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Investigation of nitrogen balance in dairy cows and steers nourished by intragastric infusion. Effects of submaintenance energy input with or without protein

BY E. R. ØRSKOV, N. A. MACLEOD, S. T. M. FAHMY*, L. ISTASSE AND F. D. DEB. HOVELL

The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

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1. Two dairy cows were maintained by intragastric infusion of volatile fatty acids and casein. Except when fasting, the casein-nitrogen was held constant, while total gross energy supply was varied from zero during fasting to 650 kJ/kg body-weight $(W)^{0.75}$.

2. One cow was estimated to attain zero N balance at an energy intake of 255 kJ/kg $W^{0.75}$ and the other at 307 kJ/kg $W^{0.75}$, which was calculated to be substantially below the estimated energy required for zero energy balance.

3. When the cows were later given an N-free infusion for a period preceding the trial, N balance occurred at 98 kJ/kg $W^{0.75}$ for one cow and 115 kJ/kg $W^{0.75}$ for the other.

4. Four steers were similarly nourished by intragastric infusion and the energy nutrient increased from 0 at fasting to 450 kJ/kg $W^{0.75}$. The protein was held constant at 1 g N/kg $W^{0.75}$ except at fasting. The energy level at which N balance occurred was 154 (SE 38) kJ/kg $W^{0.75}$ or approximately equal to the energy content of the protein. The practical implications of these findings are discussed.

A study of the relative requirements for protein and energy during undernutrition of ruminants has always been difficult. This is because at maintenance and submaintenance feeding, the retention time of digesta within the rumen is so long that little dietary protein will escape degradation (Ørskov & Fraser, 1973). Therefore, the ratio, protein:energy available to the host animal becomes relatively constant, since the protein made available is almost entirely microbial in origin and is generally proportional to the energy-yielding constituents fermented within the rumen.

The technique of complete intragastric nutrition by the infusion of volatile fatty acids (VFA) to the rumen, and of protein to the abomasum (Ørskov *et al.* 1979*b*), has enabled us to vary the protein:energy value and has made possible a detailed study of the protein requirements during periods of low energy supply.

MATERIALS AND METHODS

Expt 1

Two non-lactating and non-pregnant Friesian cows (nos. 351 and 355) were used. They weighed approximately 600 kg live weight, were in good bodily condition and were each fitted with a rumen cannula and an abomasal catheter, as described by Ørskov & MacLeod (1982). The cows were nourished by intragastric infusion as described by Ørskov *et al.* (1979*b*) whereby the VFA, buffer and mineral solutions were infused into the rumen, and protein, vitamins and trace elements into the abomasum. The VFA mixture infused into the rumen consisted of 0·65, 0·25 and 0·10 acetic, propionic and butyric acids respectively, expressed as molar proportions.

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* Present address: University of El Menia, Egypt.

Table 1. *Expt 3. The sequence of treatments for the four steers used*

Period no.	Intended gross energy of infusate (kJ/kg $W^{0 \cdot 75}$)	Intended protein in infusate (g N/kg $W^{0 \cdot 75}$)	Duration of experimental period* (d)
1	450	1·0	4
2	450	0	5
3	0	0	5
4	150	1·0	4
5	250	1·0	4
6	350	1·0	4
7	450	1·0	4

W, body-weight.

* The first day of each period was excluded from the results.

Expt 2

The same cows were used when they were dry and non-pregnant, but preceded by a pregnancy and a lactation period. They weighed 700 kg at the start of the experiment. The composition of the VFA mixture was the same.

Expt 3

Four Friesian steers were used. They were approximately 10-months-old and weighed on average 264 kg at the start of the experiment. They were prepared with rumen and abomasal cannulas as described by Ørskov *et al.* (1979*b*) and MacLeod *et al.* (1982). They were adapted to the intragastric infusion procedure as described by Ørskov *et al.* (1979*b*) and MacLeod *et al.* (1982).

