Concentrations of amino acids and urea in the plasma of the ruminating calf and estimation of the amino acid requirements

By A. P. WILLIAMS and R. H. SMITH

National Institute for Research in Dairying, Shinfield, Reading RG2 9AT

(*Received* 14 *January* 1974 – *Accepted* 26 *February* 1974)

1. A study was made of factors affecting the plasma concentrations of free amino acids (PAA) and urea (PU) in calves receiving approximately equal daily amounts of concentrates (flaked maize and protein supplements) and straw, the former at 10.00 and 17.00 hours, the latter at 17.00 hours only.

2. For calves receiving a diet containing 20 g nitrogen/kg dry matter in which the protein supplement was decorticated, extracted groundnut meal (DCGM) (diet A) there were marked increases in PAA and PU about 1–2 h after a morning feed, then a fall in these values 2 h later to a level which was maintained for the next 3 h. No similar changes occurred after the evening feed. Samples taken 3 h after the morning feed were used in subsequent comparative experiments. There was much more variation between animals than within animals in total PAA, PU and the concentrations of most individual amino acids in these samples.

3. Total PAA and most individual amino acid concentrations were not appreciably affected when the DCGM intake was reduced to give 10 g N/kg dry matter in the diet (diet C), but PU was halved. When maize gluten replaced DCGM as the protein supplement at the higher N intake (diet B) then PU doubled, but again total PAA and most individual amino acid concentrations were little affected. Exceptions were arginine, which was halved, and leucine, which was doubled.

4. Infusions of more than 4·4 g L-methionine/d into the abomasums of calves (110–160 kg live weight) receiving diet A led to a marked increase in plasma methionine concentration. This was considered to correspond with the point at which methionine requirements were met. Using a chromic oxide marker to estimate flows of methionine and cystine from the rumen to the duodenum, it was calculated that under these conditions the methionine requirement was 9·8 g/d, with a cystine flow of 4·9 g/d. Similar calculations showed the corresponding value to be 7·5 g/d with a cystine flow of 2·8 g/d for calves receiving diet C.

5. Infusion of increasing levels of L-lysine into the abomasums of calves (110–160 kg live weight) receiving diet B led to a progressive increase in plasma lysine concentration. There was no consistent change in the rate of increase with increasing amounts infused. Estimated lysine requirement appeared therefore to be less than the flow of lysine from the rumen to the duodenum under these conditions (18·8 g/d).

Experiments with sheep have shown that wool growth can be substantially increased by infusing cystine or methionine into the abomasum (Reis & Schinckel, 1963, 1964; Reis, 1967) or by feeding high-quality protein treated with formaldehyde to reduce its degradation in the rumen (Ferguson, Hemsley & Reis, 1967). This work indicated that sheep do not receive enough sulphur-containing amino acids on normal dietary regimens to sustain maximum wool growth, and stimulated a number of attempts to estimate the requirements of the sheep for these and other amino acids (e.g. Kaminski, Hatfield & Owens, 1970; Nimrick, Hatfield, Kaminski & Owens, 1970; Wakeling, Lewis & Annison, 1970; Tao, Asplund, Wolfrom & Kappel, 1972; Brookes, Owens, Brown & Garrigus, 1973; Mercer & Miller, 1973). No comparable studies have been carried out for the growing calf, although Hutton & Annison (1972)

Table 1. *Daily amounts (kg) of the major components of the stall diets given to the calves at the time that they weighed 114–135 kg. For animals at different live weights these amounts were increased or decreased by about 12% for each 20 kg increment in live weight*

	Diet		
	A	B	C
Straw	1·36	1·36	1·36
Flaked maize	0·51	0·51	0·51
Decorticated, extracted groundnut meal	0·46	—	0·14
Maize gluten	—	0·34	—
Maize starch	0·40	0·40	0·52
Glucose	0·23	0·23	0·29

produced estimates of the requirements of the calf by calculation from known requirements of the pig. Apart from wool growth there is little evidence of improvements in ruminant production resulting from the use of formaldehyde-treated protein, or from the abomasal infusion of amino acids. Part of the present work is an investigation into the amino acid requirements of the ruminating calf based upon the measurement of changes in plasma amino acid (PAA) and plasma urea (PU) concentrations in response to the supplementation of digesta by the infusion of amino acids into the abomasum. Studies with single-stomached animals (Zimmerman & Scott, 1965; Lewis & Speer, 1973) have shown that the response of PAA and PU concentrations to dietary supplementation may be used to determine amino acid requirements. Other studies (reviewed by Munro, 1970) have shown that the response is modified by such factors as animal to animal variation and time of sampling relative to food ingestion. Similar studies on the ruminating calf are limited, but are clearly important, and the present paper describes work designed to obtain additional information on factors influencing PAA and PU concentrations in an attempt to assess the value of using these measurements to estimate amino acid requirements. A preliminary report of the work has been published (Williams & Smith, 1974*a*, *b*).

EXPERIMENTAL

Animals and feeding

Castrated male Friesian calves, which had been weaned at 5–8 weeks onto a normal calf-rearing mixture and hay, were used in these experiments. Operations were performed at 8–15 weeks of age, when the calves were fitted with a simple abomasal cannula (i.d. 15 mm, length 80 mm, of which 30 mm was inside the abomasum) made of Kematal (ICI Plastics Division Ltd). The cannulas were sited in the lateral aspect of the abomasum, in the fundus, near to the pyloric-fundal junction. Periods of at least 3 weeks after the operation and at least 10 weeks after weaning were allowed before experiments were begun. During the experimental periods, the calves (live weight 110–160 kg) were given a variety of diets, all providing energy intakes for a growth rate of about 0·4 kg/d, as shown in Table 1. Concentrates, which were given in two equal amounts at 10.00 and 17.00 hours, also contained a vitamin and mineral

supplement. The straw was given at 17.00 hours only. At least 3 weeks were allowed between changing a diet and taking samples. Shredded paper impregnated with chromic oxide (kindly supplied by Dr J. F. D. Greenhalgh, Rowett Research Institute, Aberdeen) was added to the concentrates, as a non-absorbable marker, in two equal amounts at 10.00 and 17.00 hours to give a daily intake of 1·9 g/kg dry matter intake per d.

