INTRAVENOUS GLUCOSE INFUSION AFFECTS NITROGEN RETENTION IN SHEEP

PRAPTI MAHYUDDIN

Research Institute for Animal Production
P.O. Box 221, Bogor 16002, Indonesia

(Recieved by the editor 28 January 1997)

ABSTRAK

MAHYUDDIN, PRAPTI. 1997. Infusi glukosa secara intravenus berpengaruh terhadap retensi nitrogen pada domba. *Jurnal Ilmu Ternak dan Veteriner* 2 (4): 225-232.

Untuk mengetahui pengaruh glukosa terhadap retensi nitrogen (N), 2 tahap percobaan telah dilakukan. Tahap pertama (Exp 1) dilakukan untuk menetapkan waktu yang diperlukan untuk menstabilkan pemasukan glukosa, tahap kedua (Exp 2) dirancang untuk mempelajari pengaruh tingkat infusi glukosa terhadap retensi N. Pada Exp 1, digunakan 4 ekor domba jantan masing-masing dilengkapi dengan kateter pada kiri dan kanan vena jugular, dan diberi pakan *lucern* untuk memenuhi energi hidup pokok. Domba tersebut diinfus dengan garam faal (Hari 0) selama 2 hari kemudian dengan cairan glukosa pada kecepatan 21.8 mmol/jam selama 6 hari dan sekali lagi dengan garam faal pada hari ke-7. Laju pemasukan glukosa (LPG) diukur dengan menginfus D-[6-^{3}H] glukosa pada hari ke-2 infusi garam faal (Hari 0) dan pada periode yang sama setiap hari selama 7 hari berikutnya. Infusi glukosa menurunkan LPG endogenous sebanyak 30% pada Hari 1 dan 2, 13% pada Hari 3 tetapi pada Hari 4 kembali normal. Konsentrasi insulin mencapai puncaknya pada hari pertama infusi glukosa dan kemudian menurun. Konsentrasi urea menurun sejalan dengan hari infusi glukosa. Dapat disimpulkan bahwa untuk domba yang diberi pakan untuk kebutuhan hidup pokok dan diinfus glukosa, diperlukan 4 hari untuk metabolisme glukosa mencapai keseimbangan. Pada Exp 2, dipergunakan 4 ekor domba jantan seperti pada Exp 1. Domba tersebut diinfus terus-menerus selama 5 hari dengan glukosa pada kecepatan 0 (garam faal), 10, 20 dan 30 mmol/jam dalam rancangan Latin Square (4 laju infusi x 4 periode). Kenaikan endogenous LPG yang tinggi ditemukan pada tingkat infusi yang tertinggi. Laju pemasukan glukosa adalah 28,8 , 48,3 , 54,7 dan 86,1 mmol/jam masing-masing untuk laju infusi glukosa 0, 10, 20 dan 30 mmol/jam. Konsentrasi glukosa dan urea tidak berubah dengan infusi glukosa. Retensi N naik dengan naiknya tingkat infusi glukosa ($P< 0,05$) yang disebabkan oleh turunnya kadar N dalam urin. Percobaan ini menunjukkan pentingnya glukosa dalam pembentukan protein.

Kata kunci: Infusi glukosa, laju pemasukan glukosa, retensi N

ABSTRACT

MAHYUDDIN, PRAPTI. 1997. Intravenous glucose infusion affects nitrogen retention in sheep. *Jurnal Ilmu Ternak dan Veteriner* 2 (4): 225-232.

To investigate the effect of intravenous glucose infusion on nitrogen (N) retention, two experiments were conducted in 2 phases. The first phase (Exp 1) was to establish the time required for a stable glucose entry and the second phase (Exp 2) was designed to study the effect of different levels of glucose infusion on N retention. In Exp 1, four wethers were used, each prepared with catheters in the left and right jugular veins, continuously fed lucerne chaff at calculated maintenance energy level. The animals were infused continuously with saline for 2 days and thereafter with glucose solution at the rate of 21.8 mmole/h for 6 days and again with saline on the seventh day. Glucose entry rate (GER) were measured using constant infusion of D-[6-^{3}H] glucose, on the second day of saline infusion (Day 0) and at the same period each day for the next seven days. Infusion of glucose reduced endogenous glucose entry by 30% on Day 1 and 2, by 13% on Day 3, but by Day 4 onwards endogenous glucose entry had returned to normal levels. Plasma insulin, reached its peak value on the first day of glucose infusion and subsequently declined. Plasma urea concentration declined with ensuing days of glucose infusion. It was concluded that for sheep fed maintenance energy level infused with glucose, it takes approximately 4 days for glucose metabolism to reach equilibrium. In Exp 2, four wethers were used, each prepared with catheters and fed lucerne pellets at calculated maintenance energy level. The sheep were infused continuously for five days with glucose at a rate of either 0 (saline), 10, 20 and 30 mmole/h in a Latin Square design (4 infusion rate x 4 periods). A large increase in endogenous glucose entry was found with the highest level of infusion. Glucose entry rates were 28.8, 48.3, 54.7 and 86.1 mmole/h for glucose infusion of 0, 10, 20 and 30 mmole/h respectively. No significant changes in both plasma glucose and urea concentration with infusion rate of glucose. N retention increased with increasing level of glucose infused ($P<0.05$) and was mainly due to the reduction of urinary N. This experiment suggests the important of glucose in protein deposition.

