

Effect of glucose supply on fasting nitrogen excretion and effect of level and type of volatile fatty acid infusion on response to protein infusion in cattle

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Two experiments were carried out on cattle nourished entirely by intragastric infusion, to determine the extent to which glucose or a glucose precursor determines the response to protein infusion in energy-undernourished animals. In order to determine the requirement for glucose in 1-year-old fasting cattle, glucose was infused at increments to supply 0, 1.5, 2.5, 3.5, 4.5, 5.5 and 6.5 g/kg metabolic body weight ($W^{0.75}$) and the effects on plasma β -hydroxybutyrate and N excretion were measured. At 5.5 g glucose/kg $W^{0.75}$ plasma β -hydroxybutyrate was reduced to a basal level of 1.65 mmol/l and fasting N excretion reduced from 529 to 280 mg N/kg $W^{0.75}$. No further reduction was observed with the higher level of 6.5 g glucose/kg $W^{0.75}$. In the second trial, three steers were used in a 3×3 Latin square design and infused with a volatile fatty acid mixture of 65, 27 and 8 mol acetic, propionic and butyric acids respectively/100 mol, either at an estimated maintenance energy level of 450 kJ/kg $W^{0.75}$ and supplying a calculated glucose equivalent level of 13.0 g/kg $W^{0.75}$ (M1A), or at $1.5 \times$ maintenance supplying a glucose equivalent of 20 g/kg $W^{0.75}$ (M1.5A). Another mixture infused at the maintenance energy level contained 49, 43 and 8 mol acetic, propionic and butyric acids respectively/100 mol but with a glucose equivalent of 20 g/kg $W^{0.75}$ (M1P). Casein was infused at each of these energy treatments to supply 0, 200, 400, 800, 1600 and 2500 mg N/kg $W^{0.75}$ daily, and N balance and blood metabolites were measured. N retention increased linearly (r 0.98) with casein infusion. The coefficients for N retention were 0.55, 0.57 and 0.64 for M1A, M1.5A and M1P respectively. The mean efficiency of N utilization was 0.58. The results suggest that provided the glucose need is met there is no relationship between energy supply and efficiency and level of protein retention. However, the results also indicate that glucose requirement in cattle may be higher than that previously observed in sheep.

Glucose requirement: Intragastric nutrition: Nitrogen excretion: Volatile fatty acids: Protein retention

Work in this laboratory using intragastric nutrition in ruminants (Ørskov *et al.* 1979) has challenged the concept that protein requirement is dependent on energy supply (Balch, 1967). According to this concept animals fed below their maintenance energy requirement would not attain positive retention. However, later work (Ørskov *et al.* 1983) showed that fasted sheep responded to protein, and were in positive protein balance while in substantial negative energy balance. This concept was further elaborated by Fattet *et al.* (1984), showing that lambs in substantial negative energy balance on straw diets increased their carcass weight when a protected protein source was given. Evidently protein deposition was fuelled by oxidation of body fat.

In more recent work Chowdhury *et al.* (1997a) compared responses in fasted sheep with those of sheep given a mixture of volatile fatty acids (VFA) to supply 250 kJ/kg

metabolic body weight ($W^{0.75}$). They observed that at zero protein intake there were substantial differences in N loss, depending on whether energy was given or not. However, when casein was also infused it did not influence N retention, as the casein apparently provided some glucose precursors so that no effect of energy was apparent. It was postulated that the effect of energy supply was due to supply of glucose precursors, since β -hydroxybutyrate (BHB) was elevated in the fasted sheep. Subsequently, Chowdhury *et al.* (1997b) showed that by supplying a VFA mixture high in propionic acid at 91 kJ/kg $W^{0.75}$ the fasting N excretion and plasma BHB were in the normal basal range and energy supply had no effect on N retention. While this work was done with sheep, some preliminary work reported by Liu *et al.* (1997) on cattle maintained by intragastric infusion indicated that energy level influenced responses to protein, but there were also indications that the glucose requirement

Abbreviations: BHB, β -hydroxybutyrate; VFA, volatile fatty acids; $W^{0.75}$, metabolic body weight.

