

Omasal Sampling Technique for Assessing Fermentative Digestion in the Forestomach of Dairy Cows^{1,2}

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ABSTRACT: A procedure allowing digesta sampling from the omasum via a ruminal cannula without repeated entry into the omasum was developed. The sampling system consisted of a device inserted into the omasum via the ruminal cannula, a tube connecting the device to the ruminal cannula, and a single compressor/vacuum pump. Eight cows given ad libitum access to a total mixed diet were used in a crossover design to evaluate the effects of the sampling system on digestive activity, animal performance, and animal behavior. Results indicated that the omasal sampling system has minimal effect on normal digestive and productive functions of high-producing dairy cows. Dry matter intake was reduced (24.0 vs 21.8 kg/d; $P < .02$) and seemed related more to the sampling procedures than to the device in the

omasum. Observations of animal behavior indicated that cows with the sampling device were similar to control cows, although rumination and total chewing times were reduced slightly. The composition of digesta samples was biased toward an over-abundance of the liquid phase, but using a double-marker system to calculate digesta flow resulted in fairly small coefficients of variation for measurements of ruminal digestion variables. This technique may prove useful for partitioning digestion between the fermentative portion of the forestomach and the lower gastrointestinal tract. The omasal sampling procedure requires less surgical intervention than the traditional methods using abomasal or duodenal cannulas as sampling sites to study forestomach digestion and avoids potentially confounding endogenous secretions of the abomasum.

Key Words: Omasum, Digesta, Digestibility Markers, Dairy Cows, Cannulas

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Introduction

Abomasal and duodenal fistulas in sheep and cattle have been used as digesta sampling sites to study digestion in the ruminant forestomach. Abomasal secretions can interfere with some measures of forestomach digestion when digesta are obtained from these sites. Also, abomasal or intestinal surgical procedures are often more difficult and involve longer

animal recovery times than ruminal cannulation, and cannulas at these locations require extensive maintenance.

Several procedures have been used to collect digesta from the omasum (Ash, 1962; Engelhardt and Hauffe, 1975; Rupp et al., 1994). These procedures require either omasal cannulation or collection of digesta passing the omaso-abomasal orifice via a sleeve that is secured to the orifice and exteriorized through an abomasal cannula. These procedures are even more involved than abomasal or duodenal cannulation and have had limited success. The procedure of Punia et al. (1988) involves inserting a tube through the reticulo-omasal orifice via the ruminal cannula and withdrawing digesta with a vacuum pump. However, this procedure requires insertion of the sampling tube at each sampling time.

This experiment tested the feasibility of inserting a sampling device into the omasum for up to 3 wk via the ruminal cannula for the purpose of sampling digesta leaving the reticulo-rumen. Measures of digestive activity, animal performance, and animal behavior were made.

¹Trade names and the names of commercial companies are used in this report solely to provide specific information. Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the USDA or endorsement over products not mentioned.

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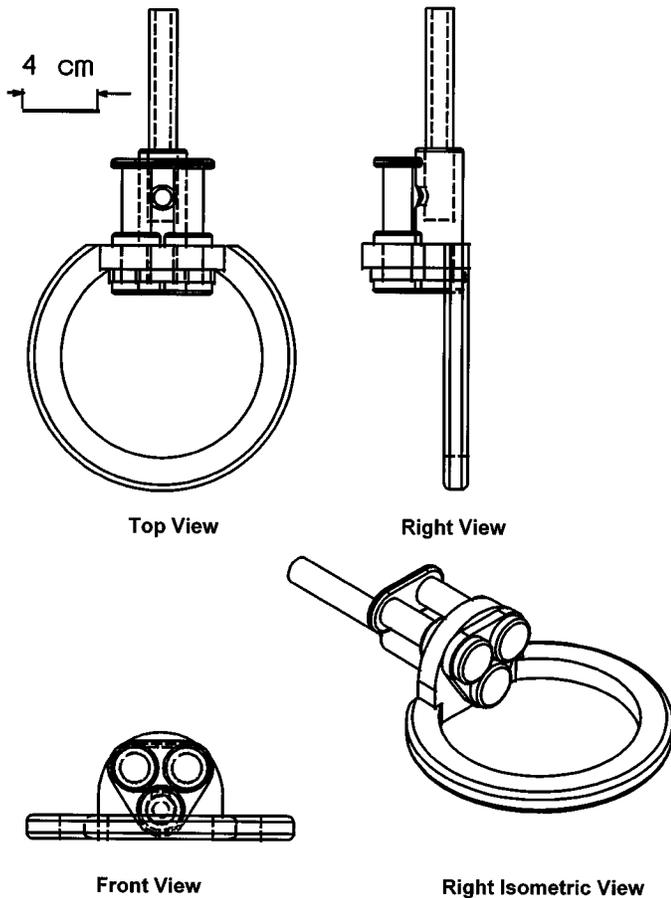


Figure 1. Sampling device that resides in the omasum. The opening through which digesta flows is between two protective fingers shown on the top and isometric view.

Materials and Methods

Sampling Device. The omasal sampling device was molded from polyvinyl chloride (PVC) polymer (Plastisol®). A drawing of the device is shown in Figure 1. The device consisted of a round plate (6 cm in diameter by 1.5 cm thick) with a perpendicular ring (15 cm o.d. by 12 cm i.d.) fused to the edge of the plate. The ring was used to keep the sampling fixture in the omasum. Two fingers made of PVC rod 1.6 cm in diameter (Durometer hardness: Shore A, 55) were placed in front of the opening to the sampling tube to prevent omasal leaves from blocking the tube. The sampling tube was 1.2 m long by 1.6 cm o.d. and .95 cm i.d. The sampling device was inserted into the omasum by compressing or folding the ring. The ring, held with a tie, was released once it was in place in the omasum. The other end of the tube was pushed through a 1.6-cm hole in the plug of the ruminal cannula. The tube was stoppered at the ruminal cannula when not used for sampling.

