Determination of reticulo-rumen and whole-stomach digestion in lactating cows by omasal canal or duodenal sampling

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(Received 19 March 1998 – Revised 10 May 1999 – Accepted 16 July 1999)

Four ruminally and duodenally cannulated multiparous Finnish Ayrshire cows were fed on diets consisting of grass silage (0·6 kg/kg DM) and one of four concentrates: barley, barley + urea, barley + rapeseed meal and barley + rapeseed cake. The objective of the present study was to compare omasal canal and duodenal digesta flows. Values for digesta flow into the omasal canal and duodenum were determined using a triple-marker method based on Co-EDTA, Yb-acetate and indigestible neutral-detergent fibre (NDF) markers. Microbial non-NH_3 N (NAN) flow was assessed by purine flow. Microbial samples to determine the bacterial purine : N ratio were harvested from the rumen, omasum and duodenum. Organic matter flow was significantly lower into the omasum than the duodenum, indicating an endogenous organic matter secretion into the abomasum. In contrast, NDF and acid-detergent fibre flows were significantly higher into the omasum indicating digestion of fibre in the omasum. Microbial NAN flows were significantly different ($P < 0·001$) when estimates were based on bacterial samples harvested from different sites. Differences in total NAN, microbial NAN and dietary NAN flows entering the omasal canal and duodenum were non-significant. The results indicated that the omasal sampling technique provides a promising alternative to the duodenal sampling technique to investigate forestomach digestion in dairy cows and offers an alternative means to study rumen N metabolism.

Ruminant: Digestibility: Markers: Omasum

Physiological studies of digestion in ruminants often require digestive and absorptive processes in the rumen to be quantified. The variety of methods to measure compartmental nutrient flows within the alimentary tract has been limited to the use of cannulas fitted in the omasum, abomasum or duodenum (Faichney, 1993; Harmon & Richards, 1997). Currently, the most widely used method to measure digesta flow from the rumen relies on sampling through a simple T-cannula fitted either in the proximal duodenum or abomasum (Titgemeyer, 1997). Reports on ruminal digesta outflow measured without post-ruminal cannulation are limited. Punia *et al*. (1988) estimated the flow of N-fractions from the rumen by obtaining digesta samples from the omasal canal by aspiration through a plastic tube. Huhtanen *et al*. (1997) developed an omasal sampling technique for obtaining spot samples of ruminal digesta outflow through the reticulo-omasal orifice. The potential advantages of this technique compared with post-ruminal sampling techniques include (1) only ruminal cannulation of animals is needed, (2) substantially less endogenous N is secreted into the rumen than into the duodenum (Ørskov *et al*. 1986) and (3) since rumen microbes are not digested in the abomasum, digesta N flow can be separated into particle- and liquid-associated bacteria, protozoa and soluble and insoluble dietary N fractions.

The objective of the present experiment was to evaluate the validity of the omasal sampling technique to estimate digesta flow. To produce valuable quantitative information on ruminal digestive processes digesta flow measurements must be both accurate and precise. The precision of measured values can be evaluated in terms of within-animal variation but assessment of the accuracy requires reliable reference values. In this study, a duodenal sampling technique was used as an alternative method to provide comparative values. However, since omasal and abomasal digestive and absorptive processes may affect the flow of digesta between the omasal canal and the proximal duodenum (Matthews *et al*. 1996; Faichney *et al*. 1997) some differences may be expected. Comparative effects of the different dietary treatments used in this study are documented in another publication (Ahvenjärvi *et al*. 1999).

Abbreviations: ADF, acid-detergent fibre; FP, fluid phase; LP, large-particle phase; NAN, non-ammonia nitrogen; NDF, neutral-detergent fibre; OM, organic matter; SP, small-particle phase.

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Materials and methods

Experimental procedures

Four multiparous dairy cows fitted with 100 mm i.d. ruminal cannulas and T-shaped cannulas in the duodenum proximal to the pancreatic duct were used in a 4×4 Latin-square experiment. Cows were fed on silage (0·60 kg/kg DM) supplemented (0·40 kg/kg DM) with either barley, barley + urea, barley + solvent-extracted rapeseed meal or barley + moist-heat-treated rapeseed cake. Silage was prepared from a first-cut sward containing timothy (*Phleum pratense*), meadow fescue (*Festuca pratensis*) and red clover (*Trifolium pratense*). Herbage was wilted after cutting, then harvested using a precision-chop forage harvester and ensiled with 5 litres/tonne of a formic-acid-based additive. Barley–rapeseed meal and barley–rapeseed cake treatments were not entirely isonitrogenous, since equal quantities of rapeseed meal and rapeseed cake were included in the concentrate portion (0·25 kg/kg DM). Urea content of the barley + urea diet was adjusted so that the diet was isonitrogenous with respect to the mean N content of the barley–rapeseed meal and barley–rapeseed cake treatments. Each animal was also offered 300 g/d of a commercial mineral mixture (Viher-Minera, Suomen Rehu Oy, Helsinki, Finland). Intake of feed ingredients is shown in Table 1, while chemical composition is presented in Table 2. Cows averaged 74 (SD 22·3) d in milk and 560 (SD 68·5) kg live weight at the beginning of the study. Each experimental period lasted 21 d consisting of a 16 d adaptation period and a 5 d sample collection period.

Cows had free access to silage during adaptation periods, but during sample collection periods intake was restricted to 0·95 of that during the adaptation period. Intake restriction was used to minimize between- and within-day variations in intake during intensive sampling. Diets were offered twice daily at 06.00 and 18.00 hours, with cows being milked at 07.00 and 17.00 hours. Intake and milk production were recorded daily. Cows were weighed at the beginning and the end of the study.