Keywords: Glucose infusion, glucose entry rate, N retention

INTRODUCTION

Glucose has been known to spare amino acids from being catabolised and to facilitate the redirection of the acids towards protein synthesis. When glucose was infused with or without by-pass protein into duodenum of sheep, glucose did not only increase growth rate but it also improved the feed conversion ratio (LENG *et al.*, 1978). This suggests that increased glucose availability may spare the deamination of amino acid and thus increase the efficiency of protein accretion by the animal. Results of other studies which consistent with this have been reported by ESKELAND *et al.* (1973; 1974). These workers compared glucose and volatile fatty acids, and found that glucose followed by propionate were the most effective in improving nitrogen (N) retention.

The introduction of exogenous glucose into the blood of a ruminant animal could result in a suppressed rate of gluconeogenesis. JUDSON *et al.* (1968) observed that when ruminant animals were fed a high grain diet in which there was a high rate of glucose absorption from the gut, gluconeogenesis from propionate was suppressed. Inhibition of gluconeogenesis in sheep was also observed when glucose was infused intravenously into the animal (ANNISON and WHITE, 1961; ANNISON *et al.*, 1963; BARTLEY and BLACK, 1966; WEST and PASSEY, 1967; JUDSON and LENG, 1973; CHAIYABUTR *et al.*, 1983). The period of infusion in these studies ranged from 2-6 h. It was suggested (TELENI *et al.*, 1989) that the entry rate of endogenous glucose might be suppressed more effectively in shorter term glucose infusions such as mentioned above than in longer term infusions *e.g.* 17 h (TELENI *et al.*, 1989) or 4 d (LEENANURUKSA and MCDOWELL, 1982). During short term intravenous glucose infusion (2-6 h), it is possible that the initial surge in plasma insulin concentration inhibits gluconeogenesis and that with longer term infusions, the decline in plasma insulin concentration, the initial inhibitory effect of insulin is overriden by the stimulatory effect of glucagon on gluconeogenesis (see TELENI *et al.*, 1989).

In growing animal, protein is preferentially deposited therefore it is logical to investigate the balance of nutrients that would optimise the process. In this regard, the balance between protein-yielding (amino acids) and energy-yielding nutrients (particularly glucose) would be the more important consideration.

The present study was designed first : to establish the length of time of continuous intravenous glucose infusion during which glucose entry rate and presumably hormone and substrate relationship would be established, and second: to investigate the effect of different levels of glucose infusion on endogenous glucose entry rate and on N retention. These studies were preliminary to subsequent studies designed to investigate the effect of intravenously infused glucose on the metabolism of N in sheep.

MATERIALS AND METHODS

Experiment 1. The establishment of time required for a stable glucose entry

Animals and management

Four wethers (mean liveweight 24 kg) were fed lucerne (*Medicago sativa*) chaff in metabolism crates at calculated maintenance energy level (MAFF, 1975). The feed was dispensed continuously into feed troughs, over each 24 h period, by automatic feeders. The animals had been accustomed to the diet 3 weeks before the experiment commenced. Each animal was prepared with chronic indwelling catheters in the left and right external jugular veins 2 days before the infusion. Polyethylene catheters (1.0 mm ID x 1.5 mm OD) (Dural Plastic and Engineering, NSW) filled with sterile heparinised saline (250 IU/ml) was surgically inserted into the vein (under local anaesthesia).

Glucose infusion

The glucose infusate (187.5 mg/ml) was prepared by dissolving D-glucose in sterile distilled water, and filtering the solution through a bacteriological filter (EKWIP D9, Sizw 14) into sterile 5 L bottles. Glucose solutions were stored at 4°C untill used.

