Determination of 
Ruminal Feed Digestibility and Microbial Synthesis 
Based on Digesta Sampling from the Omasal Canal

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Academic dissertation

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the Faculty of Agriculture and Forestry of the University of Helsinki, 
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Helsinki 2002
ABSTRACT

The main objective of the current thesis was to evaluate use of the omasal sampling technique to assess the flow of digesta entering the omasal canal of lactating dairy cows. Digesta flow entering the omasal canal and the proximal duodenum was compared in publication I. The flow of OM entering the omasal canal was lower than in the duodenum that reflects the contribution of endogenous secretions into the abomasum. A higher flow of NDF entering the omasal canal indicated that fibre was digested in the omasum. No differences were detected in total non-ammonia N (NAN) or undegraded dietary N flows between the omasal canal and the duodenum, while microbial N entering the omasal canal tended to be slightly higher. Compared with the omasal canal, measurements at the duodenum indicated a smaller effect of rapeseed meal supplementation on undegraded dietary N flow. Differences in nutrient flow between the omasal canal and the duodenum were primarily due to metabolism and absorption in the omasum and abomasum. In general, the precision of flow measurements was similar for both sampling sites. However, the composition of digesta samples obtained from the omasal canal deviated from true digesta to a greater extent than samples obtained from the duodenum.

Supplementation of a grass silage and barley based diet with urea, rapeseed meal or rapeseed cake on ruminal digestibility, microbial protein synthesis and milk yield was studied in publication II. Urea and rapeseed feeds had no effect on microbial N flow entering the omasal canal, despite a predicted deficiency in N availability in the rumen. Both rapeseed meal and cake increased undegraded dietary N flow entering the omasal canal that was reflected as improved milk production.

The effect of heterogeneity in particulate matter composition entering the omasal canal on the accuracy of fibre flow measurements was studied in publication III. Digesta particles entering the omasal canal were larger than those in the omasum, duodenum or in faeces, suggesting that samples collected from omasal canal did not provide a reflection of particle size distribution escaping the rumen. Since the concentration of Cr decreased and that of INDF increased with decreasing particle size, NDF flow entering the omasal canal was slightly overestimated based on INDF and slightly underestimated using Cr. However, the results indicated that the omasum may have a greater role in fibre digestion than the intestines.

The effects of barley and rapeseed meal supplementation of grass-red clover silage on ruminal digestibility and microbial flow were assessed in publication IV. Barley supplementation increased the flow of liquid associated bacterial and protozoal N, but had no effect on ruminal ammonia concentration or net absorption of N from the rumen. Barley increased daily N retention but decreased ruminal and total tract NDF digestibility. Rapeseed meal supplementation increased non-microbial N flow, net absorption of N from the rumen, urinary N excretion and daily N retention. Barley and rapeseed meal increased milk yield, the effects being additive. Responses attained with barley were explained by increased energy supply to the animal and microbes, whilst the effect of rapeseed meal was mediated through increased supply of ruminally undegradable protein. The contribution of liquid and particle associated bacteria and protozoa to total microbial N flow from the reticulorumen was modified by diet and accounted for proportionately between 0.64-0.71, 0.20-0.30 and 0.04-0.10, respectively.

The effect of four indigestible markers (LiCoEDTA, Yb-acetate, Cr-mordanted straw and INDF) and three marker systems on the flow of digesta entering the omasal canal of lactating dairy cows was assessed in publication V. Digesta flow was calculated according to single markers or using the reconstitution system based on combinations of two or three markers. It appears that digesta consists of at least three phases which tend to separate during sampling. As such a triple marker system based on Co and Yb in combination with Cr or INDF is required in order to accurately measure the flow of water-soluble nutrients, OM, N and NDF.

Current data demonstrated that precise and accurate measurements of ruminal feed digestibility and microbial synthesis can be attained based on the flow of digesta entering the omasal canal.
ACKNOWLEDGEMENTS

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LIST OF ORIGINAL PUBLICATIONS

This thesis consists of a general discussion and the following original publications referred to in the text by their Roman numerals:


The author was responsible for planning and conducting the experiments documented in publications I-IV. Publication V documented the results of four experiments, for which the author was responsible for planning and conducting three of the four. The author took full responsibility for calculation of the results, statistical analysis and preparation of the manuscripts.
**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAT</td>
<td>Amino acids absorbed from the intestine</td>
</tr>
<tr>
<td>ADF</td>
<td>Acid detergent fibre</td>
</tr>
<tr>
<td>CP</td>
<td>Crude protein (N × 6.25)</td>
</tr>
<tr>
<td>DM</td>
<td>Dry matter</td>
</tr>
<tr>
<td>INDF</td>
<td>Indigestible neutral detergent fibre</td>
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<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NAN</td>
<td>Non-ammonia N</td>
</tr>
<tr>
<td>NDF</td>
<td>Neutral detergent fibre</td>
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<tr>
<td>OM</td>
<td>Organic matter</td>
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<td>PBV</td>
<td>Protein balance in the rumen</td>
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<td>VFA</td>
<td>Volatile fatty acids</td>
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PUBLICATIONS I-V
1. INTRODUCTION

In ruminant animals the major proportion of nutrients absorbed from the alimentary tract are either end-products of microbial fermentation (such as VFA) or derived from enzymatic digestion of microbial OM synthesised in the forestomach. Prediction of nutrient supply to the ruminants relies on in vivo measurements of nutrient flow leaving the forestomach (NRC, 2001). In addition, the accuracy of mechanistic models of ruminal digestion based on compartmental analysis and measurements of digestion and passage kinetics requires validation using independent in vivo data (Firkins et al., 1998). Therefore, the accuracy of empirical predictions and mechanistic models are dependent on the accuracy of digesta flow measurements. In addition, these measurements should be sufficiently precise to allow biologically significant differences to be distinguished within the constraints of only limited numbers of observations.

Typically, digestibility of nutrients in the rumen is assessed through the collection of digesta via simple-T or closed-T shaped duodenal cannula (Harmon and Richards, 1997). Anatomically, the duodenum is an ideal sampling site because digesta flow is tubular by nature. However, endogenous secretions into the duodenum contribute to substantial proportion of non-ammonia N (NAN) flow entering the duodenum (NRC, 2001). Because non-microbial N is difficult to partition between dietary and non-dietary sources endogenous secretions introduce significant errors in measurements of true N flow derived solely from the diet. Furthermore, because digesta entering the duodenum is exposed to digestion in the abomasum, the integrity of microbial cells is not maintained (Larsen et al., 2000), such that N in digesta is unable to be reliably fractionated into microbial (liquid and particle associated bacteria, protozoa), soluble and non-soluble dietary N sources. Direct measurements of various N fractions leaving the rumen is fundamental to the development of dynamic models that attempt to predict the nutrient supply available to the ruminant animal.

