that there are significant correlations between *in vitro* GP and DMI (e.g. Blummel & Becker, 1997; Hetta et al., 2007). It seems that most of the results are revealed in the beginning of the incubation and this is also supported by other researchers (Van Soest et al., 1978, Blummel et al., 1997). Rodriguez et al. (2002) found that the first 12 h of fermentation were the most important hours for prediction of forage intake and also Blummel et al. (1997) concluded that it was in the beginning of the incubation that most information

regarding the relationship between GP and feed intake was revealed. Blummel et al. (1997) has also suggested that a combination of gas volume measurements (4-8 h) and the amount of substrate degraded (>24 h) is superior to the models based on only GP kinetics. With all these results it seems obvious that an incubation time of 72 h up to 96 h is not necessary in GP studies. As the GP data gives information about the relationship of forages to silage intake at the beginning of incubation, there is no need for that long incubation times. This will only decrease the analytical capacity of the GP technique.

Regression models for prediction of silage intake

The results presented indicate that the combination of chemical characteristics and GP data gives the best information for the prediction of silage intake. The models were improved when both type of characteristics were included; one factor does not exclude the other. Values on the coefficient of determination were high and S.D. values were very low. Also here, it is obvious that 24 h incubation is enough for studies on high quality forages as the results were comparable with the results from 72 h incubation.

Conclusion

There is a great potential for improving the analytical capacity of the technique by reducing the length of incubation from 72 h to 24 h for studies on high quality forages. Shorter incubation time will lead to an improved analytical capacity as more experiments can be conducted per time unit (week) in the laboratory, making the technique less time consuming.

This study has shown that 24 h incubation gives informative results with high reproducibility of the measurements, clear relationships and high correlations between different parameters, and also reliable models with high values on the coefficient of determination. All these results support the fact that shorter incubation time is possible and that it in many aspects will be favorable in GP studies.

Further experimental work is required, with wider spectra on forages where different types of high quality forages are analyzed. The term “high quality forage” should also be declared, as the degradability of forages will have an impact on the incubation time. This is an important aspect that should be considered and defined. It is known that forages with low degradability require longer incubation times, compared to forage of high quality. The quality of the forage will decide how long the appropriate incubation time is, as it will differ between different forages and forage qualities.

It would be interesting to make incubations on different feed samples in the same run, with different incubation times on each batch to get a more exact comparison on how the results are affected by incubation time. It would also be interesting to study how different types of forages, with both low and high degradability, respectively, would react on this. This could be a way to define high quality forages, and what forages that will fit in this term.

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