



## A comparison of ruminal or reticular digesta sampling as an alternative to sampling from the omasum of lactating dairy cows

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### ABSTRACT

The objective of this study was to develop and compare techniques for determining nutrient flow based on digesta samples collected from the reticulum or rumen of lactating dairy cows with estimates generated by the omasal sampling technique. Pre-experimental method development suggested, after comparing with the particle size distribution of feces, application of primary sieving of ruminal and reticular digesta from lactating cows through an 11.6-mm sieve, implying that digesta particles smaller than this were eligible to flow out of the rumen. For flow measurements at the different sampling sites 4 multiparous, lactating Nordic Red cows fitted with ruminal cannulas were used in a Latin square design with 4 dietary treatments, in which crimped barley was replaced with 3 incremental levels of protein supplementation of canola meal. Digesta was collected from the rumen, reticulum, and omasum to represent a 24-h feeding cycle. Nutrient flow was calculated using the reconstitution system based on Cr, Yb, and indigestible neutral detergent fiber and using <sup>15</sup>N as microbial marker. Large and small particles and the fluid phase were recovered from digesta collected at all sampling sites. Bacterial samples were isolated from the digesta collected from the omasum. Several differences existed for digesta composition, nutrient flows, and estimates of ruminal digestibility among the 3 different sampling sites. Sampling site × diet interactions were not significant. The estimated flows of DM, potentially digestible neutral detergent fiber, nonammonia N, and microbial N were significantly different between all sampling sites. However, the difference between DM flow based on sampling from the reticulum and the omasum was small (0.13 kg/d greater in the omasum). The equality between the reticulum and the omasum as sampling sites was supported by the following regression: omasal DM flow = 0.37 (±0.649) + 0.94 (±0.054) reticular DM flow ( $R^2 = 0.96$  and root mean square error = 0.438 kg/d). More deviating nutrient-flow es-

timates when sampling digesta from the rumen than the reticulum compared with the omasum suggested that sampling from the reticulum is the most promising alternative to the omasal sampling technique. To definitively promote sampling from the reticulum as an alternative to the omasal sampling technique, more research is needed to determine selection criteria of reticular digesta for accurate and precise flow estimates across a range of diets.

**Key words:** dairy cow, digesta flow, microbial protein synthesis, rumen digestibility

### INTRODUCTION

Accurate estimation of nutrient outflow from the rumen is a prerequisite for proper ration formulation to meet the nutrient requirements of dairy cattle (NRC, 2001; Fox et al., 2004). The abomasum or duodenum in fistulated sheep and cattle has commonly been used as a digesta sampling site to study digestion in the ruminant fore-stomachs. Abomasal or intestinal surgical procedures are often more difficult and involve longer animal recovery times than ruminal cannulation and may shorten the lifetime of the experimental animals, and cannulas at these locations require extensive maintenance (Faichney, 1993; Harmon and Richards, 1997). Huhtanen et al. (1997) proposed an omasal sampling technique for obtaining spot samples of ruminal digesta through the reticulo-omasal orifice. Improved accuracy of digesta flow measurements using a triple-marker system (Ahvenjärvi et al., 2003) and reduced interference from endogenous N flow and abomasal digestive processes are the advantages of the omasal sampling technique compared with sampling digesta from the duodenum (Ahvenjärvi et al., 2000). However, sampling from the omasum can reduce feed intake (Ahvenjärvi et al., 2002), and compared with samples collected from the duodenum, composition of omasal samples deviated more from calculated true digesta (Ahvenjärvi et al., 2000). This could increase errors in cases of unrepresentative digesta sampling if using less than 3 markers.

Björnhag et al. (1984) introduced a reticular sampling device to replace duodenal sampling in sheep. Hristov