To overcome these recognised shortcomings, digesta samples should be collected prior to digestion in the abomasum. Ideally, samples should be obtained at the omaso-abomasal orifice because digesta flowing through the reticulo-omasal orifice is not exposed to digestion in the omasum. Various techniques have been proposed to allow collection of samples from the omaso-abomasal (Kameoka and Morimoto, 1959; Engelhardt and Hauffe, 1975) or reticulo-omasal orifice (Bouckaert and Oyaert, 1954; Harmeyer and Michalowski, 1991). However, these techniques have not been widely adopted, possibly due to the invasive nature of these techniques and the reduction in experimental animal longevity (2-3 months, Engelhardt and Hauffe, 1975). Punia et al. (1988) obtained digesta samples from the omasal canal by aspiration through a plastic tube passed into the omasal canal via rumen cannula. Huhtanen et al. (1997) proposed a method for sampling of digesta entering the omasal canal using a sampling device, which resided in the sampling site for the entire collection period. Huhtanen et al. (1997) concluded that the sampling technique decreased DM intake of cows but had only minor effects on normal feeding behaviour, nutrient digestion and milk production. Digesta samples appeared to contain excessive amounts of liquid phase compared with particulate matter, while use of the double marker method of Faichney (1975) resulted
in flow measurements with acceptable precision. Because the accuracy of digesta flow measurements is the primary factor governing extensive implementation of this novel technique, the main focus of the studies documented in this thesis was to evaluate the relative merits and demerits of this approach compared with conventional methods. More specifically, the studies were conducted to assess the accuracy of

- estimates of ruminal digestibility and microbial synthesis based on measurements of digesta flow entering the omasal canal and the proximal duodenum
- measurements of fibre flow entering the omasal canal
- readily available markers and marker methods to determine the flow of digesta entering the omasal canal

and to evaluate the effects of

- concentrate supplementation of grass silage based diets on nutrient digestibility and microbial synthesis in the rumen
2. MATERIALS AND METHODS

The current work documented in publications I to V was conducted as five separate experiments (Table 1). All experiments were performed using lactating Finnish Ayrshire dairy cows fitted with rumen cannulas, and for Exp. 1 and 2 cows also had simple-T shaped cannulas in the proximal duodenum. Diets were formulated using feed ingredients typical of those fed on Finnish dairy farms. Because experimental procedures have been described in detail in publications I-IV, only a brief summary is presented herein. Digesta flow entering the omasal canal and the proximal duodenum was determined in Exp. 1. Comparison of flow measurements between the sampling sites is presented in I. The effects of N supplementation of a grass silage and barley based diet on digesta flow entering the omasal canal and milk production in the same experiment are documented in II. Experiment 2 assessed the impact of heterogeneity of particulate matter composition in omasal canal digesta on measurements of ruminal NDF digestibility (III). In Exp. 3, the effects of N and energy supplementation of a sole forage diet on the flow of microbial fractions entering the omasal canal were assessed (IV). Measurements of digesta flow entering the omasal canal of lactating dairy cows were conducted in two additional experiments. In Exp. 4, grass silage was gradually replaced with whole crop barley silage, whilst each diet was supplemented with 8.8 kg/d DM of a typical concentrate. For Exp. 5, cows were offered a basal diet of grass silage and cereal based concentrate at a restricted level of intake supplemented with 500 g/d of linseed, rapeseed or soybean oil. Data obtained in Exp. 1, 3, 4 and 5 were used to evaluate the effect of various indigestible markers and marker methods on the measurements of digesta flow entering the omasal canal (V).

Digesta flow entering the omasal canal was determined based on the composition of pooled spot samples obtained from the omasal canal using the omasal sampling technique of Huhtanen et al. (1997) incorporating the minor modifications described in I and III. Digesta flow was determined using indigestible markers [LiCoEDTA and Cr-mordanted straw (Udén et al., 1980); Yb-acetate or Yb-chloride, and indigestible NDF (INDF)] in combination with the reconstitution technique (France and Siddons, 1986) that allows the composition of true digesta to be calculated irrespective of unrepresentative sampling. Use of markers and calculation procedures are discussed in more detail (V).

The relationship between non-ammonia CP flow entering the omasal canal and milk protein synthesis, and that between the flow of NDF entering the omasal canal and in faeces was assessed using the regression model described by St-Pierre (2001). The model assumes each experiment has a random effect on the overall intercept and slope of the fitted regression. Model adjusted values were used to examine these relationships within experiments. Relationships based on measured and adjusted values are presented in figures 1a, 1b, 2a and 2b.
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<td>V</td>
<td>1, 3, 4, 5</td>
<td>Four 4 × 4, 4 Latin square, 4 studies studies</td>
<td>Exp. 4: Grass silage (S), whole crop barley silage (BS), concentrate mixture (C)</td>
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3. GENERAL DISCUSSION

3.1. Determination of digesta flow entering the omasal canal

Ruminal metabolism of ingested nutrients can be quantified either by direct measurements of digesta flowing from the reticulorumen or on the basis of mechanistic models that integrate measurements of pool sizes, passage rates and digestion kinetics (Firkins et al., 1998). Flow measurements do not provide information on the kinetics of ruminal metabolism but are invaluable as independent reference measurements that allow the predictive accuracy of mechanistic models to be evaluated. Huhtanen et al. (1997) introduced a technique for aspirating digesta samples from the omasal canal using a specific sampling device. The underlying assumption is that digesta flow entering the omasal canal is representative of that leaving the reticulorumen. Owing to different passage rates of liquid and various particle size fractions, the composition of digesta flowing out of the reticulorumen is different from that of ruminal contents. Because the mechanisms responsible for differential passage kinetics are attributable to the actions of the reticulum, reticulo-omasal orifice and the omasum (Lechner-Doll et al., 1991; Mathison et al., 1995), the composition of digesta entering the omasal canal is apparently more heterogenous than digesta entering the abomasum or the duodenum. Therefore, the probability of obtaining samples of an erroneous composition is inherently higher at the omasal canal compared with the duodenum.

Duodenal samples have typically been collected using simple-T cannulas (Harmon and Richards, 1997). However, the simple-T cannula has been criticised with respect to the inability of collecting representative digesta samples (Faichney, 1993; Harmon and Richards, 1997). In order to avoid unrepresentative sampling, closed-T cannulas (Komarek, 1981) have been designed, which allow complete diversion of digesta flow. Closed-T cannulas probably allow collection of samples that are representative of digesta entering the sampling sites at the time of sampling but owing to variation in composition of digesta flow do not necessarily provide samples that are representative of the daily mean composition (Gill et al., 1999). This problem can to a large extent be overcome by frequent sampling throughout the day (Firkins et al., 1998). Because digesta samples from the omasal canal are obtained by aspirating through a plastic tube, digesta phases are prone to more extensive separation than encountered with duodenal sampling (I). Furthermore, because the omasal canal sampling technique only permits the collection of spot samples flow measurements have to be based on the use of indigestible and non-absorbable external markers or internal digesta components. In theory, measurements of digesta flow assume steady state conditions, whereas in practise, unless the animals are fed continuously or at hourly interval, this situation is rarely attained due to substantial diurnal variation (Gill et al. 1999). In animals fed two equal meals at 12 h intervals steady state conditions are assumed to apply to the mean flow during 12 or 24 h. Provided that the average composition of frequently collected spot samples are representative, digesta flow can be determined using any indigestible component that can be accurately measured. However, owing to differential flow
characteristics of liquid and particulate matter, these phases may separate during sampling such that relative proportions in collected samples are unrepresentative compared with true digesta flowing past the sampling site (Faichney, 1975; 1993). Faichney (1975) suggested that digesta can be considered as two distinct phases, such that the collection of unrepresentative samples occurs due to a tendency of these phases to separate during sampling. Provided that the assumption of two homogenous phases is valid, unrepresentative samples can be reconstituted to represent true digesta using markers which exhibit selective affinities to individual phases (Faichney, 1975). The validity of the reconstitution method is dependent on the assumption that digesta sample is representative of true digesta when each marker indicates similar estimates of digesta flow. The reconstitution method can be extended to include additional phases (e.g. liquid, small and large particles) provided that markers that associate with each phase of unique flow characteristics are available (France and Siddons, 1986).

