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Amino Acid Imbalance in the Liquid-fed Lamb

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Abstract

Eleven Poll Dorset × Merino crossbred female lambs 4 weeks of age were trained to suck liquid diets from bottles. In three separate experiments liquid diets providing 14·2% (expt 1) 10·6% (expt 2) or 8·0% (expt 3) of gross energy as protein and amino acids were fed. Responses in voluntary intake, growth rate and changes in plasma amino acid concentrations were studied when complete or incomplete mixtures of amino acids were added to the liquid diet. These mixtures supplied either: (1) all amino acids in quantities to bring the total of protein plus amino acids to provide more than 20% of dietary gross energy, the amino acids being provided in proportions estimated to meet adequately the lamb's requirements ('complete'); or (2) as the same total amount of amino acids but with the amino acid supplement devoid of threonine ('low-threonine', expts 1 and 2) or isoleucine ('low isoleucine', expt 3).

In experiment 1, there was no food intake or growth depression after feeding the amino acid mixture lacking threonine.

In both experiments 2 and 3, voluntary food intake was depressed to about 50% of that observed in lambs fed the low protein diet, when the amino acid mixture devoid of threonine or of isoleucine, respectively, was fed. Addition of the missing amino acid to the low threonine and low isoleucine diets resulted in recovery of voluntary intake in experiments 2 and 3 respectively, but no significant improvement above that found after feeding the low protein (basal) diet.

In experiments 1 and 2, after feeding the low threonine diet the threonine concentration in the blood plasma decreased markedly, while concentrations of total amino acids were elevated. Although there was no improvement in growth or food intake, the feeding of the diet containing the complete amino acid mixture resulted in an elevation of all essential amino acids including threonine. Similarly in experiment 3, plasma isoleucine concentration decreased in the lambs fed the isoleucine imbalanced diet.

Results indicate that the suckled, preruminant lamb exhibits sensitivity to dietary amino acid imbalance, in a manner analogous to that found in simple-stomached animals. These results also clearly illustrate a depression in food intake associated with the deletion of a specific essential nutrient from the diet of the lamb.

Introduction

Amino acids added in a proportionally inadequate pattern to a low-protein diet fed to rats causes a rapid reduction in voluntary food intake and growth (Harper *et al.* 1970). The specific nutritional situation in which one or more essential amino acid(s) are added to a low-protein diet, and the resulting depressions of food intake and growth are corrected by the addition of the one dietary-limiting essential amino acid has been referred to as an amino acid imbalance (Harper *et al.* 1970). Although such imbalances are often discernible in amino acid supplementation studies with rats and other simple-stomached animals, the most severe imbalance arises in experimental situations in which all essential amino acids except the one already limiting in the diet are added to a low-protein diet.

There have been no reports concerning amino acid imbalances in sheep or other ruminants. Since the amino acid requirements of ruminants have not been satisfactorily determined, the extent of departure from the 'optimal' amino acid composition of the proteins normally digested by the preruminant lamb (milk protein) and the ruminant sheep (bacterial, protozoal, and plant protein) is not known. While the biological value of milk protein is high (about 98%) estimates of the biological value of proteins yielded to the ruminant animal range from 70 to 80% (McNaught *et al.* 1954; Purser and Beuchler 1966; Egan and Walker, unpublished data). The extent of adaption of the ruminant, in both the evolutionary sense and the individual developmental sense, to the amino acid disproportionalities thus imposed on the one hand, and to the relative constancy of the digested amino acid pattern on the other, could conceivably influence the physiological capacity of the ruminant to respond to an experimentally imposed amino acid imbalance. In this respect the suckled lamb may be more sensitive to an amino acid imbalance than a ruminant lamb. In the present paper, amino acid imbalances were created in the suckled lamb by adding amino acid mixtures lacking either threonine or isoleucine to a low-protein liquid diet.

Table 1. Composition of liquid diets

Components expressed as grams per kilogram of liquid diet. Diets 1–3 used in experiment 1, diets 4–6 in experiment 2 and diets 7–10 in experiment 3

