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Degradation- and passage kinetics of concentrate particles and solubles in the rumen

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ABSTRACT

This thesis deals with three actual issues of ruminant degradation and escape of dietary components:

One of the main emphasis has been on how particle size distribution of concentrate feed affects the degradation and ruminal escape. Feed manufacturers producing compound feeds for ruminants want to increase the fraction of undegraded starch and protein feed passing out of the rumen, and the particle size distribution of the feed may be used to increase this fraction. The residence time distribution of concentrate particles was not much affected by the particle size in the range from 0.1 to 2.8 mm, but the degradation kinetics of barley and maize was substantially depressed by increased particle size. For maize the ruminal degradability was strongly dependent of the particle size, but for barley this effect was much weaker because the degradation kinetics was much faster than the passage kinetics. However, expander treatment of barley to reduce protein degradability would probably increase the effects of particle size on degradability since this treatment decrease the degradation rates.

The second part of this thesis deals with the possibilities to simplify the laborious *in situ* technique used for ruminal degradation measurements in Norway. This study showed that the number of incubation times could be reduced to two or three without sacrificing much accuracy. The amount of human work and the total incubation time could therefore be reduced by this method.

In the last part of this thesis we study the degradation of soluble nitrogen fractions of fresh grass and grass silage, which are the main forages in Norway. Half or more of the total N in

grass silage is in the form of soluble non-protein nitrogen – mainly small peptides and free amino acids. Therefore, it is important to know how much of the soluble peptides and free amino acids that escapes the rumen to improve the accuracy of determination of the protein value of grass and grass silage. The results obtained showed that approximately 10 % of the soluble dietary nitrogen escaped the rumen, and the data also indicated that the synthesis of microbial protein from fresh grass was more efficient than silage, caused by a better access to energy for this synthesis.

ACKNOWLEDGEMENTS

I wish to express my gratitude to everyone that have supported and helped me during these years:

My supervisor Professor Norvald Nesse for your great support, our constructive discussions, and for all your proof readings.

My co supervisor Dr. Agric Harald Volden for being so enthusiastic to this project and for your contributions during our discussions and for practical help with my experiments.

Thor Mejdell at SINTEF Kjemi for important contributions to two of the papers, and to SINTEF Kjemi for giving financial support.

Leidulf Nordang for participating in the advisory group meetings and to Felleskjøpet Fôrutvikling for financing parts of this project.

Philip André Reme for helping with the microscopic setup and implementation of the image analysis program and Kai Hjarbo for helping with the Laser diffraction instrument.

To my office colleague Beranhu Demessie and the rest of my colleagues for good working environment.

My wife's family here in Trondheim for helping with baby sitting and picking up our children in school and kindergarten when it has been needed.

Finally I will thank my dear wife, Rut, and my four lovely children, Johanne, André, Emil August, and Elise Kristine for being such a great family to live in. I will also thank you Rut for your patience when I was finishing the writing of the thesis.

This project has been partly financed by Norwegian Research Council over Husdyrprogrammet 1996 – 1999, and I will thank them for their financial support

LIST OF PAPERS

I. Olaisen, V., Nesse, N. and Volden, H. (2001) Technical note: Use of laser diffraction for particle size measurements in duodenal digesta. *J. Anim. Sci.* **78**, 761-756.

II. Olaisen, V., Volden, H., and Nesse, N. (2001) The effect of particle size and sedimentation velocity on the flow resistance out of the rumen.

III. Olaisen, V., Volden, H., Mejdell, T. and Nesse, N. (2001) The effect of concentrate particle size on degradation and passage rate in the rumen. To be submitted for publication in *Anim. Feed Sci. Technol.*

IV. Olaisen, V. (2001) Modelling the barley and maize effective degradability of starch and protein as a function of particle size.

V. Olaisen, V., Mejdell, T., Volden, H. and Nesse, N. (2001) Simplified *in situ* methods for estimating rumen degradability of concentrates. To be submitted for publication in *J. Anim. Sci.*

VI. Volden, H., Mydland, L.T., and Olaisen, V. (2001) Apparent ruminal degradation and rumen escape of soluble nitrogen fractions in grass and grass silage administrated intraruminally to lactating cows. To be submitted for publication in *J. Anim. Sci.*

LIST OF ABBREVIATIONS AND SYMBOLS

A	"Soluble" fraction in the first order degradation model	%
AAT	Aminosyrer absorbert i tarm	
B	Potential degradable particle fraction in the first order degradation model	%
C	Concentration	mg/mL
c	Degradation rate in the first order degradation model	h^{-1}
CP	Crude protein	
CSTR	Continous stirring tank reactor	
D	Disappearance from nylon bags	%
DM	Dry matter	
E	Residence time distribution function	dimensionless, total area of one
ED	Effective degradability	%
FAA	Free amino acids	
GI	Gastrointestinal (tract)	
k_p	Passage rate	h^{-1}
LD	Laser diffraction	
MIA	Microscopic image analysis	
MRT	Mean residence time	h
NAN	Non-ammonia nitrogen	
PBV	Proteinbalansen i vomma	

PFR	Plug flow reactor	
PLP	Proteins and long chain peptides	
RMSE	Root mean square error	
ROO	Retiulo-omasal orifice	
RTD	Residence time distribution	
s	Particle size	mm
sol	True solubility	%
SP	Small peptides	
t	Time	h
w	Wash fraction from the nylon bags	%
τ	Time delay	h

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

The background for this thesis was a corporation project between Felleskjøpet Fôrutvikling and SINTEF Kjemi. The aim was to study the effects of different processing conditions of expander treated cereals on rumen degradability and to develop cheaper *in vitro* methods for degradability measurements. My work has been a continuation of this project, and the main objectives were to study the effects of feed particle size on the rumen degradability and the possibility of using a simplified *in situ* technique for degradability estimations. In the following paragraphs I give a more extensive discussion of the background and motivation for this thesis.

Ruminant animals differ from non-ruminants by being able to break down fiber components into simple sugars that can be utilized by the rumen microbes. This degradation is a slow process, and therefore ruminants have evolved a large fermenting chamber (reticulorumen) to achieve effective cell wall digestion. However, much of the components in concentrate feeds such as starch and protein do not need to be broken down in the rumen but can be directly digested in the small intestine. For two reasons it is optimal that a large fraction of those components escape the rumen undegraded:

1. Degradation in the rumen requires energy, and degradation in the rumen represent nutrient loss for components that can be digested in the small intestine.
2. A very rapid degradation of starch gives a unfortunate pH drop in the rumen, which may lead to acidosis. This is a problem for typical Norwegian cereals such as barley and oats.

There are in principle, two strategies to improve the amount of dietary components to pass out of the rumen: the first is to alter the microbial degradation kinetics, and the second is to alter the residence time distribution (RTD) of the feed particles. The degradation kinetics has been altered by chemical and/or thermal treatments of the feed (Prestløkken, 1999). In addition both degradation kinetics and residence time distribution may be dependent on the particle size, and changing the particle size distribution in the feed may also alter the ruminal degradability. The particle size distribution of the feed will be determined by the processing conditions, for instance the milling type. It is therefore of interest to study the effect of concentrate particle size on degradation kinetics and RTD of concentrate cereals.

In Norway the *in situ* method is widely used to estimate the degradation kinetics of a feed. The method requires a large number of nylon bags for each feed sample, and therefore a substantial amount of human work. It is therefore quite expensive to carry out degradability measurements. Several *in vitro* methods have been tried with the attempt to replace the *in situ* method, but at present, no *in vitro* method that is generally accepted as a satisfactory alternative seems to exist (Stern *et al.*, 1997; Hvelplund and Weisbjerg, 1998). Trying to simplify the *in situ* procedure can therefore be an alternative approach to make this method cheaper.

Protein in grass and grass silage, the main forages for ruminants in Norway, are very susceptible to microbial breakdown and are often poorly utilized by the rumen microbes. During ensiling, the protein fraction has been impaired by extensive proteolysis. On an average, half or more of the total N in grass silage is in the form of soluble non-protein nitrogen – mainly small peptides and free amino acids. Therefore, it is important to know how much of the soluble peptides and free amino acids that escapes the rumen to improve the accuracy of determination of the protein value of grass and grass silage. Moreover, lower efficiency in microbial protein synthesis on grass silage diets compared to grass diets may be due to the asynchronous release of energy and N in grass silage (Siddons *et al.*, 1985). Therefore, recent studies (Beever and Cottrill, 1994) indicated that synchronizing the N and carbohydrate degradation rate in the rumen might improve the efficiency of microbial protein synthesis. It is vital to know the rate of degradation to identify the potential benefits from improved synchronization.

1.2 Outline of the Thesis

The main results in the thesis are presented in the six attached papers denoted with roman letters. In addition this thesis consists of two main chapters; **Chapter 2** gives a review of the relevant literature, and in **Chapter 3** the results in the papers are summarized and discussed.

The literature review present deals with the following topics:

- a very briefly presentation of rumen anatomy and the rumen motility
- factors affecting the particulate residence time in the rumen
- residence time distribution models used to estimate particulate flow out of rumen
- protein degradation mechanism and kinetics models to estimate degradation parameters
- the main experimental techniques used in the papers, which were labeled particles used to estimate residence time distribution, and the *in situ* technique to estimate degradation kinetics.

Paper I is a technical note which compares laser diffraction (LD) and wet sieving for particle size measurements of duodenal digesta. Laser diffraction was also compared with microscopic image analysis, which is often used as an absolute method. The study showed that particle size distribution measured by LD were comparable to wet sieving.

The laser diffraction method was used in **paper II** to measure the relative resistance as a function of particle size and sedimentation velocity for particle to pass out of rumen.

Paper III gives data on the residence time distribution and degradation kinetics of different concentrate particle sizes. For barley and oats the degradation rate and the soluble fraction decreased by increasing particle size. There was no large variation in mean residence time (MRT) between the different particle sizes.

In paper IV the results obtained in paper III are used in combination with literature data to model the degradability of starch and protein as a function of initial particle size. These results were used to illustrate how different grinding techniques can affect the degradability of a feed.

Paper V presents a simplified *in situ* procedure for degradability estimates of concentrates. The number of time-points could be reduced substantially without loosing much accuracy.

In **paper VI** *in vivo* degradation of soluble nitrogen fraction from fresh grass and different grass silage is studied. The results showed that approximately 10 % of the soluble non-ammonia nitrogen fraction escaped the rumen.

CHAPTER 2

LITERATURE OVERVIEW

2.1 THE DYNAMICS OF DIGESTA IN THE RUMEN

2.2.1 ANATOMY OF THE RUMINANT FORESTOMACH

Ruminant animals have three digestion sites; the reticulorumen, the small intestine, and the large intestine. The reticulorumen is the site that differentiates ruminants from non-ruminant animals. The main function of the rumen is to degrade fiber components in cell wall into simple sugars that can be utilized by the rumen microbes. Non- ruminant animals cannot take advantage of the fiber in the feed. Fiber components are not digested in the small intestine, and only a very small amount of the fiber is digested in the large intestine. The degradation of fiber is a slow process; therefore ruminants have evolved a large fermentation chamber (the reticulorumen) in their stomachs to achieve effective cell wall digestion, and they retain feed particles substantially longer than fluid in that compartment of the stomach (Lechner-Doll *et al.*, 1990). The long retention time restricts feed intake because of rumen fill (Van Soest, 1994).

