

UTILIZATION OF RUMEN BACTERIA BY RUMINANTS

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Bacterial protein synthesised in the rumen from dietary nitrogen (N) constitutes the major amino acid (AA) supply for the ruminant animal. It has been demonstrated that the AA composition of samples of rumen bacterial populations obtained from very different dietary regimes differ very little (Hungate, 1966). This could be due to the uniformity in composition of the many different bacterial species and forms usually present.

Previous experiments designed to investigate the nutritive value of microbial protein have been conducted with rats, the assumption being that rats and ruminants utilize bacterial protein to the same extent. Apart from possible differences in the digestive and absorptive abilities in rats and ruminants, there may also be differences in the degree of utilization of absorbed AA's. Furthermore, it is possible that the AA composition for the maintenance and synthesis of rat tissue may be different from that needed for the synthesis of ruminant tissue, wool and milk. Also, ruminants use volatile fatty acids (VFA) as their main energy source and not glucose, as the rat and other monogastrics do.

An infusion technique has recently been developed by Ørskov *et al.* (1978) whereby ruminants have been sustained entirely by the intra-gastric infusion of VFA and minerals into the rumen and casein and vitamins

into the abomasum. This seemed a possible method by which digestibility and utilization for tissue maintenance and synthesis could be measured directly in a metabolically normal ruminant.

The present experiments were conducted to study the utilization of bacterial protein isolated on a large scale from whole rumen content. The bacterial protein preparation was infused abomasally at various input levels as the sole N source to ruminant lambs which were otherwise sustained entirely by intraruminal infusion of VFA and minerals.

Materials and Methods

Isolation of bacterial protein

A small continuous flow isolation plant was set up at a local slaughterhouse whereby whole rumen contents from cattle and sheep which were starved overnight were fractionated according to particle size and relative density by a series of filters with decreasing pore size down to 5 μ m (Endecotts Ltd., London), followed by 3 differential centrifugations from 1200 gN (MSE 3000 basket centrifuge MSE Ltd., London) for 3 min. through 19 000 gN (Sharples N° 6 Super Centrifuge, Pennwalt, Surrey) for 3 min., up to 19 000 gN for 12 min. Micros-

copric examination of filtered rumen liquor and of the 3 supernatants showed that protozoa and obvious dietary particles were present before but not after the first centrifugal treatment and that the bacterial population density was appreciably lowered and virtually absent in the second and third supernatants respectively. By difference therefore the sediments retained in the second and third centrifugations was taken to consist of rumen bacterial cells.

The sediments were immediately frozen, freeze-dried and stored separately in airtight polythene bags at 1 °C. Every 3 months the polythene bags of each individual centrifugal fraction were bulked, mixed and analysed.

The N content and content of AAN in the first fraction was similar to values obtained by other workers (Mason and Palmer, 1971) for proportions obtained from whole rumen content, whereas estimates of the second and third fractions appear very similar to figures obtained from pure and mixed cultures grown in various laboratories (Hobson, 1969).

The range of N concentration for the first, second and third and a mixture of the second and third fractions were 8.5 to 8.8, 10.1 to 10.4, 10.0 to 10.3 and 10.0 to 10.3 respectively. The respective percentages of amino acid N (AAN), of total N ranged from 83.1 to 84.9, 73.3 to 75.3, 74.9 to 76.4 and 75.5.

Based on these chemical analyses and microscopic examinations, the second and third sediments were combined and considered the best approximation to pure rumen bacteria.

Animals

Four female and three castrated male Suffolk x (Finnish Landrace x Dorset Horn) lambs, weighing between 25 and 30 kg, previously fed a conventional hay and whole barley diet, were surgically fitted with rumen cannulae and abomasal catheters.

Infusion procedures

After two weeks post-surgical recovery the lambs were infused intra-uminally with VFA and minerals and intra-abomasally with casein and vitamins at increasing levels every second day so that they reached their maintenance level in eight days and twice their

maintenance level in eighteen days. The method is described in detail by Ørskov *et al.* (1979). The level of VFA infusion was adjusted to keep the lambs at either once or twice (430 or 860 KJ/kgW^{0.75}) their energy maintenance requirement.

