INFLUENCE OF PROTEIN DIGESTION ON PLASMA AMINO ACID LEVELS IN SHEEP

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Summary

A study was made of the effect of increased protein digestion in the intestines on the concentration of amino acids in the peripheral blood plasma. Plasma samples were analysed from three sheep offered a basal diet of wheaten hay either alone or supplemented during successive 14-day periods by the infusion per abomasum of 50, 100, and 150 g/day of calcium caseinate. In addition, plasma was analysed from five sheep fed diets of dried ryegrass (*Lolium perenne* × *L. multiflorum*) which provided 7–43 g/day of dietary nitrogen.

The total concentration of essential amino acids in the plasma increased in response to each successive casein infusion, but the increase after the third infusion was less than after the preceding two. The levels of most of the individual essential amino acids also rose. However, as a proportion of the total essential amino acids, only the levels of valine, leucine, and phenylalanine rose, whereas those of the sulphur-containing amino acids remained steady and those of isoleucine, lysine, histidine, and arginine fell. Similar trends in the plasma essential amino acids were observed with increasing intestinal digestion of protein with the ryegrass diets except that the rise in phenylalanine was small.

In response to increased protein digestion, there was little change in the sum of the plasma levels of the six non-essential amino acids studied. Relative to total essential amino acids, the level of proline rose, but glycine, alanine, tyrosine, aspartate, and glutamate levels fell. The fall in the ratio of glycine to total essential amino acids from 1·4 with the basal diet to 0·3 with the highest casein infusion was particularly striking.

It is suggested that the ratios of amino acids such as valine, leucine, histidine, lysine, and glycine to total essential amino acids in the plasma may provide a basis for predicting the extent of protein digestion in the intestines.

I. Introduction

Widespread investigations have been made of the factors controlling the levels of individual amino acids in the plasma of man in normal and diseased states (e.g. Holden 1962). An indication that events in the digestive tract influence the amino

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acids in the peripheral plasma came from Denton, Gershoff, and Elvehjem (1953) and Denton and Elvehjem (1954), who showed that the ingestion of protein by dogs was followed within a few hours by substantial increases in the concentration of amino acids in the portal vein and by lesser increases in amino acid concentration in the peripheral circulation. Similar observations have since been made with man (Stein, Bearn, and Moore 1954; Frame 1958). However, no quantitative relationship has been demonstrated between levels of amino acids in peripheral plasma and the amounts of amino acids absorbed from the intestines. This relates partly to the fact that few studies have been reported in which both parameters were measured and partly to difficulties of interpretation of data when long and often irregular intervals occurred between meals.

The relationship between protein digestion and the levels of amino acids in the plasma of ruminants is even more obscure. Recently, Theurer, Woods, and Poley (1966), working with sheep, showed that with the exception of the sulphur-containing amino acids (cystine and methionine) changes in the concentration of amino acids in blood in the portal vein were reflected in changes of concentration in blood from the jugular vein. This tended to confirm the significance of observations by Leibholz (1965) of substantial changes in the amino acid levels in peripheral plasma of sheep fed once daily. However, little information is available on the quantities and proportions of amino acids absorbed from the intestines of ruminants, because of the effects on dietary protein of microbial activity in the rumen. Thus, under some conditions, large amounts of protein are digested in the rumen, whence much of the nitrogenous portion is transferred to the blood in the form of ammonia. Under other conditions, more protein passes through the pylorus than is consumed in the diet (e.g. Clarke, Ellinger, and Phillipson 1966; Hogan and Weston 1967). However, it is known that wide variation occurs in the amounts of protein digested in the intestines of the sheep; the range with sheep offered roughage diets near *ad libitum* has been reported to be 30–160 g/day (Hogan and Weston 1967; Weston and Hogan 1968). It is clearly important in studies of protein metabolism to know the effect of the digestion of such widely varying amounts of protein on the levels and pools of plasma amino acids.

