



Comparison of the rumen passage rates of different forages using rare-earth markers

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**Institutionen för husdjurens
utfodring och vård**

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**Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management**

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Abstract

The passage rate of nine different forages (diploid perennial ryegrass, tetraploid perennial ryegrass, Italian ryegrass, hybrid ryegrass, meadow fescue, tall fescue, timothy, cocksfoot and white clover) was evaluated with help of two markers (Yb and Dy) in a small-scale study at IGER, Aberystwyth, UK. Five dry Holstein-Friesian cows were used in a 4-period incomplete changeover design experiment. Cows received a mix of 30 kg maize silage (30% DM), 1 kg barley straw, 1 kg soyabean meal and dry cow mineral. At the beginning of each measurement period the cows were offered small amounts of two marker-labelled feeds which were randomly assigned to them, one Yb-labelled and one Dy-labelled. Grab samples of faeces were taken twice-daily over the next nine days. Faeces were dried, ground and analysed for concentration of markers. The concentrations were plotted against time and an equation was fitted and analysed statistically. There were no significant differences between passage rate of the different forages or markers. The highest passage rates were for diploid perennial ryegrass (Dy-labelled) and white clover (Yb-labelled).

Sammanfattning

Passagehastigheten av nio olika foder (diploidt engelskt rajgräs, tetraploidt engelsktrajgräs, Italienskt rajgräs, hybridrajgräs, ängssvingel, rörsvingel, timotej, hundäxing och vitklöver) undersöktes med hjälp av två markörer (Yb och Dy) i en liten studie. Fem Holstein -sinkor användes i ett ofullständigt 4-periods försök på IGER, Aberystwyth, UK. Korna fick en blandning av 30 kg majsensilage (ts ca 30%), 1 kg havrehalm, 1 kg sojamjöl och sinkomineral. På den första morgonen i varje försöksperiod fick korna en mindre mängd av de två markörmärkta foder som var lottade till dem, ett Yb-märkt och ett Dy-märkt. Gödselprover togs två gånger per dag under de nästkommande nio dagarna. Gödseln torkades, maldes och analyserades för innehållet av markörer. Koncentrationen av markörer plottades mot tid och ekvationen från regressionslinjen användes för att beräkna om det var skillnad i passagehastighet mellan foder. Det var ingen signifikant skillnad i passagehastighet mellan olika foder eller markörer. Högst beräknade passagehastighet hade diploidt engelskt rajgräs (Dy-markör) och vitklöver (Yb-markör).

Abbreviation key: Diploid perennial ryegrass =DPRG, Tetraploid perennial ryegrass =TPRG, Italian ryegrass=IRG, Hybrid ryegrass=HRG, Meadow fescue=MF, Tall fescue=TF, Timothy=T, Cocksfoot=C, White clover=WC, Dysprosium=Dy, Ytterbium=Yb.

Literature review

Introduction

Dairy production, and other animal management systems, cause unavoidable losses to the environment; these include CO₂, CH₄ and NH₃. These emissions may contribute considerably to environmental problems, particularly in areas which have a high concentration of intensive animal systems. Nutritional management of farm animals has, until recently, been used as a tool to maximise the output of products like milk and meat. This has been done with little or no attention to and urinary excretion of N and P, or the losses of CO₂ and CH₄ (Tamminga, 1992). Farmers of today have political as well as public pressure to reduce nitrogen loss to the surroundings. At the same time, the costs of protein supplements and nitrogen fertiliser have been rather low. However, recent trends have included an interest in self-sufficiency from home-grown forages and concentrate feeds (Dewhurst *et al.*, 2003). By mixing grass or grass-based products with forages or concentrates with low N content an important reduction in N intake and losses can be realised (Tamminga, 1992). Any improvements in the utilisation of forages will be favourable, both for the soil, which will have to cope with fewer leaking nutrients, and for the farmer, who can be more independent of world trade (Dewhurst *et al.*, 2003).

The utilisation of N by dairy cows can be improved by increasing milk protein output. This can be achieved either by an increased milk protein content or an increased milk production. Unfortunately, it is rather difficult to manipulate milk protein percentage by nutrition management and effects are only small. One factor that reduces milk protein percentages is underfeeding. If negative energy balance occurs, the dairy cow reduces both the milk yield and the milk protein content. Energy intake is an important determinant of milk protein content as well as milk production. Conversely, there are no positive effects of increasing the dietary protein content on milk protein content, but there are positive effects on the milk yield. Another factor is when non-structural carbohydrates are replaced with structural carbohydrates, for example when concentrates are replaced with forage. This occasionally leads to reduced milk protein content (Tamminga, 1992).

The protein in ruminant feeds is considered to consist of an undegradable and a degradable part. The extent of degradation of protein in the rumen may be influenced by factors such as stage of maturity, forage species and preservation method (Hoffman *et al.*, 1993). Some forage protein is degraded to a large degree in the rumen and ruminants fed fresh forage have responded positively to supplementation with slowly degraded protein. Other forage proteins are more resistant to ruminal degradation. Legumes, for example, are known to have a higher quantity of protein and also some of these are more or less undegradable in the presence of for example tannins, and might therefore be more favourable for the ruminant. Legumes also contain more total nitrogen and less NDF (Brown & Pitman, 1991). It is well established that animals have a higher intake of legumes than of grasses, due to legumes lower cell wall contents and maybe also as a result of the high content of nitrogen (Hoffman *et al.*, 1993; Dewhurst *et al.*, 2003). Legume silage has a big potential for high intake and milk production. For example Auld *et al.* (1999) and Dewhurst *et al.* (2003) have shown that white clover silage have a high feeding value for dairy cows, with high rates of passage and fermentation in the rumen and high levels of voluntary intake. Thomson *et al.* (1985) found, when dairy

cows grazing either ryegrass or white clover, that cows grazing white clover had a higher milk production.

The use of legumes in ruminant production has generally been declining due to the low cost of N fertiliser and difficulties of ensiling legumes. However, there is now growing interest in organic and low-input production systems and expanding use of legumes (Dewhurst *et al.*, 2003). Dewhurst *et al.* (2003) have found that white clover silage led to significantly higher yields of milk, milk fat, milk protein and milk lactose and also larger silage meals compared with red clover, alfalfa and ryegrass. Milk from these cows had a lower fat per cent and a considerably higher protein percent. Intake and urinary nitrogen were highest for cows offered white clover silage but the efficiency could not have been as low as expected because of the high yield of milk protein. The high nitrogen utilisation might be correlated to rapid rate of fermentation in, and the high rate of passage from, the rumen. White clover silage seems superior to give milk yield, in comparison with other legumes and grass silages. However white clover is probably not a viable monoculture because of its low yields.

The proportion of a given pool of digesta that passes a point along the gastro-intestinal tract in a given time or leaves a particular pool, such as the reticulo-rumen, is described as the rate of passage (Kotb & Luckey, 1972). Different feeds have different passage rate and for the understanding of aspects of digestion it is important to know these passage rates (Owens & Hanson, 1992). Using labelled feed can be used do this. Passage rate of digesta affects, for example, degradability of the feed (Robinson *et al.*, 1987) and microbial efficiency (Sniffen & Robinson, 1987). As early as 1979, Ørskov & McDonald showed that with higher the outflow rates, less is degraded. Results from Dewhurst *et al.* (2000) show that in this situation the efficiency of microbes increases. There are also a lot of factors that affect passage rate, such as surrounding temperature and feeding level (Miaron & Christopherson, 1992).

Little work has been done on differences in passage rates between different grass species. In this work I review effects of passage rates and identify other factors that influence passage rates. The aim of the present work was to investigate if there are different passage rates of dissimilar crops, and with the results from the experiment try to fill this gap of information between different passage rates and grasses. This study was a pilot experiment in a bigger project, which will consider passage rate and if it is correlated to nitrogen utilisation in dairy cows.

Estimating rumen passage rate

Knowing the passage rates of feeds from the rumen is important for understanding different aspects of digestion, for example a correct estimation of protein utilisation and of digesta flow. Passage rates through the gastro-intestinal tract depends on a lot of factors. The larger a particle is, the longer it takes to pass through the rumen, and fluids are known to have the highest passage rates. When the dietary protein or feed intakes increase all passage rates tends to increase. Also in the later stages of gestation the dilution rates increase. The disappearance from the digestive tract occurs because of digestion or outflow. Depending of the particle's rate of passage or its rate of digestion is the disappearing different between particles (Owens & Hanson, 1992).

Methods for measuring passage rates

Digesta flow can be measured without markers. As an example can faecal output be measured with faecal bags and the digestive pool size can be calculated after slaughter or by evacuation through cannula. All these measurements can often be simplified by using markers. Markers are less invasive to the animal and the animal can behave quite normally (Owens & Hanson, 1992). A number of different markers, for example stained particles and rare earth elements, have been used in investigations of digesta flow (Huhtanen & Kukkonen, 1994). In order to make good measurements on the passage rate through the gastro-intestinal tract some particles or liquid have to be marked (Udén *et al.*, 1980). The ruminal digesta consists of a number of interacting fractions, for example free fluid, small dense and raft particles and large dense and raft particles. Microbes can move between these different pools and the marker may or may not do so. To label and follow each pool is therefore a quite complex thing to do. A marker which, is suitable for one pool, may not be for another (Owens & Hanson, 1992). A good marker is one that doesn't separate from the labelled fractions (Udén *et al.*, 1980). An ideal marker must not be absorbed and be physically similar to or intimately associated with the labelled material. The digestive tract or its microbes must not be affected or affect the marker. Good markers have a specific and sensitive method of estimation (Owens & Hanson, 1992). By labelling the feed with a marker and collecting faeces a period after feeding, the passage rate can be measured. Faeces is analysed for markers and the natural logarithm of marker concentration in faeces is plotted against time of sampling. The descending portion of the curve is used for regression analysis and the regression line represents the passage rate described by Elimam & Ørskov (1984) based on the work of Grovum & Williams (1973).