Omasal canal and duodenal digesta flows were assessed using indigestible neutral-detergent fibre (NDF), LiCoEDTA (Udén *et al.* 1980) and Yb-acetate as markers. LiCoEDTA (12 g/d per cow) and Yb-acetate (3·7 to 4·2 g/d per cow) were dissolved in 6 litres distilled water and infused continuously into the rumen. Continuous infusion of Co and Yb was started on day 13. To determine digesta flow into the omasal canal and duodenum, 400 ml spot omasal canal and duodenal samples were collected three times daily at 4 h intervals on days 17–20. Sampling started at 07.00 hours and advanced 1 h every day to cover a 12 h period, which was considered representative of the entire feeding cycle. Samples were collected from the omasal canal using the system described by Huhtanen *et al.* (1997), with the exception that the three-way stainless steel ball valve controlling alternating pressure and vacuum in the omasal sampling device was replaced by solenoid valves controlled by an adjustable relay. In addition, the tube of the sampling device was larger (14 *v.* 9·5 mm i.d.). Omasal canal and duodenal spot samples from each cow were pooled on an equal volume basis over the sampling times to provide composite omasal canal and duodenal samples. Pooled samples were stored frozen at −20°. Once thawed at room temperature, samples were separated into three different phases as follows. Whole digesta was first squeezed through one layer of cheesecloth. Solids retained on the cheesecloth were defined as the large-particle phase (LP). The filtrate was separated into small particle (SP) and fluid phases (FP) by centrifugation at 10 000 ***g*** for 15 min and removing the supernatant fraction by aspiration. LP, SP and FP were frozen and freeze-dried for chemical analysis.

Feed digestibility was determined by total faecal collection on days 18–21. Urine was separated from faeces by means of a light harness attached to the vulva of each cow and a flexible tube attached to a container residing behind the animal. On day 21, 500 ml samples of ruminal and omasal canal and 250 ml duodenal digesta were collected immediately before feeding and at 4 and 8 h post-feeding. Omasal canal and duodenal samples were collected using the method previously described. Ruminal samples were evacuated by a vacuum pump using a tube with holes on the side inserted into the rumen via the rumen cannula. This procedure allowed some particle flow into the sample. Samples were chilled on ice immediately after collection. Ruminal and omasal canal samples were divided into two 250 ml subsamples.

Bacterial samples were prepared from ruminal, omasal canal and duodenal samples by differential centrifugation. Samples were first centrifuged at 200 ***g*** for 7 min and the supernatant fraction was decanted through two layers of cheesecloth. The pellet was then suspended in 50 ml NaCl solution (9 g/l) and agitated for 10 s (Ultra-Turrax, model T-25, Janke & Kunkel GmbH & Co KG, Staufen, Germany)

Table 1. Intake of feed ingredients (kg DM/d) by cows fed on different diets

	Diet			
	B	BU	BRM	BRC
Silage	8·47	8·46	8·65	8·89
Barley	5·70	5·22	4·50	4·51
Rapeseed meal			1·54	
Rapeseed cake				1·50
Urea		0·128		
Minerals	0·277	0·257	0·292	0·293
Total	14·4	14·1	15·0	15·2

B, barley; BU, barley + urea; BRM, barley + solvent-extracted rapeseed meal; BRC, barley + moist-heat-treated rapeseed cake.

Table 2. Chemical composition of experimental feeds (g/kg DM)

	Silage*	Barley	Rapeseed meal	Rapeseed cake
DM (g/kg)	211	872	897	910
Ash	78	23	73	75
Nitrogen	22·7	17·9	64·9	56·2
NDF	638	260	268	290
ADF	385	65	193	198
Ether extract	ND	ND	39	102

NDF, neutral-detergent fibre; ADF, acid-detergent fibre; ND, not determined.
* Silage fermentation quality: pH 4·13; in DM (g/kg): lactic acid 39·1, acetic acid 20·8, propionic acid 0·5, butyric acid 2·5, reducing sugars 24·9; in total N (g/kg): soluble N 490, ammonium N 58.

to detach bacteria adhering to particles. This suspension was further centrifuged at 200 ***g*** for 7 min. The supernatant fraction was decanted through two layers of cheesecloth and pooled with the supernatant fraction from the first centrifugation. The supernatant fraction was centrifuged again at 200 ***g*** for 7 min. Following centrifugation, the supernatant fraction was decanted and centrifuged at 10 000 ***g*** for 30 min to sediment bacteria. The bacterial pellet was subsequently suspended in distilled water and freeze-dried.

Chemical analysis

Feed and faecal DM values were determined by oven-drying at 105° for 18 h. Silage DM was corrected for the loss of volatiles (Huida *et al*. 1986). Silage and digesta samples were freeze-dried for analysis. Organic matter (OM) was determined by ashing at 600° for 18 h. NDF and acid-detergent fibre (ADF) were determined according to Van Soest *et al*. (1991) and Robertson & Van Soest (1981) respectively. Difficulties in filtering after refluxing in NDF-solution were encountered during NDF analysis of omasal SP. This resulted in an increase in SP NDF content, and consequently increased the NDF : ADF ratio in SP in relation to that of LP (8·5 *v*. 2·0). Therefore, the NDF content in SP was estimated from the ADF content assuming the same NDF : ADF ratio of LP. Silage and ruminal fluid volatile fatty acids were measured according to Huhtanen *et al*. (1998). Silage was analysed for lactic acid (Barker & Summerson, 1941) and water-soluble carbohydrates (Somogyi, 1945). NH_3-N concentrations in silage and digesta were analysed according to McCullough (1967). NDF- and ADF-associated N contents were assessed according to Licitra *et al*. (1996). Purines in bacterial samples and digesta, expressed as yeast RNA equivalents, were determined according to the method of Zinn & Owens (1986). Crude protein content of feeds, dried digesta and bacterial samples was determined using a Dumas-type N analyser (Leco FP-428; Leco Corporation, St Joseph, MI, USA). The soluble N content of silage and the N content of undried silage and faecal samples were analysed by the Kjeldahl method. Co and Yb, prepared as described by Williams *et al*. (1962), were analysed by atomic absorption spectrophotometry. Indigestible NDF was determined in LP, SP and faeces but not in FP since particles were absent in this phase. Samples were weighed in duplicate (4 g) into 6 μm pore size nylon bags, incubated in the rumen of two cows for 12 d, then rinsed in a household washing machine, incubated for 60 min in boiling NDF solution, rinsed again and dried to a constant weight at 60°. Since indigestible residue was not ashed after NDF extraction, indigestible NDF contains inorganic matter, unlike NDF.