Infusion

The animals were continuously infused, intravenously, with sterile physiological saline for two days and thereafter with the glucose solution for six days followed by sterile saline on the seventh day. The glucose solution was infused into each sheep through a Gilson peristaltic pump (Gilson, Minipuls 2, France) at the rate of 0.32 ml/min.

For the estimation of glucose entry rate (GER), D-[6-^{3}H] glucose was infused at a rate of 4.44 lBq/min through the same jugular catheter used for infusing the cold glucose. The isotope was continuously infused for 9 h. Blood samples were collected at half-hourly intervals for 3 h via unused venous catheter, from the 6th h of isotope infusion. Glucose entry rates were estimated on the second day of saline infusion (Day 0) and each day for the next seven days.

Analysis

Plasma was deproteinised using Somogyi's reagent (SOMOGYI, 1945). The resulting supernatant was analysed for glucose concentration and its radioactivity and urea concentrations. The determination of glucose SRA was carried out using glucose pentaacetate derivative method of JONES (1965).

Plasma insulin concentration was analysed using radioimmunoassay technique described by ROSSELIN *et al* (1966). Ovine insulin antiserum and purified antiserum were used as a standard. The ^{125}I-labelled insulin was iodinated, according to the Chlorine-T method of HUNTER and GREENWOOD (1962) and purified by gel filtration (JORGENSEN and LARSEN 1972). All samples were analysed in one assay to avoid the effect of between-assay variation. The within-assay precision was determined by assaying triplicate sets of standards several times within the one assay. This assay was considered valid if the within-assay coefficients of variation did not exeed 15%. The sensitivity of the assay was 2.4 mU/L. The sample was analysed for radioactivity using a Packard Auto-Gamma Scintillation Spectrometer.

Experiment 2. The effect of different levels of glucose load on GER and N retention

Animals and management

Four wethers, with a mean liveweight of 40.1 ± 2.32 kg were fed lucerne pellets, continuously from automated feeders, at calculated maintenance energy level (MAFF, 1975). Each animal was prepared with catheters in the left and right jugular veins (as described in Experiment 1).

Glucose infusion

The glucose solutions used were 0 (saline), 93.7, 187.5 and 281.2 mg/ml, and prepared similar to Experiment 1. The animals were intravenously infused with glucose solution at nominal rates of 0, 10, 20 and 30 mmole/h in a Latin Square design (4 sheep x 4 glucose levels). Solutions were infused, using the Gilson peristaltic pump, at the rate of 0.32 ml/min for 6 days in 4 periods. Each period consisted of 5 resting days to avoid possible carry-over effects of previous infusion treatments.

Glucose entry rate was estimated using D-[6-^{3}H] glucose (as described in Experiment 1), on Day 5 of glucose infusion.

Sampling

Feed offered and feed residues, faecal and urinary output were measured at 10.00 hours each day. Urine was collected from each sheep into plastic bottle containing 10 ml of 9 M sulphuric acid. Subsamples (10% of daily output) from each sheep were stored in an airtight bottle at -20°C. Faeces and feed residues were also subsampled daily and dried at 60°C to a constant weight for dry matter (DM) determination. The dried samples were ground to pass through a 1 mm screen and stored in airtight 100 ml jars at room temperature untill analysed. A subsample of bulk feed samples was treated similarly.

Analysis

Plasma concentration of glucose and its radio-activity, plasma urea and plasma insulin concentration were analysed as described in Experiment 1.

For organic matter determination, DM was determined by drying the sample to a constant weight at 105°C in a drying oven and ashing the dried sample at 600°C, in a muffle furnace for at least 8 h. Organic matter was calculated by difference.

Nitrogen was determined by digesting the sample (approximately 0.5 g faeces, 0.5 ml urine) using the Kjeldahl digestion method. The digested sample was diluted with an appropriate amount of distilled water and analysed according to the method of CLARE and STEVENSON (1964) using the Technicon Autoanalyser.

RESULTS AND DISCUSSION

Experiment 1. The establishment of time required for a stable glucose entry

During glucose infusion the plasma glucose concentration remained stable during the first three days, lower on Day 4 but increased again on Day 5 and 6 (see Figure 1). The measured rate of glucose infusion was 21.8 mmole/h. The mean GER at Day 0 was 22.9 mmole/h. Glucose entry rate was relatively low on Day 1 and 2 of glucose infusion, but increased by Day 3 and remained stable after Day 4 (see Figure 1).