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in cattle was higher than that in sheep, and that these results were perhaps influenced by the supply of a glucose precursor. The work reported here tests the hypothesis that when the glucose requirement of cattle is met there will be little or no response in protein retention to different levels of energy and glucose supply. In the first experiment the level of glucose infusion needed to reduce plasma BHB and urinary N excretion to basal levels was determined. A higher level of glucose was then supplied at each of two different VFA mixtures to supply estimated maintenance and $1.5 \times$ maintenance energy requirement.

Materials and methods

Animals

Five steers (Limosine \times Friesian), each about 12 months old with an average body weight of 340 kg, were used in the infusion trial. Each animal was fitted with a rumen cannula and abomasal catheter as described by MacLeod *et al.* (1982). They were housed in individual pens and offered a mixed hay-concentrate diet for 5 weeks before and after surgery. They were then transferred to open-circuit respiration hoods where normal diets were discontinued and nutrients supplied by intragastric infusion (Ku Vera *et al.* 1990). Nutrient intakes were then stepped up from a basal level of 0.25 maintenance to reach maintenance level (defined as $450 \text{ kJ/kg W}^{0.75}$; Ørskov *et al.* 1979a) over a period of 10 d. Individual weights were recorded at the start of the trial and converted to their corresponding $W^{0.75}$, from which calculated levels of infusates were determined.

Expt 1. Treatments and design

Two steers were infused with maintenance levels of VFA and casein-N (defined as $450 \text{ kJ/kg W}^{0.75}$ and $400 \text{ mg N/kg W}^{0.75}$ daily; Ørskov & McLeod, 1982) for four consecutive days. This treatment was then followed by a full 2 d fast before six consecutive daily infusion periods of glucose at 1.5, 2.5, 3.5, 4.5, 5.5 and $6.5 \text{ g/kg W}^{0.75}$ respectively. The trace minerals and vitamins required according to MacLeod *et al.* (1982) were given at all times. The infusions were terminated when plasma BHB stabilized.

Expt 2. Treatments and design

This experiment was arranged as a 3×3 Latin square using three animals allocated to each of three VFA energy treatments in three periods. These treatments consisted of two at estimated maintenance, one high-propionic acid with 49, 43 and 8 mol acetic, propionic and butyric acids respectively/100 mol and one low-propionic acid with 65, 27 and 8 mol acetic, propionic and butyric acids respectively/100 mol. The third treatment was infusion at $1.5 \times$ estimated maintenance requirement, such that the amount of glucogenic precursor supplied by propionic acid was similar to that supplied by the high-propionic acid mixture infused at maintenance energy requirement. The calculated yields of glucose derived from propionic acid between the different mixtures were 13 and $20 \text{ g/kg W}^{0.75}$. It is assumed here that 2 mol propionic acid is equal to 1 mol glucose, although it is

known that not all propionic acid absorbed reaches the liver in the form of propionic acid (Weekes, 1989). These glucose yields were in excess of the glucose infusion required to reduce BHB to basal levels, in order to establish whether additional levels of glucose caused any further reduction. Six different levels of casein (i.e. 0, 200, 400, 800, 1600 and $2500 \text{ mg N/kg W}^{0.75}$), each given for 3–4 d duration, were then infused with each of the energy levels within the Latin square design. In each case values from the first day at each casein level were deleted when calculating respective N balances. Hovell *et al.* (1983) earlier showed that the responses to small changes in the infusion of energy and protein were rapid, and that the values even on the first day of each treatment were typical of true responses. However, we chose to omit the first day to avoid any possibility of a carry-over effect. It would have been desirable to have randomized the levels of casein. However, this would result in large changes between infusion levels and would increase the length of the experiment; since the main question was about differences due to type and level of VFA infusion, the method chosen was considered to be the best.