Calculations

Digesta flows into the omasum and duodenum were calculated using a triple-marker method (France & Siddons, 1986). Residual NH_3 in freeze-dried omasal and duodenal samples was subtracted from total N to calculate non-NH_3 N (NAN) content. Freeze-dried duodenal digesta samples were devoid of residual volatile fatty acids but omasal samples, which had a higher pH, contained substantial amounts. Therefore, freeze-dried FP samples were analysed for volatile fatty acid concentration and omasal OM flow was corrected for residual volatile fatty acids. Microbial NAN flows into the omasal canal and duodenum were calculated using purine bases as a marker. Endogenous NAN flow was calculated according to Ørskov *et al*. (1986) assuming 0·085 and 0·195 g NAN/kg live weight$^{0·75}$ per animal per d entering the omasal canal and duodenum respectively.

Microbial NAN flow (g/d) =
NAN in bacteria (g/g)/purines in bacteria (g/g) × purine flow (g/d).

Total NAN flow (g/d) = microbial NAN flow (g/d) + dietary NAN flow (g/d) + endogenous NAN flow (g/d).

Marker recoveries in faeces averaged 1·03, 1·01 and 0·98 g/g for indigestible NDF, Yb and Co respectively. However, estimates of Yb and Co flows into the omasal canal and duodenum were based on their appearance in faeces. Co flow beyond the sampling site was not corrected for potential absorption from the digestive tract.

Statistical analysis

The effect of digesta sampling site was assessed by the following split-plot model using the general linear models procedure within SAS (version 6, 1987; Statistical Analysis Systems Institute Inc., Cary, NC, USA).

$$Y_{ijkl} = \mu + A_i + P_j + D_k + e_{ijk} + S_l + AS_{il} + PS_{jl} + DS_{kl} + e_{ijkl},$$

where A, P, D and S are animal, period, diet and sampling site effects. Interactions between treatment, digesta sampling site and bacterial sampling site were assessed by the following split-split-plot model:

$$Y_{ijklm} = \mu + A_i + P_j + D_k + e_{ijk} + S_l + (AS)_{il} + (PS)_{jl} + (DS)_{kl} + e_{ijkl} + B_m + (AB)_{im} + (PB)_{jm} + (DB)_{km} + e_{ijkm} + (SB)_{lm} + (ASB)_{ilm} + (PSB)_{jlm} + (DSB)_{klm} + e_{ijklm},$$

where A, P, D, S and B are animal, period, diet, digesta sampling site and bacterial sampling site effects. The significance of pairwise differences between means was evaluated using Tukey's test in SAS (1987; Statistical Analysis Systems Institute Inc.). CV within animal were calculated as the square root of the mean square (e_{ijk}) divided by the mean. Standard error of the mean for each digesta sampling site was calculated as the square root of the mean square (e_{ijkl}) divided by the square root of the number of observations (n) per mean. Standard error of the mean for each bacterial sampling site was calculated as the square root of the mean square (e_{ijklm}) divided by the square root of the number of observations (n) per mean.

Table 3. Intake and flow of digesta chemical components (g/d) into the omasal canal and duodenum of cows

(Mean values for fifteen observations, with pooled standard errors)

	OM	NDF	ADF
Intake	13890	6818	3670
Omasal canal flow	6948[a]	3174[a]	1609[a]
Duodenal flow	7469[b]	2872[b]	1478[b]
SEM	112	43·0	23·4
In faeces	4102	2749	1440
SEM	89	61·4	30·5
Apparent digestibility in the			
Reticulo-rumen*	0·504[a]	0·538[a]	0·563[a]
Whole stomach†	0·466[b]	0·582[b]	0·600[b]
SEM	0·0074	0·0065	0·0072
Total digestibility	0·706	0·599	0·610
SEM	0·0058	0·0088	0·0086

OM, organic matter; NDF, neutral-detergent fibre; ADF, acid-detergent fibre.
[a,b] Mean values within a column with different superscript letters were significantly different, $P < 0·05$.
* Based on omasal canal flow.
† Based on duodenal flow.

Results

Sample quality

In period 2, one cow stopped eating during the collection period and consequently one observation was lost. The compressible ring of the omasal sampling device was found to be stretching the reticulo-omasal orifice and probably accounts for this lack of appetite. According to reconstitution factors the proportions of different phases (g/g DM) in pooled omasal canal spot samples and in calculated true digesta were 0·36 and 0·54 for LP, 0·25 and 0·20 for SP, 0·39 and 0·26 for FP respectively. The proportions of different phases in pooled duodenal spot samples and in calculated true digesta were 0·66 and 0·65 for LP, 0·07 and 0·10 for SP, 0·26 and 0·25 for FP respectively.