Treatments

Expt 1. The cows were adapted from a normal diet to total alimentation by infusion over a period of 2 weeks. They were then given an infusion with gross energy (VFA+protein) at a predetermined level of 675 kJ/kg body-weight $(W)^{0 \cdot 75}$. The infusion of protein consisted of casein supplying nitrogen at 750 mg/kg $W^{0 \cdot 75}$, equivalent to 118 kJ/kg $W^{0 \cdot 75}$ using a determined value of 157 kJ/kg N in the casein. The gross energy infused was then decreased in four steps from 675 to the energy value of the protein alone (118 kJ/kg $W^{0 \cdot 75}$). This sequence was followed by a period of fasting (i.e. the VFA and the casein were omitted from the liquid infusates). Each infusion lasted for a period of 6 d. The first day of each period was not included in the mean value. During all periods the amount of liquid infused was the same and was similar in amount to that reported by MacLeod *et al.* (1982).

Expt 2. In order to investigate the effect of the previous protein nutrition on the response to protein infusion, the cows were first given a period of 6 d with estimated maintenance energy and protein (450 kJ/kg $W^{0 \cdot 75}$ and 972 mg N/kg $W^{0 \cdot 75}$ respectively). This was followed by a maintenance energy but protein-free period, then a period of 5 d during which the energy was halved, after which the fasting measurements were made of 5 d followed by a complete restoration of the casein-protein alone for 5 d which was given at 938 mg N/kg $W^{0 \cdot 75}$ or 147 kJ/kg $W^{0 \cdot 75}$. The protein infusion was then held constant and the infusion of VFA was increased in eight equal increments, each of 3 d, to a total of 675 kJ/kg $W^{0 \cdot 75}$. Since the changes in input for each level were small, all values are included in the means plotted in Fig. 2 (see p. 102).

Expt 3. The steers were given a sequence of seven experimental periods as described in Table 1, with variation in energy supply from zero to an estimated maintenance energy of 450 kJ/kg $W^{0 \cdot 75}$.

Table 2. *Expt 1. Effect of level of energy nutrition on daily nitrogen, urea-N and creatinine excretion in dairy cows*

(Values for N are expressed as mg/kg body-weight $(W)^{0\cdot 75}$)

Cow no.	Gross energy infused (kJ/kg $W^{0\cdot 75}$)	N infused	Urinary N	Faecal N	Total-N excretion	N balance	Urea-N excretion	Creatinine excretion (g/d)
351	672	762	644	21	665	97	504	13·3
	461	752	711	22	733	19	590	13·0
	238	780	798	13	811	−31	653	13·5
	120	767	901	26	927	−160	716	13·0
	0	0	425	13	438	−438	294	12·4
355	665	744	626	26	652	92	501	12·9
	462	756	695	47	742	14	590	12·2
	237	751	775	32	807	−56	670	11·9
	120	765	880	29	909	−144	718	12·0
	0	0	393	11	404	−404	194	11·3

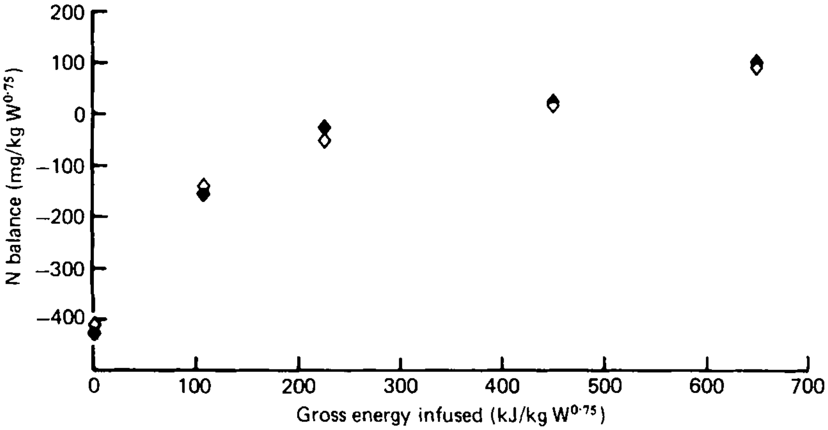

Fig. 1. Expt 1. The effect of the level of infusion of energy on nitrogen balance in dairy cows. The first increment of energy consisted of proteins alone. Cow no. 355 (◇), cow no. 351 (◆). W, body-weight.