In popular parlance, we say that the ruminant animals have four stomachs. In reality they have only one, the abomasum. The other three "stomachs" (rumen, reticulum, and omasum) are fermenting chambers on the esophagus. The rumen and reticulum are often considered as one single organ (the reticulorumen or the forestomach) separated by the reticuloruminal fold. The separation is only partial; free exchange of contents is still possible. Pillars divide

the rumen into discrete sacs: dorsal sac, ventral sac, cranial sac and reticulum (Figure 2.1). The reticulorumen is a large organ, up to 110 liters in dairy cattle.

The reticulorumen is separated from the omasum by the reticulo-omasal orifice (ROO). Passage of digesta to the omasum can occur only when the reticulo-omasal orifice is open and the pressure in the reticulum exceeds that in the omasum. Those conditions are met during the peak of the second phase of the reticular contraction (Wyburn, 1980).

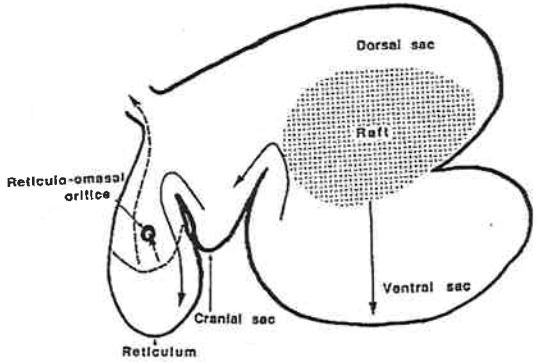

Figure 2.1 The discrete sacs of the reticulorumen (From Kennedy and Doyle, 1993).

2.2.2 Rumen Motility and Digesta Flow

As I already mentioned, pillars divide the rumen into discrete sacs. During contraction of these pillars, sacs become smaller and the ingesta are circulated in the rumen (Wyburn, 1980). One stream circulates in the dorsal sac, and another stream circulates in the ventral sac. The digesta in the reticulum and cranial sac is tipped back and forth across the reticuloruminal fold. The contracting motion can be divided into a primary contraction, which affects the whole reticulorumen, and a secondary contraction, which affects only a part of the organ. During the peak of the secondary phase of the reticular contraction, the reticulo-omasal orifice is opened and digesta is allowed to flow into the omasum. This contracting

pattern repeats 1-2 times/min; the contractions are most frequent during eating (Matison *et al.*, 1995).

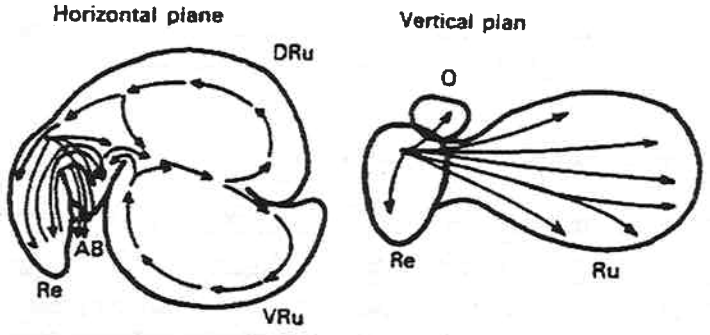

Figure 2.2 The movement pattern of digesta in the reticulorumen in a horizontal plane (left) and a vertical plane (right). Ab-Abomasum, DRu-Dorsal rumen, O-Omasum, Re-Reticulum, Ru-Rumen, VRu-Ventral Rumen. (From Van Soest, 1994, after Waghorn and Reid, 1977).

Particles in the rumen are separated according to their sedimentation/flotation properties. Buoyant particles tend to float and make a "fiber mat" in the dorsal sac. Newly ingested particles are often coarse and buoyant, and these particles tend to float in the particulate fibre mat. As they get denser during breakdown and hydration, they tend to get lower in the rumen until they enter the ventral rumen cycle (Figure 2.2). In the ventral rumen cycle these particles can be transported to the cranial sac and then to the reticulum. Probably, the reticular motility plays a major role in particle separation with respect to particle gravity. Kaske and Engelhardt (1990) placed plastic particles of three densities and three sizes into the reticulum of sheep, and after 30 minutes (during which animals did not ruminate) recovered them from the reticulum and ventral rumen. Rejection of particles to the ventral sac was substantially greater for the lowest density (1.03 g/ml) than for medium (1.22 g/ml) and high (1.44 g/ml) densities, and the rejection was independent to length. Sutherland (1988) also

found substantially more dense digesta particles in the reticulum than in ventral and dorsal rumen. These findings indicate that the reticular contractions discriminate particles with respect to density. The particles are discriminated with respect to size in ROO, the omasum, or in the dorsal rumen since smaller particles have higher passage velocity out of the retiulorumen (Kennedy, 1995; Dixon and Milligan, 1985) and the particle size distribution is similar in both ventral rumen and retiulum (Sutherland, 1988).

2.2 Factors Affecting the Particulate Residence Time in the Rumen

2.2.1 Particle Density

The density of digesta particles affects their ability to sedimentate in the reticulorumen and pass out of the reticulorumen. Functional gravity is a concept often used in the literature, which Lechner-Doll *et al.* (1991) defined as the sum of all factors contributing to the effective buoyancy of a digesta particle. These factors include the structural components of the particle, the fluid and the gas inside the particle, as well as attached gas bubbles.

Residence time of inert particles with different densities has been extensively studied (Campling and Freer, 1962; Durkwa, 1983; desBordes and Welch, 1984; Ehle, 1984; Ehle and Stern, 1986; Murphy *et al.*, 1989; Kaske and Engelhardt, 1990). These studies showed that particles with densities in the range of 1.3-1.5 g/ml had the lowest residence times (Figure 2.3). The variation in the data between the studies can be explained by different particle sizes, different feeding situations and different measuring techniques. desBordes and Welch (1984) also measured the rumination activity on the particles. They found that the ruminating activity increased when the density increased from 0.9 g/ml to 1.1 g/ml, and then the rumination activity decreased when the particles became denser. Murphy *et al.*, (1989) measured a decreasing ruminating activity as the particle density increased from 1.1 g/ml to 1.77 g/ml.

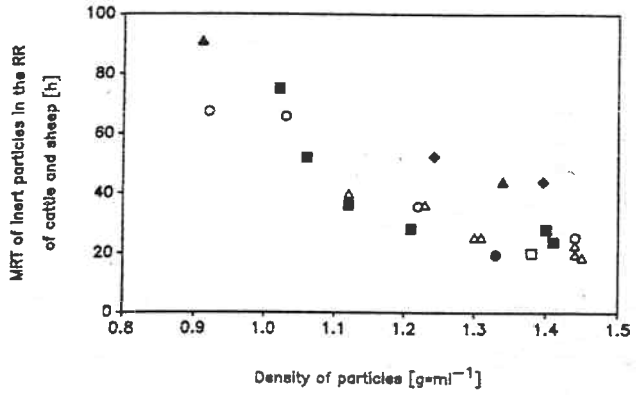

Figure 2.3 Mean ruminal residence time for inert particles as a function of particle density. (From Lechner-Doll *et al.*, 1991).

The functional gravity of digesta particles change during their time spent in the reticulorumen. There are two factors that alter the gravity of the rumen particles: (1) Hydration by the rumen saliva, which makes the particles denser, and (2) gas production under the rumen fermentation, which makes the particles more buoyant. Hooper and Welch (1985a) studied changes in gravity *in vitro* both with water and with autoclaved rumen saliva as the solvent (1985b). They found that the gravity increased more rapidly in the autoclaved rumen saliva than in water. Gravity changes studied *in vivo* (Hooper and Welch, 1985a) showed an even more rapid change than for the autoclaved ruminal saliva used in the *in vitro* studies. They concluded that this more rapid change possibly be caused by microbial digestion or by ruminal contractions that mix the digesta.

The gravity change is more rapid for small particles (Hooper and Welch, 1985a) than large one. Different feeds have also different density changes under hydration. Van Milgen *et al.* (1993) studied gravity changes for different roughages; and Wattiaux *et al.* (1992a) studied gravity changes for hay and silage of alfalfa. Ramanzin *et al.* (1994) studied the gravity changes for different concentrate feeds. They found high gravities (1.4 - 1.6 g/ml), but the gravity change under hydration was small. Bhatti and Firkins (1995) also studied the gravity

changes under hydration for different concentrate feeds. They found generally lower values than Ramanzin *et al.* (1994). Udén (1992) studied the sedimentation of concentrates during in vitro fermentation. Generally the sedimentation of concentrate particles were high initially.

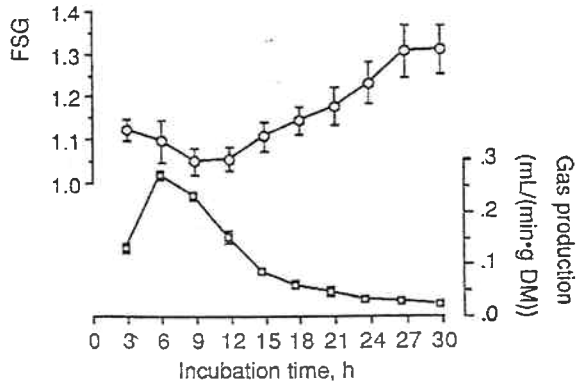

Figure 2.4 Change in functional specific gravity (FSG) and gas production of alfalfa hay particles during *in vitro* digestion (From Wattiaux *et al.*, 1992b).

Wattiaux *et al.* (1992b) studied the gravity changes and gas production under microbial fermentation, and they observed a negative correlation between gravity and gas production (Figure 5). The high gas production the first hours led to a decreased gravity, whereas the gravity increased again when the gas production decreased. Silicano-Jones and Murphy (1991) and Hooper and Welch (1985a) did not observed this decrease in gravity during the first hours of the microbial fermentation.

2.2.2 Particle Size

Several authors have investigated the particle size distribution of digesta particles leaving the reticulorumen and they have found that very few large particles are allowed to leave the reticulorumen (Poppi *et al.*, 1980, 1985; Dixon and Milligan 1985; Waghorn *et al.*, 1989;

Kovacs *et al.*, 1997). These findings have lead to the concept of a critical particle size, a size where very few larger particles are allowed to leave the reticulorumen. For sheep and cattle, this size is suggested to be in the range of 1-2 mm (Poppi *et al.*, 1980, 1985; Lechner-Doll and Engelhardt, 1989). The concept of a critical particle size has been used in modelling of digesta flow (France *et al.*, 1985), and these models divide reticulorumen particles into two homogeneous pools; a large- particle pool which cannot pass out of the reticulorumen and a small-particle pool which leave the reticulorumen with no resistance.

Three main techniques have been used to study the effect of particle size on the passage rate:

1. Comparing particle size distribution in rumen with the particle size distribution in lower gut.
2. Study the RTD of labeled particles
3. Study the RTD of inert particles (indigestible particles of plastic material).