Organic matter and fibre flow

Interactions between diet and digesta sampling sites (omasal canal and duodenum) were not significant ($P > 0·05$) for OM, NDF and ADF flows. OM flow was lower ($P = 0·033$) into the omasal canal than the duodenum (Table 3). In contrast, ash flow was higher ($P < 0·001$) into the omasal canal (2211 *v.* 1913 g/d, SEM 26·2 g/d). Within each sampling site, the CV in OM flow was similar for the omasal canal and duodenum (0·065 *v.* 0·067). The correlation coefficient between omasal canal and duodenal OM flow was 0·92 (Fig. 1).

NDF flow was 302 g/d higher ($P = 0·005$) into the omasal canal than the duodenum (Table 3). Of this difference, hemicellulose-associated protein (N × 6·25) accounted for 85 g/d, protein-free hemicellulose 86 g/d, and ADF 131 g/d. Relative proportions of NDF digested in the rumen, omasal canal and intestines were 0·90, 0·07 and 0·03 respectively. The CV for NDF flow within each sampling site was slightly higher for the omasal canal compared with the duodenum (0·084 *v.* 0·070). The correlation coefficient between the omasal canal and duodenal NDF flow was 0·93 (Fig. 2).

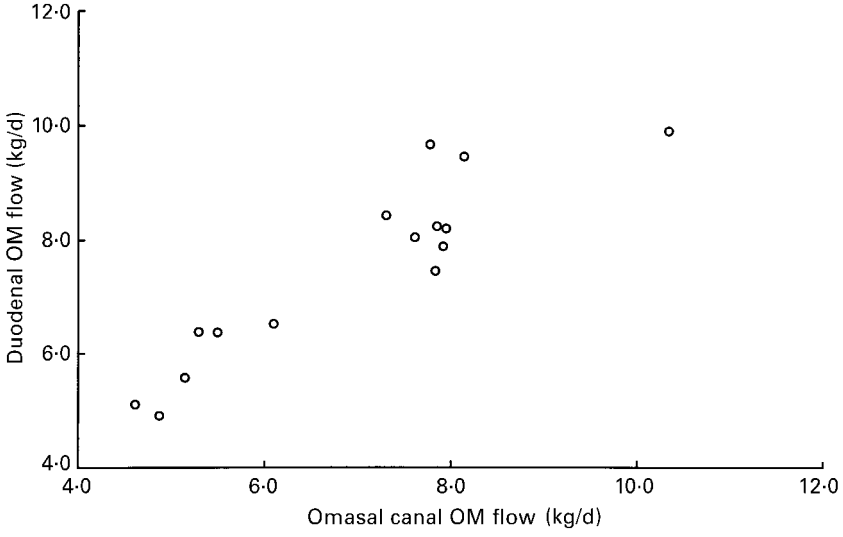

Fig. 1. Relationship between flows of organic matter (OM) into the omasal canal and duodenum (kg/d) in cows (r 0·92).

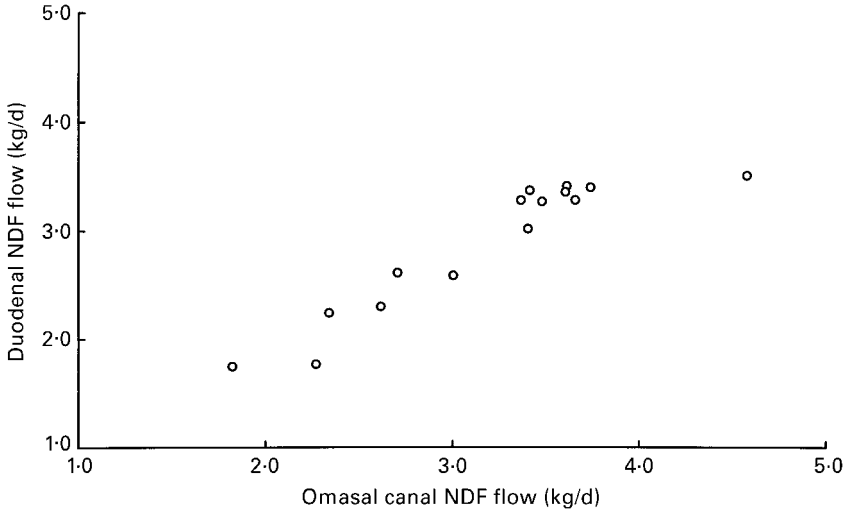

Fig. 2. Relationship between flows of neutral-detergent fibre (NDF) into the omasal canal and duodenum (kg/d) in cows (*r* 0·93).

ADF flow was higher ($P = 0{\cdot}012$) into the omasal canal than the duodenum (Table 3). The proportions of ADF digested in the rumen, omasal canal and intestines were 0·92, 0·06 and 0·02 respectively. CV in ADF flow within each sampling site was slightly higher for the omasal canal than the duodenum (0·087 *v.* 0·075). The correlation coefficient between the omasal canal and duodenal ADF flows was 0·96 (Fig. 3).

Nitrogen fractions

Three-way interactions between diet, sampling site and bacterial sampling site for microbial NAN flow, dietary NAN flow or true N digestibility were non-significant. Two-way interactions between diet and bacterial sampling site in microbial or dietary NAN flow or true N digestibility were also non-significant.

Bacterial samples collected from the rumen, omasal canal and duodenum had significantly different ($P < 0{\cdot}001$) purine : N ratios. Bacterial purine content was highest in bacteria harvested in the rumen and lowest in those collected from the duodenum. Purine content of bacteria from the omasal canal was intermediate (Table 4). Differences in bacterial purine : N ratios between harvesting sites resulted in significant differences ($P < 0{\cdot}001$) in microbial and dietary NAN flow and true rumen N digestibility. Microbial NAN flow was lowest based on the purine content of bacteria harvested from the rumen and increased for samples harvested from the omasal canal and duodenum (Table 4). CV in calculated microbial NAN flow were similar when based on bacteria collected from the rumen (0·070), omasal canal (0·071) and duodenum (0·076) respectively.