The present experiment was designed to determine whether the levels of amino acids in the plasma of sheep were related to the amounts of protein digested in the intestines and, accordingly, to indicate whether plasma amino acid levels could be used to predict the quantity of amino acid nitrogen that becomes available to the sheep following digestion in the intestines. A basal low-protein diet, wheaten hay, was supplemented by the infusion into the abomasum of three levels of the highly digestible protein, casein. The small amounts of amino acids derived from the basal diet were thus augmented by substantially more amino acids from the protein supplement. A constant supply of protein was provided for intestinal digestion by offering the sheep equal amounts of food each 3 hr and by infusing protein into the abomasum at a steady rate. Plasma samples were also examined from five sheep fed ryegrass diets that provided 7–43 g/day of dietary nitrogen.

II. Materials and Methods

(a) *Sheep and Feeding*

The sheep used were Merino wethers with permanent cannulae in the rumen and abomasum. Every 3 hr they were fed either 80 g of wheaten hay taken from the supply used in previous studies (Weston and Hogan 1967*a*; Hogan and Weston 1967) or 45–160 g of dried ryegrass which had been harvested at several different stages of maturity (Weston and Hogan 1968).

(b) *Experimental Plan*

Three sheep were offered the basal diet for 3 weeks. This diet was then supplemented with casein (calcium caseinate, Casinal, Glaxo) infused in solution into the abomasum at approximately 50 g/day for 2 weeks, 100 g/day for the following 2 weeks, and 150 g/day for the final 2 weeks. Small variations occurred in the rates of infusion and it was estimated that the three levels of infusion supplied 6·3, 12·9, and 19·5 g nitrogen/day. The sheep were then fed the basal diet alone. The four diets of dried H1 ryegrass (*Lolium perenne* × *L. multiflorum*), which have been described in more detail elsewhere (Weston and Hogan 1968), represented three advancing stages of maturity from the 1965 harvest and one late stage of maturity from the 1964 harvest. The four diets (designated 1–4) were each fed to one sheep at levels approaching *ad libitum* and provided 4·6, 8·7, 9·6, and 20 g amino acid nitrogen/day respectively. A fifth sheep, offered the most immature diet at approximately one-half the *ad libitum* level (diet 5), received 10 g nitrogen/day.

(c) *Collection of Samples*

In the main experiment, three blood samples were collected from the jugular vein of each sheep on two successive days at the end of each period and on the fifth day after the casein infusions ended. Three blood samples were collected after the five sheep had eaten the ryegrass diets for 14 days. The samples were collected at times corresponding to 30, 90, and 150 min after the commencement of feeding.

(d) *Analytical*

The blood samples were transferred from the syringes to heparinized flasks kept in an ice bath. They were promptly centrifuged for 10 min at 3000 *g* in a refrigerated centrifuge and the proteins were precipitated from the plasma with cold picric acid (1% w/v) without delay, to minimize the binding of free cyst(e)ine on plasma proteins (Downes, Sharry, and Till 1965). The precipitated proteins were removed by centrifuging as before and the supernatant fluid was stored at −10°C to await analysis. Before analysis, the picrate was removed and the samples prepared for chromatography by the technique of Stein and Moore (1954). A sample corresponding to 1·0 ml plasma was analysed with a Technicon Amino Acid Analyser (Technicon Co., New York), using a 145 by 0·6 cm column with Technicon type A resin and the standard 21-hr gradient elution (pH 2·875–5·000). For calculation of amino acid concentrations, norleucine was used as an internal standard. Evidence was obtained that methionine underwent variable oxidation; hence, the concentration of methionine was estimated as the sum of methionine, methionine sulphoxide, and, when present, methionine sulphone. The estimation of small amounts of methionine as two or three peaks on the chromatogram was subject to unknown but probably substantial errors. Casein was hydrolysed with 6N HCl for 24 hr under reflux. The acid was removed by repeated evaporation almost to dryness under reduced pressure. The amino acids were separated with the amino acid analyser on a 70 by 0·6 cm column of Technicon type C2 resin and the Technicon standard 4½-hr elution (pH 2·75–6·10). In this paper, the amino acids cystine and cysteine will be referred to, for simplicity, as cysteine. The system used to analyse plasma did not separate threonine from serine and hence values for threonine are not presented with those for the essential amino acids. In addition, the values for histidine may also include tryptophan. In this paper, the term "essential amino acids" has been applied to valine, methionine, cysteine, phenylalanine, isoleucine, leucine, lysine, histidine + tryptophan, and arginine. Cysteine has been included in this classification because it can spare methionine for the synthesis of wool keratin (Reis and Schinckel 1963). Arginine has also been included because it is regarded as an essential amino acid for some species (see, for example, Meister 1965), though there is evidence to the contrary for cattle (Black *et al.* 1957) and sheep (Downes 1961). The inclusion or exclusion of arginine from the essential amino acids makes only minor differences to the calculations of ratios of individual amino acids to total essential amino acids.