Studies by Kennedy (1995) and Dixon and Milligan (1985) based on comparing particle size distributions showed that the turnover rate in cattle increased as the particle size decreased. The data from Kennedy (1995) indicated that the increased turnover rate was an exponential function of the particle size. Turnbull and Thomas (1987) studied the residence time distribution in steers for three different labeled particle sizes of dry-cracked and two sizes of stem-rolled maize. Cracked maize passed from the rumen at 33% faster rate than steam-rolled maize with the same particle size. Furthermore, they found that particles of size 0.5 - 1.0 mm passed out of the rumen at 19 and 21 % faster rate than particles of size 1.0 - 2.0 mm and 2.0 – 4.0 mm, although the differences were not significant. Also in the study of Worell *et al.* (1986) meadow hay particles smaller than 0.85 mm had shorter MRT than particles of sizes 0.85 – 1.68 mm and particles larger 1.68 mm. However, in both these studies different markers were used for the different particle sizes, which may have lead to confounding of particle size and marker. Quiroz *et al.* (1988) recommended avoiding this confounding if the objective of a study was to determining passage kinetics of different particle sizes. Studies with inert particles have also shown that the ruminal residence increases with increasing particle size (Durkwa, 1983; Ehle and Stern, 1986; Murphy *et al.*, 1989; Kaske and Engelhardt, 1990; Prigge *et al.*, 1993). Kaske and Engelhardt (1990) found that particles with length of 10 mm of different densities were retained in the reticulorumen averagely 22.7 h longer than particles with length of 1 mm. The data in these four studies with inert particles

was consistent with one exception, Ehle and Stern (1986) found, surprisingly, that particles with a diameter of 3.2 mm had a longer retention time than particles with a diameter of 6.4 mm.

Feed particulate matter may be reduced in particle size by four processes: chewing during eating, chewing during rumination, microbial attack, and the action of rumen contractions. The rumination is a process where boli with digesta particles are regurgitated, chewed, mixed with saliva, and then reswallowed. Chewing during eating and rumination are the two most important processes to comminute coarse particles in the rumen (Ulyatt *et al.*, 1986) where the ruminative chewing contributes more than eating chewing (Kennedy, 1985). Large particle breakdown during rumination has been estimated to account for 71-85% of post-ingestive particle comminution in cattle (Kennedy, 1985; McLeod and Minson, 1988). Ulyatt *et al.* (1986) proposed that chewing during eating and chewing during rumination have different functions with respect to particle size reduction. Chewing during eating is responsible for preparing feed for swallowing, releasing soluble constituents, and damaging plant tissue for microbial degradation. Chewing during rumination has the primary function of reducing the particle size of refractory material so that it can be cleared from the rumen. The primary function of the rumination is to facilitate clearance of digested particles from the reticulorumen by reduction of particle size. Total rumination time in sheep and cattle rarely exceeds 10 h/day (Welch, 1982), and this limitation in rumination time is a restricting factor for the intake of roughage feed. Inert particles have also been used to study the rumination activity with respect to particle size and density (Durkwa, 1983; desBordes and Welch, 1984; Murphy *et al.*, 1989), and large particles with low density was most ruminated whereas there was practically no rumination activity on small particles with high density.

2.2.3 Feeding Level and Concentrate to Roughage Ratio

Many reviewers have stressed the feeding level and concentrate to roughage ratio as factors that strongly affect the particulate retention time in the rumen (Owens and Goetsch, 1986, Sauvant and Archimède, 1990; Cannas and Van Soest, 2000; NCR, 2001). Based on literature data these reviewers have derived correlations for concentrate and roughage passage rate as a function of feed intake and concentrate to roughage ratio. Increasing feeding level leads to

decreased ruminal retention time, and both concentrate and roughage retention time decreases as the roughage fraction increases.

2.3 Digesta Residence Time Distributions

Using a pulse dose of labeled feed particles and assuming a constant volumetric flow rate and constant rumen volume, RTD is given by the following equation (Scott Fogler, 1992):

$$E(t) = \frac{C(t)}{\int_0^\infty C(t)dt} \tag{2.1}$$

where C(t) is the marker concentration as a function of time and the integral in the denominator is the area under the C(t) curve. The RTD function is therefore a normalized dimensionless outflow concentration profile. The mean residence time (MRT), which is the centroid of the RTD, is defined by

$$MRT = \int_0^\infty t \cdot E(t)dt \tag{2.2}$$

2.3.1 Residence Time Distribution Models

Many different mathematical RTD models have been used to model particulate flow in the ruminant gastrointestinal (GI) tract. A continuous stirring tank reactor (CSTR) is the simplest rumen RTD model, and this model is much used in degradability calculations (Ørskov and McDonald, 1979). The model assumes ideal mixing and that all particles have the same probability to escape the reticulorumen. None of these assumptions are valid in the reticulorumen, and this model is therefore an oversimplification. The mixing in the rumen is not instantaneous, and as already mentioned, large and buoyant particles need to be broken down before they can pass out of the reticulorumen. Blaxter *et al.* (1956) used a model with

two CSTR and a plug flow reactor (PFR) in series to model the fecal output in sheep (Figure 2.5), and this model has later been used to model ruminal particulate flow (Huhtanen and Kukkonen, 1995; Stensig *et al.*, 1998). Matis (1972) noted that feedstuff fragments in the rumen are subjected to many digestive processes that require time before such fragments acquire properties required to increase the probability of their escape. He therefore introduced the family of gamma functions with integer shape factor (Erlang family) to model effects of such an age-dependent process (Figure 2.5). This family of RTD models have been extensively used in the modelling of digesta flow in ruminants (Pond *et al.*, 1988; Quiroz *et al.*, 1988; Poore *et al.*, 1991; Moore *et al.*, 1992; Stensig *et al.*, 1995; Bernard *et al.*, 1998). Reese *et al.* (1995) developed a mathematical procedure for solving gamma functions with non-integer values of the shape factor of the gamma model. Different multi-compartment models (Figure 2.5) have also been used (Matis and Wehrly, 1979; Dhanoa *et al.*, 1985; France *et al.* 1985), and Ellis *et al.* (1994) have discussed all these models extensively.

2.3.2 Marker Methods

Digesta markers are of two general types. Inherent markers are component part of a feedstuff, whereas external markers consist of a variety of inert compounds. External markers are either bound to feed particles or inert plastic particles are used directly as marker.

From their literature review Owens and Hanson (1992) have listed four requirements for an ideal marker

1) It must not be absorbed,
2) It must not affect or be affected by the digestive tract or its microbial population,
3) It must flow parallel with or be physically similar to or intimately associated with the material it is to mark, and
4) it must have a specific and sensitive method for estimation.

The consequence of these requirements is that an ideal marker should be bound to the original marked particles during their time in the rumen, and it should not change the properties of the particles such as digestibility and gravity. No single marker fulfils all these criteria, and the chose of marker will therefore depend of which of the requirements most critical to fulfil.

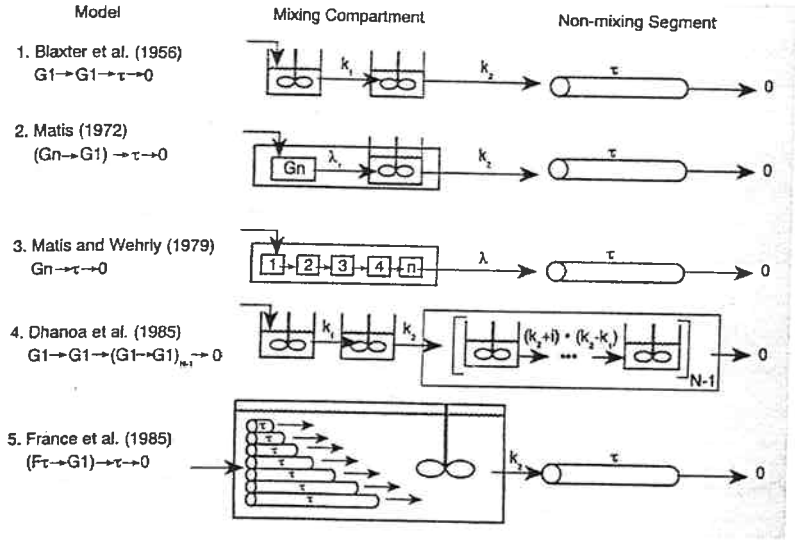

Figure 2.5 Five types of proposed models for digesta flow in the ruminants and their representation by physical models of mixing compartment and non-mixing flow. G1 = CSTR, k_1 and k_2 = passage rates of CSTR compartments, Gn = age-dependent distributed residence times, λ = age-dependent turnover rate, τ = time delay, O = output (From Ellis *et al.*, 1994).

Rare-earth elements and chromium mordant are the most commonly used markers used to label particulate matter for passage studies. Cr can form stable complexes with practically all compounds containing free alcohol groups (Van Soest *et al.*, 1988), and starch, sugars and other potentially soluble substances needs to be removed to avoid producing soluble complexes. The Cr complexes of plant cell wall and protein are highly insoluble and totally stable in the rumen (Uden *et al.*, 1980). Rare earth elements include the lanthanide transition series (atomic numbers, Z = 57-71) plus yttrium and sometimes scandium. Totally sixteen elements can be used as particulate markers since element 61, promethium, has no stable isotope.

The feed particles can be labeled with an immersion technique or a spray technique. Using the immersion technique the particles are soaked in aqueous solution of the marker for at least 12 h and thereafter washed with water and an acetic acid solution (pH = 4) to remove loosely bound marker. Using the spray techniques the marker is sprayed on dry particles, which afterward are dried to the original dry matter content. Mader *et al.* (1984) compared the spray and immersion methods and concluded that immersion was preferred. The immersion technique does allow for greater exposure to binding sites and a better distribution of marker of interest. The immersion technique should also prevent localized concentration of unbound or loosely bound marker.

Migration of rare earth elements from labeled feeds may occur in the gastrointestinal tract. Teeter *et al.* (1984) measured detachment of Yb during water dialysis and found that feed-Yb complexes dissociated with 0.1 to 0.6 % of bound Yb being released each hour. The same authors calculated *in vivo* migration rates of 0.43 to 0.73 %/h using the data of Hartnell and Satter (1979). Turnbull and Thomas (1987) found that 89.2 % of the recovered Yb was still bound to the corn particles after 48 h of *in vitro* fermentation. Beauchemin and Buchanan-Smith (1989) reported that only 57.7 % of Yb was recovered on silage particles after 48 h ruminal *in situ* incubation, and Stensig *et al.* (1998) reported even lower recovery; 53 and 15 % after 24 and 48 h, respectively. However, Beauchemin and Buchanan-Smith (1989) found much higher marker recovery in the bags when the feed was labeled with Cr. Also other investigators have found that Cr binds more tightly to the particles than rare earth elements (Minde and Rygh, 1997; Udén *el al.* 1980). However, not all of the Yb disappearance was necessarily caused by migration, Yb could also be disappeared by loss of small particles from the bags.

The degree of migration for rare-earth elements has been shown to increase with decreasing pH (Ellis *et al.*, 1983). Therefore much of the marker will disappear when the particles enters the acid condition in abomausum. Allen (1986) recommended that rare-earth marker should be used in gastrointestinal that are near neutral such as rumen, and that samples should be taken from the rumen via a fistula or, to reduce sampling problems, from an abomasums fistula or duodenal cannula. In contrast Ellis *et al.* (1994) claimed that post-ruminal displacement of marker was of little consequence because the ruminal digesta is the primary

if not sole source of variation in flow of particulate matter and solutes. Components of the digesta also have comparable flow rates subsequent to abomasum (Silicano-Jones and Murphy, 1986). Other investigators have compared ruminal passage rates from duodena and faecal sampling and reported that ruminal passage rate could be accurately predicted from fecal sampling (Prange *et al.*, 1982; Pond *et al.*, 1988; Poore *et al.*, 1991; Huhtanen and Kukkonen, 1995).

