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Effect of abomasal glucose infusion on alanine metabolism and urea production in sheep

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The effect of abomasal infusion of glucose (120 kJ/d per kg body weight $(BW)^{0·75}$, 758 mmol/d) on urea production, plasma alanine-N flux rate and the conversion of alanine-N to urea was studied in sheep offered a low-N diet at limited energy intake (500 kJ/d per kg $BW^{0·75}$), based on hay and grass pellets. Glucose provision reduced urinary N ($P = 0·040$) and urea ($P = 0·009$) elimination but this was offset by poorer N digestibility. Urea-N production was significantly reduced (822 *v*. 619 mmol/d, $P = 0·024$) by glucose while plasma alanine-N flux rate was elevated (295 *v*. 342 mmol/d, $P = 0·011$). The quantity of urea-N derived from alanine tended to be decreased by glucose (127 *v*. 95 mmol/d) but the fraction of urea production from alanine was unaltered (15 %). Plasma urea and alanine concentrations (plus those of the branched chain amino acids) decreased in response to exogenous glucose, an effect probably related to enhanced anabolic usage of amino acids and lowered urea production.

Glucose: Alanine: Ureagenesis: Gluconeogenesis: Sheep

In ruminants fed roughage, most of the dietary starch is fermented in the rumen and, in consequence, little or no glucose is available for absorption from the small intestine. When grain-based concentrate diets, which contain a greater fraction of less-fermentable starches are offered, glucose absorption from the small intestine is enhanced, as is the supply to the liver (Seal & Reynolds, 1993). In both dietary situations, however, insufficient glucose is available from the diet to meet the needs of the animal. This shortfall is met by gluconeogenic sources, notably propionate, lactate and amino acids.

When exogenous glucose supply to ruminants is increased by infusion into the abomasum or the blood, then urinary N excretion is reduced and whole-body N retention increases (Eskeland *et al*. 1974; Matras & Preston, 1989; Obitsu *et al*. 1993). This N-sparing effect of glucose may arise though several mechanisms. For example, hepatic gluconeogenesis may be inhibited if sufficient exogenous glucose is supplied (Judson & Leng, 1973). Alternatively, the hyperglycaemia-induced hyperinsulinaemia may stimulate the uptake of amino acids into peripheral tissues (Brockman *et al*. 1975; Ahmed *et al*. 1983). Furthermore, glucose may inhibit the activity of the urea cycle (Jahoor & Wolfe, 1987). Although any, or all, of these mechanisms may account for the improvement of N use with increased glucose supply, there are limited quantitative data on the fate of amino acid N in response to altered glucose supply. Of the amino acids extracted by the liver, alanine is the major gluconeogenic source (Wolff & Bergman, 1972*a*; Reynolds & Tyrell, 1991). Use of alanine-C to synthesise glucose releases the amino-N, which may be used to aminate oxo-acids or enter the ornithine cycle. These alternative fates have consequences for the availability of N for anabolic purposes. Furthermore, any reduction in requirement of amino acid-C for gluconeogenesis may be directed at essential amino acids, such as threonine (MacRae & Egan, 1983), leaving alanine metabolism relatively unaffected. Alternatively, a general reduction in catabolism of all gluconeogenic amino acids may occur. Although the transfer of alanine-C across the liver in fed sheep has been studied with radioactive (Wolff & Bergman, 1972*a*) and stable (Lobley *et al*. 1996) isotopes, the fate of the amino-N has not been elucidated clearly in ruminants.

In the current study, the fate of the ^{15}N-amino group of alanine to the urea production has been studied in response to abomasal infusion of glucose in sheep.

Materials and methods

Animals

Six Suffolk-cross wether sheep (average body weight (BW) 47 (SE 3·4) kg, 9–12 months of age) prepared with

Abbreviation: BW, body weight.

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an indwelling aortal catheter (Lobley *et al.* 1995) plus a polyvinyl chloride catheter in the abomasum were used. For infusion of labelled metabolites temporary polyvinyl chloride catheters (0·8 mm i.d.; 1–2 mm o.d.; Critchley Electrical Products Pty. Ltd., Auburn, New South Wales, Australia) were inserted into each external jugular vein. One of these was inserted into the right ventricle of the heart to provide mixed systemic blood as a substitute if the arterial catheter failed.