Based on the ratio between particulate and liquid phase markers (Cr:Co and Yb:Co) in omasal canal digesta and faeces, Huhtanen et al. (1997) concluded that digesta samples contained excessive amounts of liquid relative to true digesta. In order to assess the composition of digesta samples relative to that of true digesta entering the omasal canal, collected samples were fractionated into liquid, small and large particulate phases (V). Furthermore, the particle size and chemical composition of particulate matter entering the omasal canal was assessed relative to that in the reticulorumen, omasum, duodenum and faeces (III). The heterogenous nature of digesta was reflected as substantial variation in the concentration of chemical components and markers between digesta phases (V). Soluble minerals, VFA and Co were mainly associated with free liquid, nitrogenous compounds and Yb were concentrated in small particulate matter that contains small feed particles and microbes, whereas fibrous material, Cr and INDF were primarily associated with large particulate matter (V). The composition of large particulate matter was shown to be heterogenous with respect to physical and chemical characteristics (III). A similar pattern was noted for all sampling sites such that concentrations of NDF, potentially digestible NDF and Cr decreased and INDF increased with reductions in particle size (III).

Calculation of digesta flow using single markers (Co, Yb, Cr and INDF) resulted in substantial differences between estimates of OM flow suggesting that digesta samples were not representative of true digesta (V). Estimates of the reconstitution factors (i.e. the quantity of liquid and small particle DM that must be either removed or added to digesta) based on triple marker systems suggested that omasal canal digesta consisted of at least three distinct phases that tended to separate during sampling (V). Furthermore, particles in omasal canal samples were larger than those in the duodenum or faeces suggesting that these were not truly representative of particles escaping the reticulorumen (III). Owing to the unrepresentative composition of particulate matter in the collected samples, the concentration of Cr appeared to be slightly overestimated and that of INDF marginally underestimated. This resulted in a respective overestimation or underestimation of NDF flow based on INDF and Cr (III).
Evaluation of marker systems suggested that the reconstitution of true digesta should be based on a triple marker system that contained markers which associated with liquid (Co), small particle (Yb) and large particle phases (Cr or INDF; V). Since the liquid phase contained relatively low amounts of OM, the flow of organic nutrients estimated using triple marker systems were primarily dependent on particle phase markers. Sensitivity analysis suggested that variations in Cr and INDF concentration is largely reflected in estimates of fibre flow, whilst N flow is primarily determined by Yb concentration (V). Overall, the results indicated that triple marker systems based on Co and Yb in combination with Cr or INDF are required for accurate measurements of water-soluble nutrients, OM, N and NDF flow. Differences between Cr and INDF distribution across particle size fractions suggested that use of both Co.Yb.Cr and Co.Yb.INDF systems could further improve the accuracy of digesta flow measurements (III).

3.2. Passage dynamics of reticulorumen digesta

Because the quantity and composition of digesta passing through the reticulo-omasal orifice is determined by digestion and passage kinetics in the reticulorumen and net synthesis of microbial matter, consideration of these dynamic processes should indicate factors that potentially affect the accuracy of digesta flow measurements. Contents of the reticulorumen may be classified as liquid and particulate matter. Liquid matter is comprised of water and soluble substances, whereas particulate matter consists of insoluble matter. In terms of passage dynamics, liquid and particulate matter have to be considered as two distinct compartments, since the mean retention time of particulate matter is generally much greater (Lechner-Doll et al., 1991). Liquid matter is homogenous in composition such that the passage kinetics of this phase can be described by a single compartment model. In contrast, particulate matter is physically heterogeneous such that small feed particles and microbial cells may be suspended in liquid and flow in association with free liquid (Faichney, 1986), whilst freshly ingested large particulate matter may not be escapable prior to digestion and comminution (Poppi et al., 1980).

*Liquid matter*

Although a variable proportion of ruminal water (0.27-0.86, Teeter and Owens, 1983) is absorbed onto particulate matter and cannot be considered as free liquid, Teeter and Owens (1983) noted that soluble markers rapidly equilibrated between free liquid and particle associated liquid pools suggesting that the assumption of a single rumen liquid pool is adequate. Based on the assumption of simple first order dynamics for the liquid pool, numerous studies have determined ruminal outflow of soluble substances or liquid associated fractions according to measurements of rumen pool size and fractional passage rates determined using soluble markers (Poutiainen, 1968; Chen et al., 1987; Hristov and Broderick, 1996). However, owing to anatomical and functional compartmentation within the reticulorumen, incomplete mixing of the liquid phase may occur. Because saliva and drinking water may not be completely mixed with ruminal contents before entering the omasal canal (Faichney, 1993), the flow of liquid passing through the reticulo-omasal orifice may be greater than that measured using liquid
passage rates and rumen pool size. Choi et al. (2002) observed a significantly higher concentration of soluble non-ammonia N in liquid samples obtained from the omasal canal compared with the rumen (96 vs. 73 mg/l) tentatively indicating that soluble substances entered the omasal canal without completely mixing with rumen contents. It appears that sampling at the omasal canal rather than the rumen results in more reliable estimates of liquid phase passage kinetics.

**Particulate matter**

Rumen particulate matter consists of a heterogenous compartment with respect to chemical composition and physical characteristics. The size of particles varies from a few microns (e.g. bacteria and cell organelles) to several centimeters as in the case of forage particles. The passage of particulate matter from the reticulorumen is a selective process such that the probability of particle passage is inversely related to the concentration of digestible matter (Sutherland, 1988; Allen, 1996). Within the constraints of finite rumen size selective passage allows ruminants to maximise retention of potentially digestible fibre and minimise that of indigestible fibre (Huhtanen and Kukkonen, 1995). The mechanisms of selective passage have not been unequivocally demonstrated, but it is evident that particle size and functional specific gravity are the primary factors influencing particle passage (Lechner-Doll et al., 1991). The probability of particle passage increases with increases in density and decreases in particle size (Lechner-Doll et al., 1991). Observations that few large particles (>1-2 mm) appear in digesta collected distal to the reticulo-omasal orifice (Poppi et al., 1980; Waghorn, 1986) has evolved the concept of a critical particle size necessary for passage. Critical particle size has been defined as a sieve size that retains 5% of faecal particulate matter (Kennedy and Poppi, 1984). However, the reduction in particle size appears to be a prerequisite rather than the rate limiting step for particle passage from the reticulorumen (Ulyatt et al., 1986; Mathison et al., 1995). Faichney (1986) hypothesized that the passage of particles eligible to escape the rumen may be retarded owing to entrapment within the raft of large particulate matter. Lechner-Doll et al. (1991) suggested that the discrimination between escapable and non-escapable particles occurs in the reticulum, where the muscular contractions propel buoyant particles into the ventral rumen, whereas sedimenting particles settle in the ventral reticulum prior to entering the omasal canal. Endoscopic observations have indicated backflow of digesta from the omasum, suggesting that large particles could be preferentially returned back into the reticulum (McBride, 1984). Dardillat and Baumont (1992) suggested that when reticular digesta flows through an opening (either artificial or the reticulo-omasal orifice) particulate matter tends to accumulate which effectively blocks the entry of large particles into the omasal canal.

Description of excretion patterns of particle associated markers determined in the duodenum or faeces according to a single first order compartment model is clearly inadequate (Pond et al., 1988). Compartmental analysis has demonstrated that particle passage is delayed through a selective passage that can be described as an age dependent process, such that the probability of particle escape increases with retention time (Pond et al. 1988). Previous studies have consistently demonstrated that digesta flow beyond the abomasum is non-selective. Faichney and Boston (1983) and Huhtanen
and Kukkonen (1995) noted similar passage kinetics for soluble and particle associated markers distal to the abomasum. However, previous studies have not unequivocally identified the site beyond which particle passage is non-selective. Results from a slaughter study (Ulyatt et al., 1986) suggested that selection occurs prior to the omasum. Waghorn (1986) observed a higher proportion of large particles in the omasum than the abomasum favoring the hypothesis that large particles may be returned from the omasum back into the reticulum.