	Diet No.:									
Component	1	2	3	4 (LTB)	5 (LTBC)	6 (TI)	7 (LIB)	8 (LIBC)	9 II	10 (TC=IC)
Denkavit	77·3	77·3	77·3	40	40	40	40	40	40	40
Lactose	34·4	28·5	28·5	50	50	43	51·7	51·7	43	43
Butterfat	32·5	26·6	26·6	44	44	37	45·7	45·7	37	37
Amino acids	6·5[B]	26·0[A]	26·0[B]	12·2[A]	12·2[B]	36·8[A]	6·1[C]	6·1[A]	36·8[C]	36·8[A]
Monoglyceryl stearate	—	—	—	5·0	5·0	5·0	5·0	5·0	5·0	5·0
Vitamin B mixture	—	—	—	0·75	0·75	0·75	0·75	0·75	0·75	0·75
Vitamin A and D mixture	—	—	—	0·13	0·13	0·13	0·13	0·13	0·13	0·13
Mineral mixture	—	—	—	5·0	5·0	5·0	5·0	5·0	5·0	5·0
Antibiotic	—	—	—	0·75	0·75	0·75	0·75	0·75	0·75	0·75
Total	150·7	158·4	158·4	157·8	157·8	168·4	155·1	155·1	168·4	168·4
				Estimated composition of diets (as % dry wt)						
Protein	14·5	13·76	13·76	7·10	7·10	6·65	7·22	7·22	6·65	6·65
Carbohydrate	48·0	41·96	41·96	44·10	44·10	37·1	45·96	45·96	37·17	37·17
Fat	30·26	25·06	25·06	35·35	35·35	28·97	37·07	37·07	28·97	28·97
Minerals	1·55	1·46	1·46	3·92	3·92	3·68	3·99	3·99	3·68	3·68
Amino acids	4·31	16·41	16·41	7·73	7·73	21·85	3·93	3·93	21·85	21·85
Protein as % of gross energy	11·2	11·6	11·6	5·35	5·35	5·34	5·35	5·35	5·33	5·34
Protein and amino acids as % of calories	3·01	12·53	12·53	5·27	5·27	15·88	2·63	2·63	15·86	15·88
Total	14·21	24·13	24·13	10·62	10·62	21·22	7·98	7·98	21·19	21·22

[A] Complete amino acid mixture proportions as shown in text.

[B] No threonine was included in this mixture.

[C] No isoleucine was included in this mixture.

Experimental Procedure

Animals

Eleven female crossbred lambs (sired by Poll Dorset from first cross Dorset Horn × Merino ewes) were selected from an experimental flock at 4–5 weeks of age on the basis of uniformity of weight and appearance. Each had been vaccinated against enterotoxaemia and tetanus at 1–2 weeks of age. The lambs were housed indoors close together in individual pens with all lambs visible to each other.

Diets and Feeding Procedure

All lambs were trained to suck from bottles with a rubber teat (Lambar, Farmers Union) initially using a calf milk replacer (Denkavit, Hall Sandford & Co., Torrens Park, S.A.) mixed at 10% dry matter and offered at first three times and later twice daily to provide a total of 120 g dry matter per day. Lambs, once trained, sucked eagerly, adopting the position described as associated with closure of the oesophageal groove (Watson 1944; Ørskov and Benzie 1969). All lambs were then tested with a defined purified liquid diet composed of safflower oil, casein, and lactose with added minerals and vitamins. This diet was intermittently rejected and average daily intake provided inadequate energy for growth. The liquid diets used were thereafter based on a dilution of the milk replacer (Denkavit) with butter oil and lactose, minerals, and vitamins. This approach improved the acceptability of the diets and increased average daily voluntary intake to satisfactory levels. The basal low-protein diets were therefore formulated on this basis (Table 1) from the following components: Denkavit calf milk replacer, lactose B.P. grade (Wydale, Lactose Co. of New Zealand Ltd.), butter fat (anhydrous) (Southern Farmers, Adelaide), crystalline L-amino acid mixture (Tanabe Seiyaku Co. Ltd, Osaka, Japan), glycerol monostearate (Amscol, Adelaide), vitamins A and D_3 (APAC, Nicholas Pty. Ltd., Chadstone, Vic), vitamin B mix (BP grades), and a mineral mixture.

The amino acids were mixed in the following proportions (g/100 g mixture):

Alanine	4·18	Histidine.HCl	3·55	Proline	4·18
Arginine.HCl	14·61	Isoleucine	5·64	Serine	4·18
Asparagine	4·18	Leucine	7·93	Threonine	5·22
Glutamine	6·26	Lysine.HCl	8·77	Tryptophan	1·25
Glutamic acid	4·18	Methionine	6·26	Valine	5·64
Glycine	6·26	Phenylalanine	7·72		

There were 10 000 i.u. vitamin A and 2 000 i.u. of vitamin D_3 per gram of mixture, and the vitamin B mix contained the following components in the following proportions (g/kg mixture in a carrier of 985 g glucose:

Folic acid	6·0	Calcium pantothenate	2·5	Vitamin B_{12}	1·0
Niacin	3·5	Thiamine	1·0	Riboflavine	0·5
				Pyridoxine	0·3

The complete mineral mixture contained essential elements in the following proportions (g/100 g mixture):

NaCl	40	Na_2SO_4	2·38	$ZnSO_4$	0·09
$CaHPO_4$	20	$FeC_6H_5O_7.3H_2O$	0·45	Na_2MoO_4	0·01
$CaCO_3$	17	$CuSO_4.5H_2O$	0·14	KI	0·007
$MgSO_4.7H_2O$	12	$MnSO_4$	0·03	H_2SeO_3	0·002
KCl	8				

The diets used in experiments 1, 2 and 3 were compounded from the above components; the proportions of components used in the respective diets is described in Table 1. The liquid diets were homogenized in a Kenwood blender and were fed warm.

In experiment 1, lambs were fed twice daily, offering as much as they would ingest up to 500 g of liquid diet at 9.30 a.m. and up to 600 g at 4.30 p.m.

