Protein utilization during energy undernutrition in sheep sustained by intragastric infusion

Effect of body fatness on the protein metabolism of energy-restricted sheep

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The effect of body fat content on the protein metabolism of energy-restricted sheep has been studied in two experiments. In the first experiment, six Suffolk-cross wether sheep, three weighing about 39 kg and three of about 61 kg, were given progressively increasing amounts of casein-N from 0 to 3000 mg N/kg metabolic body weight ($W^{0.75}$) daily with constant energy, 91 kJ/kg $W^{0.75}$ daily, from a high-propionic acid mixture of volatile fatty acids (VFA). In the second experiment, two lean and two fat sheep of similar body weights were given progressively increasing amounts of casein with the same VFA mixture. All the animals attained a positive N balance when they were in negative energy balance. N balance was not affected by body fatness of the magnitude studied, although lean animals utilized increasing levels of standard VFA (acetate–propionate–butyrate 65:25:10, molar proportions) infusion for N accretion more efficiently than fat animals. Endogenous energy was utilized for protein accretion with an efficiency of 0·56. Supply of glucogenic VFA equivalent to 28 mmol glucose/kg $W^{0.75}$ reduced fasting N excretion by 39 %. Fasting heat production decreased from 335 to 300 kJ/kg $W^{0.75}$ with the infusion of casein and glucogenic VFA. It is argued that fasting induces additional heat losses due to raised protein metabolism and is unsuited as a baseline for dietary assessment.

Body composition: Energy restriction: Protein utilization: Fasting metabolism

It has been shown, using intragastrically-nourished sheep, that infusion of volatile fatty acids (VFA) to provide additional exogenous energy (250 kJ/kg metabolic body weight ($W^{0.75}$) daily) had little effect on the N balance when protein was infused in progressively increasing amounts (Chowdhury *et al.* 1997). Even when not given any VFA, and in negative energy balance, the sheep were able to retain N. It was concluded that endogenous energy reserves (body fat) were mobilized to provide the energy required for protein retention. However, when protein was supplied in progressively decreasing amounts, the animals not supplemented with VFA showed lower N balance than those supplemented with VFA (250 kJ/kg $W^{0.75}$). It was posulated that the body fat content in the animals not supplemented with VFA might have been responsible for lower N retention. In a second experiment (Chowdhury, 1992), sheep were given 1000 mg casein-N/kg $W^{0.75}$ and VFA

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infusions were either stepped up or stepped down between 250 and 560 kJ VFA energy/kg $W^{0.75}$. The higher level of VFA infusion was found to raise the N balance in both situations, and the effect was more pronounced in the light animals (38–44 kg; presumably leaner) than the heavy (46–50 kg; presumably fatter) animals. Hovell & MacPherson (1977) showed a similar effect in lean and fat sows. In the present experiment we have studied the effect of body fat content on energy and protein metabolism in energy-restricted sheep. In addition, the effect of different levels of exogenous energy from VFA on protein metabolism was also examined.

MATERIALS AND METHODS

Animals and their management

In Expt 1, six Suffolk-cross wether sheep, approximately 15 months of age, were used. Each was fitted with a rumen cannula and an abomasal catheter under general anaesthesia. The animals were paired on the basis of their initial body weight. All animals were given 1 kg/d of a 50 : 50 (w/w) mixture of chopped hay and concentrates. One sheep from each pair was given in addition 1 kg whole barley/d. After 35 d, the mean body weights of the two groups of animals were 61 (SD 2·2) kg and 39 (SD 4·5) kg respectively. Two heavy and two light animals were accommodated in four closed-circuit respiration chambers, while the other two animals (one fat, one lean) were housed in metabolism crates. Thus, N balance was measured on six animals, and energy balance on four. In Expt 2, four Suffolk-cross wether sheep of approximately 11 months of age and mean live weight of 42 (SD 1·11) kg, were used. They were divided into fat and lean pairs on the basis of their fat cover over the spinous and transverse processes of the lumbar vertebrae (Alliston, 1983). The animals were then fitted with a rumen cannula and an abomasal catheter. In both experiments, the initial overnight-fasted live weights were used for calculation of the quantity of nutrients to be supplied. At the end of Expt 2 the animals were slaughtered and their carcasses minced and analysed.