Overall, data reported in the literature seems to indicate that when digesta flowing through the reticulo-omasal orifice is sampled, the liquid phase is likely to be representative of that leaving the rumen. In contrast, if large particulate matter passing through the reticulo-omasal orifice is selectively returned, particulate matter obtained from the omasal canal may not be truly representative of that leaving the reticulorumen.

### 3.3. Flow of chemical components entering the omasal canal

**Organic matter flow**

Organic matter entering the omasal canal consists of microbial matter, undegradable feed and endproducts of ruminal fermentation (ammonia and VFA). The pH of digesta entering the omasal canal is similar to that of rumen contents (pH > 6), whereas the pH of digesta entering the proximal duodenum approaches that of abomasal digesta (pH 2-3). Therefore, VFA (pKa between 4.75-4.87) in digesta entering the omasal canal are largely dissociated and therefore do not evaporate during lyophilization. In contrast, the low pH of duodenal digesta results in only negligible concentrations in lyophilized duodenal digesta. Because VFA can be considered as end products of rumen fermentation, those entering the omasal canal were subtracted from total OM. For Exp. I and IV OM flow calculated in this manner was proportionately 0.13 and 0.19 lower than total OM entering the omasal canal, respectively.

Higher OM flow entering the duodenum than the omasal canal (6.95 vs. 7.47 kg/d, I) is potentially explained by endogenous OM secreted into the abomasum. Consistent with this suggestion Punia et al. (1988) reported a proportionately 0.03 lower flow of OM entering the omasum than the abomasum (2.3 vs. 2.4 kg/d). However, Punia et al. (1988) did not account for the contribution of VFA to OM flow, such that endogenous contributions were likely to be underestimated. In sheep, a large proportion of VFA (0.50) is apparently absorbed during passage through the omasum (Engelhardt and Hauffe, 1975). The role of the omasum in digestion is probably greater for the bovine than the ovine, since the weight of wet contents of the reticulorumen, omasum and abomasum as a proportion of total stomach contents was 0.85, 0.12, and 0.04 in bovines and 0.91, 0.02, and 0.08 for ovines (Warner and Flatt, 1965). On this basis, the relative retention time of digesta in the omasum would be expected to be higher in the bovine.

**Inorganic matter flow**

Inorganic matter flow entering the omasal canal consists of minerals consumed in feed and those secreted in saliva. Inorganic matter flow entering the omasal canal was
marginally higher than that in the duodenum (2211 vs. 1913 g/d, I). This finding is consistent with other studies, which have indicated net absorption of minerals from the omasum. Punia et al. (1988) reported higher inorganic matter flow entering the omasum compared with the duodenum (1295 vs. 872 g/d). Data presented by Engelhardt and Hauffe (1975) indicated that net absorption of sodium, potassium, and phosphate (3.1 g/d) and net secretion of chloride (3.2 g/d) occurred in the omasum of sheep. In ruminating calves, Edrise et al. (1986) reported a net absorption of Na and K (31 and 3 g/d respectively) and net secretion of Cl (14 g/d) in the omasum.

Nitrogen flow
Nitrogen flow entering the omasal canal consists of ammonia-N, microbial N, undegradable feed N and minor fractions of endogenous N. The concentration of ammonia-N (pKa 9.02) in lyophilized omasal canal digesta was considerably lower than that observed in digesta samples collected from the duodenum (0.02 vs. 0.06 of total N) suggesting that during freeze-drying ammonia is lost through evaporation in omasal digesta but is retained in duodenal digesta. Total NAN flow entering the omasal canal and the duodenum was similar (319 vs. 324 g/d, I). However, partitioning of total NAN between microbial and non-microbial N fractions was different between the sampling sites. Microbial NAN flow entering the omasal canal was higher than that entering the duodenum (186 vs. 176 g/d, I). Based on the assumption of lower endogenous NAN flow entering the omasal canal than the duodenum (9.8 vs. 22.4 g/d, Ørskov et al., 1986) no differences in undegraded dietary N flow were found between these sites (123 vs. 126 g/d, I). Because microbial N flow was determined using purine bases, the higher microbial flow entering the omasal canal could reflect a net disappearance of purine bases (either of microbial or dietary origin) between the omasal canal and the duodenum. In sheep, Faichney et al. (1997) noted that both microbial synthesis and degradation occurs in the omasum. Three out of four observations suggested net synthesis, whilst the other indicated net degradation of microbial N in the omasum (Faichney et al., 1997). Another explanation for higher purine flow entering the omasal canal could be due to the lack of specificity of methods used to determine purine base concentrations in digesta. Makkar and Becker (1999) reported a substantial effect of sample matrix on the concentrations of purines measured according to standard procedures (Zinn and Owens, 1986). Digestion of NDF occurred in the omasum (302 g/d) which based on the assumption of a similar efficiency for the microbial synthesis as in the reticulorumen (20.6 g/kg OM truly digested) suggests a net 6 g of microbial N synthesis in the omasum (I).

Microbial N flow. Microbial N entering the omasal canal is comprised of a mixture of microbial populations present in the reticulorumen. Rumen microbes can be classified as bacteria, protozoa and fungi, with each class containing a wide range of species. Rumen bacteria have been described as 1) free-living bacteria associated with rumen liquid phase, 2) bacteria loosely associated with feed particles, 3) bacteria firmly adhered to feed particles, 4) bacteria associated with rumen epithelia and 5) bacteria attached to the surface of protozoa or fungal sporangia (Miron et al., 2001). A major proportion of ruminal bacteria are associated with particulate matter (0.70-0.80, Craig et al., 1987; 0.74, Hristov and Broderick, 1996), but owing to slower passage rate of
particulate matter compared with liquid (0.11 vs. 0.02/h; Huhtanen and Jaakkola, 1992; Jaakkola and Huhtanen, 1992) the proportion of particle associated bacteria in microbial N flowing out of the rumen is likely to be considerably smaller. Protozoa represent a major proportion of total ruminal microbial biomass (0.46-0.87, Harrison and McAllan, 1980; 0.38-0.62, Faichney et al., 1997), but owing to selective retention in the rumen the contribution to total microbial N flowing out of the rumen is considerably smaller (0.22-0.41, Steinhour et al., 1982; 0.20-0.43, Punia et al., 1988; 0.26-0.29, Punia and Leibholz, 1994). The mean retention time of ruminal protozoa is four to six times that of CrEDTA (Michalowski et al., 1986), and considerably greater than that of digesta particles (Faichney et al., 1997). Anaerobic fungi represent only a minor component of total microbial N in the rumen (0.01-0.04) and duodenum (0.01-0.03, Faichney et al., 1997).

Measurements of microbial flow are compromised due to marker concentrations (e.g. RNA, purines, $^{15}$N) being substantially different between microbial populations (Broderick and Merchen, 1992). Differences in marker concentrations relative to that of N between liquid and particle associated bacteria have often been reported (Martin et al., 1994; Hristov and Broderick, 1996, IV), whilst the ratio of purines to N and $^{15}$N enrichment are consistently lower for protozoa than bacteria (Firkins et al., 1987; Martin et al., 1994; Hristov and Broderick, 1996, IV). These observations suggest that microbial N flow can become substantially underestimated if the contribution of protozoa is not taken into account, and yet the flow of microbial N is typically estimated based on the composition of rumen bacteria. In order to isolate a representative sample, microbes should be recovered from samples of digesta that are representative of that leaving the rumen. In this respect sampling from the duodenum would be ideal. However, lower RNA and higher DAPA concentration in bacteria harvested from the duodenum relative to the rumen, suggests that bacteria are subject to lysis in the abomasum (Larsen et al. 2000). Consistent with this observation, Dobson et al. (1984) noted lysozyme activity in the abomasum of ruminants. Provided that a representative digesta sample has been obtained prior to the abomasum, differences in recovery between microbial pools presents an additional problem. Detachment of particle associated bacteria is often incomplete (Craig et al., 1987), whilst the recovery of microbes following detachment is even lower (Martín-Orúe et al., 1998). A number of methods for the isolation of protozoa have been proposed (John and Ulyatt, 1984; Martin et al., 1994; Hristov and Broderick, 1996) which introduces variation between studies in the recovery and extent of feed particle contamination of protozoa (Neill and Ivan, 1996).