(*e*) *Nutrients Provided by Wheaten Hay and Casein*

The wheaten hay diet provided the sheep with 590 g/day dry matter and 536 g organic matter. The dry matter digestibility of the diet was 53% (Hogan and Weston 1967) and hence, from the formula of Moir (1961), the intake of digestible energy was about 1350 kcal/day. Assuming that metabolizable energy is 81% of digestible energy (Blaxter 1964), the intake of metabolizable energy was approximately 1100 kcal/day. Assuming further that the metabolizable energy derived from casein was equivalent to 31·3 kcal/g nitrogen (Blaxter and Martin 1962), the successive casein infusions provided about 200, 400, and 600 kcal/day of metabolizable energy.

In previous studies with this diet, the amount of nitrogen other than ammonia (NAN) apparently digested in the intestines was equivalent to 0·88 g/100 g organic matter intake (Hogan and Weston 1967). When allowance is made for metabolic faecal nitrogen secreted into the intestines at an approximate rate of 0·18 g/100 g organic matter passing from the stomach (Hogan and Weston 1968), the NAN truly digested in the intestines was slightly less than 1 g/100 g organic matter intake or 5·3 g/day. Clarke, Ellinger, and Phillipson (1966), in their analysis of digesta collected from the duodenum with sheep fed on a hay diet, showed that 8·5 g NAN was equivalent to 6·3 g amino acid nitrogen or approximately 380 m-moles of amino acids. To these amino acids must be added methionine, probably present at about 1·4 g methionine nitrogen/100 g nitrogen (Weller 1957). The 5·3 g NAN truly digested in the intestines in the present experiment would thus have contained about 4 g amino acid nitrogen or about 240 m-moles of amino acids. It was assumed that the proportions of amino acids in the samples of digesta with the wheaten hay diet were the same as for the hay diet of Clarke, Ellinger, and Phillipson (1966).

Casein infused into the abomasum was assumed to be completely digested in the intestines (Reis and Schinckel 1961; Blaxter and Martin 1962), with all the casein nitrogen released as amino acids.

The quantity of amino acid nitrogen supplied by the ryegrass diets has been estimated from data on the digestion of those diets described elsewhere (Weston and Hogan 1968) assuming that 75% of digested NAN was amino acid nitrogen. No attempt was made in either experiment to estimate the quantities of amino acids derived from the digestion of endogenous nitrogen entering the small intestines, although it is realized (Hogan 1957) that substantial amounts of nitrogen may be derived from this source.

III. Results

(*a*) *Amino Acid Composition of Digesta and of Casein*

A comparison of the amino acid composition of digesta and casein is presented in Table 1. The composition of casein was almost identical with that reported by Ellinger and Boyne (1965). Only minor differences were observed between digesta and casein in the proportions of the essential amino acids. The amount of cysteine in casein was low but the total levels of cysteine and methionine were similar for both samples. The main differences were observed with the non-essential amino acids — the digesta contained much higher levels of aspartate, glycine, and alanine, but appreciably less glutamate and proline.