The endogenous GER, calculated by difference between total GER during glucose infusion and GER at Day 0 (22.9 mmole/h), was reduced by 30% on Day 1 and 2, by 13% on Day 3 but from Day 4 onward the endogenous GER had returned to normal (see Figure 1). WEST and PASSEY (1967) and JUDSON and LENG (1973) reported reductions ranging from 30-89% resulting from short periods (2-6 h) of glucose infusion at rates similar to that used in the current study. ANNISON and WHITE (1961) who infused glucose at rate of 22-44 mmole/h for 3 h into sheep, were able to partially or to totally inhibit endogenous glucose entry. It would appear that effective inhibition of endogenous glucose production would occur with short term glucose infusion. It is probable that the surge in insulin secretion resulting in a dramatic increase in plasma insulin concentration in Day 1 of glucose infusion (see Figure 1) plays a central role in the inhibition of endogenous glucose production. The subsequent sharp decline of plasma insulin concentration from Day 1 onwards probably facilitated the stimulatory effect of glucagon on gluconeogenesis (BASSETT, 1978) thus resulting in the observed return to pre-infusion value of endogenous GER from Day 4 of continuous glucose infusion. The result of this study is in agreement with that of LEENANURUKSA and MCDOWELL (1982) who infused glucose at approximately 13.5 mmole/h.

Since a reduction in endogenous GER was observed for three days after the start of glucose infusion and a return to pre-infusion entry rate from the 4th day of infusion, it may be suggested that in sheep fed at a maintenance energy level and infused with glucose, it would take approximately 4 days for glucose metabolism to reach equilibrium.

There was a reduction (approximately 37%) in plasma urea concentration during glucose infusion (see Figure 2). Glucose has been known to have a "N sparing effect" (see MACRAE *et al.*, 1985). The reduction of plasma urea concentration as a result of intravenous glucose infusion is consistent with this. If a

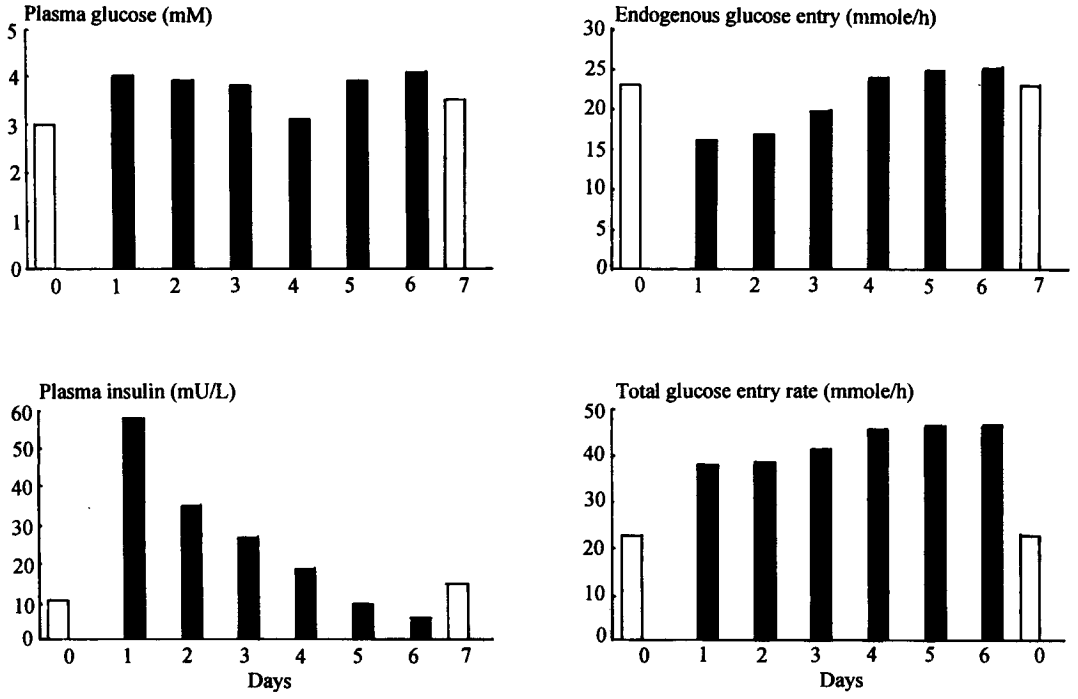

Figure 1. The mean values of plasma glucose and insulin concentrations and endogenous and total glucose entry rates in sheep during intravenous infusion of saline (□) or glucose (■)

major fraction of plasma urea were derived from amino acid catabolism in the body then a reduction in urea concentration may indicate an increased utilisation of amino acids in protein synthesis. When comparing glucose and volatile fatty acids, ESKELAND *et al.* (1973, 1974) found that glucose followed by propionate were the most effective energy-yielding substrates in improving protein deposition.