To avoid confounding effects of microbial composition due to harvesting method, omasal canal and duodenal

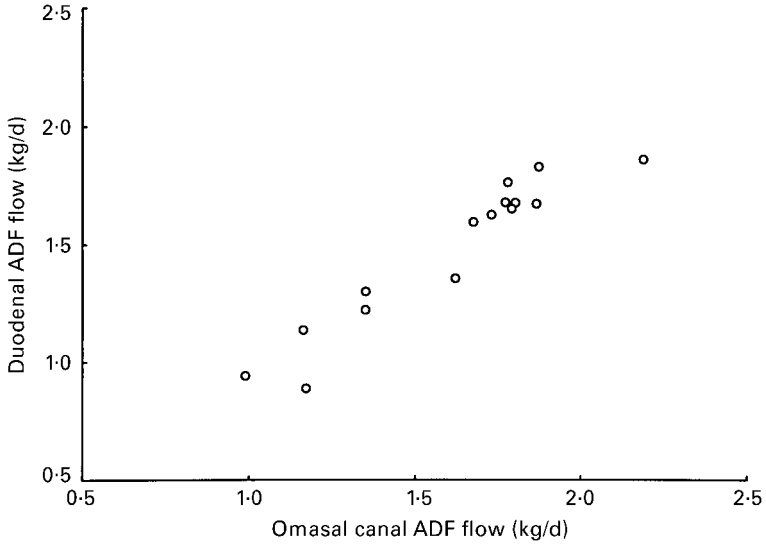

Fig. 3. Relationship between flows of acid-detergent fibre (ADF) into the omasal canal and duodenum (kg/d) in cows (*r* 0·96).

Table 4. Effect of bacterial sampling site on microbial non-ammonia nitrogen flow (g/d) and bacterial purine : N value (g/g) in cows

(Mean values with their pooled standard errors)

	Bacterial sampling site				
	Rumen	Omasum	Duodenum	SEM	*n*
Purines : N in bacteria*	1·18[a]	1·12[b]	1·02[c]	0·008	15
Microbial NAN flow	171[a]	181[b]	200[c]	0·3	30
Dietary NAN flow	134[a]	124[b]	105[c]	0·3	30
N truly digested in the forestomachs	0·622[a]	0·651[b]	0·702[c]	0·0009	30

NAN, non-ammonia nitrogen.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different, $P < 0·05$.
* Purine concentration expressed as yeast RNA equivalents.

N-fraction flows were compared based on omasal canal microbial composition. Interactions between diet and digesta sampling site were non-significant for microbial NAN ($P = 0·95$), or total NAN flow ($P = 0·29$). Interactions between these factors were also non-significant for the apparent ($P = 0·86$) and true efficiencies of microbial N synthesis ($P = 0·93$). However, interaction between diet and sampling site was significant for dietary NAN flow ($P = 0·018$) and true ruminal digestibility of N ($P = 0·036$). Compared with the control diet, rapeseed feeds increased omasal canal dietary NAN flow by 46 g/d, while duodenal dietary NAN flow increased by only 25 g/d. True ruminal N digestibilities determined in the omasal canal were 0·67 *v.* 0·62 for the control compared with diets supplemented with rapeseed feed respectively. Corresponding values determined in the duodenum were 0·62 and 0·64 respectively.

Total NAN flows into the omasal canal and duodenum (319 *v.* 324 g/d, SEM 3·1 g/d) were similar ($P = 0·35$). Microbial NAN flow tended to be higher ($P = 0·087$) into the omasal canal than duodenum (Table 5). Sampling site had no effect on the apparent or true efficiency of microbial N synthesis ($P = 0·91$ and $P = 0·81$ respectively). Since 9·8 and 22·4 g endogenous NAN/d were estimated to flow into the omasal canal and duodenum respectively, the residual NAN assigned to dietary NAN flow was similar for the omasum and duodenum ($P = 0·27$).

The CV within each digesta sampling site was lower for the omasal canal than the duodenum in total NAN flow (0·046 *v.* 0·074) and microbial NAN flow (0·092 *v.* 0·117), but higher in dietary NAN flow (0·101 *v.* 0·085). The correlation coefficient between omasal canal and duodenal microbial NAN flow was 0·94 (Fig. 4) and

Table 5. Effect of bacterial sampling site on microbial non-ammonia nitrogen (NAN) and dietary non-ammonia nitrogen flows into the omasal canal and duodenum (g/d) and on true nitrogen digestion determined in the omasal canal and duodenum in cows

(Mean values for fifteen observations, with pooled standard errors)

	Bacterial sampling site		
	Rumen	Omasum	Duodenum
Microbial NAN flow			
Omasal canal	175	186	205
Duodenum	166	176	195
SEM	3·1	3·3	3·9
Microbial N synthesis			
(g/kg OMADR)			
Omasal canal	25·6	27·2	30·0
Duodenum	26·1	27·6	30·6
SEM	0·88	0·95	1·13
(g/kg OMTDR)			
Omasal canal	19·8	20·6	22·2
Duodenum	20·1	21·0	22·6
SEM	0·50	0·51	0·55
Dietary NAN flow			
Omasal canal	133	123	104
Duodenum	135	126	107
SEM	1·2	1·3	1·6
True digestibility of N in the			
Reticulo-rumen	0·626	0·656	0·708
Whole stomach	0·618	0·646	0·696
SEM	0·0044	0·0047	0·0059

OMADR, organic matter apparently digested in the rumen; OMTDR, organic matter truly digested in the rumen.