In experiments 2 and 3, lambs were fed three times daily (9.30 a.m., 4.30 p.m. and 10.30 p.m.) with all lambs being offered 400–700 g at respective feedings to ensure the highest total daily intake for each individual with as much as they would voluntarily ingest in at least one meal per day.

No additional water was provided in any experiment and the lambs received no dry feed throughout the period.

From time to time, lambs scoured under the *ad libitum* feeding conditions. In experiment 1 the antibiotic neoterramycin was given on appearance of scouring. In experiments 2 and 3 two antibiotics, neoterramycin (Pfizer Agricare Pty. Ltd., West Ryde, N.S.W.) and Furasol (Smith, Kline & French Laboratories Ltd., French's Forest, N.S.W.) were included in the liquid diet, alternating at 5–7-day intervals throughout the experiments.

Table 2. Mean body weights of lambs in groups supplemented with complete or imbalanced amino acid mixture

Expt. No.	Group No.[A]	Mean body weight (kg) on day 0	6	7	10	12	14	17
1	1 (imbalanced)	11·3	11·9		12·5			
	2 (corrected)	12·1	12·5		12·9			
2	$1(\mathrm{LTB}^{7}\rightarrow\mathrm{TC})$	12·7		13·0		13·3	13·2	
	$2(\mathrm{LTB}^{7}\rightarrow\mathrm{TI}^{12}\rightarrow\mathrm{TC})$	12·2		13·0		13·0	13·1	
	$3(\mathrm{LTB}^{4}\rightarrow\mathrm{LTBC}^{7}\rightarrow\mathrm{TI}^{12}\rightarrow\mathrm{TC})$	12·9		14·0		13·6	13·4	
3	$1(\mathrm{LIB}^{7}\rightarrow\mathrm{IC})$	13·4		14·5			15·0	15·4
	$2(\mathrm{LIB}^{7}\rightarrow\mathrm{II}^{14}\rightarrow\mathrm{IC})$	12·9		14·2			14·0	14·3
	$3(\mathrm{LIB}^{7}\mathrm{C}\rightarrow\mathrm{II}^{14}\rightarrow\mathrm{IC})$	13·6		15·0			14·8	15·3

[A] Day of diet change indicated by superscript.

Experimental Programme and Design

In all three experiments all dietary changes were made at the 4.30 p.m. feeding and animals were weighed daily immediately prior to that feed. Blood samples were drawn at 3.30 p.m. on selected days as indicated below.

Experiment 1. Ten lambs were fed diet 1, offering 1100 g of liquid diet per day. This diet provided about 14·2% of gross energy as protein and amino acids, but was formulated to be limiting in threonine. Food intake was measured for 5 days. On the sixth day, five lambs were given diet 2 (threonine imbalanced diet) and five were given diet 3 (threonine corrected diet). These diets were fed for 6 days. Blood samples were taken while animals were on the low protein diet, on day 1 and day 5 (diet 1), and on day 6 and day 11 (diets 2 *v.* 3).

Experiment 2. Immediately following experiment 1, 11 lambs were fed the low threonine basal diet (diet 4, LTB) offering up to 1500 g per day in three meals. This diet provided about 10% of energy as protein and amino acids but was formulated to be limiting in threonine. All lambs were redistributed between three treatment groups so that two groups each of four lambs contained two lambs from each of the treatment groups in experiment 1, while one group of three lambs contained one lamb from each treatment group in experiment 1, and the one additional lamb. On day 5, three lambs were given the same diet to which 5·22 g threonine per 100 g amino acids had been added (diet 5, LTBC). On day 7, four lambs receiving diet LTB and three lambs receiving diet LTBC were given the threonine imbalanced diet (diet 6, TI). The remaining four lambs were given the threonine corrected diet (diet 10, TC). On day 12 all seven lambs fed diet TI were transferred to diet 10 for two days. Blood samples were taken on day 7 (LTB *v.* LTBC), day 8 (TI *v.* TC) and day 12 (TI *v.* TC).

Experiment 3. For experiment 3, the 11 lambs were redistributed into three new groups, with at least one lamb from each previous treatment present in each new group. Two groups of four lambs each were fed the low isoleucine basal diet (diet 7, LIB) and one group of three lambs were fed diet 7 to which 5·64 g isoleucine/100 g amino acids had been added (diet 8, LIBC) for 7 days. On day 8, four lambs previously fed diet LIB and the three lambs fed diet LIBC were fed the isoleucine imbalanced diet (diet 9, II) and the remaining four lambs were fed the isoleucine corrected diet (diet 10, IC). These respective diets were fed until day 14, after which all lambs received diet 10 for 3 days. Blood samples were drawn on day 7 (LIB *v.* LIBC), day 8 (II *v.* IC), day 10 (II *v.* IC), day 14 (II *v.* IC), and day 18 (all IC).