Intragastric infusion

The technique of intragastric infusion, the composition of infusates and the control of infusion were as described previously (Chowdhury *et al.* 1997). In addition to the standard VFA mixture (acetate–propionate–butyrate 65 : 25 : 10, molar proportions) infused previously, a glucogenic VFA mixture (acetate–propionate–butyrate 16 : 79 : 5, molar proportions) was also used.

Experimental protocol

Expt 1 was carried out using two groups of sheep (heavy and light). The animals were changed from their dry feed to total intragastric infusion over a period of 5 d, by stepping up the infusion of energy and protein (a 100 g casein/l solution) until the maintenance level (standard VFA 370 kJ/kg $W^{0.75}$ and protein 500 mg N/kg $W^{0.75}$ daily) was achieved. The animals were kept at this level for 4 d. This was followed by a 4 d fasting period when only the vitamin and mineral (both micro and macro) allowances were infused. Over a succession of 4 d periods the casein allowance was then progressively increased to provide 0, 250, 500, 750, 1500, 2250 and 3000 mg N/kg $W^{0.75}$ daily (phase 1). During this period, they also received 91 kJ/kg $W^{0.75}$ daily of the glucogenic VFA mixture (acetate–

propionate–butyrate 16 : 79 : 5, molar proportions; 13·76 kJ energy/g mixture) in order to supply the theoretical glucose requirement of about 100 g daily (Bergman, 1983). The calculation is based on the assumption that 1 mol propionate produces about 0·5 mol (90 g) glucose (Leng, 1970; Bergman, 1973); thus, for a 60 kg sheep if we provide 91 kJ/kg $W^{0.75}$ per d of the mixture (about 143 g of the mixture contain about 89 g propionate), that will provide 109 g glucose daily. However, the actual glucose requirement of a growing animal is not well defined. Lobley (1991) suggested that actual requirement for glucose is probably better defined by the loss of C moieties from the body either as secreted products (e.g. lactose in milk) or oxidized to CO_2. Lobley (1991) showed that during fasting glucose oxidation rate ranges from 0·23 to 0·33 mmol/kg $W^{0.75}$ per d in sheep. Thus, for a 60 kg sheep the irreversible loss of glucose would be 119–190 mmol (41·4–30·7 g) daily, which is far below the amount supplied in the present trial.

At the end of the incremental protein supply period the animals were given, in consecutive 1 d periods, 113, 165, 215, 269, 321, 373 and 425 kJ/kg $W^{0.75}$ as the standard VFA mixture (phase 2). During this period casein was supplied at a constant rate of 3000 mg N/kg $W^{0.75}$ daily.

In Expt 2, it was intended to repeat the incremental protein infusion adopted in Expt 1. However, because both lean animals started scouring when the amount of casein-N exceeded 1500 mg N/kg $W^{0.75}$, they were not infused with more than this amount. Since there was no such problem with the fat animals, their casein infusion was stepped up to 3000 mg N/kg $W^{0.75}$, as in Expt 1.

Measurement of heat production

Heat production was measured in closed-circuit respiration chambers as described by Chowdhury *et al.* (1997). Measurements were made for four of the six animals in Expt 1 and all four animals in Expt 2. For assessment of the change due to metabolic activity postural effects were removed from the total heat production by excluding the energy cost of standing (25 J/kg live weight per min; Brockway, 1987).

Estimation of initial body fat content

For most of the study the experimental animals were in negative energy balance. The amount of body fat mobilized by the individual animal at different levels of infusion was calculated from the respective energy balances, divided by the energy content of fat (39 kJ per g; Agricultural Research Council, 1980). The initial body fat content of each animal was then estimated by adding the cumulative fat losses during the experimental period and the fat content at slaughter.

Measurements of energy and N balances, and chemical analysis were made as described previously (Chowdhury *et al.* 1997).