Different models for estimating passage rates

Grovum & Williams (1973) described the passage rate of markers of fluid and particulate digesta through the sheep gastro-intestinal tract by linking experiments in which marker concentrations were analysed in faeces after the marker was given as a pulse oral dose to mathematical models. Their models include the following descriptions of passage rates: transit time, the time it takes digesta to pass through a section of the gastro-intestinal tract, half-time of marker in the reticulo-rumen and half-time of markers in the whole digestive tract.

Pond *et al.* (1988) as well as Grovum & Williams (1973) have reported that passage rate of the rumen could be correctly predicted from faecal sampling whereas others (Goetsch & Owens, 1985) have questioned the strength of this method. However, it is almost impossible

to take a representative sample from the rumen without disturbing the rumen environment. Faecal sampling is a less invasive method than rumen or duodenal sampling and permits a larger number of animals, high levels of production or maximal intakes (Poore *et al.*, 1991).

Because outflow rates from the rumen are faster than from caecum the rate constants for the respective compartments can be achieved by the constants of the two outflow rates are obtained for both a solute marker and a particulate marker (Dhanoa *et al.*, 1985). The model described by Dhanoa *et al.* (1985) handled two rate constants and was superior to other models that did not take two constants in account. It is discussed if Grovum & Williams (1973) model of describing the changes in marker concentration in sheep faeces following an intraruminal dose of marker who reflects the cumulative effects of marker residence time in the various sections of the digestive tract really fit faecal marker excretion curves. Dhanoa *et al.* (1985) showed with an alternative model, multicompartmental model, which described digesta flow along the gastro-intestinal tract of ruminants that Grovum & Williams (1973) was generally similar to this model.

When comparing two non-linear compartmental models with either no age dependency or with increasing gamma age dependency in the first compartment, Huhtanen & Kukkonen (1995) found that the age dependent model gave an improved fitting at passage curves. Digesta kinetic parameters calculated by linear regression of natural log transformed Yb (Ytterbium) or Cr (Chromium) marker concentrations overestimated the passage rate compared to non-linear models. Since the best fit in a non-linear models depends on the form of the increasing part of the faecal curve it is recommended to run a series of non-linear models (Huhtanen & Kukkonen, 1995).

A lot of factors influence the flow of feed particles from the rumen, for example size and specific gravity of particles. There is also selective retention of digestible fibre. This selection is necessary for the ruminant to maximise the ruminal digestion of digestible fibre. Time dependent models of marker excretion have shown that there are two compartments. It is likely that the first compartment represents the release from the non-escapable to the escapable pool and the second represents passage from the escapable pool (Huhtanen *et al.*, 1995).

Huhtanen *et al.*, (1995) compared methods to estimate NDF digestion and passage kinetics in an experiment with low and high levels of intake. Passage kinetics based on a two-compartmental model showed a higher digestibility than exponential decline in duodenal Yb concentration. This can be caused by that the exponential decline in marker concentrations underestimating the ruminal fibre digestibility. This might result from the fact that this model not takes into account the two ruminal compartments and the selective retention of fibre in the rumen.

Different markers

There are two general types of digesta markers, internal and external markers. Inherent markers are component parts of a feedstuff, for example lignin, whereas external markers are different inert compounds, for example rare earth elements, which have to be added to the feed. To estimate digesta volume or retention time in specific parts of the gut, pulse-dosing is typically used. When using a continuous or frequent marker, the digesta is labelled with the marker uniformly so that the ratio of digesta marker is constant (Owens & Hanson, 1992). One of rare earth elements advantages is that they bind tightly to plant material and might

therefore flow through the gastro-intestinal tract in close association with indigestible feed residues (Kotb & Luckey, 1972). Teeter *et al.* (1984) showed in an experiment that the binding capacity of the rare earth element ytterbium (Yb) varied with the type of particulate matter. Feeds containing high amounts of crude fibre or crude protein bound more Yb. In trials with Dy (dysprosium) and Yb labelled feed, when Co-EDTA (chromium-ethylenediamine tetraacetic acid) was included to evaluate liquid turnover rate, the concentration of rare earths peaked later and declined more slowly than that of Co-EDTA. This is evidence that rare earths remain bound to particulate matter rather than disassociating from the particles and passing with the rumen liquid phase in rumen. It is also found that specific gravity and particle size were similar for the marked particles and the unmarked particles (Poore *et al.*, 1991), which is important physical factors influencing passage of particles from the rumen (Welch, 1986). Teeter *et al.* (1984) showed that at least two types of bonds in binding affinity were present in an experimental investigation of the binding capacity of Yb. Depending on the type of particulate matter the binding capacity of particulate matter for Yb varies. This indicates that there are different functional groups that bind Yb in different feedstuffs, that the molecular environment of functional groups varies with the feed or that some types of particulate matter form multiple bonds with Yb.

Migration and absorption of markers

Migration of marker is of concern because it is the label, not the originally marked component, which will be traced. Loosely bound markers can migrate in the rumen. This is also a problem with rare earths that in excess of binding capacities. Markers that are added to feed may also be subject to microbial modification (e.g. fermentation) (Owens & Hanson, 1992). There is decreased *in vitro* disappearance of dry matter when Yb or Dy is added to feed. This is because the metal complexes are rather stable to microbial digestion because if the metal were released the digestion would proceed normally (Teeter *et al.*, 1984). Some of the rare earth elements tend to migrate from the labelled particles and also be likely to preferentially bind to small rather than to large particles (Huhtanen & Kukkonen, 1994). Compared with three lighter rare earth, samarium, lanthanum and cerium, Yb has a greater binding affinity. This can reduce the extent of migration of the rare earth elements (Teeter *et al.*, 1984). To minimise rare earth migration, weakly bound markers can be removed by an extensive washing (Owens & Hanson, 1992). If the excess not is removed, the residual marker can migrate to other feeds or to other ruminal components and complicate interpretation of marker data (Teeter *et al.*, 1984). A small amount of chelated minerals can be detected in urine but the corrections for absorption are normally low (Owens & Hanson, 1992).

Effects of altering rumen passage rate

Opposite relationships exist between passage rate and degradation. With a higher outflow of small particles from the rumen less nutrients are fully degraded. This means that the animal can eat more, but only as much as there is space for in the rumen, and the utilisation of feed is less. The passage rate also affects the microbial efficiency (Sniffen & Robinson, 1987) and N-use efficiency (Dewhurst *et al.*, 2003).

Degradability

In an experiment by Hunt *et al.* (1988) the effect of alfalfa addition to wheat straw diets on intake and digestion was evaluated. Beef steers fed *ad libitum* or at a restricted level were used to study the factors involved in diets consisting of different amounts of alfalfa and wheat straw. As the amount of alfalfa increased in the diet the dry matter intake increased linearly. With increasing levels of alfalfa the passage rate of wheat straw NDF decreased, but the total dry matter digestibility increased in the group fed *ad libitum*. There were no effects on passage rate or dry matter digestibility in the group fed at a restricted level. When Huhtanen *et al.* (1995) increased the dry matter intake the fibre digestibility decreased. Volden (1999) also reported that a reduced intake decreased the outflow from rumen but increased the digestibility. This is similar to results from Robinson *et al.* (1987) who showed that the rate of digesta flow in ruminants affects degradation. When the passage rate increases the time for digestion in the rumen is decreased, this results in a less efficient degradation. At higher feeding level the rumen outflow increases and this depresses fibre degradation.

Degradation of protein in ruminants is important for both the host animal and for its rumen microbes. Microbes of the rumen need some rumen-degradable protein for maintenance and growth, but the ruminant can make better use of proteins that are resistant to degradation in the rumen but may be available with enzymatic digestion in abomasum or intestine. Bypass protein may decrease the fermentative losses of ammonia and methane, and also the energy costs during digestion of these in rumen (Stern *et al.*, 1994). Ørskow & McDonald (1979) showed that when a restricted level of feeding was given the passage rate decreased and the protein degradability increased.

Microbial efficiency

The rumen flora consists of more than 200 species of micro-organisms (Hungate, 1966), which include bacteria, protozoa and fungi. These have enzymes that can convert fibrous feed and low-quality protein into valuable nutrients for the ruminant animal (Forbes & Barrio, 1992). With an optimal amount of degradable protein and energy, the microbes can be more efficient breaking down the feed and also yield a higher microbial biomass, which is more favourable for the animal (Dewhurst *et al.*, 2000). Microbial proteins provide a high quality supply of amino acids for the ruminant and its growth rates affect the amino acids that are available for the animal. Higher growth rates increase the outflow of microbial protein and more amino acids are available in the intestine (Stern *et al.*, 1994).

Diet composition can have a marked effect on the composition of rumen micro-organisms. Feeding of unsaturated fat can, for example, increase the efficiency of microbial protein by a defaunating action, which kills the protozoa that predate rumen bacteria (Dewhurst *et al.*, 2000). The microbial protein synthesis is dependent on the amount of available substrate and

the energy used for maintenance. This is indirect a function of the maintenance requirement and passage rate (growth rate) (Pirt, 1965) (fig. 1).

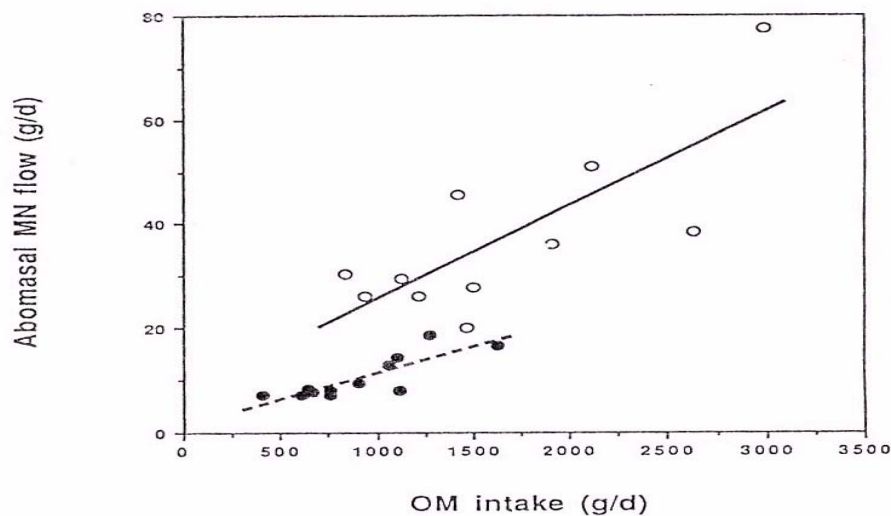


Figure 1. Organic matter (OM) intake and the microbial nitrogen (MN) flow through abomasum of ewes, ○ represent lactating ewes grazing high-quality grass, spring/summer ● represent non-lactating ewes grazing low-quality grass, autumn (Dove & Milne, 1994).