Measurements and Analyses

The calf milk replacer (Denkavit) was analysed for lipid content by ether extraction, carbohydrate by a method based on that of Bath (1958), crude protein by a Kjeldahl method (McKenzie and Wallace 1954) and gross energy by a standard bomb calorimetric method.

Intakes of liquid diets were measured throughout each experiment at each feeding; for total daily intake the 4.30 p.m. feed was considered the first meal of a 24-h period, since diet changes were made at this time. Animals were weighed daily before the 4.30 feeding.

At the times indicated above, blood samples were drawn from the external jugular by venipuncture into heparinized tubes and immediately centrifuged. Plasma was deproteinized by addition of an equal volume of 10% trichloroacetic acid within 20 min of sampling. The supernatants were analysed for amino acids using a Technicon amino acid analyser (Technicon Instrument Corp., New York). For samples taken in experiments 1 and 2, the trichloroacetic acid supernatants were heated to 85–90°C for 2 h to hydrolyse glutamine and asparagine, and thus improve resolution of the threonine peak. Four separate samples analysed before and after such treatment gave results for all amino acids (except the acidic group, poorly resolved in the untreated samples) which did not differ significantly.

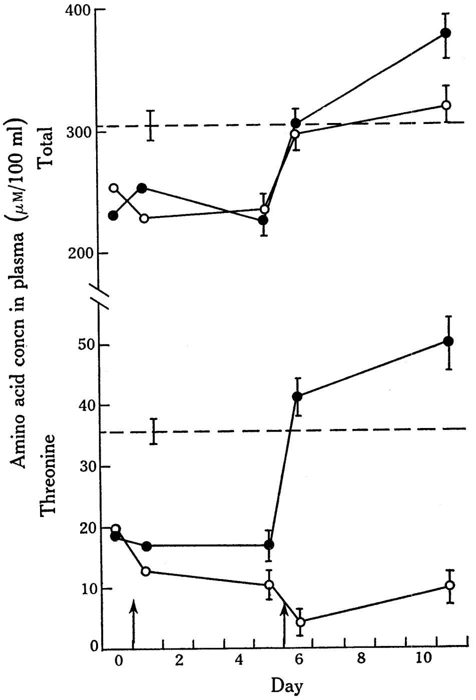

Fig. 1. Experiment 1. Total plasma amino acid and plasma threonine concentration in lambs fed liquid diets low in protein and low in threonine (○, ●, diet 1) on days 1–5, and diets with added amino acid mixtures lacking threonine (○, diet 2) or containing threonine (●, diet 3) on days 6–11. Comparative values for half-sibs suckled at pasture are shown by the dotted lines.

Results

Experiment 1

Mean daily intakes of food by the lambs (as a sum of intakes in two feeding periods daily) in the 4-day control period was 1092 g (S.D. ±48 g) of liquid diet (diet 1) containing 15·0% solids, in which protein and amino acids provided 14·2% of gross energy. Intake was not significantly increased or depressed by addition of amino acid mixture lacking threonine (diet 2) or by the addition of a complete amino acid mixture (diet 3). No significant change in rate of weight gain (*c.* 80–120 g/day) was observed (Table 2). When the threonine imbalanced diet (diet 2) was given, plasma threonine concentration (Fig. 1) was significantly lower on day 6 (49 μM) than on days 1 or 5 (all >107 μM, diet 1) but was not significantly lower on day 11 (124 μM). When the corrected amino acid mixture was given, plasma threonine levels were increased almost threefold (417 μM) within 24 h and increased further over the subsequent 5 days (to 528 μM). Total amino acid concentration was increased by 30% within 24 h of first offering the high amino acid diets, for both the threonine imbalanced mixture and the complete mixture. A further rise, greater for that of the complete mixture, was observed after a further 5 days. The ratio of essential amino acid to total amino acids in blood plasma was 0·45 on diet 1, 0·51 on diet 2, and 0·56 on diet 3.