Infusion of amino acids into the abomasum

Infusions were by means of a peristaltic pump (HR Flow Inducer MHRE 22, Watson-Marlow Ltd, Falmouth), connected to the abomasal cannula by polyethylene tubing supported on a series of pulleys to allow the animal freedom of movement. For each treatment the amino acids, dissolved in water, were infused at a constant rate of 83 ml/h for 4 d, with at least 3 d between each treatment. A control infusion of water only was also carried out. Amounts of methionine were 0, 1·8, 2·6, 3·5, 4·4, 5·3, 6·2 and 0 g/d for each treatment respectively and in that order for each calf. A corresponding series of infusions for lysine was 0, 3·6, 7·2, 9·0, 10·4, 14·4 and 0 g/d. Each infusate was at pH 7·0.

Sampling of digesta and blood

Digesta samples from the abomasum were obtained simply by unstoppering the cannulas. About 200 ml of digesta were generally obtained within 5 min. The samples were collected in vessels surrounded by ice and were homogenized in an Atomix blender (Measuring & Scientific Instruments Ltd) before sub-sampling and storing at −20° to await analysis. Samples were taken before the morning feed and at 2, 4 and 6 h after feeding. Blood samples were taken through a 150 mm nylon cannula (Portex nylon tubing, size 3v, i.d. 1·0 mm; Portex Ltd, Hythe, Kent) inserted into the jugular vein at least 30 min before feeding. Blood samples (10 ml) were collected directly into centrifuge tubes containing 50 I.U. heparin (Boots Ltd, Nottingham) immediately before the morning feed and at hourly intervals for a period of up to 24 h. Blood samples were centrifuged for 30 min at 1250 ***g*** and at 4°. Part of the separated plasma was stored at −20° for urea-nitrogen analysis. The remainder was deproteinized with 1·22 vol. (v/v) 0·47 M-sulphosalicylic acid containing 0·18 μmol L-α-amino-β-guanidino-propionic acid (Calbiochem Ltd, London)/ml and the supernatant fraction stored at −20° for amino acid analysis. The L-α-amino-β-guanidino-propionic acid was added as an internal standard to correct for losses of amino acids during deproteinization of the plasma. The concentrations of tryptophan and proline in calf plasma were too low for estimation under our conditions.

Analytical

Food ingredients and digesta. Abomasal digesta samples were freeze-dried before analysis. Total N was determined by a micro-Kjeldahl method (Smith & McAllan, 1970). Dry matter was estimated after heating at 105° for 24 h. Most amino acids were determined, after the samples had been hydrolysed with 6 M-HCl at 110° for 24 h, by the method of Spackman, Stein & Moore (1958), but cystine and methionine were

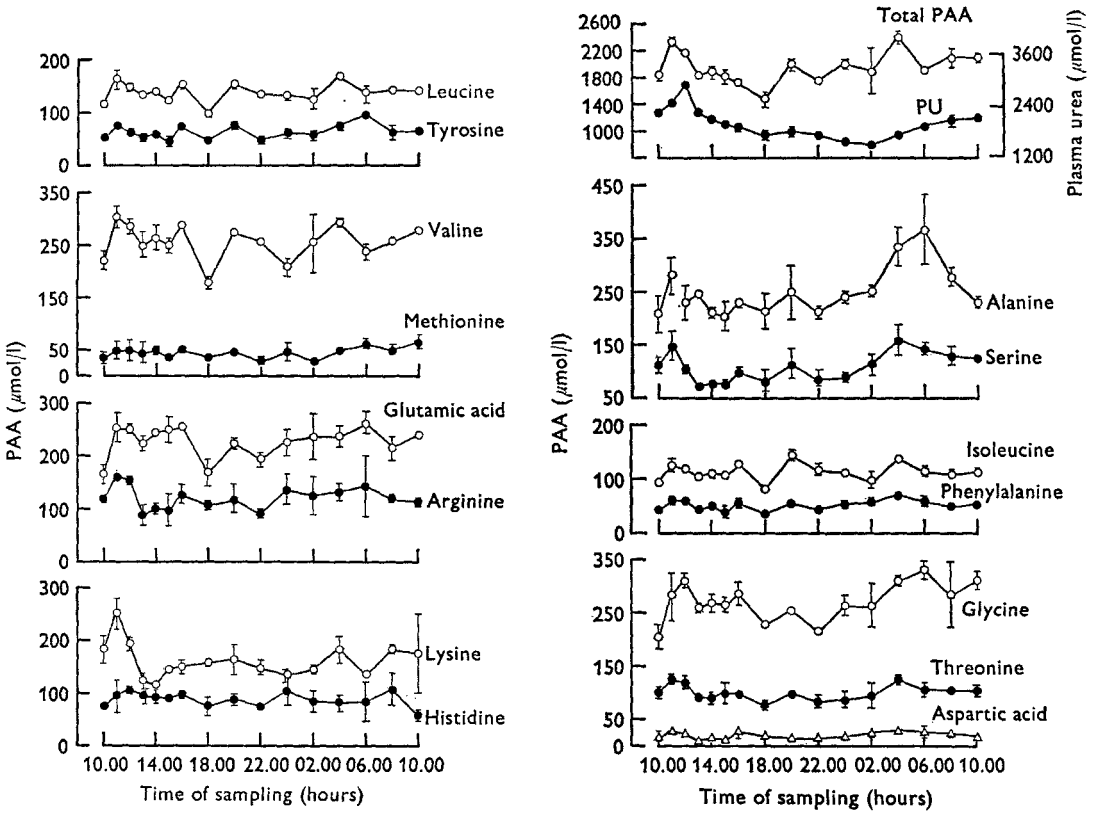

Fig. 1. Effect of time of sampling relative to food ingestion on concentrations of amino acids (PAA; μmol/l) and urea (PU; μmol/l) in the jugular blood of calves given daily equal amounts of flaked maize, decorticated, extracted groundnut meal, glucose and starch at 10.00 and 17.00 hours and straw at 17.00 hours only (diet A). Mean values for two animals; vertical bars represent the standard errors of the mean. For details of diet A see Table 1.