Microbes have higher energy maintenance requirement when they have a slower growth rate, because they need more energy to stay alive than they need for growth. Increased feeding levels often reduce the maintenance costs and increase the efficiency of the rumen microbes (Dewhurst *et al.*, 2000). The increased microbial yield may reflect of higher bacterial nitrogen flow from the rumen. Increased feed intakes give a higher outflow of particles that is in earlier stadium of degradation and this provide more attached microbes to these particles. Rumen liquid and particulate turnover rate are both positively correlated to increased intake and also to a high microbial yield (Sniffen & Robinson, 1987). It can also be a reduced microbial efficiency with a diet with high intake. This occurs for example when the microbes has to keep their cellular pH up when the rumen pH is low, perhaps through feeding high amounts of starchy concentrates (Dewhurst *et al.*, 2000). High forage levels in the diet increase microbial growth. This is because the saliva flow increase and the pH maintain stable. Saliva also increases the liquid outflow and this is suggested to enhance microbial outflow from the rumen. It will also be an improved cation exchange capacity, which leads to higher microbial attachment rate, reduced lag and increases the rate and extent of digestion. Different fibre sources have different cation exchange capacities, for example alfalfa has a high and maize has a low capacity. An enhanced hydration which reducing the lag time, and an improved microbial attachment and mat formation also occurs as a result of high forage intakes. All of these factors lead to an improved retention time and greater microbial growth, as the time of generation of microbes is reduced (Sniffen & Robinson, 1987).

Forage quality has an effect on the yield and efficiency of synthesis of microbial protein. It was a much higher efficiency when ewes grazed high-quality grasses, when comparing high-quality grass, spring/summer, with low-quality grass, autumn, though that the experiments season was confounded with lactating ewes in the spring and non-lactating in the autumn. This may account to a higher content of water-soluble carbohydrates in the high-quality grass,

and it also indicates that the rumen pH was stable (Dove & Milne, 1994). Cutting date and stage of maturity of silage do not seem to have an effect on microbial protein synthesis (Dewhurst *et al.*, 2000).

N-use efficiency

Ruminants offered white clover silage have been shown to increase milk yield or growth. Although nitrogen intake and urinary nitrogen are high for these animals the nitrogen efficiency cannot be low (Dewhurst *et al.*, 2003). By measure N-use efficiency as content of milk protein, when the effect of nitrogen intake level is taken into account, Dewhurst *et al.* (2003) found that white clover silage is superior in N-use efficiency compared to red clover, alfalfa and perennial ryegrass. This might depend on an improved rumen function with high degradability and outflow from the rumen with cows offered white clover. High passage rate from the rumen can increase the N-use efficiency because it leads to reduced rumen degradation of protein and increase microbial efficiency. It can also be a combination with high-energy supply to the mammary gland. Efficiency of use of nitrogen from alfalfa silage is low relative the nitrogen intake. This probably reflects the imbalance between rapidly available nitrogen and indigestible fibre in rumen. It can also be a combination of the imbalance and the lower energy supply from alfalfa.

Factors affecting rumen passage rate

As far as it is known, animals do not have any receptors for energy *per se*, but there are sensors for distension, osmolality, concentrations of solutes and temperature. Intake of forages cannot only be limited by distension, it may also be limited by the osmolality and concentration of short chain fatty acids (SCFA) in the digesta in the reticulum and rumen, called reticulorumen. Even abdominal temperature, uptake of propionic acid by the liver and hormones such as insulin, glucagons, gastrin and cholecystokinin can limit forage intake (Forbes & Barrio, 1992). Particle size and particle specific gravity affect passage from the rumen. Most particles leaving rumen are smaller than 1 mm, although particles of 5 cm may pass through the reticulo-omasal orifice (Welch, 1986). In the rumen exists a floating raft of digesta. This raft is maintained by entrapment of fermentation gases evolved during microbial digestion within the plant particles. The raft probably leads to a degree of entanglement of long particle, especially apply to the rough-surfaced fibres of C4 grasses (tropical grasses) because this plants buoyancy may be enhanced by entrapment of gases around the vascular bundles. Small particles may also be entrapped in the raft and that might affect the passage to intestines (Wilson & Kennedy, 1996). There are also other factors that effect the passage rate, for example, the environmental temperature and intake of feed (Miaron & Christopherson, 1992). Cold environments tend to increase the ruminal outflow (Kennedy *et al.*, 1979). Rumen outflow rates tend to be higher at high levels of feed intake (Allen, 1997).

Buffers and fibre

The pH in the rumen is influenced by many factors, including: metabolic activities of rumen micro-organisms, absorption of VFA, water flux through the rumen wall, saliva flow and its buffering effects, feed acidity and water outflow to the lower digestive tract. A buffer is something that acts against changes in pH when a strong acid or base is added and have its equivalence point (pKa) near the physiological pH of the system that it buffers (Erdman, 1988). The determinant of the rumen pH is the balance between fermentation acid and buffer secretion. It is the fibre fraction in feed that stimulate chewing, that in turn stimulates saliva secretion. Saliva, with its bicarbonate and phosphate buffers, neutralises acid produced by fermentation of organic matters in the rumen. A low pH in the rumen decrease the intake of dry matter caused by inhibition of ruminal micro fauna, fibre digestibility and microbial yield and thus decreases milk production (Allen, 1997). Depending on the diet, cattle can have a wide range of salivary flow (Erdman, 1988).

Cattle that are fed adequate amounts of roughage can produce 100 to 200 litre saliva per day (Froetschel, 1995). Cassida & Stokes (1986) showed that the resting saliva flow rates are higher in cows that produce more milk, maybe due to their increased dry matter intake. They also noticed that cows that were later postpartum, week 4 vs. 8, had a higher salivary flow. It seems like the production level and physiological state is important factors in regulation of salivary flow as also diet composition is.

Fibre from non-forage sources often ferments and passes rapidly from the rumen. As a consequence they are less effective at stimulate chewing (Oba & Allen, 2000a). In an experiment by Oba and Allen (2000a), they found that the interval between rumination was shorter and the chewing time during rumination was faster for high NDF diets, 29 vs. 38% NDF of total dry matter. There are differences in buffering capacity between different crops,

for example legumes tend to have higher buffering capacity than grasses, while cereal grains have a relatively low buffering capacity (Jasaitis *et al.*, 1987).

Results from Okeke *et al.* (1983) shows that the dilution rate of the liquid phase and the passage rate of soybean meal increase linearly with levels of sodium bicarbonate or artificial saliva salts mixture as buffers. This may be one factor that is involved in an enhanced escape of protein from rumen. Results from Thomson *et al.* (1978) showed that the dilution rate increase with higher amounts of mixed buffer salts of artificial saliva fed to sheep. Saliva increases the frequency of reticular contraction (Froetschel, 1995), which may lead to a larger outflow of small particles, less than 1 mm, from the rumen. Larger amounts of buffer have also a tendency to increase dry matter intake (Woodford & Murphy, 1988).

Temperature

The environmental temperature has a significant influence on the animals, causing them to compensate by voluntary feed intake, metabolism and heat dissipation to keep the body temperature constant (Miaron & Christopherson, 1992). When the feed is metabolized, there is also a heat production. This heat disappears through the animal to the environment and the animal has to balance the loss of heat for remain the body temperature stable (Young, 1981).

If animals are kept in cold environment they consume more feed but grow slower or produce less milk. This is because less feed energy is available for productive processes (Young, 1981). It seems like the dry matter digestibility decreases during winter conditions (Christopherson, 1976) and appears to be associated to increased gut motility, passage rate and circulating thyroid hormone. Thyroid hormone is for example interrelated with appetite, food intake and digestive functions (Young, 1981). Kennedy *et al.*, (1976) showed in a trial with sheep that the flow of dry matter and organic matter was greater through the abomasum during the cold exposure, -1 to 1°, than during the warm exposure, 18-21°. They could also see that the digestibility (rumen fermentation) was lower in the cold environment likely because of the increased passage rate. At the same time the efficiency of microbial synthesis increased, caused by an increased passage rate the maintenance cost for the microbes are reduced and efficiency of cell yield was increased.

Heifers exposed to hot environment (temperature-humidity index 94), reduced their dry matter intake and increased the water intake compared to the period under thermal comfort, (temperature-humidity index 64). Rumen passage rates were faster during the period in thermal comfort but the digestibility of dry matter, organic matter, NDF and ADF was lower. There were no significant differences in passage rate or digestibility between short, three days after heifers were placed in hot environment, or long, 24 days after heifers were placed in hot environment, exposure but there was a tendency of decreased dry matter intake and more rapid passage rate for the long exposure (Bernabucci *et al.*, 1999). Also Miaron & Christopherson (1992) found that the rumen outflow rate was lower at higher temperatures, 10 and 28 degrees compared with -10 degrees. As a result of this, there was a prolonged time for fermentation that led to a higher digestibility and the amount of bypass protein from rumen might decrease. If the animal can choose between forage and concentrate, heat-stressed cows reduce fibre intake by reducing forage consumption which is an adaptive response to balance heat production from rumen fermentation (Bernabucci *et al.*, 1999).

Feeding level

The complex set of stomachs in ruminants are well innervated and thought to generate signals which are important in the control of voluntary food intake. The rumen and reticulum have tension receptors in the muscular wall which slowly adapt and provide a measure of distension. Stomach motility and voluntary food intake are controlled by, among other things, epithelial receptors which rapidly adapt and provide information of the digesta's fibrosity. These epithelial receptors are also sensitive for digesta's chemical nature, particularly acidity. The abomasum and duodenum also contain mechano- and chemo receptors and the liver has its own chemo receptors, all is involved in the control of intake (Forbes & Barrio, 1992).

