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The nylon bag technique in the determination of ruminal feed protein degradation

Selostus: Pötsissä tapahtuvan rehuvalkuaisen hajoavuuden määrittäminen nailonpussi-menetelmällä

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Preface

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Abstract. The investigation included experiments in which factors affecting the reliability of the nylon bag method were studied. The possibility of applying the feed protein degradabilities to practical feeding conditions was also examined.

In the experiments concerning reliability, such factors as bag porosity, sample weight, sample treatment, washing procedure, diets, and differences between animals and incubation days were studied. The feed protein degradabilities were also determined by using as incubation periods the ruminal retention times for particulate matter of different feeds, evaluated as a function of DM intake/100 kg liveweight in different diets.

A nylon bag, with a pore size of 40 µm and internal dimensions of 6 × 12 cm was selected for the degradability determinations. The sample weight used in incubations was 57 – 60 mg DM/cm^2. In the determination of feed protein degradability, when sheep are used as experimental animals, it is recommended that for routine determinations only one animal be used, analyzing the contents of two bags for each incubation period during two successive days. A control sample of which degradability is determined in advance in many sheep, should be used in all incubations in order to control the digestive processes in the rumen of the experimental sheep.

The actual degradabilities analyzed by the bag method are applicable in practise, if they are determined using animals at similar feeding levels and on diets similar to those prevailing under the conditions in which the degradabilities are going to be used.

1. Introduction

Proteins are the fundamental components of all structures in an organism. The amount and turnover of proteins at different sites in an animal will therefore greatly affect its performance and production.

The protein requirement of an animal actually refers to the amount of amino acids required for maintenance and production. The body of a ruminant animal has two sources of preformed amino acids: those derived from the digestion of microbial proteins and those from rumen undegraded, digestible feed proteins.

The intake and the properties of feed protein greatly affect the overall performance of the animal. Among feed protein properties, resistance to ruminal degradation is very important. Rumen microbes have a certain requirement for ammonia and amino acids or peptides, which must be met in order to obtain maximum biosynthesis of microbial protein. The utilization of these products of proteolysis is greatly dependent on the amount of energy available to the microbes in the rumen. Therefore the degradation rate of feed protein must be balanced with the release of energy from the feeds. If this balance is not achieved, uncoupled fermentation acts to decrease the synthesis and utilization of protein in the rumen. In extreme cases, when quantities

of rumen degradable protein have been absorbed after proteolysis as ammonia through the rumen walls, infertility problems or even ammonia toxicity may result.

However, even in circumstances of maximum microbial protein synthesis, a high yield dairy cow or a young, growing ruminant has a greater amino acid requirement than can be met by microbial protein synthesis alone.

Consequently, the cow or the growing ruminant must also receive ruminally undegraded feed protein, which is well utilized by the animal. Due to this typical, dual protein metabolism of ruminants, the need for greater knowledge of ruminal feed protein degradation has been recognized. In the planning of new systems for evaluating protein utilization in ruminants, feed protein degradation was of central interest (ANON 1978, ANON 1980).

The degradability of feed proteins has been evaluated by studying protein solubility in different solvents *(in vitro)* or in the ruminally and post-ruminally cannulated animals *(in vivo)*. According to the literature, in recent years, however, the so-called nylon bag technique *(in sacco)* has come to be increasingly used in investigations.

The nylon bag method is regarded as simpler than the cannulation techniques and, unlike *in vitro* incubations allows feeds to be incubated directly in the rumen of the animal. Moreover, the nylon bag method has the additional advantage of making it possible to calculate the rate of protein degradation in the rumen. Further, many samples can be investigated at the same time in the same rumen conditions.

However, the basis for selecting the nylon bag procedure, is described in only a few cases (MEHREZ and ØRSKOV 1977, PLAYNE et al. 1978, LINDBERG 1981a, LINDBERG and KNUTSSON 1981). Neither are the possible limits of the techniques described in the literature; although such factors as i.a. bag porosity (UDEN et al. 1974), sample weight (VAN KEUREN and HEINEMANN 1962, UDEN et al. 1974), and sample treatment (VAN KEUREN and HEINEMANN 1962, McMANUS et al. 1972) have been reported as affecting the results obtained using the bag method.

The purpose of the present study was to investigate the following:

- the factors affecting the reliability of results obtained using the nylon bag technique in the analysis of protein degradation; and
- the limits within which the above results are reliable and applicable to practical feeding conditions

In principle, the degradability of crude protein is calculated according to the amount of nitrogen found in the bags before and after incubation *in sacco*. Because nitrogen is analyzed and calculated for a sample dry matter (DM) and the degradability is therefore dependent on the disappearance of DM during the incubations, the degradability of DM was evaluated and examined in all incubations. The degradability of organic matter was also examined in connection with the study of the details of the nylon bag method.

2. Review of the literature

2.1. Feed protein degradation in the rumen

Feed protein is degraded in the rumen by proteolysis and deamination. Decarboxylation is not regarded as an important way of amino acid degradation (PRINS 1977). According to PRINS et al. (1979), CRAIG and BRODERICK (1980) and NUGENT and MANGAN (1981) the first steps in proteolysis can be more important than deamination as a rate limiting factors in protein degradation.

Rumen microbes degrade feed protein by using their enzymes (BLACKBURN and HOBSON 1960). Recently HAZLEWOOD and EDWARDS (1981) reported that microbial proteinases can be stimulated by the presence of metal ions, for instance Mg^{2+}. Protein degradation is evidently caused by trypsin and chymotrypsin-like enzymes as well as other proteinases and peptidases (BROCK and FORSBERG 1980, CRAIG and BRODERICK 1980). BLACKBURN (1968a, b) suggested that the enzymes are located on the microbial cell surface or in the cell membrane and cell wall fragments.

The proteolytic enzymes of rumen microbes have a wide range of pH for their activity and pH can hence vary between 5.5 and 7.0 (BLACKBURN and HOBSON 1960, ABOU AKKADA and HOWARD 1962). Therefore it can be suggested that the pH in the rumen does not limit the enzyme activity on normal diets. Consequently, the differences found in the proteolytic activity of the rumen contents of animals on various diets can be explained by the differences of the composition of rumen microbiota (for instance BRÜGGEMAN et al. 1962).

2.1.1. Proteolytic activity of rumen bacteria

Proteolysis is caused by many bacterial species and proteolytic activity occurs in both cellulolytic and amylolytic bacteria (HUNGATE 1966). Bacterial proteases are cell bound, and proteolytic activity is directly related to cell biomass (HOGAN and HEMSLEY 1976). Proteolytic activity occurs in at least *Bacillus spp., Bacteroides, Butyrivibrio, Selenomonas* and one strain of *Streptococcus* (APPLEBY 1955, ABOU AKKADA and BLACKBURN 1963, RUSSELL and HESPELL 1981). Ammonia derived from protein degradation is an essential precursor for microbial protein synthesis in roughly 25–30 % of the rumen bacteria strains (BRYANT and ROBINSON 1962). From the total microbial N, about 50–70 % can be derived from ammonia (PILGRIM et al. 1970, AL-RABBAT et al. 1971, MATHISON and MILLIGAN 1971). CHALUPA et al. (1970) reported that amination and transamination are the mechanisms for the ammonia assimilation by rumen bacteria in sheep. Ammonia is captured in the form of amide groups of glutamine or asparagine which are used further in amination either by direct incorporation or after the release of ammonia (ERFLE et al. 1977).

In addition to ammonia, rumen bacteria need branched-chain fatty acids

and amino acids in the growth medium (ALLISON 1969, MAENG et al. 1976). This partly explains that protein degradation which takes place even when ammonia levels in the rumen are higher than suggested for maximal protein synthesis (SATTER and SLYTER 1974, NIKOLIC et al. 1975b, MEHREZ et al. 1977, SLYTER et al. 1979).

The requirement of amino acids or peptides is supported by the results of ROHR et al. (1979). They were unable to decrease protein degradation by the addition of urea to the diet. However, NIKOLIC et al. (1975a) found that urea prevented the degradation of feed protein and BRÜGGEMAN et al. (1962) suggested that change from plant protein diet to urea diet decreases the number of proteolytic organisms in the rumen.

Among the amino acids required by rumen micro-organisms are glycine, methionine, valine and histidine which are preferred in the form of peptides (PRINS et al. 1979). AL-RABBAT et al. (1971) found that rumen bacteria were able to directly incorporate preformed amino acids. PITTMAN and BRYANT (1964) had also found an incorporation system for methionine present in *Bacteroides ruminicola*.

The effects observed in connection with amino acids or protein may not always be directly due to those materials, but rather to the deaminated amino acid carbon sceletons or to oligopeptides. However, in regard to energy, carbohydrates have been suggested to be superior to proteins, and proteins superior to lipids, as energy sources for microbial protein synthesis (TAMMINGA 1978, 1979). According to BLACKBURN and HOBSON (1962) relatively few rumen bacteria are unable to utilize carbohydrates and must therefore depend on protein as an energy source.

It is thus obvious that the energy for microbial synthesis in the rumen is derived mainly from glycolytic reactions (ALLISON 1969, COLEMAN and LAURIE 1977); and that the role of amino acids as energy sources is not very important, being primarily to act as a protein factor in protein biosynthesis. The lack of an amino acid incorporation system in some bacterial strains reveals the necessity of first degrading protein to ammonia.

2.1.2. Proteolytic activity of rumen protozoa

The role of protozoa in ammonia production and protein degradation is relatively uncertain, because the protein requirements of protozoa have not been clearly defined. It is known, however, that these requirements can be met by both free amino acids in the growth medium and feed and bacterial protein present in engulfed bacterial cells or feed particles (COLEMAN 1972, COLEMAN and LAURIE 1977). Only about one half of the nitrogen present in the engulfed bacteria is utilized (COLEMAN 1975). As a result protozoa produce amino nitrogen for bacterial growth, by liberating amino acids from engulfed protein. These free amino acids pass into the rumen where they are utilized or degraded by bacteria (OWEN and COLEMAN 1977).

Among the protozoa at least *Entodinium longinucleatum* (OWEN and COLEMAN 1977) and *Polyplastron multivesiculatum* (COLEMAN and LAURIE

1977) can incorporate free amino acids. COLEMAN (1967a) hypothesized that amino acids diffuse slowly into the organism, and that the uptake mechanisms (active/passive) vary according to the total concentrations of amino acids with a specific system for each amino acids.

Recently NUGENT and MANGAN (1981) did not find strong protozoan caused proteolysis of protein in sheep on a diet of chaffed hay – crushed oats. Protozoal population had less than 10 % of the proteolytic activity of whole rumen fluid. On the other hand, ABOU AKKADA and HOWARD (1962) suggested that at least *Entodinium caudatum* was capable of hydrolyzing protein into ammonia. Moreover, in this connection ammonia is produced by oxidative hydrolysis and not by deamination (ABOU AKKADA and HOWARD 1962, COLEMAN 1967b).

2.1.3. Differences in protein degradabilities between feeds

In many papers (for instance LINDBERG 1981a, b, SIDDONS and PARADINE 1981, ZINN et al. 1981) differences in protein degradabilities between feeds have been observed. In studies on purified proteins casein has been found to be the most rapidly degradable and zein the most slowly degradable in the rumen (ANNISON 1956).

The structure and the composition of feed protein have been suggested to be the main reasons for the differences between feeds. WOHLT et al. (1973) reported that protein solubility is higher in feeds containing more albumins and globulins than prolamins and glutelins as a major protein fraction. A high solubility often means a rapid degradation of feed protein in the rumen (CRAWFORD et al. 1978). Moreover, MAHADEVAN et al. (1980) suggested that the lack of disulfide bonds in feed protein could indicate a high degradability of protein in the rumen.

The accessibility of feed protein to the microbial digestion can be reduced by different treatments, as formaldehyde (FERGUSON et al. 1967), tannic acid (NISHIMUTA et al. 1973) and heating (CHALMERS et al. 1954). In the group of special feeds, such as meat meal, protein degradation can be decreased also by the high content of hair in the meal, because the proportion of indigestible nitrogen in meal protein is increased (STOCK et al. 1981). LINDBERG (1981b) suggested that the high content of neutral detergent fibre (NDF) in both concentrates and roughage could limit ruminal protein degradation of those feeds. Therefore, under practical feeding conditions also the accessibility of non-protein organic matter in feed to the microbial digestion may determine feed protein degradation in the rumen.

2.2. *Evaluation of protein fermentation in the rumen*

2.2.1. Determination of protein solubility

A relatively simple and rapid method for evaluating the degradation of feed protein in the rumen is the determination of protein solubility. The principle involved is that rumen microbes can rapidly digest such protein as is soluble in for instance, saline solution (HUNGATE 1966).

2.2.1.1. Solubility in water

Perhaps the simplest way to evaluate feed protein solubility is to determine it in distilled water. This method is used in particular for the evaluation of the quality of nitrogen in preserved grains and silages. However, the method has only been used to a limited extent in studies of dried concentrates. LITTLE et al. (1963) found that correlation between the water solubility of proteins and the ammonia yield *in vitro* was only 0.38 when purified casein and zein, corn gluten, linseed oil meal and soybean oil meal were tested. The difference between the results obtained by determining solubility in distilled water and those obtained by determining solubility in rumen fluid can be considerable, especially in the case of casein. The solubility of purified casein was only 2 % in distilled water as compared to 81 % in rumen fluid.

A high proportion of water soluble N in the total N of grass silage will cause high ammonia concentrations in the rumen when silage is given as the only feed (SYRJÄLÄ 1972, DONALDSON and EDWARDS 1977, SYRJÄLÄ-QVIST 1982). This is in agreement with the recent finding of THOMAS et al. (1980) that silage protein is resistant to rumen degradation.

2.2.1.2. Solubility in buffer solutions

Buffer solutions have been more commonly used than distilled water in the analysis of the protein solubility of dried concentrates. The most frequently used buffers are McDougall's (McDOUGALL 1948) and Burrough's (BURROUGHS et al. 1950) solutions.

Buffer solubility of protein can be dependent on such factors as the incubation period, presence of neutral salts, and the temperature and pH of the solvent (WHITE et al. 1968, WOHLT et al. 1973). SALOBIR et al. (1969) calculated the ionic strength of rumen fluid as 0.15: it has been suggested that ionic strength is an important factor in protein solubility. However, when ionic strength varied from 0.11 to 0.15–0.19, there were no significant changes within each solvent in the solubility of proteins in wheat, oats, citrus pulp, sunflower meal, buckwheat, hominy and distiller's dried grains when Burroughs' and McDougall's buffers, and NaCl solution were used as solvents (CROOKER et al. 1978).

Incubations in buffers are generally performed at a temperature of 39° – 40°C. Within the pH ranges generally found in the rumen WOHLT et al. (1973) reported that the solubility of purified casein and isolated soy protein was significantly increased when the pH in Burroughs' solvent was changed from 5.5 to 6.5. The increase in solubility was not significant, however when

the pH was further changed to 7.5. Nonetheless, it has been suggested by LOERCH and BERGER (1980) that a significant interaction exists between protein source and pH in McDougall's solvent as the pH is changed from 5.0 to 7.0 even though there was not always a clear increase in solubility when pH was increased. For instance the protein solubility of blood meal and corn gluten meal varied as follows:

	pH 5	pH 6	pH 7
Blood meal	2.5 %	0.6 %	2.4 %
Corn gluten meal	2.9 %	5.5 %	2.5 %

CRAIG and BRODERICK (1981) found by using an incubation period of 1.5 hours that McDougall's solution gave solubilities 1.1–1.9 times higher than Burrough's solution for nitrogen in untreated or autoclaved cottonseed meal. McDougall's buffer was more accurate in predicting degradability than was Burroughs's buffer when compared to actual degradation found in rumen fluid *in vitro*.

However, protein solubility in Burroughs' buffer gave the highest positive correlation (n = 28) for the 2-hr protein degradation *in vivo*, when Burroughs' buffer, 0.15 molar NaCl solution, and autoclaved rumen fluid were compared (CRAWFORD et al. 1978). The correlations were 0.66, 0.47 and 0.54, respectively.

According to SNIFFEN et al. (1979), the solubility of crude protein in forage could not be determined with reasonable accuracy using Burrough's solution. Within class of forage (silage-hay) the variation in the results was large indicating the need of better methods of analysis.

2.2.1.3. Solubility in other solvents

In addition to distilled water and buffers, solutions of NaOH, NaCl and sterilized rumen fluid have also been used in studies of feed protein solubility.

ISAACS and OWENS (1972) showed pH to be of importance in the determination of protein solubility in sterilized rumen fluid: solubility and pH interacted so that e.g. the protein in casein and soybean meal was more soluble at a higher pH, varying from 5 to 7.

CRAIG and BRODERICK (1981) showed that for cottonseed meal 0.02 N NaOH gave protein solubilities 14.5–26.5 %-units higher than those obtained with McDougall's and Burroughs' buffers. Protein solubility in McDougall's buffer tended to decrease, when sample size increased from 104 mg to 500 mg/20 ml solvent. Compared to protein degradation *in vitro*, NaOH solution gave a 12.1 %-units higher solubility for the protein in cottonseed meal. LITTLE et al. (1963) also found that 0.02 N NaOH gave from 17 to 96 %-units higher solubilities than those obtained from incubation in rumen fluid, the difference being lowest and highest for purified casein and purified zein, respectively. A solution of 0.15 molar NaCl gave lower correlation to the results obtained *in vivo* (*in sacco*) for protein degradation than did autoclaved rumen fluid or Burroughs' buffer (CRAW-

FORD et al. 1978). The best correlations were obtained by Burroughs' solvent when concentrates and hay were studied. The authors suggested, however that soluble protein in silages could be determined by using a solution of NaCl.

Autoclaved rumen fluid has been used a standard of sorts for measuring protein solubility (see e.g. LITTLE et al. 1963, WOHLT et al. 1973, and CROOKER et al. 1978). In CROOKER et al. (1978), the correlations (n = 14) between solubilities obtained with Burrough's and McDougall's buffers and NaCl-solution to solubilities in autoclaved rumen fluid were 0.63−0.74, 0.71, and 0.68, respectively. There were several feed-solvent interactions, and the amount of nitrogen extracted from a given feedstuff by different solvents was not constant.

Autoclaved rumen fluid has given lower solubilities of protein than Burroughs' and McDougall's buffers or a solution of NaCl (CROOKER et al. 1978 and WALDO and GOERING 1979). WOHLT et al. (1973) reported that the difference was dependent on the pH of the solvents. Protein solubilities in autoclaved rumen fluid were higher than in Burroughs's buffer when pH in the solvents was 7.5, but lower at pH 5.5.

2.2.1.4. Solubility versus degradability

It has been found in many experiments (ANNISON et al. 1954, CHALMERS et al. 1954, SYRJÄLÄ 1972, NISHIMUTA et al. 1973, DONALDSSON and EDWARDS 1977) that there is a positive relationship between protein solubility and ammonia release in the rumen. However, CRAWFORD et al. (1978) and CRAIG and BRODERICK (1981) found that protein which was insoluble in Burroughs' or McDougalls' buffer was degraded in rumen fluid *in sacco* and *in vitro*. This is clear, because the proteolytic activity found in the rumen does not occur in chemical solvents.

ANNISON (1956), MANGAN (1972) and MAHADEVAN et al. (1980) showed that it is also possible that a highly soluble protein may be poorly degraded in the rumen. These differences can be explained by the composition of feed proteins. MANGAN (1972) showed that two soluble proteins, casein and ovalbumin degraded in the rumen at different rates the degradation rate being higher for casein than for ovalbumin. According to BROHULT and SANDEGREN (1954) and WHITEHOUSE (1973) protein in oats contain more globulins than proteins in barley and wheat, which have high prolamin and glutelin contents. Although it was found by WOHLT et al. (1973), using Burroughs' solvent that the protein solubility of feeds with a high albumin and globulin content was higher than the protein solubility of feeds in which the proportion of prolamins and glutelins was high, differences in ruminal degradabilities of proteins in oats and barley or wheat have not been observed (for instance CRAWFORD et al. 1978, LINDBERG 1981b and SETÄLÄ, unpublished observation). However, the protein degradation rate of feeds with a high albumin and globulin content was higher (CRAWFORD et al. 1978, and LINDBERG 1981b). Therefore PITCHARD and VAN SOEST (1977) and CRAWFORD et al. (1978) suggested that solubility is applicable only for the determination of protein fraction which is rapidly degraded in the rumen.