The system used for evacuating omasal digesta consisted of a single compressor/vacuum pump to

produce pressure and vacuum. A motorized three-way stainless steel ball valve, rotating at 30 rpm, controlled cycling through the common port on the ball valve, which was attached to the sampling device. Between the compressor and the three-way ball valve, a 23-L air tank served as a pressure reservoir. Pressure was maintained at 50 mm of Hg by a needle valve on the air tank that vented excess pressure to the atmosphere. A similar air tank and needle valve between the vacuum side of the compressor and the three-way ball valve were used to maintain the vacuum at 100 mm of Hg. A 4-L Erlenmeyer vacuum flask served as a trap between the vacuum reservoir and the three-way ball valve. The alternating pressure/vacuum resulted in net flow of digesta to the collection vessel, but the alternating pressure at half the vacuum level was sufficient to clear away any accumulated digesta at the omasal end, thus maintaining digesta flow with minimal blockage.

Animals and Diet. Eight multiparous Holstein cows fitted with permanent ruminal cannulas and averaging 71 d in milk at the beginning of the experiment were housed in a tie-stall barn with stalls covered with a rubber mat. Cows were milked twice daily at 0600 and 1700 and had free access to water throughout the trial. A local anesthetic was used during installation of the ruminal cannulas.

Cows were allocated randomly to two treatment groups in a crossover design: control, without an omasal sampling device; experimental, with an omasal sampling device. The sampling device was inserted at the beginning of each 21-d period and removed at the end of the period. Each of the two experimental periods lasted 21 d; the first 12 d were for adjustment, and the last 9 d were for sample collection. Cows were fed their total mixed ration (**TMR**) at 1000 and 2200 for ad libitum intake. The diet contained (DM basis) 50.0% alfalfa silage, 36.2% high-moisture ear corn (**HMEC**), 12.0% soybean meal (**SBM**), 1.1% dicalcium phosphate, and .7% trace mineralized salt and vitamin supplement. The latter supplied 150,000 IU of vitamin A, 35,000 IU of vitamin D, and 140 IU of vitamin E per cow per day. Diets were formulated to meet or exceed NRC-recommended nutrient allowances. Chemical composition of the dietary ingredients and TMR are in Table 1.

Experimental Procedures. Feed intake was measured daily on an individual basis. Samples of alfalfa silage, TMR, and orts were collected daily and pooled to provide a sample for each collection period. High-moisture ear corn and SBM were sampled once each period. Samples were dried in a forced-air oven at 60°C for 48 h and ground through a 1-mm Wiley mill screen before analyses. The chemical composition of the TMR was calculated from the chemical analyses of individual feed ingredients.

Milk yields were recorded daily. Milk samples were collected on d 10 and 17 from consecutive morning and afternoon milkings. Milk samples were analyzed by

Table 1. Chemical composition of dietary ingredients and total mixed ration^a

Item	Alfalfa silage	High-moisture ear corn	Soybean meal	Total mixed ration
Dry matter, %	41.5	66.0	89.8	52.3
Organic matter, % of DM	87.7	98.3	93.4	90.6
Crude protein, % of DM	20.8	9.1	48.0	19.4
Neutral detergent fiber, % of DM	37.2	17.4	13.5	26.5

^aTotal mixed ration contained (% of DM): alfalfa silage, 50; high-moisture ear corn, 36.2; soybean meal, 12.0; dicalcium phosphate, 1.1, and trace mineralized salt, .7. The latter contained (g/kg) Mn, 5.4; Zn, 5.4; Fe, 3.4; Cu, 1.4; I, .08; Se, .06; and Co, .02.

near infrared procedures for milk fat, protein, and lactose (Wisconsin DHIA Cooperative, Madison). Chewing activity and feeding behavior were monitored by recording the action of individual cows every 5 min over 24 h on d 12 of each period, except for when cows were in the milking parlor approximately 120 min/d.

Total tract apparent digestibilities were determined using chromium (Cr) and ytterbium (Yb) as external markers. Ytterbium solution, prepared as described by Hartnell and Satter (1979), was sprayed onto a portion of the HMEC. Labeled HMEC then was mixed with the TMR. The Cr-mordanted straw was prepared as described by Udén et al. (1980) and milled to pass a 4-mm screen before mixing with the TMR (30 g-cow⁻¹.d⁻¹). Concentrations of Cr and Yb in the TMR were 31.7 and 28.3 ppm, respectively. Diets labeled with Cr and Yb were fed for 6 d before the fecal and omasal sampling commenced on d 15 for each period for digestibility measurements. The marker LiCoEDTA (8 g-cow⁻¹.d⁻¹) was continuously infused into the rumen of the experimental cows using a peristaltic pump from d 10 onward, except during the time cows were in the milking parlor (approximately 120 min/d). Omasal digesta (approximately 500 mL) and feces (approximately 500 g) were collected every 3 h on d 15 to 17 of each period after the morning feeding. Collection time was advanced 1 h each 24 h. Fecal samples (approximately 200 g per sampling) were collected for wet sieving at these same times and pooled to provide one sample for each cow in each period. Fecal samples for the estimation of digestibility were dried at 60°C for 72 h, ground through a 2-mm screen, and pooled for subsequent analyses on a weight basis. Omasal samples were frozen immediately after sampling. After the collection period, samples were thawed at room temperature and divided into liquid and solid phases by filtering through one layer of cheese-cloth. The entire digesta sample used for analyses was obtained by mixing together 40% of the liquid and solid phases obtained by filtering through cheesecloth. This was done because of difficulty in obtaining representative subsamples from intact rumen contents due to settling of the solid phase during the subsampling procedure. It was thought that dividing the whole composite sample into two fractions that lend themselves to subsam-

pling would provide a more accurate whole digesta sample than would be obtained by taking a fractional sample of the composite whole digesta sample. All omasal particulate samples were dried at 60°C for at least 72 h and thereafter ground to pass a 2-mm screen.