Housing and management

The animals were housed consecutively in each of two open-circuit respiration hoods so that energy balance could be determined. The hoods were constructed on conventional concrete metabolic stalls as described by Ku Vera *et al.* (1990). These provided rubber-mat flooring with an inset metal grid, from which urine passed via a plastic tray to a plastic container underneath. Faeces were collected on a separate fibreglass tray to the rear of the animal, but because no food was given amounts were small and therefore bulked over each of the energy treatments. During the experimental period the chambers were opened daily to facilitate administration of vitamins and trace minerals to the abomasum and removal of rumen samples by syringe. Blood samples were taken on the last day of each treatment. Gases were continually exhausted from the respiration hood.

Collection and analysis of urine

Urine collections were made for each 24 h period and collected in H_2SO_4 to ensure a final pH of less than 3.8. Sub-samples of approximately 100 ml were taken for analysis and stored at 1° . Total N was determined using the automated Kjeldahl method (Davidson *et al.* 1970) and urea using the technique of Technicon Instrument Co. as modified by Marsh *et al.* (1965).

Analysis of blood samples

Blood samples (approximately 20 ml) were withdrawn by jugular venepuncture into evacuated tubes containing EDTA. They were centrifuged for 10 min at 1235 g and the plasma was stored at -70° . BHB was determined by the technique of Zavin & Snarr (1973), glucose by the method of Trinder (1969), free fatty acids using the technique of Matsubara *et al.* (1983) and urea by the method of Marsh *et al.* (1965).

Rumen samples

Rumen samples were obtained twice daily at 09.00 and 18.00 hours through the rumen cannula using vinyl tubing connecting to a 60 ml syringe. Both pH and osmolality were determined immediately with the objective of keeping values between 6.2 and 6.8, and 300 and 340 mosmol/l respectively. In all cases the total volume of infusion was kept at approximately 1.0 litre/kg $W^{0.75}$.

Results

Expt 1. Nitrogen excretion and plasma β -hydroxybutyrate

Table 1 shows the responses in urinary N excretion and plasma BHB to fasting plus incremental glucose infusions for the two steers. N excretion decreased from a fasting level of about 525 mg N/kg $W^{0.75}$ to about 250 mg N/kg $W^{0.75}$ at a daily glucose infusion level of 5.5 g/kg $W^{0.75}$. Above this level of glucose infusion N excretion showed no further reduction. At fasting the plasma BHB was about 4 mmol/l, but fell to 1.65 mmol/l at a glucose infusion of 5.5 g/kg $W^{0.75}$. With glucose infusion above 5.5 g/kg $W^{0.75}$ no further reduction in BHB was apparent. These results demonstrate a N-sparing effect of glucose on protein catabolism but also suggest a limit to this effect, with no additive effect beyond an infusion level of 5.5 g glucose/kg $W^{0.75}$ daily.

It was apparent that N excretion and BHB were correlated. BHB (Y ; mmol/l) could be predicted from the equation $Y = 0.003X + 1.13$, where X is N excretion (mg/kg $W^{0.75}$; r 0.78).

Expt 2. Nitrogen excretion and plasma β -hydroxybutyrate

Fig. 1 shows the N balance for the two treatments at estimated energy maintenance to supply 13 or 20 g glucose equivalent, or the 1.5 \times maintenance energy level also supplying 20 g glucose equivalent. There was no consistent difference in the response to casein infusions, although the coefficient of N retention for 20 g glucose equivalent at maintenance (0.64) was significantly higher than that for the other treatment ($P < 0.05$). The regression equations describing the response in N balance (Y ; mg N/kg $W^{0.75}$ per d) to casein infusion (X ; mg casein-N/d) were as follows:

maintenance, low-propionic acid infusion:

$$Y = -254 + 0.55 \text{ (SE } 0.0133)X \text{ (} r \text{ } 0.98, P < 0.01, n \text{ } 18),$$

maintenance, high-propionic acid infusion:

$$Y = -283 + 0.64 \text{ (SE } 0.00055)X \text{ (} r \text{ } 0.99, P < 0.01, n \text{ } 18),$$