The voluntary dry matter intake of ruminants consuming forages may be limited, as a result of controlled flow of digesta to the gastro-intestinal tract. Intake varies with different filling capacity of forages, which is represented by fibre mass. There is a breakpoint when the digestibility, which has limited the voluntary dry matter intake by physical fill, is replaced by limitation by satisfaction of energy demand (Allen, 1996). Many anatomical features of plants are antipathetic to high nutritive value as represented by high voluntary intake and digestion. Their purpose is for example to give leaves and stems strength and support to prevent breakage. Voluntary feed intake varies with forage type and physiological state of the ruminant. Total fibre content and ease of particle fragmentation of forages are important differences between forages in their influences on voluntary feed intake. It is the interplay between plant properties, activities of rumen microbes and handling and fate of particles for digestion in, and outflow from, rumen that influences forage intake (Wilson & Kennedy, 1996).

The limit point of intake of high-fill diets is generally regarded at the reticulorumen. With an increased dry matter intake the passage rate of small particles from reticulorumen also increases, maybe due to a higher efficiency of outflow per contraction. Small and dense particles do not wash away from the reticulo-omasal orifice when the reticulum contracts as large particles tend to do (Allen, 1996).

Forage quality affects the dilution rate from the rumen. Grasses in an early vegetative stadium have a higher outflow rate, which can be related to a higher intake (Dove & Milne, 1994). Grinding and pelleting of forages, especially of poor quality forages, decrease their particle size and this generally increases the voluntary intake as a result of volume and retention time in the reticulorumen (Woodford & Murphy, 1988) but the rate of degradation decrease when the passage rate increases (Robinson *et al.*, 1987). Okine *et al.* (1993) showed in an experiment with steers, fed hay and an increased amount of barley straw, that the daily intake and flow to duodenum decreased with an increased quantity of straw. This depends probably on a lower digestibility of straw. This is mainly associated with an increased passage rate. This is also reported by Robinson *et al.* (1987), that decreased levels of feed intake affect the rumen turnover by reducing rumen content, which cause a reduction of the rumen dilution.

Increased levels of concentrate decrease the intake of forage and the digestion of NDF. Decreased intake of forage indicates that a reduction in the ruminal rate of digestion occurs, and there is a reduction in the rate of passage of fibre or a change of physical to physiological regulation intake (Stensig & Robinson, 1997). Rumen microbes have a preference for readily fermentable carbohydrates rather than fibre components. Due to this, and that a decrease in ruminal pH occurs when rapid fermentable carbohydrates is degraded, there is a decrease in cellulolytic micro-organisms and a depressed fibre digestion (Allen, 1997).

Differences between grasses

Depending on how the plant is constructed it is more or less easy to digest. Fragmentation of leaves during digestion is controlled largely by epidermal and vascular structures. In many temperate grasses (e.g. ryegrass) the breakage will occur more easily than in tropical grasses (e.g. maize). This is caused by a strong structure, which is more common in tropical grasses, when strand in leaves are linked to the vascular tissue. Legumes do not have this structure at all. Apparently, some rumen bacteria have pectin enzymes that degrade pectin-containing unligified legumes (Wilson & Kennedy, 1996).

Hoffman *et al.* (1993) found that perennial ryegrass had the most extensive rumen degradability of crude protein, when comparing to timothy, orchardgrass, bromegrass and quack grass, cut at three different stages before flowering. This is related to a larger soluble fraction and faster degradation rate of the slow fraction. It also had less NDF and was more ruminally degradable. Quack grass contained less rumen degraded dry matter compared to the other grasses. It also seems like quack grass increased NDF content faster and the fibre was more lignified. Both this factors are negatively correlated with dry matter digestion.

Legumes

Legume NDF tend to contain more lignin than grass NDF, while grasses have a higher ADF and NDF fractions. With an advancing maturity the fibre fractions increase (Hoffman *et al.*, 1993). Ryegrass is known as the most rapidly degraded species of the grasses, but still legumes are more rapidly degraded (Waghorn *et al.*, 1989). Perennial ryegrass and alfalfa have similar nutrient contents but still dairy cows produce more milk when they are fed alfalfa, even when these feeds had similar dry matter contents. Milk protein is also lower for perennial ryegrass silage, when comparing to alfalfa silage. This might be due to a lower intake of crude protein when consuming ryegrass (Hoffman *et al.*, 1998). Results from Hoffman *et al.* (1998) also showed that dry matter intake was lower for cows fed perennial ryegrass silage and so do the passage rates. This resulted in a higher digestibility but a lower milk production. When comparing ryegrass and alfalfa, the latter has a greater particle fragility, which leads to a more rapid breakdown, and reduces the retention time in the reticulorumen. This results in a higher intake. Alfalfa also had higher protein content. Both this properties enables more feed protein to escape to the intestines (Waghorn *et al.*, 1989). A big difference between digestion of grass and legume is that grass does not have any natural discontinuities giving breakage points, and can therefore only achieve length reduction by rumination. For legumes, angular joints appear to be weak points for minor veins to fragment into short fragments. These characteristics can explain why legumes are rapidly broken down in the rumen. Another difference between grass and legume is that the lignin content in legumes is all located in the xylem, where the concentration is such that the wall is completely indigestible. In grasses, the lignin content is spread throughout all tissues except phloem. This gives a lower concentration in any particular wall than for legume xylem. As a consequence, the rate of wall digestion in grasses is slower because more, leaf, or all, stem, walls contain some lignin (Wilson & Kennedy, 1996).

Alfalfa silage tends to have a higher ADF and the NDF content and lower digestibility than red clover silage. This cause a longer retention time in the rumen and a slower outflow, which leads to a lower milk yield or growth for ruminants fed alfalfa (Dewhurst *et al.*, 2003). When comparing red clover and alfalfa, red clover has a smaller soluble crude protein fraction but is still degraded faster in the rumen. One explanation may be that crude protein associated with NDF and NDF degrades relatively slowly (Hoffman *et al.*, 1993).

When comparing white clover silage and a mix of white clover and maize silage, the rumen liquid pH was lower for white clover but still over 5.5. This may be due to a faster fermentation, possibly negatively affecting rumen function, which in turn may have led to slower fluid flow rates (Auld *et al.*, 1999).

Own investigations

Materials and methods

Forages

Nine different forage crops were cut for this work; diploid perennial ryegrass (*Lolium perenne*) (DPRG), tetraploid perennial ryegrass (*Lolium perenne*) (TPRG), Italian ryegrass (*Lolium multiflorum*) (IRG), hybrid ryegrass (*Lolium x boucheanum*) (HRG), Meadow fescue (*Festuca pratensis*) (MF), Tall fescue (*Festuca arundinacea*) (TF), Timothy (*Phleum pratense*) (T), Cocksfoot (*Dactylis glomerata*) (C) and White clover (*Trifolium repens*) (WC). The ryegrasses are all hybrids between different ryegrasses (diploid perennial ryegrass (AberDart, AverGold, AberElan, Fennema, S321, Aubisque, AberAvon, Parcour and S23), tetraploid perennial ryegrass (AgerTorch, Bastion, Merlinda, AberCraigs and Condensa), Italian ryegrass (Bb2408, AberComo, Atalja and Roberta) and hybrid ryegrass (AberStorm, AberLinnet, AberExcel, AberVisit, AberVeil, AberEcho, Polly and Augusta)).

Field management

The different crops were fertilised with NPK 23-4-13 and previous cut the 8th of May 2003. All the hays were cut the 19th of June, except the clover which were cut 10th of July, after six weeks of regrowth. Either was the hays dried immediately at 60°C or air-dried and then dried at 60°C.

Preparing labelled hay

A small amount, 500 g, of each hay was placed in a plastic container and mixed with 10 litres of a solution containing either 25 g of ytterbium acetate (Yb-Ac) or 25 g of dysprosium acetate (Dy-Ac) over night (table 1). The hays were then rinsed with tap water to remove loosely bound marker and dried at 60°C. One treatment, treatment 2, was ground before labelling through a 1 mm screen.

Table 1. The different treatments (diploid perennial ryegrass (DPRG), tetraploid perennial ryegrass (TPRG), Italian ryegrass (IRG), hybrid ryegrass (HRG), Meadow fescue (MF), Tall fescue (TF), Timothy (T), Cocksfoot (C) and White clover (WC)) and their label

Treatment	Forage	Label
1	DPRG	Dy
2	DPRG	Yb
3	TPRG	Dy
4	IRG	Yb
5	HRG	Dy
6	MF	Yb
7	TF	Dy
8	T	Yb
9	C	Dy
10	WC	Yb

Animal measurements

Five dry Holstein-Friesian cows were individually fed through tie-stalls at Trawsgoed Research Farm, IGER, Wales, UK in a four period cyclical incomplete changeover-design. The cows had an initial period of two weeks of adaptation to the maize silage-based diet followed by four 10-day periods. Every cow was offered two different hays that were labelled with either ytterbium or dysprosium (table 2).

Table 2. Labelled feed given to each cow during the different periods (for identification, see table 1)

Period	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5
1	7 + 2	1 + 4	9 + 10	3 + 8	5 + 6
2	5 + 10	9 + 2	7 + 8	1 + 6	3 + 4
3	3 + 4	7 + 6	5 + 2	9 + 10	1 + 8
4	9 + 6	3 + 8	1 + 4	5 + 2	7 + 10

The cows were fed a fixed daily allocation of about 11 kg of DM, two times a day (given half at 0900 hrs and half at 1700 hrs). The daily portion consists of 30 kg maize silage (assumes 30% DM), 1 kg barley straw, 1 kg soyabean meal and dry cow minerals at standard rate. In the beginning of each measurement period the cows were offered small amounts, 60 g, of their allocated hays before their morning feed.