2.2.2. Determination of protein degradation

Feed protein degradation has been evaluated by both *in vitro* and *in vivo* methods. Protein degradability *in vivo* is determined using cannulated animals while *in vitro* studies are generally made incubating feeds in rumen fluid – buffer solution or digesting proteins by proteolytic enzymes under controlled laboratory circumstances.

2.2.2.1. Enzymatic degradation of feed protein *in vitro*

POOS et al. (1980) recently studied the enzymatic degradation of feed proteins *in vitro* using five commercial proteolytic enzymes at a pH ranging between 5–7 and temperature varying between +35 – +45°C. These were also the conditions required for optimal enzyme activity. The enzymes studied were a bacterial protease (*Streptomyces griseus*), papain (Carica papaya), ficin (Ficus glabrata), bromelain (Ananas comosus) and fungal protease (*Aspergillus oryzaea*).

The best agreement with the results obtained for protein solubilities in phosphate buffer (pH 6.7, +39°C) was found when the fungal protease was used.

2.2.2.2. Ammonia release *in vitro*

One possibility for taking into account the proteolytic activity which occurs in the rumen is to incubate feed samples in rumen fluid in the laboratory (*in vitro*). A good relationship between ammonia release *in vitro* and ammonia concentrations in the rumen *in vivo* has been found by DEN BRAVER (1972, 1974, 1980).

It is possible to use two types of *in vitro* systems, namely continuous and noncontinuous systems. In the latter fermentation products remain in the fermentor, while in the former they are taken out. The criteria for a good *in vitro* system and the pretreatment of the rumen contents before incubation have been discussed by WARNER (1956), JOHNSON (1966), SAYRE and VAN SOEST (1973) and SENSHU et al. (1980).

The noncontinuous system is based with slight modifications on the first phase of the procedure described by TILLEY and TERRY (1963). In many experiments (SENSHU and LANDIS 1964, BERGNER et al. 1972, LANDIS and HASELBUCH 1980, SETÄLÄ 1981), it has been found that after reaching their peak value, ammonia concentrations in the fermentors decrease. This is caused by rumen microbes which utilize ammonia for their protein synthesis. The addition of energy to the fermentation medium causes more efficient microbial utilization of ammonia (WARNER 1956, BERGNER et al. 1972). However, it has also been found that ammonia could be released gradually from the experimental samples, as shown by LANDIS and HASELBUCH (1980) and SETÄLÄ (unpublished). This is more likely to be the case if "protected protein" feeds are tested (GÖRSCH and BERGNER 1978, CERESNAKOVA and SOMMER 1979, STANTON et al. 1979, SETÄLÄ 1981).

Changes in ammonia concentrations cause difficulties in calculating protein degradabilities because it is not possible to say, how much ammonia has

been released and what is the ammonia uptake of rumen microbes. It must also be assumed that "endogenous ammonia" derived from rumen fluid, remains constant during the incubation (DINIUS et al. 1974, SETÄLÄ 1981). MAHADEVAN et al. (1979) suggested that these problems could be overcome by a method of determining protein degradation in which protein first was converted to a coloured diazotized derivate. Protein was diazotized by 7-amino-1,3 naphtalene disulfonic acid, in MAHADEVAN's study.

Another possibility would be to inhibit the action of enzymes important in ammonia fixation, using e.g. hydrazine as an inhibitor (BRODERICK 1978). However, this may cause changes in fermentation and in the normal digestive functions of the fermentors (BRODERICK and BALTHROP 1979). MENKE (1980) put forward the hypothesis that it might be possible to calculate protein degradation from gas production data *in vitro*, provided that the effect of protein synthesis is eliminated.

An additional limitation in a noncontinuous system, when practical feeding is considered, is that factors such as turnover and outflow rates in the rumen are not generally taken into account. Attempts to overcome this problem have been made by BRODERICK (1978). In his work, the *in vitro* method gave lower degradabilities than those calculated using the *in vivo* method. This could be at least partly explained by an abnormal accumulation of fermentation end-products by the fermentors.

Continuous *in vitro* systems have recently been used by HOOVER et al. (1976) and CRAWFORD et al. (1980) in the evaluation of protein degradation for both diets and feeds. Undegradable protein was calculated by the amounts of NH_3–N and microbial N substracting from the input of total N.

In these systems, the effect of outflow rate of "rumen contents" can be taken into account (HOOVER et al. 1977, CRAWFORD et al. 1980). The results for protein degradabilities agree fairly well with those obtained with other methods (solubility, degradability *in vivo*), as reviewed by CRAWFORD et al. (1980), although differences between *in vitro* systems exist (HOOVER et al. 1977, STERN et al. 1978).

2.2.2.3. Determination of rumen undegradable protein post-ruminally *in vivo*

Because of the inaccuracies inherent in the techniques of solubility and NH_3 release *in vitro*, research has been directed to the use of animals with surgically modified digestive tracts. In this technique the flow of digesta from the rumen to the omasum, abomasum, and small intestine is studied. Although cannulation of the omasum can be difficult, HAUFFE and VON ENGELHARDT (1975) carried out the technique without affecting the normal functions of the omasum. According to VON ENGELHARDT and HAUFFE (1975) a representative sample of the omasal contents could also be taken using the sleeve technique which they described.

Abomasal cannulas are not extensively used, probably because of the difficulties in handling the cannula, which is disturbed by acidic conditions in the abomasum (KOWALCZYK, personal communication). THOMAS (1978) suggested that there is no great difference between the results obtained from

cannulas in the abomasum or from those in the small intestine. Intestinal cannulation has been extensively used; and cannulas and techniques have recently been developed for both sheep (IVAN and JOHNSTON 1981, KOMAREK 1981a, b) and cattle (AUSTEN et al. 1977).

The technique has the advantage over *in vitro* methods of being able to take into account the effects of the other systems in a live animal, feeding (feeding level, different diets, etc.) and "normal" digestive conditions in general. However, working with living animals also presents certain problems which must be overcome.

When feed protein degradation in the rumen is measured with the cannulation technique, three fractions (endogenous, microbial and undegraded feed protein) must be separated from the duodenal digesta. The placement of duodenal cannula anterior to the bile and pancreatin ducts is important. Otherwise the endogenous nitrogen secreted in bile and pancreatin juices can greatly affect the results calculated from duodenal samples (TASCHENOV et al. 1979, McALLAN 1981).

According to experiments with cows (KAUFMANN and HAGEMEISTER 1976), the contribution of protein secreted in digestive juices could be about 15 % of the total protein in the duodenal digesta. Expressed as nitrogen, van't KLOOSTER and BOEKHOLT (1972) estimated those values at 1–2 g N/d for sheep and 15–30 g N/d for cattle. The amount of digestive juices had a high correlation with the amount of N discharged in bile and pancreas secretions (TASCHENOV et al. 1979).

The proportion of feed protein, which remains undegraded in the rumen is calculated by subtracting the amount of microbial (and possibly endogenous) protein from the total protein flow to the duodenum. This indirect approach includes inaccuracies, because of the great differences in the results obtained by the methods used for the estimation of microbial protein synthesis (VAN NEVEL and DEMEYER 1977, CZERKAWSKI 1978, LING and BUTTERY 1978 and ALLAM et al. 1982). This can also cause differences in case degradabilities of feed proteins obtained for similar feeds in different experiments are compared using different methods, although here the effects of basal feed processing (see e.g. FIGROID et al. 1972, LAYCOCK and MILLER 1981) and feeding level (ØRSKOV and FRASER 1973, TAMMINGA et al. 1979, and ELIMAM and ØRSKOV 1982 a, b) cannot be neglected.

In practise, experiments with post-ruminally cannulated animals are quite complicated. For instance, in the case of so-called "re-entrant" cannulas, manual collection cannot be used because it changes the digesta flow in the duodenum during at least the first 25 hr collection (MACRAE 1975). Therefore, automatic sampling equipment has been developed (KAUFMANN et al. 1972, MACRAE 1975, GAUDREAU and BRISSON 1978, ZINN et al. 1980).

WENHAM (1979) suggested that re-entrant cannula can change the gut motility. It has been suggested that re-entrant cannula be replaced with T-piece cannulas, because of the reduced risk of blockage and simpler surgery (MACRAE 1975). Moreover, T-piece cannula could be more useful in versatile metabolic experiments (MACRAE et al. 1982).

In any case, the use of both types of cannulas requires specific markers for

the flow determination of solid and liquid fraction in the digestive tract (BEEVER et al. 1978, FAICHNEY 1975, 1980). For this type of experiment there is a considerable number of different markers available as reviewed by KOTB and LUCKEY (1972). In the literature, the most commonly used markers for a liquid fraction are PEG (polyethylene glycol), Cr-EDTA, and Co-EDTA and for a solid fraction Cr_2O_3, ^{103}RU-P (Ruthenium-phenantroline), ^{144}Ce (Cerium) and Yb (Ytterbium). The characteristics of an ideal marker have been described by FAICHNEY (1975).

In spite of the rather complicated technique and the inaccuracies involved, MILLER (1978) suggested that the determination of feed protein degradation could be performed with reasonable accuracy using duodenally cannulated animals. If the degradabilities to be determined are within ranges of ±5 to ±10 % of the mean (confidence limit), ten animals would be required for each sample.

2.2.2.4. Evaluation of feed protein degradation by regression analysis

In order to overcome the problems associated with the markers used in e.q. the determination of microbial protein synthesis, mathematical regressions have been used as alternative methods (HVELPLUND et al. 1976, TELLER et al. 1979, and HVELPLUND and MØLLER 1980).

HVELPLUND et al. (1976) and TELLER et al. (1979) calculated degradability using a method similar to that used in experiments with cannulated animals. Microbial protein synthesis was related to the DM intake (HVELPLUND et al. 1976) or to the intake of carbohydrates, starch and crude fibre (TELLER et al. 1979). This type of calculation can be a positive factor in these techniques because of the disadvantages of using markers for microbial protein determinations.

In the protein evaluation system devised by INRA (ANON 1978), feed protein degradation is included in the formula used for calculating the non-ammonia nitrogen flow to the duodenum. The degradability calculation is based on the assumption that 35 % of insoluble feed protein is degradable in the rumen. A similar approach for green fodder diets was suggested by HOGAN and WESTON (1981).

The regression technique did not give exactly the same results for degradability as those obtained from duodenal cannulated animals (TELLER et al. 1979). The difference varied from 3.8 to 37.9 %, calculated for the dietary N in the duodenum. Using the technique of HVELPLUND et al. (1976), TAMMINGA et al. (1979) found that duodenal cannulated animals gave lower degradabilities. The result was, however, similar with both methods, when the effect of the feeding level on protein degradation was studied.

2.2.2.5. Determination of protein degradation *in sacco*

From the results obtained in studies of solubility, ammonia release *in vitro*, and post-ruminally cannulated animals *in vivo*, it can be concluded that a method is currently needed which evaluates protein degradation *in vivo* and does not include the inaccuracies arising from indirect determination. For

this purpose BAILEY(1962), BAILEY and HIRONAKA(1970), SCHOEMANN et al. (1972), and more recently MEHREZ and ØRSKOV (1977), have used a method in which feed is incubated in the rumen in cloth bags *(in sacco)* made from nylon, polyester, or other material which is not digested by rumen microbes. After incubation, the bags are removed from the rumen, washed, and the feed residue inside the bag, is dried. Different analyses (for example, minerals, fibre fractions, protein) can be made from the residue, and the disappearance of nutrients can be calculated according to the input-output difference. The bag method was first introduced by QUINN et al. (1938) for measuring the ruminal digestibility of various feeds.

There is considerable variation in the procedures used by different laboratories in the application of the nylon bag technique to the determination of ruminal protein degradation. Among the samples for the incubations concentrates are used in rolled form (MEHREZ and ØRSKOV 1977), or they are milled through a 1–3.0 mm ∅ screen (ØRSKOV and MEHREZ 1977, LINDBERG 1981a, SIDDONS and PARADINE 1981), and a milled sample is sieved again (SCHOEMANN et al. 1972, MATHERS et al. 1977). Roughage and mixed feeds are either used as is or are further chopped (BAILEY 1962, KAUFMANN et al. 1978, NOCEK et al. 1979); or they are milled through a 1–2 mm ∅ screen (VAN HELLEN and ELLIS 1977, CRAWFORD et al. 1978, PLAYNE et al. 1978). ØRSKOV and MEHREZ (1977) used roughage samples which were first finely chopped and then milled through a 5 mm ∅ screen.

CRAWFORD et al. (1978), KAUFMANN et al. (1978), PLAYNE et al. (1978), and LINDBERG (1981a) used dried samples (at 20–70°C) in their studies. BAILEY (1962) and KAUFMANN et al. (1978) used warm (about 40°C) water in the washing of the bags, which are, according to the literature, generally washed "under a running tap".

The porosity of the cloths used in the experiments varied according to the literature from 6 μm to 150 μm. VAN HELLEN and ELLIS (1977) suggested that holes of 1.2 μm or less in the cloth would not decrease a rate of digestion of the neutral detergent fibre in forage samples. They also proposed that maximum porosity should be no greater than 10 μm for forages, because this size decreased the effect of the influx of the rumen contents on errors in analyses, compared with bags of larger porosities up to 135 μm. LINDBERG and KNUTSSON (1981) also regarded 10 μm as the best pore size. However, UDEN et al. (1974) reported difficulties in handling bags with porosities from 20 to 35 μm, because of the lack of gas release from the bags. KAUFMANN et al. (1978) had observed a very small outflow of undigested feed particles when 40 μm bags were used.

The close relationship between sample pre-treatment (especially sieving), and bag porosity is evident. It is natural that in digestibility determinations the possibility for errors caused by the washout of undigested feed particles is greater the greater the porosity of the bag, pre-treatment being the same. However, in addition to washout, feed nitrogen disappearance from the bag could also be increased by increased microbial digestion as the particle size of the feed sample decreased (MOHAMED and SMITH 1977).

The amount of sample dry matter (DM) per unit of cloth area has varied

according to the literature from 5 to 83 mg of DM/cm^2. The digestibility of the fibrous material decreases as the amount of DM/cm^2 increases, if the bag size remains constant (UDEN et al. 1974). If the ratio of DM to cloth area remains constant, the amount of DM can vary without changing forage digestibility (PLAYNE et al. 1978). MEHREZ and ØRSKOV (1977) reported that a ratio of 107 mg of DM/cm^2 was too large for proper ruminal digestion.

In some experiments (MATHERS et al. 1977, MEHREZ and ØRSKOV 1977, and ØRSKOV and MEHREZ 1977), concentrates have been studied although concentrates were not actually included in the diet of the experimental animals. Changes in the basal diet, and hence the adaptation of rumen microbes to the change (MOHAMED and SMITH 1977), and especially changes in the type and quantity of concentrates, have been shown to cause changes in the degradability of feed protein (SCHOEMANN et al. 1972, MATHERS and MILLER 1981, and SIDDONS and PARADINE 1981). Protein disappearance decreased as the amount of concentrates in the total diet increased, although according to LOERCH and BERGER (1980) this response was not similar for all feeds.

Along with various other procedures, the nylon bag technique has often been critized as giving degradabilities which only apply to those feeding and rumen conditions under which the experiment has been carried out. ØRSKOV and FRASER (1973) and TAMMINGA et al. (1979) pointed out that the feeding level can also have an influence on feed protein degradation measured using duodenal cannulated animals.

ELIMAM and ØRSKOV (1982a, b) have suggested that, as the feeding level increases, the outflow of feed particles from the rumen also increases, thus allowing rumen microbes a shorter time to degrade feed protein. Using special techniques, GANEV et al. (1979) and ØRSKOV and McDONALD (1979), have been able to take into account the effect of changes in the feeding level and the resultant changes in the outflow rate. The results obtained from nylon bag studies corrected for outflow rate changes compare well with those obtained from experiments using post-ruminally cannulated animals (see CUMMINGS et al. 1980, and MATHERS and MILLER 1981).

The nylon bag technique is relatively simple to use compared to that involving post-ruminally cannulated animals. It is also superior to laboratory methods, because it includes the digestive processes which occur in the rumen of a living animal. Because of the variations in the procedures reviewed, different (and in some cases probably erroneous) data on feed protein degradation have been obtained with the *in sacco* method. Therefore it is important to study those factors and limits which must be taken into account in the evaluation and use of the nylon bag technique and the interpretation of the results thus obtained.

3. Present investigations

3.1. Studies of the bag material

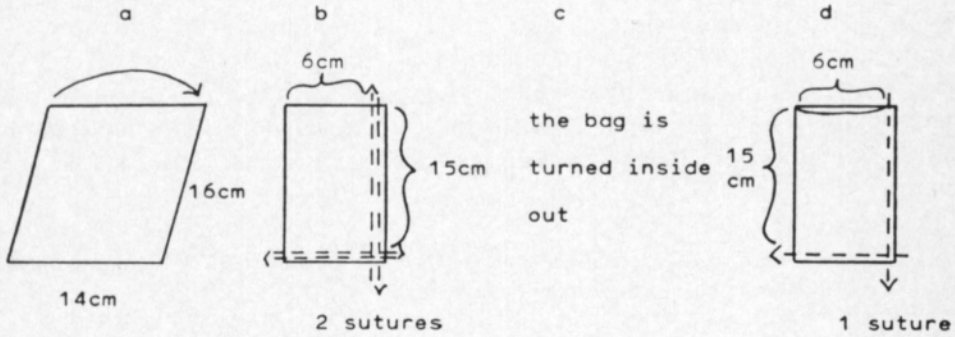
3.1.1. Washout of feed particles from the bag *in vitro*

3.1.1.1. Materials and methods

Five polyester cloths were chosen for a preliminary test. First, the structure and size of the weave was studied microscopically (400x). Two of the cloths were discarded because of their highly variable pore size and brittle structure. The porosities of the bags which were chosen for the further studies were 30–90 µm (bag A), 40 µm (bag B) and 10 µm (bag C). The porosity of bags B and C was chosen according to the recommendations of VAN HELLEN and ELLIS (1977), who suggested a maximum porosity of 10 µm; and according to the results of UDEN et al. (1974), in order to ensure proper handling of the bags.

Cloths A, B and C were further tested *in vitro* to determine the size of the error caused by washout.

Cloths cut to a size of 14×16 cm were folded in half width wise and sewn closed first with two sutures along the bottom and side, and a third suture was sewn in a similar way after the bag was turned inside out as shown below:



The internal dimensions of the bags were 6×15 cm. Exactly 4.5 grams of dry matter (DM) of hay, barley or soybean meal (see Appendix 1) were weighed and placed into the bags and the bags were tied closed, first with a narrow nylon thread and then with a nylon string, so that the actual internal dimensions of the bag were 6×12 cm. The bags were placed in the glass bottles of equal volume with rounded bottoms, and attached by the closure strings to the bottle stoppers. The lenght of the strings and the amount of cold distilled water in the bottles was adjusted so that the bags could circulate freely in the bottles.

Feed samples were fresh and undried, and they were milled through a 1.5 mm ∅ screen in order to obtain structure similar to that of ingested and partly ruminated feed. The samples were incubated in distilled water at a room temperature under a continuous shaking for 5 (hay) or 5 and 9 hours

(barley, soybean meal) and the disappearance of DM was evaluated. The bag and the feed sample it contained were removed after incubation from the bottle and dried in a 100°C oven for 18–20 hours. The disappearance of DM was calculated by subtracting the amount of DM remaining in the bag after drying from the amount of DM in the original sample placed in the bag. The difference was further calculated as a % of the DM input. Two bags of each cloth type per incubation period was used for each feed and the bag types were incubated at the same time. There were three incubations per incubation period. The results between bag types for each type of feed and incubation period were compared using the analysis of variance, one-way classification (SNEDECOR and COCHRAN 1967).