Digesta passage kinetics were estimated by using lanthanum (La) and samarium (Sm) as markers. One gram of La was sprayed onto 1 kg of alfalfa silage and 1 g of Sm onto 1 kg of HMEC (Hartnell and Satter, 1979). Marked samples were fed in a pulse-dose before the morning feeding on d 13 of each period. Fecal grab samples were taken at 6, 9, 12, 15, 18, 21, 24, 28, 32, 36, 42, 48, 51, 54, 57, 65, 73, 76, 82, and 96 h after the dose. Samples were dried at 60°C for 72 h and ground through a 1-mm screen. Concentrations of marker in the feces were fitted to a series of two-compartmental models with either no age dependency in the first compartment (G1G1) or with increasing order of gamma age dependency in the first compartment (GnG1, n = 2 to 4). Curvefitting was carried out using the iterative Marquardt method of PROC NLIN of SAS (1985). The models, in SAS programming language, were obtained from Moore et al. (1992). The models estimate the passage rate from the two compartments (k_1 and k_2) and transit time (TT). The retention time in the first compartment (CMRT₁) was calculated as n/k_1 (n = 1 to 4) and in the second, age-independent compartment (CMRT₂) as $1/k_2$. Total compartmental retention time (CMRT) was calculated as CMRT₁ + CMRT₂ and the total mean retention time as CMRT + TT. The parameters from the best fit model in terms of the smallest residual mean square were used for each animal in each period.

Liquid passage rate was determined by using LiCoEDTA (Udén et al., 1980) as a marker. Eight grams of LiCoEDTA was dissolved in 500 mL of tap water and mixed with ruminal contents 1 h before the morning feeding on d 18. Continuous Co infusion for the estimation of digestibility was stopped at this time. Nine ruminal samples were taken sequentially at 1.5-h intervals after dosing. Ruminal digesta samples were filtered through four layers of cheesecloth and stored at -20°C for subsequent Co analyses. Liquid passage rate was calculated by linear

regression of the natural logarithm of ruminal Co concentration against time.

Omasal digesta flow was calculated with a single-marker method using each of the markers (Cr, Co, and Yb) and by a graphic alternative of Faichney's (1975) double-marker method (McAllan and Smith, 1983). The Cr and Yb were used as solid-phase markers and Co as a liquid-phase marker.

Omasal digesta also were collected to study variation in composition of the samples (DM, ash, Cr, and Yb) and particle size distribution. Approximately 500 mL of omasal digesta was obtained for chemical analyses every 3 h after the morning feeding on d 13, then every 4 h for 12 h, every 6 h for 12 h, and finally at the same time that samples were obtained for digestibility measurements during d 15 to 17. Samples were dried at 60°C for 72 h and milled through a 2-mm screen. Samples (500 mL) were taken for wet sieving on d 15 to 17 at the same time as those for flow determinations. Samples were stored at -20°C before wet sieving. An additional 200 mL of omasal digesta for harvesting bacteria was collected on d 15 of each period before the morning feeding and at 3, 6, and 9 h after the morning feeding. Samples were mixed with 1% (vol/vol) formalin, pooled, and frozen at -20°C. Later the samples were thawed and filtered through eight layers of cheesecloth. Feed particles were washed with a buffer-nutrient solution to detach some of the particle-associated bacteria and filtered (Craig et al., 1984). Fluid fractions were combined and centrifuged for 10 min at $200 \times g$ to remove protozoa and feed particles. The supernatant fluid then was centrifuged for 20 min at $26,000 \times g$. The bacterial pellet was washed with .9% saline and centrifuged again. Bacterial samples were freeze-dried and analyzed for DM, nitrogen, and purines.

Ruminal samples for the determination of pH, ammonia nitrogen (NH_3N), total amino acids (AA), and VFA concentrations were taken on d 18 of each period before the morning feeding and thereafter every 1.5 h for 12 h. Ruminal fluid was filtered through four layers of cheesecloth, and pH was measured immediately. Strained ruminal fluid was diluted with formic acid (1:1; vol/vol) and stored at -20°C for subsequent analyses of VFA. Samples for NH_3N and free AA analyses were acidified with .3 mL of 50% H_2SO_4 and stored at -20°C before analyses.

Total weight of ruminal contents was determined by manual emptying on d 19 of each period at 4 h after the morning feeding. After weighing, ruminal contents were mixed thoroughly with a fork and approximately 2 kg of digesta obtained by 10 to 15 hand samples. Following sampling, digesta were returned to the rumen.

Laboratory Procedures. Samples of feed ingredients, TMR, Orts, omasal, and fecal samples were analyzed for DM, OM, nitrogen (Brotz and Schaefer, 1984), and NDF (Robertson and Van Soest, 1977). Chromium, cobalt, and ytterbium concentrations of TMR, Orts,

omasal, and fecal samples and rumen fluid (Co only) were analyzed by direct current plasma spectrometry (Spectra Span V, Fison Instruments, Valencia, CA) as described by Combs (1985). Microbial CP production was estimated from protein:purine ratios in rumen bacteria, and purine flow information. Purine content of bacterial and omasal digesta samples were analyzed according to Zinn and Owens (1986), except that a solution of .005 NH_2SO_2 and .005 M AgNO_3 (Aharoni and Tagari, 1991) was substituted for the acidic wash solution. Nitrogen contents of microbial samples were analyzed with a Carlo Erba NA 1500 nitrogen analyzer (Carlo Erba Instruments, Milan, Italy). Undegraded feed protein N was calculated as the difference between total nonammonia nitrogen (NAN) and microbial N flow without any corrections for endogenous N contribution. Ruminal fluid samples were analyzed for NH_3N and free AA according to Broderick and Kang (1980). Volatile fatty acids were analyzed by gas chromatography (Varian Vista 6000 GC, Sugarland, TX) as described by Brotz and Schaefer (1987).