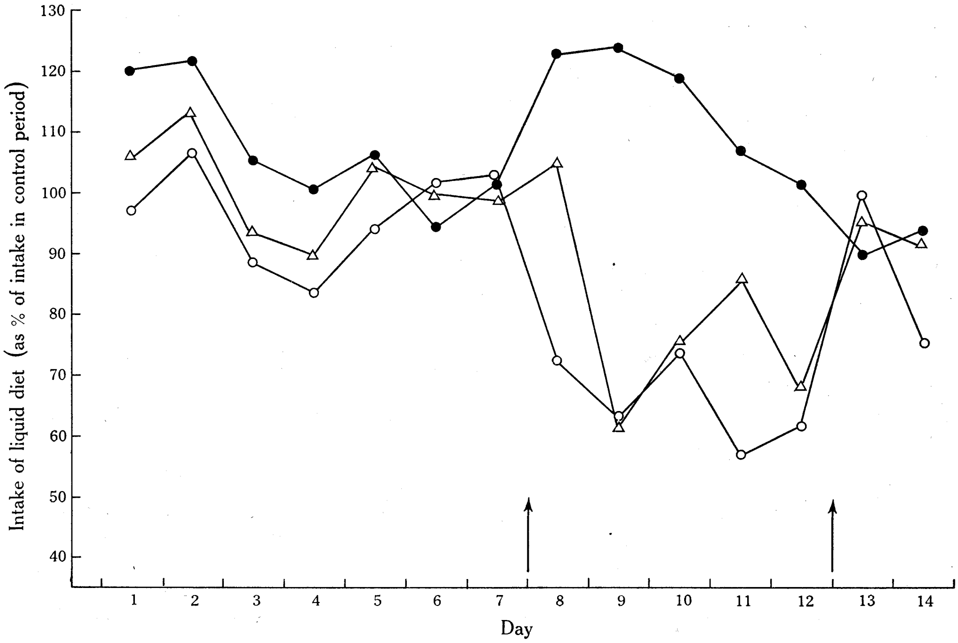

Fig. 2. Experiment 2, threonine imbalance: intake of liquid diet of groups of lambs given diets low in protein (○, ●, diet LTB; △, diet LTBC) on days 1–7, diets with added amino acid mixtures lacking threonine (○, △, diet TI) or containing threonine (●, diet TC) on days 8–12, and all given the threonine-containing diet (○, △, ●, diet TC) on days 13 and 14. Days of diet change are indicated by arrows. For each lamb intakes were expressed as a percentage of mean intake on days 4, 5, 6 and 7.

Experiment 2

Mean daily intakes of food by lambs (sum of intakes of three daily feedings) during the 3-day control period for the respective groups were 1094±36 g (LTB diet), 1327±43 g (LTB diet) and 1414±28 g (LTBC diet) of liquid diet containing 15·8% solids, in which protein and amino acids provided 10·6% of gross energy. During this period weight gain was at the rate of 50–110 g/day (LTB diet) and 150 g/day (LTBC diet).

Food intake responses to the impositions of amino acid mixtures containing (diet TC) and lacking (diet TI) threonine are shown in Fig. 2. The feeding of diet TI resulted in a food intake depression of 25% within 24 h ($P < 0{\cdot}001$) and 40% by 48 h for the LTB pretreated group ($P < 0{\cdot}001$). The low intakes were maintained until diet TC was given, when intakes rose within 24 h ($P < 0{\cdot}001$) back to levels observed during the period of feeding LTB diet. With those lambs previously receiving the LTBC diet, the feeding of diet TI did not depress food intake until the second 24 h, when intake was depressed by 40% ($P < 0{\cdot}001$). Food intake fluctuations between days were greater in this group than in the LTB pretreated group, but after correction of the threonine imbalance (i.e. on giving diet TC), intakes also returned within 24 h to levels observed in the control period (diet LTBC). The group receiving the complete amino acid mixture (diet TC) on day 8, having previously received diet LTB, showed a significant (25%) increase in intake within 24 h ($P < 0{\cdot}05$) and maintained this higher level of intake for 3 days, after which intakes declined back to the levels observed in the control period (LTB group). Maximum intakes of the TC group coincided in time with minimum intakes of both TI groups, at which stage the intakes of the TI groups were only 50% of that of the TC group.

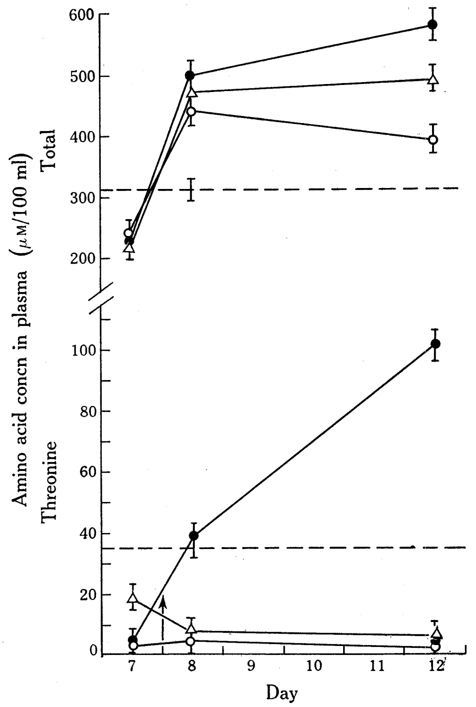

Fig. 3. Experiment 2, threonine imbalance: total plasma amino acids and plasma threonine concentration in lambs fed liquid diets low in protein (○, ●, diet LTB; △, diet LTBC) on days 1–7, diets with added amino acid mixtures lacking threonine (△, ○, diet TI) or containing threonine (●, diet TC) on days 8–12. Comparative values for half-sibs suckled at pasture are shown by the dotted lines.

Animals fed the TI diet neither gained nor lost weight, while those receiving the complete amino acid mix gained at a slow rate (60 g/day, Table 2). Concentrations of threonine and of total amino acids in plasma are shown in Fig. 3. Plasma threonine concentration was higher for the LTBC group than for either LTB groups on day 7. After change of diet on day 8, in all groups except that fed the corrected diet (TC), plasma threonine concentrations were lower than those found in experiment 1; they remained low from the 8th to the 12th day. In the LTB prefed group given the corrected amino acid mixture (TC), the plasma threonine concentration rose tenfold within 24 h to reach values similar to those found in their half-siblings blood-sampled in the field as suckling lambs. By day 12, the fourth day of feeding the threonine corrected diet (TC), the plasma threonine concentrations were extremely high (950±68 μM).