Experimental and analytical procedures

Urine and faeces were collected daily, but because the quantities of faeces excreted were small, they were bulked for each period for analysis. Urine was analysed on a daily basis; samples were analysed for total N by the procedure of Davidson *et al.* (1970). Urine was also analysed for urea by the technique of Marsh *et al.* (1965), as described by Technicon Instrument Co. Ltd. (1967), and for creatinine in Expts 1 and 3 by the method described in Hawk *et al.* (1947), as described by Technicon Instrument Co. Ltd. (1965).

RESULTS

Expt 1

The cows remained in good health throughout the experiments. Values for excretion of N, urea and creatinine are given in Table 2, and it can be seen that values from the two cows were similar. Practically all N excretion was via the urine; faecal N, which remained relatively constant, amounted to only 24 mg/kg $W^{0\cdot 75}$ and similar to that reported from

Table 3. *Expt 2. The effect of level of energy on nitrogen nutrition, N excretion and N balance in dairy cows*

(Values for N are expressed as mg/kg body-weight $(W)^{0.75}$)

Cow no.	Gross energy of infusate (kJ/kg $W^{0.75}$)	N infused	Total-N excretion	N balance
351	460	972	838	134
	450	0	319	−319
	225	0	359	−359
	0	0	375	−375
	147	938	892	46
355	460	972	832	140
	450	0	253	−253
	225	0	324	−324
	0	0	387	−387
	147	938	806	132

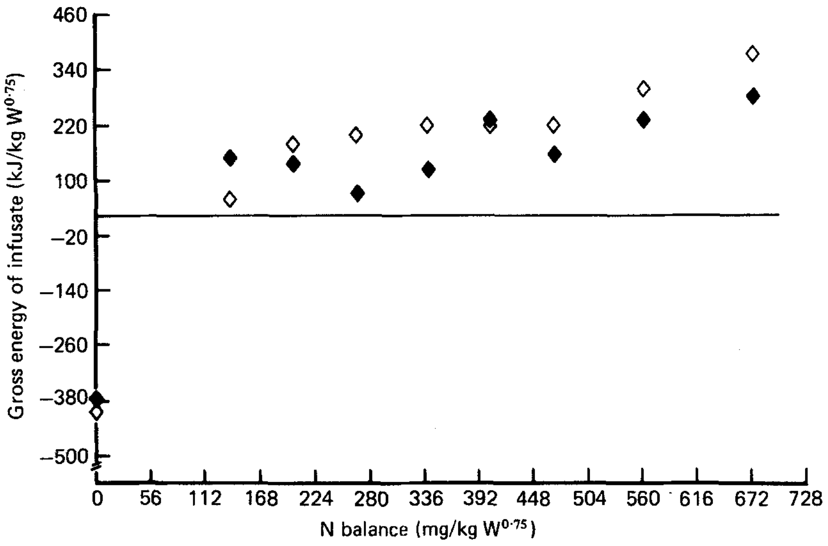

Fig. 2. Expt 2. The effect of protein and energy intake on nitrogen balance in cow no. 351 (◆) and cow no. 355 (◇) when the experiment was preceded by a period of N depletion. The first increment of energy represents casein alone. W, body-weight.

earlier work (Ørskov & MacLeod, 1982). N excretion was lowest during fasting and greatest when only protein was infused. The decrease in energy in the form of VFA resulted in an increase in N excretion. The excretion of urea N increased as urinary N increased, but the amount of urea N relative to total N was lowest during fasting. The effect of the protein and energy infused on creatinine excretion was small. The values for energy intake and N balance are shown in Fig. 1. For the purpose of obtaining an approximate estimate of the energy infused at zero N balance, an exponential equation of the form: $y = \text{A} - \text{B}e^{-cx}$ was used for each animal, where y is N balance, x is energy intake and A, B, c are constants.