determined by the method of Moore (1963). A JLC-5AH automatic analyser (Jeolco, Tokyo) was used for amino acid analysis. Chromium was determined by the method of Stevenson & Clare (1963), in samples digested according to the method of Stevenson & de Langen (1960) modified by J. F. D. Greenhalgh (personal communication). In this modification the digestion was carried out with 10 ml of an acid mixture prepared from (ml): 250, 18·0 M-H_2SO_4; 380, 14·7 M-H_3PO_4; 20, 0·45 M-$MnSO_4.4H_2O$; 350, water; after the digest was cooled 5 ml of a manganous sulphate–sulphuric acid solution (5 ml 0·45 M-$MnSO_4.4H_2O$/l 9·0 M-H_2SO_4) were added together with the diluted potassium bromate solution. After a further 4 min boiling, 100 ml hot water and 7 ml of a solution prepared from 125 g $(NH_4)_2SO_4$ and 70 ml 11·65 M-HCl/l distilled-water were added and the mixture boiled for 10 min. Filtration and further treatment were similar to that described by Stevenson & de Langen (1960).

Blood plasma. The free amino acids in blood plasma samples were determined either by the method of Spackman *et al.* (1958) or that of Benson, Gordon & Patterson (1967). Urea N was estimated on a Technicon AutoAnalyzer (Technicon Instruments

Table 2. *Concentrations (μmol/l) of amino acids and urea in the jugular blood plasma of calves 3 h after a meal of diet A**

(In column 1, mean values and standard deviations for ten calves (114–135 kg live weight); the other values are standard deviations of mean values for replicate samples taken at weekly intervals (four calves, 110–160 kg live weight) or at daily intervals (two calves, 120 kg live weight) as shown)

No. of calves	...	10	4	2
No. of samples	...	10	7§	5‖
Essential amino acids	Mean	SD	SD of mean	SD of mean
Threonine	102·9	31·6	32·4	18·5
Valine	215·0	46·2	22·2	27·1
Methionine	37·7	12·3	6·2	4·2
Isoleucine	98·0	19·3	15·5	8·3
Leucine	141·4	65·8	25·6	23·8
Phenylalanine	63·4	37·9	16·9	14·7
Lysine†	160·2	48·1	25·6	9·5
Histidine	90·8	25·6	18·3	25·7
Arginine	93·9	34·8	18·9	8·6
Non-essential amino acids				
Aspartic acid	10·8	3·8	3·7	2·5
Serine‡	120·4	36·7	22·6	25·9
Glutamic acid	185·6	85·1	27·6	24·3
Glycine	382·5	102·4	61·6	77·1
Tyrosine	52·1	16·4	9·4	11·4
Alanine	171·5	59·1	32·5	18·6
Total amino acids	1925	383	135	94
Urea	2213	904	263	239

* See Table 1 for details.
† Includes ornithine.
‡ Includes glutamine and asparagine.
§ At weekly intervals.
‖ At daily intervals.

Co. Ltd, New York) using the method of Technicon Instruments Corporation (1967) A dialyser was incorporated into the AutoAnalyzer so that it was unnecessary to deproteinize the plasma chemically.

RESULTS

Factors affecting plasma composition

Time of sampling. The amino acid and urea concentrations in jugular blood plasma at various intervals after feeding are shown in Fig. 1. Total PAA concentrations increased markedly 1–2 h after the morning feed, but returned to near pre-feeding level 2 h later. This level was maintained in further samples taken before the afternoon feed. Changes following the afternoon feed were rather irregular and there was no clear indication of the increase shown after the morning feed. Most individual amino acids and urea showed similar patterns of change. Concentrations of PAA and PU appeared to be fairly stable between 3 and 5 h after the morning feed and blood samples in subsequent comparative experiments were taken at 13.00 hours.

Variations between and within animals. The PAA and PU concentrations in calves given diet A containing 20 g N/kg dry matter are shown in Table 2. Total PAA and

PU concentrations in individual samples from ten calves (114–135 kg live weight) showed very wide variations between animals. These variations were considerably greater than variations between values for the same animal sampled at weekly intervals over 7 weeks (four calves, 110–160 kg live weight) or at daily intervals over 5 d (two calves, 120 kg live weight). A similar degree of variation was obtained with most of the individual PAA with the exceptions of threonine, histidine and aspartic acid, which showed no greater variation between than within animals. The large variation between individuals compared with variations within the same animal has been observed in studies of PAA concentrations in other species e.g. humans (Iob, McMath & Coon, 1963). The weekly observations showed no trends with time. For example, the mean values (± SE) for eight observations for four calves made when the animals were 21 weeks of age were 1818 ± 150, 2292 ± 35 and 31·1 ± 3·3 μmol/l for total PAA, PU and plasma methionine respectively. Comparable values for eight observations when the calves were 5–7 weeks older were 1897 ± 159, 2142 ± 26 and 31·3 ± 3·3 μmol/l. Within any one animal it appeared that background variation was sufficiently low to allow a satisfactory interpretation of changes (or lack of changes) induced by dietary factors or by abomasal supplementation.