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(2007) compared reticular and duodenal sampling in dairy cows and concluded that the reticular sampling technique can provide reliable estimates for ruminal digestibility. Furthermore, Krizsan et al. (2010) pointed out reticular sampling as a promising alternative to sampling from the omasum, with only a small digesta composition difference in NDF when sampling from the reticulum compared with the omasum. The composition difference resulted in a slightly higher estimated ruminal NDF digestibility when sampling from the reticulum compared with the omasum. Krizsan et al. (2010) applied the triple-marker method (France and Siddons, 1986) and sieving of the digesta through a 1-mm sieve size directly after it was collected from the reticulum. Hristov (2007) and Rotta et al. (2014) did not use any sieving to discard large particles not eligible for escape through the reticulo-omasal orifice. Krizsan et al. (2010) concluded that the selection of reticular particles below 1 mm was not completely representative of particulate matter belonging to the large-particle phase that are likely to leave the reticulum, and it appears that the triple-marker method was not able to completely correct for this unrepresentative sampling. Because flow of particulate matter from the reticulum through the reticulo-omasal orifice is associated with reductions in particle size (Ahvenjärvi et al., 2000), it was hypothesized that sieving of reticular or ruminal digesta through a sieve size yielding a particle size distribution comparable with that of fecal particles would provide a more representative sample. Poppi et al. (1980) and Udén and Van Soest (1982) suggested negligible changes in digesta particle size distribution after the abomasum. Sampling digesta from the rumen is the most reasonable alternative for estimates of ruminal nutrient outflow in small ruminants without the application of more advanced sampling equipment.

The hypothesis of this study was that the omasal sampling technique can be replaced by sampling of digesta from the reticulo-rumen of dairy cows, applying a reconstitution system based on 3 markers, and primary sieving of the collected digesta. Therefore, the objectives of this study were first to determine an appropriate sieve size selection for digesta collected from the reticulum or rumen of lactating dairy cows based on the fecal particle size distribution, and second compare nutrient flows based on sampling from these sites, applying the most appropriate sieving, with estimates based on omasal sampling.

## MATERIALS AND METHODS

All animals were registered and cared for according to guidelines approved by the Swedish University of Agricultural Sciences Animal Care and Use Committee

and the National Animal Research Authority, and the experiment was carried out in accordance with the laws and regulations controlling experiments performed with live animals in Sweden.

### *Sieve Size Selection for Rumen and Reticular Digesta*

Digesta was sampled from the rumen (the cranio-dorsal blind sac), reticulum, omasum, and rectum from 3 mid-lactation cows fed TMR consisting of on average 60% grass silage and 40% concentrate [composed of crimped barley and canola meal; ExPro-00SF (Aarhus Karlshamn AB, Malmö, Sweden) and Solid 220 (Lantmännen Lantbruk AB, Stockholm, Sweden)], averaging (mean  $\pm$  SD)  $22.6 \pm 3.8$  kg of DMI/d and  $27.6 \pm 12.6$  kg of milk/d during the sampling. Sampling was conducted at 3 different occasions. Samples of rumen and reticular digesta were collected using a 250-mL wide-necked plastic bottle with a rubber stopper according to Krizsan et al. (2010). Omasal digesta samples were collected by using the omasal sampling technique according to Huhtanen et al. (1997) and as modified by Ahvenjärvi et al. (2000). Ruminal and omasal samples were taken 4 times daily at 4-h intervals: 0600, 1000, 1400, and 1800 h. Spot samples of reticulum and feces were obtained 1 h after the omasal and rumen sampling the same 3 d at 0700, 1100, 1500, and 1900 h. Individual samples from each cow within sampling site and time point and for each applied sieve size selection were wet sieved separately. Three samples of 250 mL of rumen, reticular, and omasal digesta from each cow per sampling time per sampling site were distributed as follows: one was directly poured in a 1-L plastic bottle without primary sieving, the next 2 samples were sieved immediately after collection by custom-made sieves of 5.6 and 11.6 mm, and thereafter the filtrates were poured in 1-L plastic bottles. The sieves were made of 7-cm-high PVC tube (Uponor AB, Virsbo, Sweden) with 14-cm i.d., and the bottom of the tubes was covered by different-pore-size nets of stainless steel (Jowema, Anderstorp, Sweden). Samples were stored at  $-20^{\circ}\text{C}$  before wet sieving. Thawed samples of 50 g on a fresh-weight basis from each of the rumen, reticular, and omasal digesta samples ( $n = 4$  per cow and sampling site), respectively, were sieved through a series of sieves of 1,250, 630, and 160  $\mu\text{m}$  representing large, medium, and small particle size fractions, respectively, using a Fritsch analysette 3 PRO (Fritsch GmbH, Idar-Oberstein, Germany) sieve shaker. The subsamples were sieved for 10 min with continuous water flow of 3.5 L/min at a rate of 3,000 vibrations/min, and the amplitude was adjusted to 2.0 mm. Fecal samples were suspended in 200 mL of water before sieving by the same method as described for the

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