Statistical analysis

Data from Expt 1 were analysed by using the Genstat Statistical Programme (Lawes Agricultural Trust, 1990) to compute by ANOVA the mean of each variate with appropriate SED. In the ANOVA appropriate for the split-plot experiment, the treatment sum of squares were partitioned into weight (light or heavy) effects or treatment (levels of infusion) effects and their interaction. In addition, linear regression of the general form

$y = a + bx$ was also used. In Expt 2, since there were only two animals in each of the fat or lean groups, it was not possible to apply any formal statistical analysis.

RESULTS

Animal health

In Expt 1, one of the animals from the heavy group scoured when given the 3000 mg casein-N/kg $W^{0.75}$ infusion and so this animal was given 2250 mg N/kg $W^{0.75}$ instead of 3000 mg N/kg $W^{0.75}$ daily when the level of VFA infusion was being progressively increased. During fasting, an animal from the light group excreted blood-stained urine; a blood sample showing signs of haemolysis was found to contain 4 μg Cu/l (normal range for sheep is 0·5–1 μg/l; Larvor, 1983). Treatment with two doses of ammonium tetrathiomolybdate, 3·4 mg/kg body weight given on alternate days, immediately cured the haemolytic crisis (see Humphries *et al.* 1988). Otherwise, the animals in both experiments remained healthy during the course of infusion.

Expt 1. Nitrogen balance

Nitrogen balance and negative energy balance. The observed mean daily energy balance (of two animals) and N balance (of three animals) of the light and the heavy animals when given progressively increasing amounts of casein-N with 91 kJ/kg $W^{0.75}$ of the glucogenic VFA infusion are shown in Fig. 1. It can be seen that with 500 mg N/kg $W^{0.75}$ infusion or more, all the animals were in positive N balance even though they were in considerable negative energy balance.

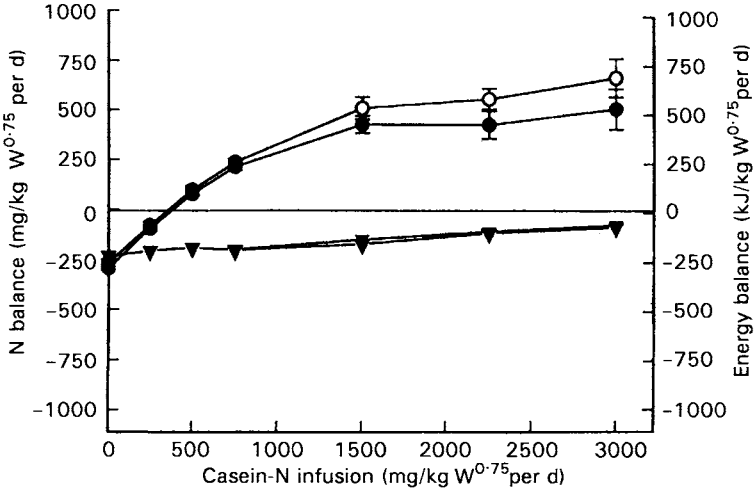

Fig. 1. Expt 1. Mean daily energy (▽, ▼; *n* 2) and nitrogen (○, ●; *n* 3) balances for the light (○, ▽) or heavy (●, ▼) sheep receiving by infusion progressively increasing amounts of casein with 91 kJ/kg metabolic body weight ($W^{0.75}$) daily of a high-propionic acid (glucogenic) mixture of volatile fatty acids (acetate–propionate–butyrate 16 : 79 : 5, molar proportions). Values are means and standard deviations represented by vertical bars for nitrogen balance. For details of animals and procedures, see pp. 274–275.

Effect of body condition on nitrogen balance. The mean estimated initial body fat contents of the heavy and the light animals were 16·0 (SD 3·56) and 6·8 (SD 0·64) kg respectively, with the corresponding values for crude protein (N × 6·25) of 9·1 and 6·1 kg. It can be seen in Fig. 1 that up to 750 mg N/kg $W^{0.75}$ infusion, N balances were almost the same in both groups. When casein infusion was further increased to 1500, 2250 and 3000 mg N/kg $W^{0.75}$, the light animals retained more N (0·182 *v.* 0·156 kg) than the heavy animals but the differences did not reach the statistical significance ($P > 0.05$).