1.5 \times maintenance, low-propionic acid infusion:

$$Y = -227 + 0.57 \text{ (SE } 0.00029)X \text{ (} r \text{ } 0.99, P < 0.01, n \text{ } 18).$$

There was little difference in plasma BHB concentration between each of the energy treatments. The overall mean value was 1.77 (range 1.45–2.3) mmol/l.

There was no significant difference ($P > 0.05$) in plasma glucose levels, which ranged between 4.2 and 4.7 mmol/l.

Plasma urea concentration increased linearly with casein infusion from 0.8 to 5.8 mmol/l, and there was no significant treatment difference ($P > 0.05$).

Expt 2. Energy metabolism in response to treatment

Fig. 2 shows the effect of changes in level and molar proportion of VFA infused on estimated heat production. There was no significant difference in heat production between the two maintenance-energy infusion levels, but as expected heat production was greater at the higher energy supply. The average heat production increased from 464 to 544 kJ/kg $W^{0.75}$ i.e. an efficiency of energy retention of 0.64.

Discussion

Glucose requirements

Glucose requirement was determined indirectly in fasting cattle as the amount of infused glucose required to reduce plasma BHB to basal levels of approximately 1.5 mmol/l (Ørskov & MacLeod, 1993). The relationship between plasma BHB and corresponding N excretion levels shows that the process of fasting is characterized by a glucose deficiency involving elevated N excretion and plasma BHB concentration. Glucose requirement determined from Expt 1 was approximately 5.5 g/kg $W^{0.75}$ daily. The second experiment, in which two VFA mixtures were infused at different energy levels supplying glucose precursor in excess of 5.5 g/kg $W^{0.75}$, showed little effect of energy level on N balance (Fig. 1); in fact the highest coefficient of N retention was observed when 20 g glucose equivalent was given at the maintenance energy level.

Previous work by Liu *et al.* (1997) showed that when cattle were infused with VFA providing 300 and 600 kJ/kg $W^{0.75}$

Table 1. Effect of abomasal glucose infusion in otherwise fasted steers on plasma β -hydroxybutyrate (BHB) and daily nitrogen excretion in the urine*

	Steer no.	Glucose infusion (g/kg $W^{0.75}$)						
		0	1.5	2.5	3.5	4.5	5.5	6.5
BHB (mmol/l)	1	4.1	3.0	2.4	2.2	2.1	1.8	1.8
	2	4.2	2.9	2.0	1.9	1.9	1.5	1.5
N excretion (mmol/kg $W^{0.75}$)	1	507	467	356	347	343	305	309
	2	550	520	371	312	260	217	227

$W^{0.75}$, metabolic body weight.

* For details of animals and procedures, see pp. 390–391.