Experiment 2. The effect of different levels of glucose load on glucose entry rate and N retention

Glucose entry rate

The actual amount of glucose infused were 0 (saline) , 10.5, 21.1 and 31.9 mmole/h. Although there was a tendency to increase plasma glucose concentration by infusing glucose intravenously, the differences between treatments were not significant. Plasma insulin concentration also did not show any significant variation on the 5th day of glucose infusion (see Table 2). As a result of glucose infusion, GER increased as the level of infusion increased. The relationship between GER and glucose infusion is shown in Fig 3. Endogenous GER, estimated by substracting glucose infusion rate from total GER, showed a large increase (88%) with the highest glucose infusion rate, but values for the other infusion rates were not significantly different from each other.

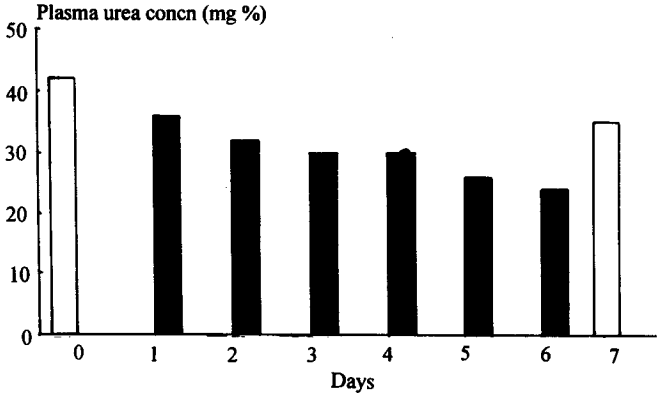

Figure 2. The mean plasma urea concentration in sheep during intravenous infusion of saline (□) and glucose (■)

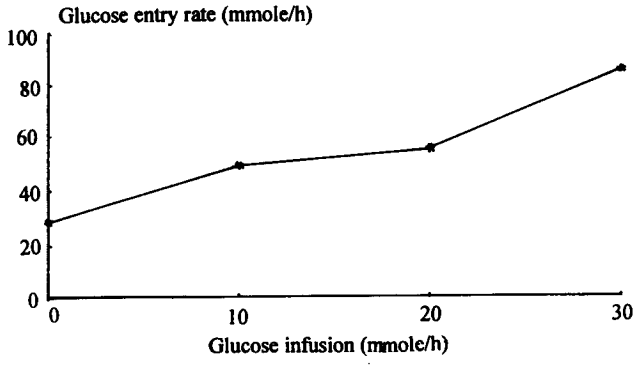

Figure 3. The relationship between glucose infusion rate and total glucose entry rate in sheep fed lucerne at maintenance energy level

Glucose entry rate increased with increasing rate of glucose infusion (Fig 3).

Intravenous glucose infusion into sheep did not suppress endogenous GER. This is reflected in increase GER observed with increasing levels of glucose infusion. The results of this experiment are in agreement with those in the Experiment 1 and LEENANURUKSA and MCDOWELL (1982). However, there was a large increase in estimated endogenous glucose entry in animals at the highest level of glucose infusion. Increased endogenous production of glucose by intravenous glucose infusion was observed by TELENI *et al.* (1989). In this study, it was observed that plasma insulin concentration increased curvilinearly as glucose infusion rate increased. It is possible that the "rebound" effect of glucagon as the insulin concentration is stabilised (as observed in this study) would be relatively greater at higher glucose infusion rate which stimulates correspondingly higher insulin secretion which presumably results in greater degree of inhibition on gluconeogenesis.

N retention

The data for intake and digestibility of the feed for different infusion treatments are shown in Table 1. Since the differences in the liveweights of the experimental animals were small and they were fed to calculated maintenance energy level, the DM and organic matter intakes were similar across treatment groups. The small differences in DM and crude protein digestibility, but not organic matter digestibility, between groups were statistically significant. Although the differences between treatment groups in DM digestibility were significant, the small differences (0.2-1.2% digestibility) may not be biologically significant, particularly when the digestibility of organic matter was not different between treatments (see Table 1).