Effect of volatile fatty acid energy supply on nitrogen balance. Fig. 2 shows the regressions of N balances (dependent variable) of the light and the heavy animals *v.* their respective VFA energy supply (independent variable). It can be seen that the increment in the N balance of the light animals was more than double that of the heavy animals in response to increasing amounts of the standard VFA supply at a constant high level (3000 mg/kg $W^{0.75}$) of casein-N infusion.

Expt 1. Effect of glucogenic volatile fatty acid infusion on fasting nitrogen excretion

The mean urinary total N and urea-N excretion of the heavy and light animals during fasting and during the infusion of 91 kJ/kg $W^{0.75}$ daily of glucogenic VFA infusion but no casein are presented in Table 1. Infusion of the glucogenic VFA significantly ($P < 0.05$) reduced the fasting total N and urinary N excretion to a similar extent in both light and heavy animals.

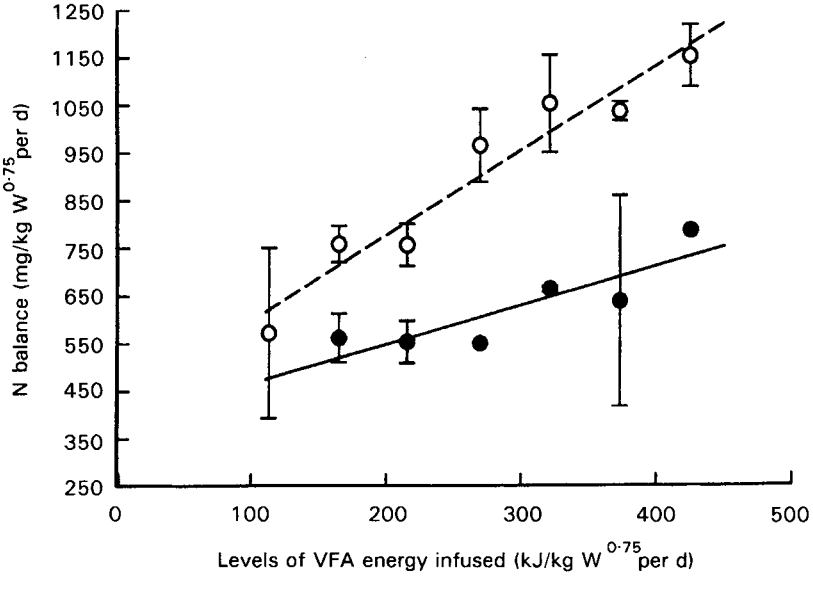

Fig. 2. Expt 1. Mean nitrogen balance of the heavy (●, *n* 2, data set from animal that received 2250 mg N/kg metabolic body weight ($W^{0.75}$) per d was not included) and the light (○, *n* 3) sheep receiving by infusion progressively increasing amounts of a standard volatile fatty acid (VFA) mixture (acetate–propionate–butyrate 65 : 25 : 10, molar proportions) with 3000 mg casein-N/kg $W^{0.75}$ daily. Values are means and ranges (for the heavy sheep) or standard deviations (for the light sheep) represented by vertical bars. The fitted linear regression between the VFA-energy infusion (X) and the N balance (Y) was $Y = 0.823X + 385$ (r^2 0·75, $P < 0.03$) for the heavy sheep and $Y = 1.791X + 419$ (r^2 0·93, $P < 0.003$) for the light sheep. The data point at 113 kJ VFA infusion for the heavy sheep was missing due to loss of urine. For details of animals and procedures, see pp. 274–275.