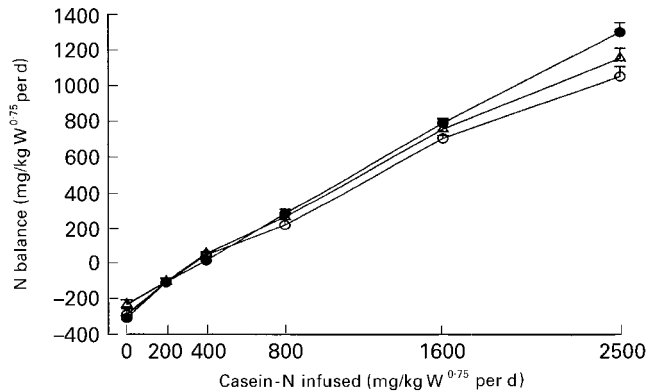


Fig. 1. Expt 2. Effect of infusion of different energy levels and types of volatile fatty acid in steers on responses in nitrogen retention to incremental casein infusion (0, 200, 400, 800, 1600 and 2500 mg/kg metabolic body weight ($W^{0.75}$)). (○), Maintenance (450 kJ/kg $W^{0.75}$; Ørskov *et al.* 1977) on a low-propionic acid mixture (65, 27 and 8 mol acetic, propionic and butyric acids respectively/100 mol); (●), maintenance on a high-propionic acid mixture (49, 43 and 8 mol acetic, propionic and butyric acids respectively/100 mol); (△), 1.5 × maintenance on a low-propionic acid mixture. Values are means and standard deviations (represented by vertical bars) for three determinations. For details of animals and procedures, see pp. 390–391.

daily at six increments of casein-N from 0 to 2500 mg N/kg $W^{0.75}$ daily the response in N balance was almost linear. At 300 kJ/kg $W^{0.75}$ daily, glucose precursor supply amounted to 7.17 g/kg $W^{0.75}$ daily. Subsequent work by Chowdhury *et al.* (1997b), using sheep infused with 91 kJ/kg $W^{0.75}$ as glucogenic VFA (16, 79 and 5 mol acetic, propionic, butyric acids/100 mol respectively) equivalent to 1.6 g glucose/kg $W^{0.75}$, showed a reduction in BHB to 1.4 mmol/l. Given that in the experiments reported here an infusion of glucose as precursor at 5.5 g/kg $W^{0.75}$ was required to reduce BHB to 1.5 mmol/l, it is reasonable to suggest that glucose requirements may be greater in cattle than in sheep, although more direct comparisons are needed to substantiate this contention.

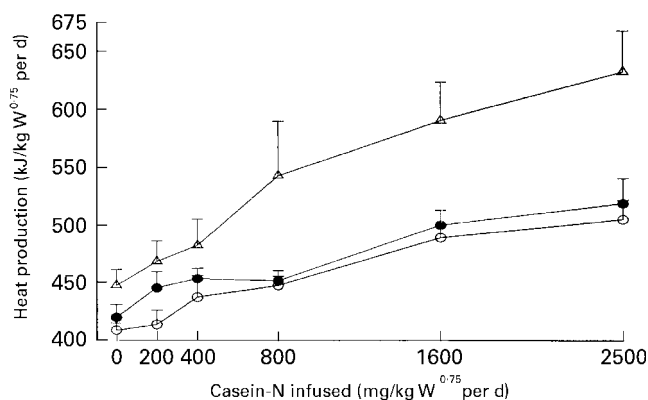


Fig. 2. Expt 2. Effect of infusion of different energy levels and type of volatile fatty acids in steers on changes in heat production with incremental infusion of casein (0, 200, 400, 800, 1600 and 2500 mg/kg metabolic body weight ($W^{0.75}$)). (○), Maintenance (450 kJ/kg $W^{0.75}$; Ørskov *et al.* 1977) on low-propionic acid mixture (65, 27 and 8 mol acetic, propionic and butyric acids respectively/100 mol); (●), maintenance on a high-propionic acid mixture (49, 43 and 8 mol acetic, propionic and butyric acids respectively/100 mol); (△), 1.5 × maintenance on a low-propionic acid mixture. Values are means and standard deviations (represented by vertical bars) for three determinations. For details of animals and procedures, see pp. 390–391.

A recent comparison between fasted sheep, cattle and camels (*Camelus dromedarius*) showed that cattle had higher plasma levels of BHB than sheep, while in camels there was no increase in BHB during fasting (J Wensvoort, DJ Kyle, ER Ørskov and DA Bourke, unpublished results).