The degradability may be depressed and the marker labeling may alter the physiochemical properties of the feedstuff. The consequence is that the labeled particles conduct different than unlabelled particles of the same feedstuff. Teeter *et al.* (1984) found that addition of Yb or Dy to feed components decreased *in vitro* disappearance of dry matter compared with extracted with either water or acid. Yb labeling also decreased extent of dry matter in situ digestion. Moreover the Yb disappearance averaged 54 % of dry matter disappearance and Yb concentration of residual undigested feed increased over time. This would be expected if Yb is bound to the fibrous, less digestible fraction of feedstuff or if Yb decreases feedstuff digestibility. Also Ledoux *et al.* (1985) reported that their data indicated that 90 % of the Yb binds to the seed coat of corn kernels rather than to the more digestible starch components. Other studies (Allen and Van Soest, 1982; Stensig *et al.*, 1998) have revealed depression in degradation when the feedstuff was labeled with rare earth markers. Degradation of Cr mordanted feeds are more depressed than rare earth labeled feeds (Minde and Rygh, 1997), and their physiochemical properties may be greatly affected (Ramanzin *et al*, 1991). Ehle (1984) found that specific gravity of feedstuff particles increased with increasing marker concentration when the feed was mordanted with chromium. In contrast, Poore *et al.* (1991) found no effects on functional gravity when particles were labeled with Dy and Yb.

2.4 Protein Degradation in the Rumen

2.4.1 Degradation Mechanism

All three main categories of rumen microorganism; bacteria, protozoa, and fungi have been found to be proteolytic, and they contain a variety of different types of proteolytic enzymes (Wallace *et al.*, 1997). Bacteria are the principal microorganisms involved in protein

metabolism (Broderick *et al.*, 1991). Ciliate protozoa, and probably to a lesser extent anaerobic fungi, also carry out proteolysis, peptidolysis, and deamination, but are generally considered to be less important than bacteria. The first step in breakdown by bacteria is adsorption, either of soluble protein to the bacterial surface or of bacteria to insoluble protein. The access to the protein by the proteolytic enzymes is affected by the physiochemical structure of the protein molecule and it seems that this accessibility is the most important factor influencing protein degradation in the rumen (NCR, 1985). Soluble proteins are in general more susceptible to degradation than insoluble proteins (Wallace *et al.*, 1997). Proteins with extensive cross-linking, such as disulfide bounds are less accessible to proteolytic enzymes and thus relatively resistant to degradation (NCR, 1985). After initial proteolysis, the large polypeptides are broken down to oligopeptides, which are broken further down to dipeptides and amino acids. The major peptidase activity in the rumen is aminopeptidase, which is characterized by dipeptides rather than single amino acids being cleaved from the peptide chain (Wallace *et al.*, 1997). The principal form in which peptides or amino acids enter the bacterial cell has not yet been clearly defined. Some experiments have indicated that the mixed microbial populations preferentially take up peptides rather than free amino acids (Wright, 1967; Chen *et al.*, 1987), whereas other investigators (Armstead and Ling, 1993; Ling and Armstead, 1995) found that several bacterial species preferred free amino acids rather than peptides. In the microbes the peptides and amino acids are hydrolyzed, and microbial protein is synthesized from amino acids, ammonia, and possibly peptides. The synthesizing of microbial protein requires energy, and in situations where energy is limiting, peptide catabolism may lead to excessive ruminal ammonia production (Wallace, 1996). Ammonia is transported out of the microbes and absorbed through the rumen wall. Figure 2.6 shows a model of the different nitrogen transactions in the rumen.

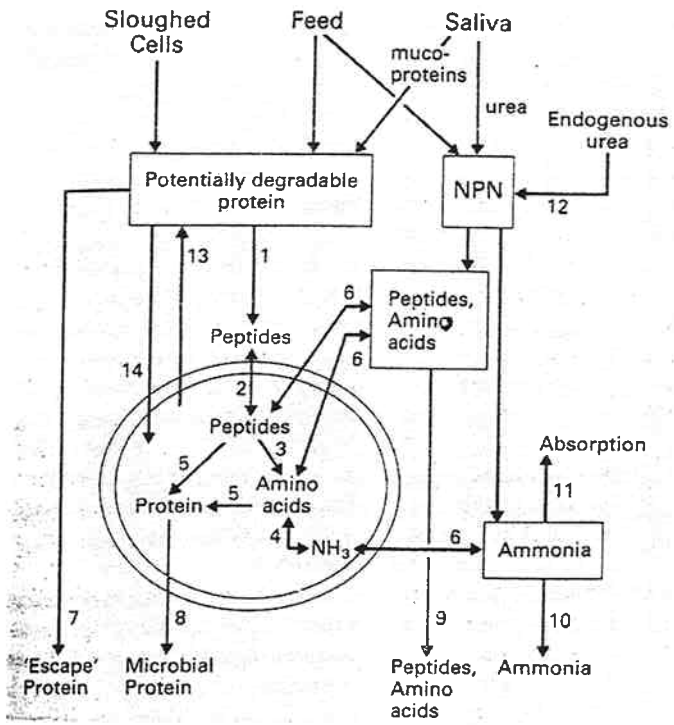

Figure 2.6 A model of nitrogen transactions in the rumen. The ovals delineate the microbial cell wall and numbers adjacent to arrows refer to individual pathways as follow: 1 = proteolysis by bacterial, protozoal and fungal proteases, 2 = carrier-mediated peptide uptake across microbial cell walls, 3 = peptidolysis, 4 = amination / deamination, 5 = protein synthesis, 6 = microbial assimilation / excretion or equilibration of amino acids and ammonia, 7 = protein not hydrolysed before efflux from rumen, 8 = microbial protein efflux, 9 = efflux of extracellular peptides and amino acids, 10 = efflux of extracellular ammonia, 11 = absorption of ammonia through rumen wall, 12 = movement of endogenous urea through the rumen wall, 13 = N compounds excreted by living cells and debris of lysed cells, 14 = engulfment of proteinaceous particles by protozoa. (From Nolan, 1993).

2.4.2 Degradation Models and Estimation of Effective Degradability

The firs order model is the simplest and one of the most commonly used models used to estimate degradation parameters from *in vitro* and *in situ* measurements. This model assumes that the degradation rate is a constant fraction of the available substrate, and the model is normally presented on the following form (Ørskov and McDonald, 1979):

$$D(t) = A + B(100 - e^{-ct}) \tag{2.3}$$

where D(t) is the percentage degraded as a function of time, A is the soluble fraction, B is the potential degradable fraction, and c is degradation rate (h^{-1}) of B.

Many feeds have showed a sigmoid degradation profile, and several models for fitting sigmoid degradation responses have been proposed. McDonald (1981) added a discrete time delay to the first order model by Ørskov and McDonald (1979). The model of McDonald was by Dhanoa *et al.* (1995) extended to permit the fractional degradation rate to vary with time, and the residuals for the early stages of incubation were significantly reduced compared to the model of McDonald (1981). Van Milgen and Baumont (1995) compared logistics and Gomperetz-like models with the discrete time lag model of McDonald (1991), and they found similar residual patterns for all three models. López *et al.*, (1999) used the equations above in addition to generalized Michaelis Mentens and Von Bertalanffy models. Based on statistical criteria, the models of McDonald, (1981), Dhanoa *et al.*, (1995), generalized Michaelis Mentens and Von Bertalanffy models were better than the other for most statistical tests and disappearance curves. However, when the extent of degradation was calculated for each forage and feed component, differences between the estimates obtained with the different models were of little nutritional significance for the animal. Thus, if the primary objective is to determine effective degradability, the choice of model seems to become less important.

In order to calculate effective degradability (ED) which describe the mean degradability of particles escaping the rumen, we can average the degradability of the digesta particles in small time intervals (Scott Fogler, 1992):

$$\begin{pmatrix} ED \text{ of the} \\ \text{particles in} \\ \text{the exit stream} \end{pmatrix} = \sum_{\substack{\text{all elements} \\ \text{of exit stream}}} \begin{pmatrix} \text{Degradability} \\ \text{achived after} \\ \text{spending a time} \\ t \text{ in the rumen} \end{pmatrix} \times \begin{pmatrix} \text{Fraction of feed} \\ \text{particles in the exit} \\ \text{stream which is of age} \\ \text{between } t \text{ and } t + \Delta t \end{pmatrix}$$

When $\Delta t \rightarrow 0$, we have

$$ED = \int_{t=0}^{\infty} D(t) \cdot E(t)\ dt \tag{2.4}$$

where D(t) is the degradation model and E(t) is the RTD model. This general expression may be used for any combinations of degradation and RTD models.

2.4.3 Different Techniques for Measuring Ruminal Degradation

Ruminal degradation are estimated from three main techniques:

In vivo: The total non-ammonia N and microbial N flow in duodenum are measured, and feed N is calculated as the difference between these two fractions.

In situ: The feed samples are incubated in porous bags and placed in the rumen through the rumen fistula. The samples are then taken out at different times, and kinetic parameters can be estimated from the disappearance curves.

In vitro: Samples are incubated in tubes or flasks with buffer solution and ruminal fluid as an inoculum.

The *in vivo* measurements are the most expensive, labor intensive and time-consuming of these three main techniques. Also it is almost impossible to use this technique to estimate degradation of individual feeds or to take up kinetic time series data. The *in situ* technique is much lower in cost compared to the *in vivo* technique and has been used as a reference technique to estimate protein degradation in several protein evaluation systems for ruminants (Madsen, 1985; Vérité *et al.*, 1987; Tamminga *et al.*, 1994). The main advantage with this technique compared to the *in vitro* technique that it involves degradation processes that occur in the rumen of a living animal (Stern *et al.*, 1997). Several *in vitro* techniques have been introduced (see reviewes by Stern *et al.*, 1997 and Hvelplund and Weisbjerg, 1998).

2.4.4 THE *IN SITU* TECHNIQUE

The *in situ* technique has been used extensively for measuring ruminal degradation of feedstuffs. However, this technique has large variation, both within and between laboratories (Madsen and Hvelplund, 1994). The *in situ* technique have been discussed very extensively by the reviews of Lindberg (1985), Noek (1988), Michalet–Doreau and Ould-Bah (1992), and Vanzant *et al.* (1998), and the different factors that may affect the degradability estimates are listed in Table 2.1.

Table 2.1 The different factors that affects the degradability estimates by the in situ technique.

Bag and sample characteristics	Diet effects	Procedural considerations
Bag porosity	Diet composition	Preincubation
Feedstuff particle size	Feeding level	Timing of insertion and removal
Ratio of sample size to bag surface area	Frequency of feeding	Bag location within the rumen
		Rinsing procedures
		Microbial correction
		Correction for small-particles loss
		Incubation times and mathematical models

The effects listed in table 2.1 should be standardized to decrease the variability with this technique and to make easier comparisons between laboratories. Some of these factors such as ratio of sample size to bag surface area and bag porosity have been extensively studied and similar values are used across different recommended procedures (Lindberg, 1985; Noeck, 1988; AFRC, 1992; Michalet-Doreau and Ould-Bah, 1992; Madsen and Hvelplund, (1994); Vanzant *et al.*, 1998). Other factors such as rinsing procedure and quantifying microbial contamination are not well standardized, and these effects provide the greatest benefits to

increasing the precision of the procedure (Vanzant *et al.*, 1998). As already mentioned, which degradation model to choose seems not to be important if the objective is to estimate the degradability (Vanzant *et al.*, 1998; López *et al.*, (1999).