Table 4. *Expt 3. The effect of level of infusion of energy and protein and of fasting on nitrogen balance and urea and creatinine excretion in steers*

(Mean values for N, for four observations, are expressed as mg/kg body-weight $(W)^{0\cdot75}$)

Period no.	Energy-yielding nutrients (kJ/kg $W^{0\cdot75}$)	Protein N	Faecal N	Urinary N	N balance	Urea-N excretion	Creatinine-N excretion
1	466	952	35	763	154	515	33·7
2	450	0	21	324	−345	148	33·7
3	0	0	25	617	−641	384	31·5
4	156	993	36	969	−12	683	30·3
5	246	987	37	948	2	699	27·0
6	361	1013	29	937	47	650	26·6
7	474	1009	38	798	173	548	28·1
Approximate SEM			4·6	16·3	19·3	15·7	1·5

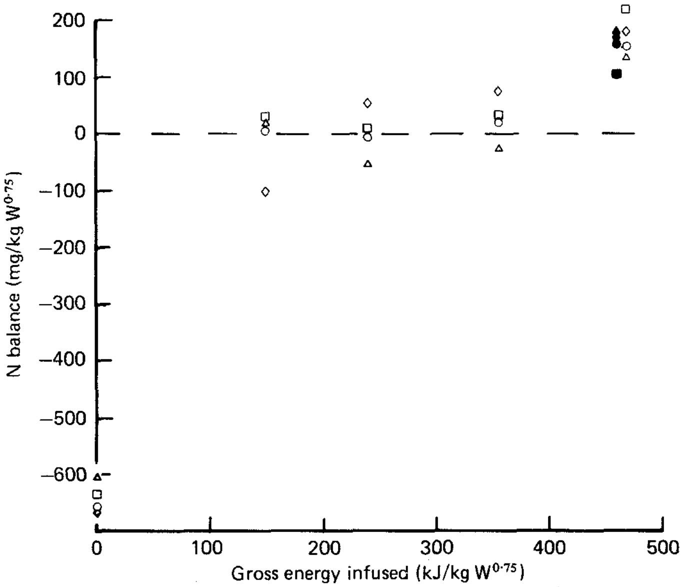

Fig. 3. Expt 3. The effect of the level of energy infused on nitrogen balance in steers. The first increment of energy consisted of protein alone. (△, □, ○, ◇, ▲, ●, ◆, ■), individual steers; ▲, ●, ◆, ■, measurement made before the fasting period began.

This allowed an analysis of the estimates from each animal to ascertain the precision. Using this exponential equation it was calculated that the cows achieved N balance at 255 and 307 kJ/kg $W^{0\cdot75}$. The mean value was 281 kJ/kg $W^{0\cdot75}$.

Expt 2

The results of Expt 2 are given in Table 3 for the first five periods. The values from the fasting period onwards are shown in Fig. 2, similar to that shown in Fig. 1. It can be seen that N balance was positive for both cows when only protein was infused. As for Expt 1 the values were described by the exponential equation $y = \text{A} - \text{B}e^{-cx}$ to derive and estimate x when y was zero. The value of x at N balance was 131 and 98 kJ/kg $\text{W}^{0\cdot75}$ for cows nos. 351 and 355 respectively, and the mean value was 115 (SE 16) kJ/kg $\text{W}^{0\cdot75}$.

N balance was further increased as levels of energy increased and appeared to plateau at approximately 200 mg N/kg $\text{W}^{0\cdot75}$.

Expt 3

All the steers completed their experimental periods in apparently good health. On some occasions urine was spilt; in such cases another day was added to the period. When the experiment was terminated the steers were given 2 l rumen inoculum from other animals and given free access to hay. Normal feed intakes were established in 2–4 d.

The results obtained are summarized in Table 4. The values were calculated by omitting the results from the first day of each period. From previous experience, only 1 d was sufficient to establish a new equilibrium (Ørskov & MacLeod, 1982).

As in previous work with cows, the creatinine excretion remained fairly constant regardless of the level of VFA or of protein infusion. The faecal N excretion amounted to an average of 32 mg N/kg $\text{W}^{0\cdot75}$ and did not vary consistently with VFA or casein infusion.

The relationship between urea N excretion (y) and urinary N excretion (x) could be described by the equation: $y = -124 + 0\cdot836x$ (r 0·99). The proportion of urea N varied from 0·46 on the protein-free diet at energy maintenance to more than 0·70 with the highest urinary-N excretion.