Experimental treatments

The freeze-dried bacterial preparations were reconstituted with 3.6 l water and mixed with the appropriate vitamins and trace-elements, in a blender each day for each animal, and then infused continuously into the abomasum as the sole nitrogen source to give in sequence approximately 2.00, 1.50, 1.00, 0.75, 0.50, 0.25 and 0.00 g N/kgW^{0.75}/d.

Collection procedures

Faeces were collected daily, bulked for each animal and stored at 0 °C. Urine was collected in plastic containers containing 400 ml 4NH₂SO₄ to maintain pH below 3. Urine output was measured and sampled daily.

Analytical

Samples of the bacterial preparation and faeces were dried at 100 °C to constant weight to determine dry matter (DM) content. The bacterial preparation, faeces and urine were analysed for total N by the micro Kjeldahl method. The amino acid content of the bacterial preparations were estimated on a Technicon Auto Analyser after hydrolysis at 110 °C for 24 h under reflux.

Statistical

All results and comparisons are expressed on a metabolic body weight basis (kg W^{0.75}). The maintenance nitrogen requirement and rate of utilization of each lamb was calculated by linear regression of nitrogen balance (NB) on input of apparently digested N (DNI). The NB was calculated as the apparent DNI minus the urinary N output. The maintenance requirement was taken as that N input which supported zero N balance. Regression lines were compared statistically by analysis of covariance. Other parameters were compared between animals by analysis of variance and simple t-test.

Results

Experiment 1

The total energy input was maintained at 430 KJ/kgW^{0.75}/d. Increasing the input of digestible N (DNI) from 0.20 to 0.85 g N/kgW^{0.75}/d gave linear increases in N retention.

There were no significant differences in this relationship between animals, neither in slope or intercept. A single regression equation could therefore be fitted, where $y = N$ retention and x the DNI.

$$y = 0.61x - 0.43 \quad \text{RSD} = 0.028$$

The intercept with zero N balance ($y = 0$) estimates maintenance requirement and was 0.70 gW/kgW^{0.75}/d. Apparent digestibility of dry matter (DMD), faecal N concentration and apparent digestibility of N were 71.6±1.78, 7.2±0.20 and 78.2±1.24 respectively. There were significant differences between lambs.

Experiment 2

The total energy input was maintained at 860 kJ/kgW^{0.75}/d. Increasing DNI levels from 0.20 to 1.70 g N/kgW^{0.75}/d gave linear increases in N retention. There were no significant differences in this relationship between animals, neither in slope or intercept. A single regression equation could therefore be fitted:

$$y = 0.66x - 0.41 \quad \text{RSD} = 0.044$$

Maintenance requirement ($y = 0$) is estimated at 0.62 g N/kg W^{0.75}/d. At zero DNI ($X = 0$) the mean was -0.34 g N/kg W^{0.75}/d. The rate of utilization of DNI for retention was 0.66. Apparent DMD, FN % and apparent ND was 70.92 ± 2.44, 7.2 ± 0.14 and 77.6 ± 1.63 respectively. There were significant differences between lambs.

Discussion

It would appear from the work so far that the apparent digestibility of rumen microbial N by ruminants is about 78%. Since by the nature of the experiment very little substrate entered the large intestine, the apparent N digestibility measure is likely to be very close to the true N digestibility.

The utilization of 66% of the digestible N is probably close to expected values since it must be taken into account that the digestible N contains a considerable fraction of nucleic acid N.

The amount of digestible N required for zero N balance is considerably more than expected from published values of endogenous urinary N. It agrees well however with recent reassessment of the tissue N maintenance and the philosophy that metabolic faecal N and endogenous urinary N are both components of the tissue N maintenance (Ørskov and Grubb, 1979).

References

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