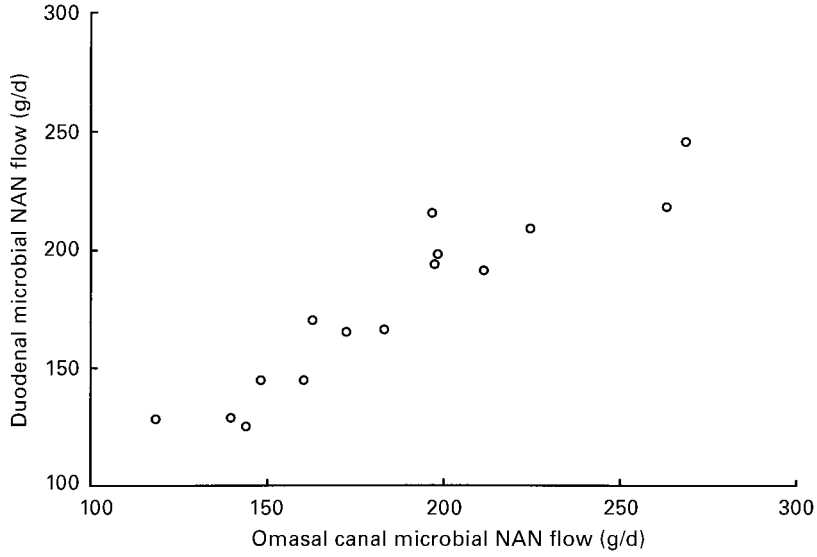

Fig. 4. Relationship between flows of microbial non-ammonia nitrogen (NAN) into the omasal canal and duodenum (g/d) in cows (*r* 0·94).

between omasal canal and duodenal dietary NAN flow 0·79 (Fig. 5).

Discussion

Sample quality

When omasal canal and duodenal digesta flows are compared some differences might be expected due to omasal and abomasal digestive processes. Correlation coefficients between omasal canal and duodenal flows reflected sampling errors, precision of chemical analysis and the extent of endogenous secretions and metabolism of nutrients between sampling sites. Generally, observed variations in nutrient flows into the omasal canal or duodenum were relatively similar for each site and were acceptable compared with published values (Titgemeyer, 1997). Since a small proportion of Cr-EDTA has been shown to be absorbed from the digestive tract and excreted in urine, correction for this absorption has been suggested (Faichney, 1975*b*). In the present study, daily faecal outputs of markers were assumed to represent the true quantity of markers passing

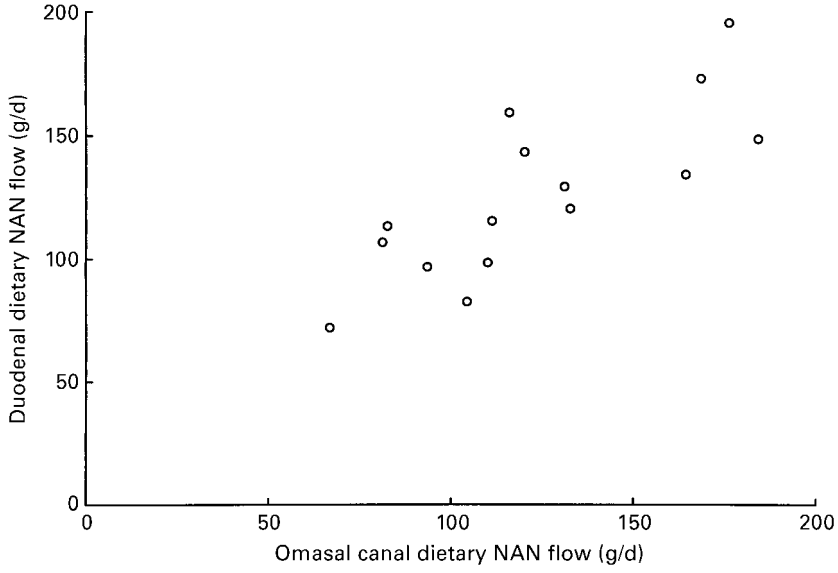

Fig. 5. Relationship between flows of dietary non-ammonia nitrogen (NAN) into the omasal canal and duodenum (g/d) in cows (*r* 0·79).

the sampling sites. However, if post-ruminal marker absorption occurred, the calculated marker dose should have been corrected for the quantity of the marker absorbed posterior to the sampling site. If 0·04 of Co-EDTA had been excreted in urine (Faichney, 1975*a*; Siddons *et al*. 1985), and assuming that Co-EDTA absorption is related to mean sectional retention time (Faichney, 1975*a*) and that the retention times are similar in the rumen and post-ruminal tract (Huhtanen & Kukkonen, 1995), in the current study the daily dose of Co would have been underestimated by 0·02 units. This would have resulted in a 0·01 unit underestimate of DM flow, but flows of other chemical components would have remained essentially unchanged.

The composition of omasal canal samples deviated more from calculated true digesta than that in duodenal samples. Large deviations from the true composition may seriously challenge marker systems used to correct for unrepresentative sampling. Theoretically, non-uniform distribution of a marker in digesta particle phase in combination with an erroneous particle size distribution could result in an erroneous marker concentration in the sample and its particle phase. Faichney (1993) demonstrated that non-uniform distribution of markers in digesta, i.e. between liquid and particle phases, had little effect on digesta DM flow. However, this conclusion cannot be extended to a situation where marker concentration was biased within particle phase due to sampling errors. A triple-marker method (France & Siddons, 1986), an extension of the double-marker method (Faichney, 1975*b*), was used to reconstitute true digesta. Omasal canal and duodenal digesta were separated into three phases (LP, SP and FP) and analysed for marker concentrations. Two of these phases (SP and FP) appeared to be homogeneous and should therefore not challenge the marker system used. In contrast, the LP phase containing more heterogeneous large particulate matter was probably more prone to sampling errors due to less uniform marker distribution. Therefore, more detailed studies on marker distribution in omasal canal digesta and potential effects of erroneous particle composition on the chemical composition of omasal canal LP phase are required.