Table 1. *Expt 1. The effects of glucogenic volatile fatty acid (VFA) infusion (91 kJ/kg $W^{0.75}$ daily)* on mean fasting or basal urinary nitrogen excretion (mg nitrogen/kg $W^{0.75}$ daily) in heavy and light sheep*†

(Mean values for three animals)

	Experimental group	Fasting‡	Basal§	Proportion spared	SED (df 4)	Statistical significance: $P <$
Total N	Heavy	423	264	0·38	21·7	0·01
	Light	408	249	0·39	41·7	0·05
Urea-N	Heavy	270	162	0·40	24·2	0·05
	Light	248	117	0·53	25·4	0·05

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

* Acetate–propionate–butyrate (16 : 79 : 5, molar proportions).

† For details of animals and procedures, see pp. 274–275.

‡ Animals infused with vitamins and minerals only.

§ Animals infused with 91 kJ/kg $W^{0.75}$ daily supplied as glucogenic VFA, plus vitamins and minerals (no casein infused).

Heat production

Heat production by the two groups of animals is shown in Fig. 3. At the various levels of infusion, daily heat production on a per kg $W^{0.75}$ basis was very similar in the heavy and light animals. The heat production tended to decline up to the 500 mg casein-N/kg $W^{0.75}$ infusion and then increased gradually with the higher level of infusion.

Efficiency of endogenous energy utilization

The energy cost per kJ protein (N × 6·25) accretion was estimated from the regression of protein-energy accretion *v.* the respective corrected heat production in the following equation. In addition to the correction for postural effect, heat production was also corrected for the energy cost incurred in urea excretion, assuming 4 mol ATP (1 mol ATP = 77 kJ) per mol urinary urea (Blaxter, 1989).

$$Y = 296 + 0{\cdot}781\ (\text{SE}\ 0{\cdot}2034)X \qquad r^2\ 0{\cdot}45,\ P < 0{\cdot}01,\ n\ 20,$$

where, Y is the corrected heat production (kJ/kg $W^{0.75}$ per d) and X is the protein-energy accretion (kJ/d). For each kJ protein accretion, the experimental animals utilized 0·781 kJ endogenous energy (presumably body fat) with an efficiency $(1/(1+0{\cdot}781))$ of approximately 0·56.

Expt 2. Nitrogen balance

Fat compositions of lean and fat animals were estimated at the start of the experiment as 7·2 and 9·0 kg respectively. The energy and N balances of the individual animals are plotted in Fig. 4. All the animals attained positive N balance despite being in negative energy balance. Up to 1500 mg casein-N/kg $W^{0.75}$ infusion, N balance responses were similar for all the animals. When casein infusion increased to 2250 mg N/kg $W^{0.75}$ daily, both the lean animals started scouring.