3.1.1.2. Results and discussion

The bags were compared first using hay samples. After the water used in incubation had been passed through a Whatman 4 filter paper, clearly visible feed particles were found on the paper from Bag A. Following this discovery, Bag A was eliminated from the incubations for concentrates, which were carried out using only Bags B and C.

No visible losses of DM were found in the incubations of hay in Bags B and C. The disappearance of concentrate DM was significantly higher when Bag B was used in the incubations (Table 1). However, it was difficult to wash Bag C when soybean meal and especially barley (see also FIGROID et al. 1972) were used as test samples. Water tended to remain in the bag, because the pores of the cloth were obviously blocked by starch from the samples.

NOCEK et al. (1979) suggested that there were no particle losses after two hours' incubation in distilled water, when soybean meal was incubated in 50–150 μm bags. The samples were ground to the size normally offered to the animals. EHLE et al. (1982) found disappearances of 27 and 17 % for DM and

Table 1. Comparison of the disappearance (%) of feed DM from bags made from three types of cloth (6 observations/incubation period/feed/cloth type)

Incubation time, hours	Test sample		Type of cloth		
			A, 30–90 μm	B, 40 μm	C, 10 μm
5	Hay	$\bar{x}$	14.7	13.2	13.7
		SEM	2.5	1.1	2.0
5	Barley meal	$\bar{x}$	–	65.2	18.6***
		SEM	–	1.1	0.9
9	Barley meal	$\bar{x}$	–	67.6	19.2***
		SEM	–	2.1	0.9
5	Soybean meal	$\bar{x}$	–	31.6	27.6**
		SEM	–	0.1	1.2
9	Soybean meal	$\bar{x}$	–	35.2	26.1***
		SEM	–	1.2	1.4

SEM = standard error of the mean
** $P < 0.01$
*** $P < 0.001$

crude protein in soybean meal, when particles larger than 1180 μm were incubated *in vitro* for 30 min. in 70 μm bags. When PLAYNE et al. (1978) studied different forage plant species in 25 μm bags; DM losses of 2.3–5.3 % or 5.0–10.6 % were found when oven-dried samples were sieved through either a 2.0 mm or 1.0 mm screen, respectively. DAFAALLA and KAY (1980) found particulate losses of 2–4 % after 1 hr incubation, in which timothy-ryegrass hay had particle sizes of 2, 5 and 20 mm or hay was used in a chopped form. Dry matter losses of 15–16 % were found by FIGROID et al. (1972) when barley was incubated in water at 39°C for 8 hours. In the incubations with hay LINDBERG and KNUTSSON (1981) observed particulate losses of 9.4–15.7 %, and 4.5–8.5 % when using the bags having pore sizes of 36 μm, and 10 μm, respectively.

3.1.2. The effect of sample weight on dry matter degradabilities in the rumen

3.1.2.1. Materials and methods

Barley meal, soybean meal, and field-dried hay were used as test feeds, and pretreated for the incubation as described above.

Samples of 22–24 mg DM/cm^2, 35–36 mg DM/cm^2, and 57–60 mg DM/cm^2 of each feed were incubated in bags with porosities of 10 μm and 40 μm. Incubation periods of 2 and 7 hours were carried out in rumen-cannulated sheep on Diet 1 (see Appendix 1 and 2). Most of the experiments concerning the procedure of the nylon bag method *in vivo* were made with sheep on a hay diet which was regarded as a safe feeding for these studies.

The bags were attached to the top of the cannula so that the free length of the attachment string was 20–25 cm. The incubations were made as follows: (BM = barley meal, SBM = soybean meal):

Bag B (40 μm)									Bag C (10 μm)								
BM			SBM			HAY			BM			SBM			HAY		
s1	s2	s3	s1	s2	s3	s1	s2	s3	s1	s2	s3	s1	s2	s3	s1	s2	s3

Samples of different weights (s1, s2, s3) for each feed were incubated at the same time during two successive days so that there were two observations/each sample weight. Samples in different bag types were incubated separately. In all incubations there was a control bag containing hay (57 mg DM/cm^2) so that there were four bags (control, 1s, s2 and s3) in the rumen at the same time. This program was followed in both 2 and 7 hours' incubations.

After incubation the bags were removed from the rumen. The material in the bag was gently crushed by hand and washed under cold, running tap water until the water was clear; the water remaining in the bag was gently squeezed out by hand. The bags were opened and dried at 100°C in an oven overnight without removing the feed residue. Before the incubations each empty bag had been put into a 100 ml decanter, dried at 100°C overnight, and tared. The incubated feed sample plus bag was also dried in a 100 ml decanter

after rumen incubation, and then tared. The DM content of feed samples was also determined after drying at 100°C overnight. Hence the DM degradability during incubations could be calculated as follows:

$$\text{DM-degradability, \%} = \frac{(fs-fr)\times 100}{fs}$$

fs = amount of DM in feed sample, grams
fr = amount of DM in feed residue after the incubation, grams

The degradability of the control sample was taken into account so that the average degradability of the control sample determined during the whole experiment was related to the DM degradability of the control sample for each day. After these calculations the degradabilities of each test sample were multiplied by the factor obtained when the degradability-% of DM for the control sample was used as described above. The average degradabilities of DM in the control sample were in 40 µm bags 7.75±0.21 % and in 10 µm bags 6.27±0.36 % after two hours' incubation, respectively. The corresponding values after 7 hours' incubation were 8.50±0.36 % and 6.90±0.33 %.

Differences in the results between sample weight for each type of feed and within each incubation period, and for different bags were tested using the t-test (MATTILA 1964, SNEDECOR and COCHRAN 1967, and MÄKINEN 1978):

1) test of the variances using the F – test:

$$F = \frac{\text{larger variance } (s_a^2)}{\text{smaller variance } (s_b^2)}, \quad v_1 = n_a - 1,\ v_2 = n_b - 1$$

2) variances not significantly different

$$t = \frac{|\bar{x}_a - \bar{x}_b|}{s\sqrt{2/n}}, \quad s = \sqrt{\frac{(n_a-1)s_a^2 + (n_b-1)s_b^2}{n_a + n_b - 2}}$$

$$df = n_a + n_b - 2$$

3) variances significantly different

$$t = \frac{|\bar{x}_a - \bar{x}_b|}{s_d}, \quad s_d = \sqrt{\frac{s_a^2}{n_a} + \frac{s_b^2}{n_b}}$$

$$df = v, \quad \frac{1}{v} = \frac{c^2}{n_a - 1} + \frac{(1-c^2)}{n_b - 1}, \text{ in which}$$

$$c = \frac{\frac{s_a^2}{n_a}}{\frac{s_a^2}{n_a} + \frac{s_b^2}{n_b}}$$

3.1.2.2. Results and discussion

The lengths of the incubation periods were chosen in order to study the effect of sample weight on the rate of DM degradation. A longer incubation period would be likely to reduce differences in degradabilities caused by differences in sample weight.

Both VAN KEUREN and HEINEMANN (1962) and UDEN et al. (1974) reported that an increased sample weight decreased digestibility in the rumen, especially, if the ratio of sample weight to bag area was not constant.

Sample weight most clearly affected the results obtained with the 10 μm bag (Bag C) (Tables 2–3); but the largest differences were found between bags (Bags B and C) as has been noted earlier in this study. An increase in sample weight tended to give lower degradabilities for barley and soybean meal also with the 40 μm bag (Bag B). This was especially true when a sample weight of 57–60 mg DM per cm^2 was used: the results agreed well with those of VAN KEUREN and HEINEMANN (1962) and UDEN et al. (1974).

The higher (not significantly) digestibility of roughage DM obtained after 7 hours with larger samples could be explained by the increased substrate available for microbial digestion in the rumen. Results of this kind were also obtained by PLAYNE et al. (1978) with hay samples, although they increased sample weight so that its ratio to bag surface remained the same.

Table 2. DM degradability (%) of different sample weights in bags of two different porosities after ruminal incubation for 2 hours.

Sample	Bag pore	Sample weight, mg DM/cm^2		
		22–24	35–36	57–60
Barley meal	40μm	64.4[a]	60.4[b]	60.7[b]
	10μm	11.5[c]	5.1[d]	−1.5[e]
Soybean meal	40 μm	20.2	19.8	19.9
	10 μm	14.9[c]	12.8[c]	7.6[d]
Hay	40 μm	8.0	7.3	7.3

a–b $P < 0.05$, for differences in degradabilities of different sized samples within the same bag type.
c–e, $P < 0.01$, as above

Table 3. DM degradability (%) of different sample weights in bags of two different porosities after ruminal incubation for 7 hours.

Sample	Bag pore	Sample weight, mg DM/cm^2		
		22–24	35–36	57–60
Barley meal	40μm	85.6	87.0	81.5
	10μm	21.5[a]	28.6[b]	15.5[c]
Soybean meal	40μm	44.9	44.4	39.7
	10μm	29.8	28.7	28.7
Hay	40μm	7.7	9.0	9.4

a–c $P < 0.05$, see Table 2.

As stated above, the sample weight reviewed in the literature varied from 5 to 83 mg DM/cm^2 in studies of both concentrates and roughage. MEHREZ and ØRSKOV (1977) suggested that a weight of 33 mg DM/cm^2 was adequate for proper digestion, and samples with similar weights have been used in many other studies (BAILEY 1962, MATHERS et al. 1977, NOCEK et al. 1979, and DEMEYER et al. 1982).

In the *in sacco* technique used in the present study, the degradability of DM in the rumen can be determined from a sample residue weighing only a few hundred milligrams. However, when calculating the degradability of crude protein, in particular its various fractions (amino acids, pepsin-HCl soluble protein, etc.), it is necessary to have a larger sample particularly in the case of duplicate determinations done to ensure the accuracy of the analysis. Moreover, when samples are incubated for longer periods than those used in this study, there will generally be less residual DM left in the bags, unless the sample becomes completely undigestible after the first 2–3 hours of the incubation period.

Owing to the calculation technique, the degradability of crude protein is dependent on both the degradability of DM and the amount of crude protein in the residual DM. It is clear that DM plays a very important role, but errors in crude protein determinations can also have a very strong effect on crude protein degradabilities.

For the above reasons, it was decided to use a sample weight which would correspond to 57–60 mg DM/cm^2. Furthermore, such a sample weight gave a significantly ($P < 0.05$) lower dry matter degradability only with barley meal incubated for 2 hours in 40 μm bags compared to a sample weight corresponding 35–36 mg DM/cm^2 which is close to the suggested "optimal" weight of 33 mg DM/cm^2. A sample size of c. 50–60 mg DM/cm^2 was also suggested by FIGROID et al. (1972) according to incubations with barley.

3.1.3. Applicability of bags with 10 and 40 μm porosities for rumen incubations

3.1.3.1. Materials and methods

Cloths B and C were studied microscopically to determine the pore size (magnifications 400x and 900x). After 10–12 incubations in the rumen, the shape and size of the pores in the 40 μm bag (B) were again studied microscopically (400x) to see if rumen digestion had affected the bag material.

The incubation periods of 2 and 5 hours were chosen according to VERITE (1979) in order to determine protein fractions which would be poorly (degraded after 2 hours) or probably efficiently (degraded within 3 to 5 hours) utilized by rumen microbes. Incubation period of 9 hours is the time between morning and afternoon feeding of the animals. These incubation periods were generally used in the following studies.

Barley meal, soybean meal, and hay were incubated in the rumen of one

sheep on Diet 2 (Appendix 1 and 2). The incubations were made according to following design:

	Barley meal		Soybean meal		Hay	
Bag	10μm	40μm	10μm	40μm	10μm	40μm
Hours	2 5 9	2 5 9	2 5	2 5	5	5

The incubation periods varied from 2 to 9 hours and the samples for each type of feed within an incubation period were incubated as follows:

	Cloth type	
	10μm	40μm
Day 1	2 bags	2 bags
Day 2	2 bags	2 bags

The incubation was performed and the degradation of feed DM was calculated as described in Chapter 3.1.2.1.

Organic matter (OM) in the feed samples was calculated indirectly from the ash content, which was determined by placing the sample in a 700°C oven for 2.5 hours. In the calculation of OM as a % of DM, moisture absorbed from the air was analyzed by keeping vacuum-dried (+50°C for 2–3 days, milled through a 1.0 mm ∅ screen) samples at 100°C for 24 hours ("secondary DM"). This correction was also made for samples incubated in the rumen.

The crude protein (CP) content of both unincubated and incubated samples was analyzed according to the Kjeldahl method (6.25 × N), calculated and corrected for "secondary DM". The OM and CP degradabilities were calculated as follows:

$$\text{OM-degradability } \% = \frac{[(OM_f\text{–}\% \times DM_f) - (OM_{fr}\text{–}\% \times DM_{fr})] \times 100}{OM_f\text{–}\% \times DM_f}$$

OM_f–% = OM, % in feed sample DM
OM_{fr}–% = OM, % in feed residue DM
DM_f = amount of feed sample DM
DM_{fr} = amount of feed residue DM after incubation

$$\text{CP-degradability } \% = \frac{[(CP_f\text{–}\% \times DM_f) - (CP_{fr}\text{–}\% \times DM_{fr})] \times 100}{CP_f\text{–}\% \times DM_f}$$

CP_f–% = crude protein, % in feed sample DM
CP_{fr}–% = crude protein, % in feed residue DM
DM_f, DM_{fr} = as above

3.1.3.2. Results and discussion

The results obtained from the two types of bags differed more clearly when bags were incubated in the rumen (Table 4) than when incubated in water (Table 1). Difficulties in washing the 10 μm bag (C) became more evident. Because of these difficulties, very small and even negative

Table 4. Degradability (%) of dry matter, organic matter, and crude protein, after incubation of barley meal, soybean meal, and hay for 2, 5 or 9 hours in 40 µm (B) or 10 µm (C) bags in the rumen of sheep

Incubation period			Barley meal		Soybean meal		Hay	
			B	C	B	C	B	C
2 hours	Dry matter	$\bar{x}$	77.3	5.8	39.9	7.7	–	–
		SEM	0.19	1.05	0.45	1.59		
	Organic matter	$\bar{x}$	76.4	5.7	38.0	7.3	–	–
		SEM	0.16	0.95	0.44	1.56		
	Crude protein	$\bar{x}$	72.9	3.1	39.1	11.4	–	–
		SEM	0.65	1.15	0.89	0.71		
5 hours	Dry matter	$\bar{x}$	83.8	4.9	43.9	11.5	19.0	9.2
		SEM	0.50	0.78	1.41	0.89	0.41	0.48
	Organic matter	$\bar{x}$	81.2	1.4	42.3	11.4	15.3	5.2
		SEM	0.54	0.84	1.40	0.94	0.39	0.40
	Crude protein	$\bar{x}$	87.6	0.7	43.1	−3.9	39.0	27.3
		SEM	1.29	1.29	0.41	0.91	0.85	1.50
9 hours	Dry matter	$\bar{x}$	86.2	38.4	–	–	–	–
		SEM	0.26	2.0				
	Organic matter	$\bar{x}$	84.3	35.0	–	–	–	–
		SEM	0.13	2.1				
	Crude protein	$\bar{x}$	91.6	33.5	–	–	–	–
		SEM	1.15	1.2				

degradabilities were found when the concentrates were studied. That is, difficulties arose similar to those reported by UDEN et al. (1974) when they used 20–35 µm bags.

Although VAN HELLEN and ELLIS (1977) and LINDBERG and KNUTSSON (1981) recommended a maximum porosity of 10 µm for bags used in forage digestibility experiments, the present study found that in 10 µm bags less DM was degraded after 5 hours in the rumen than in the water incubations when hay from the same source was incubated in both experiments. Similar observations were found also for barley and soybean meal after 5 hours' incubation.

Table 4 shows that the proper digestion of fibrous material (hay) was enhanced when bags of 10 µm were used. This stresses the importance of a porosity which allows microbes to enter the bag and attach themselves to feed particles. According to HUNGATE (1966), a porosity of 10 µm is too small and would prevent the entrance of most of the protozoa. In the group of rumen bacteria, this is most likely to affect three strains: *Bacteroides ruminicola*, *Clostridium longisporum*, and *Clostridium locheadii*, of which

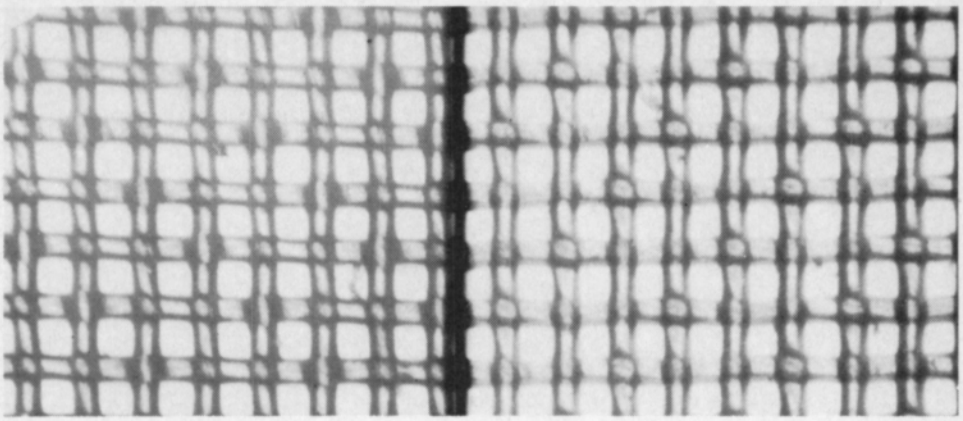


Figure 1: Changes in the structure of type B cloth (40 μm) after a bag made of this cloth has been used in 12 incubations (magnification 400 X). Left = unincubated, right = incubated.

the last two strains are cellulolytic.

It is rather difficult to say, which porosity is better, because the superiority of one over another can be changed e.g. by different pretreatment of the samples, differences in sample weight, etc. In addition to the problem of washout, which has caused variable results regarding the superiority of a particular porosity (VAN HELLEN and ELLIS 1977, NOCEK et al. 1979, LINDBERG and KNUTSSON 1981, present study), there may also be an influx of rumen contents into the bags (VAN HELLEN and ELLIS 1977). According to MADDOX and POLAN (1980) the influx was more of a problem in bags of 50–150 μm than in 30–70 μm bags.

After 12 incubations, the structure of the cloth (type B, 40 μm) pores was still the same as that of unincubated cloth (Fig. 1). However, it is not recommended that the same bag be used in more than 4–5 incubations, because the cloth tears easily, especially if a drying temperature of 100°C is used.

On the basis of these findings, it was decided to use bags made from cloth B (40 μm porosity, PES 40/27, Franz Eckert Cie, West Germany) in further investigations. Because the DM losses from such bags were quite high in water incubations, it was necessary to perform a more detailed study in order to determine the reasons for the losses. This study is described in the following chapter.

3.1.4. Comparison of DM and crude protein disappearances from 40 μm bags *in vitro* and *in vivo*

3.1.4.1. Materials and methods

The incubation procedure and chemical analyses in the degradability determinations were similar as previously described; but only one-day incubations were made and only bags with a porosity of 40 μm were used. In

addition to hay, barley and soybean meal, rapeseed meal was also incubated (see Appendix 1).

The incubations *in vivo* (in sheep on Diet 2, Apppendix 2) and *in vitro* (see Chapter 3.1.1.) were made so that each feed was incubated for 2,5, and 9 hours as follows:

	in vitro	*in vivo*	
2 hours	4 bags	4 bags	Day 1
5 hours	4 bags	4 bags	Day 2
9 hours	4 bags	4 bags	Day 3

In order to make a more detailed investigation of the degradation of DM, the disappearance of starch *in vitro* in barley was also determined for the 9 hr incubations. Starch was analyzed as decribed by SALO (1965), and its disappearance was calculated as made for crude protein.