Table 1. The intake of dry matter, organic matter, nitrogen and estimated metabolisable energy (ME), the digestibility of dry matter, organic matter and crude protein in sheep infused with different levels of glucose solution

	Glucose infused (mmole/h)					
	0	10.5	21.1	31.9	SE	P
Intake (g/d):						
Dry matter	801	792	804	797		
Organic matter	737	729	740	734		
ME * (MJ/d)	7.67	7.52	7.59	7.41		
Digestibilty (%):						
Dry matter	64.6	64.4	65.1	63.9	0.45	
Organic matter	63.9	63.1	63.4	62.0	0.52	NS
Crude protein	78.1	77.4	79.9	79.3	0.48	<0.05

Mean values (within rows) with different superscripts differ significantly (P<0.05)

* ME (MJ/d) = 0.15 x organic matter digestibilty (% DM) x DM intake (kg) (MAFF, 1975)

Data on N transaction are summarised in Fig 4. Nitrogen retention increased (P<0.05) as the rate of infused glucose increased (see Fig 4). Glucose infusion did not affect faecal N but reduced urinary N (P <0.05) with increasing rates of glucose infused (see Fig 4).

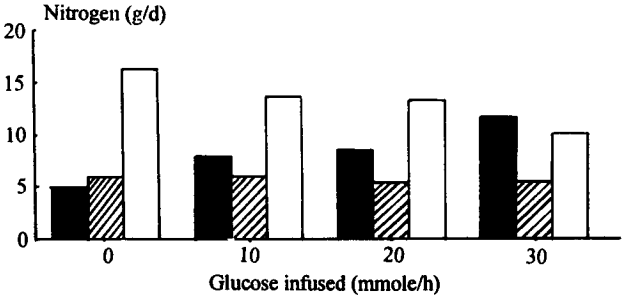

Figure 4. Nitrogen retention (■), faecal nitrogen (▨) and urinary nitrogen (□) output in sheep fed lucerne at calculated maintenance energy level and intravenous infused with different levels of glucose

The increased N retention observed with increasing rates of glucose infusion was mainly due to a reduction in urinary N excretion rate. Similar results were found by ESKELAND *et al.* (1974) with concentrated diet and MATRAS and PRESTON (1989). Evidence from MATRAS and PRESTON (1989) showed that a maximum reduction of urinary N could be achieved by

infusing glucose at a rate of 36.8 mmole/h. They concluded that this is the rate where possible maximum N retention could be achieved without disturbing insulin and blood glucose concentrations. Animals without glucose infusion had a mean N retention of 4.9 g/d presumably because the animals were fed diet with higher energy value (9.6 MJ ME/kg DM; see Table 1) than had been estimated (8.6 MJ/kg DM).

ESKELAND *et al.* (1973, 1974) who infused glucose approximately 30.7 mmole/h intravenously into lambs on concentrate diet, at a rate of 2.09 MJ/d found that at this level of glucose infusion, approximately 4.5-5.0 g/d of N was retained. With reference to Fig 4, the regression equation for the relationship between the rate of N retention and the rate of glucose infusion: $Y = 4.84 + 0.18 X$ would predict that for a glucose infusion rate of 30.7 mmole/h, the N retention would be 10.4 g/d. This value is 1.8 times higher than the value reported by ESKELAND *et al.* (1974).

The relationship established in this study, between N retention and intravenous glucose infusion rate, would suggest that the ratio of protein-yielding: energy yielding nutrients in the body of sheep fed a lucerne diet would not be optimal for protein synthesis. It is likely therefore that some amino acids would be utilised as direct energy sources or as glucose precursors in sheep fed such a diet. The mean dietary N:DOM (digestible organic matter) ratio of 0.05, which is higher than the optimal value of 0.04 suggested by HOGAN and WESTON (1970) for microbial synthesis, would tend to support this suggestion although the amount of protein reaching the small intestine for absorption as amino acids is uncertain.