Ruminal, omasal, and fecal samples were wet-sieved for 10 min with a vibrational sieve shaker using a continuous water spray on the top sieve. Five sieves with screen openings of 2.36, 1.18, .6, .3, and .15 mm were used for omasal and fecal samples. An additional sieve with screen openings of 4.75 mm was used for ruminal samples. Sixty grams of ruminal digesta and feces were sieved in duplicate. Omasal samples of approximately 500 mL (12/cow) were sieved without replication. Residues retained on each sieve were rinsed on to preweighed 11-cm Whatman 54 filter paper and dried at 60°C for 48 h. Dry matter retained on each sieve was expressed as a proportion of DM applied to the sieves. Dry matter of the samples used for wet sieving was assumed to be the same as those used for chemical analyses. Dry matter passing the .15-mm screen was calculated as the difference between total dry matter applied to the sieves and the amount retained on the sieves.

Statistical Analyses. Data were analyzed using the General Linear Models procedures of SAS (1985). The statistical model used was $y_{ijk} = m + T_i + P_j + C_k + e_{ijk}$, where y_{ijk} = dependent variable for cow k on treatment i during period j , m = population mean, T_i = treatment effect ($i = 1, 2$), P_j = period effect ($j = 1, 2$), C_k = cow effect ($k = 1$ to 8), and e_{ijk} = error. Ruminal fermentation parameters were tested using split-plot analyses of variance. The model was $y_{ijkl} = m + T_i + P_j + C_k + e_{ijk} + H_l + (\text{TH})_{il} + (\text{PH})_{jl} + (\text{CM})_{kl} + e_{ijkl}$, where H_l = time effect ($l = 1$ to 9) with respective interactions, and m , T , P , and C are as defined above. The error term, e_{ijkl} , was used to test the main effects. Coefficient of variation between cows was calculated as the standard deviation between cows divided by the overall mean. Coefficient of variation between sampling times was calculated as the standard deviation

Table 2. Feed intake and milk production of cows with or without the omasal sampling device

Item	Control	Experimental	SE	<i>P</i> -value ^a
Dry matter intake, kg/d	24.0	21.8	.46	.02
Milk, kg/d	36.9	35.2	.45	.06
3.5% FCM, kg/d ^b	36.8	35.3	.78	.23
Milk fat, %	3.47	3.52	.096	.72
Milk fat, kg/d	1.29	1.24	.041	.45
Milk protein, %	3.10	3.02	.028	.12
Milk protein, kg/d	1.14	1.05	.016	.01
Milk lactose, %	4.88	4.88	.031	.99
Milk lactose, kg/d	1.81	1.72	.029	.09

^aProbability of treatment effect.

^b3.5% fat-corrected milk = .432 (kg milk) + 16.2 (kg fat).

between sampling times divided by the overall mean. The CV within cows represents the variation that was not explained by either cow or sampling time effects.

Results

The control cows (**C**) had a higher ($P < .02$) DMI than the experimental cows (**E**) (Table 2). The difference was smaller (1.2 kg/d) before the collection period began than during the whole period (2.2 kg/d). Milk yield tended ($P = .06$) to be less for E than C. This trend, together with a slightly lower milk protein content, resulted in less ($P = .01$) protein yield for E than for C cows. Fat and fat-corrected milk yields were similar between the groups.

Eating time was similar between the groups, but time spent ruminating and total chewing time were longer for the control cows (Table 3). However, when expressed per unit of DM intake, the omasal sampling device did not affect total chewing time. Time spent standing and lying down and the number of times that the cows lay down were similar between the groups.

Ruminal fermentation data are shown in Table 4. No treatment \times time interactions were observed,

suggesting that diurnal variation in ruminal fermentation was similar between the groups. There were no differences between the groups in ruminal pH and concentrations of NH₃ and total VFA. The concentration of total AA in ruminal fluid was higher ($P < .05$) in the control cows. Molar proportion of acetate was lower ($P < .01$) and that of propionate higher ($P < .04$) in the control cows. Small differences existed between the groups in the molar proportions of valerate and isovalerate.

There were no differences in total tract digestibility of dietary constituents between the groups (Table 5). Values were slightly higher for the cows with the omasal sampler when Yb was used as a marker.

Experimental cows had a slower passage rate of La-labeled alfalfa from the rumen (Table 6). Retention time in the age-independent compartment and total mean retention time were longer for E than for C cows. Transit time and retention time in the age-dependent compartment was not affected by the treatments. Differences between passage kinetics of Sm-labeled corn were similar to those for La-labeled alfalfa, but differences did not always reach statistical significance. Liquid passage rate was faster for C cows than for E cows (16.4 vs 13.7%/h; SEM = .87, $P = .056$).

Table 3. Effect of the omasal sampling device on chewing activity

Item	Control	Experimental	SE	<i>P</i> -value ^a
Eating, min/d	222	201	9.4	.17
Ruminating, min/d	479	423	15.4	.04
Total chewing				
min/d	701	624	10.2	.01
min/kg DM intake	31.3	29.5	1.33	.37
Rumination periods	13.8	12.6	.39	.09
Lying, min/d	604	621	15.6	.49
Left side, %	52	50	7.8	.85
Right side, %	48	50	7.8	.85
Lying periods	8.0	8.5	0.75	.65
Standing, min	738	722	15.6	.49

^aProbability of treatment effect.

Table 4. Effect of the omasal sampling device on ruminal fermentation

	Control	Experimental	SE	P-value ^a
pH	6.04	6.05	.076	.96
Ammonia N, mM	17.5	16.9	.60	.60
Free amino acids, mM	2.3	1.8	.13	.05
Total volatile fatty acids, mM	136	138	3.2	.64
Volatile fatty acids, mol/100 mol				
Acetate	61.5	63.0	.25	.01
Propionate	21.5	20.0	.38	.04
Isobutyrate	1.17	1.17	.016	.67
Butyrate	11.8	11.6	.17	.52
Isovalerate	1.94	2.17	.041	.01
Valerate	1.99	1.91	.017	.02

^aProbability of treatment effect.