TABLE 1

AMINO ACID COMPOSITION OF HYDROLYSED SAMPLES OF DUODENAL CONTENTS FROM SHEEP FED ON HAY* AND OF CASEIN

Amino Acid	Amount (moles/100 moles analysed) in:	
	Duodenal Contents	Casein
Valine	6·9	7·1
Cysteine	1·9	0·4
Methionine	2·2†	4·1
Isoleucine	5·4	5·2
Leucine	7·6	8·7
Phenylalanine	4·0	4·1
Lysine	6·2	6·8
Histidine	1·6	2·5
Arginine	3·5	2·7
Threonine	6·0	4·2
Aspartate	10·5	6·5
Glutamate	10·5	18·0
Proline	5·0	11·5
Glycine	9·7	3·3
Alanine	10·0	4·5
Tyrosine	3·3	4·1
Serine	5·7	6·3

* Calculated from data of Clarke, Ellinger, and Phillipson (1966).

† Calculated from Weller (1957).

(b) *Effect of Casein Infusion on Essential Amino Acids in Digesta and Plasma*

It was estimated that, with the basal ration, approximately 97 m-mole/day of essential amino acids were released into the intestinal digesta (Table 2), the mean total concentration of these amino acids in the plasma being 55 μmole/100 ml, with values for individual sheep of 42, 60, and 63 μmole/100 ml. There was no clear relationship between the amounts of individual essential amino acids supplied in the digesta and the concentrations of amino acids in the plasma. Thus, of the amino acids present in the plasma at low concentrations, cysteine, methionine, and phenylalanine reflected the small amounts supplied from the digesta, but arginine did not do so. By contrast, histidine was present in the plasma at a substantial concentration despite a low dietary supply.

Casein infusions increased the supply of essential amino acids to about 240, 390, and 550 m-mole/day, while the total levels in the plasma rose to 94, 135, and 152 μmole/100 ml respectively. Thus, the first two levels of casein infusion successively increased the mean total concentration of essential amino acids in the plasma by about 40 μmole/100 ml above the basal level but there was a rather smaller additional response, 17 μmole/100 ml, to the highest casein infusion. The individual sheep differed widely in the extent to which the successive casein infusions altered the levels of plasma amino acids. However, the sheep which had the lowest level of amino acids with the basal diet continued to have lower levels than the others following the casein infusions. Although the total concentration of essential amino acids increased with each infusion, several different patterns of change were observed within the individual amino acids. Thus, considering only the first two levels of casein infusion, the concentrations of cysteine + methionine, leucine, isoleucine, and valine in the plasma

TABLE 2

ESSENTIAL AMINO ACIDS RELEASED IN THE INTESTINES AND THE LEVELS OF THESE AMINO ACIDS IN THE PLASMA OF THREE SHEEP

Amounts of amino acids released in the intestines are expressed as m-moles/day, and levels in the plasma as μmoles/100 ml $\pm$ S.E. based on variance between sheep. The basal hay diet offered was supplemented during periods 2–4 with calcium caseinate infused into the abomasum to supply 6·3, 12·9, and 19·5 g of nitrogen/day

Amino Acid	Amounts Found during:				
	Period 1	Period 2	Period 3	Period 4	Period 5
Valine					
Digesta	17	42	68	94	17
Plasma	10·3±1·9	21·2±4·0	34·4±3·4	39·5±9·2	12·0±1·1
Cysteine + methionine					
Digesta	10	26	42	59	10
Plasma	2·9±0·6	5·3±2·0	8·7±1·9	7·0±1·0	4·3±0·9
Isoleucine					
Digesta	13	31	50	69	13
Plasma	6·1±1·0	9·1±1·4	11·3±0·6	12·6±1·6	5·6±0·4
Leucine					
Digesta	19	49	81	112	19
Plasma	6·0±1·1	11·5±2·5	17·5±3·6	23·7±3·4	7·2±1·2
Phenylalanine					
Digesta	10	24	39	54	10
Plasma	2·1±0·2	4·5±1·0	20·8±13·9	33·9±15·4	4·0±0·1
Lysine					
Digesta	15	39	64	89	15
Plasma	10·7±2·3	20·6±3·6	21·7±3·5	19·0±4·7	10·4±1·0
Histidine					
Digesta	4	13	22	31	4
Plasma	12·9±1·9	13·8±1·7	12·5±0·3	8·3±0·3	9·8±1·1
Arginine					
Digesta	9	18	28	38	9
Plasma	3·9±0·5	8·4±1·3	8·4±0·6	7·7±1·0	5·0±0·4
Total levels					
Digesta	97	242	394	546	97
Plasma	55±6·5	94±16·4	135±23·2	152±26·9	58±5·9