Sampling and chemical analysis

Samples of each hay, bulked samples of maize silage and soyabean meal were taken for chemical analysis during the experiment. Faeces were collected twice a day over a nine days long period after that the experimental period had started. The faeces were then low temperature dried, ground and submitted for analysis of Yb or Dy. This analyse was done by inductively coupled plasma emission spectroscopy, using a Liberty Series 2 Axial Spectrometer made by Varian. After the analyses, results were plotted with marker concentration against sampling times. The points after the peak on the descending straight line were used for regression analysis, as described by Grovum & Williams (1973).

Statistical analysis

All statistics were made in the SAS system for Windows (SAS 6.12, TS level 020, SAS Institute, Cary, NC, USA). Data were either analysed by the mixed model procedure (Littell *et al.*, 1996) or the general linear model in SAS. For both analyses the following SAS-model was used:

$$Y = \mu + a + c + p + e$$

Where Y= dependent variable; μ = overall mean; a= fixed effect of treatment; c= fixed effect of time; p = fixed effect of period; e = random residual error. The dependent variables used are shown in table 3. Results for the two markers (Yb and Dy) were analysed separately.

Results

A simple method was used to estimate rumen passage rates from the marker excretion curves- the linear descending portion of curves was identified by eye from plots of log marker concentrations against time, these points were then used in a linear regression analysis and the slope of these lines taken to be the rate of passage from the main pool in the animal (i.e. the rumen).

The goodness of fit of linear regressions lines is indicated by R^2 values. R^2 - values for individual experimental units are given in appendix 1 (table 1), all values were greater than 0,73- indicating a good level of fit. The goodness of fit can also be seen in the figures below.

LS Means for different treatments, concentrations of marker (Yb or Dy) and NDF are given in table 3.

Table 3. Used label (Yb or Dy), passage rate LS Means, marker and NDF concentrations in the different forages

Forages	Label	LS Means (% per hour)	Marker conc. (mg/100g DM)	NDF (g/kg DM)
DPRG	Dy	2,329	1071	554
DPRG-ground	Yb	1,931	2189	555
TPRG	Dy	2,372	736	547
IRG	Yb	1,852	726	570
HRG	Dy	2,091	759	574
MF	Yb	1,850	763	601
TF	Dy	2,244	472	592
T	Yb	1,782	617	645
C	Dy	2,453	562	642
WC	Yb	2,093	858	394

The results of analysis of variance for estimated rumen passage rates are shown in the following two tables. Cows and periods did not affect the results. Within groups of forages that were labelled with the same rare earth, there were no significant differences in estimated rumen passage rates. Estimated rumen passage rates tended to be higher for forages labelled with Dy.

Thought there were no statistically significant differences at the 5% confidence interval, C had the highest passage rate followed by TPRG and DPRG of the Dy-labelled grasses. Of the Yb-labelled had WC the highest passage rate followed by MF and IRG. Of the ryegrasses, TPRG had the most rapid passage rate and HRG the slowest (table 4 and 5). The cows different passage rates are shown in table 6.

Table 4. Estimated rumen passage rates (% per hour) of Dy-labelled forages

Forage:					SEM	Sig.
DPRG	TPRG	HRG	TF	C		
2,329	2,372	2,091	2,239	2,454	0,272	Not significant

Table 5. Estimated rumen passage rates (% per hour) of Yb-labelled forages

Forage:					SEM	Sig.
DPRG ground	IRG	MF	T	WC		
1,932	1,852	1,850	1,782	2,093	0.139	Not significant

The variation in per cent of LS Means was for Dy-labelled forages around 12 and for Yb-labelled forages around 7, which is a normal variation in a biological material.

Table 6. Passage rates (% per hour) for the cows on the different forages

Forages	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5
DPRG	-	2,73	1,75	2,33	2,15
DPRG-ground	1,75	2,17	1,49	2,43	-
TPRG	3,67	2,61	-	1,52	2,14
IRG	1,81	1,93	1,54	-	1,80
HRG	1,77	-	1,74	2,53	2,13
MF	1,88	1,65	-	2,27	1,71
TF	2,54	2,17	1,78	-	2,55
T	-	1,87	1,66	2,14	1,61
C	2,60	2,66	2,04	2,53	-
WC	1,67	-	2,52	2,13	2,00

The passage rates for the different forages and cows are presented in figure 2-11 and the equation of the regression lines are presented in appendix 1, table 2a and 2b.

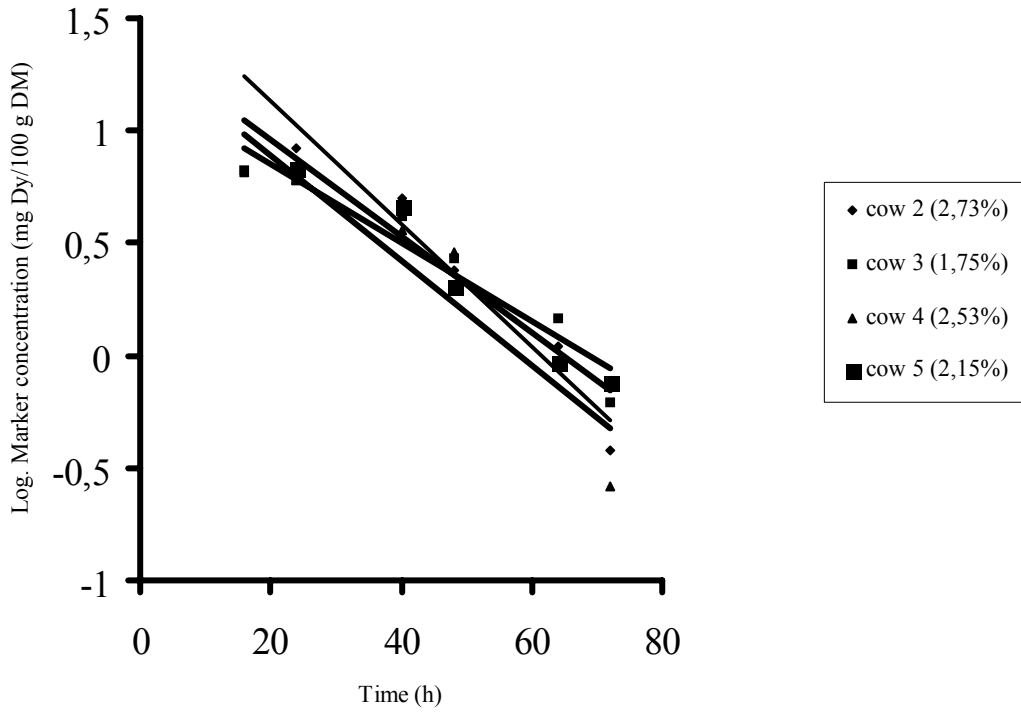


Figure 2. Passage rate (% per hour) for DPRG (Dy).

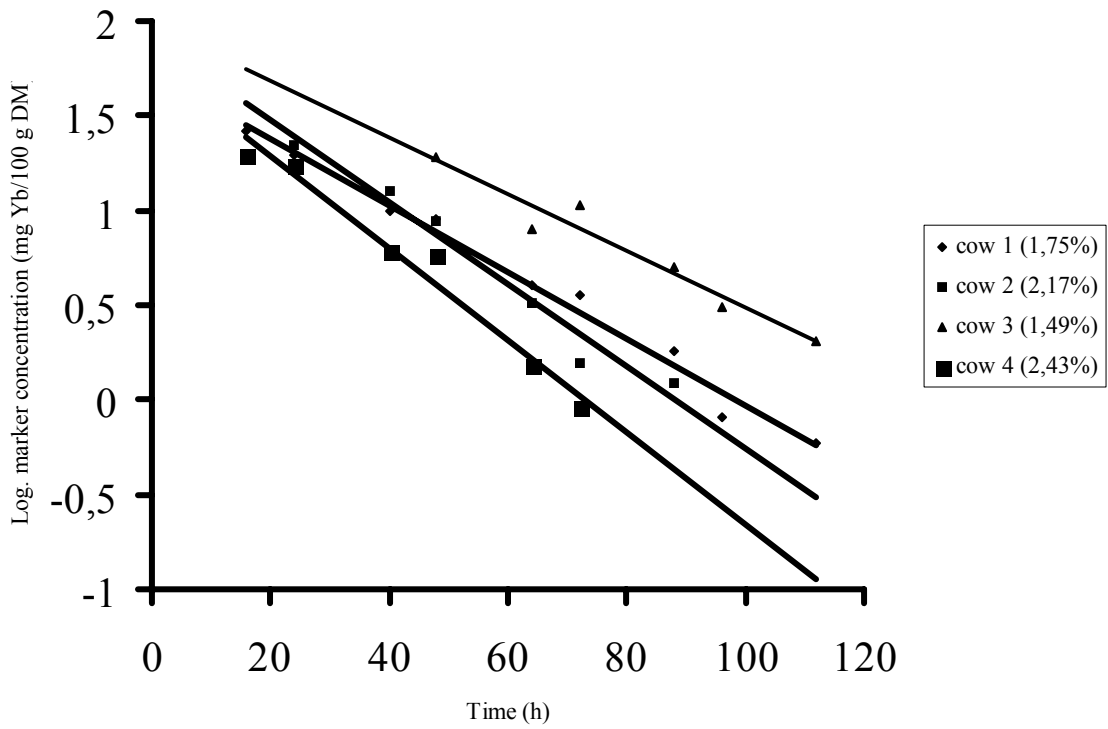


Figure 3. Passage rate (% per hour) for DPRG-ground (Yb).