Relationship between glucose supply, plasma β -hydroxybutyrate and glucose, and urinary nitrogen excretion

The high fasting daily urinary N excretion observed during the first experiment (529 mg N/kg $W^{0.75}$) is similar to the values of 518 mg N/kg $W^{0.75}$ obtained by Liu *et al.* (1997), 510 mg N/kg $W^{0.75}$ reported by Lobley *et al.* (1987), but lower than that reported by Ørskov *et al.* (1983; 641 mg N/kg $W^{0.75}$). The difference between the values reported here and those of other workers is probably due to age differences. Ku Vera (1989) showed that daily fasting urinary N excretion was higher in young immature steers (727 mg N/kg $W^{0.75}$) compared with mature fat steers (539 mg N/kg $W^{0.75}$). Chowdhury *et al.* (1997b) observed a lower fasting N excretion with adult castrate sheep than young sheep.

The fact that incremental levels of glucose infusion (1.5, 2.5, 3.5, 4.5, 5.5 and 6.5 g/kg $W^{0.75}$) resulted in proportional reductions in urinary N excretion by 0.06, 0.31, 0.38, 0.43, 0.50 and 0.49 mg N/d respectively demonstrates the N-sparing effect of glucose on body protein catabolism. Corresponding reductions in plasma BHB with successive glucose infusions were 0.29, 0.47, 0.51, 0.52, 0.60 and 0.60 mmol/l. These results followed closely those of non-esterified fatty acids and urinary N, and were inversely related to trends in plasma glucose, demonstrating clearly the anti-ketogenic effects of exogenous glucose in the fasted state (Ku Vera, 1985, 1989; Chowdhury *et al.* 1997a). The absence of any further reduction in N excretion when glucose infusion was raised to 6.5 g/kg $W^{0.75}$ suggests a limit to the N-sparing effect. This is supported by the absence of any further reduction in BHB at the highest infusion level, and is in agreement with the finding of Ku Vera (1989) that large amounts of glucose (above 400 g/d) failed to reduce N excretion any further. This finding supports the observation that there is a minimum protein turnover even during periods of total food deprivation (Lobley *et al.* 1987).

Efficiency of utilization of volatile fatty acids

While our work was not designed to study utilization of VFA, the efficiency when calculated as the increment in heat production as a result of increasing VFA infusion from 1.0 to 1.5 × maintenance was 0.64, which is very similar to values from much more extensive work reported with sheep (Ørskov *et al.* 1979) and with cattle (Ørskov & MacLeod, 1993).

Practical implications

The apparent absence of any clear response of N retention to energy supply suggests that the earlier model proposed by Balch (1967) was affected by a glucose deficiency. He suggested that at any given N intake, N retention is a function of both N intake and exogenous energy intake,

and that, irrespective of N intake, N retention would always be negative when energy supply is low. Earlier work by Ku Vera (1989), Meehan (1994) and Chowdhury *et al.* (1997b), showed that not only can a positive N balance be achieved when in negative energy balance ($159 \text{ kJ/kg W}^{0.75}$), but that substantial increases in tissue gain can occur when in negative energy balance fuelled by endogenous energy, provided glucose requirements are met. Within the limits imposed in these experiments, no further N-sparing effect seems apparent when glucose supply is increased above that required to reduce to BHB to basal levels.

Marston (1948), quoted by Webster *et al.* (1974), showed that fasting heat production depended on the level of nutrition before fasting. We have identified, in agreement with Chowdhury & Ørskov (1994), yet another reason why fasting metabolism is an inappropriate basis for measuring the utilization of nutritionally-balanced diets for ruminants, and that such measurements should be based at a level of glucose supply equivalent to approximately $5.5 \text{ g/kg W}^{0.75}$ or more daily when plasma BHB is normal. The data reported here, compared with those for sheep (Chowdhury *et al.* 1997b), suggest that there is a species difference between cattle and sheep in glucose requirement and glucose metabolism. Fasting N excretion was consistently higher in cattle than sheep, and for a given input in glucogenic VFA infusion, cattle seem to respond more than sheep. However, differences in age, weight, maturity and physiological state will confound cross-species comparisons. Further research is needed to substantiate these findings.

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