The relationship between energy nutrition and N balance is shown in Fig. 3 omitting the values for N excretion at estimated zero energy balance when no protein was infused. By fitting exponential equations to the results, as for Expts 1 and 2, it could be calculated that when N balance was zero the energy level infused was 154 (SE 38) kJ/kg $\text{W}^{0\cdot75}$, which is almost equal to the energy value of protein infusion alone (156 kJ/kg $\text{W}^{0\cdot75}$; Table 3).

DISCUSSION

Creatinine excretion

The small effect on creatinine excretion is in agreement with earlier work using the procedure of intragastric infusion (Ørskov & MacLeod, 1982), and with many reports in the literature which indicate that creatinine excretion is relatively constant. This was shown many years ago by Palmer *et al.* (1914). Graystone (1968) showed that about 1 g creatinine/20 kg muscle mass was excreted daily, and Ørskov & MacLeod (1982) suggested that the basal requirement for N by intact animals could possibly be predicted from creatinine excretion. If the equation by Ørskov & MacLeod (1982) of $y = 6\cdot86x + 6\cdot38$ (where y is N excretion at zero N intake at energy maintenance (g/d) and x is the creatinine-N excretion (g/d)) is used for the values from steers, a value of 21·5 g N/d or 328 mg/kg $\text{W}^{0\cdot75}$ is calculated while the value found was 22·6 g N/d or 345 mg/kg $\text{W}^{0\cdot75}$ (Table 4).

N excretion during fasting and energy balance

The difference between Expts 1 and 2 in the energy level at which N balance occurred is probably due to the attempts by an N-free period and a period of fasting to cause some protein depletion in the animals. While we suspect that this was the main reason, it cannot be completely distinguished from the body condition of the cows as they weighed 100 kg more at the start of Expt 2 than at the start of Expt 1. The effect of body condition may also in part determine the difference between fasting-N excretion and N excretion at estimated energy maintenance.

The mean total N excretions during fasting were 421 mg/kg $\text{W}^{0\cdot75}$ for Expt 1 and 381 mg/kg $\text{W}^{0\cdot75}$ for Expt 2, and compare with a value of 372 mg/kg $\text{W}^{0\cdot75}$ for adult sheep

(Blaxter, 1962) and with values for fasting cows, goats and sheep of 333, 387 and 404 mg/kg $W^{0.75}$ respectively (Morris & Roy, 1939). They are greater than the excretions occurring at an infusion of 450 kJ/kg $W^{0.75}$ with N-free diets which were 286 mg/kg $W^{0.75}$ in Expt 2 and 329 mg/kg $W^{0.75}$ in the work of Ørskov & MacLeod (1982).

The difference between fasting-N excretion and excretion at estimated energy balance was much greater for the young steers where the value was 641 mg N/kg $W^{0.75}$ at fasting and 345 mg N/kg $W^{0.75}$ at energy maintenance. It is possible that the difference between the cows and the steers with respect to the differences in fasting-N excretion has similarities with the observations published by Jakobsen (1958). He observed that the difference in N excretion between fasted cockerels and cockerels fed at energy equilibrium was small in fat birds but large in lean birds. The cows used here were visually very fat while the steers, apart from being younger, were visually only moderately fat.

Effect of energy on protein balance

The observation that protein balance can be achieved at calculated submaintenance energy levels with steers is in good agreement with our observation on lambs (Hovell *et al.* 1982). In an experiment with lambs similar to the trial reported here, N balance occurred at an energy input of less than 150 kJ/kg $W^{0.75}$. The result here indicates that both in adult and young animals this is possible. In the adult, however, a period of protein depletion appears to alter the energy level at which N balance occurs (Expts 1 and 2). It should be pointed out that the energy balance was estimated here, and it could, in fact, be lower than 450 kJ/kg $W^{0.75}$ because of the reduced energy costs for eating and digestion in infused cattle (Webster, 1980). However, a value of 450 kJ/kg $W^{0.75}$ was obtained in other experiments with lambs maintained on intragastric nutrition (Ørskov *et al.* 1979*a*).