Substantial variation still exists among animals, across days within the same animal, and between replicate bags within these animals on a given day even if all effects listed in Table 2.1 is well standardized. Volden and Harstad (unpublished data, see Volden 1999) introduced a control sample of soybean meal and repeated the control sample 27 times over a period of 15 months. They found that the variations attributed to animal effects were 22 % and to repetitions 43 %. In their literature review Vanzant *et al.* (1998) found lower variation caused by repetition than Volden and Harstad (Volden 1999), which may be explained by much shorter repetition period in the studies reviewed by Vanzant *et al.* (1998). In their study, Volden reported water soluble protein fraction ranging from 3 to 23 % although the same washing machine was used. The results by Volden (1999) agreed with those of Vanzant *et al.* (1998) who observed that much of the variation in degradability between days was due to differences in washing effectiveness.

In *in situ* measurements the fraction disappearing from the bag after washing only, is normally assumed to be instantaneously degraded. However, this assumption is questioned, and the so-called soluble fraction is probably more precisely divided into three fractions (López *et al.*, 1994; Madsen and Hvelplund, 1994; Dhanoa *et al.*, 1999):

1. A true soluble fraction
2. An escaped insoluble fraction, consisting of mainly small particles, which probably are degraded at a faster rate than the particles remaining in the bag
3. An insoluble non-degradable fraction.

We have to make some assumptions for the escaped wash fraction in the degradability estimations since the degradation kinetics of this fraction is not measured:

1. It consists of a degradable and non-degradable fraction of the same ratio as for the particles remaining in the bag after washing.
2. The degradation rate was the same for the particle loss fraction as for the particles remaining in the bag.

3. The passage rate was the same for the particle loss fraction as for the particles remaining in the bag.

Dhanoa *et al.* (1999) discussed these assumptions and also suggested other such as using the liquid passage rate for the wash fraction. Table 6 present estimated protein degradability corrected for the escaped wash fraction in three different ways:

Correction 1: Using all assumption above

Correction 2: Using the liquid passage rate ($k_p = 0.12\ h^{-1}$) of the escaped wash fraction

Correction 3: Using double degradation rate of the escaped wash fraction.

Correction 2 gave the largest deviations from the uncorrected values, whereas correction 3 gave the smallest deviations. Madsen and Hvelplund (1994) reported large variability in the measure true solubility in a European ring test, and they suggested that the filter paper should be standardized to reduce this variability.

Table 2.2 Estimated protein degradability[a] for different assumptions for correction for small particle loss of the wash fraction.

	Uncorrected	Correction 1	Correction 2	Correction 3
Fish meal	46.0	36.7	35.5	38.0
Barley	74.2	66.9	64.8	70.3
Oats	91.2	81.1	78.1	87.0
Soybean meal, extracted	62.7	59.0	57.4	60.1
Rape seed meal	71.6	64.8	62.7	67.6
Rape seeds	63.0	59.2	58.7	60.2
Lupine seeds	86.5	82.7	81.0	84.8
Peas	86.4	81.0	78.8	82.4
Concentrate mix	71.0	56.9	53.6	61.8
Maize	32.5	28.7	27.8	28.4
Dried beet pulp	56.8	55.3	55.0	55.8
Maize gluten meal	23.6	19.6	19.1	19.8
guar meal	68.3	66.4	65.5	67.1

[a] Using passage rate of $0.06\ h^{-1}$

Some simplified *in situ* techniques have been proposed for degradability measurements. Wilkerson *et al.* (1995) used only the 16 h ruminal incubation time to estimate the ruminal protein degradability in roughages by assuming that the 16 h sample directly estimated the escape protein. Broderick (1994) proposed a model that was based on estimating the degradation rate from the wash sample (0 h) and one sample incubated in the rumen (16 h) and using acid detergent insoluble protein (ADIP) as an estimate of the total undegradable fraction. Vanzant *et al.* (1996) used this simplified technique to calculate the protein degradability in prairie hay and alfalfa. Using only the 0- and 16 h points to construct degradation rates resulted in similar estimates of protein degradation when compared with full time-series calculations. Calsamiglia *et al.* (1994) observed that degradation rate and degradability based on double point incubations (0 and 24 h or 2 and 24 h) explained at least 99% of the variation in measurements determined from curves based on seven time-points in the range from 0 to 24 h.

2.4.5 The Effect of Particle Size on Ruminal Degradation Kinetics

As already mentioned the first degradation step is adsorption of rumen microorganism to the particle surface, and the degradation processes occur on the surface of the particles. It is reasonable to expect a faster degradation rate of small particles since smaller particles have larger specific surface area than larger particles. Both *in vitro* and *in situ* techniques have been used to study the effect of particle size on degradation kinetics. The general picture is that most investigators found decreasing degradability with increasing particle size (Bayourthe *et al*, 2000; Beuchemin *et al.*, 1994; Bjorndal *et al.*, 1990; Cerneau and Michalet-Doreau, 1991; Cone *et al.*, 1989; Lykos and Varga, 1995; Michalet-Doreau and Cerneau, 1991; Nordin and Campling, 1976; Stern *et al.*, 1994; Wadhwa *et al.*, 1998). Cerneau and Michalet-Doreau (1991) and Michalet-Doreau and Cerneau (1991) studied *in situ* degradation of starch and protein for both concentrates and roughage feeds grinded on 0.8 mm, 3.0 mm, and 6.0 mm screens, respectively. Both degradation rate and solubility decreased with increasing particle size for barley and pea. Also Bayourthe *et al.* (2000) found that particle size of pea strongly influenced protein and starch degradation. Beauchemin *et al.* (1994) studied the in situ degradation of whole, chewed, halved and quartered particles of barley, maize and wheat, and they found that the particle size affected the degradation kinetics for all feeds. Neither Cerneau and Michalet-Doreau (1991), Michalet-Doreau and Cerneau (1991),

and Wadhwa *et al.* (1998) found any effects of particle sizes on the starch, protein, and DM degradation rates in maize. Cone *et al.* (1989) found decreasing in vitro starch degradation after 6 h by increasing particle size for maize. Both Lykos and Varga (1995) and Stern *et al.* (1994) reported of increased degradability for smaller soybean particles.

CHAPTER 3

SUMMARY OF THE RESULTS IN THE PAPERS

3.1 USE OF LASER DIFFRACTION FOR DIGESTA PARTICLE SIZE MEASUREMENTS (PAPER I)

Wet and dry sieving are the dominant techniques for particle size measurements in ruminant research. These techniques have been used for several purposes in research of ruminant digestion, such as particle breakdown (Ehle *et al.*, 1982; Luginbuhl *et al.*, 1990), passage (Kennedy, 1995), and resistance to flow (Poppi *et al.*, 1980, 1985). We wanted to study the resistance to flow as a function of both particle size and sedimentation velocity (paper II). Two factors with this experimental set up made sieving techniques inappropriate for particle size measurements:

- The samples were too small to get reliable results by sieving
- A large number of samples, which would require a substantial amount of human labour if the wet sieving technique should be used.

We therefore wanted to test if particle size distributions measured by wet sieving and laser diffraction (LD) were comparable. A further objective was to compare particle size distributions measured by LD and microscopic image analysis (MIA). MIA is often used as an absolute method for particle size analysis because it is the only method whereby the individual particles are observed and measured.

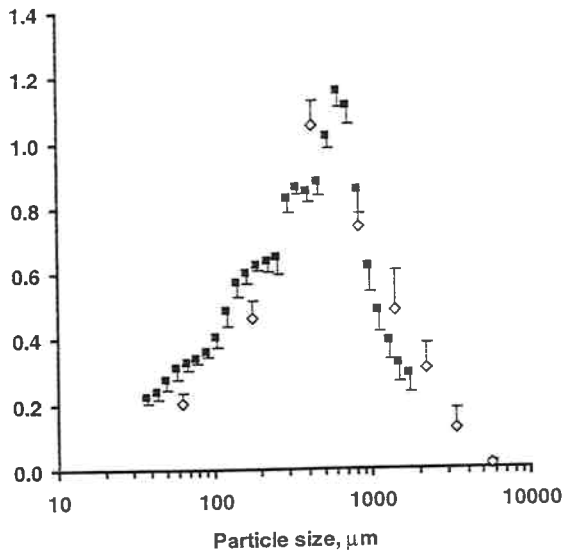

Figure 3.1. Duodenal particle size distribution from one cow measured by wet sieving (◊) and laser diffraction (■). Standard error of mean is represented by vertical bars pointing upward for wet sieving and downward for laser diffraction. The dimensionless ordinate is scaled to give areas under the graphs equal to one when the logarithmic values of the particle sizes are used.

Duodenal samples from three lactating cows were used to measure the particle size distribution by wet sieving and by laser diffraction (LD). The median particle size was calculated in the size range where the methods overlapped, and the median particle size was not different between the methods ($P = 0.98$). The particle size data were also fitted to a log normal distribution function (Figure 3.1). The logarithmic mean particle size ($\log_{10}$mean) tended to be larger for wet sieving ($P = 0.06$), and the logarithmic standard deviation ($\log_{10}$SD) was similar for the two methods ($P = 0.32$). In addition, duodenal samples from the same three animals were fractionated into five different size classes by wet sieving. Particle

size distributions were measured by LD and by microscopic image analysis (MIA) for each of the size classes. The $\log_{10}$mean particle size calculated by LD was inside the sieve size range of the four smallest size classes. Log_{10}mean particle size calculated by LD was smaller than $\log_{10}$mean calculated by MIA for two size classes (106 to 300 µm, and 300 to 600 µm), and LD gave generally broader particle size distributions than MIA. This study showed that duodenal particle size distributions measured by LD and wet sieving were similar and LD is therefore an alternative method for particle size distribution measurements in post ruminal digesta.

3.2 The Effects of Particle Size and Density on the Rumen Residence Time (Papers II and III)

As mentioned in chapter 2.2.2 three main techniques have been used to study the effect of particle size on the passage rate:

1. Comparing particle size distribution in the rumen with the particle size distribution in the lower gut.
2. Study the excretion curves of labeled particles
3. Study the excretion curves of inert particles (indigestible particles of plastic material).

In the first method the residence time distribution is not measured directly. However, if MRT of a given particle size is known MRT, for other particle sizes can be calculated by comparing the particle size distribution in the rumen and the lower gut. We used the two first techniques to study the effect of particle size and sedimentation velocity on rumen RTD.