Organic matter and fibre flow

The higher OM flow into the duodenum than the omasal canal indicated a net secretion of OM into the abomasum. Whether this conclusion is valid could not be verified with the current data since endogenous OM secretion into the alimentary tract is difficult to quantify. Punia *et al*. (1988) observed a 0·03 higher OM flow into the abomasum than the omasal canal, slightly lower than the 0·07 observed in the present study. Comparison of ash flows into the omasal canal and the duodenum indicated a net absorption of 298 g minerals/d between the omasal canal and proximal duodenum, which agrees with previous observations (Punia *et al*. 1988). However, this is not a true estimate of mineral absorption, because it is underestimated by the extent of abomasal secretion of minerals. Omasal absorption of Na and K and secretion of Cl^- ions have been observed in sheep and goats (Engelhardt & Hauffe, 1975).

The large decrease in NDF flow between the omasal canal and duodenum (302 g/d) indicated an important role of the omasum in total NDF digestion. Furthermore, NDF flow decreased much less (123 g/d) as digesta passed from the duodenum through the lower tract. This suggests that more NDF was digested in the omasum than in the intestines. Another possible explanation for the large proportion of NDF digested in the omasum is that the sample contained particles which, under normal conditions, are incapable of escaping the reticulum. Reticular contractions and potential backflow from the omasum discriminate on the basis of particle length and specific gravity which particles are capable of leaving the rumen (Lechner-Doll *et al*. 1991; Kaske & Midasch, 1997). Particles with a low probability of escaping the rumen are likely to contain more digestible matter than particles with a high probability of leaving the rumen (Sutherland, 1988). In the present experiment, the omasal sampling device was located very close to or at the reticulo-omasal orifice, so that light particles close to this area during a reticular contraction may have been included in the sample, leading to unrepresentative sampling. This type of error cannot be ameliorated by any marker method. However, Huhtanen *et al*. (1997) observed no differences in particle size distribution between omasal canal and faecal samples. Similar particle size distributions suggest that errors associated with particle composition of omasal canal samples are relatively minor.

Smith (1984) speculated that the proportion of digestible fibre fermented in the omasum could be about 0·10 in bovines, which is slightly higher than the 0·07 currently reported. Assuming a 0·07/h digestion rate of potentially digestible NDF (Huhtanen & Jaakkola, 1993) a mean omasal retention time of 3·5 h would have been required for the digestion of 300 g NDF/d. In a slaughter study (Mäkelä, 1956), the lowest omasal mean retention time of lignin, estimated for a cow with the highest hay intake (10·7 kg DM/d), was 6 h. Compared with the present study a similar or slightly greater role for the post-duodenal tract in total NDF digestion has been observed in bulls: 0·04 (Khalili & Huhtanen, 1991) and 0·08 (Tesfa, 1993) and non-lactating cows: 0·05 (Vanhatalo *et al*. 1992). In dairy cows a smaller contribution of the intestines to total fibre digestion appears justified due to low retention times (Poore *et al*. 1991). In addition, abomasal conditions may induce a lag effect on post-abomasal cell-wall digestion. In contrast, no lag effect is likely to precede omasal cell-wall digestion.

Digestion of NDF-associated protein (NDF-N × 6·25) was disproportionally high between the reticulum and the duodenum (0·43), compared with that of the rumen (0·44) and post-duodenal tract (0·12). The proportion of NDF-associated protein digested in the omasum and abomasum (0·43) as a proportion of total digestion was much higher than the proportion of protein-free hemicellulose (0·06) or ADF (0·06) digested in these organs. These findings are consistent with those from mobile bag studies, which have indicated that post-ruminal disappearance of NDF-N is considerably greater than that of hemicellulose or cellulose (Vanhatalo *et al*. 1996; Huhtanen & Vanhatalo, 1997). Susceptibility of NDF-N to post-ruminal digestion might be due to acid hydrolysis of linkages between protein and cell walls rather than enzymic digestion.

Nitrogen

The site used to harvest the bacterial samples to assess the bacterial purine : N ratio had a clear impact on microbial and dietary NAN flows. Our procedure for bacterial sample preparation involved an attempt to detach particle-adherent bacteria. The degree of detachment was not verified but it is likely to have been incomplete (Craig *et al*. 1987). A deficiency of large particles in omasal canal digesta samples would promote a further decrease of the relative proportion of particle-associated bacteria in omasal canal bacterial samples. Since fluid-associated bacteria may have a higher purine : N ratio than particle-associated bacteria (Cecava *et al*. 1990; Perez *et al*. 1996*b*, 1997), a low recovery of particle-associated bacteria would result in an underestimate of microbial NAN flow. Ruminal bacterial samples had consistently higher ($P < 0{\cdot}05$) purine : N values than omasal canal or duodenal samples, which probably reflects a smaller proportion of particles in ruminal than omasal canal samples obtained by our procedures. Moreover, as duodenal digesta samples appeared to contain more particulate matter than omasal canal samples, the purine : N value was lower ($P < 0{\cdot}05$) for duodenal than omasal canal bacteria. An alternative explanation for this difference is that duodenal bacterial composition was modified due to abomasal digestion (Dobson *et al*. 1984), which has led some scientists to discourage the use of bacteria harvested from the abomasum or duodenum (Titgemeyer, 1997).

Using ^{35}S or ^{15}N as microbial markers Siddons *et al*. (1982) found lower microbial NAN flow into the duodenum when bacterial samples were collected from the rumen compared with the duodenum. Perez *et al*. (1996*a*) observed lower microbial flows into the duodenum estimated using purine bases compared with ^{15}N. In both experiments differences were attributed to unrepresentative sampling of bacteria leaving the rumen.