Effect of dietary protein source and level of intake. The effects of varying the types and amounts of protein supplements in the diet were studied by giving individual calves different diets in alternate periods. Table 3(*a*) shows the effect on PAA and PU of replacing diet A with diet B so that DCGM was replaced by an isonitrogenous amount of maize gluten. PU was almost doubled. Total PAA were slightly but not significantly higher with diet B, and the only individual amino acids to show significant ($P < 0.05$) changes were arginine and alanine, which decreased by 72% and 22% respectively, and leucine, which increased by 105%. Although lysine concentration appeared to decrease, the change was not significant. The amounts of arginine and lysine in digesta leaving the rumen also decreased while the amount of leucine increased (Table 4). These changes apparently reflected differences in dietary amino acid composition despite the masking effect of microbial protein synthesis although other changes in amounts of amino acids in digesta (e.g. a decrease in histidine concentration) were not reflected in PAA changes. Table 3(*b*) shows the effect on PAA and PU of replacing diet A with diet C so that the N intake was decreased from 20 to 10 g N/kg dry matter. The PU concentration was halved, but there were no appreciable changes in total PAA and only minor changes in individual amino acids (e.g. arginine and isoleucine, which decreased by 35% and 18% respectively, and glutamic acid and glycine, which increased by 52% and 18% respectively). Only the change in glutamic acid was significant ($P < 0.05$).

Effects of abomasal infusion of amino acids on plasma composition. There was no significant difference between PAA concentrations in comparable samples taken when water alone was infused into the abomasum. For example, for six calves given diet A, the plasma methionine concentrations (mean values ± SE) were 33·9 ± 2·9 and 29·6 ± 2·9 μmol/l for the initial and final water infusions respectively. As an additional check on 'carry-over' effects some infusions of amino acids were repeated after the series of treatments described above had been carried out. When this was done there were

Table 3. *Concentrations of total amino acids (PAA), of individual amino acids (% total PAA) and urea in jugular blood plasma of (a) two calves given diet A* (decorticated extracted groundnut meal) and then diet B* (maize gluten) or (b) two calves given diet A (20 g nitrogen/kg dry matter) and then diet C* (10 g N/kg dry matter)*

(Mean values with standard errors of differences between means)

	(a)				(b)			
	Diet A	Diet B	(A−B)	SE of (A−B) (1 df)	Diet A	Diet C	(A−C)	SE of (A−C) (1 df)
Essential amino acids (% total)								
Threonine	4·3	4·8	−0·5	0·30	6·4	5·4	1·1	1·00
Valine	10·3	13·0	−2·7	1·20	10·4	9·4	1·0	0·40
Methionine	2·1	1·8	0·3	0·10	2·3	1·9	0·4	0·60
Isoleucine	4·8	4·8	—	0·3	4·9	4·0	0·9	0·20
Leucine	5·6	11·5	−5·9	0·50	4·6	4·7	−0·1	1·20
Phenylalanine	2·4	2·7	−0·3	0·28	2·6	2·6	—	0·10
Lysine†	7·5	5·3	2·2	2·00	7·4	7·0	0·4	0·30
Histidine	4·8	4·9	−0·1	0·56	4·1	3·5	0·6	0·70
Arginine	7·9	2·2	5·7	0·70	5·7	3·7	2·0	0·50
Non-essential amino acids (% total)								
Aspartic acid	1·5	1·2	0·3	0·10	0·7	0·7	—	0·10
Serine‡	5·5	5·8	—	0·10	7·1	7·6	−0·5	—
Glutamic acid	13·0	12·1	0·9	1·60	8·1	12·3	−4·2	0·40
Glycine	19·6	20·7	−1·1	3·90	23·1	27·3	−4·2	1·20
Tyrosine	2·6	2·9	−0·3	0·1	2·4	2·1	0·3	0·70
Alanine	8·5	6·6	1·9	0·2	10·5	9·0	1·5	0·40
Total PAA (μmol/l)	2216	2413	−197	101	1762	1796	−34	9·80
Urea (μmol/l)	2078	3613	−1495	215	1917	882	1035	378

* See Table 1 for details of diets.
† Includes ornithine.
‡ Includes glutamine and asparagine.

no appreciable differences between the original and repeat PAA concentrations. For example, the plasma methionine concentrations (mean values ± SE) for two calves given diet A at a methionine infusion level of 5·3 g/d were 89·1 ± 1·9 and 90·6 ± 5·4 μmol/l, for the original and repeat infusions respectively. Comparable values for plasma methionine at an infusion level of 6·2 g/d were 125·7 ± 2·2 and 126·6 ± 9·5 μmol/l.

Plasma methionine concentrations, in six calves, given diet A, showed little response to increasing amounts of infused methionine when the amounts infused were small, but rose markedly when greater amounts were infused. Linear regression lines expressing these different types of response and based upon mean values for the different calves (Fig. 2(*a*)) intersected at a methionine infusion rate of 4·4 g/d. When the results for the individual calves were treated separately intersection points from 3·9 to 5·0 g/d with a mean value (± SE) of 4·4 ± 0·2 g/d were obtained. In other experiments with two calves given diet C, the response of plasma methionine (Fig. 2(*b*)) was similar to that in Fig. 2(*a*). Linear regression lines intersected at a methionine