In order to avoid problems associated with the isolation of representative microbial samples, the flow of each microbial fraction was measured separately (IV). Flows of particle associated bacteria N were calculated according to NAN flow in the large particle phase and $^{15}$N-atom% excess in bacteria recovered from large particulate matter. Protozoal N flow was determined based on the quantity of protozoa recovered from composite samples containing both liquid and small particulate matter. Liquid associated bacteria N flow was calculated based on $^{15}$N-atom% excess which is not accounted for in protozoal N. Differences between microbes in $^{15}$N-atom% excess
suggested that use of liquid associated bacteria composition alone would underestimate microbial N flow by proportionately 0.08 (IV). Errors would be reduced if microbial N flow was calculated based on mixed bacteria composition. However, the extent of error is subject to variation since the contribution of individual populations to total microbial N flow will be dependent on the diet. Currently, variation between diets in the proportion of liquid and particle associated bacteria and protozoa was between 0.64-0.71, 0.20-0.30 and 0.04-0.10, respectively (IV).

Table 2. Endogenous non-ammonia N entering the omasal canal and the proximal and distal duodenum.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species, n</th>
<th>Diet</th>
<th>Omasal canal</th>
<th>Abomasum or proximal duodenum</th>
<th>Distal duodenum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ørskov et al. (1986) Dairy cow, 2</td>
<td>Intragastric infusion</td>
<td>8.3 g/d or 85 mg/kg BW&lt;sup&gt;0.75&lt;/sup&gt;</td>
<td>51 mg/kg BW&lt;sup&gt;0.75&lt;/sup&gt;</td>
<td>13.4 g/d or 195 mg/kg BW&lt;sup&gt;0.75&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Steer, 2</td>
<td></td>
<td>5.1 g/d or 58 mg/kg BW&lt;sup&gt;0.75&lt;/sup&gt;</td>
<td></td>
<td>2.9 g/d or 181 mg/kg BW&lt;sup&gt;0.75&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Steer, 4</td>
<td></td>
<td>5.8 g/d or 85 mg/kg BW&lt;sup&gt;0.75&lt;/sup&gt;</td>
<td></td>
<td>2.9 g/d or 181 mg/kg BW&lt;sup&gt;0.75&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Lamb, 4</td>
<td></td>
<td>1.3 g/d or 76 mg/kg BW&lt;sup&gt;0.75&lt;/sup&gt;</td>
<td></td>
<td>2.9 g/d or 181 mg/kg BW&lt;sup&gt;0.75&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Hart and Leibholz (1990) Steer, 3</td>
<td>Straw, sucrose, urea, minerals</td>
<td>6.2 g/d or 2.2 g/kg DMI</td>
<td>23.4 g/d or 8.6 g/kg DMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15% of total N or 41% of total N</td>
<td></td>
<td>2.2 g/kg DMI or 8.6 g/kg DMI</td>
<td></td>
</tr>
<tr>
<td>Van Bruchem et al. (1997) Sheep, 3</td>
<td>Hay, concentrates</td>
<td>3.1 g/d or 10.2 g/d or 12% of total N</td>
<td>33% of total N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.1 g/d or 10.2 g/d or 12% of total N</td>
<td></td>
<td>33% of total N</td>
<td></td>
</tr>
<tr>
<td>Larsen et al. (2000) Dairy cow, 3</td>
<td>Straw, molasses, starch, urea, minerals</td>
<td>121 g/d or 121 g/d or 35% of total N</td>
<td>35% of total N</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>121 g/d or 35% of total N</td>
<td></td>
<td>35% of total N</td>
<td></td>
</tr>
<tr>
<td>Vérité and Peyraud (1989) Dairy cow</td>
<td>NDOM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3 g/kg</td>
<td>5.3 g/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRC (2001) Dairy cow</td>
<td></td>
<td>1.9 g/kg DMI</td>
<td>1.9 g/kg DMI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Non-digestible organic matter intake.

Endogenous N. Sources of endogenous protein contributing to N flow entering the proximal duodenum listed by NRC (2001) include 1) mucoproteins in saliva, 2) epithelial cells from the respiratory tract, 3) cellular debris from epithelial tissue of the mouth, oesophagus and reticulorumen, 4) cellular debris from epithelial tissue of the omasum and abomasum and 5) enzyme secretions into the abomasum. Additionally, in the case that the cannula is located in the distal duodenum, bile and pancreatic
secretions also contribute to endogenous secretions (Van Bruchem et al., 1997). As a result of microbial metabolism, the first three fractions may be at least partly degraded in the rumen. However, earlier studies have shown that endogenous secretions provide a substantial amount of the total NAN entering the duodenum (Table 2). Because endogenous N is difficult to distinguish from undegradable dietary N for typical on-farm diets, measurements have been conducted in animals nutritionally maintained by intragastric infusion (Ørskov et al., 1986) or fed semipurified diets that provide little or no rumen undegradable N (Hart and Leibholz, 1990; Larsen et al., 2000). Limited observations tend to indicate that endogenous N entering the omasal canal represents approximately one third of that entering the proximal duodenum (Ørskov et al., 1986; Hart and Leibholz, 1990). On the basis of a 600 kg dairy cow ingesting 20 kg DM per day, respective flows of 500, 300, and 200 g of total, microbial and non-microbial NAN entering the proximal duodenum and the assumption that endogenous N secretion into the duodenum amounts to 1.9 g/kg DMI (NRC, 2001), an estimated 38 g/d of endogenous N would be predicted to enter the duodenum. In the case that omasal endogenous N flow is one third of that in the duodenum, then a contribution of 13 g/d would be expected. Thereby, endogenous N would account for proportionately 0.07 and 0.19 of non-microbial NAN entering the omasal canal and duodenum, respectively. Because of the substantial variation in endogenous flow between and within individual animals (Larsen et al., 2000) use of predicted values increases errors associated with measurements of undegraded dietary N flow. The contribution of endogenous secretions is related to the site at which it is measured and should therefore be markedly lower for samples collected from the omasal canal than the duodenum.

**Precision of N flow measurements.** The relationship between non-ammonia CP entering the omasal canal and milk protein yield was consistent within studies, whilst considerable differences between individual experiments were noted (Figure 1a). Adjusting milk protein yield for between-study differences (Figure 1b) indicated a linear relationship between protein flow and output in milk ($R^2 = 0.98$). Since observations of milk protein yield are independent of digesta flow measurements this consistency would tend to suggest that observed differences in NAN flow within studies was a true reflection of between-diet differences. Variation attributed to between-experiment differences probably reflect differences in the nutritional balance of experimental diets and variations in the stage of lactation and utilisation of absorbed nutrients. Consistent with the current data, Thomas and Rae (1988) noted that relationships between non-ammonia CP flow in the duodenum and milk protein yield were entirely consistent within but substantially different between studies.