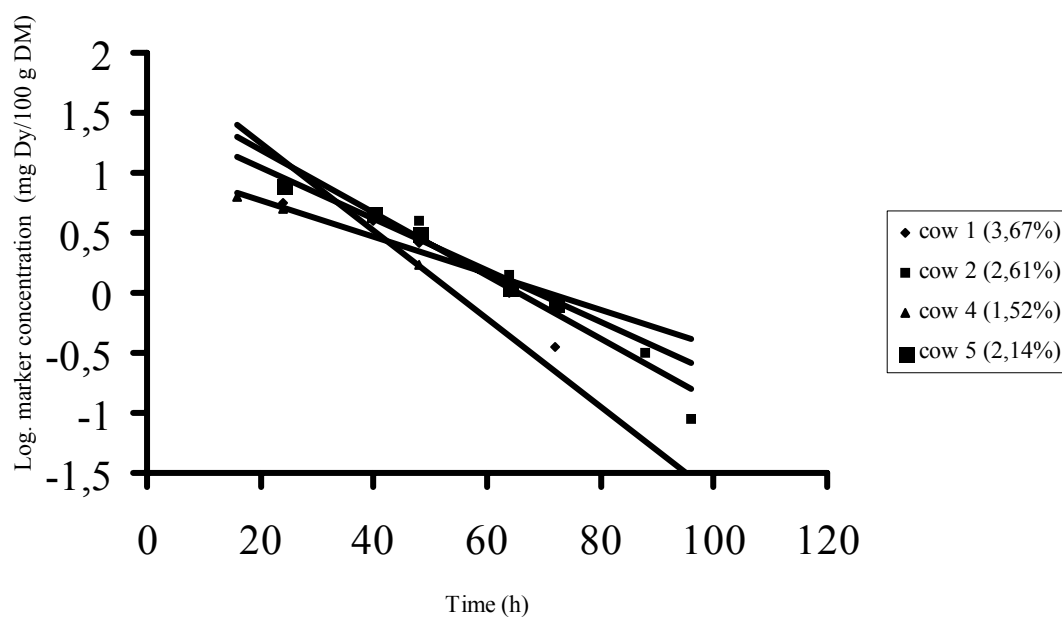


Figure 4. Passage rate (% per hour) for TPRG (Dy).

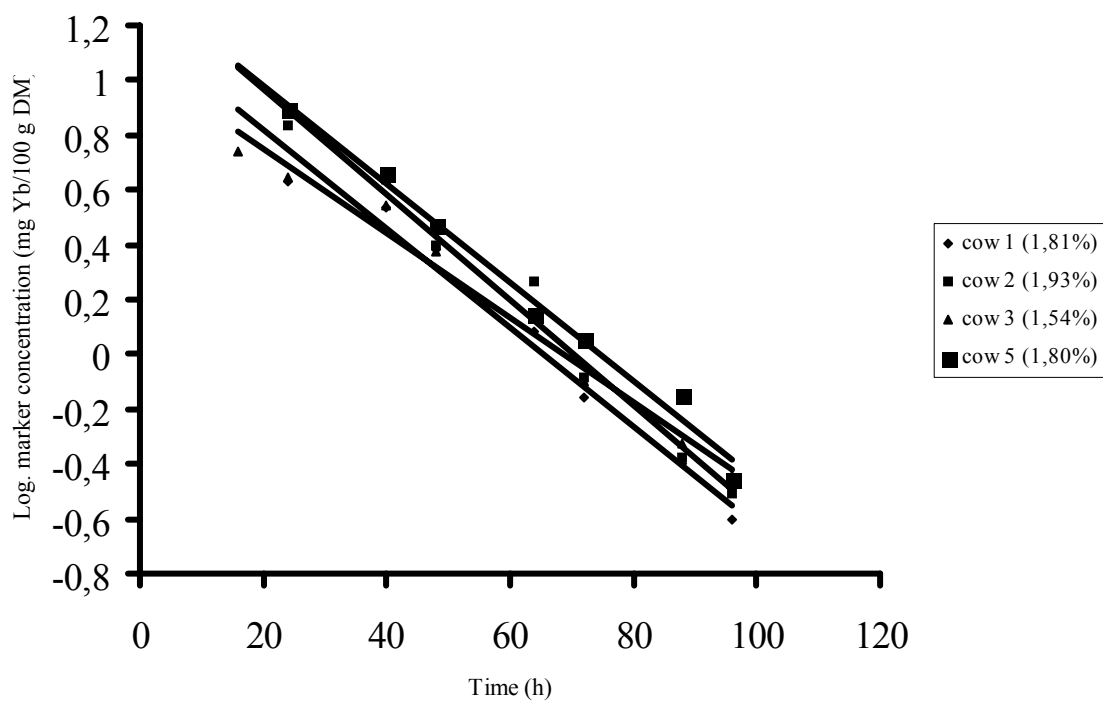


Figure 5. Passage rate (% per hour) for IRG (Yb).

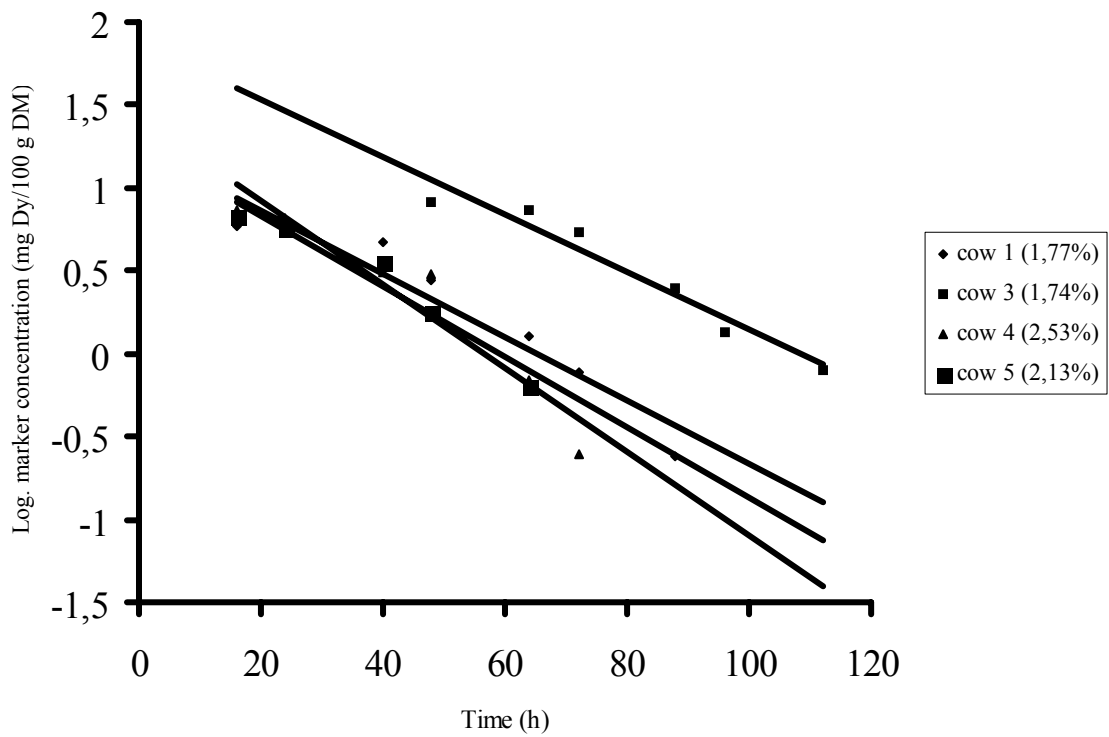


Figure 6. Passage rate (% per hour) for HRG (Dy).

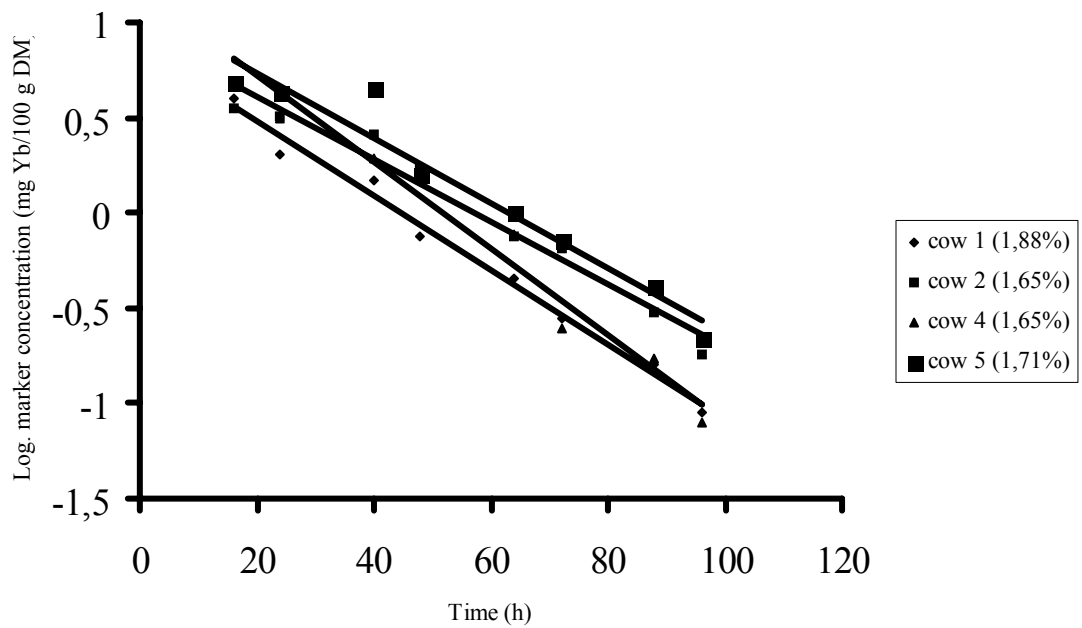


Figure 7. Passage rate (%) for MF (Yb).