The results between *in vivo* and *in vitro* within each feed and incubation period were compared using the t-test (see Chapter 3.1.2.1.).

3.1.4.2. Results and discussion

The disappearance of barley DM *in vitro* was similar to that found in earlier experiments; it remained constant after 2 hours of incubation (Fig. 2). The difference was significant ($P < 0.001$) between *in vitro* and *in vivo* results, clearly showing that as well as highly soluble material, fractions insoluble in water are also digested in the rumen.

Results similar to those for barley were also found for soybean meal. However, for rapeseed meal and hay the difference between *in vitro* and *in vivo* became significant ($P < 0.001$) only after 9 hours of incubation. There was a clear lag in the digestion of those feeds of up to five hours.

A similar lag was also found by GRUMMER and CLARK (1980), when corn silage, and corn concentrate with soybean meal or defatted soybean flakes was incubated as a complete feed and by ROOKE et al. (1982) when soybean meal was incubated. MEHRA et al. (1981) suggested that the degradability of hay DM over the time of incubation can be divided into two phases: a phase followed by a secondary phase beginning only after 10 hrs of incubation. The first phase would include the almost complete degradation of non-fibrous DM.

In the present investigation, the lag in digestion became more evident when the concentration of crude fibre (see Apppendix 1) in the sample DM increased. This is also in agreement with the findings of AKIN et al. (1974), AKIN (1976) and AKIN (1979), who suggested that rumen bacteria degrade thin mesophyll and phloem cell walls with extra-cellular enzymes. Thick-walled bundle sheath and epidermal cells are digested after bacterial attachment, which can cause a lag in digestion. On the other hand, one bacterial strain can digest a substrate after the other strain has degraded some fractions in the substrate (HUNGATE 1966). This can also cause a lag in digestion.

The water solubilities of crude protein that were found (Figure 3), remained within the ranges given in the literature (LITTLE et al. 1963, WOHLT

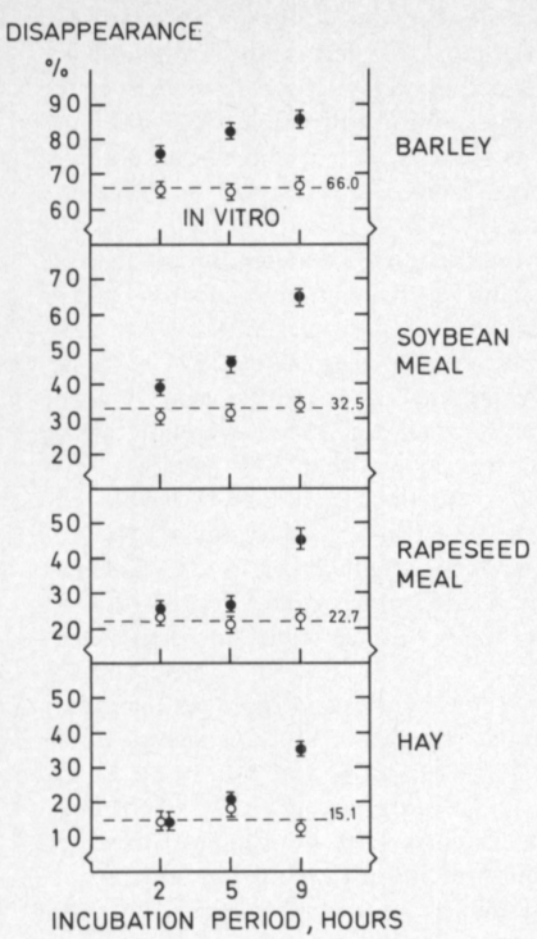


Figure 2. Disappearance of DM from samples of barley, soybean meal, rapeseed meal, and hay after *in sacco* incubation both *in vivo* ● and *in vitro* ○.

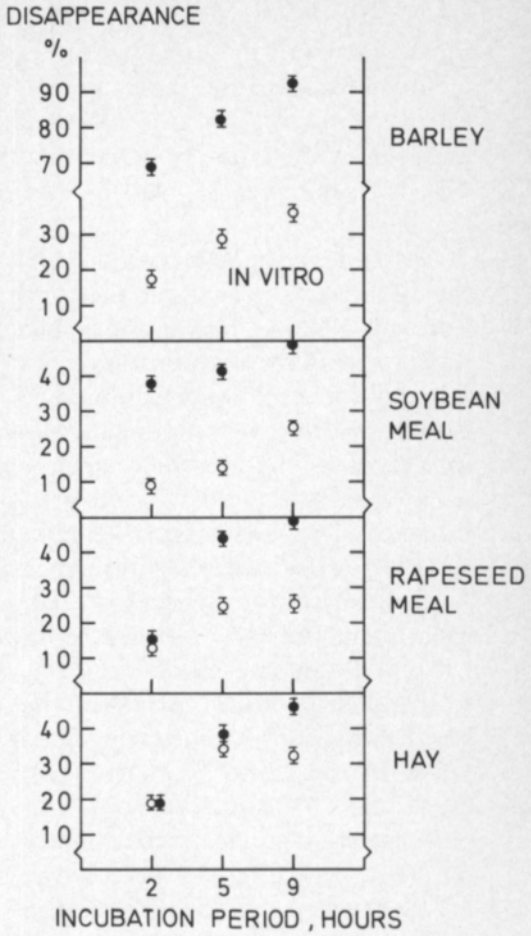


Figure 3. Disappearance of crude protein from samples of barley, soybean meal, rapeseed meal, and hay after *in sacco* incubation both *in vivo* ● and *in vitro* ○.

et al. 1973, FIGROID et al. 1972, ANON 1978, and CRAWFORD et al. 1978). Only the solubility of rapeseed protein was slightly lower. The reported solubilities either in water or buffer solutions were: soybean meal 13–30 %, rapeseed meal 30–39 %, barley 17–45 %, and timothy hay 30–35 %.

The results in the present study clearly demonstrated that solubility of crude protein is very different from degradability. Crude protein disappearances were significantly ($P < 0.001$) higher *in vivo* than *in vitro* for barley and soybean meal. In the case of rapeseed meal and hay, significant ($P < 0.001$) differences were not found until the samples had been incubated for 5 and 9 hours, respectively. In the case of both rapeseed meal and hay, solubility during 2 hours' incubation indicated the fraction of the most rapidly rumen degradable protein as was also suggested by PITCHARD and VAN SOEST (1977), and CRAWFORD et. al. (1978).

However, it must be observed that the disappearance of barley DM *in vitro* was relatively high. Barley was the most difficult sample to handle in incubations, because the sample remained quite heterogenous even after milling through a 1.5 mm screen. The distribution of particles after milling, evaluated with different screens, was as follows: > 1.0 mm screen 0.2 %, 0.5–1.0 mm screen 26.3 %, 0.1–0.5 mm screen 65.1 % and < 0.1 mm screen 8.4 %.

Possible particulate losses of DM were therefore evaluated by calculating the disappearance of starch the concentration of which in unincubated barley DM was 57.2 %. The disappearance of starch *in vitro* was 92.8 %; and this together with the disappearance of soluble crude protein caused 91–92 % of the total losses of water soluble DM during incubations of 9 hours. Because the effects of other components, ether extract and crude fibre, were not taken into account, the loss of particulate matter was less than 8–9 %.

The particulate loss can be assumed to be mainly the loss of very small feed particles which is in agreement with some of the observations of EHLE et al. (1982). However, they did not find any consistent pattern to the influence of particle size on the rates of dry matter and crude protein degradation in nylon bag studies *in vitro* and *in vivo,* when samples were sieved through 150–1180 μm screens.

EHLE et al. (1982) also found that rumen microbes decreased feed particle size by digesting them during the incubations. According to the results of FIGROID et al. (1972), MOHAMED and SMITH (1977) and WEAKLEY et al. (1977, ref. NETEMEYER et al. 1980), it can be suggested that small feed particles are degraded more rapidly and extensively in the rumen than large particles, providing that they have similar retention time in the rumen.

Hence it may be possible that the actual loss of undigested particles from the bag is small, because microbes may start their digestion work before the washout of the particles out from the bag has begun. In that case, feed particles lost from the bag by washout will be at least partly degraded. It is nonetheless necessary that microbes are able to enter the bag and make enzyme contact with the feed particles.

PLAYNE et al. (1978) suggested that nutrient degradabilities should be corrected by those results found for particulate losses, but at least in the case of roughage the losses should be measured for each sample because the extent of the losses varies with plant species. According to FIGROID et al. (1972) it is questionable to correct nutrient degradabilities of grains *in vivo* by the nutrient solubilities *in vitro,* because grains, bags, and particle sizes of the samples may interact differently *in vitro* versus *in vivo.*

For the above reasons it was decided not to correct degradabilities according to particulate losses.

3.1.5. The effect of the washing procedure on the degradabilities of DM and crude protein

The nylon bag technique is based on the assumption that all digested and hence water-soluble material is washed away in running water. On this basis,

it might also be assumed that the amount of water used in washing has an effect on the results especially if undigested particles are washed out during rinsing.

3.1.5.1. Materials and methods

Samples of both barley meal and hay (see Appendix 1) were pretreated and incubated in the rumen of sheep on a hay diet (Diet 2, see Appendix 2) as described earlier, for 5 and 9 hours. The incubations were made as follows (a = running water, b = controlled amounts of water):

	Barley meal		Hay	
	a	b	a	b
5 hours	5 bags	5 bags	5 bags	5 bags
9 hours	5 bags	5 bags	5 bags	5 bags

The washing procedures within each feed and incubation period were compared in two successive incubations so that there were 5 bags in the rumen at the same time (2a + 3b, and 3a + 2b).

After the incubations, the bags were washed either under running water until the water was clear or in a controlled (4–5 l) amount of rinsing water. After washing, the bags, feed samples, and sample residues were treated as described above (Chapter 3.1.1.), and the degradability of DM was determined.

The statistical treatment of the results within each feed and incubation period was made using the analysis of variance, one-way classification (SNEDECOR and COCHRAN 1967).

3.1.5.2. Results and discussion

No significant differences were found between washing methods after the results from a total of 40 determinations of DM degradability (20 for hay and 20 for barley) were compared. The differences varied from 0.006 to 0.69 %-units.

The results agreed with those of CRAWFORD et al. (1978), who suggested that there were no additional particle losses due to extensive rinsing when bag porosity varied from 20 to 70 μm.

VAN KEUREN and HEINEMANN (1962) also postulated that the washing method did not affect the variability of DM degradability. However, McMANUS et al. (1972) found a significant increase in DM degradability, when the rinsing period was increased from 0.5 to 3.0 min, and roughage-type substrates were incubated in the rumen. The porosity of the bag cloth was not given. On the other hand, they observed no sifting of the sample through the cloth while the substrate was suspended in the bag for 12 hr in gently running water.

It seems very important to wash the rumen fluid colour from the bag and substrate. When limited amounts of water were used, the water from the bag did not run clear after washing. Limited rinsing probably left some material from the rumen inside the bag, which prevented proper drying under the

conditions used. This improper drying sometimes gave larger degradabilities of DM, compared to those from bags rinsed in unlimited amounts of water; because according to the analyses, "less" DM was left in the bag.

3.1.6. Observations from the sample treatment before and after the incubations and the effects of these treatments on the nutrient degradability in the rumen

The most important task in treatment procedure is to prepare samples which would have a physical structure similar to that of extrusa. Therefore, concentrates, and field-dried hay or straw samples were milled through a screen with a weave of 1.5 mm ∅. All materials which contained relatively high amounts of water, such as silage etc., were chopped with scissors so that the lenght of the particles was not longer than 0.5 cm.

When using the treatment procedures described above, difficulties appeared in the case of field bean or pea meal, industrially processed concentrates, and occasionally barley meal. For example, if pelleted concentrates or whole peas were milled, the structure of the sample became so fine that much of the sample escaped from the bag as dust. Therefore, pellets were crushed when possible in a pounding mill, and peas or beans were minced and milled in a GWB-mill (Arthur and Thomas Co, Philadelphia, USA), which gave a structure coarse enough to minimize physical loss from the bag.

VAN KEUREN and HEINEMANN (1962) found occasional differences in digestibility of DM when forage ground to different degrees of coarceness was studied. SCHOEMANN et al. (1972) and MATHERS et al. (1977) regarded a homogenous sample as being so important that they used carefully sieved (between 0.25 and 1.5 mm) samples in their study of the ruminal digestion of concentrates. MEHREZ and ØRSKOV (1977) also reported that heterogenous samples were more likely to cause variations in the results.

However, if the sample is sieved, part of the original feed is discarded, which could change the chemical composition of the sample (FIGROID et al. 1966, ref. FIGROID et al. 1972) and the digestibility of the sample, especially when nutrients are distributed among different parts of the feed. Therefore, it is debatable if an homogenous sample can be achieved by sieving. However, if attempts at milling or crushing do not give a homogenous sample, it is reasonable to try sieving the sample to find the proportions of different sized particles. Pretreatments can be continued until the sample is homogenous in terms of particle size. Then the incubation can be made with an unsieved sample which has been pretreated by the method, found appropriate.

Any significant ($P > 0.05$) differences in DM, organic matter, or crude protein degradabilities were not found when fresh or vacuum-dried concentrates (Appendix 1) were milled through a 1.5 mm screen and incubated in the rumen of sheep on diet 2 (Appendix 2) for 5 hours (Table 5). However, vacuum drying tended to cause greater variation in the results, especially those from the protein-rich feed, soybean meal. In addition, if the feed sample must be similar to actual feeds eaten by the animal, vacuum drying is

Table 5. The effect of vacuum drying (+50°C for 2–3 days) of the sample on the degradability –% of dry matter, organic matter, and crude protein. Incubation period 5 hours, 3 observations / treatment / feed.

	Barley[1)]		Soybean meal[1)]	
	Not vacuum dried	Vacuum dried	Not vacuum dried	Vacuum dried
Dry matter	85.8 ±1.1	85.7 ±1.3	51.0 ±0.3	52.0 ±4.2
Organic matter	86.0 ±1.2	86.1 ±1.3	49.2 ±0.1	50.7 ±4.6
Crude protein	85.7 ±4.7	85.4 ±5.5	38.2 ±0.5	39.5 ±6.9

[1)] Sample milled through 1.5 Ø mm screen

not recommended, although it would make storage of dry samples for the analyses much easier.

After incubation and washings, it is important to ensure that the sample is properly dried before the amount of DM in the bag is determined. It must be stressed that a fixed time of drying does not always guarantee that all the water has evaporated from the sample residue. This is especially important when additional analyses (e.g. amino acids) are made from the protein fraction and a low drying temperature (+60°C) is used.

3.2. Factors causing variability in determinations of nutrient degradabilities

3.2.1. Differences between bags

3.2.1.1. Materials and methods

Determinations of degradabilities of DM and crude protein (CP) in sheep on different diets from the years 1979–81 were compared. In the analysis of the differences between bags from the same incubation, the material consisted of determinations from the sample residue in 184 (DM) and 140 (CP) bags *in vivo*, and 59 (DM) bags *in vitro*.

The daily results *in vivo* from bags containing the same feed, incubated for the same length of time, within the same sheep, were compared. The differences between bags were tested using a one-way analysis of variance (SNEDECOR and COCHRAN 1967). The daily results *in vitro* were treated in a similar way, substituting results from each day and laboratory incubation for those from each sheep.

3.2.1.2. Results and discussion

No significant differences were found between bags from the same incubation either *in vitro* or *in vivo*.The coefficient of variation was much

Table 6. Coefficient of variation (CV %) from the comparisons of differences in the results from different bags for the disappearance of DM in different feeds, incubation *in vitro*.

	Grain concentrates[1)]	Protein concentrates[1)]	Hay[1)]
n	24	27	8
CV %	2.9	4.8	9.5

[1)] incubations from 2 to 9 hours

Table 7. Coefficient of variation (CV %) from the comparisons of differences in the results from different bags for the degradabilities of DM or crude protein (CP) in different feeds, incubation *in vivo*.

	Grain concentrates[1)]		Protein concentrates[1)]		Hay[2)]		Grass silage[2)]	
	DM	CP	DM	CP	DM	CP	DM	CP
n	38	32	96	62	34	34	16	12
CV %	1.0	0.9	3.2	5.3	3.9	2.6	3.2	2.0

[1)] incubations from 2 to 9 hours
[2)] incubations from 2 to 24 hours

higher in *in vitro* than *in vivo* incubations, when hay was compared to concentrates (Table 6 and 7).

There are three factors in the bag itself which could cause differences in the results from bags in the same incubation. These are: 1) differences in the structure and 2) in the placement of bag sutures leading to 3) differences in the open cloth area (internal dimensions). In the incubations *in vivo* some residues of either rumen contents or feed particles was also found inside the seams. The N-content of the bag cloth also increased, but both of these changes could only be regarded as important after the bags had been used more than 3–4 times (Fig. 4). However, these findings were not connected

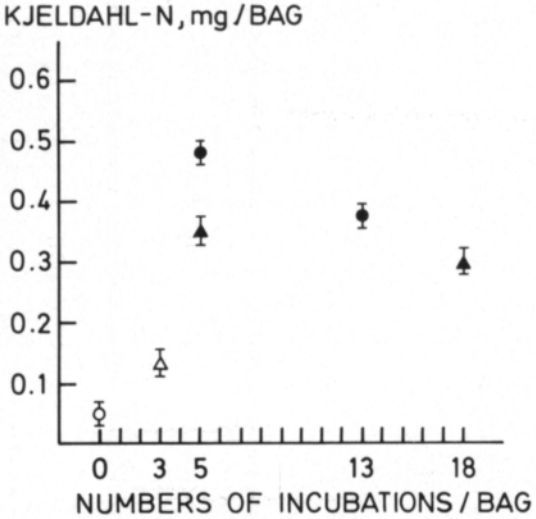


Figure 4. The effect of the number of incubations on the amount of residual nitrogen in the bags (○ unincubated bags, ● incubated bags, ▲ bags, when seams are opened and cleaned, △ bags with seamed edges cut off). Each point is the average of three determinations.

with any special type of bag, and they should be considered a factor common to all bags. On the other hand, it has been recommended that one bag should not be used more than 3–4 times (see page 29).

All the results explained above (see Fig. 4) were from concentrates. However, it is not obvious that feed particles from concentrates would react differently from those in hay samples in this respect. Therefore the highest coefficient of variation obtained from the DM disappearances of hay *in vitro* could also be explained by the variable homogenity of the sample. Despite careful mixing, it is always possible that larger amounts of more poorly digestible or soluble material would be placed in one bag than in another. However, this seems to be important only under *in vitro* conditions, because the variations in the degradabilities of either DM or crude protein between feeds *in vivo* were not as great as the variations in the *in vitro* disappearances.

3.2.2. Differences between animals and days

3.2.2.1. Materials and methods

Experiment A

This experiment was made using 2×2 factorial approach according to following design:

	Sheep 1		Sheep 2	
	Day 1	Day 2	Day 1	Day 2
Replications	2	2	2	2

This program was followed in incubations for each feed and incubation period (2,5, and 9 hours).

The sheep were fed on Diet 2; and samples of barley, soybean meal, and hay (see Appendix 1) were incubated in the rumen for 2, 5 and 9 hours. The degradabilities of DM and crude protein were determined and calculated as described in Chapters 3.1.2.1. and 3.1.3.

The statistical analysis of the results was done according to SNEDECOR and COCHRAN (1967), treating results within each incubation period (2, 5, 9 hours) seperately.

Experiment B

The material was collected from 74 incubations made with sheep on hay, or grass silage and hay diets. The samples of hay, grains (barley, oats), protein concentrates (soybean meal, rapeseed meal) were incubated in the rumen as described earlier (Chapters 3.1.2.1. and 3.1.3.). The incubation periods for concentrates and grains were 2, 5 and 9 hrs; and for hay 2, 5, 9, 12 and 24 hours.

The effect of different days or sheep on variations in the degradabilities of dry matter and crude protein was evaluated by the coefficient of variation.