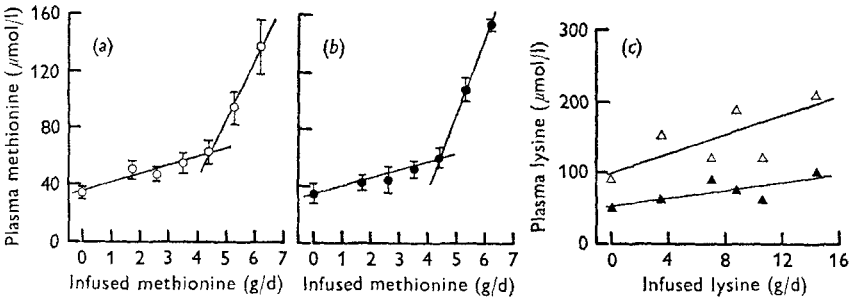

Fig. 2. Effect on plasma amino acid concentrations (PAA) of infusions of different amounts of amino acids into the abomasum of the calf. (*a*) Effect of methionine infusion for six calves given diet A, (*b*) effect of methionine infusion for two calves given diet C and (*c*) effect of lysine infusion for two calves given diet B. Mean values with standard errors (shown as vertical bars) for methionine; for lysine values for two experiments with individual calves; calf 234 (△ — △); calf 241 (▲ — ▲). For details of diets see Table 1.

infusion rate of 4·4 g/d. Similar experiments were carried out with two calves, but with maize gluten providing the dietary protein supplement (diet B) and with increasing amounts of L-lysine infused into the abomasum. Plasma lysine concentrations showed no definite changes with increasing amounts of infused lysine and a two-phase response like that for plasma methionine shown in Fig. 2(*a*) and 2(*b*) was not observed. The changes in plasma lysine concentrations were therefore represented as a linear increase over the whole range of abomasal infusions by calculation of the best straight line through the points by linear regression. PU concentrations were not affected appreciably by either methionine or lysine infusions.

Amino acid requirements. PAA response curves have been used to determine amino acid requirements in several species, e.g. Zimmerman & Scott (1965) and Mitchell, Becker, Jensen, Harmon & Norton (1968) by using the fact that with increasing supplementation the plasma concentration of a limiting amino acid generally remains at a low, relatively constant level until the requirement is met, after which it increases rapidly. The point of intersection of linear regression lines expressing these two different types of response is assumed to represent the requirement. In the present study this type of response was observed for plasma methionine as a result of abomasal infusions of methionine. For calves given diets A and C both intercepts were 4·4 g infused methionine/d. In addition to the quantities of methionine infused into the abomasum, sulphur amino acids were also provided by the digesta leaving the rumen. These quantities, together with other amino acids, were estimated from the amino acid: chromic oxide ratios in the abomasal digesta (Table 4). Chromic oxide is associated with the solid phase of the digesta (Harris & Phillipson, 1962) but some of the amino acids may have been present in a soluble form in the liquid phase which may have biased the results for the quantities of amino acids entering the abomasum. However, no marked variations in amino acid : chromic oxide ratio were observed over the four sampling periods. For example, for four calves given diet A the methionine: chromic oxide ratios (mean values ± SE) at 0, 2, 4 and 6 h after feeding were 1·32 ± 0·16, 1·04 ± 0·07, 1·03 ± 0·06 and 1·11 ± 0·09 respectively. Estimated quantities of

Table 4. *Quantities of amino acids ingested in the food and quantities leaving the rumen of calves (g/24 h)*

(Mean values with their standard errors for amount leaving the rumen, which were calculated from amino acid : chromic oxide ratios in samples of abomasal digesta taken 0, 2, 4, and 6 h after feeding, and total intakes of chromic oxide)

	Diet*								
	A			B			C		
	Food	Rumen		Food	Rumen		Food	Rumen	
No. of animals		4			2			2	
Essential amino acids		Mean	SE		Mean	SE		Mean	SE
Threonine	9·4	16·2	1·5	10·1	11·9	0·6	5·5	6·8	0·7
Valine	13·7	17·2	1·8	13·7	11·4	1·0	7·2	7·7	0·4
Methionine	3·6	5·4	0·2	6·1	4·9	0·1	2·0	3·1	0·2
Cystine	5·2	4·9	0·2	3·6	4·6	0·1	2·3	2·8	0·1
Isoleucine	11·1	14·7	1·1	11·9	10·5	1·2	5·7	6·4	0·2
Leucine	22·6	23·5	1·8	42·1	29·5	1·6	13·1	11·2	1·0
Phenylalanine	16·1	14·7	0·9	17·7	14·1	0·8	7·7	6·7	0·4
Lysine	11·1	20·4	1·2	6·9	18·8	0·2	5·7	9·8	0·4
Histidine	7·8	7·3	0·9	6·8	4·7	0·2	3·8	3·2	0·4
Arginine	26·3	13·4	1·1	10·0	8·3	0·1	10·4	5·9	0·5
Non-essential amino acids									
Aspartic acid	31·5	33·0	2·5	19·4	22·8	0·8	14·3	14·5	1·0
Serine	14·3	14·5	1·1	14·5	13·3	0·3	7·3	6·6	1·0
Glutamic acid	56·2	40·8	4·0	59·8	41·4	2·8	27·3	19·4	1·0
Proline	16·8	13·6	1·6	17·8	14·8	3·6	9·3	5·9	0·8
Glycine	17·2	18·9	2·5	9·8	10·1	0·3	8·2	6·9	0·3
Tyrosine	9·8	13·1	1·9	12·3	10·2	0·2	4·3	5·2	0·6
Alanine	15·9	21·4	2·1	25·9	19·5	1·2	9·6	9·0	0·8

* See Table 1 for details of diets.

methionine and cystine entering the abomasum from the rumen were 5·4 and 4·9 g/d respectively for calves given diet A. With this cystine supply the estimated methionine requirement for calves of 110–160 kg live weight growing at a rate of 0·4 kg/d was therefore 9·8 g/d. Cystine is included because the amount of methionine an animal requires will depend on the cystine level since cystine, if not adequately supplied, can be synthesized from methionine (Lewis & Boorman, 1970). No attempt was made to correct these values for the apparent digestibility of amino acids in the small intestine (Coelho da Silva, Seeley, Thomson, Beever & Armstrong, 1972) since such information is not available for the calf. Clearly such a correction would be desirable, since the availability of the amino acids infused into the abomasum would probably be greater than that of the amino acids of the digesta leaving the rumen. Estimated quantities of methionine and cystine entering the abomasum from the rumen were 3·1 and 2·8 g/d respectively for calves given diet C. With this cystine supply the estimated methionine requirement for these calves was therefore 7·5 g/d.