The ability to reach N balance well below energy maintenance has also been shown in growing pigs (30 kg live weight) by Fuller & Croft (1977). When the pigs were given N at 1 g/kg $W^{0.75}$, N balance occurred at a metabolizable energy intake of 388 kJ/kg $W^{0.75}$, while 471 kJ/kg $W^{0.75}$ was required for energy maintenance. Similar results were reported by Close *et al.* (1978). Experiments with humans are more difficult to interpret as most investigators have studied protein needs at adequate, or even above, the energy needs for energy equilibrium (Calloway & Spector, 1954).

Protein: energy value and submaintenance feeding

If we accept that the protein required for maintenance can be met below energy maintenance, for instance at 200 kJ/kg $W^{0.75}$, depending on previous nutrition, body fatness, etc., as referred to earlier, then it also follows that diets providing microbial protein only cannot meet this need. According to the Agricultural Research Council (1980) a microbial N yield of 1·25 g N/MJ could be expected and, using a value of 0·54 for net efficiency of microbial N (Storm & Ørskov, 1982), the net contribution would be $0{\cdot}200 \times 1{\cdot}25 \times 0{\cdot}54 = 0{\cdot}135$, or 135 mg/kg $W^{0.75}$, while the net need is above 300 mg (Ørskov & MacLeod, 1982). It is also clear that the extent of inadequacy depends on the extent of undernutrition. A ruminant is likely to lose protein when in negative energy balance unless an undegraded protein source is supplied.

A very good example of the phenomenon of protein loss during undernutrition may be calculated from the results of Burton *et al.* (1974). They showed that sheep in the process of losing live weight from 70 to 50 kg also lost 1·3 kg protein which, assuming approximately 200 g protein/kg dry matter, must have accounted for a considerable proportion of the live-weight loss. When the same animals were later given a high-protein diet they showed a compensatory increase in protein content so that they contained more protein at the same body-weight during the realimentation phase than when they were continuously grown or when they were in the period of submaintenance. Similar observations were reported by Keenan *et al.* (1969). They showed that weight loss during 11 weeks was restored in 5 weeks but that only 71 % of body energy loss was restored. Evidence of protein loss during a period of undernutrition was also obtained when fish meal was compared with urea for lambs given barley diets after a period of undernutrition. Such lambs, weighing 40 kg and supplemented with fish meal, grew much faster initially than lambs supplemented with urea. Normally-grown lambs of the same breed and weight did not require protein in excess of the microbial protein supply and showed no difference in live-weight gain with urea or fish-meal supplementation (Ørskov & Grubb, 1979). The higher requirement for protein at maintenance than that previously estimated (Ørskov & MacLeod, 1982, present work), may also help to explain the anomalous observations by Andrews & Ørskov (1970) that lambs fattened at a continuous low level of feeding contained more fat and less protein in their bodies than lambs fattened at higher levels of feeding.

Implication for fasting metabolism

It should finally be pointed out that the very efficient use of protein for protein synthesis in fasted animals probably means that fasting metabolism, or for that matter basal metabolism, represents the metabolism during gross protein deficiency. It could be argued, therefore, that the use of fasting metabolism and basal metabolism as a basis for nutrient requirement in animals and humans respectively must be questioned.

Practical implications

The efficient use of protein, both for tissue maintenance and indeed for net synthesis in adult cattle and young steers, as well as in young lambs (Hovell *et al.* 1982), has important practical implications as it suggests that ruminants, and possibly humans too, with stores of fat, can enter a period of severe energy undernutrition with little or no losses in live weight, possibly even gains, provided that protein is given of the kind which in the ruminant escapes the rumen without being degraded. The efficient capture of protein during fasting demonstrates that cattle, like sheep (Hovell *et al.* 1982), distinguish between protein and energy-yielding nutrients and do not readily utilize protein as a source of energy. For instance, in Expt 3 infusion of 993 mg N/kg $W^{0.75}$ increased N balance by 629 mg, i.e. the utilization of the protein by otherwise fasting steers was 0·63.

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