The coefficient of variation was calculated separately for the results from each type of feed within each incubation period. The average values of these coefficients were used in comparisons of the combinations and numbers of days and/or sheep in degradability studies.

3.2.2.2 Results and discussion

Experiment A

When either barley or soybean meal were incubated, significant ($P < 0.05-0.01$) differences in DM degradability between both days and sheep were observed (Tables 8–9). However, such differences were not found when hay was incubated. This indicates that if the diet of the experimental animals is composed of feeds similar to those, which are to be studied, less variation in the results can be expected. The significance ($P < 0.05, 0.01, 0.001$) of the sheep × day factor implies that the digestive capasity and ability may change from day to day and from sheep to sheep without following any regular pattern.

Table 8. The significance of days and animals as the source of variaton, when the degradability of DM was measured.

Incubation period		Barley			Soybean meal			Hay		
		S	D	SxD	S	D	SxD	S	D	SxD
2 hours	df	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4
	F	0.05	0.52	0.73	3.70	1.36	1.92	0.02	0.05	0.21
5 hours	df	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4
	F	1.46	13.2*	0.16	55.6**	1.32	0.60	1.68	2.39	4.16
9 hours	df	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4
	F	1.14	18.51*	0.51	6.31	23.72**	46.1**	0.01	2.80	0.29

df = degrees of freedom, S = sheep, D = days
* $P < 0.05$ ** $P < 0.01$

Table 9. The significance of days and animals as the source of variation, when the degradability of crude protein was measured.

Incubation period		Barley			Soybean meal			Hay		
		S	D	SxD	S	D	SxD	S	D	SxD
2 hours	df	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4
	F	41.7**	0.26	0.58	3.22	0.01	0.14	11.67*	6.67	15.33*
5 hours	df	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4
	F	0.21	2.55	0.24	116.11***	0.28	0.59	4.05	5.77	0.70
9 hours	df	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4	1,4
	F	0.95	1.85	0.18	3.00	7.13	21.67**	0.15	1,73	80.00***

df, S, D see Table 8.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

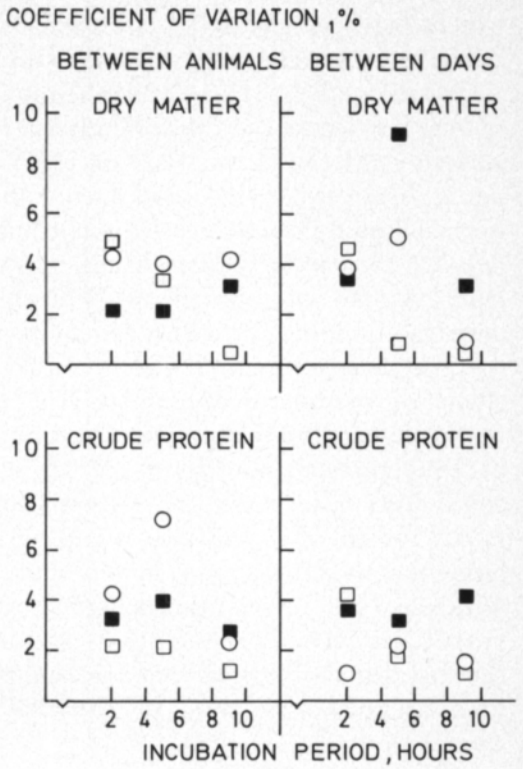


Figure 5. Estimated coefficients of variation (%) for dry matter and crude protein degradabilities between animals and days (○ barley, ■ soybean meal, □ hay).

The variations in the results among both animals and days tended to decrease as the length of the incubation period increased (Fig. 5). In microbial digestion of feed, enzymes are used for the digestion of feed particles, which are degraded (HUNGATE 1966). Occasionally the inner part of the sample in the bag obviously had no contact with the rumen liquor during the first hours, which may have caused at least part of the differences observed. This is supported by the finding that when barley samples *in sacco* were gently sprayed with water in the laboratory, the innermost part of the sample occasionally remained fairly dry.

Another reason for the observed differences can be found in the changes in the amount and composition of rumen microbiota between animals (WARNER 1962, THOMSON et al. 1978) and from day to day (WARNER 1962) within the same animal. If it is assumed that the digestive capacity of the rumen is dependent on the number and activities of rumen microbes, then the amount and quality of ingested energy and variations in pH, and the dilution rate in the rumen are factors which need further discussion (LAMPILA 1959, THOMSON et al. 1978, RUSSELL et al. 1979).

Because the sheep ate similar amounts of hay all the time and feeding was not *ad libitum*, there were no obvious differences in the energy intakes between animals or days. On similar diets LAMPILA (1964) also showed that there were only slight differences in rumen pH between animals and between the different parts of their rumens. If the bag did not move around freely in the rumen, it is possible that the bag might remain in one part of the rumen in

which, for instance, a very low pH could decrease microbial activity and hence cause variations in the degradability results.

Moreover, POUTIAINEN (1968) showed with cows that on the same level of long hay intake there was significant difference in saliva secretion between animals. HARTNELL and SATTER (1979) found great differences in ruminal liquid turnover between cows even with a similar DM intake. The saliva and ruminal liquid flow also varied according to DM intake (POUTIAINEN 1968). Because the rate of hay comsumption of the sheep could also have varied from day to day in the present experiment, the differences in DM degradabilities observed between animals and days could partly be explained by changes in ruminal dilutions caused by changes in saliva flow and their effects on the microbial population in the rumen.

WARNER (1962) reported variations in the counts of rumen microbes between animals and days in the hay diet. He also suggested that variation could most easily occur, when there were very similar microbial populations in the rumen. This was also found when the effect of the dilution rate on fermentation and microbes in the rumen was studied (THOMSON et al. 1978). WARNER (1962) also found a higher variation in the composition of rumen microbiota between animals than between days. Moreover, in the studies for ruminal degradability of feed DM and protein MEHREZ and ØRSKOV (1977) found a greater variation in degradabilities between sheep than between days.

Those facts which have just been discussed above also concern the degradation of protein in the rumen. Variations in protein degradation were evidently caused by temporary changes in rumen microbiota. BLACKBURN and HOBSON (1962) suggested that proteolytic activity can be found in both bacteria (see also APPLEBY 1955, and ABOU-AKKADA and BLACKBURN 1963) and protozoa (see also ABOU-AKKADA and HOWARD 1962, COLEMAN 1967b). It is possible that changes in the dilution rate also variously affect the concentrations of bacteria and protozoa, because bacteria, important for proteolysis, flow out from the rumen together with the liquid fraction, which also includes fine feed particles (BERGEN and YOKOYAMA 1977). However, according to WELLER and PILGRIM (1974), compared with bacteria, only 6–29 % of protozoa are present in this fraction. Therefore if the proteolytic activity of protozoa varies as could be deduced from the disagreement between the results of ABOU-AKKADA and HOWARD (1967), COLEMAN (1967b), and NUGENT and MANGAN (1981) this might also cause some variation in the results obtained in nylon bag studies.

Some attachment of rumen bacteria to the surface of feed particles (AKIN et al. 1974, AKIN 1976) may occur by means of the complicated glycocalyx fixation system (COSTERTON et al. 1978) or by means of the fibrous capsules (CHENG et al. 1977). Owing to these there are difficulties in washing away the microbial material (PEKKARINEN et al. 1982), which can also cause variations in the results when the degradability of crude protein is determined. This is especially true when feeds which have low DM degradabilities are studied (see e.g. SETÄLÄ and SYRJÄLÄ-QVIST 1982). MATHERS and AITCHISON (1981) suggested that the greatest underestimation of protein

degradability is likely to be made when feeds of low protein content but high protein degradability are studied.

Experiment B

The average degradabilities of DM and crude protein in the feeds used in the incubations are presented in Tables 10–11. An increase in the number of experimental animals caused higher coefficients than an increase in days on hay diet (Figure 6). This was especially true when the number of sheep used was increased from one. If more than one sheep was used, the smallest variation in the incubations of concentrates was obtained when the sample was incubated in two sheep during 10 successive days so that there was 2 bags/sample/incubation period. However, in the case of hay the best combination was four sheep, two days, and two bags. The coefficient of variation obtained in one-sheep and one-day incubations was due to variation caused by differences between bags and their treatments.

Observations similar to described above were found in the incubations in sheep on grass silage and hay diet, except when degradabilities were determined in 2-days or one-day incubations using two bags/hay/period, and the results obtained in one sheep or two sheep were compared (Figure 7). However, variation in the degradabilities was smaller in the incubations in one sheep than in two sheep when the number of days was increased in incubations.

A fairly low coefficient of variation is obtained if the incubations are made with one or two sheep, over 1–2 days with 2–4 bags/feed/incubation

Table 10. The degradabilities of dry matter in the feeds used in incubations.

Incubation period, hours	Degradability-% of dry matter					
	Barley		Soybean meal		Hay	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
2	75.2	3.7	39.3	1.2	14.5	0.4
5	81.9	1.4	44.5	3.9	19.3	1.0
9	84.9	0.5	64.8	2.3	34.4	1.4

Table 11. The degradabilities of crude protein in the feeds used in incubations.

Incubation period, hours	Degradability-% of crude protein					
	Barley		Soybean meal		Hay	
	$\bar{x}$	s.d.	$\bar{x}$	s.d.	$\bar{x}$	s.d.
2	68.3	3.8	38.8	1.1	28.0	1.9
5	84.7	1.8	40.9	1.2	38.4	2.0
9	92.2	1.5	55.4	4.0	46.8	0.6

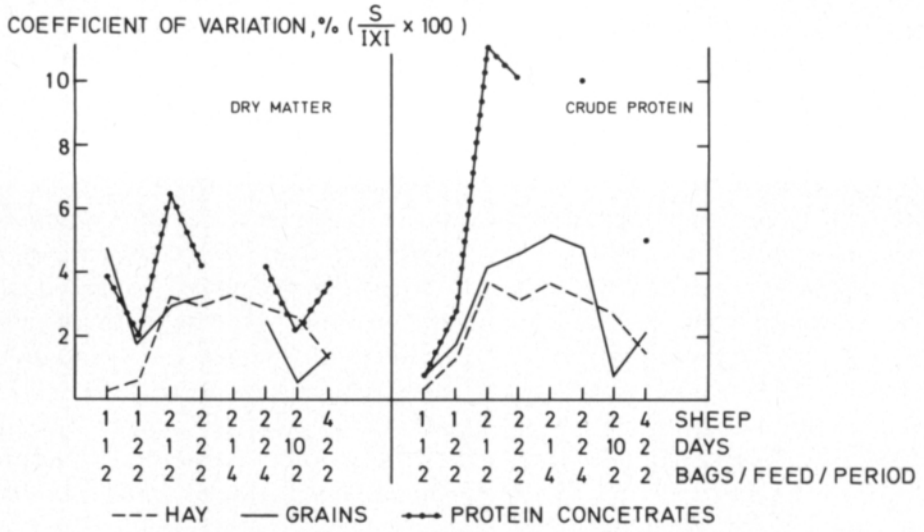


Figure 6. Estimated coefficients of variation (%) for dry matter and crude protein degradabilities using the nylon bag technique for various numbers of days, bags, and sheep on a hay diet.

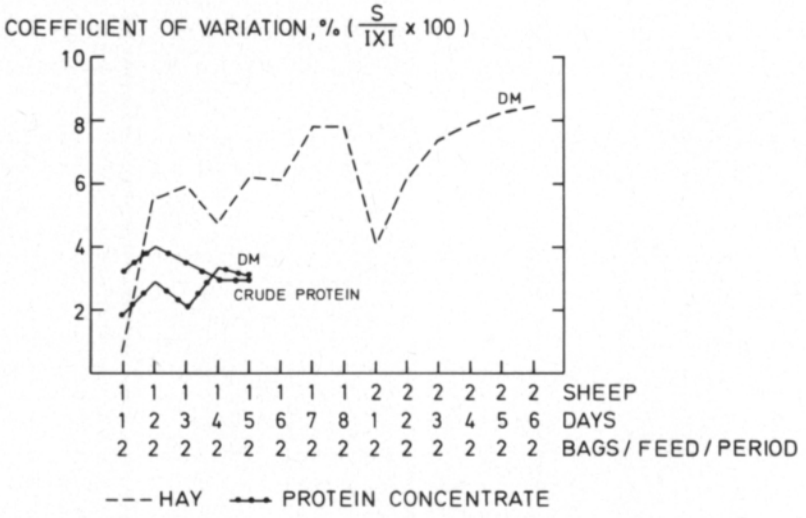


Figure 7. Estimated coefficients of variation (%) for dry matter and crude protein degradabilities using the nylon bag technique for various numbers of days, bags, and sheep on a diet of grass silage and hay.

period. Because there were no great differences in coefficients of variation within these combinations, it seems relevant to choose that combination which takes the shortest time and uses the smallest number of bags and animals: i.e. one sheep, two successive days, and two bags/feed/incubation period.

Variation can also be decreased and repeatability (intraclass correlation, calculated according to SNEDECOR and COCHRAN 1967) increased, if a control sample is used in incubations. The results in Table 12 were obtained

Table 12. Intraclass correlation and the coefficient of variation when the DM degradability of a protein concentrate mixture was evaluated using a control sample in incubations during successive days. Incubations in sheep on grass silage and hay diet (Diet 3, Appendix 2) for 9 hours.

	Coefficient of variation, %				
	Sample 1	Sample 2	Sample 3	Sample 4	Intraclass correlation
No control	1.2	3.7	1.3	4.1	92.6
With control	0.9	2.4	2.1	1.2	96.3
Incubation days	3	3	2	2	–

The average degradability for DM in a control sample was 63.6 % and for the test samples 60.0 %.

incubating a protein concentrate mixture (soybean meal 30.0 %, rapeseed meal 15.0 %, brewers' dried grains 15.0 %, molassed beet pulp 15.0 %, molasses 5.0 %, skimmilk powder 9.0 % and urea 3.0 %) in the rumen as a control sample together with test samples of the same mixture treated with formaldehyde. The incubations were made with sheep on grass silage and hay diet (Appendix 2) so that there were 5 bags (control and 4 test samples) in the rumen at the same time the incubation period being 9 hours. Samples 1 and 2 or 3 and 4 were incubated in 3 or 2 successive days, respectively.

When the correction system used in Chapter 3.1.2.1. was used in this experiment, the results for the test samples were remarkably changed. Therefore the degradabilities of the test samples were corrected using degradabilities found for the control sample as follows:

1. uncorrected degradabilities ($a_1 - a_{10}$) of test samples were related to the average ($\bar{c}$) degradability of the control sample found in the experiment
2. regression was calculated so that $y = a_1 \ldots a_{10}$ and $x = a_1/\bar{c} \ldots a_{10}/\bar{c}$
3. final correction was made by relating the uncorrected degradability of each test sample to the degradability of the control sample in the corresponding day, and by calculating the corrected values according to the regression ($x = a_1/c_1 \ldots a_{10}/c_{10}$) above

It is obvious that the corrections should be made if samples, which have to be compared together, cannot be incubated in the rumen at the same time.

Degradabilities of DM in test samples followed changes in degradability of DM in a control sample when different types of control samples were used in many other incubations. The corresponding correlations were

Control sample	*Test sample*	*Correlation*
Concentrate	Concentrate	$r = 0.750^{***}$ ($n = 14$)
Hay	Concentrate	$r = 0.777^{***}$ ($n = 17$)
Hay	Hay	$r = 0.986^{***}$ ($n = 12$)

Hay was a suitable control sample in incubations for both concentrates and roughage.

Dry matter degradability of a control sample could vary ±2.0 %-units (concentrates as a control) or ±1.5 %-units (roughage as a control) from the average degradability in incubations and this variation did not indicate such a big change in digestive processes in the rumen that it would have changed DM degradability of test samples significantly. In the case of a greater variation test samples must be incubated again.

If the results are to be applied to general recommendations for the degradabilities of DM or crude protein of a certain feed, the use of a control sample is particularly necessary, especially, when the combination of animals, days, and bags used in the experiment is similar to that suggested earlier. The necessity of the use of a control sample in incubations has also been postulated by ZINN et al. (1981). CRAWFORD et al. (1978) have used wheat middlings as a control sample in incubations in which both concentrates and roughage were studied. EHLE et al. (1982) used a reference sample containing a composite of experimental feeds.

The results obtained for a control sample can be used in two ways:

1. for corrections of the results obtained for the test samples as described above
2. to decide if the incubations must be made again which is obviously a better way to control different incubations, and to make incubations comparable

3.2.3. Differences between diets

3.2.3.1. Materials and methods

Experiment A

The experiment was made with two rumen cannulated sheep, one of which was fed on grass silage, and the other was on a hay diet (Diet 4, Appendix 2). Grass silage and hay was harvested from different growth stages (Table 13). This experiment was part of a larger project in which the digestibility of various silages and hays (TUORI et al. 1981) and the effect of

Table 13. The stages of growth of the timothy grasses harvested for silage or hay (see TUORI et al. 1981).

Silage I	—— harvested 10. 6. 1980, lenght of the grass 47 cm, heads not yet visible in the grass.
Silage II Hay I	—— harvested 17. 6. 1980, length of the grass 69 cm, heads partly visible in the grass.
Silage III Hay II	—— harvested 24. 6. 1980, length of the grass 67 cm. heads entirely visible in the grass.
Silage IV Hay III	—— harvested 1. 7. 1980, lenght of the grass 91 cm, about 25 % of the grasses were blooming.

the diet on rumen fermentation (OJALA et al. 1981, SETÄLÄ et al. 1981) were studied according to two 4×4 Latin Square designs. The sheep were fed twice a day; and for each new diet according to experimental design they had a 7 days' adaptation period followed by the experimental period of 9 days. On the 6th and 7th days of the experimental period, samples of rumen fluid from both sheep were taken before and 1.5, 3.0, and 4.5 hours after the morning feeding. Samples were treated as described by SYRJÄLÄ (1972), and pH and NH_3-N in the rumen fluid were analyzed according to SETÄLÄ et al. (1980).

On the 8th and 9th days of the experimental period, soybean and rapeseed meals (see Appendix 1) were incubated as duplicate samples for 9 hours in the rumen of sheep on grass silage (4b) or hay (4a) diet:

Period	LATIN SQUARE 1 GRASS SILAGE [1]				LATIN SQUARE 2 HAY [1]			
	Sheep				Sheep			
	A	B	C	D	E	F	G	H
1	I	II^{NB}	III	IV	I	II^{NB}	III	IV
2	II	III^{NB}	IV	I	II	III^{NB}	IV	I
3	III	IV^{NB}	I	II	III	IV[2]	I	II
4	IV	I^{NB}	II	III	IV	I^{NB}	II	III

[1] Silage or hay I–IV, see Table 13
[2] nylon bag analyses were not made because of a leakage of the rumen cannula of the sheep
NB = nylon bag incubations were performed

Both of these samples were pretreated for the incubations in a manner similar to that used in earlier experiments. The degradabilities of DM and crude protein were then analyzed.

The results were analyzed by the analysis of variance, one-way classification (SNEDECOR and COCHRAN 1967) and the differences between sample means by the Tukey-test (STEEL and TORRIE 1960) so that the degradabilities of DM and crude protein for either soybean meal or rapeseed meal were compared between different growth stages of forage within the diet of grass silage or hay.

Experiment B

This experiment was made with one sheep being fed on diets with different proportions of concentrates (Diets 5a–d, Appendix 2). The ratio of hay/barley meal in the diet DM was 100/0 (5a), 60/40 (5b), 40/60 (5c) and 30/70 (5d). The sheep was fed twice a day at an interval of 8 hours. The four experimental periods on the different diets are described in detail in Appendix 5.

Samples from the rumen contents of the sheep were taken before and 1, 2, 3, 5 (for NH_3-N), and 6 and 7 hours (pH) after the morning feeding. Rumen samples were treated and analyzed as explained in the above experiment (3.2.3.1.A). NH_3-N was determined for Diets 5a, 5c, and 5d; and pH for diets 5a–d.