Experimental design

Animals were maintained in metabolism crates and offered a mixed diet containing ryegrass pellets (estimated metabolizable energy 10·5 MJ/kg DM; 22·0 g N/kg; 893 g DM/kg) and chopped hay (estimated metabolizable energy 9·2 MJ/kg DM; 9·7 g N/kg; 945 g DM/kg) in a ratio of 1 : 1, supplied as 24 × 1 h portions by means of automated feeders. The daily amount of diet supplied 500 kJ metabolizable energy/d per kg $BW^{0·75}$ equivalent to approximately 1·25 × maintenance energy requirement. Estimated metabolizable protein supply (620 mg N/d per kg $BW^{0·75}$) was 15 % lower than the energy provided, based on the need to support live-weight gain (Agricultural and Food Research Council, 1993). The experiment was conducted as a crossover design in which three sheep were started with a water infusion (control) followed immediately by glucose administration. The other three sheep were allotted to these treatments in reverse order. In each treatment, sheep were continuously infused with water or a glucose solution (120 kJ/d per kg $BW^{0·75}$; 137 (SE 8·0) g glucose/d) into the abomasum for 9 d. The amount of glucose infused was adjusted to increase energy supply by 0·3 × maintenance energy per d. From days 6 to 9, the animals were harnessed to allow total faeces (by bag) and urine (by suction from the rubber cup connected to the harness and set under the abdomen) to be collected for measurement of N balance and urinary urea excretion. From day 7, a [^{14}C]urea solution (11 kBq/g dissolved in sterile 0·15 M-NaCl with 1 mM-urea as carrier) was infused into the jugular vein for 52 h at a rate of 44 kBq/h. During the final 4 h of the [^{14}C]urea infusion, urine was collected hourly to allow estimation of urea production rate. On day 9, [^{15}N]alanine (99 atom %; 40 mM in sterile 0·15 M-NaCl; Isotec Inc., Miamisburg, OH, USA) was infused into the jugular vein for 9 h at the rate of 0·4 mmol/h. From 3 h after starting the [^{15}N]alanine infusion, arterial blood from the aorta (or mixed blood from the heart) was collected hourly. For 2 d before [^{15}N]alanine infusion, unlabelled alanine (40 mM in sterile 0·15 M-NaCl) was infused at the same rate to counteract any change of alanine flux as a consequence of the [^{15}N]alanine infusion. Immediately before each isotope infusion, the spot urine and blood samples were collected to determine the natural abundance of ^{15}N (background).

Sample preparation and chemical analyses

A portion of total faeces was subsampled for each animal and freeze-dried. Urine for N balance was acidified with H_2SO_4 and the sub-samples were frozen (−20°C) until later analysis. Plasma was separated from the hourly blood samples and frozen at −20°C until later analysis.

DM contents of feed and faeces were measured after drying at 105°C for 4 h. The N contents of the feed and faeces were determined following the Dumas method, using an automated procedure (Foss Haraeus Macro Nitrogen Analyser, York, UK). Urine N was quantified by a micro-Kjeldahl procedure. The urea concentration in urine and plasma were measured on a Technicon Auto Analyser (Technicon Instruments Corporation, Tarrytown, NY, USA) by the method of Marsh *et al.* (1965). Plasma glucose concentration was determined by a glucose oxidase procedure on a Kone Dynamic Selective Analyser (Kone Instruments, Espoo, Finland). Free amino acid concentrations in plasma was measured by a LKB Alpha Plus Amino Acid Analyser (LKB Pharmacia, Uppsala, Sweden) using appropriate buffer combinations for physiological fluids. Specific radioactivity of urinary urea was determined as described previously (Lobley *et al.* 1996).

For the determination of plasma alanine and urea enrichment, stored plasma was deproteinized with 0·15 volumes sulfosalicylic acid (480 g/l) and centrifuged at 13 000 ***g*** for 5 min. The supernatant fraction was desalted by application to 2 ml cation exchange resin (AG-50, 100–200 mesh, × 8, H^+ form; Biorad, Richmond, CA, USA). After washing with 5 ml water, urea was eluted with 30 ml water (this recovers approximately 90 % of the urea), followed by application of 3 ml 4 M-NH_4OH to collect the alanine. These two eluates were concentrated under reduced pressure and by freeze-drying respectively. The freeze-dried alanine eluate was prepared as the tert-butyldimethylsilyl derivative (Calder & Smith, 1988), the enrichment of which was determined by GC–mass spectrometry using a Trio 1 (VG Mass Lab., Manchester, UK).