To evaluate the precision of N flow measurements, variation in apparent ruminal N digestibility and N flow entering the omasal canal for Exp. 1 to 7 were compared to data reported in the literature (Titgemeyer, 1997). With the exection of Exp. 5, measurements based on omasal canal sampling were found to be more precise than the majority of measurements based on sampling at the duodenum (Table 3).
Figure 1. The relationship between non-ammonia CP flow entering the omasal canal and milk protein yield (g/d) in six studies. Figure a) measured values and Figure b) values adjusted for between-study effects. [Exp. 1 to 5 (refer to Table 1), Exp. 6 (Korhonen et al. 2002), Exp. 7 (Korhonen et al., unpublished)]

\[ Y = 0.25 \text{(SE 0.11)} \times + 213 \text{(SE 241)} \]

\[ R^2 = 0.98 \]
Table 3. Variation in apparent ruminal N digestibility and N flow entering the omasal canal based on Exp. 1 to 7 or measurements at the duodenum reported in the literature (Titgemeyer, 1997).

<table>
<thead>
<tr>
<th>Study</th>
<th>SD for RNDa</th>
<th>CV for N flowb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1</td>
<td>5.0</td>
<td>5.7</td>
</tr>
<tr>
<td>Exp. 3</td>
<td>5.4</td>
<td>8.8</td>
</tr>
<tr>
<td>Exp. 4</td>
<td>6.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Exp. 5</td>
<td>12.6</td>
<td>13.1</td>
</tr>
<tr>
<td>Exp. 6</td>
<td>7.2</td>
<td>6.5</td>
</tr>
<tr>
<td>Exp. 7</td>
<td>9.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Previous studies (Titgemeyer, 1997)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2.0-32.6</td>
<td>1.2-60.9</td>
</tr>
<tr>
<td>Percentilec</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>6.0</td>
<td>7.6</td>
</tr>
<tr>
<td>50</td>
<td>9.6</td>
<td>11.0</td>
</tr>
<tr>
<td>75</td>
<td>15.2</td>
<td>17.8</td>
</tr>
</tbody>
</table>

aStandard deviation for apparent ruminal N digestibility.
bCoefficient of variation for N flows entering the omasal canal or duodenum.
cPercentage of studies with measurement less than listed values.

Exp. 1 to 5 (refer to Table 1), Exp. 6 (Korhonen et al. 2002), Exp. 7 (Korhonen et al., unpublished).

**Fibre flow**

Owing to the heterogenous nature of large particulate matter entering the omasal canal (III), the accuracy and precision of measured fibre flows are likely to be lower than that of other nutrients associated with other phases (V). The flow of NDF entering the omasal canal was substantially greater than that entering the duodenum (302 g/d; I), whilst relative proportions of NDF digested in the reticulorumen, omasum and intestines were 0.90, 0.07, and 0.03, respectively (I). These observations suggest that either NDF has a higher digestibility in the omasum compared with the hind gut or that the flow of NDF entering the omasal canal was overestimated. Measurements of particle size distribution of digesta in the reticulorumen, omasal canal, duodenum and in faeces indicated that the size of particles entering the omasal canal was slightly overestimated and, as such, the flow of NDF in the omasal canal could have been overestimated using INDF as a marker (III). However, the concentration of potentially digestible NDF for similar size particles was higher in digesta entering the omasal canal than the duodenum, suggesting that the contribution of the omasum to total NDF digestion was greater than that of the intestines (III).

Problems were encountered in the determination of small particle NDF content in digesta using crucibles, owing to slower than recommended rate of filtration of detergent extracted samples (Undersander et al., 1993). The ratio of NDF to ADF was considerably higher for small relative to large particulate matter (8.5 vs. 2.0, I). Filtration problems were not experienced during the analysis of duodenal small particulate matter resulting in a substantially lower NDF to ADF ratio (2.7 vs. 1.9 for small and large particulate matter). The repeatability of NDF analysis of small particulate matter was improved using an ANKOM 220 FiberAnalyzer (ANKOM Technology, Fairport, NY). However, the NDF to ADF ratio of small particles was
much higher than that of large particles (5.7 vs. 1.8, IV). The difference was assumed to be an artifact, as indicated by the concentration of NDF associated N in small particulate matter and, therefore, the NDF concentration of small particulate matter was calculated based on ADF concentration assuming the same NDF to ADF ratio for large and small particulate matter. Use of direct NDF measurements would have resulted in a proportionally 0.19 higher NDF flow (IV). In order to accurately assess the NDF content of small and large particulate matter, five samples from Exp. 3 were extracted with neutral detergent solution using the ANKOM system, while polysaccharide composition of NDF was determined using the Uppsala method (Theander et al., 1995). The results indicated that a major proportion of NDF in small particulate matter was in the form of protein, whilst less than half was as true fibre (Table 4). Based on $^{15}$N-atom% excess of particle associated bacteria (IV) proportionately 0.71 of N associated with NDF was derived from bacteria. This suggests that neutral detergent does not dissolve all bacteria firmly attached to small feed particles. In contrast, a major fraction of large particulate matter NDF consisted of polysaccharides, whereas the protein concentration was considerably lower. Furthermore, protein was primarily of dietary origin (0.58). Overall, based on assumption that the NDF concentration of large particles was accurately determined the ratio of NDF to ADF in small particles of approximately 2.6 is estimated which is consistent with that in duodenal digesta (Exp. 1). This implies that NDF flow was proportionately underestimated by 0.04 in IV.

Table 4. Chemical composition of small and large particulate matter in omasal canal digesta.

<table>
<thead>
<tr>
<th>Component</th>
<th>Small particulate matter (n=5)</th>
<th>Large particulate matter (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>NDF, g/kg DM</td>
<td>256 (23.2)</td>
<td>618 (22.0)</td>
</tr>
<tr>
<td>ADF, g/kg DM</td>
<td>48 (8.4)</td>
<td>335 (23.0)</td>
</tr>
<tr>
<td>NDF/ADF</td>
<td>5.45 (0.906)</td>
<td>1.85 (0.108)</td>
</tr>
<tr>
<td>Estimated NDF, g/kg DM</td>
<td>88 (13.3)</td>
<td></td>
</tr>
<tr>
<td>NDF composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (N × 6.25), g/kg DM</td>
<td>448 (37.3)</td>
<td>95 (17.2)</td>
</tr>
<tr>
<td>Cellulose, g/kg DM</td>
<td>109 (12.4)</td>
<td>282 (6.2)</td>
</tr>
<tr>
<td>Noncellulose polysaccharides, g/kg DM</td>
<td>160 (17.7)</td>
<td>315 (14.1)</td>
</tr>
<tr>
<td>Klason lignin, g/kg DM</td>
<td>95 (2.1)</td>
<td>158 (24.2)</td>
</tr>
<tr>
<td>Total fibre, g/kg DM</td>
<td>364 (4.0)</td>
<td>755 (29.1)</td>
</tr>
<tr>
<td>Unidentified residue, g/kg DM</td>
<td>188 (31.1)</td>
<td>149 (32.6)</td>
</tr>
</tbody>
</table>

$^1$Estimated NDF concentration assuming the same ratio of NDF to ADF in small and large particulate matter.

$^2$Microbial protein constituted proportionately 0.71 (SD 0.065) and 0.42 (SD 0.046) of NDF associated protein in small and large particulate matter, respectively.

Digestibility of NDF associated protein (0.43) between the omasal canal and the duodenum as a proportion of total tract digestibility was considerably higher relative to that of protein free hemicellulose (0.06) or ADF (0.06; I). This observation is consistent
with those of Vanhatalo et al. (1996) and Huhtanen and Vanhatalo (1997) indicating more extensive post-ruminal digestion of NDF associated N compared with hemicellulose or cellulose. However, the composition of small particulate matter entering the omasal canal suggested that this finding was probably due to abomasal digestion of protein, hence decreasing NDF content of particulate matter entering the proximal duodenum.