Use of bacterial purine : N ratios to calculate microbial NAN flow underestimates this value due to the contribution of protozoal NAN, since protozoa have been reported to have a considerably lower purine content than bacteria (Firkins *et al*. 1987). In cattle, estimates of the protozoal contribution to microbial NAN flow have varied from 0·2 to 0·5 (Firkins *et al*. 1987; Steinhour *et al*. 1982; Punia *et al*. 1988; Punia & Leibholz, 1994). Therefore, assuming that the protozoal purine : N value was 0·55 of that for bacteria (Firkins *et al*. 1987) and the proportion of protozoa in microbial NAN was between 0·1 and 0·3 of microbial NAN flow, this value would have been underestimated by between 0·05 and 0·14 in this experiment due to the exclusion of protozoa.

Ruminal degradation of RNA and purine bases is often assumed to be complete (McAllan & Smith, 1973). However, Perez *et al*. (1996*b*) estimated that the proportion of purines escaping rumen degradation ranged between 0·02 and 0·22 for a range of feedstuffs assuming a 0·02/h outflow rate. This would be a compensating error in our estimates but may have also masked true differences in microbial flow between diets due to variation in dietary purine escape.

Rather than trying to draw conclusions about the most reliable bacterial sampling site, a more suitable approach to measure microbial NAN flow out of the rumen should be advocated. First, the contribution of protozoa to N leaving the rumen should be quantified. Second, since using mixed populations of liquid- and solid-associated bacteria may not be an adequate approach due to difficulties in quantitative detachment of particle-associated bacteria (Craig *et al*. 1987; Cecava *et al*. 1990), the purine concentrations of fluid- and particle-associated bacteria should be determined separately. DM flow in liquid and solid phases can be estimated separately if an appropriate double- or triple-marker method is used. If purine concentrations are also separately analysed for liquid and solid phases then the respective bacterial purine : N ratios can be used to calculate microbial NAN flow in both phases separately. Faichney *et al*. (1997) used this type of procedure in combination with [^{14}C]choline-labelled protozoa and ^{15}N to determine duodenal flows of protozoa, liquid-associated bacteria and solids-associated bacteria.

Total NAN flows were similar into the omasum and duodenum (319 *v*. 324 g/d). These values agree with those of Punia *et al*. (1988), who observed similar NAN flows into the omasum and abomasum (60 *v*. 63 g/d) in 300 kg heifers. Similar total NAN flows for the sampling sites despite lower endogenous NAN flows reported into the omasum than into the duodenum (Ørskov *et al*. 1986; Hart & Leibholz, 1990) might indicate NAN absorption from the omasum, which is consistent with indirect evidence indicating peptide absorption from the omasum (Matthews *et al*. 1996).

Slightly higher microbial NAN flow into the omasum than the duodenum is in contrast with a slightly higher bacterial N flow into the duodenum than from the rumen in sheep (12·6 *v*. 13·4 g/d; Faichney *et al*. 1997). Since some fibre fermentation seemed to occur in the omasum, some microbial synthesis might be expected. The extent of dietary purine digestion was not estimated but some purines may have been digested in the omasum thereby decreasing duodenal purine flow. If duodenal microbial NAN flow were calculated using duodenal bacterial composition, omasal microbial NAN flow would be lower than duodenal flow (186 *v*. 195 g/d respectively). However, this procedure assumes that no bacterial lysis occurred in the abomasum and that the different proportion of particles in digesta samples did not affect the bacterial composition but that differences were derived from microbial synthesis in the omasum. Similar efficiencies of microbial N synthesis estimated in the omasum and duodenum suggest that omasal N metabolism had a small effect on total microbial synthesis. Efficiencies observed in the present study were within the range estimated to be reasonable for high-quality diets (20–25 g N/kg OM truly fermented; Titgemeyer, 1997).

The accuracy of calculated dietary NAN flow depends on the accuracy of microbial NAN and endogenous NAN flow. Endogenous NAN flow was estimated based on results with cattle nourished with protein-free nutrients by intragastric infusion (Ørskov *et al*. 1986). If calculated on a per kg live weight$^{0{\cdot}75}$ basis, Hart & Leibholz (1990) reported a similar omasal endogenous NAN flow as Ørskov *et al*. (1986) but an abomasal endogenous NAN flow that was 0·22 units higher. Omasal endogenous NAN flow seemed to be positively correlated to DM intake whereas abomasal flow

appeared to be unrelated to DM intake (Hart & Leibholz, 1990). If a duodenal endogenous NAN flow of 0·22 units higher were used, dietary NAN flow would be similar into the omasum and duodenum (123 and 121 g/d, $P = 0{\cdot}22$). Since endogenous NAN flow is considerably lower into the omasum than the duodenum, a more accurate estimate of dietary NAN flow could be expected.

Conclusions

The omasal canal sampling technique provides a promising alternative to duodenal sampling as a means of investigating ruminal digestion in cattle. However, accuracy and precision of omasal canal flows are more dependent on marker techniques than duodenal flows. Omasal canal sampling enables quantification of soluble N-fractions leaving the rumen, since abomasal degradation of microbial N can be avoided. Comparison of omasal canal and duodenal digesta flows indicated absorption of minerals from the omasum and OM secretion in the abomasum. The extent of NDF and ADF digestion appeared to be greater in the omasum than in the intestines. Whether this was an unbiased estimate cannot be elucidated in the present trial. The role of the omasum in NDF digestion requires further evaluation. Differences between N fraction flows entering the omasal canal and duodenum were non-significant.

Acknowledgements

The authors wish to thank Mr Aaro Hakkarainen for building the omasal sampling device, Mrs Aino Matilainen and her staff for care of the animals and Mr Vesa Toivonen and his staff for the laboratory analysis.

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