There were no differences between treatments in the amount of digesta in the rumen and ruminal digesta DM content (C = 80.1 kg and 16.0%; E = 77.8 kg and 15.8%). Ruminal turnover time of DM was similar for the groups (13.0 vs 13.7 h). The effect of the treatments on the percentages of ruminal digesta and fecal DM retained on each screen were small and not significant (Table 7). More than 35 and 50% of the DM of ruminal digesta and feces, respectively, passed through a .15-mm screen.

The average DM content of omasal samples was 4.52% (CV within cows was 11.7%). Dry matter concentration was highest during and after the morning feeding. Another peak in omasal digesta DM content was observed at approximately 1900; this was when cows returned from the milking parlor and showed some eating activity. There was considerable variation within cows in marker concentrations (CV for Cr, Yb, and Co were 23.0, 20.8, and 23.6%, respectively). Diurnal variation was smaller for Cr than for Yb and Co; the CV were 8.6, 14.7, and 18.0%, respectively. Particle size distribution of omasal digesta is shown in Table 8. More than 75% of DM passed through a .15-mm screen. The largest source of

variation in DM distribution of omasal digesta was within cows, and the smallest generally was between sampling times.

The Cr:Co and Yb:Co ratios were lower in the dry matter of omasal digesta samples than in feces (.552 and .550 vs .726 and .695), suggesting that the samples were not representative of the digesta flowing from the rumen. The estimates of omasal OM flow were highest when Cr was used as a single marker, and estimates of OM flow based on Yb were higher than those based on Co (Table 9). Calculation of omasal OM flow using a double-marker method, with Cr and Co as markers, generally resulted in smaller standard errors in ruminal OM digestion, OM digestion in the intestines, and ruminal OM digestion as a proportion of total OM digestion than single marker methods. In contrast, a double-marker method with a combination of Yb and Co as markers produced much higher omasal OM flow and lower ruminal OM digestion than values based on either Yb or Co alone. This may be related to the fact that Yb concentration was higher in the liquid than in the solid phase of the digesta (50.5 vs 38.4 ppm). Because Yb was behaving more like a liquid than like a solid phase marker,

Table 5. Effect of the omasal sampling device on total apparent digestibility

Item	Control	Experimental	SE	P-value ^a
	%			
Chromium marker				
Dry matter	73.1	73.8	.60	.51
Organic matter	74.1	74.8	.63	.47
Crude protein	75.2	75.6	.71	.72
Neutral detergent fiber	52.1	51.2	1.02	.58
Ytterbium marker				
Dry matter	73.6	75.5	.74	.10
Organic matter	74.5	76.5	.77	.11
Crude protein	76.0	77.5	.75	.18
Neutral detergent fiber	52.3	55.3	1.57	.37

^aProbability of treatment effect.

Table 6. Effect of the omasal sampling device on the passage kinetics of lanthanum-labeled alfalfa silage and samarium-labeled high-moisture ear corn

Item	Control	Experimental	SE	P-value ^a
Lanthanum—alfalfa				
Passage rate, %/h	7.22	5.85	.338	.04
CMRT ₁ , h ^b	3.7	5.9	1.10	.21
CMRT ₂ , h ^c	14.2	17.8	.87	.03
CMRT, h ^d	17.9	23.7	1.65	.06
Transit time, h	8.8	7.4	1.04	.41
TMRT, h ^e	26.7	31.1	1.20	.05
Samarium—ear corn				
Passage rate, %/h	8.04	6.40	.446	.05
CMRT ₁ , h ^b	5.0	5.5	.72	.63
CMRT ₂ , h ^c	12.9	16.6	1.11	.07
CMRT, h ^d	17.9	22.1	1.45	.10
Transit time, h	7.1	7.5	.86	.81
TMRT, h ^e	25.1	29.6	1.49	.09

^aProbability of treatment effect.

^bCMRT₁ = mean retention time in the age-dependent compartment.

^cCMRT₂ = mean retention time in the age-independent compartment, generally considered to be the reticulo-rumen.

^dCMRT = mean compartmental retention time (CMRT₁ + CMRT₂).

^eTMRT = total mean retention time (CMRT + transit time).

omasal OM flow and OM digestion also were calculated using a combination of Cr and Yb in a double-marker system. Flow of OM from the rumen averaged .72 kg/d greater with Cr:Yb than with Cr:Co, and consequently apparent ruminal OM digestion was lower and intestinal OM digestion higher with Cr:Yb.

Omasal flow of NAN, expressed as a fraction of N intake, was .689 (SE = .0165) when Cr and Co were used in a double-marker method. Efficiency of microbial CP synthesis, using total purines as a microbial marker, averaged 21.8 (SE = 1.33) g/kg of OM apparently digested in the rumen. Apparent ruminal protein degradation was .643 (SE = .0151).

Discussion

Obtaining Digesta Samples. Few problems were encountered during sampling of digesta. An occasional stoppage in the sampling tube was cleared by gentle blowing with compressed CO₂. It took approximately 15 to 20 min to collect two 500-mL samples from a total of four cows. Digesta were obtained only after a reticular contraction, and no digesta flowed between reticular contractions. This might indicate that the digesta sampled were that which were flowing from the reticulum, and not digesta present in the omasum or backflowing from the abomasum. Based on odor and

Table 7. Effect of the omasal sampling on particle size of rumen digesta and feces

Screen size, mm	Control	Experimental	SE	P-value ^a
	% of Total DM added to the sieve			
Rumen digesta				
4.75	18.8	19.1	.53	.62
2.36	14.5	13.7	.61	.35
1.18	8.4	7.9	.22	.14
.6	11.6	10.6	.38	.09
.3	7.2	7.7	.33	.32
.15	3.9	3.4	.18	.11
<.15	35.6	37.6	.90	.15
Feces				
2.36	15.7	13.6	.98	.17
1.18	6.3	6.7	.19	.17
.6	7.3	7.5	.31	.67
.3	9.7	10.3	.55	.44
.15	7.9	8.2	.42	.64
<.15	53.1	53.7	.82	.62

^aProbability of treatment effect.