The relationship between NDF flow entering the omasal canal and faeces indicated small but consistent differences between studies (Figure 2a). The differences may be attributed to imprecision in marker analysis and true differences in the extent of NDF digestion in the intestines. Adjusting faecal NDF output for between-study differences (Figure 2b) indicated a linear relationship ($R^2 = 0.97$) with a tendency for an increase in the proportion of NDF digested in the intestines with increased NDF flow. Because faecal excretion of NDF can be accurately determined the consistency in the relationship suggests that measurements of NDF flow entering the omasal canal based on the mean derived using Co.Yb.Cr and Co.Yb.INDF marker systems were reasonably accurate.

Table 5. Pool size and estimated mean retention time of lignin at various sites of the gastrointestinal tract of dairy cows (Paloheimo and Mäkelä, 1959; n = 21)

<table>
<thead>
<tr>
<th>Item</th>
<th>Lignin intake</th>
<th>Reticulum-rumen</th>
<th>Omasum</th>
<th>Abomasum and small intestine</th>
<th>Large intestine$^3$</th>
<th>Whole tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin pool size, kg</td>
<td></td>
<td>0.45</td>
<td>0.95</td>
<td>0.14</td>
<td>0.07</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean retention time, h</td>
<td></td>
<td>60.1</td>
<td>7.6</td>
<td>3.9</td>
<td>8.1</td>
<td>79.6</td>
</tr>
<tr>
<td>Paloheimo and Mäkelä (1959)$^1$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huhtanen and Hristov (2001)$^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$Mean retention time was determined using the model described in Figure 3, such that simulated pool size corresponds to published measurements (Paloheimo and Mäkelä, 1959).

$^2$Mean retention time was calculated assuming a total mean retention time of 47 h (Huhtanen and Hristov, 2001) and the proportion of total retention time remained similar for each site, i.e. passage rate induces no effect on relative differences between pool sizes.

$^3$Gut contents and faecal excretion during transportation to the slaughter.

Dynamic model of NDF digestibility. In order to assess the contribution of different sites of the gastrointestinal tract to fibre digestion a dynamic model presented in Figure 3 was used in combination with passage kinetics data (Paloheimo and Mäkelä, 1959; Huhtanen and Hristov, 2001). The model assumes that rumen particulate matter is comprised of two pools that can be described as non-escapable and escapable pools (Allen and Mertens, 1988). Paloheimo and Mäkelä (1959) determined lignin pool sizes at various sites of the digestive tract in slaughtered dairy cows (Table 5). The mean retention time of lignin at various sites of the gastro-intestinal tract was estimated using the dynamic model assuming ingested lignin was completely recovered in faeces. Owing to the low DM intake (1.5% SD 0.39 of body weight) the total mean retention time estimated from the data of Paloheimo and Mäkelä (1959) was substantially higher
Figure 2. The relationship between NDF flow entering the omasal canal and faeces (kg/d) in four studies. Figure a) measured values and Figure b) values adjusted for between-study effects.

Y = 0.794 (SE = 0.039) X + 0.408 (SE = 0.122)  
R² = 0.97
than that in late lactation dairy cows (80 vs. 47 h; Huhtanen and Hristov, 2001). Therefore, a total mean retention time of 47 h was used, whilst proportionate decreases in retention time were assumed to be the same for all sites. Digestibility of NDF in the reticulorumen, omasum, and intestines was calculated assuming a similar rate of digestion for potentially digestible NDF across all sites. The rate of digestion was estimated iteratively such that observed and predicted total tract digestibility converged. Rates of 0.055/h (I) and 0.085/h (III) for potentially digestible NDF were estimated. The model predicted marginally lower ruminal NDF digestibility than that observed in I and slightly higher digestibility in the intestines, whereas the predicted value at the omasum was in good agreement with the direct measurements (Table 6). Digestibility of NDF predicted for the various sites was consistent with those reported in III, although the model suggested a small shift in digestibility from the omasum to the intestines. The tendency for the model to predict higher intestinal digestibility relative to observed values is potentially attributable to a 1) longer than assumed retention time in the caecum and colon, or 2) that the rate of NDF digestion was higher in the rumen than the intestines, due to higher microbial activity in the rumen (Mauricio et al., 2001) or heterogeneity of NDF such that the intrinsic rate of NDF digestion was higher in the rumen than the intestines.

3.4. Effect on experimental animals

Based on welfare considerations, sampling from the omasal canal compared with the duodenum is advantageous due to less invasive and complicated surgery, allowing more animals to be used for experimental purposes. In order to reduce variation in DM intake during digesta collection, silage intake was restricted to proportionately of 0.95 of ad libitum intake in Exp. 1, 3 and 5. In Exp. 4, the animals were offered (on a DM basis) 8.9 kg/d of a concentrate mixture and were allowed to consume silage ad libitum. Intake recorded during digesta collection periods averaged 0.95 (21.4 vs. 22.6 kg/d) of that recorded during the period prior to fitting the sampling device into the omasal canal. In a previous study (Korhonen et al. unpublished) cows were given (on a DM basis) either 7.4 or 11.0 kg/d of concentrates and grass silage ad libitum. In this case intake during sample collection averaged 0.90 (18.6 vs. 20.6 kg/d) of that recorded in the absence of the sampling device. These observations indicate that sampling procedures tend to reduce DM intake of experimental animals. Huhtanen et al. (1997) also reported a lower DM intake for cows equipped with a sampling device compared with control cows (21.8 vs. 24.0 kg/d, respectively). However, Huhtanen et al. (1997) noted that the difference was smaller before (1.2 kg/d) than during sample collection (2.2 kg/d) indicating that intake is partly affected by the presence of the sampling device and partly by the sampling procedures per se. Reduced feed intake was reflected as lowered rumination and total chewing time and reduced digesta passage rate (Huhtanen et al., 1997). Fitting of the sampling device had no effect on the time cows spent lying or standing. Furthermore, feed digestibility, rumen digesta content and particle size distribution in rumen digesta and faeces was unaffected. It appears that the presence of the sampling device does not alter the normal passage of digesta through the alimentary tract. Detrimental effects on feed intake could possibly be minimised through assembling and removing the sampling device at each sampling interval.
Figure 3. Dynamic model of NDF digestion in the digestive tract of the dairy cow. The model assumes that the rumen is comprised of two compartments, the age-dependent non-escapable pool (Rumen_NEP) and the age-independent escapable pool (Rumen_EP).
Table 6. Measured (I and III) and predicted NDF digestibility at various sites of the gastrointestinal tract.

<table>
<thead>
<tr>
<th>Item</th>
<th>Measured NDF digestibility</th>
<th>Predicted NDF digestibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestibility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rumen</td>
<td>0.538</td>
<td>0.514</td>
</tr>
<tr>
<td>Omasum</td>
<td>0.044</td>
<td>0.049</td>
</tr>
<tr>
<td>Intestines</td>
<td>0.017</td>
<td>0.036</td>
</tr>
<tr>
<td>Total tract</td>
<td>0.599</td>
<td>0.599</td>
</tr>
<tr>
<td>Proportion of total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rumen</td>
<td>0.90</td>
<td>0.86</td>
</tr>
<tr>
<td>Omasum</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Intestines</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestibility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rumen</td>
<td>0.650</td>
<td>0.655</td>
</tr>
<tr>
<td>Omasum</td>
<td>0.066</td>
<td>0.050</td>
</tr>
<tr>
<td>Intestines</td>
<td>0.023</td>
<td>0.035</td>
</tr>
<tr>
<td>Total tract</td>
<td>0.740</td>
<td>0.740</td>
</tr>
<tr>
<td>Proportion of total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rumen</td>
<td>0.88</td>
<td>0.89</td>
</tr>
<tr>
<td>Omasum</td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td>Intestines</td>
<td>0.03</td>
<td>0.05</td>
</tr>
</tbody>
</table>

3.5. Evaluation of diet effects

Supplementing grass silage based diets with energy and protein rich concentrates has consistently improved milk production of dairy cows (Huhtanen, 1998). However, marginal yield responses generally decline with increased concentrate feeding suggesting that the quantity of nutrients supplied by each feed ingredient is dependent on the composition of the basal diet and level of DM intake (Huhtanen, 1998). Therefore, developing dynamic models of ruminal nutrient metabolism has the potential to improve the efficiency of nutrient utilisation in dairy cows. In order to provide a better understanding and generate data for dynamic modelling, the effects of typical ingredients in concentrates fed to Finnish dairy cows on the supply of nutrients entering the omasal canal was studied in II and IV.