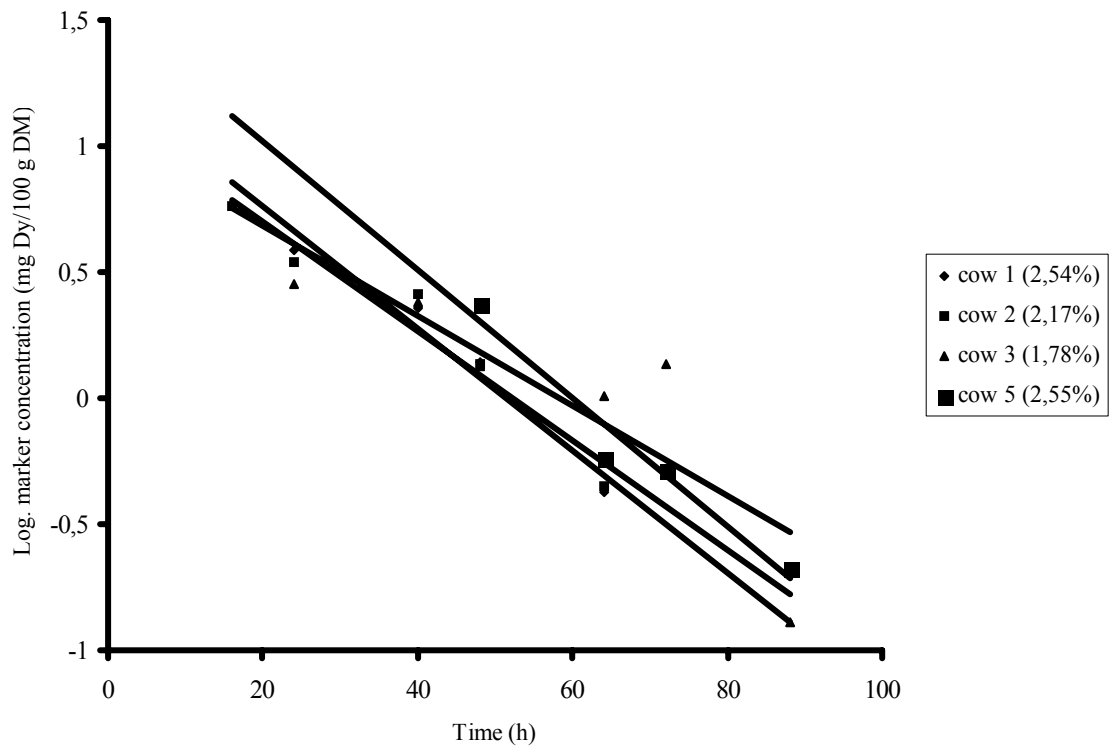


Figure 8. Passage rate (%) for TF (Dy).

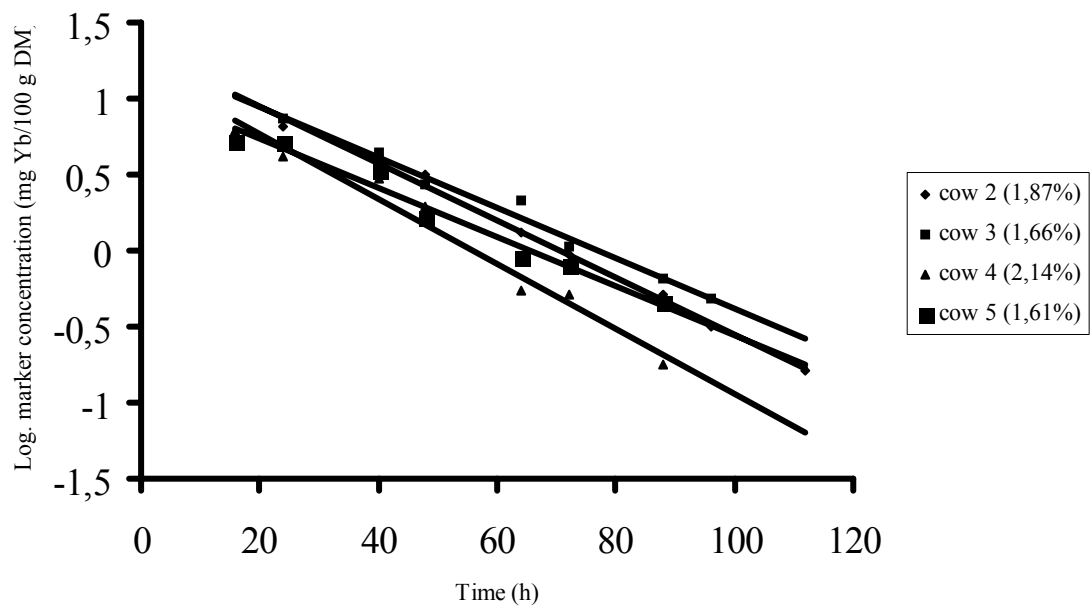


Figure 9. Passage rate (%) for T (Yb).

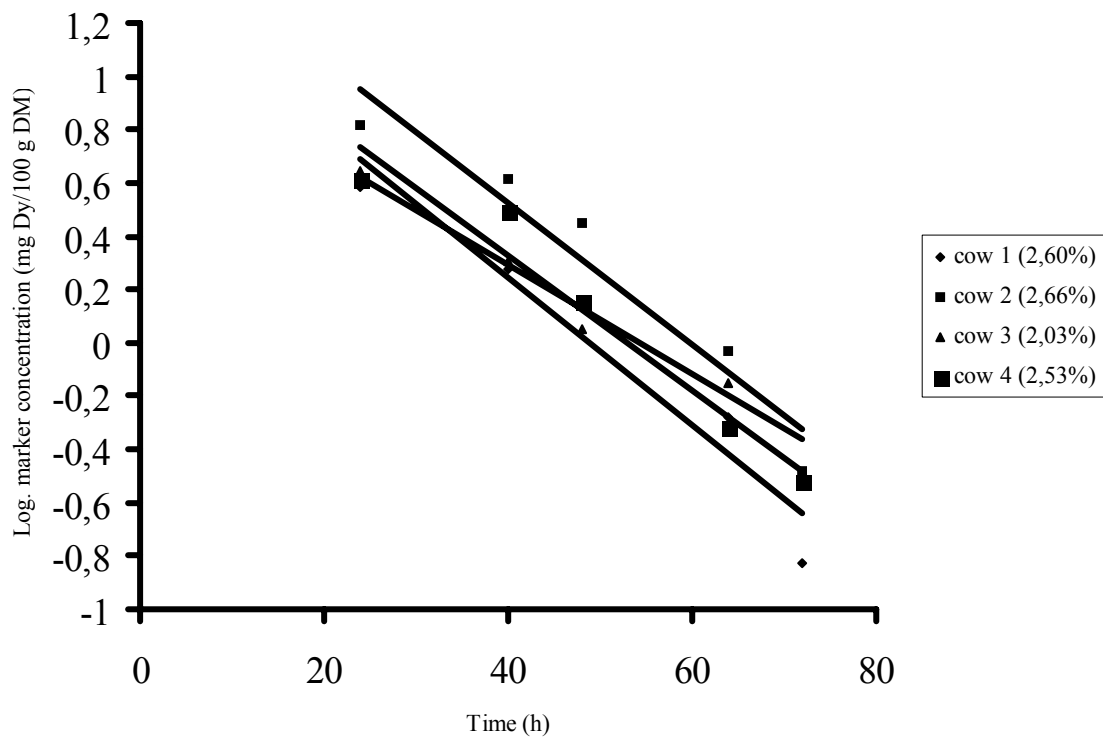


Figure 10. Passage rate (%) for C (Dy).

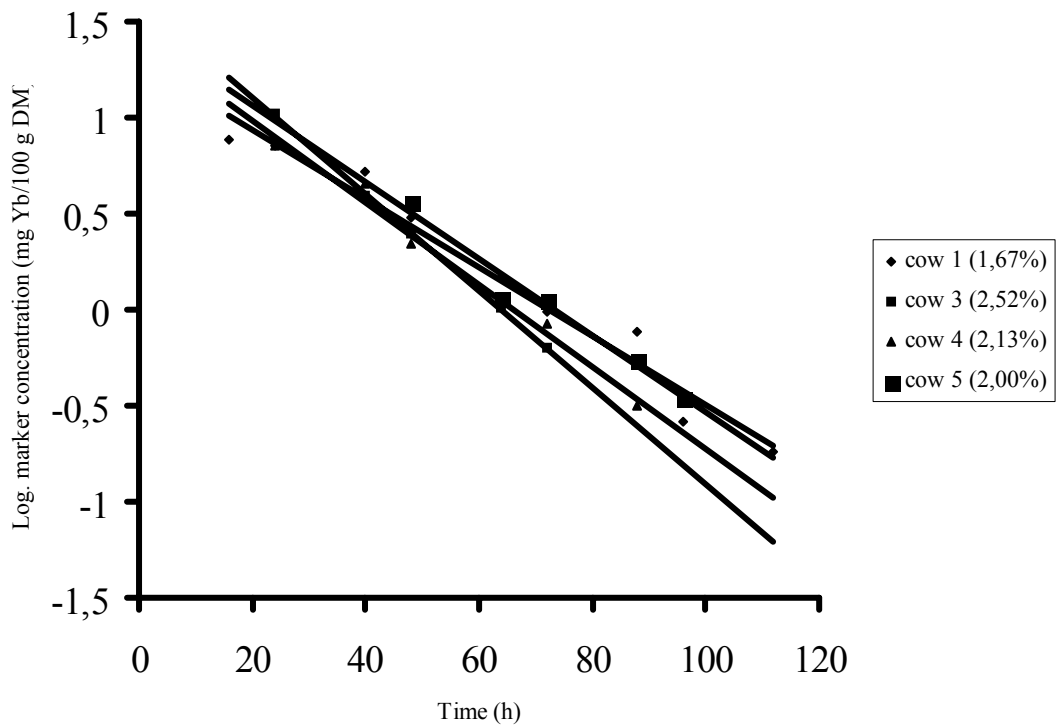


Figure 11. Passage rate (%) for WC (Yb).

Discussion

The high values of R^2 for the fit of regression lines shows that there were not a lot of error in the estimation of passage rates and the technique worked well.