Table 8. Particle size of omasal digesta

Screen size, mm	DM retained on screen %	Coefficient of variation, %		
		Between cows	Between samplings	Within cows
		----- % of Total DM -----		
2.36	6.7	24.6	20.4	49.5
1.18	3.8	18.3	12.2	31.1
.6	3.6	16.0	14.4	28.7
.3	4.5	16.4	13.7	33.9
.15	4.2	12.1	16.0	31.5
<.15	77.3	4.4	3.8	8.8
		----- % of DM retained on screens -----		
2.36	28.3	16.2	10.6	26.2
1.18	16.9	4.2	7.3	12.8
.6	15.7	6.9	7.3	12.8
.3	20.3	13.2	10.5	29.7
.15	18.9	26.8	15.3	30.1

visual appearance, digesta did not seem to be of abomasal origin. Unfortunately, pH of the digesta was not recorded. Cows did not stop eating or ruminating during sampling.

Digestion, Animal Performance, and Animal Behavior Measurements. Reduction in feed intake of cows with the omasal sampling device was greater during the 9-d collection period than during the 12-d adaptation period. This suggests that sampling procedures depressed feed intake more than the mere presence of the device in the omasum. Two explanations may be offered for this. The sampling tube sometimes was blocked by pieces of corn, and it was opened by blowing carbon dioxide through the tube. This, along with frequent sampling, may have disrupted the cows' normal routine. Another possibility is that digesta sampling may cause a temporary imbalance in the supply of sodium. Sodium content of digesta flowing from the omasum is approximately 2.5 g/L (Edrison et al., 1986). At a sampling rate of 6 L/d, an amount of

sodium equal to 25 to 30% of the daily sodium supplementation would be removed. In a subsequent study, we observed no reduction in feed intake during the collection period when smaller volumes of sample were collected and extra sodium supplement was given during the collection periods (Huhtanen, Brotz, and Satter, unpublished data). High feed intake (90 to 95% of that for control cows) and maintenance of high milk yield suggest that the indwelling sampling device can be used with minimum impact on the animal. Depression of feed intake in sheep fitted with simple T-piece or reentrant duodenal and ileal cannulas can be equal to or exceed that observed in the present study (MacRae and Wilson, 1977).

Cow behavior did not seem to be influenced by the presence of the sampling device. Total chewing time was slightly reduced in cows with the sampling device, but that might be related to the reduced feed intake. Treatment and control cows spent a similar amount of time lying down, on both the left and right side.

Table 9. Organic matter digestion as calculated with different markers

Item	Single marker			Double marker		
	Cr	Co	Yb	Cr:Co	Yb:Co	Cr:Yb
OM intake, kg/d ^a	19.47	19.47	19.47	19.47	19.47	19.47
OM flow at omasum, kg/d	11.09	8.34	9.86	9.53	12.63	10.25
SE	.54	.40	.52	.42	.79	.42
OM digestion						
In rumen as % of intake	43.0	57.2	49.4	51.1	37.3	47.3
SE	1.74	1.22	1.76	1.26	2.17	0.96
Intestines						
% of Omasal flow	54.6	39.1	52.9	47.3	61.7	50.7
SE	1.67	2.45	1.46	1.47	1.68	1.80
Rumen/total %	57.6	76.5	64.7	68.3	48.9	63.4
SE	2.28	1.62	2.16	1.51	2.95	1.33

^aOrganic matter intake for only those days when digesta were sampled from the omasum.

Frequency of lying down also was similar. These observations suggest that the sampler was not causing discomfort.

The sampling device had no effect on digestion of dietary constituents. Similarly, abomasal or intestinal cannulas have shown no effect on diet digestion in sheep (MacRae and Wilson, 1977) or in cattle (Hayes et al., 1964). Similar particle size distribution in ruminal digesta and fecal DM indicates that the sampling device had no specific effect on the retention or flow of feed particles. If the device in the omasal orifice had prevented free flow of particles, it should have resulted in a smaller proportion of large particles in the feces. An increased proportion of large particles might have been observed if the device kept the orifice open, provided of course that large particles were present in the reticulum. Reticular contractions were not measured in the present study, but Harmeyer and Michalowski (1991) recorded normal reticulo-ruminal motility when they sampled the digesta flowing to the omasum through a tube inserted via the abomasal cannula. They observed digesta flow only after the second reticular contraction, which we also observed in this study.

The slightly higher molar proportion of acetate and lower proportion of propionate in cows with the sampling device may be associated with the reduced DMI during the collection period. These changes in ruminal fermentation pattern agree with studies in which a given diet has been fed at different levels (Staples et al., 1984; Sutton, 1985).

Longer total retention time and ruminal retention time of La-labeled alfalfa silage and Sm-labeled HMEC, as well as slower passage rate of CoEDTA, can be related to reduced feed intake. Numerous studies have shown that ruminal as well as total retention time of digesta decreases with increasing feed intake (Grovmum and Williams, 1977; Colucci et al., 1990). However, it was not possible in this experiment to conclude whether the sampling device prevented free outflow of digesta from the rumen and thus reduced DMI, or whether reduced DMI related to collection procedures increased marker retention times. The small depression in DMI during the adaptation period (1.2 kg/d) suggests that both factors were involved. In sheep fed a fixed amount of feed, intestinal T-piece cannulas or reentrant cannulas had no effect on marker retention times (MacRae and Wilson, 1977).