Plasma urea enrichment was determined by GC–combustion–isotope ratio mass spectrometry (GC–C–IRMS). In order to improve the GC separation, urea was first converted to 2-hydroxypyrimidine (Wolthers *et al.* 1994). Samples were dried in v-vials to which 100 μl 4 M-HCl and 50 μl malondialdehyde bis-dimethylacetal (diluted 1 : 20 (v/v) with water) were added and the reaction allowed to proceed at room temperature overnight. The reagent was then removed in a stream of N_2 at room temperature before addition of 100 μl *N*-tert-butyldimethylsilyl-*N*-methyltrifluoracetamide–acetonitrile (1 : 1, v/v). The vials were heated in a dri-block at 100°C for 30 min, leading to the formation of the tert-butyldimethylsilyl derivative of 2-hydroxypyrimidine. Of this 1 μl (equivalent to approximately 30 nmol N) was injected, in splitless mode, onto an HP 5890 gas chromatograph (Hewlett Packard, Avondale, PA, USA) connected to an ORCHID module (Europa Scientific, Crewe, Ches., UK). During passage though the ORCHID the sample is oxidised in a quartz tube containing platinised Cu wire, to produce CO_2, N_2, nitrogen oxides and water. The nitrogen oxides were reduced to N_2 in a second quartz tube containing Cu metal. H_2O and CO_2 were removed by a Nafion membrane (Perma Pure Inc., Toms River, NJ, USA) and a liquid N_2 trap respectively. The resulting pure N_2 gas was admitted to a SIRA Series II (VG Isotech, Middlewich, Ches., UK) mass spectrometer and ratio of m/z 29/28 measured against that in reference O_2-free N_2 gas.

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Calculation and statistics

Urea-N production rate (UNPR, mmol/d) was calculated from:

$$UNPR = I_u/SA_u \times 2 \times 1{\cdot}44,$$

where I_u is the rate of [^{14}C]urea infusion (Bq/min); SA_u is the specific radioactivity of urinary urea (Bq/μmol urea); 2 is the number of N in one urea molecule; and 1·44 is conversion factor of time scale. Urea-N entering the gastrointestinal tract was calculated as the difference between daily UNPR and the amount of urea-N eliminated in the urine. The appearance rate of plasma alanine (plasma alanine-N flux rate, Ra_{ala}, mmol/d) was calculated from:

$$Ra_{ala} = ((99/E_{ala}) - 1) \times I_{ala} \times 1{\cdot}44,$$

where E_{ala} is the plasma alanine enrichment (atom % excess) at pseudo-plateau; I_{ala} is the rate of infusion (μmol/min) of [^{15}N]alanine; and 99 is the atom % of infused [^{15}N]alanine.

Because [^{15}N]urea enrichment did not reach isotopic steady state during the 9 h of the [^{15}N]alanine infusion, urea enrichment data were fitted by a non-linear regression according to a one-pool model (Model Maker, Cherwell Scientific Publishing Ltd, Oxford, Oxon., UK):

$$E\text{-urea}_t = A(1 - e^{-kt}),$$

where $E\text{-urea}_t$ is atom % excess ^{15}N for plasma urea-N at time t; k is the rate constant; and A is the ^{15}N enrichment of urea-N at isotopic steady state.

The rate of transfer of alanine-N to urea N was calculated as follows:

$$\text{urea-N derived from alanine-N (\%)} = A/E_{ala} \times 100;$$

and the rate of urea-N production from alanine-N (mmol/d) = UNPR × urea-N derived from alanine-N (%). Because [^{15}N]alanine was infused for less than two half-lives of the body urea pool, there will be an uncertainty for the value obtained for the rate constant of urea-N-labelling (k). This uncertainty will extend to the estimation for the plateau enrichment and subsequent calculations.

The results were analysed as a crossover design using the GLM procedure of SAS (SAS/STAT User's Guide (1995) version 6, 4th ed.; Statistical Analysis Systems Institute Inc., Cary, NC, USA) with animals as blocks and treatment and period as factors. If period effects were found to be not significant then the data were reanalysed with this factor excluded.

