### Intragastric infusion of nutrients in cattle

# BY N. A. MACLEOD, W. CORRIGALL, R. A. STIRTON AND E. R. ØRSKOV

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

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1. A method of continuous alimentation of cattle by total infusion of nutrients has been developed. Friesian steers within the weight range 100-400 kg live weight and dairy cows were used.

2. A multi-channel peristaltic pump was used to infuse solutions of volatile fatty acids (VFA), minerals, and buffer through a cannula in the rumen and a casein-vitamin solution into the abomasum.

3. The method described was successfully used with two cows and four steers in a series of trials over intervals of approximately 2 months. The levels of infusion were up to twice maintenance and with various relative proportions of VFA and protein. Blood metabolite levels, rumen osmotic pressure and pH were monitored and effectively controlled.

In ruminants where there are two interrelated sets of requirements: those of the microbial population and those of the host animal, interpretation of information collected from studies on normally-fed animals is difficult. These difficulties arise in predicting the fate of nutrients in the rumen, and in determining quantitatively the contribution made by bacteria and protozoa to the host animal's requirements.

In order to reduce the contribution of the rumen fermentation to the animal's requirement, diets have variously been supplemented with salts of volatile fatty acids (VFA) added to the diet, or with infusions of VFA directly into the rumen and infusions of protein into the abomasum. Armstrong & Blaxter (1957a, b) used infusions of acetic, propionic and butyric acids to supply part of the energy of a basal diet of dried grass and Martin & Blaxter (1963) infused protein only, in fasting sheep. Hovell (1972) conducted studies with lambs using salts of VFA, and Tao & Asplund (1975) developed a method by which they infused partially-neutralized VFA into the rumen of sheep and intravenously-infused amino acids. Difficulties were sometimes encountered however, primarily with the control of rumen pH and with electrolyte imbalances. Furthermore, with the exception of very low levels of infusion, the approach was generally that of supplementation of a normal basal diet. A method was developed however, using lambs (Ørskov, Grubb, Wenham et al. 1979) in which all nutrient requirements were met by infusion. Relatively few difficulties arose except with a VFA mixture containing a high proportion of acetic acid (mmol/mol: 850 C<sub>2</sub>, 50 C<sub>3</sub>, 100  $C_4$ ), when problems of metabolic disturbance and of controlling rumen pH were encountered (Ørskov, Grubb, Smith et al. 1979). The technique reported here is an adaptation of the method used for lambs.

#### MATERIALS AND METHODS

Rumen and abomasal cannulas. The rumen cannulas fitted to the cattle (Fig. 1) were Vulcathene waste-drain fitments modified by smoothing out the internal grid-shoulder and by forming an exterior flange and locking nut (McKenzie & Kay, 1968). A tight-fitting rubber stopper was inserted into the cannula and held in position with a threaded blanking cap. Transparent vinyl tubing, 3.5 mm bore and 6.5 mm external diameter, for the VFA, buffer, and water infusates were inserted through the stopper into the rumen and a fourth hole was provided in the rubber bung to facilitate sampling of rumen fluid by means of



Fig. 1. Arrangement for ruminal infusions. (a) Transparent vinyl tubing, (b) rubber stopper, (c) stoppered hole for sampling rumen fluid, (d) threaded retaining cap, (e) threaded flange and locking nut, (f) body wall, (g) perforated polythene disc, (h) rumen wall, (i) volatile fatty acid and water outlets, (j) buffer outlet.

a syringe and tube. The VFA and water tubes inside the rumen were fused at their outlets inside a small perforated  $60 \times 25$  mm polythene vial in order to effect a more rapid dilution and to avoid the possibility of irritation of the rumen wall at the point of outflow. The tubes extended into the rumen approximately 250 mm to ensure the outlets would be immersed in rumen fluid.