Sample Composition. The average DM content of omasal digesta obtained with the sampler was 4.52%. This is lower than reported (Combs, 1985) for DM content of duodenal digesta of dairy cows (6.3%). Lower DM content of omasal digesta, obtained as it flows into the omasum, is related partly to the absorption of water from the omasum (Engelhardt and Hauffe, 1975; Barry et al., 1985; Edrison et al., 1986) and partly to sampling errors. The marker ratios indicate that the samples of Combs (1985) were

rich in solids whereas ours contained too few solids. In the present study, DM content of omasal digesta seemed to be higher during eating than during resting or ruminating, in agreement with the observations of McSweeney (1986). Dry matter contents of digesta were higher in samples taken after feeding at 1000 and 2200 than the average of other samples (4.86 vs 4.48%), and also slightly higher during feeding activity at 1900 when cows returned from the milking parlor. The actual flow of digesta cannot be measured with our technique, but McSweeney (1986) reported a twofold increase in flow of digesta from the omasum during the first .5 h after feeding in sheep. The volume of flow was sustained at a high level for the next 1.5 h after feeding, even if eating was stopped, but DM content in digesta declined gradually. Collecting digesta flowing through the omasal orifice by a tube inserted via the abomasal cannula, Harmeyer and Michalowski (1991) reported DM contents of 3.7 and 4.9% in sheep. These values are similar to results of Punia et al. (1988), who measured DM content of 3.3 to 4.1% in cattle omasal digesta obtained from a tube inserted via the ruminal cannula. Rupp et al. (1994) reported markedly higher values for DM content of digesta sampled from the omaso-abomasal orifice using the sleeve system. This system allows for complete collection of digesta leaving the omasum. Higher values in their study may be attributed to water absorption in the omasum, and also to the high proportion of concentrate in two of their three diets.

There was wide variability in marker concentrations of omasal spot-samples within animals. Part of this variation may be associated with methods of marker administration to the cows. The Cr-mordanted straw and Yb-labeled corn were mixed with the TMR, but a variable eating pattern can cause variation in marker concentration in ruminal digesta, and consequently in omasal digesta. The passage rate of Sm-labeled corn was 6.4%/h, which corresponds to a decrease of 17.5% in marker concentration if the cow does not eat for 3 h. Infusion of CoEDTA was disconnected twice daily for approximately 1 h when the cows were at the milking parlor. This explains part of the variation in Co concentration in omasal samples. However, the variation in marker concentrations of fecal samples was smaller than in omasal samples (CV for Cr, Yb, and Co in fecal DM was 12.1, 15.3, and 22.0%, respectively). Variation in fecal marker concentration has also been observed in continuously fed animals (Faichney, 1972) or with continuous infusion of markers (Faichney and Griffiths, 1978). It also occurs when complete digesta collection has been made using duodenal reentrant cannulas (Harris and Phillipson, 1962; MacRae and Armstrong, 1969; van't Klooster et al., 1969; Offer et al., 1972). Variability in marker concentrations of omasal samples cannot be attributed only to sampling errors.

There is considerable evidence that the passage of small particles from the reticulo-rumen is accelerated during feeding (Kennedy and Doyle, 1993). This probably is because the number and the duration of reticular contractions are enhanced during eating (Grofum, 1986). Higher DM content in omasal samples during eating is consistent with these observations. Weston (1989) observed that the OM content of digesta flowing from the reticulum was only 55% of that in the reticulum, suggesting that flow to the omasum is selective. There may be variation in digesta composition between digesta flushes. However, it is noteworthy that DM concentration of digesta in the omasum (Engelhardt and Hauffe, 1975) and abomasum (Faichney and Barry, 1986) is much higher than in the digesta flowing out of these organs. Digesta flow to the abomasum consists of two kinds of material, one with relatively low DM content, and the other with much higher DM. The latter appears at irregular intervals (Smith, 1984). Combs (1985) reported a much wider range in duodenal DM content than we observed in the present study.

Little reduction in size of digesta particles occurs between the abomasum and feces (Poppi et al., 1980; Udén and Van Soest, 1982). Therefore, particle size distribution in omasal and fecal particulate matter should be similar if a representative sample of digesta was obtained. In contrast, small particles might be preferentially digested, leading to an increase in particle size distribution with passage through the intestine. In our study, particle size fractions of omasal and fecal DM were similar. However, HMEC was included in the TMR, and it was apparent from visual observation that the samples contained a sizeable amount of large corn particles. It is likely that digestibility of corn is higher in the lower gastrointestinal tract than that of cell wall carbohydrates in large particles. This suggests that the average particle size of omasal digesta should be larger than the particle size of fecal DM. Because this was not the case, it implies that the omasal samples did not contain enough large particles, as the marker ratios also suggested. The proportion of total DM retained on 2.36- and 1.18-mm screens (6.7 and 3.8%) was smaller than the values of 8.6 and 9.1% reported by Combs (1985). He used slightly smaller screens (2.0 and 1.0 mm), and based on marker ratio there was an over-abundance of particulate DM, in contrast to our study. Compared with our study, the proportion of large particles in DM was much more variable in duodenal samples (Combs, 1985). Particulate matter tends to accumulate in the fundic region of the abomasum, so DM content of abomasal digesta may be several times greater than that of digesta that flows through the pylorus (Faichney, 1980). Enhanced intensity or frequency of propulsive contractions in response to physical manipulation of the cannula can periodically increase DM content of abomasal or

duodenal samples. Increases in DM content are related to increased proportions of large particles in the sample (Egan and Doyle, 1984; Combs, 1985). The proportion of large particles in omasal digesta DM was greater than in abomasal or fecal DM (Waghorn, 1986). This indicates either entrapment of large particles between the laminae and occasional backflow of particles to the reticulum (Smith, 1984) or differential rates of passage of liquid and small and large particles. Based on the foregoing discussion, it is possible that the composition of digesta flowing from the reticulum may be more homogenous than that of digesta flowing from the omasum or abomasum.