Results

Glucose infusion did not affect DM intake, except for one sheep that showed poor appetite during the period of [^{15}N]alanine infusion. Glucose infusion resulted in a slight increase in faecal DM output with a corresponding decrease in apparent DM digestibility ($P < 0{\cdot}11$; Table 1). As expected, plasma glucose concentration increased ($P < 0{\cdot}01$) with the glucose infusion (Table 1).

The glucose infusion did not affect N intake, but slightly increased ($P < 0{\cdot}15$) faecal N and reduced ($P < 0{\cdot}10$) digested N (Table 2). In contrast, urinary N excretion was reduced ($P < 0{\cdot}05$) by the glucose infusion. In consequence, N retention was unaltered.

Glucose infusion reduced both urea-N production rate ($P < 0{\cdot}03$) and plasma urea concentration ($P < 0{\cdot}05$). Although there was also a decrease in the absolute amount of urea-N entering the gastrointestinal tract ($P = 0{\cdot}1$), the proportion of urea-N that entered the gastrointestinal tract was unaltered.

The plasma concentrations of aspartate, alanine, isoleucine and leucine decreased ($P < 0{\cdot}05$) as a result of glucose infusion, with downward trends ($P < 0{\cdot}1$) also for valine and lysine (Table 3). In contrast, the concentrations of glycine increased ($P < 0{\cdot}05$), while other amino acids were unaltered by treatment.

Plasma [^{15}N]alanine enrichments attained isotopic steady state for both treatments (Fig. 1(a)) and tended to be lower ($P < 0{\cdot}15$) for sheep infused with glucose (Table 4). Although Ra_{ala} increased ($P < 0{\cdot}05$) with the glucose infusion, there was an associated period effect ($P < 0{\cdot}05$). The Ra_{ala} in sheep allotted to the control treatment first increased markedly with the glucose infusion (from 316 to 393 mmol/d). Sheep infused with glucose first exhibited only a slight reduction (from 289 to 272 mmol/d) during the control period.

Although plasma urea-N enrichment did not reach the isotopic steady state by the end of the blood sampling (Fig. 1(b)), there were no differences in the enrichments between the treatments at any time. Consequently, estimated urea-N enrichment at isotopic steady state, calculated by fitting the data to a one-compartment model, was only slightly lower during the glucose infusion. Glucose infusion tended to reduce the rate of alanine-N transfer to urea-N from 126 to 95 mmol/d. Excluding the data of one sheep that had a poor appetite, the trend was shown more clearly ($P < 0{\cdot}15$). Although the proportion of urea-N derived from alanine-N

Table 1. Dry matter intake, faecal output and digestibility and plasma glucose concentration in sheep fed a ration of grass pellets plus hay, with or without abomasal infusion of glucose*

(Mean values for six sheep)

			ANOVA†	
Item	Control	Glucose-infused	SEM	*P*
Dry matter (g/d)				
Intake (g/d)	885	880	8·9	NS
Faecal output (g/d)	355	381	11·0	NS
Digestibility (%)	59·7	56·3	1·01	0·107
Plasma glucose concentration (mM)	3·74	4·38	0·095	0·003

* For details of procedures see p. 158.
† One-way ANOVA with sheep treated as blocks, 5 residual degrees of freedom.

Table 2. Nitrogen balance (g/d) and urea-N production (g N/d) in sheep fed a ration of grass pellets plus hay, with or without abomasal infusion of glucose*

(Mean values for six sheep)

N transfer (g N/d)	Control	Glucose-infused	ANOVA†	
			SEM	*P*
Intake	14·2	14·1	0·15	NS
Faecal output	6·3	7·1	0·30	NS
Digested	7·9	7·0	0·30	0·080
Urinary excretion				
Total N	5·4	4·4	0·28	0·040
Urea N	3·0	2·0	0·18	0·009
Retained	2·4	2·6	0·30	NS
Urea-N production	11·5	8·8	0·57	0·024
Urea-N recycled into the gut	8·5	6·7	0·59	0·101
% of urea production	73·6	77·4	1·90	NS
Plasma urea (mM)	2·20	1·66	0·152	0·041

* For details of procedures see p. 158.
† One-way ANOVA with sheep treated as blocks, 5 residual degrees of freedom.