Protein supplementation

According to the Nordic AAT/PBV protein evaluation system (Madsen et al., 1995) the basal diet, formulated from grass silage and barley, was predicted to be marginally deficient in rumen degradable N (-20 g/d) such that increases in rumen microbial N synthesis would be expected in response to supplements of rumen degradable N (II). However, NAN flow entering the omasal canal was equal to N intake for the basal diet indicating that the amount of N degraded in the rumen was equal to that synthesized by
Rumen microbial synthesis may be constrained by limitations in the supply of rumen degradable amino acids (Russell et al., 1992; Griswold et al., 1996). However, supplementation of diets comprised of silage and barley (II, IV) or silage alone (IV) with rapeseed meal or rapeseed cake had no effect on microbial N flow suggesting that increases in the supply of ruminally degradable amino acids did not enhance microbial N synthesis. Rapeseed meal (II, IV) and cake (II) increased NAN flow entering the omasal canal, milk yield and milk protein yield. Furthermore, supplementation of diets with rapeseed meal increased daily N retention but had no effect on N utilisation (IV).

Heat treatment of expelled rapeseed has been shown to decrease ruminal N degradability determined *in situ* (0.82 vs. 0.64, Rinne et al., 1999) and, as a result would be expected to increase the supply of amino acids entering the duodenum (Rinne et al., 1999). However, ruminal N degradability estimated based on flow measurements indicated lower degradabilities than those determined *in situ* and small differences between rapeseed meal and cake (0.50 vs. 0.41, respectively; II). Due to the lower N concentration of rapeseed cake compared with rapeseed meal, NAN flow entering the omasal canal was similar for diets supplemented with both protein sources (II). This finding is consistent with observations that milk yield or milk protein output were not different in cows offered rapeseed meal or rapeseed cake (II; Rinne et al., 1999).

Current observations demonstrated that production responses to rapeseed supplements were attributable to increases in rumen undegradable protein supply, since rapeseed had no effect on microbial protein synthesis or fibre digestibility. Linear increases in milk production in response to rapeseed supplements (Rinne et al., 1999) clearly suggest that the utilization of rapeseed protein could be improved through reductions in rumen degradability. However, attempts to reduce degradability by heat treatment have proved ineffective in practice (II; Rinne et al., 1999).

*Energy supplementation*

Supplementation of grass-red clover silage diets with barley provided more fermentable energy to rumen microbes and, thereby, increased energy and protein supply to the host animal (IV). Energy supplements also increased omasal canal NAN flow due to higher flows of liquid associated bacteria and protozoa. Increased protozoal N flow is consistent with observations that supplementing diets based on grass silage with concentrates increase the number of protozoa in the rumen (Chamberlain et al., 1985; Jaakkola and Huhtanen, 1992). However, improved microbial N synthesis did not decrease net absorption of N from the rumen because barley also increased ruminal N degradability. This is in agreement with barley having no effect on rumen ammonia
concentrations or urinary N excretion. Barley supplementation increased milk yield, milk protein output, daily N retention and improved the efficiency of N utilisation (IV).

Current feed tables (Tuori et al., 2000) document an AAT and PBV content of barley of 105 and -46 g/kg DM, respectively. These values suggest that supplementation of diets with 5.1 kg/d of barley should increase NAN flow by 140 g/d and decrease ruminal net absorption of N by 38 g/d. However, barley only increased NAN flow by 54 g/d and had no effect on net N absorption from the rumen (IV). These discrepancies can be explained by reduced silage DM intake (-2.3 kg/d) and decreases in NDF digestibility, such that barley supplementation resulted in a marginal increase in digestible OM intake of only 2.5 kg/d. When combined these effects were able account for approximately 50 g/d less NAN flow than that predicted. Furthermore, increased protozoal mass in the rumen may have reduced the efficiency of microbial N synthesis for barley supplemented diets, since rumen defaunation has consistently increased the amount of microbial N entering the duodenum (Jouany et al., 1988; Koenig et al., 2000).

Milk production responses to barley supplements can be explained by increases in energy supply both to the host animal and rumen microbes resulting in improved protein and energy availability in the duodenum. However, negative effects on silage intake and fibre digestibility caused a substantial reduction in energy intake relative to prediction based on tabular values (Tuori et al., 2000). Furthermore, utilisation of additional energy for microbial synthesis may be limited by the increase in rumen protozoal numbers.
5. CONCLUDING REMARKS

1. The current results suggest that ruminal feed digestibility and microbial synthesis can be determined accurately and precisely based on measurements of the flow of digesta entering the omasal canal. Lower endogenous contributions in digesta from the omasal canal than the duodenum implies that measurements of N flow are potentially more accurate using the omasal canal sampling technique. However, since microbial N may be synthesised in the omasum estimates of microbial N flow entering the omasal canal may be marginally lower than that available for absorption in the duodenum.

2. The omasal canal sampling technique allows the contribution of soluble N components (protein, peptides and free amino acids), microbial populations (liquid and particle associated bacteria, protozoa and fungi) and insoluble dietary N components to N flow from the reticulorumen to be determined.

3. Composition of digesta aspirated from the omasal canal is not representative of true digesta flowing from the reticulorumen. Therefore, determination of digesta flow entering the omasal canal is highly dependent on the use of reliable digesta reconstitution systems. An adequate marker system should be comprised of markers associated with free liquid (e.g. LiCoEDTA), small particulate matter (Yb or other rare earth metals) and large particulate matter (Cr-mordanted fibre or INDF). Ideally the distribution of particle phase marker concentrations between particle size fractions should be homogenous.

4. The accuracy of digesta flow measurements are dependent to a large extent on errors in the determination of indigestible marker concentrations. Evaluation of the accuracy of methods used to determine marker concentrations in the presence of various sample matrices warrants specific attention.

5. The contribution of liquid associated bacteria, particle associated bacteria and protozoa to total microbial N flow from the reticulorumen was dependent on the diet and accounted for proportionately between 0.64-0.71, 0.20-0.30 and 0.04 to 0.10, respectively.

6. Supplementation of grass silage based diets with rapeseed meal had no effect on microbial N synthesis in spite of low rumen ammonia concentrations. Positive milk production responses to rapeseed feeds were due to increases in undegradable dietary N flow leaving the reticulorumen.

7. Supplementation of grass silage based diets with barley increased the flow of liquid associated bacteria and protozoa. In spite of increased microbial N synthesis, barley supplements had no effect on the net absorption of N from the rumen. This may be related to increased protozoal synthesis that could reduce the efficiency of microbial N synthesis. It appears that current AAT and PBV values assigned to barley are over- and underestimated, respectively.

8. The contribution of the omasum to fibre digestion appeared to be greater than that of the intestines. Because the relative size of the omasum in bovine is considerably larger than that in ovine, the quantitative importance of the omasum to nutrient digestion in the bovine requires further investigation.
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