The estimated quantity of lysine entering the abomasum from the rumen for calves given diet B was 18·8 g/d. If it is assumed that lysine was not limiting under these conditions, as abomasal infusions of lysine did not result in a two-phase response curve for

plasma lysine, then the lysine requirement for these calves was less than 18·8 g/d. Similar assumptions were made by Wakeling *et al.* (1970) in estimating the lysine requirements of sheep.

DISCUSSION

Several workers (Leibholz, 1965, 1969; Sibbald, Loughheed & Linton, 1968) have reported increases in PAA in ruminants after feeding, but others have reported no change (Champredon, Pion & Fauconneau, 1969; Halfpenny, Rook & Smith, 1969; Prior, Milner & Visek, 1972) or even decreases (Theurer, Woods & Poley, 1966; Fenderson & Bergen, 1972; Mangan & Wright, 1973). The reason for these differences is not clear, but it should be noted that in most of these investigations the first sample after feeding was not taken until after 3 h, so that an early change such as we observed would not have been detected. Our results, in showing increased PAA and PU after feeding, are similar to responses reported for single-stomached animals (Porter & Williams, 1963; Eggum, 1970). This may have been because, even in the ruminant, feeding would most probably be followed by a period of increased flow of digesta into the duodenum. Nevertheless the synthesis and flow of microbial protein from the rumen could be expected to smooth out fluctuations and to mask differences between diets of different amino acid composition. Indeed many workers have failed to find much effect of dietary amino acid composition on PAA concentrations (Ogilvie, Bray, Hauser & Hoekstra, 1960; Oltjen, Kozak, Putnam & Lehman, 1967; Slyter, Oltjen, Williams & Wilson, 1971; Shimbayashi & Yonemura, 1972; Burris, Bradley & Boling, 1973; Redd, Boling, Bradley & Ely, 1973), although others have shown such effects (Leibholz, 1966; Leibholz & Moss, 1967; Amos, Little, Ely & Mitchell, 1971), with differences in dietary amino acid composition. It appears that for a dietary change to affect PAA it must first affect the composition of digesta entering the duodenum. Differences between results of different workers may be partly explained by the different natures of the diets used and perhaps the conditions under which they were given. For example, maize gluten is resistant to microbial degradation in the calf rumen (Smith & McAllan, 1973) and might be expected to influence duodenal composition more than a readily degraded protein such as decorticated groundnut meal. This is probably partly responsible for the differences in abomasal amino acids which we have observed between diets containing different proteins (Table 4) and consequently for the differences in PAA shown by calves receiving these diets (Table 3(*a*)). Amos *et al.* (1971) observed similar results in steers given diets containing supplements of maize gluten, distillers' dried solubles or soya-bean meal. The results of Liebholz (1966) and Leibholz & Moss (1967) showing marked differences in PAA, apparently related to dietary amino acid composition even though readily degradable proteins (dried skim milk, meat meal) were used, may have been partly due to the fact that the calves were only 11 weeks old and not fully ruminating. Even for maize-gluten diets our results suggest that any effect of dietary protein was masked by rumen synthesis of microbial protein except for the amino acids, arginine and leucine, the amounts of which differ considerably between the two protein sources.

Results in the literature on the effect of level of protein intake on PAA concentrations are also conflicting. Even in the single-stomached animal the effects of such changes are unclear and depend on such factors as the period for which a particular diet is given and the differences in sites of transamination of PAA (Munro, 1970). Our results support those of Ogilvie *et al.* (1960) and Mangan & Wright (1973), for ruminants, in showing little effect with increasing protein intake, although some individual amino acids showed variations. The reason why these results differed from those of Schelling, Hinds & Hatfield (1967), Hogan, Weston & Lindsay (1968), Leibholz (1970) and Nimrick, Owens, Hatfield & Kaminski (1971), who showed marked increases in most PAA concentrations for sheep with increasing protein intake, may have been that at the lower intake levels the sheep used by the latter groups of workers were overtly protein deficient whereas our own calves and the sheep of Mangan & Wright (1973) were not. In interpreting these effects, however, the relative resistance of different proteins to microbial degradation must again be taken into account.

The effect of dietary protein source and level on PU concentrations shown in this study confirm the results of other studies on calves (Oltjen *et al.* 1967; Boling, Young & Bradley, 1972; Kennedy & Siebert, 1972; Young, Boling & Bradley, 1973) which showed that PU increased with increasing protein intake for a given protein, but is dependent on the nature of the protein. In sheep this dependence was shown by Lewis (1957) to be related to the extent to which ammonia is formed from a protein in the rumen rather than the quality of the protein. These considerations and the effect of urea-recycling on PU concentrations in ruminants (Weston & Hogan, 1967) probably explain the lack of response of PU to abomasal infusions of methionine and lysine in our experiments. Mercer & Miller (1973) have suggested that the response of PU to abomasal infusions of methionine may be of value in estimating the methionine requirements of sheep, but our results do not support this suggestion.