(15 %) was unaltered, there was a smaller proportion of plasma alanine-N flux (28 *v*. 42 %; $P < 0{\cdot}06$) transferred to urea-N when glucose was infused. This was partly a consequence of the higher plasma alanine-N flux during glucose infusion.

Discussion

The diet was formulated to minimise glucose absorption and, thus, glucose requirements for the sheep would need to be met by gluconeogenesis. Furthermore, the predicted fermentation and digestion would provide energy in excess relative to N supply (Agricultural and Food Research Council, 1993). Therefore, any response to glucose provision should not be a consequence of an increase in energy availability but rather reflect direct aspects of glucose metabolism *per se*.

The amount of glucose infused which was absorbed from the small intestine was not quantified in the current study, but in cattle, 85 % of glucose infused into the abomasum disappeared from the small intestine, with 73 % of this recovered as net portal glucose flux (Kreikemeier & Harmon, 1995). The increase in plasma glucose concentration observed with infusion in the present investigation probably reflects a similar increase in glucose absorption.

Table 3. Arterial plasma free-amino-acid concentrations (μM) in sheep fed a ration of grass pellets plus hay, with or without abomasal infusion of glucose*

(Mean values for six sheep)

Amino acids (μM)	Control	Glucose-infused	ANOVA†	
			SEM	*P*
Aspartate	9·0	7·5	0·33	0·024
Threonine	100·2	80·1	9·96	NS
Serine	75·0	82·5	3·23	NS
Asparagine	71·5	69·4	3·21	NS
Glutamate	75·8	72·5	5·34	NS
Glutamine	295·6	272·2	12·47	NS
Glycine	489·1	571·3	23·97	0·043
Alanine	202·9	156·2	10·58	0·028
Citrulline	139·2	142·4	5·57	NS
Valine	229·3	185·9	10·98	0·078
Methionine	20·3	17·4	1·45	NS
Isoleucine	91·2	66·8	5·19	0·034
Leucine	119·8	94·2	5·25	0·031
Tyrosine	69·8	67·6	3·60	NS
Phenylalanine	54·5	53·4	1·68	NS
Ornithine	72·8	56·6	2·96	0·070
Lysine	109·7	75·7	8·40	0·052
Histidine	60·5	57·7	2·96	NS
Tryptophan	45·2	46·8	1·82	NS
Arginine	135·2	104·6	6·10	NS
Total	2643	2430	57·81	NS

* For details of procedures see p. 158.
† One-way ANOVA with sheep treated as blocks, 5 residual degrees of freedom.

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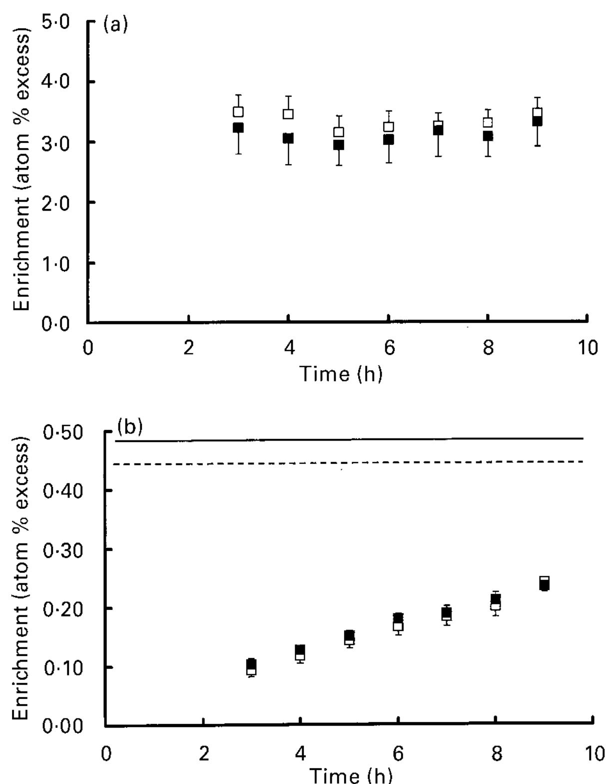

Fig. 1. Plasma enrichment of (a) [^{15}N]alanine and (b) [^{15}N]urea during infusion of [^{15}N]alanine into the jugular vein of sheep given (□), water (control) or (■), glucose into the abomasum. Values are means for six lambs with standard errors indicated by vertical bars. (b): (——), plateau enrichment for the control, (- - - -), glucose infusion.