In paper II we measured the relative resistance to escape the rumen as a function of the size and sedimentation velocity of the particles. These measurements were carried out by comparing the particle distributions in duodenum and rumen. Particles with a size and sedimentation velocity with high concentration in duodenum compared to rumen will escape the rumen at a high passage rate, and particles with a size and sedimentation velocity not found in duodenum will not escape the rumen. The relative resistance can be used to measure the passage rate under the assumptions of steady state, representative sampling, and no

changes in particle properties between the rumen and duodenum. Figure 3.2 shows the estimated passage rate as a function of particle size and square root of sedimentation velocity. Particles with a diameter of 0.3 mm had the highest passage rate, and the passage rate increased with increasing sedimentation velocity. In particular, the passage rate was very low for particles with low sedimentation velocity (0.38 mm/s) and particle size larger than 0.75 mm. The effect of sedimentation velocity on the passage rate was stronger when the animals were fed at low feeding level. At low feeding level there was no fibrous mat on the top of the rumen, and the particles were therefore able to settle more freely than at a high feeding level. Therefore, the rumen may separate particles with respect to their sedimentation/flotation properties more effectively at a low feeding level.

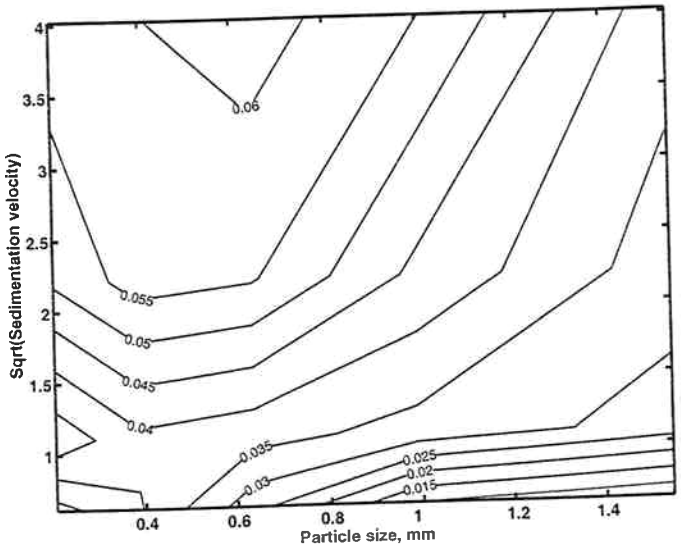

Figure 3.2 Contour plot of the estimated passage rate as a function of particle size and square root of sedimentation velocity (mm/s). The distance between the contour lines are equally distributed with a distance of 0.005 h^{-1}

Barley ground on a hammer mill with sieve size of 3 mm was labelled with Yb (see paper III for more details), dried and sieved into four different size classes: 106 – 355 µm, 355 – 850 µm, 850 – 1400 µm, and 1700 – 2800 µm. The labelled particles were pulse dosed into the rumen via the rumen fistula simultaneously with the morning feeding, and duodenal samples were obtained at 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 24, 28, 32, 36, 42, and 50 h after administration of the dose.

We evaluated a RTD model with two CSTR series and a RTD model with a gamma distributed and a mixing compartment in series to fit the experimental data. Both models were used with or without a time delay incorporated in the model. The RTD model with two mixing compartments in series with a time delay fitted the experimental data best for the duodenal Yb excretion curves, and this model is expressed by:

$$E(t) = \frac{k_1 k_2}{k_2 - k_1}\left[e^{-k_1(t-\tau)} - e^{-k_2(t-\tau)}\right], \qquad \text{for } t \geq \tau \qquad (3.1)$$

$$E(t) = 0 \qquad \text{for } t < \tau$$

where k_1 and k_2 are the passage rates (h^{-1}) of compartment 1 and 2 ($k_1 \neq k_2$), and τ is the time delay (h).

Generally, there was no large variation in MRT between the different particle size classes (Table 3.1). The MRT increased by increasing particle size for the three smallest size classes, but decreased surprisingly for the largest particle size. By visual observation, much of the particles in the largest size class consisted of spherical pieces of kernels, whereas the smaller size classes consisted of much more elongated shell fractions. This difference in particle shape may explain why MRT decreased for the largest particle size.

Figure 3.2 shows that the passage rate decreases with increasing particle size, but the figure also shows that the passage rate increases as the sedimentation velocity increases.

The relationship between sedimentation velocity and particle size is given by Stokes law (Perry *et al.*, 1984), which states that sedimentation increases proportional to the square of the particle size for particles with equal size and density:

$$v = K_1 \frac{g\ s^2(\rho_p - \rho)}{18\mu} \qquad (3.2)$$

$$K_1 = 0.843\ \log(\psi / 0.065)$$

where ψ = the sphericity (surface area of a sphere having the same volume as the particle divided by the surface area of the particle), g = the local acceleration due to gravity, s = "particle size" (diameter of a sphere of equal volume), ρ_p = particle density, ρ = fluid density, and μ = fluid viscosity. Figure 3.2 indicates that we can expect small variations in passage rate for different sized particles of a feedstuff where the particles have equal density and shape over the particle size. Turnbull and Thomas (1987) found that cracked maize passed from the rumen at 33% faster rate than steam-rolled maize with the same particle size, which indicate that different particle shape can give different MRT.

Table 3.1 Model parameters (equation 3.1) and mean residence time for the residence time distribution model with two mixing compartments in series with a time delay.

Particle size, mm	k_1	k_2	τ	MRT
0.21 – 0.35	0.32	0.064	2.1	21.0[a]
0.35 - 0.85	0.33	0.057	2.9	24.4[ab]
0.84 – 1.4	0.31	0.051	2.3	26.0[b]
1.7 – 2.8	0.45	0.057	3.0	23.6[ab]

[a,b] Significant differences ($P < 0.05$) for different superscript letters inside same column.

3.3 The Effect of Particle Size on Estimated Degradability (Papers III and IV)

In paper III we measured DM, starch, and protein *in situ* degradation parameters for three particle sizes of barley, oats, and maize. The degradation rate was low for maize compared to the other two feeds. The degradation rate and soluble fraction decreased by increasing particle size for all feed components of barley and maize. In contrast to barley and maize the middle particle size had the highest degradation rate for oats. Also the soluble fraction was very high for the different feed components for the smallest particle size in oats. In the ED calculations we used the same RTD model for all feeds and particle sizes. The starch degradability of maize decreased sharply as the particle size increased, from 79.9 % to 42.0 % between the smallest and largest particle size. This effect of particle size was also large for protein degradability with a difference of 18 percentage units between the smallest and largest particle size. The starch and protein degradability of barley decreased only slightly from the smallest to the middle particle size, but decreased by 7.4 and 7.6 percentage units from the middle to the largest particle size. The starch degradability of oats was very high for all particle sizes, whereas the protein degradability of the middle particle size was 2.8 and 6.7 percentage units larger than the smallest and largest particle size.

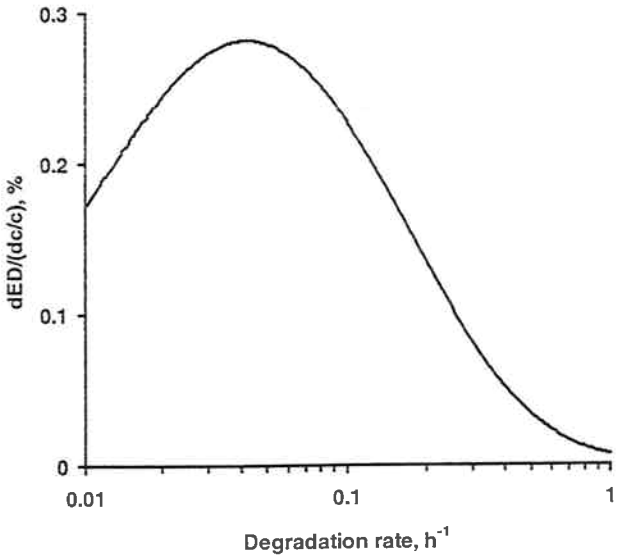

Figure 3.3 The effect of a relative increase in degradation rate on the estimated degradability for different degradation rates. The calculations were carried out assuming that A = 0 % and B = 100 %.

The drop in degradation rate for starch and protein with increasing particle size was substantial for both barley and maize. However, the drop in ED was much larger for maize than barley. Figure 3.3 shows the effect of a relative increase in the degradation rate on the change of the degradability. The figure shows that the effect is largest the degradation and passage rates are in the same size order (approximately 0.04 h^{-1}). The starch degradation rates of barley were high for all particle sizes and thereby the degradability was also high. In contrast, the starch degradation rates of maize varied in the range 0.028 - 0.080 h^{-1}, which is around the maximum point of the curve in figure 3.3. Therefore, changing the degradability by altering the particle size distributions will have largest effect on feeds with relatively slow degradation rates.

In paper IV the data in paper III and some literature data are used to develop models that describe the effect of particle size (s) on degradation kinetics and RTD. These models were used to describe the degradability as a function of particle size. Three different models were used for MRT as a function of particle size:

1. MRT constant across particle size $MRT(s) = 19.8$ (3.3)
2. MRT increased linearly: $MRT(s) = 19.8 + 1.41 \cdot s$ (3.4)
3. MRT increased more steeper $MRT(s) = 19.8 + 1.41 \cdot s + 0.4 \cdot s^2$ (3.5)

Figure 3.4 shows the effect of particle size on estimated starch and protein degradability of barley and maize. The difference in ED between the three RTD models is small for small particles, but increases for larger particles.

Laurinen *et al.* (2000) presented data for barley particle size distribution from different grinding techniques. These data were used to estimate ED from different grinding techniques. Table 3.2 shows estimated protein degradability from hammer mill and crimping roller mill. The degradability was lower for crimping roller mill, but the difference in ED was small between the degree of grinding fineness within each milling type.

Table 3.2 Estimated degradability for different grinding techniques using the RTD models described in equations (3.3), (3.4) and (3.5)

		Hammer mill[a]		Crimping roller mill[b]	
Feed fracion	RTD model	Fine	Coarse	Fine	Coarse
Protein	1	84.7	82.7	77.7	74.1
	2	84.1	81.8	75.4	70.6
	3	84.7	83.0	78.7	76.2
Starch	1	95.8	94.0	89.0	84.6
	2	96.2	94.6	90.2	86.2
	3	96.1	94.6	91.0	88.5

[a] Sieve size 3 and 5 mm for fine and coarse, respectively.
[b] gap between rolls 0.5 and 0.8 mm for fine and coarse, respectively.