increased steadily with increasing dose rates. By contrast, with lysine and arginine there was an increase with the first level of infusion but no subsequent change. Histidine + tryptophan showed little change. The mean concentration of phenylalanine rose slightly with the first level of casein infusion, but quite substantially with the second level, because of large increases in concentration in the plasma of two of the three sheep.

The third level of casein infusion generally produced smaller increases in concentration per gram of casein infused than the previous infusions (valine, isoleucine), or even a fall in concentration (cysteine, methionine, lysine, histidine, arginine). Leucine continued to rise in proportion to dose while phenylalanine again rose sharply. All three sheep showed greatly elevated levels of phenylalanine in the plasma above those observed with the basal diet, but wide variation was observed between sheep.

Five days after the casein infusions were terminated, the levels of plasma amino acids had returned almost to those observed during the first period.

(c) *Relative Proportions of Individual Essential Amino Acids*

The proportions of individual amino acids in the total essential amino acids in digesta and plasma are presented in Table 3. With the basal diet, valine alone was

TABLE 3

INDIVIDUAL ESSENTIAL AMINO ACIDS IN INTESTINAL DIGESTA AND IN PERIPHERAL PLASMA OF SHEEP, EXPRESSED AS PERCENTAGE OF TOTAL ESSENTIAL AMINO ACIDS IN THE RESPECTIVE MEDIA

The basal hay diet offered was supplemented during periods 2–4 with calcium caseinate infused into the abomasum to supply 6·3, 12·9, and 19·5 g of nitrogen/day

Amino Acid	Proportions Found during:				
	Period 1	Period 2	Period 3	Period 4	Period 5
Valine					
Digesta	17·5	17·4	17·3	17·2	17·5
Plasma	18·8	22·6	25·4	26·0	20·6
Cysteine + methionine					
Digesta	10·3	10·7	10·7	10·8	10·3
Plasma	5·3	5·6	6·4	4·6	7·4
Isoleucine					
Digesta	13·4	12·8	12·7	12·6	13·4
Plasma	11·1	9·6	8·4	8·3	9·6
Leucine					
Digesta	19·6	20·2	20·6	20·5	19·6
Plasma	10·9	12·2	12·9	15·6	12·3
Phenylalanine					
Digesta	10·3	9·9	9·9	9·9	10·3
Plasma	3·8	4·8	15·4	22·3	6·9
Lysine					
Digesta	15·5	16·1	16·2	16·3	15·5
Plasma	19·5	21·8	16·0	12·5	17·8
Histidine					
Digesta	4·1	5·4	5·6	5·7	4·1
Plasma	23·5	14·5	9·3	5·5	16·8
Arginine					
Digesta	9·3	7·4	7·1	7·0	9·3
Plasma	7·1	8·9	6·2	5·2	8·6

present in the same proportions in the plasma as in the digesta, lysine and histidine were relatively higher in the plasma, and the remaining amino acids were lower. Casein infusions produced only relatively small changes in the proportions of the individual essential amino acids in the digesta. In response to the infusions, there was also little change in the relative proportions of cysteine + methionine in the plasma essential amino acids. However, the proportions of valine, leucine, and phenylalanine rose in the plasma while those of isoleucine, lysine, histidine, and arginine fell. When the composition of the essential amino acids in plasma is compared with that in digesta, it may be seen that valine represented a consistently higher proportion of essential amino acids in the plasma, whereas cysteine, methionine, isoleucine, leucine, and arginine were lower. Phenylalanine, which initially represented a lower proportion of essential amino acids in plasma than in digesta, changed with the higher casein infusions to form a much higher proportion of essential amino acids in plasma. The reverse change was observed with lysine and histidine.