Table 2. Glucose entry rate and plasma glucose and insulin concentrations in sheep fed pelleted lucerne at calculated maintenance energy level and intravenously infused with different levels of glucose

	Glucose infusion (mmole/h)					
	0	10.5	21.1	31.9	SEM	P
Plasma glucose (mM)	4.9	4.1	4.8	5.1	0.46	NS
Plasma insulin (mU/L)	47.1	45.9	54.4	38.8	8.9	NS
Total glucose entry rate (mmole/h)	28.8	48.3	54.7	86.1	3.5	<0.05
Endogenous glucose entry rate (mmole/h)*	28.8	37.7	33.6	54.2	3.5	<0.05
Plasma urea (mg/100 ml)	43.7	43.7	42.7	40.6	3.22	NS
N retention (g/d)	4.9	7.8	8.4	11.6	0.29	<0.05

* Estimated by substracting glucose infusion rate from total glucose entry rate

Table 3. The effect of different sources and amount of energy supplement on plasma urea concentration in sheep fed different diets

Basal diet	Source	Amount (g/d)	Plasma urea (mg/ml) C	S	References
Grass	Flaked barley	300	47	27	NORTON *et al.* (1982)
Grass	Sucrose	150	39.4	27.8	KENNEDY *et al.* (1981)
Grass	Sucrose	300	39.4	16.3	KENNEDY *et al.* (1981)
Concentrate	Glucose intravenous infusion	133	26.9	28.3	ESKELAND *et al*. (1974)
Roughage	Glucose intravenous infusion	133	24.2	18.9	ESKELAND *et al*. (1974)
Maintain on VFA infusion	Glucose intragastric infusion	118	72.3	42.8	ASPLUND *et al*. (1985)
Lucerne	Glucose intragastric infusion	97	52	49	JUDSON & LENG (1973)
Lucerne	Glucose intragastric infusion	143	44	41	JUDSON & LENG (1973)
Lucerne	Glucose intragastric infusion	191	30	28	JUDSON & LENG (1973)
Wheat	Glucose intragastric infusion	24	39	39	JUDSON & LENG (1973)
Wheat	Glucose intragastric infusion	147	34	29	JUDSON & LENG (1973)
Wheat	Glucose intragastric infusion	197	51	48	JUDSON & LENG (1973)
Pellet	Glucose intragastric infusion	149	63	49	TELENI *et al.* (1989)
Concentrate	Glucose intragastric infusion	79.2	20.8	23.4	MATRAS & PRESTON (1989)
		118.8	20.8	18.4	MATRAS & PRESTON (1989)
		158.4	20.8	20.9	MATRAS & PRESTON (1989)
		237.6	20.8	17.5	MATRAS & PRESTON (1989)

C = control animal S = supplemented animal

The importance of glucose in N retention in sheep at least, has been strongly evident in this study. The strenght of the relationship, obviously will depend on the degree of imbalance between available amino acids and energy-yielding nutrients (particularly glucose) in the body. Results of other work which are consistent with this statement might be gleaned from Table 3 where plasma urea concentration appears to be inversely related to glucose availability. On roughage (ESKELAND *et al.*, 1974; Table 3) and wheat (JUDSON and LENG 1973; Table 3) diets on which sheep may have relatively lower and higher GER respectively, increasing availability of glucose in the body by intravenous or intragastric infusion resulted in a reduction in plasma urea concentration to a higher and lower degree, respectively. Although there was no statistically significant effect on the rate of glucose infusion on plasma urea concentration in sheep in this study, there was an apparent trend of decreasing urea concentrations with increasing rate of glucose infusion.

CONCLUSIONS

Long term glucose infusion in sheep fed maintenance level results in suppressed endogenous glucose production which last for 3 days. On the 4th day, it return to pre-infusion value, therefore it may be suggested that 4 days is the length of time required for a stable glucose entry.

Insulin play an important role in inhibition of endogenous glucose production as reflected by the sharp increase of plasma insulin when the inhibition was highest, and subsequently declined when endogenous glucose production start to return to normal value.

Infusion of glucose with different levels results in increase glucose entry rate and N retention as the level of glucose increased. The increase in N retention is mainly due to the reduction of urinary N which an indication of increased utilisation of amino acids for protein deposition.

REFERENCES

ANNISON, E.F. and R.R. WHITE. 1961. Glucose utilisation in sheep. *Biochem. J.* 80: 162-169.

ANNISON, E.F., D.B. LINDSAY, and R.R. WHITE. 1963. Metabolic interrelations of glucose and lactose in sheep. *Biochem. J.* 88: 243-251.

ASPLUND, J.M., E.R. ORSKOV, F.D.DEB. HOVELL, and N.A. MACLEOD. 1985. The effect of intragastric infusion of glucose, lipids or acetate on fasting N excretion and blood metabolites in sheep. *Br. J. Nutr.* 54: 189-195.

BARTLEY, J.C. and A.L. BLACK. 1966. Effect of exogenous glucose on glucose metabolism in dairy cows. *J. Nutr.* 89: 317-328.