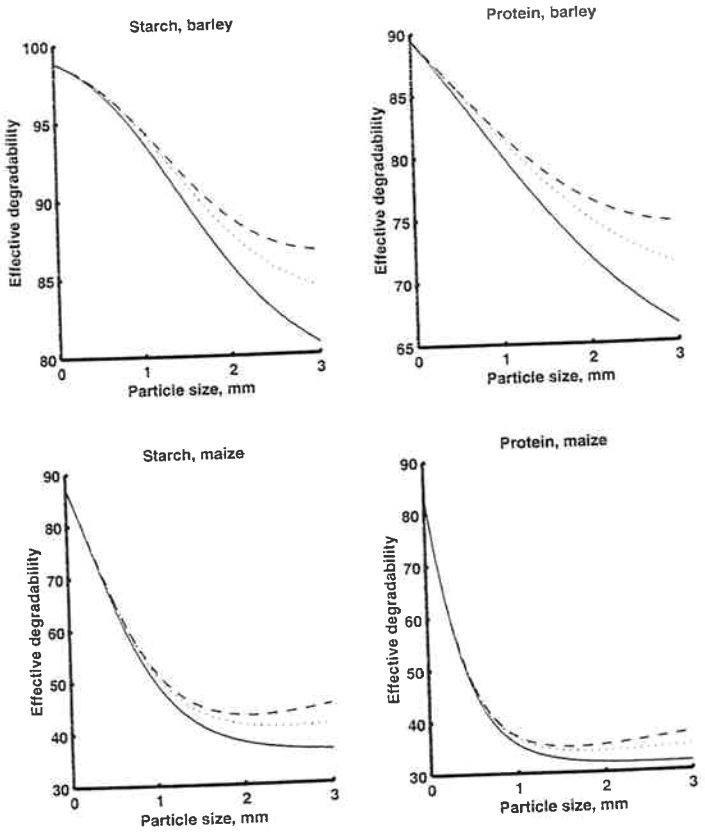

Figure 3.4 Estimated starch and protein degradability of barley and maize as a function of particle size. ····· = RTD model 1, —— = RTD model 2, ----- = RTD model 3

In conclusion, different grinding treatments of barley did not give very variable degradability estimates. However, in Norway it is normal to treat the cereal with an expander. This treatment decreases substantially the degradation kinetics of protein (Prestløkken, 1999). The degradability would be strongly particle size dependent if the degradation kinetics were depresses to the range we observed for starch degradation of maize (Figure 3.3). Therefore, it is reasonable to believe that we would observed a much stronger particle size dependency in expander treated barley.

3.4 The Effect of the Residence Time Distribution Model on the Estimated Degradability (Paper III)

The CSTR model is the most commonly used RTD model in degradability calculations. This model is based on the assumption of instantaneous and complete mixing of all particles entering the rumen, constant input, outflow and rumen mass, and same probability for escape of all particles, which gives a pure exponential RTD function. Many studies have shown that particulate and liquid rumen RTD functions requires more complex flow models, and therefore several RTD models have been proposed (See Chapter 2.3.1). The effective degradability is estimated by integrating the RTD and degradation curves (Equation 2.4). Therefore, it is important to use a RTD curve with a correct shape to get precise estimates of the degradability. Table 3.3 shows estimated degradability for concentrates using the RTD model expressed in equation 3.1 (M1), a CSTR model (M2) with equal MRT, and the difference (Δ) between the two models. The difference varied from 0.4 to 8.3 percentage units and was generally largest for barley and smallest for maize. The CSTR model overestimates the outflow rate the first hours and gives therefore an underestimation in degradability. This underestimation is largest in feeds where most of the degradation takes part over the time period where the RTD models differ most. Figure 3.5 shows how the degradation parameters affect the difference in ED from the two RTD models. The difference between the two models is largest at a degradation rate of 0.18 h^{-1}.

Table 3.3 Effective degradability for different fractions of barley, oats and maize estimated using different RTD models.

Feed	Particle size, mm	Dry matter			Starch			Protein		
		M1[a]	M2[b]	Δ[c]	M1[a]	M2[b]	Δ[c]	M1[a]	M2[b]	Δ[c]
Barley	0.21 – 0.36	78.5	74.1	4.4	98.6	94.6	4.0	84.2	77.9	6.3
	0.85 – 1.12	83.9	77.8	6.1	97.0	90.4	6.6	84.0	76.9	7.1
	1.70 – 2.80	84.6	76.8	7.8	89.6	81.3	8.3	76.4	69.5	6.9
Oats	0.21 – 0.36	87.7	86.1	1.6	98.6	96.6	2.0	89.4	87.5	1.9
	0.85 – 1.12	70.9	68.1	2.8	99.5	96.8	2.7	92.2	87.7	4.5
	1.70 – 2.80	84.1	79.2	4.9	98.4	93.8	4.6	85.5	79.4	6.1
Maize	0.21 – 0.36	76.5	72.3	4.2	79.9	75.1	4.8	52.2	51.4	0.8
	0.85 – 1.12	52.8	50.0	2.8	55.2	51.9	3.3	39.1	38.4	0.7
	1.70 – 2.80	48.2	46.0	2.2	42.0	39.8	2.2	34.2	33.8	0.4

[a] RTD model with two CSTR in series with a PFR.

[b] RTD model with one CSTR

[c] The difference in estimated degradability between the two RTD models

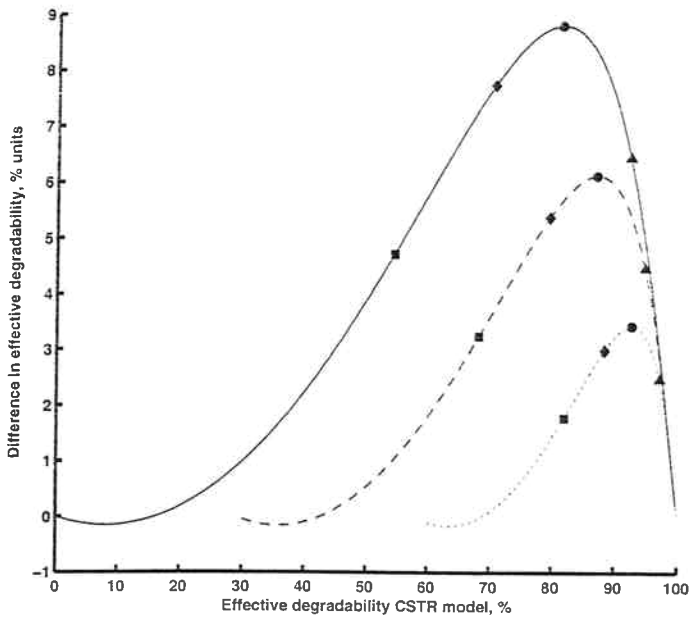

Figure 3.5. The effect of various first order degradation parameters on the difference in ED calculated with a single CSTR and two CSTR in series with a PFR. Each curve is obtained by keeping the A-values constant ((—— A = 0), (- - -, A = 30) and (····, A = 60)) and let the c-values vary (A + B = 100% for all curves). Values for c = 0.05 (■), c = 0.1 (◆), c = 0.18 (●), and c = 0.5 (▲) are also shown.

3.5 Simplified in situ Technique (Paper V)

Several methods are used to estimate degradation of dietary organic matter (OM) in the rumen (Hvelplund and Weisbjerg, 1998). The in situ method has achieved the widest application. In spite a number of limitations, no other in vitro method has been generally accepted as a satisfactory alternative (Stern *et al.*, 1997; Hvelplund and Weisbjerg, 1998). Therefore, the in situ technique has been chosen as the reference method to measure rumen protein degradation in several protein evaluation systems for ruminants (Madsen, 1985; Vérité and Peyraud, 1989; Tamminga *et al.*, 1994; NCR, 2001). However, the method requires a large number of nylon bags for each feed sample, and therefore a substantial amount of human work. It is therefore a need for simpler in situ techniques, in particular for routine analysis (Vanzant *et al.*, 1996).

In paper V we used dry matter (DM) and crude protein (CP) data from 135 different concentrate feeds was used to test if the number of incubation times in the in situ procedure could be reduced without losing too much accuracy. Effective degradability (ED) was estimated both with and without correction for particle loss of the wash fraction. The protein ED corrected for particle loss was widely distributed in a range from 16 to 90 % with an overall mean value of 60.4 %, and the dry matter ED was distributed in the range from 22.7 to 80.7 % with a mean value of 56.9 %. The simplified method was based on bilinear regression models where the disappearance values were used to estimate ED directly by

$$ED = a_0 + \sum_{i=1}^{n} a_i \cdot D(t_i), \qquad (3.6)$$

where a_0 and a_i are the parameters, $D(t_i)$ are the percentages of DM or CP disappearance from the bags at the times t_i, and n is the number of incubation times used in the model. For uncorrected data this general model was used to test all one (7), two (21), and three time-points (35) model combinations in the range from 0 (wash sample) to 48 hours to determine the time-points optimal for estimating ED. Both the wash- and the true soluble fractions had to be measured when using corrected disappearance values, and the true solubility was therefore incorporated in all bilinear models using disappearance values corrected for particle loss of the wash fraction:

$$ED = a_0 + a_1 s + \sum_{i=2}^{n} a_i \cdot D^{corr}(t_i) \qquad (3.7)$$

where $D^{corr}(t_i)$ and is the corrected the percentages of DM or CP disappearance and s is the true solubility (%).The corrected disappearance values were corrected by:

$$D^{corr}(t_i) = \frac{D(t_i)(100 - sol) - (w - sol)}{100 - w} \qquad (3.8)$$

where $D(t_i)$ and $D^{corr}(t_i)$ are the measured and corrected percentage disappeared at time t_i, sol is the true solubility (%), and w is the wash fraction (%).

More than 97 % of the variation in ED was explained at passage rate at 0.06 h^{-1} when the one incubation-time (8 h) bilinear model was used. For passage rates lower than 0.05 h^{-1} the model based on the 16 h incubation time was best for one time-point. However, with 17 percent of the residuals larger than 4 percentage units (uncorrected model) in absolute value the errors seemed to be too large for using only a single time-point model in routine analyses. Using a two time-points model only 2 percent of the residuals were larger than 4 percentage units in absolute value. The model based on 4 and 24 h incubation times generally showed the best results for the two points models across the passage rates tested. Using uncorrected data the model based on 2, 8, and 24 h incubation times was best of the three time-points models for passage rates higher than 0.047 h^{-1} and the model based on 2, 16, and 48 h was best for lower passage rates. The best combination of ruminal incubation times was similar for the corrected data with the exception that the 2-h incubation was replaced with a 4-h incubation.

Poncet *et al.* (1995) reported that the passage rate of labeled concentrate particles are usually in the range from 0.04 to 0.06 h^{-1} in dairy cows. The Dutch protein evaluating system (DVE/OEB) use a passage rate of 0.06 h^{-1} for concentrates (Tamminga *et al.*, 1994), whereas the Nordic AAT/PBV system use passage rates from 0.04 h^{-1} up to 0.08 h^{-1} depending on the country (Madsen *et al.*, 1995). Both two and three time-points bilinear regression models fitted the data well in the actual passage rate range (0.04 to 0.08 h^{-1}). The estimates were substantially more precise for the three time-points model than the two time-points model(Figure 3.6 and 3.7), and RMSE was also very stable in the most actual passage rate range for the three time-points model. Which of the model to choose is therefore a question of what to stress the most: either simplicity or precision. The bilinear regression models reduced the number of nylon bags substantially compared to the reference in situ method. The amount of manual human work and thereby costs can therefore be considerably reduced. In addition the reduction of total incubation time to 24 h will increase the number of samples possible to be analyzed in each cow. Additional measurements of the wash value and true solubility have to be carried out if the degradability shall be corrected for small particle loss. Still there will be a substantial reduction of human work compared to the reference method.

In paper V we used only a simple mixing compartment RTD model in the calculations. However, corresponding simplified *in situ* models can be developed for other RTD models such as two CSTR in series

These models can also be applied to other feed fractions such as starch, fiber and individual amino acids although the models were developed for CP data only. Simplified *in situ* models can also probably be used to estimate degradability in roughage feeds, i.e. bilinear regression model based on 2, 16, and 48 h incubation times gave excellent estimates at low passage rates (0.03 h^{-1} – 0.045 h^{-1}).