Rapeseed meal and barley were incubated in the rumen for 2, 5, 9, and 12 hours; for hay an incubation period of 24 hours was also used (samples, see Appendix 1). The degradability of DM and crude protein was analyzed following the incubation. The incubations were made so that all incubation periods for each feed were incubated during one day. The samples were incubated when the sheep had been on the experimental diet for at least 14 days.

Digestive processes in the rumen of the sheep were controlled comparing degradability of hay DM after 9 hours' incubation (on Diet 5a) with the average degradability obtained with other sheep ($\bar{x}$ 32.9 %, n = 4) for the same hay on similar diets. The degradabilities did not differ significantly.

3.2.3.2. Results and discussion

Experiment A

The diet affected the degradability of DM and crude protein of both soybean and rapeseed meals. Although significant differences between diets were found for both meals (Table 14), the effect of the diet was most evident when soybean meal was incubated in the rumen.

The degradabilities of DM and crude protein of the feeds were relatively low. The reason for these low values remained unclear. Obviously a low digestive capasity of the cannulated sheep was not the reason, because such differences between the sheep in the experiment were not found when the

Table 14. The effect of diet on the degradability of dry matter and crude protein in soybean meal or rapeseed meal incubated in the rumen. (DM = dry matter, CP = crude protein)

	Growth stages of forage[1)]			
	I	II	III	IV
Grass silage diet				
Soybean meal				
Degradability of DM, %	46.5^{c}	45.5^{c}	40.1^{ad}	37.9^{bd}
Degradability of CP, %	38,8^{c}	30.8^{d}	24.9^{e}	21.4^{f}
Rapeseed meal				
Degradability of DM, %	33.9^{a}	34.3^{a}	35.2^{a}	33.1^{a}
Degradability of CP, %	26.1^{a}	22.9^{b}	23.0^{b}	22.6^{b}
Hay diet				
Soybean meal				
Degradability of DM, %	50.1^{a}	48.5^{b}	52.8^{a}	–
Degradability of CP, %	38.8^{a}	37.3^{a}	38.9^{a}	–
Rapeseed meal				
Degradability of DM, %	38.7^{a}	38.3^{a}	39.5^{b}	–
Degradability of CP, %	27.1^{c}	29.5^{d}	29.0^{d}	–

[1)] see Table 13

a, b, $P < 0.05$ or c-f, $P < 0.01$, means with different letters between growth stages of forage differ significantly

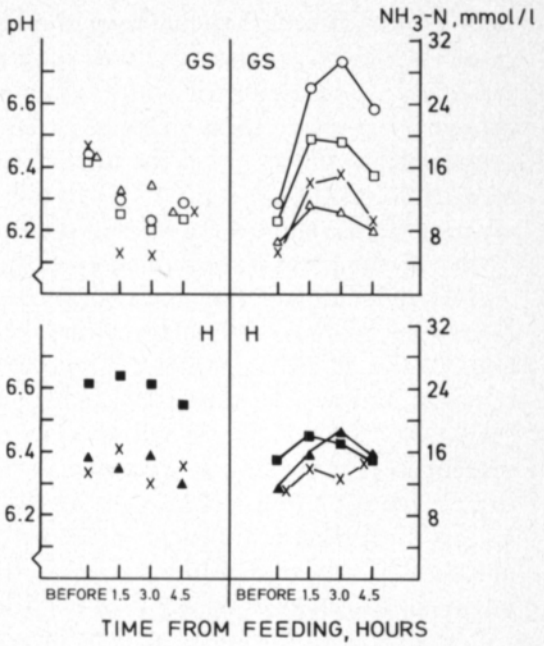


Figure 8. The pH and NH_3–N concentrations in the rumen fluid of sheep fed on diets of timothy-grass silages (GS) or hay (H) harvested at different stages of growth I–IV, see Table 13. (○● stage I, □■ stage II, △▲ stage III, X stage IV)

digestibility of roughage DM *in vivo* was determined. However, it is possible that exceptional processings caused lower degradabilities than usually in the case of both soybean meal (SETÄLÄ, unpublished observation) and rapeseed meal (SETÄLÄ and SYRJÄLÄ-QVIST 1982).

It is unlikely that changes in DM degradabilities of the soybean and rapeseed meals between diets were caused by a shortage of ammonia which affects microbial digestion in the rumen. The ammonia concentration in the rumen was lower than the suggested requirement (12–13 mmol NH_3-N/l) for maximum digestion of DM (MEHREZ et al. 1977, SETÄLÄ et al. 1982) only when grass silage from stage III cutting was included in the diet (Figure 8).

As suggested by THOMSON et al. (1972) for roughage; and by GANEV et al. (1979), ØRSKOV and McDONALD (1979) and TAMMINGA et al. (1979) for concentrates, the rate of passage of feed particles in the rumen may change the ruminal digestibility of feed. When the passage rate increased, digestibility decreased; these changes are typical at least for certain concentrates (ØRSKOV et al. 1980). However, changes in the rate of passage cannot be the reason for changes in the degradabilities of DM or crude protein of meals which remained inside the bags.

The quality of silages was quite poor. Although butyric acid was found only in silage II (0.26 % butyric acid in fresh weight of silage), considerable degradation of silage proteins had already occurred in the silos. The average percentages of water soluble N and NH_3-N in the total N contents were 65.6 %, 66.8 %, 62.1 % and 71.0 %, 6.1 %, 12.1 %, 5.5 %, and 8.8 % in the silages I, II, III and IV, respectively. The average concentrations of sugars, determined according to SALO (1965), were 4.4 % (Silage I), 3.7 % (Silage II), 5.8 % (Silage III) and 8.0 % (Silage IV) of silage dry matter. Because of this

high concentration of sugars more rapid fermentation and lower pH in the rumen were found when late-cut silage (Silage IV) was fed to the sheep.

A high proportion of water soluble N indicates a low proportion of protein N in silage total nitrogen (SETÄLÄ 1982). In the present study, the proportion of protein N in the total N contents of the diets varied from 29.0 to 37.9 %. MAENG et al. (1976) found that for optimal microbial protein synthesis in the rumen, the ratio of protein N to non-protein N should be 1:3 in diet. In the present study, however, the protein in both of the concentrates was degraded during the incubations. Because rumen ammonia did not limit microbial protein synthesis in the rumen (SATTER and SLYTER 1974, NIKOLIC et al. 1975b and SLYTER et al. 1979), rumen microbes might have required additional protein N in their growth medium. SETÄLÄ and SYRJÄLÄ-QVIST (1982) suggested that methionine, histidine, and valine are present in that fraction of rapeseed protein which is easily degraded in the rumen. Since LAMPILA (1967) and PRINS et al. (1979) have hypothesized that at least these three amino acids promote the growth of rumen microbes, their presence in rapeseed protein may be the reason it was degraded without incurring the changes in degradablity found for soybean meal protein.

According to the t-test analysis the degradability of soybean protein was significantly higher than the degradability of rapeseed protein in the Diet containing Silage I ($P < 0.001$) and Silage II ($P < 0.01$). The reason for the higher degradability of soybean protein is relatively unclear because such a release of amino acids found in the case of rapeseed meal has not been observed in soybean meal (GANEV et al. 1979 and VARVIKKO et al. 1982). When the organic matter digestibilities of the silages were determined *in vitro* according to the method described by TILLEY and TERRY (1963), it was found that the sheep received more digestible organic matter from diets containing early-cut silages. The corresponding digestibilities for Silages I, II, III and IV were 74.1 %, 69.9 %, 62.4 % and 64.4 %. It can therefore be concluded that sheep fed on early cut silages received more ruminally digestible energy, which may cause a higher rate of microbial protein synthesis in the rumen. Under these circumstances, the degradation of soybean protein probably released peptides and amino acids which were not released from the protein of rapeseed. Moreover, the degradation of DM and crude protein in soybean meal was closely related to bacteria counts in the rumen. The amount of bacteria tended to decrease when late cut silages were fed to the sheep, but no clear change was found in the counts of protozoa (OJALA et. al. 1982).

It is also possible that rumen microbes degraded soybean meal in order to obtain more energy for their activity and growth. The relationship between rumen degradable nitrogen (RDN) and the amount of ruminally fermented organic matter (RFOM) was 3.60 (Silage I), 3.05 (Silage II), 2.43 (Silage III), and 2.35 (Silage IV), expressed as grams RDN/100 grams RFOM. In these calculations, it has been assumed that 80 % (SETÄLÄ 1982) of the crude protein in the silages, and all of the digestible organic matter *in vitro,* were degraded in the rumen. According to ALLEN and MILLER (1976) and McMENIMAN and ARMSTRONG (1977), the ratio of grams RDN/100 grams

RFOM should be 2.0–3.0 for optimal microbial protein synthesis in the rumen. Therefore, rumen microbes required additional energy to that available from diets containing Silage I and Silage II.

In the case of hay the effect of the diet was relatively small but sometimes significant. The differences in degradabilities of dry matter and crude protein in soybean and rapeseed meals could also be similarly explained as for grass silage diets. However, any clear changes in the counts of bacteria and protozoa between diets were not found (OJALA et al. 1982). On the hay diets, amino-N requirements of rumen microbes might have been relatively higher than on silage diets. Organic matter digestibilities *in vitro* in the total diets were 69.0 %, 63.4 % and 60.9 % for Hay I, II and II, respectively. According to t-test the differences in degradabilities of dry matter and crude protein between the two types of meal were significant ($P < 0.001$) on each of the hay diets. Such a difference was not observed earlier in the experiments performed in this study. It is possible that the results obtained were typical for these dietary circumstances. When the same meals were incubated in the rumen of a sheep receiving a hay (fibre content 34.2 % and crude protein content 8.6 % in dry matter) harvested one week after Hay III, no significant ($P > 0.05$) differences in the crude protein degradability were observed between soybean and rapeseed meals.

Experiment B

Diet also affected the degradabilities of dry matter and crude protein in the test samples in this experiment (Tables 15, 16 and 17). An increase in the proportion of concentrates decreased most clearly the degradabilities of dry matter in barley and hay, and crude protein in hay and rapeseed meal, respectively.

The results from this experiment are in agreement with the observations of SCHOEMANN et al. (1972), MOHAMED and SMITH (1977), GANEV et al. (1979), and LINDBERG (1981b) for protein concentrates, and SIDDONS and PARADINE (1981) for barley. Using 10 μm bags LINDBERG (1981b) found no decrease in degradability of barley nitrogen when the ratio of hay to oats was changed from 70 : 30 to 30 : 70. When TAMMINGA (1981) used duodenal cannulated cows, he found no clear effect of the roughage/concentrate ratio on protein degradation in the rumen.

The variations in the degradabilities of DM and crude protein were most evidently caused by changes in rumen microflora. At least this was the case when hay was incubated in the rumen. The proportion of cellulolytic bacteria in the total microflora decreases when the amount of carbohydrates (concentrates) increases in the diet (LATHAM et al. 1974, SCHWARTZ and GILCHRIST 1975). This was especially true when pH in the rumen falls near pH 5 (STEWART 1977). LINDBERG (1981b) hypothesized that a decrease in the digestion of the NDF fraction in the feeds could decrease the degradabilities of both DM and crude protein in the case of both concentrates and roughage.

A shortage of ammonia (Fig. 9) which might be needed for proper ruminal degradation of rapeseed meal and hay DM and hence also of crude protein, is also possible (MEHREZ et al. 1977, SETÄLÄ et al. 1982) especially

Table 15. Dry matter degradability (%) of barley meal, rapeseed meal, and hay in the rumen of sheep on four diets.

Diet	Incubation, hours	Barley meal	Rapeseed meal	Hay
I hay:barley 100:0,	2	76.5	34.0	18.1
2.0 kg DM/100 kg liveweight	5	83.7	44.6	25.5
	9	87.8	55.6	31.0
	12	89.1	66.3	37.3
	24	–	–	46.2
II hay:barley 60:40	2	74.2	36.1	18.1
1.5 kg DM/100 kg liveweight	5	80.6	45.4	22.9
	9	82.6	57.1	35.2
	12	86.1	68.2	38.6
	24	–	–	42.1
III hay:barley 40:60,	2	72.4	35.7	18.8
2.0 kg DM/100 kg liveweight	5	80.7	45.6	20.7
	9	83.4	52.8	27.1
	12	86.4	51.7	25.2
	24	–	–	39.7
IV hay:barley 30:70,	2	75.6	33.8	18.0
2.5 kg DM/100 kg liveweight	5	81.9	40.2	19.1
	9	83.4	53.4	27.5
	12	84.9	53.6	30.0
	24	–	–	40.2

Table 16. Crude protein degradability (%) of barley meal, rapeseed meal, and hay in the rumen of sheep on four diets.

Diet	Incubation, hours	Barley meal	Rapeseed meal	Hay
I hay:barley 100:0,	2	68.0	37.1	36.0
2.0 kg DM/100 kg liveweight	5	89.5	48.8	35.6
	9	92.4	–	37.9
	12	95.9	74.2	48.9
	24	–	–	59.0
II hay:barley 60:40,	2	71.9	37.4	28.4
1.5 kg DM/100 kg liveweight	5	78.5	49.4	25.7
	9	80.2	60.5	48.4
	12	83.5	72.3	45.0
	24	–	–	51.0
III hay:barley 40:60,	2	75.2	35.8	31.9
2.0 kg DM/100 kg liveweight	5	83.7	47.8	32.2
	9	85.9	56.7	42.2
	12	90.7	55.2	48.2
	24	–	–	47.7
IV hay:barley 30:70,	2	68.5	38.4	34.8
2.5 kg DM/100 kg liveweight	5	79.0	48.7	30.6
	9	88.4	60.0	38.0
	12	88.0	60.3	43.9
	24	–	–	49.3

Table 17. Correlation between feed dry matter or crude protein degradability (%) and the proportion of concentrates (% in dry matter) in the diet.

	Incubation period, hours				
	2	5	9	12	24
Barley meal					
Dry matter	– 0.546	– 0.703	– 0.843	– 0.943	–
Crude protein	0.418	– 0.763	– 0.407	– 0.576	–
n	4	4	4	4	–
Rapeseed meal					
Dry matter	0.175	– 0.448	– 0.613	– 0.772	–
Crude protein	0.123	– 0.335	– 0.306[1)]	– 0.829	–
n	4	4	4	4	–
Hay					
Dry matter	0.277	– 0.982*	– 0.500	– 0.709	– 0.979*
Crude protein	– 0.272	– 0.405	0.113	– 0.621	– 0.954*
n	4	4	4	4	4

[1)] n = 3
* $P < 0.05$

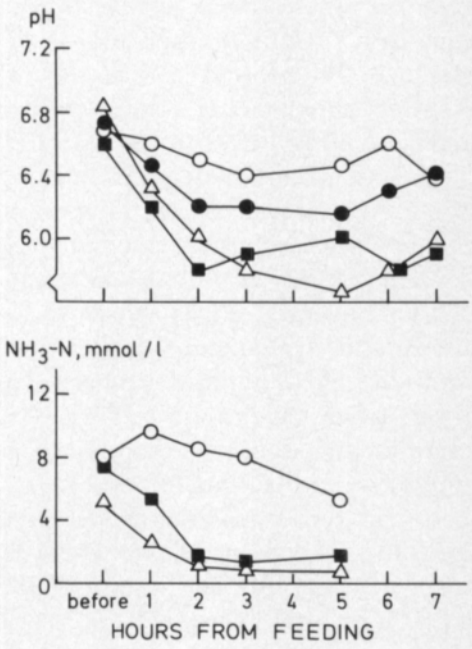


Figure 9. The pH and NH_3–N concentrations in the rumen fluid of sheep on four types of diet (○ hay: barley 100:0, ● hay: barley 60:40, △ hay: barley 40:60, ■ hay: barley 30:70).

when the two highest levels of concentrates were used. On the other hand, the increase in the proportion of starch-rich concentrate (barley) and fermentable energy may have increased microbial protein synthesis and caused more efficient utilization of ammonia, resulting in lower ammonia levels in the rumen (OFFER et al. 1978, STERN et al. 1978, HAGEMEISTER et al. 1980).

3.3. *Applicability of the in sacco* results to various feeding programmes

The effect of the basal diet can be taken into account when the experiments are carried out in an animal on the diet to which the results will be applied. Because the outflow rate and hence the retention time of feed particles in the rumen may be dependent on the feeding level (TAMMINGA et al. 1979, ØRSKOV and McDONALD 1979, and ELIMAM and ØRSKOV 1982a, b), possibilities for taking this into account need further clarification.

In nylon bag studies, the effect of the outflow rate can be taken into account by calculating the effective rate of degradation (see GANEV et al. 1979, MATHERS and MILLER 1981, or KRISTENSEN et al. 1982). One possibility is to try to evaluate the retention time of the feed particles in the rumen in different feeding levels and adjust the incubation period according to this evaluation.

3.3.1. Materials and methods

Experiment A

In order to arrive at the formulas for the evaluation of the rumen retention time for feed particles from different feeds, material was collected from the literature (Appendix 4). The measured retention time in each reference was plotted against the DM intake/100 kg liveweight, because retention time is better associated with the intake/body weight than intake/metabolic body size (EVANS 1981). The main purpose of the evaluation was to calculate ruminal retention time of feed particles under dietary conditions normally used for cattle. However, experiments with sheep had to be included when ruminal retention time of grass was evaluated.

Using data from PALOHEIMO and MÄKELÄ (1959) the rumen retention time of concentrates was chosen to be the retention time of valuable N-free nutrients in the rumen. On diets including roughage and concentrates the retention time of lignin was regarded as the time roughage particles remained in the rumen. According to SALO (1958), the method by which lignin was determined gives only the amount of indigestible lignin, although the DM of samples should contain about 7 % or more lignin.

In part of the material (MÄKELÄ 1956, PALOHEIMO and MÄKELÄ 1952, 1959), the net weights of the cows were converted to total weights according to TULLOH (1966). The weight of the digestive organs was calculated as 7 and 5 % of total liveweight for lactating and dry cows, respectively. These values were obtained for cows with liveweights of 330–460 kg.

In the collected material, the fractional outflow rate constant (k=%/100/hr) for the concentrate particles was calculated from the following formula given by ØRSKOV and McDONALD (1979):

$$t = \frac{1}{c} \ln \left(\frac{c + k}{k}\right)$$

in which t = retention time in the rumen, hours (Fig. 11)
c = factor obtained from the formula
$P = a + b\,(1-e^{-ct})$, if
P = crude protein degradability (%) after the incubation period of t hours
a = fraction of rapidly degradable crude protein (%) (after incubation of two hours)
b = fraction of crude protein, which is subjected to degradation (100 % − a)

Because barley was used as the experimental concentrate by PALOHEIMO and MÄKELÄ (1959), c was calculated from the degradabilities found for barley in Experiment 3.2.3.1.B. From these calculations and the results of LINDBERG (1981c) 0.10 was chosen for the value of c.

Experiment B

The digestibility of roughage was compared *in vivo* and *in sacco*. The experiment included determinations in four digestibility trials (Appendix 2) with sheep. The trials were as follows:
Trial 1, Diets 4a, 4b (TUORI et al. 1981, see Chapter 3.2.3.1.)
Trial 2, Diet 6 (TUORI and SETÄLÄ 1980)
Trial 3, Diets 7a, 7b (TUORI 1981)
Trial 4, Diets 8a–d (SYRJÄLÄ-QVIST et al. 1982)

The digestibilities of roughage *in vivo* were determined according to 4 × 4 Latin square design in Trials 1, 3 and 4. Trial 2 was performed with three sheep, each of which received the same diet for the same length of time. The digestibilities of roughage were compared *in vivo* and *in sacco* using only one silage (Silage II), and one hay (Hay II) in Trial 1 (see Table 13), and two (A, B) of the hays in Trial 3.