Table 4

NON-ESSENTIAL AMINO ACIDS RELEASED IN THE INTESTINES AND THE LEVELS OF THESE AMINO ACIDS IN THE PLASMA IN THREE SHEEP

Experimental details and units of measurement as for Table 2

Amino Acid	Amounts Found during:				
	Period 1	Period 2	Period 3	Period 4	Period 5
Aspartate					
Digesta	26	49	72	96	26
Plasma	2·3±0·3	2·3±0·3	2·4±0·5	2·8±0·4	1·4±0·1
Glutamate					
Digesta	26	89	155	221	26
Plasma	8·9±1·7	6·3±1·4	12·3±0·2	10·4±1·3	6·2±0·4
Proline					
Digesta	12	52	94	136	12
Plasma	7·5±0·9	29·4±3·3	46·2±4·8	56·3±3·0	8·8±0·8
Glycine					
Digesta	24	35	47	59	24
Plasma	78·7±8·1	62·7±2·0	52·0±0·7	46·0±1·8	66·4±1·1
Alanine					
Digesta	25	41	57	73	25
Plasma	21·3±2·3	21·4±2·6	15·4±0·6	14·7±0·8	19·0±0·9
Tyrosine					
Digesta	8	22	38	53	8
Plasma	4·1±1·0	9·2±1·0	9·7±0·9	9·7±0·9	5·6±0·5
Total levels					
Digesta	121	288	463	638	121
Plasma	123±11·4	131±9·5	138±5·2	140±1·9	107±3·6

(*d*) *Effects of Casein Infusions on Non-essential Amino Acids in Digesta and Plasma*

It was estimated that approximately 121 m-mole/day of the six non-essential amino acids studied were released into the intestinal digesta with the basal ration (Table 4) the total concentration of these amino acids in the plasma being 123 μmole/100 ml. The absence of relationships for individual amino acids between the amount supplied by the diet and the level in the plasma was even more apparent for the non-essential than for the essential amino acids. Thus, of the four amino acids supplied by the digesta at 24–26 m-mole/day, glycine was present in the plasma at 79, alanine at 21, glutamate at 9, and aspartate at 2 m-mole/100 ml. The plasma levels of tyrosine and proline were not inconsistent with a lower supply from the digesta.

The casein additions increased the supply of these non-essential amino acids to about 290, 460, and 640 m-mole/day. However, the increases in total concentrations in the plasma were only slight, namely 8, 7, and 2 μmole/100 ml for the three successive infusions. As with the essential amino acids, the individual non-essential amino acids showed variable responses to the increasing casein additions. Thus, aspartate and glutamate showed no consistent change, while proline rose with each level of infusion; tyrosine rose with the first infusion and then maintained a steady level, whereas glycine and alanine declined. Within 5 days of the end of the infusions, the individual amino acids approached the pre-infusion levels.

Table 5

INDIVIDUAL NON-ESSENTIAL AMINO ACIDS IN INTESTINAL DIGESTA AND IN PERIPHERAL PLASMA OF SHEEP, EXPRESSED AS A RATIO OF THE TOTAL ESSENTIAL AMINO ACIDS IN THE RESPECTIVE MEDIA

Experimental details as for Table 3

Amino Acid	$10^2 \times$ Ratio of Non-essential to Total Essential Amino Acids during:				
	Period 1	Period 2	Period 3	Period 4	Period 5
Aspartate					
Digesta	27	20	18	18	27
Plasma	4	2	2	2	2
Glutamate					
Digesta	27	37	39	41	27
Plasma	16	7	9	7	11
Proline					
Digesta	12	22	24	25	12
Plasma	14	31	34	37	15
Glycine					
Digesta	25	15	12	11	25
Plasma	143	67	39	30	114
Alanine					
Digesta	26	17	15	13	26
Plasma	39	23	11	10	33
Tyrosine					
Digesta	8	9	10	10	8
Plasma	14	10	7	6	10