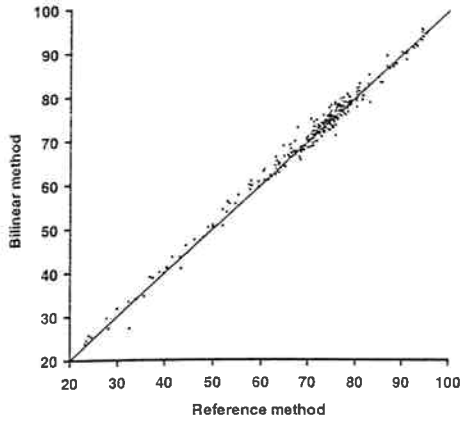

Figure 3.6 Correlation plot for estimated degradability by the reference method and two incubation-times (4 and 24 h) bilinear regression model at a passage rate of 0.06 h^{-1}. The data is not corrected for particle loss of wash fraction.

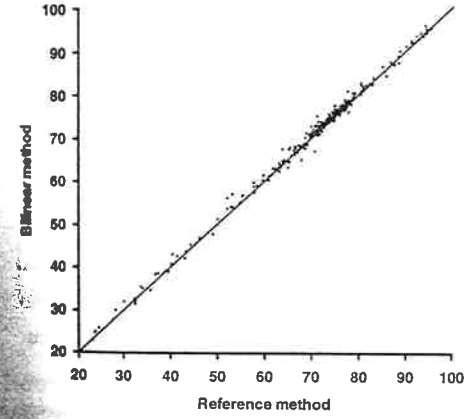

Figure 3.7 Correlation plot for estimated degradability by the reference method and three incubation-times (2, 8, and 24 h) bilinear regression model at a passage rate of 0.06 h^{-1}. The data is not corrected for particle loss of wash fraction.

3.6 Degradation and Escape of Dietary Soluble Nitrogen Fractions (Paper VI)

The extent of ruminal degradation of soluble dietary N is determined by the rate of degradation and by the rate of passage of liquid out of the rumen. Nevertheless, in the Nordic AAT/PBV protein evaluation system for ruminants (Madsen *et al.*, 1995), the soluble dietary N-fractions are assumed to be 100 % degraded in the rumen. However, studies of Chen *et al.* (1987) and Volden *et al.* (1998) showed that small peptides (SP) and free amino acids (FAA) escaped rumen degradation. During ensiling, the protein fraction has been impaired by extensive proteolysis, and on average, half or more of the total N in grass silage is in the form of soluble non-protein nitrogen - mainly small peptides and free amino acids (McDonald, 1981). Therefore, it is important to know how much of the soluble N that escapes the rumen to improve the accuracy of determination of the protein value of grass and grass silage. Moreover, lower efficiency in microbial protein synthesis on grass silage diets compared to grass diets may be due to the asynchronous release of energy and N in grass silage (Siddons *et al.*, 1985). It is vital to know the rate of degradation as well as rumen escape of soluble protein, peptides and free amino acids in grass and grass silage to identify the potential benefits from improved synchronization.

In paper VI we studied *in vivo* ruminal degradation and rumen escape of soluble nitrogen fractions in grass and grass silage. Soluble protein and long chain peptides (PLP), SP and FAA were obtained from fresh grass and grass silage fertilized with different levels of nitrogen. Soluble extracts from the forage were pulse dosed into the rumen of three cannulated lactating dairy cows, and a simple or complex (mechanistic) model was used to examine the ruminal kinetics of soluble N-fractions. When soluble extracts from silage was investigated, pulse-dosages of total non-ammonia N (NAN) was 21, 27, and 32 g, whereas from fresh grass only one dosage of 20 gram was ruminally administered. A schematic description of the complex model is shown in figure 3.8, and we assumed that all processes were of first order. Figure 3.9 shows an example of soluble N profiles in the rumen after administration of a pulse dose of dietary soluble NAN. All three N-fractions decreased rapidly and FAA-N and PLP-N reached equilibrium after 2 h, whereas for SP-N equilibrium was reached after approximately 3 h. The profiles presented in the figure were very typical

for all treatments, which indicated that all three dietary soluble N fractions were very rapidly degraded in the rumen.

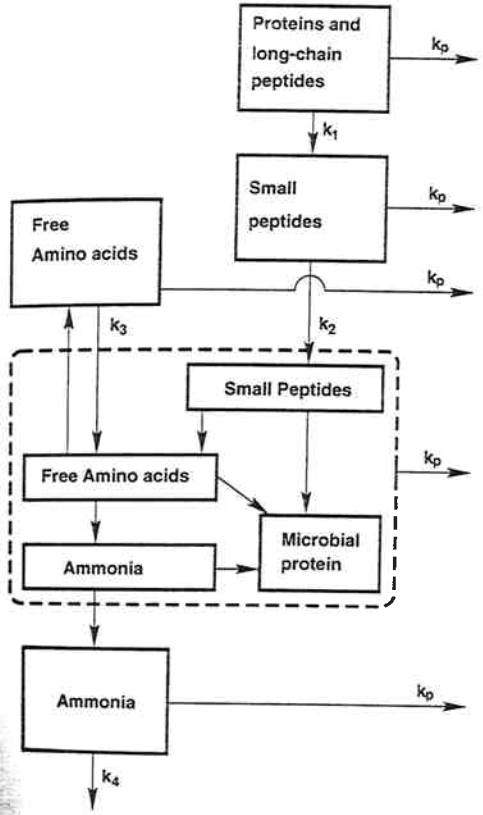

Figure 3.8 Flow diagram of the complex model for prediction of ruminal degradation of soluble N-fractions. The intracellular pools are inside the dashed rectangle. k_p = passage rate, k_1 = degradation rate of proteins and long-chain peptides, k_2 = transport rate of extracellular small peptides into the microbe, k_3 = net transport rate of extracellular free amino acids into the microbe, k_4 = absorbtion rate of extracellular ammonia through the rumen wall.

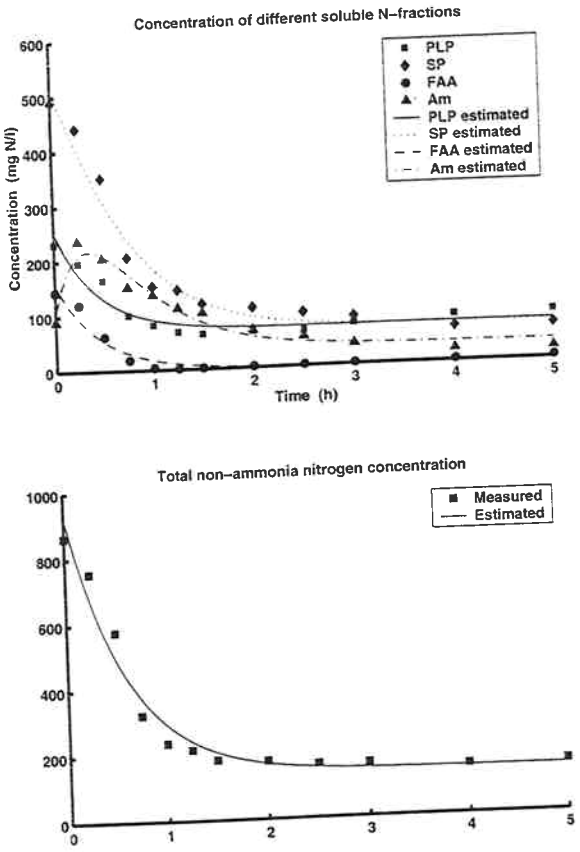

Figure 3.9 Upper: Measured and estimated concentrations of soluble N-fractions in the rumen after intraruminally administered dietary soluble nitrogen (27 g NAN og SN1) ■, proteins and long-chain peptides; ♦, short-chain peptides; ●, amino acids; ▲, ammonia Lower: Measured and estimated total NAN.

Ruminal degradation rate and rumen escape of dietary soluble N-fractions in silage administered to the rumen at three dosages is presented in Table 3.4. No interaction ($P > 0.05$) was observed between soluble N-fraction and dose-size, and the degradation rate decreased linearly ($P < 0.05$) with increased dosage. Across soluble N-fraction, dosage and model, mean escape (% of dose) of soluble dietary NAN in the silages was 8.5 % and because of the slower degradation rate, the ruminal escape was higher for SN1 than for SN0 and SN3. Therefore, this studies indicate that when taking into account all soluble N-fractions, approximately 10% of the dietary soluble N will escape the rumen and thus contribute to the intestinal amino acid flow and affect the protein values of the feed.

For the GN1, only 24 % of the N dose were converted to ammonia, whereas for the silage it was three times higher. For all silage, across dosage, on average 76 % of the N dose was converted to ammonia. Wallace (1996) state that if energy is insufficient supplied, or when the rate of peptide degradation exceeds the rate of amino acids used for microbial protein synthesis, peptide catabolism leads to excessive ammonia production. This means that low conversion efficiency in grass silage could be explained by an imbalance of dietary nitrogen and energy. These results show that it is important to feed adequate amounts of easily fermentable carbohydrates when grass silage is the main forage in the diet.

Table 3.4 Rumen fractional degradation rate and ruminal escape of intraruminally administered soluble nitrogen fractions from grass silage in dairy cows at three levels of dosage. Parameterized according to a simple or complex model for describing ruminal degradation of soluble dietary N-fractions

	Soluble N-fraction[a]			Soluble nitrogen dosage, mg			SEM	Contrast[b]	
Item	PLP-N	SP-N	FAA-N	21	27	32		1	2
Simple model									
Degradation rate, %/h	222[x]	127[y]	281[z]	252	206	171	9.4	< 0.01	< 0.01
Ruminal escape, % of dose	8.1[x]	13.6[y]	5.9[z]	7.9	9.4	10.3	0.5	< 0.01	< 0.01
Complex model									
Degradation rate, %/h	230[x]	214[x]	334[y]	318	235	225	14.6	< 0.01	< 0.01
Ruminal escape, % of dose	7.1[x]	11.2[y]	5.0[z]	6.6	8.2	8.5	0.4	< 0.01	< 0.01
Proportion of N-dose converted to ammonia				0.69	0.85	0.75	0.05		0.409

[a]Soluble N-fraction: PLP-N = protein and long-chained peptides; SP-N = short-chained peptides; FAA-N = free amino acids.

[b]Probability of contrast: 1 = Soluble N-fraction across dosage; 2 = linear response of increased dosage of intraruminally administered soluble nitrogen extracted from grass silage

[x,y]Means within the same row with different superscript differ ($P < 0.05$)

3.7 Main Conclusions

1. This study showed that duodenal particle size distributions measured by LD and wet sieving were similar and LD is therefore an alternative method for particle size distribution measurements in ruminant digesta.

2. Rumen particles with a size of 0.3 mm had the lowest resistance to flow out rumen, and therefore the highest passage rate. The resistance decreased by increasing sedimentation velocity.

3. There were no large variations in rumen MRT of Yb labeled barley particles in the size range from 0.1 to 2.8 mm.

4. The starch and protein degradation rates of barley and maize decreased strongly with increasing particle size. However, the drop in degradability was larger for maize than barley because the degradation rates of barley were in the same size range as the passage rates.

5. The *in situ* technique for degradability estimations could be substantially simplified by reducing the number of rumen incubation-times to two or three. Still the accuracy of the method is good compared to the full incubation-time procedure.

6. A significant amount of dietary soluble N of grass silage escapes ruminal degradation, and thus contributes to intestinal amino acid supply.

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