Omasal Digesta Sampling Procedures. Sampling omasal rather than abomasal or duodenal digesta can provide several advantages. The technique is less invasive, because only a ruminal cannula is required. Animals fitted with a ruminal cannula can be maintained for years without any adverse effects. Radiological studies (Wenham and Wyburn, 1980) have indicated some disruption of normal digesta flow in sheep fitted with intestinal cannulas, causing retention of digesta and distension of intestines around the cannula. The simple T-piece cannula caused the least disturbance. Problems of poor appetite and general unthriftiness sometimes are reported for surgically prepared animals, especially for those fitted with reentrant cannulas (Wenham and Wyburn, 1980). Because less surgery is needed, and ruminally cannulated animals are easier to maintain than intestinally cannulated animals, experiments can be conducted with a larger number of animals, thereby increasing the possibility of detecting treatment differences. In the present study, results for OM and N digestion showed rather small coefficients of variation, especially when compared with studies using duodenally cannulated cows and a single-marker method to calculate digesta flow. Using a sampling tube with a wider inside diameter probably could reduce the sampling error and further reduce variability of the estimates.

Most of the methods developed to sample digesta from the omasum require more surgery than duodenal or abomasal cannulation (McSweeney, 1986; Michalowski et al., 1986; Rupp et al., 1994) and therefore are not very practical. To our knowledge, these techniques have not been widely adopted. The technique used by Harmeyer and Michalowski (1991) is less invasive than techniques requiring an omasal cannula or a sleeve secured around the omaso-abomasal orifice. They inserted a sampling tube via an abomasal cannula through the omasal canal and into the reticulo-omasal orifice with the aid of a teflon tube permanently fixed between the abomasal cannula and reticulo-omasal orifice. This method probably interferes less with digesta movement in the rumen and reticulo-omasal orifice than our system. However, their approach requires an abomasal cannula, and it is

also necessary to insert the collection tube into the cannula before each sampling. This could be cumbersome in studies requiring frequent sampling. It is noteworthy that a very similar approach to our sampling device has been used successfully for abomasal nutrient infusions (Spires et al., 1975).

Punia et al. (1988) and Punia and Leibholz (1994) obtained omasal samples from cattle by suction through a tube passed before each sampling into the omasal canal via the rumen. This system probably interferes with digesta movements to a lesser extent than our method, but passing the tube before sampling through ruminal contents probably will bring large particles into the vicinity of the reticulo-omasal orifice. This may result in biased composition of the sample. It seemed to us that there was more particulate matter in omasal samples immediately after we inserted the device into the omasum.

Effect of Sampling Site on Digestion Measurements. Using omasal sampling to partition digestion in ruminants between the fermentative area of the forestomach and the rest of the gastrointestinal tract can result in measurements differing somewhat from those obtained at the abomasum or duodenum due to absorption from the omasum and secretion in the abomasum. Sampling site is extremely crucial in studies examining water, mineral, and VFA absorption (Engelhardt and Hauffe, 1975; Smith, 1984). In cattle, the absorption of minerals and water in the omasum is more efficient (Edrize et al., 1986) than in sheep (Engelhardt and Hauffe, 1975), probably because of differences between sheep and cattle in the relative size and complexity of the omasum. Dry matter flow to the duodenum was less than to the omasum (Punia et al., 1988), mainly because of absorption of minerals. Although a large amount of water is absorbed from the omasum (Edrize et al., 1986), digesta flow to the duodenum was only slightly less than that to the omasum (Punia et al., 1988; Punia and Leibholz, 1994). This is due to the secretion of fluid into the abomasum (Barry et al., 1985; Rupp et al., 1994).

There is microbial activity in the omasum (Smith, 1984), but protozoal numbers are much smaller in the omasum than in the rumen (McSweeney, 1986; Punia et al., 1988; Punia and Leibholz, 1994). Smith (1984) calculated that 10% of the potentially digestible fiber entering the omasum can be digested. This estimate was based on a 5-h turnover time, which probably is too long for dairy cows with high feed intake. Therefore, the contribution of the omasum to fiber digestion is likely to be of minor importance.

Omasal instead of duodenal sampling probably has a greater effect on values obtained for CP digestion than for those of OM or cell wall digestion. Endogenous secretions to the abomasum can cause substantial errors in estimating degradation of dietary protein. Flow of endogenous N to the abomasum was

17.2 g/d in cattle of 300 kg live weight (Hart and Leibholz, 1990). Ørskov et al. (1986) reported a mean value of 195 mg of endogenous N/kg BW^{0.75} flowing from the abomasum in cattle nourished completely by intragastric infusion of nutrients. This value is most likely an underestimate for animals fed normal diets. Webb et al. (1992) made observations on ruminal, and especially omasal, absorption of peptides. If the absorption of peptides from the omasum is of quantitative importance for protein nutrition, omasal sampling should be preferred in studies examining ruminal N metabolism. However, markedly greater N flow to the duodenum than to the omasum (Hart and Leibholz, 1990) does not agree with the observations of Webb et al. (1992). The omasal sampling technique also enables more accurate estimates of the amount of nonmicrobial, nonammonia N (soluble protein N, peptide N, and amino N) flowing out from the reticulo-rumen. Endogenous secretions and hydrolysis in the abomasum compromise these measurements made on abomasal or duodenal samples. Recent data of Hristov and Broderick (1996) suggest that the nonmicrobial, nonammonia N fraction can be quantitatively important.

Implications

A technique for digesta sampling from the omasum via a ruminal cannula was developed. This method may prove useful for partitioning digestion between the fermentative portion of the forestomach and the lower gastrointestinal tract. The omasal sampling device is easily installed in ruminally cannulated cows and can be left in place for the entire sampling period. In the present study, the composition of omasal digesta samples was biased, but using a double-marker method resulted in fairly small coefficients of variation for measurements of ruminal digestion variables.

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