BASSETT, J.M. 1978. Endocrine factors in the control of nutrient utilisation in ruminants. *Proc. Nutr. Soc.* 37: 273 - 280.

CHAIYABUTR, N., A. FAULKNER, and M. PEAKS. 1983. Effects of exogenous glucose on glucose metabolism in the lactating goat *in vivo*. *Br. J. Nutr.* 49 (1): 159-165.

CLARE, N. J. and A. E. STEVENSON. 1964. Measurement of feed intake by grazing cattle and sheep. x. Determination of nitrogen in faeces and feed using an autoanalyser. *NZ. J. Agric. Res.* 7: 198-204.

ESKELAND, B., W.H. PFANDER, and R.L. PRESTON. 1973. Utilisation of volatile fatty acids and glucose for protein deposition in lambs. *Br. J. Nutr.* 29: 34-355

ESKELAND, B., W.H. PFANDER, and R.L. PRESTON. 1974. Intravenous energy infusion in lambs: effects on nitrogen retention, plasma free amino acids and plasma urea nitrogen. *Br. J. Nutr.* 31: 201-211.

HUNTER, W.M. and F.C. GREENWOOD. 1962. Preparation of Iodine-131 labelled growth hormone of high specific activity. *Nature* 194: 495-496.

JONES, G.B. 1965. Determination of specific activity of labelled blood glucose by liquid scintillation using glucose pentaacetate. *Anal. Biochem.* 12: 249-258.

JORGENSEN, K.H. and U.D. LARSEN. 1972. Purification of I ^{125}-glucagon by ion exchange chromatography. *Horm. Metab. Res.* 4: 223-224.

JUDSON, G.J., E. ANDERSON, J.R. LUICK, and R.A. LENG. 1968. The contribution of propionate to glucose synthesis in sheep given diets of different grain content. *Br. J. Nutr.* 22: 69-75.

JUDSON, G.J. and R.A. LENG. 1973. Studies on the control of gluconeogenesis in sheep: effect of glucose infusion. *Br. J. Nutr.* 29: 175-195.

KENNEDY, P.M., R.T.J. CLARKE, and L.D. MILLIGAN. 1981. Influences of dietary sucrose and urea on transfer of endogenous urea to the rumen of sheep and numbers of epithelial bacteria. *Br. J. Nutr.* 46 : 533-541.

LEENANURUKSA, D. and G.H. MCDOWELL. 1982. The importance of glucose supply at the initiation of artificial lactation in the ewe. *Aust.J.Biol.Sci.* 35: 629-636.

LENG, R.A., S. ECONOMIDES, and F.M. BALL. 1978. The effect on growth of supplying glucose in to duodenum of lambs on low-protein diets. *Proc. Aust. Soc. Anim. Prod.* 12: 134.

MACRAE, J.C., J.S. SMITH, P.J.S. DEWEY, D.S. BREWER, and A. WALKER. 1985. The efficiency of utilisation of metabolisable energy and apparent absorption of amino acids in sheep given spring-and autumn-harvested dried grass. *Br.J. Nutr.* 54: 197-209.

MAFF. 1975. Energy allowances and feeding systems for ruminants. Ministry of Agric.Fisheries and Food (HMSO): London.

MATRAS, J. and R.L. PRESTON. 1989. The role of glucose infusion on the metabolism of nitrogen in ruminants. *J. Anim. Sci.* 67: 1642-1647.

NORTON, B.W., J.B. MACKINTOSH, and D.G. AMSTRONG. 1982. Urea synthesis and degradation in sheep given pelleted grass-diet containing flaked barley. *Br. J. Nutr.* 48: 249-263.

ROSSELIN, G., R. ASSAN, R.S. YALLOW, and S.A. BENSAN. 1966. Separation of antibody-bound and unbound peptide hormones labelled with iodine-131 by talcum powder and precipated sulica. *Nature (London)* 212: 355-357.

SOMOGYI, M. 1945. Determination of blood sugar. *J. Biol. Chem.* 160: 69-73.

TELENI, E., W.R. KING, J.B. ROWE, and G.H. MCDOWELL. 1989. Lupins and energy-yielding nutrient in ewes. I. Glucose and acetate biokinetics and metabolic hormones in sheep fed a supplement of lupin grain. *Aust. J. Agric. Res.* 40: 913-924.

WEST, C.E. and R.F. PASSEY. 1967. Effect of glucose load and insulin on the metabolism of glucose and palmitate in sheep. *Biochem. J.* 102: 58-64.