The abomasal cannula used for casein and vitamin infusion (Fig. 2) consisted of a 1 m long surgical non-toxic, translucent vinyl tubing  $5 \cdot 0$  mm bore and  $7 \cdot 0$  mm external diameter (Portex Ltd, Hythe, Kent). The end inserted into the abomasum was prepared by cementing a vinyl collar approximately 5 mm deep to the extremity and cementing on to this a 25 mm diameter by 2 mm thick disc of Vitrathene polythene (Stanley Smith & Co., Islesworth, Middlesex). The prepared end was then inserted in the mid-lateral body of the abomasum and the incision closed by a 'purse-string' suture. The tubing was drawn up inside the body cavity and exteriorized immediately behind the last rib, care being taken to ensure that there was no tension which might cause displacement of the abomasum. The exteriorized portion was trimmed to approximately 300 mm and fixed to the back of the animal with adhesive patches.

Plate 1 shows the arrangement of infusion tubes and cannulas. It was found better to connect the main infusion lines (shown taped together in Plate 1) to the tubes inserted into the rumen and abomasum via connectors. This created a weak point which could break fairly easily thus lessening the possibility of damage or discomfort to the animal should there be excess tension on the flow lines.

Control of infusion. Four calibrated infusion reservoirs (WCB Containers Ltd, Stalybridge, Cheshire) each of 30 l capacity, were used per animal (Plate 2). These contained respectively a solution of casein, one of the VFA and major minerals, the buffer solution, and water. Solutions to be infused were prepared daily from more concentrated bulk preparations

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Fig. 2. Abomasal cannula. (a) Body wall, (b) vinyl infusion tube, (c) wall of abomasum, (d) polythene disc, (e) vinyl collar.

(Table 1) and pumping speeds were set to give the required volumes within the 24 h period. A variable speed peristaltic metering pump with multi-channel pumping module (Watson and Marlowe Ltd, Falmouth, Cornwall) was used to infuse the solutions. Control of flow-rate was effected by selecting an appropriate bore-size of silicone rubber tubing for the module and by adjusting the pump speed. Plastic nipple connectors were used to join the module onto the main infusion circuit which consisted of 3.0 mm bore, 4.2 mm external diameter transparent vinyl tubing. There were slight differential flow rates, in particular with casein at higher concentrations where the rate of flow was slowed by the high viscosity. In order to ensure that all the diluted casein was infused over the same period of time it was found more convenient to reduce its volume slightly in comparison to the other infusates rather than to introduce various tube sizes on the pump module. When the amounts infused were sufficient to cover maintenance requirements, at approximately 10 d from commencement, the normal feed offered the animals was removed and eight plastic pan scrubbers inserted into the rumen via the cannula. These were used in an attempt to ensure that rumen motility and muscle tonus were maintained. The pH and osmotic pressure of rumen fluid were routinely checked twice daily and blood samples were removed once weekly to enable the metabolic profiles to be measured (Table 2). pH was determined on a Model 3030 portable pH meter with combination electrode (Electronic Instruments Ltd, Chertsey, Surrey). The check on osmotic pressure was maintained using a model 3L Advanced Osmometer (Advanced Instruments Inc., Needham Heights, Massachusetts, USA).

Preparation of solutions (Table 1). A series of concentrated solutions were prepared and diluted to a standard volume to give the solutions for infusion. In practice it was best to maintain the volume infused more or less constant, and to vary the concentration in order to change the level of nutrition. The volumes of VFA and buffer as infused into the rumen

# Table 1. Composition (g/kg) of concentrated preparations

(These were further diluted to give a combined rumen and abomasal infusate volume of approximately 0.8 1/kg body-weight<sup>0.75</sup> per d)