Alanine metabolism

Due to transamination reactions involving the amino-N, plasma alanine-N fluxes obtained in the present study may not equal the total flux of the C-skeleton. Comparisons of the fluxes obtained by the use of different labelled atoms would give some indication of the transamination of the amino-N. Plasma alanine-N fluxes obtained in the present study (control treatment, 205 μmol/min; 11·8 μmol/min per kg BW$^{0·75}$) were greater than for the plasma alanine-C flux observed in both adult (192 μmol/min, 9·6 μmol/min per kg BW$^{0·75}$; Wolff & Bergman, 1972*b*) and young sheep (142 μmol/min; 9·7 μmol/min per kg BW$^{0·75}$; Lobley *et al.* 1996*a*). These data suggest transamination exceeds C-skeleton flux by approximately 20–25 %. These calculations contrast with observations in post-absorptive human subjects, where alanine-N flux was lower than for alanine-C (Yang *et al.* 1984). The authors explained this apparent anomaly as conservation of the labelled amino-N through compartmentalized pyruvate–alanine interchange.

The increment of plasma alanine-N flux (47 mmol/d) by glucose infusion was equivalent to 6 % of infused glucose (760 mmol/d) in the present study, although this value would be only 3 % if supply of three C units was considered. This increase in plasma alanine-N flux by glucose infusion supports similar observations in human subjects (Robert *et al.* 1982; Wolfe *et al.* 1986). Reasons offered for these observations include: (1) an increase in alanine absorption; (2) stimulation of protein degradation in tissues; (3) enhanced alanine synthesis *de novo*. Short-term infusion of glucose into either the duodenum or blood of sheep fed dried grass led to increased net portal absorption of certain non-essential amino acids (Piccioli Cappelli *et al.* 1997), notably citrulline, glycine and ornithine, but not alanine. In other studies with sheep fed a diet of cereal and straw, net portal absorption of amino acids decreased as a result of glucose infusion into the jugular vein (Balcells *et al.* 1995). In human subjects, intravenous glucose infusion increased alanine-N flux though stimulation of alanine synthesis *de novo*, with protein breakdown unaltered (Robert *et al.* 1982). Thus, the increase in the alanine-N flux with glucose infusion in the present study was probably due to an increase in alanine synthesis.

Plasma alanine concentration was reduced with abomasal glucose infusion, in spite of the increase in the whole-body alanine flux. Similar reductions were noted for the branched-chain amino acids. These may be caused by insulin-mediated mechanisms because intraduodenal or intravenous infusion of glucose stimulates insulin secretion

Table 4. [^{15}N]Alanine flux and urea kinetics in sheep fed a ration of grass pellets plus hay, with or without abomasal infusion of glucose (758 mmol/d)*

(Mean value for six sheep)

Item	Control	Glucose-infused	ANOVA†	
			SEM	*P*
Alanine kinetics				
Plateau enrichment (ape)	3·3	3·0	0·11	NS
Plasma alanine-N flux (mmol/d)	295	342	7·3	0·033‡
Urea-N kinetics				
Production rate (mmol/d)	820	619	40·9	0·024
Predicted plateau enrichment (A; ape)	0·485	0·444	0·0218	NS
Rate constant of urea-N labelling (k; %/h)	7·13	8·92	0·0218	NS
Urea-N production derived from alanine-N (mmol/d)	126	95	14·2	NS
% of total urea-N production	15·1	15·4	1·41	NS
% of plasma alanine-N flux	42·2	27·6	3·14	0·059

ape, atom percent excess.
* For details of procedures see pp. 158–159.
† One-way ANOVA with sheep treated as blocks, 5 residual degrees of freedom.
‡ Period effect was observed ($P = 0·046$).

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in a dose-dependent manner in ruminants (Balcells *et al.* 1995; Piccioli Cappelli *et al.* 1997). Elevation of plasma insulin concentration by intravenous infusion of the hormone lowered plasma amino acid concentrations but increased amino acid uptake to peripheral tissues (Brockman *et al.* 1975; Ahmed *et al.* 1983). This stimulation of the peripheral uptake of amino acids may reduce the hepatic inflow of amino acids for urea synthesis.