When the digestibility of roughage was evaluated *in vivo,* a collection period of 7 days was used. The degradability of DM was analyzed, using the retention time of roughage particles in the rumen, calculated according to the formulas in Figures 10–11, as incubation periods. Trials 1 and 4 included rumen cannulated sheep which were used for *in sacco* studies.The *in sacco* studies were made on two successive days with one replication (Trial 1) or on one day with one replication (Trial 4) immediately after the collection period with incubation samples taken from the diet of the cannulated sheep.

The feeds from trials 2 and 3 were analyzed with rumen cannulated sheep on a hay diet. Two replications were made, either of the one-day incubation or of 2-days incubations made with two sheep.

Experiment C -1

In this experiment the outflow rate of the liquid and solid fractions in the rumen was followed in an animal on diets with different feeding levels and ratio of roughage: concentrate.

The trial was made with one rumen-cannulated sheep, which was fed on Diets 5a – d (Appendix 2) according to the following design (see also Chapter 3.2.3.):

	kg DM/100 kg liveweight			
On DM basis	2.0	1.5	2.0	2.5
Roughage[1]: concentrate,[2]	100:0	60:40	40:60	30:70

[1] hay
[2] barley

The outflow rate for the liquid fraction was calculated using polyethylene glycol (PEG, Carbowax 4000) as a marker. The outflow rate for the solid fraction was measured with the chromium mordant technique (GANEV et al. 1979, and ØRSKOV and McDONALD 1979). Hay, barley, and rapeseed meal were used as marker feeds; they were treated with chromium according to the method described by GANEV et al. (1979) and UDEN et al. (1980).

Cr-feed (50 g) milled through a 2.0 mm sieve was diluted in 250 ml of PEG and the mixture was given as a single dose into the rumen of the sheep before the morning feeding. The sheep was fed twice a day, at 0800 a.m. and 1600 p.m. Water intake was restricted during the collection period so that the sheep had water available only during one hour after feeding. The lengths and details of the experimental periods are presented in Appendix 5.

The grab samples from the rumen contents were taken before, and 1, 2, 3, 5, 7, 8, 9, 10, and 12 hours after the morning feeding. One part of the sample was squeezed and the PEG analyzed from the liquor according to ULYATT (1964). Another part was dried in a 100°C oven overnight in order to determine the DM content of the rumen contents. This dried and crushed sample was also used when chromium analyses from the rumen contents were made according to the method presented by PETRY and RAPP (1971).

The outflow rates were calculated using the regression technique described by ØRSKOV and McDONALD (1979), and GANEV et al. (1979) for a solid fraction and HYDEN (1961) and POUTIAINEN (1968) for a liquid fraction.

Experiment C-2

In this experiment the outflow rate of solid fractions in the rumen was followed using three sheep on diets with two feeding levels and ratios of roughage: concentrate. In the trial the sheep were fed on Diets 9a–b (Appendix 2) according to the following design:

Kg DM/100 kg liveweight	Sheep 1	Sheep 2	Sheep 3
1.5	Roughage: concentrate 60:40 on DM basis		
3.0	Roughage: concentrate 40:60 on DM basis		

Hay and barley (see Appendix 3) were used as a roughage and a concentrate, respectively. The length of each experimental period was 19 days, and the feeding of the animals was performed as described in Experiment C-1.

The outflow rate for the solid fraction was evaluated using chromium treated rapeseed meal as a marker. The excretion of the marker in the faeces

was followed by taking faecal samples 2, 3.5, 5.5, 7.5, 24, 26, 27.5, 29.5, 31.5, 48, 50, 51.5, 53.5 and 55.5 hours of the dose of a marker in the rumen of the sheep. The technique for dosing and treating of a marker was similar to the technique described in Experiment C-1. Faecal samples were collected during the last three days of the experimental period.

Outflow rate for the solid fraction (k) was calculated according to GROVUM and WILLIAMS (1973). The formula used in calculations was as follows:

$$Y = A_1 e^{-k_1(t-TT)} - A_2 e^{-k_2(t-TT)}$$

where Y, A_1, A_2 are concentrations of a marker in faecal DM

k_1 is a rate constant describing kinetics of a marker movement in the reticulorumen

k_2 is a rate constant describing kinetics of a marker movement in the caecum and colon

t is a time since a marker was dosed

TT is a time of the first appearance of a marker in the faeces

Chromium concentrations of the faecal samples were analyzed according to PETRY and RAPP (1971, see Experiment C-1).

3.3.2. Results and discussion

Experiment A

The retention times for the particles of roughage and concentrates indicated differences between them and between Diets (Fig. 10–11). In animals on the same feeding level, the retention times obtained from the literature for grass were lower than times for hay or grass silage. When roughage and concentrates are given to the animal in different proportions, the retention time of roughage was calculated as being the same in diets including various amounts of concentrates on the same feeding level. The retention time of roughage was longer on diets including concentrate and roughage than on diets including roughage alone.

The retention time of concentrates changes if the proportion of concentrates in the total DM of the diet is altered: In Figure 11 this proportion was 30 or 50 %, for the Formula y_1 and y_2, respectively. This type of change also occurs when the outflow rate constants (k) are calculated (Table 18).

The material concerning grass silage and hay was collected from the papers of MÄKELÄ (1956), PALOHEIMO and MÄKELÄ (1959), WALDO et al. (1965), CAMPLING (1966) and HARTNELL and SATTER (1979). WALDO et al. (1965) suggested that there were no difference in the retention time between hay or grass silage in animals on the same feeding level.

The retention times obtained for roughage agree fairly well with the findings of SRISKANDARAJAH et al. (1981). The use of the formula for the

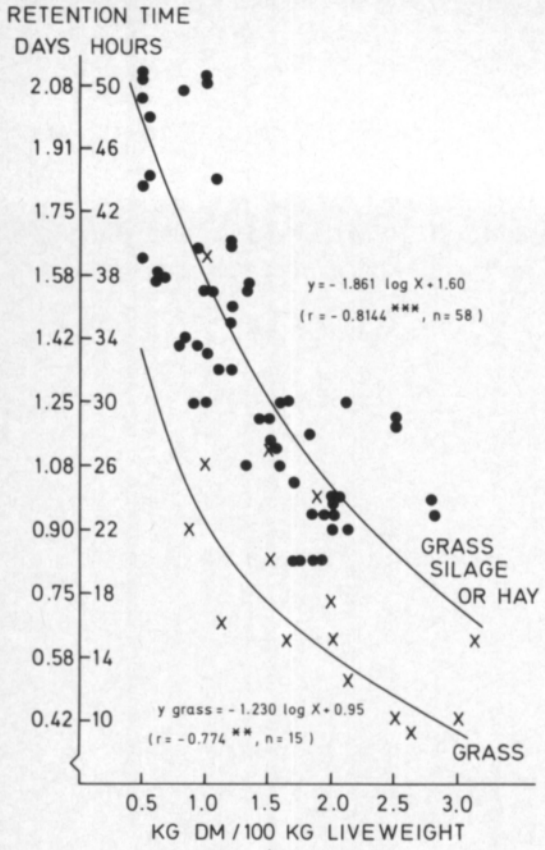


Figure 10. Ruminal retention times of hay, grass silage, and grass at different feeding levels of an animal on roughage diets (y = retention time, days , x = kg DM/100 kg liveweight). Material is based on data collected from the literature, for references, see Appendix 4.

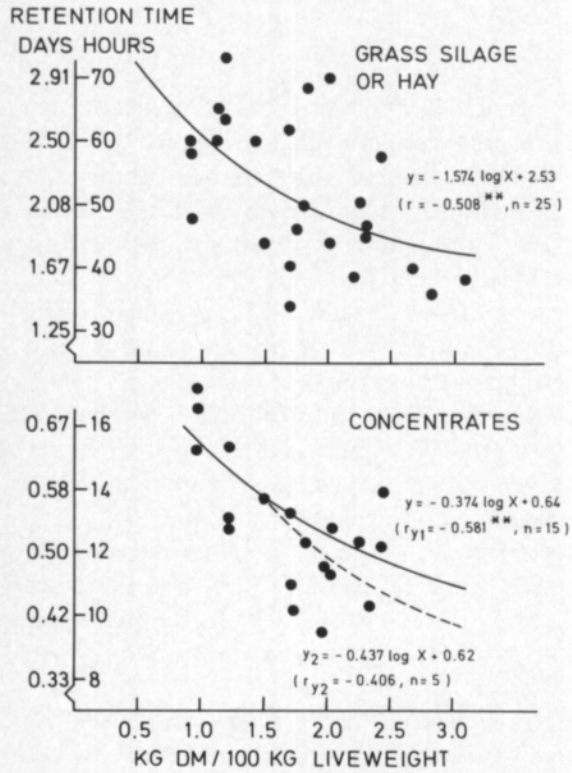


Figure 11. Ruminal retention times of hay, grass silage and concentrates when the feeding level of an animal and the ratio of roughage to concentrate (y_1, 70:30, y_2, 50:50) in the diet varied (y and x as in Figure 10). Material is based on data collected from the literature, for references, see Appendix 4.

Table 18. Calculated fractional outflow rates (k = %/100/hr) of concentrate particles in animals on different feeding levels

Roughage : concentrate	kg DM/100 kg liveweight				
on DM basis	1.0	1.5	2.0	2.5	3.0
70:30 (y_1)	0.056	0.060	0.064	0.067	0.071
50:50 (y_2)	–	0.064	0.068	0.073	0.077

evaluation of the retention time of hay and grass silage on diets including concentrates (Fig. 11) assumes that the proportion of forage to concentrate remains unchanged at different feeding levels. In practise this is probably not always true, because the proportion of concentrates generally increases at the higher feeding levels used for e.g. dairy cows unless the "flat rate feeding" (ØSTERGAARD 1979) or complete feed system (OWEN 1982) are used.

There was a great difference in the retention times of hay and grass silage between diets containing different amounts of roughage or roughage and concentrate (Fig. 10–11). LATHAM et al. (1974) and SCHWARTZ and GILCHRIST (1975) suggested that the proportion of cellulolytic bacteria will decrease as the proportion of carbohydrates in the diet increases. This means that fibrous material in the rumen is digested at a slowlier rate on a concentrate – roughage diet than on a roughage diet. The separation of crude, undigested material takes place at the reticulo-omasal orifice; and undigested feed particles are returned to the rumen (BALCH 1958a). NETEMEYER et al. (1980), POPPI et al. (1980), and UDEN and VAN SOEST (1982) suggested that particle size was critical to the outflow rate of feed particles from the rumen. On the other hand, there was still, although with great difficulty, some outflow with particles larger than the "appropriate" size (POPPI et al. 1980), indicating that other factors, such as specific gravity, may also be important (CAMPLING and FREER 1962, BALCH and CAMPLING 1965).

Lower outflow rates for hay than for grains were also found by HARTNELL and SATTER (1979), and PRANGE et al. (1979), when both hay and grains were included in the diet of dairy cows. However, the ruminal turnover of hay decreased from 7.2 %/hr to 4.6 %/hr, if the proportion of concentrates was increased from 17 to 71 % and the DM intake of the lactating cows was kept on the same level (PRANGE et al. 1979). This finding disagrees with the results obtained in the present investigation, because according to Figure 11 the retention time of hay was not changed by the increase of the proportion of concentrates in the total diet. The outflow rate of concentrates did, however, increase when the proportion of concentrates in the total diet increased, which is in agreement with results obtained using the formula for the outflow rate of concentrates in the present investigation.

The retention times, which can be calculated by the formula y_2, are in agreement with the results found for rolled barley by MATHERS and MILLER (1981) when intestine – cannulated sheep were used as experimental animals. ELIMAM and ØRSKOV (1982a,b) reported higher outflow rates for concen-

trates when the feeding levels of the sheep or dairy cows increased. In their study, the k values were calculated on the basis of marker excretion in faeces, and were similar (ELIMAM and ØRSKOV 1982b) or lower (ELIMAM and ØRSKOV 1982a) than the values obtained in this study (Table 18.). Generally the k values in this study were higher than those found for sheep on a maintenance feeding level (LINDBERG 1982b, k 0.0252–0.0499); or those for dry (k 0.020–0.048) or lactating (k 0.025–0.055) dairy cows (HARTNELL and SATTER 1979); and those for dairy cows on a maintenance (k 0.0338–0.0433) or three times maintenance (k 0.0494–0.0616) feeding level (LINDBERG 1982a).

The calculated rumen retention times for fresh grass (Fig. 10) were much shorter than the times calculated for hay or grass silage. This can at least partly be explained by the fact that values for grass were calculated from the results of experiments with sheep. Although MANSBRIDGE and ØRSKOV (1980) i.a. showed no consistent differences in the flow rates of the protein concentrates between sheep and cattle at similar feeding levels, EVANS (1981) suggested that k-values for forage might be higher if determined with sheep than with cattle. On the other hand, it must be noted that mainly legume grasses were used as feed in the material from which the retention times were obtained. INGALLS et al. (1966) reported that the retention time of grasses could be up to 30 % longer than the times of legumes; although this is not probably the exact difference, due to a slight interaction between feed intake and retention time in that experiment.

On the other hand, MATHERS and MILLER (1981) found even shorter retention times. They reported that chopped alfalfa remained 8.3 hours in the rumen of sheep on a diet of 2.0–2.2 kg DM/100 kg liveweight.

Experiment B

When the digestibility of DM *in vivo* and *in sacco* were compared, there was fairly good agreement between the results (Fig. 12). The *in sacco* method tended to give lower digestibilities. This difference can be explained by the fact that cellulose and hemicellulose can be digested in the lower digestive tract of ruminant. ARMSTRONG and BEEVER (1969) reported that about 10–30 % of cellulose and hemicellulose were digested after the forestomachs with digestion taking place mainly in the caecum and colon. If it is assumed that in hays and silages, or in straw, the corresponding concentrations of cellulose plus hemicellulose would be about 35 or 60 % of DM (see i.a. THEANDER and ÅMAN 1980), the differences between *in sacco* and *in vivo* results can be explained by post-ruminal digestion.

From the above results, it can be concluded that, by using the incubation period (= assumed retention time) obtained with the formulas in Fig. 10–11, DM digestibility of roughage can be determined with reasonable accuracy.

When the digestibility of DM can be correctly analyzed the degradability of crude protein can also be fairly accurately determined, because much of the protein degradation is dependent on the digestibility of DM. Finally it must be taken into account that any attempts to correct the retention times

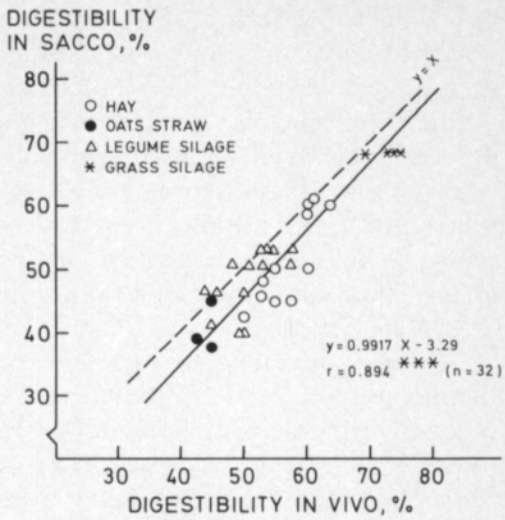


Figure 12. Comparison of the *in vivo* and *in sacco* methods in the determination of dry matter digestibility of roughage.

for roughage presented in Fig. 10–11 according to particle size of the feed were not made. The possible effects of special kind of additives, e.g. sodium monensin were not taken into account either, although LEMENAGER et al. (1978) showed that monensin reduces solid turnover rate in the rumen.

Experiment C–1

Good agreement between the observed and calculated (Fig. 10) retention times for hay was also found in this experiment (Table 19) in sheep on the hay diet. The outflow rate of hay clearly differed from that of the different concentrates.

An increase in the feeding level affected the outflow rate, particularly that of barley. This is in agreement with the formulas in Fig. 11 and with the results of PRANGE et al. (1979); and with the observations of the effect of feeding level generally reported in the literature (i.a. GANEV et al. 1979, ELIMAM and ØRSKOV 1982a, b). However, HARTNELL and SATTER (1979) reported no clear effect of changes in feeding level on the outflow rate of concentrates when the ratio of roughage to concentrates was changed at the same time.

Table 19. Fractional outflow rate (%/100/hr) of liquid (k_l) or solid (k_s) fraction, and the retention time (RTT) of feed particles in the rumen for chromium treated hay, barley, and rapeseed meal.

Kg DM/100 kg liveweight	Ratio of roughage[1] to concentrate	^{Cr}Hay			^{Cr}Barley		^{Cr}Rapeseed meal		$\bar{x}$	$\bar{x}$
		k_l	k_s	RTT	k_s	RTT	k_s	RTT	k_s	RTT
2.0	100:0	0.124	0.035	22.7	0.055	8.5	0.077	9.5	–	–
1.5	60:40	0.125	–	–	0.085	7.4	0.051	13.5	0.068	10.5
2.0	40:60	0.172	–	–	0.135	5.8	0.041	16.7	0.088	11.2
2.5	30:70	0.147	–	–	0.165	4.6	0.096	8.8	0.130	6.7

[1] roughage = hay, concentrate = barley meal
RTT = hours

Part of the differences between barley and rapeseed meal can be explained by the finding that some of the chromium labelled rapeseed meal was found in the rumen after the 4 o'clock feeding, which indicates an unstable flow of the marker. This was not found in the case of barley: the reason for this difference between the two markers remained unclear. One explanation could be that rapeseed meal tended to sequestrate on the bottom of the glass tube when rumen samples were treated and analyzed at the laboratory. This type of sequestration might also occur in the rumen. Increase in the specific gravity of rapeseed meal after the chromium treatment was suggested also by LINDBERG (1982a).

The outflow rate constants (k) were much higher than those reported in the literature or found in the present investigation (see Table 18). This was especially the case with barley. This can partly be explained by the results of EVANS et al. (1980), according to which the particulate turnover rate in the rumen can be significantly higher in animals fed twice daily (0.028 g/g/hr) than in those fed continuously (0.0175 g/g/hr). In most of the experiments cited from the literature, the animals had been fed more often than twice a day.

On the other hand, the results presented here have been obtained for one sheep only, and it is therefore possible that they are typical for this particular sheep. It should be noticed, however, that wide variation has been found between animals in the same experiments (BINES and DAVEY 1970, MATHERS and MILLER 1981) and even on the same level of DM intake (PALOHEIMO and MÄKELÄ 1952, SKRISKANDARAJAH et al. 1981). It is clear that the individual differences between animals have less effect when the results are expressed as an average value for many animals. However, the most important point in the present experiment is the result that the change in feeding level affected the outflow of the particulate material in a manner similar to that calculated from the curves in Figure 11.

A similar change to that observed in the present experiment in the liquid flow, caused by a decrease in the amount of long roughage in the diet, was also observed in dairy cows by POUTIAINEN (1968). The reason for this is evidently decreased secretion of saliva (BALCH 1958 b).

The increased outflow rate of the solid fraction can also partly explain those changes found in ammonia levels in the rumen (see Fig. 9). Because crude protein in barley is easily degradable in the rumen, the increased outflow may reduce the degradability of barley protein in the rumen and hence its effect on rumen ammonia.

Experiment C–2

The results obtained in Experiment C–1 were confirmed in Experiment C–2; higher feeding level increased the outflow rate of solid fraction in the rumen when chromium treated rapeseed meal was used as a marker (Table 20). The results for the outflow rate also agreed better with the rates presented in Table 18 than those found in Experiment C–1. The outflow rates were, however, higher than found by LINDBERG (1982a) with cattle fed

Table 20. Fractional outflow rates (k = %/100/hr) of the solid fractions in the rumen (k_1) and in the lower digestive tract (k_2) measured using chromium treated rapeseed meal as a marker.

Kg DM consumed/100 kg liveweight	Sheep 1 k_1	Sheep 2 k_1	Sheep 3 k_1	$\bar{x}$ k_1	$\bar{x}$ k_2
1.5[1)]	0.0613	0.0566	0.0684	0.0621	0.0309
2.7[2)]	0.0929	0.0571	nd	0.0750	0.0161

[1)] hay:barley 60:40 on DM basis
[2)] hay:barley 40:60 on DM basis
nd = not determined because of diarrhea of the sheep

twice a day and when chromium treated rapeseed meal was used as a marker.