Earlier studies (e.g. Dewhurst *et al.*, 2003) showed that differences in rumen function and the efficiency of nutrient use between forages may be related to differences in rumen passage rates. The high rumen passage rate of white clover was particularly noticeable. Contrary to earlier findings, the results of this study showed no significant differences between passage rates of different forages. Legumes have a propensity to higher buffering capacity compared to grasses (Jasaitis *et al.*, 1987). This might be one reason why white clover had a higher passage rate in the experiment by Dewhurst *et al.* (2003) when cows consumed the forages that were being evaluated but not in the present study, when cows consumed a standard basal diet. When rumen pH is stable at a normal level without any reduction the ruminal microflora are not inhibited and the fibre digestibility can be faster. More generally, it appears that forages affect rumen passage rates mainly through their effects on the rumen environment rather than through inherent properties of the particles themselves. Thus in the work of Dewhurst *et al.* (2003), the formation of a thick retentive mat of fibrous material on top of rumen contents when grass silage was fed may be the major reason for lower passage rates with these diets. Future studies must feed the cows on the same forages as those being evaluated with marked feed.

The fibre fraction of feeds stimulates chewing and saliva production (Allen, 1997). A high saliva production increase the frequency of reticular contraction (Froetschel, 1995). All grasses in this study were cut at the same day except WC, which was cut later. The growth stage of plants can affect the outflow rate from the rumen. Grass in an early vegetative stage has a higher passage rate (Dove & Milne, 1994). Content of NDF can be a kind of measurement of the vegetative stage. The more mature the grass is the higher amount of NDF, but there is also a difference in NDF content between different grasses. The NDF content of the forages was quite similar between different forages (around 600 g/kg DM) except for the WC which had a lower NDF content (390 g). This would have affected the passage rate of WC to be the fastest, and so was also the case when comparing the Yb-labelled forages. T and C were in this study the forages with the highest content of NDF. T had one of the slowest passage rates for the Yb-labelled grasses. In contrast from T had not C, as expected one of the slowest passage rates of the Dy-labelled, but instead one of the fastest. The amount of the labelled forages is small compared to the whole feed intake and therefore is it difficult to say how much the other feeds affected the passage rate of the grasses evaluated. This small amount of each forage could not have affected the chewing and saliva flow, which may had lead to a higher outflow of small particles from the rumen and an increased dry matter intake.

During this experimental period it was not an extreme surrounding temperature, which could have affected the results of the passage rate. As Bernabucci *et al.* (1999) found, the higher surrounding temperature it is, the tendency is that the outflow rate from the rumen is slower and the animal reduces fibre intake if it is possible. The feed in this experiment was mixed and it could have been difficult for the animals to sort different feed ingredients, but they had a good appetite and ate all their rations so that might prove that they not were stressed by heat.

In this study, the feed amount kept constant over all days and cows, so the effect of different feeding level did not appear. If it had been increasing amount forages it is suggested that the passage rate of small particles would have enhanced.

It is well established that ryegrass is most degradable of the grasses, and one reason is the lower content of NDF. The results from this experiment show that the different type of ryegrasses has the fastest passage rate, which might depend on fast rumen degradation. It was also the ryegrasses who had the lowest content of NDF of the grasses. TPRG had the fastest passage rate of the Dy-labelled forages and HRG the slowest. Of the Yb-labelled forages, WC had the fastest passage rate and DPRG-ground the slowest. In legumes exists naturally a breakage point in form of angular joints, which leads to a rapid breakage in the rumen (Waghorn *et al.*, 1989). Legumes have also all lignin concentrated in the xylem, markedly different from grasses which have the lignin spread throughout all tissues. The consequence of this is that the digestion of grasses is slower (Wilson & Kennedy, 1996). WC has the highest passage rate of the Yb-labelled feeds in this study, but it is not so large effect seen by Dewhurst *et al.* (2003), comparing grasses silage and white clover silage. This might depend on that the propensities of the rumen affect passage rate more then propensities of the added feeds. There are a need to do a large-scale study on this with cows fed the same forages as are being evaluated to find out more about passage rates of different forages.

Because of big differences between the different markers it is not so easy to say if the ground DPRG has a higher passage rate than unground DPRG, which was expected. When comparing the Yb-labelled grasses, the ground DPRG had the second fastest passage rate and of the Dy-labelled the DPRG had a passage rate in the middle. It seems, by then, as the ground DPRG has a quit the same passage rate as DPRG. Woodford & Murphy (1988) found that especially forages of poor quality have a tendency to an increased passage rate when they were ground. The amount of labelled grass in this work might be too small for these effects to occur. Maybe the measured passage rates were not the results of the inherent propensities of particles, but instead related to effects on rumen, such as fill, dry matter content and mat formation. Passage rates of the different cows consuming DPRG-ground had a high variation in passage rates and it is therefore difficult to draw any specific conclusion from this treatment.

The markers used in this study, Yb and Dy, were chosen because they are known to be rather stable and have a good affinity. The differences in marker concentration between different forages could be explained by cows consuming less marker or more feed. Since feed intake was fixed and they ate the labelled hays, this seems unlikely. Maybe was not all the excess gone by the washing and residual marker could have migrated to other particles and complicated the marker data. Differences between variation of markers can also be caused by animal differences, environmental effects or genetic differences. It is also easy to mix and contaminate the forages with markers. Because of the high R^2 was probably not the forages mixed or contaminated. There might even be different effects between the added markers on feed fermentability. Maybe have the markers anti-microbial effects or effects on the sites of microbial attachment to forages. Teeter *et al.* (1984) found that the binding capacity of Yb increase with higher amounts of crude fibre or crude protein. Maybe is the protein content in WC a reason why it bound so much Yb. The ground DPRG bound most Yb and DPRG most Dy. Huthanen & Kukkonen (1994) found that rare earth preferentially bound to small rather than to large particles which might explain the high concentration of Yb in the ground DPRG. It would be interesting to do a study of forages labelled with both Yb and Dy to make it possible to compare these markers and try to sort out the differences between them.

The strengths with the model and technique used in this experiment is that it is a less invasive method and it do not disturb the rumen environment when taking representative samples. One

disadvantage is that the passage rate cannot be measured in one particular part of the gastrointestinal tract and this makes it difficult to follow the feed during a specific part.

This study was made as a pilot study for a bigger project whereas passage rates will be linked to the utilisation of nitrogen in dairy cows. Big-scale studies of the forages evaluated are needed to find out more about passage rate of different forages, different markers, particle size and its effects on rumen.

Conclusion

This study evaluated passage rates for different forages evaluated in a small-scale study. The R^2 -values for the fitting of linear regressions to marker excretion results were high, indicating a good model fit and that the technique worked well. There were no significant differences in passage rate between different forages when comparing 10 different crops. It seems likely that the measured passage rates reflected the similar rumen conditions from the cows receiving the same basal diet- and that the small amount of test feed used did not allow differences between forages to be expressed. Larger-scale studies are necessary for further evaluation of differences in passage rates between different forages, particle size and markers.

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Appendix 1.

For abbreviation key see page 2.

Table 1. R²-values for the cows on the different forages

Forages	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5
DPRG	-	0,9538	0,9272	0,8828	0,9643
DPRG-ground	0,9875	0,9672	0,9442	0,9704	-
TPRG	0,8687	0,9491	-	0,7723	0,9814
IRG	0,9769	0,9796	0,979	-	0,9872
HRG	0,9502	-	0,9451	0,9126	0,9439
MF	0,9937	0,9654	-	0,9558	0,9471
TF	0,9255	0,948	0,7318	-	0,9667
T	-	0,9947	0,9836	0,9717	0,9748
C	0,903	0,9334	0,9745	0,9518	-
WC	0,9539	-	0,9995	0,9869	0,9566

Table 2a. R²-values for the cows on the different forages

Forages	Cow 1	Cow 2	Cow 3	Cow 4
DPRG	-	$y=-0,0273x + 1,6782$	$y=-0,0175x + 1,2042$	$y=-0,0233x + 1,3585$
DPRG-ground	$y=-0,0175x + 1,7407$	$y=-0,0217x + 1,9129$	$y=-0,0149x + 1,9812$	$y=-0,0243x + 1,7732$
TPRG	$y=-0,0367x + 1,9940$	$y=-0,0261x + 1,7101$	-	$y=-0,0152x + 1,0752$
IRG	$y=-0,0181x + 1,1828$	$y=-0,0193x + 1,3555$	$y=-0,0154x + 1,0587$	-
HRG	$y=-0,0177x + 1,2101$	-	$y=-0,0173x + 1,8800$	$y=-0,0253x + 1,4275$
MF	$y=-0,0188x + 1,0617$	$y=-0,0165x + 0,9395$	-	$y=-0,0227x + 1,1785$
TF	$y=-0,0254x + 1,0103$	$y=-0,0217x + 1,1346$	$y=-0,0178x + 1,0352$	-
T	-	$y=-0,0187x + 1,3189$	$y=-0,0166x + 1,2786$	$y=-0,0214x + 1,2002$
C	$y=-0,0260x + 1,2808$	$y=-0,0126x + 1,5935$	$y=-0,0204x + 1,1114$	$y=-0,0253x + 1,3404$
WC	$y=-0,0167x + 1,2667$	-	$y=-0,0252x + 1,6138$	$y=-0,0213x + 1,4131$

Table 2b. R²-values for the cows on the different forages

Forages	Cow 5	Cow 6	Cow 7
DPRG	$y=-0,0215x + 1,3938$	-	$y=-0,0273x + 1,6783$
DPRG-ground	-	$y=-0,0175x + 1,7408$	$y=-0,0217x + 1,9130$
TPRG	$y=-0,0214x + 1,4689$	$y=-0,0367x + 1,9950$	$y=-0,0261x + 1,7102$
IRG	$y=-0,0180x + 1,3407$	$y=-0,0181x + 1,1829$	$y=-0,0193x + 1,3556$
HRG	$y=-0,0213x + 1,2561$	$y=-0,0177x + 1,2102$	-
MF	$y=-0,0171x + 1,0776$	$y=-0,0188x + 1,0618$	$y=-0,0165x + 0,9396$
TF	$y=-0,0255x + 1,5301$	$y=-0,0254x + 1,0104$	$y=-0,0217x + 1,1347$
T	$y=-0,0161x + 1,0551$	-	$y=-0,0187x + 1,3190$
C	-	$y=-0,0260x + 1,2809$	$y=-0,0126x + 1,5936$
WC	$y=-0,0200x + 1,4623$	$y=-0,0167x + 1,2668$	-

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