Urea synthesis

In the present study, the increase in glucose supply (136 g/d, 760 mmol/d) though the abomasal infusion reduced urea-N production by 200 mmol/d. Because short-term intraduodenal infusion of glucose did not decrease NH_3 absorption from the portal-drained viscera (Piccioli Cappelli *et al.* 1997) and hepatic extraction of NH_3 has a higher priority than amino acid removal (Lobley & Milano, 1997), the reduced ureagenesis probably reflects lower amino acid catabolism.

An increase in exogenous glucose supply reduced the apparent endogenous glucose production from gluconeogenic precursors, other than ruminal propionate (Judson & Leng, 1972). Thus, gluconeogenesis from alanine is probably also reduced with glucose infusion. Consequently, it would be expected that N transfer from alanine to urea would be reduced correspondingly.

To test this hypothesis, the N transfer from alanine to urea was predicted from the ^{15}N enrichment of plasma alanine and urea after the intrajugular infusion of [^{15}N]alanine. Based on the results of Judson & Leng (1973), the increase in glucose supply through the abomasal infusion in this study (136 g/d) should reduce endogenous glucose production by 36 g/d (200 mmol/d). Assuming that 5·4 % of glucose C was derived from alanine (Wolff & Bergman, 1972*a*), then this reduction in gluconeogenesis could spare alanine by 10 mmol/d. A similar reduction in alanine amino-N transfer to urea would be expected. In the present study, however, the glucose infusion reduced alanine-N transfer to urea by a much greater extent (30 mmol/d).

The proportional contribution of alanine-N to urea-N (15 %) did not appear to be affected by the glucose infusion in the present study, although this contribution was higher than that based on calculations of net hepatic movements of alanine and urea-N in sheep fed a similar level of roughage (6·9 %, Lobley *et al.* 1995). NH_3 may contribute 59–72 % of urea-N in sheep fed similar rations (Lobley *et al.* 1995, 1996*b*, 1998) and thus a maximum of 28–41 % would arise from amino acid-N. Under these circumstances alanine would make a disproportionately large contribution to ureagenesis (36–54 %) compared to other amino acids. This would suggest that a substantial portion of the alanine extracted by the liver is destined for 'catabolic' fates, oxidation, gluconeogenesis and ureagenesis, rather than 'anabolic' end products, synthesis of constitutive and export proteins. An alternative explanation for the large contribution of alanine-N to urea-N is that entry of alanine-N into the ornithine cycle (via aspartate) is more indirect and involves transamination reactions with other amino acids. In this case, the transfer of ^{15}N in alanine to urea may represent gross rather than net N movements.

If it is assumed that exogenous glucose provision did not alter hepatic extraction of NH_3 (Lobley & Milano, 1997) then the decrease in non-NH_3-N inflow into the ornithine cycle was quite substantial (probably > 48 %). If this were due solely to a reduced need for precursor C for glucose synthesis then disproportionate reductions in metabolism of those amino acids known to be gluconeogenic in sheep, i.e. alanine, glutamate, glutamine, glycine, serine (Wolff & Bergman, 1972*a*) and threonine (MacRae & Egan, 1983) might be expected. This did not occur for alanine in that the fractional contribution to urea-N synthesis remained unaltered. One possible reason is that 'sparing' occurred for the other amino acids, but the linkage between alanine and glucose is maintained, as observed in non-ruminants where the two metabolites are involved in C and N transfers between the liver and peripheral tissues (Perriello *et al.* 1995). Alternatively, the decrease in urea-N entering the gastrointestinal tract and the slight increase in faecal-N output with glucose infusion may reduce the absorption and hepatic removal of NH_3 as well as amino acids. However, short-term glucose supply did not affect net portal absorption of NH_3 (Piccioli Cappelli *et al.* 1997). Thus, the reduction in urea-N recycling may be another reason for the unaltered contribution of alanine-N to urea-N with glucose infusion.

In conclusion, glucose infusion into the abomasum caused a reduction of urea synthesis and urinary excretion in sheep. This depression in urea synthesis was accompanied by a trend for smaller absolute transfer of alanine-N to urea. Thus, increases in glucose absorption from the small intestine may contribute to an increase in flow of amino acids to peripheral tissues and reduce the wastage as urinary N excretion.

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