Total amino acid concentration in plasma increased twofold within 24 h after feeding either the TI or TC diets. Though concentrations remained high, by day 12 the plasma total amino acid concentration was lower for the two TI groups than for the TC group. The ratio of essential amino acids to total amino acids in blood plasma was 0·41 for all groups on low protein diets, but rose to 0·60 for both TI and TC groups.

Experiment 3

Mean daily intakes (the sum of intakes in three daily feedings) of liquid diet containing 15·5 % solids, in which protein and amino acids contributed 8 % of gross energy, during the 4-day control period for the respective groups were 1553±50 g (LIB diet), 1475±49 g (LIB diet) and 1591±38 g (LIBC diet). During this period rate of weight gain was 150–180 g/day (LIB diet) and 200 g/day (LIBC diet) (Table 2). Daily food intakes for the various groups are shown in Fig. 4.

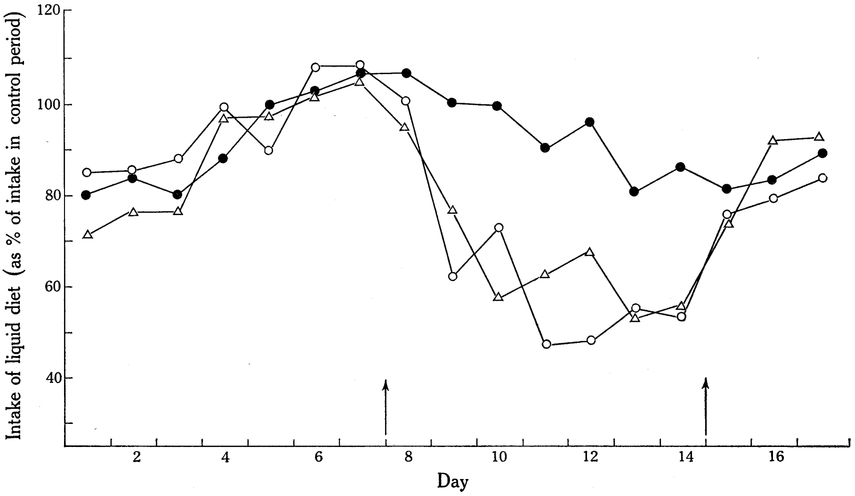

Fig. 4. Experiment 3, isoleucine imbalance: intake of liquid diet of groups of lambs given diets low in protein (○, ●, diet LIB; △, diet LIBC) on days 1–7, diets with added amino acid mixtures lacking isoleucine (○, △, diet II) or containing isoleucine (●, diet IC) on days 8–14, and all given the isoleucine-containing diet (○, △, ●, diet IC) on days 15 and 17. Days of diet change are indicated by arrows. For each lamb intakes are all expressed as a percentage of the mean intake on days 4, 5, 6 and 7.

In experiment 3, the addition of the complete amino acid mixture (diet IC) resulted in a downward trend, food intakes becoming 25 % lower ($P < 0{\cdot}05$) by the sixth day after the dietary change and then showing no further significant change in the subsequent four days. Addition of the amino acid mixture lacking isoleucine (diet II) resulted in no significant depression in intake in the first 24 h, but a significant 30–40 % ($P < 0{\cdot}001$) decrease in intake within 48 h. After 4 days, intake has reached minimum levels of 48 and 60 % of the intake level during the control period (diets LTBC and LTB respectively). At this point relative intakes for the two isoleucine imbalance groups (diet II) were about 65 % of that of the group receiving the complete amino acid mixture. After correction of the isoleucine imbalance (diet IC) on day 14, intakes of the two groups returned within 24 h to levels not significantly different from the group receiving the corrected diet (IC) throughout, and remained at those levels for the subsequent 3 days. Rate of body weight gain for the IC group was 90–120 g/day, and for the imbalance groups small weight losses were observed (Table 2).