In Experiment C−1 it was noticed that it was very difficult to take a representative sample from the rumen contents. Therefore sampling of the faeces was used in Experiment C−2 and it was found a fairly convenient way to take the samples with a high repeatability of the analyses. This technique has also been used earlier by many other researchers e.g. ELIMAM and ØRSKOV (1982a, b) and LINDBERG (1982a), and it was suggested by ELIMAM and ØRSKOV (1981) that k-values comparable could be obtained when the samples were taken either from the rumen contents or from the faeces.

There were differences between sheep in the results for outflow rate of solid fractions in the rumen. The differences were greater when the highest feeding level was used. Therefore it could be suggested that processes in the digestive tract between animals are more evidently different when high levels of feeding are used. The greatest differences were also found in k-values between Experiment A (Table 18) and C−1 (Table 19) when the highest feeding levels were compared.

Finally, the actual protein degradation for barley and rapeseed meal was calculated with four systems presented in the literature and by the regression for log protein degradabilities of barley and rapeseed meal (from Table 16, Diet III) plotted against time. These results were compared with degradabilities which were calculated directly (one value for the degradability) from the curves drawn from degradabilities presented in Table 16 (Diet III). Time was evaluated from Figure 11 (Table 21). The outflow rate constants (k 0.068, 0.077) were obtained from Formula y_2 in Table 18 (see Figure 11), at a respective feeding level of 2.0 and 3.0 kg DM/100 kg liveweight.

The results calculated directly from the curves agreed well with all the systems and best with the system of MILLER (1980). Both Miller's system and that of ØRSKOV and McDONALD (1979) gave protein degradabilities comparable to those obtained *in vivo* from duodenal cannulated animals (MATHERS and MILLER 1981). This means that if protein degradation is determined for an incubation period covered by the formulas for the calculation of the retention time for a particulate matter, the results obtained describe the true degradability in those feeding conditions. This seems to be the case for concentrates as well as for roughage (Experiment 3.3.2.B).

Table 21. Comparison of the calculation of the present system and four other methods for the actual degradation of feed protein in the rumen (P = actual degradation, %).

	k	1a	1b	2a	2b	3	4	5	$\bar{x}$
Barley	0.068	91.1	91.0	88.7	90.7	88.7	88.9	86.3	89.6
	0.077	88.0	88.0	87.9	89.1	88.0	88.1	84.9	87.7
Rapeseed meal	0.068	59.7	56.0	60.8	62.0	60.9	58.3	60.3	59.7
	0.077	54.8	56.0	59.1	58.6	59.1	56.5	55.1	57.0

1) a. calculated by the regression for log degradation of crude protein (%) plotted against time, which was 10 hours (k 0.077) or 12 hours (k 0.068); k was taken from Table 18 and time from Figure 11 (y_2)
b. estimated directly from the curves drawn from the degradability values presented in Table 16 (Diet III)

2) ØRSKOV & McDONALD 1979 : $P = a + b\,(1 - e^{-ct})$
a) t was calculated from the formula

$$t = \frac{1}{c} \ln \left(\frac{c + k}{k} \right)$$

c was calculated from the degradability values in Table 16 (Diet III) as was explained in Chapter 3.3.1.A
b) t was 10 or 12 hours (see 1a.)

3) ØRSKOV et al. 1980 : $P = a + \frac{bc}{c + k}$

a and b were received from the degradabilities in Table 16 (Diet III) as explained in Chapter 3.3.1.A

4) MILLER 1980 : $P = a + (a - 1) \frac{k_d}{k_r + k_d}$,

in which k_d is a rate constant for degradability from the values in Table 16 and k_r is a constant for outflow rate (calculations, see MILLER 1980)

5) Calculated by the regression for log EPD (Efficient Protein Degradation according to KRISTENSEN et al. 1982, values from Table 16) plotted against time, which was 10 hours (k 0.077) or 12 hours (k 0.068)

3.4. Summary and conclusions

Feed protein degradation in the rumen is one of the most central factors to be determined before protein utilization by the ruminant can be evaluated. The nylon bag technique provides the possibility of analyzing feed protein degradation under *in vivo* conditions without involving complicated surgical procedures or equipment.

The size and shape of the bag pores i.e. the weave of the cloth from which the bag is made, is important for proper degradability with minimum particulate loss. Cloth pore size of 40 µm ensures proper feed digestion when sample weight is 57–60 mg DM/cm^2 and shape and size of the pores are not changed by fermentation processes in the rumen. However, it is not recommended to use the same bag more than 3–4 times.

Pretreatment of the sample before incubation, and the washing technique of the bag and sample after incubation, are very critical points in the procedure. Chopping of coarse, wet material such as silage, and milling of fresh hay or straw through a 1.5 ∅ mm screen were found to be suitable pretreatments for roughage. Milling of fresh samples is also suitable for most of the concentrates. Large seeds, e.g. peas or beans, and industrially processed concentrates need special attention. The main point of the treatment is to obtain a sample with a structure similar to extrusa, but which will result in the smallest particulate losses from the bag during incubation. Washing of the sample after incubation can be carried out with an unlimited amount of water. It is important that washing is continued until the rinse water is clear and that after washing, the sample is properly dried before the amount of DM in the bag is determined.

Variation in the degradabilities of DM and crude protein occurs both between animals and incubation days. The effect of the animals is greater even under standardized feeding conditions. It is recommended that only one animal be used, analyzing the contents of two bags for each incubation period during two successive days.

The use of a control sample in all incubations is necessary in order to make different incubations comparable. Degradability of a control sample must be analyzed in advance in many sheep. Test samples must be incubated again if the degradability of a control sample is significantly different from the average degradability obtained in many sheep or in successive incubations.

In order to obtain results which could also be applied to practical feeding conditions, such factors as the type of diet and the feeding level of the animal must be taken into account. Incubations have to be made in animals on the diet for which the degradabilites will be applied. In this way, the possible effects of rumen fermentation will be taken into account. Moreover, it is important to know the feeding level of the experimental animal, that is, are the degradabilities to be applied to high-producing or low-producing animals. Feeding level determines the length of incubation period, when actual nutrient degradability is analyzed. The length of the incubation period can be calculated as follows (x = kg DM/100 kg liveweight, y = incubation period, days):

(1) for silage or hay on diets of roughage alone
$y = -1.861 \log x + 1.60$
(2) for silage or hay on diets of roughage and concentrates
$y = -1.574 \log x + 2.53$
(3) for concentrates, diet as in (2)
$y_1 = -0.374 \log x + 0.64$ (roughage : concentrates = 70:30 on DM basis in the diet)
$y_2 = -0.437 \log x + 0.62$ (roughage : concentrates = 50:50 on DM basis in the diet)

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SELOSTUS

Pötsissä tapahtuvan rehuvalkuaisen hajoavuuden määrittäminen nailonpussi-menetelmällä

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Nailonpussi-menetelmässä tutkittavaa rehua inkuboidaan eläimen pötsissä erikoisvalmisteisissa pusseissa. Pussit valmistetaan kankaasta, jota pötsimikrobit eivät pysty hajottamaan (esim. keinokuitukangas) ja jonka huokoskoko mahdollistaa mikrobien vapaan liikkumisen pussin sisään. Rehun sulavuus pötsissä lasketaan erotuksena, joka saadaan vähentämällä pussiin pannusta rehumäärästä inkubaation jälkeen pussissa ollut rehumäärä. Ennen tarvittavia määrityksiä pussi rehujäännöksineen pestään inkubaation jälkeen juoksevalla vedellä, jotta pussiin tullut pötsin sisältö saataisiin poistetuksi pussista. Rehun sulavuus lasketaan rehun ja rehujäännöksen kuiva-ainemäärän erotuksen perusteella. Raakavalkuaisen hajoavuusmäärityksessä erotuslaskelmia varten rehun ja rehujäännöksen raakavalkuaispitoisuus analysoidaan myös prosentteina rehun ja rehujäännöksen kuiva-aineesta.

Tässä tutkimuksessa selvitettiin
- tekijöitä, jotka vaikuttavat nailonpussi-menetelmällä rehuvalkuaisen hajoavuudelle saatujen tulosten luotettavuuteen
- rajoituksia, joiden mukaan rehuvalkuaisen hajoavuudelle saatuja tuloksia voidaan soveltaa käytännössä vaihteleviin ruokintaolosuhteisiin

Tutkimuksessa selvitettiin pussikankaan huokoskoon, näytteen koon ja näytteen esikäsittelyn vaikutusta nailonpussi-menetelmällä rehun kuiva-aineen, orgaanisen aineen ja raakavalkuaisen hajoavuusasteelle saatuihin tuloksiin. Lisäksi tarkasteltiin yksittäisten pussien, koeeläinten ja inkubointipäivien merkitystä vaihtelulähteinä rehujen kuiva-aineen ja raakavalkuaisen hajoavuusmäärityksissä. Eri ruokinnoista tarkasteltiin korsirehu: väkirehu -suhteen sekä säilörehun tai heinän eri kasvuasteiden vaikutusta rehujen hajoavuuteen pötsissä. Kaikki tutkimukset suoritettiin pötsifistelöidyillä lampailla ja inkuboitavina rehuina käytettiin pääasiassa heinää, kauranolkea, säilörehua, ohraa, soijarouhetta ja rypsirouhetta.

Laboratorio *(in vitro)*- ja eläinkokeiden *(in vivo)* perusteella pussin materiaaliksi valittiin 40 µm:n polyesterkangas (PES 40/27, Franz Eckert Cie, West Germany), jonka huokoset olivat samansuuruisia eivätkä pötsin käymistapahtumat vaikuttaneet kankaan rakenteeseen. Inku-

boitavan rehunäytteen suuruudeksi suhteessa kankaan pinta-alaan valittiin 57–60 mg näytteen kuiva-ainetta/cm^2, kun nailonpussin sisämitat inkubaation aikana olivat 6× 12 cm.

Inkubointia varten tuoreet (ei kuivattu vakuumissa) näytteet heinästä, oljesta, viljaväkirehusta sekä rouheesta esikäsiteltiin jauhamalla näyte 1.5 mm:n ∅ seulan läpi. Säilörehu silputtiin saksilla siten, että näytteen partikkelikoko oli alle 0.5 cm.

Koe-eläinten väliset erot olivat suurempia kuin inkubointipäivien väliset erot määritettäessä rehujen hajoavuutta pötsissä. Rehun sulavuus tietyllä aikavälillä voidaan määrittää riittävällä varmuudella inkuboimalla yhdellä lampaalla kahtena peräkkäisenä päivänä rehusta kaksi rinnakkaisnäytettä (pussia)/inkubaatioaika. Käytetyissä aineistoissa vaihtelukertoimet kuiva-aineen ja raakavalkuaisen hajoavuustuloksille olivat tällöin vastaavasti 0.6–5.5 ja 1.3–3.0 %.

Säilörehu- ja heinäruokinnalla eri kasvuasteiden välillä todettiin tilastollisesti merkitseviä eroja soija- ja rypsirouheen kuiva-aineen ja raakavalkuaisen pötsihajoavuudessa. Väkirehun osuuden lisääminen 60–70 prosenttiin rehuannoksen kuiva-aineessa vähensi ennen kaikkea rypsirouheen raakavalkuaisen hajoamista pötsissä, kun vertailuruokinnoissa väkirehun osuus vaihteli 0–40 prosenttiin kuiva-aineessa. Tulosten perusteella rehun raakavalkuaisen hajoavuus pötsissä tulisi määrittää aina niissä ruokintaolosuhteissa, joihin tuloksia sovelletaan käytännössä.

Rehuannoksen koostumuksen lisäksi ruokinnan voimakkuus vaikuttaa rehujen sulavuuteen pötsissä siten, että ruokintatason (kg ka/100 elopaino-kg) noustessa rehun viipymisaika pötsissä lyhenee mikä vähentää rehun kuiva-aineen ja raakavalkuaisen hajoamista pötsissä. Ruokinnan voimakkuus voidaan ottaa huomioon laskemalla inkubaatioajan pituus seuraavasti (× = kg ka/100 elopaino-kg, y = inkubaatioaika, d):

(1) säilörehu ja heinä, vain korsirehua ruokinnassa
$y = -1.861 \log X + 1.60$

(2) säilörehu ja heinä, ruokinnassa korsirehua ja väkirehua
$y = -1.574 \log X + 2.53$

(3) väkirehu, ruokinta kuten kohdassa (2)
$y_1 = -0.374 \log X + 0.64$ (korsirehu : väkirehu = 70:30 kuiva-aineen perusteella laskettuna)
$y_2 = -0.437 \log X + 0.62$ (korsirehu : väkirehu = 50:50 kuiva-aineen perusteella laskettuna)

Appendix

Appendix 1
Chemical composition[1)] of feeds used as test samples in *in sacco* incubations with sheep on Diets 1–5 (see Appendix 2).

Feed	Chapters in the text	DM, %	Ash	Crude protein	Crude fibre	Ether extract	N-free extracts
			% in DM				
Hay	3.1.1.–3.1.4., 3.2.2.A.	82.7	6.9	7.7	35.4	2.0	48.0
Barley	3.1.1.–3.1.2., 3.2.2.A.	87.2	2.9	12.6	6.2	3.7	74.6
Barley	3.1.3.–3.1.6.	89.1	2.6	10.7			
Soybean meal	3.1.1.–3.1.2., 3.2.2.A.	89.0	6.7	54.1			
Soybean meal	3.1.3.–3.1.6.	91.2	6.4	50.0			
Rapeseed meal	3.1.4.	87.0	7.4	37.9	13.4	4.0	37.3
Hay	3.1.5.	83.0	5.4	10.3	36.4	1.9	46.0
Soybean meal	3.2.3.1.A	83.6	7.0	51.6			
Rapeseed meal	3.2.3.1.A	85.5	10.6	35.9			
Hay	3.2.3.1.B	85.8	7.5	11.1	38.0		
Barley	3.2.3.1.B	90.5	2.6	12.2	5.4		
Rapeseed meal	3.2.3.1.B	89.8	7.7	38.6	13.9		

[1)] Chemical composition of feeds was determined using standard methods.

Appendix 2
Average composition of experimental diets (g DM/kg$W^{0.73}$/d)

Diet	Hay	Grass silage	Barley	Oats straw	Molasses beet pulp	Urea	Oats-horse bean silage
1	61.1						
2	75.9						
3	23.5	41.9					
4a	46.2						
4b		45.1					
5a	69.3						
5b	31.2		20.8				
5c	27.7		41.6				
5d	26.0		60.7				
6				45.8	24.2	1.8	
7a	59.0						
7b	53.2						
8a							50.6
8b							51.3
8c							52.4
8d							53.4
9a	27.0		17.2				
9b	33.8		48.7				

Minerals were offered ad libitum in diets 1–3, 5, 9 and 25–35 g/d in diets 4, 6–8. The composition of the mineral mixture was as follows (g/kg):

Ca 175, P 80, Na 95, Mg 50, Se 0.01

Appendix 3
Chemical composition[1] of the feeds used in experimental diets

Diet	Feed	DM %	Ash	Crude protein	Crude fibre	Ether extract	N-free extracts
			% in DM				
1	Hay	83.6	7.0	8.2	35.0	2.0	47.8
2	Hay	88.1	8.1	12.2	31.8	3.1	44.8
3	Hay	88.5	6.0	9.6	34.8	2.0	47.6
	Grass silage	26.3	9.3	15.6	28.4	5.4	41.3
4	Hay I	83.9	7.9	14.2	32.5	2.8	42.6
	II	84.3	7.0	12.1	34.6	2.5	43.8
	III	85.3	6.8	10.4	35.3	2.4	45.1
	Grass silage I	17.1	7.9	19.4	28.1	6.1	38.5
	II	20.7	8.4	15.3	29.7	4.8	41.9
	III	19.5	6.1	11.1	35.0	4.5	43.4
	IV	25.2	6.9	11.0	33.6	3.9	44.6
5	Hay	85.8	7.5	11.1	38.0	2.3	41.1
	Barley	90.5	2.6	12.1	5.4	2.4	77.5
6	Oats straw	80.0	9.1	3.7	44.8	1.6	40.7
	Urea	99.7	–	46.5[2]	–	–	–
	Molasses beet pulp	85.5	13.9	15.3	13.6	0.7	56.4
7	Hay A	83.6	4.9	7.7	36.2	1.9	49.2
	Hay B	83.0	8.0	8.7	36.0	2.4	45.0
8	Oats-horse bean silage						
	I	21.0	9.8	12.2	34.5	3.3	40.2
	II	19.2	8.0	14.1	36.9	3.1	37.9
	III	25.4	8.1	11.0	33.1	3.1	44.7
	IV	23.6	7.4	11.8	33.3	3.1	44.4
9	Hay	87.8	6.9	12.4	36.1	1.7	42.9
	Barley	87.4	3.1	12.3	6.0	2.3	76.3

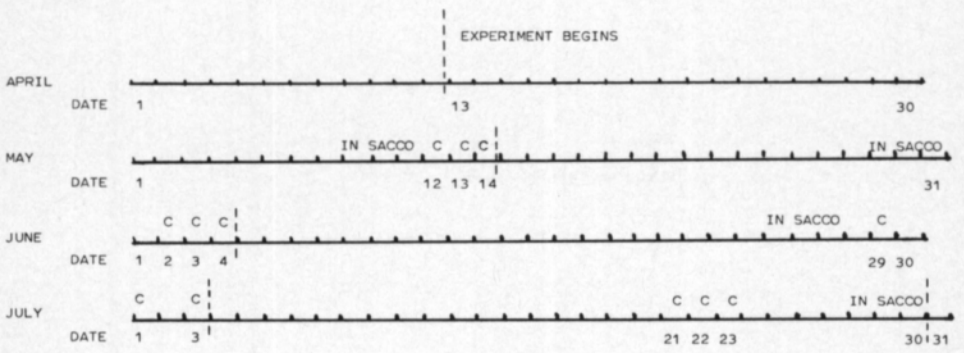
[1] Chemical composition was determined using standard methods
[2] % nitrogen in DM

Appendix 4
Details of references used in arriving at the formulas for evaluating rumen retention time (RTT) of feed particles

Diet	Animal	Method for RTT calculation	References
Hay + concentrates	Cattle	Stained particles	PALOHEIMO & MÄKELÄ 1952
Hay	Cattle	Slaughter technique [1]	MÄKELÄ 1956
Hay + concentrates	Cattle	Slaughter technique[1]	PALOHEIMO & MÄKELÄ 1959
Hay	Cattle	DM content in the rumen/DM intake	WALDO et al. 1965
Hay + concentrates			
Hay + grass silage			
Grass silage			
Hay + concentrates	Cattle	Stained particles	CAMPLING 1966
Grass	Sheep	Slaughter technique[1]	INGALLS et al. 1966
Grass	Sheep	OM content in the rumen/OM intake	MINSON 1966
Grass	Sheep	DM content in the rumen/DM intake	LAREDO & MINSON 1973
Grass	Sheep	^{144}Ce, ^{144}Pr	GROVUM & WILLIAMS 1977
Hay + concentrates	Cattle	Samarium (Sa), Lanthanium (La)	HARTNELL & SATTER 1979

[1] The animals were kept on a regular feeding schedule and slaughtered at a time midway between two feedings. The contents of the reticulorumen, other stomachs, and the small and large intestine were investigated separately.

Appendix 5



C = sample collection for analysis of chromium, PEG, pH, and NH_3-N

IN SACCO = measurements of the degradations of DM and crude protein in hay, barley, and rapeseed meal

Period 1 : 100 % hay, 13.4. - 14.5., Diet 5a

Period 2 : 60 % hay, 15.5. - 4.6. , Diet 5b

Period 3 : 40 % hay, 5.6. - 3.7. , Diet 5c

Period 4 : 30 % hay, 4.7. - 30.7. , Diet 5d