Casein solution		Comments		
Casein (89% DM) Na <sub>2</sub> CO <sub>3</sub> Vitamin solution Water	100·0 5·3 25·7 869·0	Casein: 133 g N/kg, 20·239 kJ/g. Final solution infused into abomasum at approximately 10% less volume than each of rumen infusates because of reduced rate of flow. In the instance of dairy cows the volume was 23 l/d.		
VFA solution: mixture 'C' Acetic ( $C_2$ ) Propionic ( $C_3$ ) Butyric ( $C_4$ ) CaCO <sub>3</sub> Water	388·2 183·9 87·8 18·0 322·1	Mixture 'C' (mmol/mol): $650 C_2 \cdot 250 C_3$ , $100 C_4$ . Energy concentration $11 \cdot 66 \text{ kJ/g}$ . This $10 \text{ m-VFA}$ concentrate was diluted four to twenty-five times before infusion depending on the level of alimentation.		
Mineral solution $Ca(H_2PO_4)_2 \cdot H_2O$ $MgCl_2 \cdot 6H_2O$ Water	15·0 7·5 977·5	Mixed daily with the VFA solution at the rate of 40 g/kg body-weight <sup>0.75</sup> per d at maintenance level and 80 g at twice maintenance. Particular care required to get $Ca(H_2PO_4)_2$ . $H_2O$ into solution 18 ml/l soluble at 30°.		
Buffer solution NaHCO <sub>3</sub> KHCO <sub>3</sub> NaCl Water	73·0 38·0 7·0 882·0	Daily quantities of these concentrates were approximately related to the amount of acids infused as discussed on p. 551 and within the range 2.3 times acid concentrate at maintenance level to 3.7 times acid concentrate at maintenance		
Vitamin preparation Thiamine hydrochloride Riboflavine Nicotinic acid Choline chloride Pyridoxin hydrochloride P-amino-benzoic acid Calcium DL-pentothenate Folic acid Cyanocobalamin Myo-inositol D-biotin 2-Methyl-1,4-Napthaquinone DL-α-tocopheryl acetate Linoleic acid	0.76 3.04 3.04 113.89 0.30 0.08 2.28 0.01 0.01 113.89 0.05 0.38 3.04 759.23	The vitamin-linoleic acid mixture was homogenized at a rate of 1 kg in 7.59 l ethanol:water mixture (30:70, v/v) and the homogenate incorporated in the casein concentrated solution at 25.7 g/kg. A further intramuscular injection of vitamins A, D and E was administered at 14 d intervals according to manufacturer's recommendation.		
Trace minerals $FeSO_4 \cdot 7H_2O$ $ZnSO_4 \cdot 7H_2O$ KI $MnSO_4 \cdot 4H_2O$ $CuSO_4 \cdot 5H_2O$ $CoSO_4 \cdot 7H_2O$ NaF	822-79 48-26 43-91 22-94 22-15 8-70 31-25	The trace minerals were dissolved at a concentration of 253 g in 101 water and the solution then used at the rate of 1 ml/kg body-weight <sup><math>75</math></sup> per d at maintenance level. It was injected on a daily basis as one dose through the abomasal cannula preferably flushed with water to avoid blockage of cannula with casein precipitate.		

were each in the order of 0.2-0.3 l/kg body-weight<sup>0.75</sup> per d, with a similar amount of diluted case in solution directed to the abomasum.

Casein was prepared as a 100 g/l solution by adding 53 g sodium carbonate/kg air-dry lactic casein and homogenizing for 20 min in warm water. During homogenization the vitamin solution was added in amounts calculated to meet the animal's requirement at maintenance. In the event of animals being fed submaintenance levels of protein, supple-

mentary vitamins were added directly to the infusate reservoirs. Vitamins A, D and E were given by the intramuscular injection of Vetrivite at fortnightly intervals according to the manufacturer's recommendations (C-Vet Ltd, Minster House, Western Way, Bury St Edmunds, Suffolk).

Calcium carbonate was added to the concentrated VFA solution while the remainder of the major minerals, calcium tetrahydrogen diorthophosphate and magnesium chloride, were prepared as a separate concentrated solution and added to the VFA only when daily infusates were prepared. The calculations of the total energy to be supplied were based on the assumption that the requirement was 450 kJ/kg body-weight<sup>0.75</sup> per d at maintenance. Observations were made using various molar proportions of VFA up to levels of twice maintenance energy.

The amounts of buffer solution containing sodium and potassium bicarbonates and sodium chloride were adjusted to maintain the rumen pH between 6.0 and 6.5. The amounts required were calculated in relation to the level of VFA infused. Increases in the level of infusion of VFA generally resulted in a greater proportion of buffer being needed. Expressed as concentrated buffer: VFA solutions (see Table 1) these ranged from approximately 2:1 at lower levels of infusion to approximately 4:1 at the higher levels. During the changeover from a normal diet to intragastric alimentation the buffer requirements were less, presumably because the residual food debris in the rumen stimulated rumination and salivation. In this respect an interesting observation (E. Storm, unpublished results) indicated that when salivation was artifically stimulated by encouraging the animals to chew on rubber tubing no additional buffer was required at the maintenance level and at twice maintenance only minimal amounts.