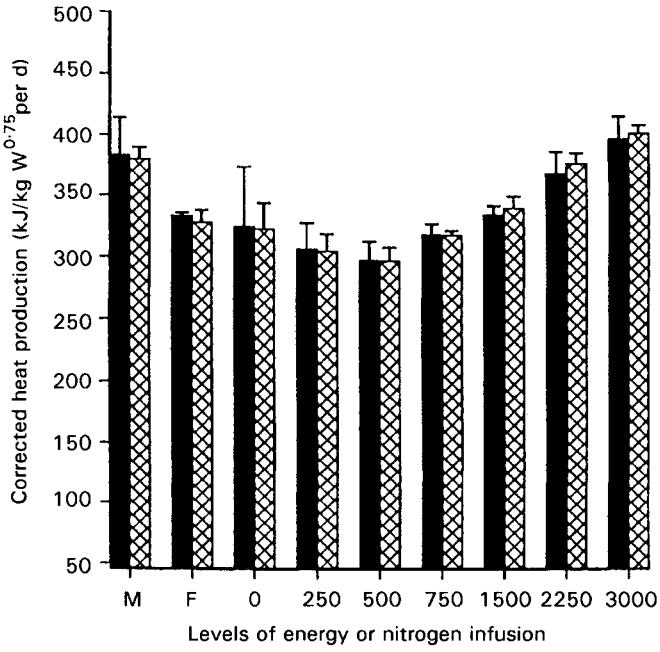

Fig. 3. Heat production of heavy (■) and light (⊠) sheep at different levels of energy and nitrogen infusion. Values are means for two animals with their ranges represented by vertical bars. M, F, maintenance (370 kJ/kg metabolic body weight ($W^{0.75}$) per d as volatile fatty acids (VFA; acetate–propionate–butyrate 65 : 25 : 10, molar proportions) and 500 mg casein-nitrogen/kg $W^{0.75}$ per d with vitamins and minerals) and fasting (no energy or protein infusion except vitamin and minerals) levels of infusion respectively. 0–3000, Levels of daily infusion of casein-nitrogen (mg/kg $W^{0.75}$) with 91 kJ/kg $W^{0.75}$ as a high-propionic acid (glucogenic) mixture of VFA (acetate–propionate–butyrate; 16 : 79 : 5, molar proportions). Heat production was corrected for postural effects. For details of animals and procedures, see pp. 274–275.

DISCUSSION

Effect of body fat content

The anticipated model of N balance of the light and the heavy animals was that animals with a higher initial body fat reserve (heavy animals) will have a higher N balance than those with a lower body fat reserve (light animals). Therefore, during infusion of progressively increasing amounts of casein-N, heavy animals should have higher N balances than the light animals. The experiments showed that the hypothesis of higher N retention in heavy animals was not valid. Despite lower fat reserves the light animals in fact tended to show the highest N balances during infusion of progressively increasing amounts of casein (Fig. 1), contrary to our expectations. It is possible that the light animals had been subjected to restricted feeding before the experimental period and as a result they may have shown compensatory N repletion when infused with casein (see Hovell *et al.* 1983, 1987). It is also possible that the heavy animals will have been closer to their target body composition and weight (about 60–70 kg for Suffolk-cross sheep), which may have resulted in lower N retention than was anticipated; whereas the light animals (about 39 kg) may have a greater potential to grow, and thus to retain N, than was anticipated.

However, the greater response to energy of the light animals (Fig. 2) during infusion of progressively increasing amounts of standard VFA was in accord with expectations.

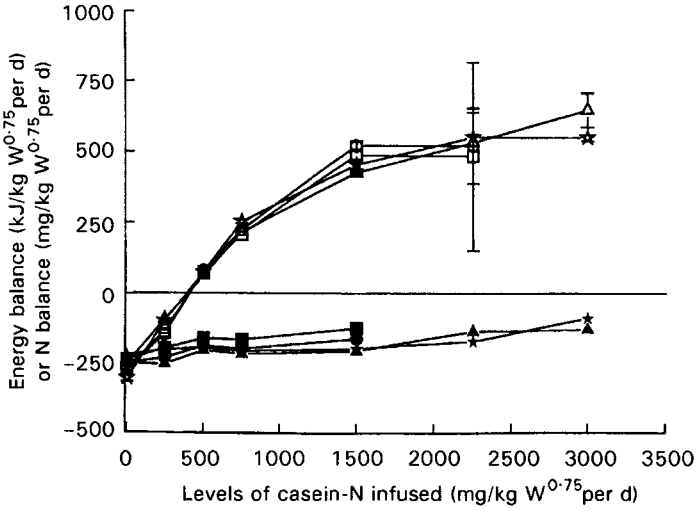

Fig. 4. Expt 2. Energy (●, ▲, ■, ★) and nitrogen (○, △, □, ☆) balances of the individual lean and fat animals receiving by infusion progressively increasing amounts of casein-nitrogen with 91 kJ/kg metabolic body weight ($W^{0.75}$) per d as a high-propionic acid (glucogenic) mixture of volatile fatty acids (acetate–propionate–butyrate 16:79:5, molar proportions). Values are means for observations for 3 d. The two lean animals started scouring when the amounts of casein-nitrogen infusion exceeded 1500 mg N/kg $W^{0.75}$ per d, but there was no such problem with the fat animals. For details of animals and procedures, see pp. 274–275.