Accepting that the PAA-response method is valid our results suggest that, under the conditions used, methionine was the first limiting amino acid. This conclusion agrees with the results of Linton, Loughheed & Sibbald (1968) from the response of PAA in steers given an encapsulated methionine product, of Sibbald *et al.* (1968) in which increases in N retention and weight gain occurred when methionine was infused into the abomasum of steers, and of Burroughs, Ternus, Trenkle, Vetter & Cooper (1970) in which increases in weight gain occurred when methionine hydroxy-analogue was given in the diet. Other workers (Steinacker, Devlin & Ingalls, 1970; Chalupa & Chandler, 1972) have failed to demonstrate any improvement in N retention with abomasal methionine supplementation. Steinacker *et al.* (1970) suggested that this could have been due to another amino acid being limiting under these conditions or to an amino acid imbalance occurring at the high levels of methionine infused. Some workers (Devlin, 1966; Chandler, 1970; Boila & Devlin, 1972; Chalupa & Chandler, 1972) have suggested that other amino acids such as lysine, threonine or isoleucine may, under some conditions, be limiting for calves, but evidence supporting these views is limited.

In only one report (Hutton & Annison, 1972) have values for the amino acid

requirements of the calf been estimated. These estimates were based on a comparison of the duodenal requirements of amino acids for the steer, calculated by a factorial method using the known requirements of the young pig, with estimated values for the synthesis of amino acids by the rumen bacteria of the calf. Although acknowledging certain weaknesses in their method, Hutton & Annison (1972) calculated that the methionine and lysine requirements (g/d) in digesta entering the duodenum for a 200 kg steer growing at 1 kg/d were 12·9 and 17·9 respectively. A value for cystine was not calculated. Our values for methionine (9·8 g/d) and lysine (less than 18·8 g/d) for 110–160 kg calves growing at 0·4 kg/d are consistent with their values although the differences in size and growth rate of the animals prevent an exact comparison. Our results strongly support the view, however, that the quantity of methionine synthesized in the rumen, together with that surviving from the diet, is insufficient for the needs of the animal under some conditions. In applying these and other estimates of amino acid requirements to practical situations certain facts need to be borne in mind. We have specified our values for methionine requirements as daily amounts of methionine entering the duodenum for calves receiving energy intakes for a certain growth rate and with certain cystine flows into the duodenum. Under different conditions these requirements would be different. For example, if cystine were absent from the digesta it is probable that the methionine flow would need to be increased by 4·9 g/d to compensate for this. Further, our requirements are expressed in terms of amino acids entering the duodenum and take into account therefore a factor for the digestibility of the protein and availability of the amino acids in the small intestine. Over-all digestibility of microbial protein may vary between about 0·40 and 0·70 (Bird, 1972; Salter & Smith, 1974) under different conditions and there is no evidence about the apparent digestibilities of individual amino acids for the calf as there is for the sheep (Coelho da Silva *et al.* 1972). It seems that requirements at the tissue level may be appreciably less than those required in the duodenum, but exact figures are not available.

We thank Dr H. L. Buttle and Mr S. C. Watson for carrying out all surgical operations, Mrs S. J. Askew for supervising the care of the animals, Mr J. E. Cockburn for carrying out amino acid analyses, and Dr D. Hewitt for advice on statistical analyses.

REFERENCES

Amos, H. E., Little, C. O., Ely, D. G. & Mitchell, G. E. (1971) *Can. J. Anim. Sci.* **51**, 51.
Benson, J. V., Gordon, M. J. & Patterson, J. A. (1967). *Analyt. Biochem.* **18**, 228.
Bird, P. R. (1972). *Aust. J. biol. Sci.* **25**, 195.
Boila, R. J. & Devlin, T. J. (1972). *Can. J. Anim. Sci.* **52**, 681.
Boling, J. A., Young, A. W. & Bradley, N. W. (1972). *J. Nutr.* **102**, 1247.
Brookes, I. M., Owens, F. N., Brown, R. E. & Garrigus, U. S. (1973). *J. Anim. Sci.* **36**, 965.
Burris, W. R., Bradley, N. W. & Boling, J. A. (1973). *J. Anim. Sci.* **36**, 219.
Burroughs, W., Ternus, G. S., Trenkle, A. H., Vetter, R. L. & Cooper, G. C. (1970). *J. Anim. Sci.* **31**, 1037.
Chalupa, W. & Chandler, J. E. (1972). *Tracer Studies on Non-protein Nitrogen for Ruminants* p. 107. Vienna: International Atomic Energy Agency.
Champredon, C., Pion, R. & Fauconneau, G. (1969). *C. r. hebd. Scéanc. Acad. Sci., Paris* **269**, 2029.
Chandler, P. T. (1970). *Proceedings of Virginia Feed Convention and Nutrition Conference* p. 22.

