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Measurement of microbial nitrogen flow in the duodenum in sheep

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The microbial nitrogen (N) in the duodenum is normally estimated by the marker ratio technique, using the concentration of the marker in the liquid associated bacteria (LAB) isolated from the rumen or sometimes from the duodenal contents as reference. It implicitly assumes that LAB are representative of the total microbial mass present in the rumen, which is now known to be incorrect (Merry and Mc Allan, 1983; Yang *et al,* 1989). We report here the preliminary data which allow an estimation of the error resulting from this assumption.

Four 2-yr-old wethers fitted with rumen and duodenal cannulae were successively given the same amounts (1210 g DM/d in equal meals at 3-h intervals) of 4 isonitrogenous diets composed of good-quality orchard grass hay and concentrate–either dehydrated beet pulp or corn grain–in the ratios 70/30 and 40/60 on a (DM basis). Chemical composition and distribution of LAB and adherent biomasses expressed as "solid adherent bacteria equivalent" (SABeq, assuming sampled SAB were representative of the entire adherent population of SAB and fungi) in each wet-sieved particle pool were determined in the rumen and in the duodenum as described by Yang *et al* (1989). Cr-EDTA and ^{15}N, continuously infused for 5 d and 3 d respectively before sampling, were used as flow and microbial markers. LAB biomasse in the rumen effluent was estimated as: rumen LAB pool x rumen water fractional outflow rate, and that of SABeq as the concentration of SABeq on each size of rumen particles x duodenal DM flow of these particles. ^{15}N enrichments of the microbial matter flowing from the rumen was then calculated as the sum of the enrichments in LAB and SAB from each particle pool, weighted according to their proportion in the total biomass. Dividing the NA-^{15}N duodenal flow by this enrichment (Method 3) gave an estimate of the actual flow which was compared to that calculated using rumen (Method1) or duodenal (Method 2) enrichment of LAB alone.

As expected, the more reliable Method 3 always provided higher microbial N flow than the currently used Method 1 (18.4 *vs* 15.6 g N/d; 18% increase, $P < 0.05$; means of 4 diets). Method 2 gave the same estimates (18.4 g N/d) as Method 3 because of the shift in duodenal LAB-^{15}N enrichment towards that of the more representative microbial sampling of Method 3. The 3 methods showed the same diet effect.

In conclusion, Method 3 is not a reference method because it assumes that protozoa and fungi have the same ^{15}N enrichment as SAB. However, it can be concluded that the commonly-used method is inadequate and must be improved upon.

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