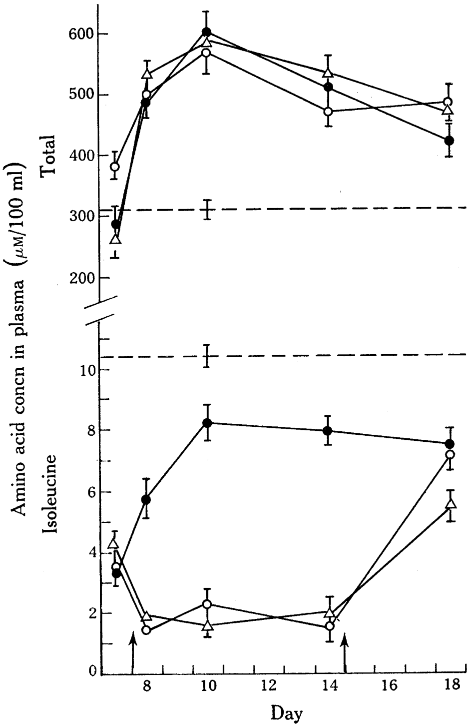

Fig. 5. Experiment 3, isoleucine imbalance: total plasma amino acids and plasma isoleucine concentration in lambs fed liquid diets low in protein (○, ●, diet LIB; △, diet LIBC) on days 1–7, diets with added amino acid mixtures lacking isoleucine (○, △, diet II) or containing isoleucine (●, diet IC) on days 8–14, and all given the isoleucine-containing diet on days 15–18. Comparative values for half-sibs suckled at pasture are shown by the dotted lines.

Plasma isoleucine concentration (Fig. 5) was reduced to 30% of control values within 24 h of giving the isoleucine imbalanced diet (II), and was maintained at this low level until the isoleucine corrected (IC) diet was given. The feeding of the IC diet produced a threefold increase in plasma isoleucine concentration within 24 h and on the third day concentrations were six times greater in the plasma of lambs fed the IC diet than that in lambs fed the II diet. Nevertheless, the concentrations which were maintained over the subsequent 8 days did not reach the values found in the suckling half-sibs, sampled in the field. Total amino acid concentration rose rapidly, increasing by 50 to 100% within 24 h after the high amino acid diets, either complete or isoleucine imbalanced, were first fed. No significant differences between treatments were seen, and in all cases, values at day 14 were significantly lower than at day 10 ($P < 0 \cdot 05$). The ratio of essential to total amino acids was 0·25 for the low protein diets, 0·50 for the isoleucine imbalanced and complete diets when first fed, falling to 0·42 for the complete diet and 0·35 for the isoleucine imbalanced diet on the sixth day of feeding those diets.

Discussion

The data from experiments 2 and 3 provide evidence that the lamb, when fed entirely on a low-protein liquid diet, responds to the imposition of an essential amino acid imbalance by reducing food intake. The physiological responses themselves are indirect but convincing evidence of the effective delivery of the respective dietary mixtures to absorptive sites, without substantial loss or modification in the poorly developed reticulo-rumen. Responses were found with both threonine and isoleucine imbalance. This was not the effect of an excess of free amino acids in the diet since intake was not reduced when the corrected amino acid mixture was given. Differences between diets in flavour or smell could not have served as primary influences affecting the level of intake since there was no evidence of detection of the absence or presence of threonine or isoleucine in the amino acid mixtures at the first meal after a change in diet. The fact that intake recovered after adding the missing essential amino acid further indicates a mechanism of metabolic origin, sensitive to direct or indirect effects of the relative lack of a single amino acid. The rapidity with which intake depression occurred on administering the imbalanced diet, and recovery occurred with the correction of the imbalance gives an indication of the sensitivity of the mechanism. These responses are all similar to those of classical amino acid imbalance effects reported for the rat (Harper *et al.* 1970). However, no significant adaption towards improved intake was observed to occur with increasing time of imposition of the imbalance. Because of the size of the lamb, however, longer-term studies will be required before appropriately concluding that the lamb lacks the ability to adapt to amino acid imbalance.

Two observations on the experimental conditions need to be considered when comparing the responses of the lambs to responses of rats in other studies. Firstly, the growth rates of lambs were low (80–120 g/day) on the low protein diet, as would be expected (Walker and Norton 1971; Black *et al.* 1973). When given the complete amino acid mixture, growth rates were not measurably improved, although growth rates were further reduced in the imbalance treated animals. Interpretation of short-term change in body weight during changing nutritional conditions is difficult, since changes in water balance and compensatory changes in fat and protein deposition cannot be ruled out. However, one simple interpretation of both the intake and body weight data would be that the complete amino acid mixture is itself not optimally balanced with respect to one or more amino acids; this might result in the slow appearance (expt 2) of a mild imbalance. Because cyst(e)ine and tyrosine are of low solubilty in water, the amino acid mixture for each experiment was formulated to provide a supply of methionine and phenylalanine which was assumed sufficient to account for requirements of cysteine and tyrosine respectively, and these two amino acids were not included in the amino acid mixture. The assumption may be incorrect, and cysteine and tyrosine may not be completely dispensable in the growing lamb, the requirement being greater than that provided by the basal protein supply. Further studies on these two amino acids appear warranted. It is also possible that another amino acid was in relative undersupply. Isoleucine, for example, was in relatively low concentration in plasma on the IC diet when compared with the values for the suckled half-sib lambs sampled in the field. Such inadequacies arising from the limited information upon which the estimates of the amino acid requirements of the lamb were based do not negate the point that a greater departure from requirements for a single amino acid in the imbalance treatments had a direct effect in reducing significantly the intake of the liquid diet.