Coelho da Silva, J. F., Seeley, R. C., Thomson, D. J., Beever, D. E. & Armstrong, D. G. (1972). *Br. J. Nutr.* **28**, 43.
Devlin, T. J. (1966). *Diss. Abstr.* B **27**, 942*B*.
Eggum, B. O. (1970). *Br. J. Nutr.* **24**, 983.
Fenderson, C. L. & Bergen, W. G. (1972). *J. Anim. Sci.* **35**, 896.
Ferguson, K. A., Hemsley, J. A. & Reis, P. J. (1967). *Aust. J. Sci.* **30**, 215.
Halfpenny, A. F., Rook, J. A. F. & Smith, G. H. (1969). *Br. J. Nutr.* **23**, 547.
Harris, L. E. & Phillipson, A. T. (1962). *Anim. Prod.* **4**, 97.
Hogan, J. P., Weston, R. H. & Lindsay, J. R. (1968). *Aust. J. biol. Sci.* **21**, 1263.
Hutton, K. & Annison, E. F. (1972). *Proc. Nutr. Soc.* **31**, 151.
Iob, V., McMath, M. & Coon, W. C. (1963). *J. surg. Res.* **3**, 85.
Kaminski, J., Hatfield, E. E. & Owens, F. N. (1970). *J. Anim. Sci.* **31**, 1042.
Kennedy, P. M. & Siebert, B. D. (1972). *Aust. J. agric. Res.* **23**, 45.
Leibholz, J. (1965). *Aust. J. agric. Res.* **16**, 973.
Leibholz, J. (1966). *Aust. J. agric. Res.* **17**, 237.
Leibholz, J. (1969). *J. Anim. Sci.* **29**, 628.
Leibholz, J. (1970). *Aust. J. agric. Res.* **21**, 723.
Leibholz, J. & Moss, F. P. (1967). *Aust. J. agric. Res.* **18**, 157.
Lewis, A. J. & Speer, V. C. (1973). *J. Anim. Sci.* **37**, 104.
Lewis, D. (1957). *J. agric. Sci., Camb.* **48**, 438.
Lewis, D. & Boorman, K. N. (1970). In *Proteins as Human Food* p. 448 [R. A. Lawrie, editor]. London: Butterworths.
Linton, J. H., Loughheed, T. C. & Sibbald, I. R. (1968). *J. Anim. Sci.* **27**, 1168.
Mangan, J. L. & Wright, P. C. (1973). *Proc. Nutr. Soc.* **32**, 53A.
Mercer, J. R. & Miller, E. L. (1973). *Proc. Nutr. Soc.* **32**, 87A.
Mitchell, J. R., Becker, D. E., Jensen, A. H., Harmon, B. G. & Norton, H. W. (1968). *J. Anim. Sci.* **27**, 1327.
Moore, S. (1963). *J. biol. Chem.* **238**, 235.
Munro, H. N. (1970). In *Mammalian Protein Metabolism* Vol. 4, p. 299 [H. N. Munro, editor]. New York: Academic Press.
Nimrick, K., Hatfield, E. E., Kaminski, J. & Owens, F. N. (1970). *J. Nutr.* **100**, 1301.
Nimrick, K., Owens, F. N., Hatfield, E. E. & Kaminski, J. (1971). *J. Dairy Sci.* **54**, 1496.
Ogilvie, M. L., Bray, R. W., Hauser, E. R. & Hoekstra, W. G. (1960). *J. Anim. Sci.* **19**, 1281.
Oltjen, R. R., Kozak, A. S., Putnam, P. A. & Lehman, R. P. (1967). *J. Anim. Sci.* **26**, 1415.
Porter, J. W. G. & Williams, A. P. (1963). *Biochem. J.* **87**, 7P.
Prior, R. L., Milner, J. A. & Visek, W. J. (1972). *J. Nutr.* **102**, 1223.
Redd, T. L., Boling, J. A., Bradley, N. W. & Ely, D. G. (1973). *J. Anim. Sci.* **36**, 219.
Reis, P. J. (1967). *Aust. J. biol. Sci.* **20**, 809.
Reis, P. J. & Schinckel, P. G. (1963). *Aust. J. biol. Sci.* **16**, 218.
Reis, P. J. & Schinckel, P. G. (1964). *Aust. J. biol. Sci.* **17**, 532.
Salter, D. N. & Smith, R. H. (1974). *Proc. Nutr. Soc.* (In the Press.)
Schelling, G. T., Hinds, F. C. & Hatfield, E. E. (1967). *J. Nutr.* **92**, 339.
Shimbayashi, K. & Yonemura, T. (1972). *Jap. J. zootech. Sci.* **43**, 677.
Sibbald, I. R., Loughheed, T. C. & Linton, J. H. (1968). *Proceedings of 2nd World Conference on Animal Production, Urbana* p. 453. Washington, DC: American Dairy Science Association.
Slyter, L. L., Oltjen, R. R., Williams, E. E. Jr & Wilson, R. L. (1971). *J. Nutr.* **101**, 839.
Smith, R. H. & McAllan, A. B. (1970). *Br. J. Nutr.* **24**, 545.
Smith, R. H. & McAllan, A. B. (1973). *Proc. Nutr. Soc.* **32**, 84A.
Spackman, D. H., Stein, W. H. & Moore, S. (1958). *Analyt. Chem.* **30**, 1190.
Steinacker, G., Devlin, T. J. & Ingalls, J. R. (1970). *Can. J. Anim. Sci.* **50**, 319.
Stevenson, A. E. & de Langen, H. (1960). *N.Z. Jl agric. Res.* **3**, 314.
Stevenson, A. E. & Clare, N. T. (1963). *N.Z. Jl agric. Res.* **6**, 121.
Tao, R. C., Asplund, J. M., Wolfrom, G. W. & Kappel, L. C. (1972). *J. Anim. Sci.* **35**, 1135.
Technicon Instruments Corporation (1967). *Technicon Method Sheet N-1c.* Tarrytown, New York: Technicon Instruments Corp.
Theurer, B., Woods, W. & Poley, G. E. (1966). *J. Anim. Sci.* **25**, 175.
Wakeling, A. E., Lewis, D. & Annison, E. F. (1970). *Proc. Nutr. Soc.* **29**, 60A.
Weston, R. H. & Hogan, J. P. (1967). *Aust. J. biol. Sci.* **20**, 967.
Williams, A. P. & Smith, R. H. (1973). *Proc. Nutr. Soc.* **32**, 51A.
Williams, A. P. & Smith, R. H. (1974*a*). *Proc. Nutr. Soc.* **33**, 34A.
Williams, A. P. & Smith, R. H. (1974*b*). *Proc. Nutr. Soc.* **33**, 35A.
Young, A. W., Boling, J. A. & Bradley, N. W. (1973). *J. Anim. Sci.* **36**, 803.
Zimmerman, R. A. & Scott, H. M. (1965). *J. Nutr.* **87**, 13.

Printed in Great Britain