Possibly the heavy animals had sufficient body fat reserves (16 kg) to utilize the supplied protein with near maximum efficiency or, as mentioned previously, may be closer to mature protein content. Therefore, the supply of exogenous energy from VFA resulted in significantly less response than in light animals. It is evident that within the normal range of protein supply during exogenous energy undernutrition, even an apparently lean animal can efficiently utilize the supplied protein by oxidizing its body fat reserves. This is probably one of the mechanisms by which depleted animals show rapid protein gain (see Ryan, 1990) during the initial re-alimentation period of compensatory growth. As a result, a higher proportion of protein and a lower proportion of fat appears to be the common feature of the compensatory carcass weight of empty-body-weight gain (Ørskov *et al.* 1976; O'Donovan, 1984; Ryan, 1990; Iason *et al.* 1992).

The reason(s) for scouring by the thin animals in Expt 2 is not clear.

In Expt 1, the supply of 91 kJ/kg $W^{0.75}$ per d as glucogenic VFA reduced the 'fasting' N excretion (no casein infused) by approximately 39 %. Intra-abomasal infusion of glucose to otherwise fasted lambs was also found to reduce the fasting N excretion by 40 % (Asplund *et al.* 1985). The infusion of 91 kJ/kg $W^{0.75}$ as glucogenic VFA was equivalent to about 28 mmol glucose/kg $W^{0.75}$, and may thus have spared protein oxidation by an amount (159 mg N/kg $W^{0.75}$) equivalent to 3 mmol glucose/kg $W^{0.75}$ (assuming 0·55 g glucose per g protein (N × 6·25) oxidized; Krebs, 1964). The requirement for similarly large amounts (over 100 g) of glucose to spare relatively small amounts of protein oxidation (equivalent to 12–15 g glucose) have also been reported in sheep (Ku Vera, 1983; Asplund *et al.* 1985) and steers (Ku Vera *et al.* 1989). Lobley (1991) suggested that the actual requirement for glucose for a fasted sheep could be as low as 5·52–7·92 mmol/kg $W^{0.75}$ daily. Thus, 28 mmol glucose/kg $W^{0.75}$ supplied in the present trials could be well in excess of the animal's actual requirement. It thus appears that during fasting gluconeogenesis from amino acids (3 mmol/kg $W^{0.75}$ daily) represents about 38 %

$((3 \times 100)/7{\cdot}92 = 37{\cdot}9)$ of the glucose requirement. These data, therefore, support the hypothesis that the major part (over 50 %) of the fasting N loss is not due to the specific requirements for the glucose precursors (Asplund *et al.* 1985; Lobley, 1991).

When 91 kJ/kg $W^{0.75}$ as VFA with 0, 250 and 500 mg N/kg $W^{0.75}$ were infused daily there was no increase in heat production, although under normal feeding conditions about 40 % of the VFA energy would be dissipated as heat. This is no doubt due to the compensatory effect of the decrease in N excretion. Similar decreases in fasting heat production due to casein-N infusion from 0 up to 500 mg/kg $W^{0.75}$ daily was also observed in our previous work (Chowdhury *et al.* 1997). It has been suggested (Ku Vera, 1988; Chowdhury, 1992) that the decreased energy cost of proteolysis, lipolysis and ketogenesis are probably responsible for the reduced heat production. The energy cost of protein oxidation can contribute from 22 % (sheep; Chowdhury, 1992) to 26 % (cattle; Ku Vera, 1988) of the extra heat production during fasting. This observation provides further evidence that fasting is an particular metabolic adaptation which increases heat production and, therefore, it should not be used as the basis for measuring the utilization of a nutritionally-balanced diet (Ørskov & Ryle, 1990).

The main objective of the present study was to investigate the effects of body fatness on the protein metabolism of energy-restricted sheep. Results from the two experiments indicate that during exogenous energy restriction, with infusion of up to 1500 mg casein-N/kg $W^{0.75}$ (approximately 3 × maintenance requirement of protein). N balance is not affected by body condition (leaness or fatness) of the magnitude studied here, at least in animals of our age, weight and nutritional history. There was no clear indication of there being a common threshold of body fatness necessary before body fat can be utilized to support protein retention during exogenous energy undernutrition, although obviously there must be a cut-off point at very low levels of body fat. This may imply that even an apparently-lean animal can gain protein at the cost of body fat provided it has an adequate supply of protein and has the potential to grow.

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