Secondly, in experiment 1, in which no significant decrease in intake was observed, there were several features of the diets and experimental conditions which were subsequently changed and which could have contributed to the lack of response. The protein plus amino acid concentration in the low protein diet was 50 and 70% greater than in experiments 2 and 3 respectively. Growth rates were greater than in the subsequent experiments. By feeding only twice per day in the first experiment it is possible that energy demand was not fully met on a 24-h basis, despite the fact that intake of liquid diet was maximized at the two discrete times of food availability. In this way meal feeding may reduce to some extent either the severity of metabolic effects of imbalance, or the ability of the animal to express a response. Once-a-day meal feeding has been reported to decrease the severity of an amino acid imbalance (Leung *et al.* 1968*a*). Though not statistically significant it was noted in experiment 1 that the only occasions on which small decreases in intake were observed with all animals in the TI group were at the afternoon feeding periods on days 7 and 9, 48 and 96 h, respectively, after the change to the imbalance diet. The interval between morning feeding and afternoon feeding was 7 h, so that it is possible that at the afternoon feeding the intake of the liquid diet came closest to meeting any deficit in energy supply expressed at that time. Absolute intakes were lower for experiment 1 than in the control period or the corrected periods of experiments 2 and 3, but during the period of imposition of the threonine imbalanced diet, were maintained some 30% higher than in the imbalance periods in the later experiments. A higher level of protein supply in the basal diet or a smaller input of the imbalanced amino acid mixture may remain possible factors which could have contributed to the lack of response. Certainly the threonine concentration in the plasma did not fall as low in the imbalanced group in experiment 1 (Fig. 1) as in the two imbalanced groups in experiment 2 (Fig. 3).

Plasma amino acid patterns in all three experiments were typical of imbalance effects (Leung *et al.* 1968*b*). Threonine concentration (expts 1 and 2) and isoleucine concentration (expt 3) were reduced markedly, and rapidly, reaching their lowest levels within 6 h after first feeding the imbalanced mixture. In experiment 1 the level of the missing amino acid rose after several days ingestion of the diet, a possible indication of an adaptation. Although it is often observed as a later response in imbalance studies with rats (Leung *et al.* 1968*b*; Pant *et al.* 1974) this effect was not apparent in experiments 2 or 3. In these two experiments, concentration in the basal diet was much lower than in experiment 1, and hence the threonine and isoleucine deficits were relatively greater. Total plasma amino acids were invariably elevated by both the corrected and the imbalanced amino acid mixtures. The order of the elevation was greater in experiments 2 and 3 than in experiment 1 and resulted in a narrower ratio between essential and non-essential amino acids.

The results of these studies indicate that the preruminant liquid-fed lamb is sensitive to an amino acid imbalance. The extent of the effect appears dependent upon the low basal level of the limiting amino acid and upon the quantity of the amino acid mixture added to cause the imbalance. If the food intake results of experiment 2 are examined carefully, during the feeding of diet LTB it appears that the amino acid mixture added to the milk replacer diet to assure that threonine was dietarily limiting, caused a mild imbalance. That is, all three groups of lambs decreased their food intake and after 2–3 days increased their intakes back to normal, whether additional threonine was added or not. The reduction of voluntary food intake of animals ingesting amino acid imbalanced or deficient diets is thought to be a protective behavioural response—a part of a homeostatic mechanism within the animal body (Harper *et al.* 1970). Force-feeding a diet devoid of a single essential amino acid results in pathological lesions and death. The abnormal plasma amino acid pattern, particularly the lowering of the limiting amino acid, has been postulated to be the signal for the central food intake regulatory mechanisms which results in the decrease in intake of amino acid deficient and imbalanced diet (Leung and Rogers 1969; Rogers and Leung 1973). While the exact mechanisms remain to be elucidated for any species, the fact that the lamb shows essentially the same response as rats implies that the food intake control system, which contains elements sensitive to amino acid deficiencies, is present in animals of diverse dietary habit. The physiological significance of this mechanism in the lambs under normal nutritional circumstances is yet to be established in its true perspective. The amino acid proportions yielded by the diet are relatively uniform as provided to the suckled lamb in milk, and are less well balanced but still relatively uniform as provided to the ruminant lamb in a mixture of plant and microbial protein digested (Egan and Walker, unpublished data). Perhaps modification of the level and the pattern of intake of herbage and milk may accompany changes in the partitioning of dietary protein entry into the rumen or directly into the abomasum, in a manner which enhances the lamb's survival potential. The change in proportional amino acid supply, such as occurs in the change from the suckled to the ruminant habit, may affect levels of energy intake or adaptations may permit accommodation of those nutritional changes without effects on level of energy intake. In either case, studies in the ruminant lamb should hold clues to a better understanding of food intake regulatory mechanisms and their relation to homeostatic mechanisms. It is, however, already clear that in attempts to modify amino acid supply to the lamb by physical or chemical protection of protein or amino acid (Ferguson *et al.* 1967) or by ruminal bypass via the oesophageal groove (Ørskov and Fraser 1969), the sensitivity to amino acid imbalance is a factor which must be considered where levels and proportions of essential amino acids may be substantially altered.

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