#### **RESULTS AND DISCUSSION**

There were a few problems initially in adapting the same techniques used with lambs to mature cows and steers. One of these was related to controlling osmotic pressure and pH of rumen fluid. Increases in osmotic pressure to approximately 400 mosmol/l were generally associated with a fall in pH and it was therefore found better to maintain an osmotic pressure below 350 mosmol/l (Engelhardt, 1969). In the event, simply diluting the rumen contents by an additional infusion of water was usually sufficient to correct the imbalance. Although water was freely available, the animals did not respond to osmotic pressure rises in rumen fluid by drinking more. In terms of total infusates, aqueous solutions had to be given at approximately  $0.7-1.0 \text{ l/kg body-weight}^{0.75}$  per d and in the instance of the dairy cows under observation this amounted to total daily infusates in excess of 100 kg/d. The quantities were 87 l infused into the rumen and 26 l as dilute casein to the abomasum. Sheep and lambs on the other hand appeared to tolerate lower infusate volumes of approximately  $0.5-0.7 \text{ l/kg body-weight}^{0.75}$  per d.

The blood metabolites in both steers and cows were monitored (Table 2) and they were similar to those reported for sheep by Ørskov, Grubb, Wenham *et al.* (1979). Observations have now been made over prolonged periods of time on steers, and on dairy cows at different stages of pregnancy, lactation and when dry; and at varying inputs of nutrients up to a calculated level of twice maintenance for both energy and protein.

Reproduction appeared to be perfectly normal with regular oestrous cycling. The cows were artificially inseminated to an Aberdeen Angus bull for their third gestation while still being maintained by infusion, held to first service and gave birth to healthy 30 kg live-weight calves. The cows have again been inseminated for their fourth gestation and pregnancy diagnosis confirms that they are in calf to first service.

The Friesian steers used ranged in weight between 100 and 400 kg live weight and the only difficulties encountered were those associated with leakage from the cannulas. In our experience one of the essentials is to have a tight seal between the rumen cannula and

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	Accepted range	Cows on infusion	SD for infusion observations
Red blood cells (10 <sup>12</sup> /l)	5–9	6.3	0.71
White blood cells $(10^9/1)$	4-10	9.2	3.15
Packed cell volume (1/1)	0.24-0.40	0.3	0.30
Haemoglobin (g/l)	80-140	117	12-2
Sodium (mmol/l)	136-144	136.0	5-81
Potassium (mmol/l)	4.3–5.7	4.5	0.35
Magnesium (mmol/l)	0.8-1.3	1.3	0.08
Calcium (mmol/l)	2.0-2.5	2.4	0.16
Phosphate (mg/l)	43–77	51.0	9.3
Total reducing sugars (mg/l)	600-700	812.0	90.0
Ketones (qualitative test)	Negative	Negative	—

# Table 2. Accepted values in normal dairy cow metabolic profiles compared to values derived from Friesian cows sustained on infusion

(Mean of forty observations)

the fused rumen and body wall to ensure that there are no leakages which will lead to loss of metabolites.

The volumes of urine were closely related to the amounts of liquids infused. In general, the mean daily excretion, over all treatments, of faecal dry matter with the dairy cows ammounted to 100 g/d. The nitrogen and ash contents of faeces were usually approximately 30 and 550 g/kg dry faeces respectively.

Changing animals from total infusion to normal feeds was effected easily and there were no apparent problems or disturbances to the animals. When an infusion period was completed, a large rumen fluid inoculum from normally-fed animals was introduced via the cannula and normal feed was offered. In the instance of the dairy cows, the inoculum amounted to 4 l on two successive days. The amount of inoculum was purely arbitrary and probably not required in this quantity since microscopic examination on the second day indicated a thriving mixed rumen microflora. The animals established an appetitie for solid food immediately and food intakes were back to normal within one week.

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#### EXPLANATION OF PLATES

Plate 1. Arrangement of infusion tubes on 600 kg Friesian dairy cow.

Plate 2. Peristaltic pumps and infusion reservoirs.

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Plate 1

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Plate 2

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