(e) *Proportions of Non-essential Amino Acids Relative to Essential Amino Acids*

The ratios of individual non-essential amino acids to total essential amino acids in the digesta and plasma are presented in Table 5. In the digesta, the ratio of tyrosine was little affected by the infusions. With the other amino acids, the first level of infusion increased glutamate and proline and decreased aspartate, glycine, and alanine relative to total essential amino acids, and further casein infusions continued these trends but only to a small extent. In the plasma, the most striking effect of the infusions was the decline relative to the essential amino acids of glycine and, to a lesser extent, of alanine and tyrosine. The relative proportions of aspartate and glutamate also declined with the first level of infusion but showed no subsequent decline. Proline alone of the six amino acids increased its plasma level relative to essential amino acids. These data also indicate that there was no correspondence in the relative proportions of the non-essential amino acids in the plasma with those in the digesta.

Table 6

EFFECT OF DIFFERENT DIETS ON THE LEVELS OF ESSENTIAL AMINO ACIDS IN THE PERIPHERAL PLASMA

Dried ryegrass diets 1–5 [see Section II(*b*)] were fed to five sheep, one diet per sheep. For comparison, data from Tables 2 and 3 are presented for three sheep fed on wheaten hay with and without the infusion of 19·5 g nitrogen/day into the abomasum as calcium caseinate. Amino acids are expressed as concentrations (μmoles/100 ml), and relative to total essential amino acids

Amino Acid	Amount in Peripheral Plasma of Sheep fed:						
	Wheaten Hay	Ryegrass Diet 1	Ryegrass Diet 2	Ryegrass Diet 3	Ryegrass Diet 5	Ryegrass Diet 4	Hay + Casein
Valine							
Concn.	10·3	10·6	8·3	17·0	19·2	25·1	39·5
Rel. amount	18·8	18·3	17·5	23·0	26·3	29·0	26·0
Cysteine + methionine							
Concn.	2·9	2·1	2·2	4·5	4·5	6·1	7·0
Rel. amount	5·3	3·6	4·6	6·1	6·2	7·1	4·6
Isoleucine							
Concn.	6·1	4·6	4·1	6·4	6·8	8·0	12·6
Rel. amount	11·1	8·0	8·6	8·7	9·3	9·3	8·3
Leucine							
Concn.	6·0	7·3	5·3	9·4	12·3	15·5	23·7
Rel. amount	10·9	12·6	11·2	12·7	16·8	17·9	15·6
Phenylalanine							
Concn.	2·1	3·2	2·3	3·7	6·6	5·3	33·9
Rel. amount	3·8	5·5	4·9	5·0	9·0	6·1	22·3
Lysine							
Concn.	10·7	13·6	10·7	14·2	10·1	12·9	19·0
Rel. amount	19·5	23·5	22·6	19·2	13·8	14·9	12·5
Histidine							
Concn.	12·9	11·6	8·5	8·5	5·9	5·0	8·3
Rel. amount	23·5	20·1	17·9	11·5	8·1	5·8	5·5
Arginine							
Concn.	3·9	4·8	6·0	10·2	7·7	8·7	7·7
Rel. amount	7·1	8·3	12·7	13·8	10·5	10·0	5·2
Total essential amino acids (concn.)	55	58	47	74	73	87	152

(*f*) *Plasma Amino Acid Levels in Sheep Fed Ryegrass*

The plasma amino acid levels observed with sheep fed ryegrass are presented in Tables 6 and 7. For comparison, some of the results obtained with the wheaten hay diets (Tables 1–4) are again presented. The estimated amounts of amino acid nitrogen (g/day) released into the intestinal digesta from the various diets were as follows:

Diet	Wheaten Hay	Ryegrass Diet 1	Ryegrass Diet 2	Ryegrass Diet 3	Ryegrass Diet 5	Ryegrass Diet 4	Hay+ Casein
Amino acid nitrogen	4·0	4·6	8·7	9·6	10·0	20·0	22

There was a tendency for the concentration of the total plasma essential amino acids to increase for the unsupplemented roughage diets with increases in nitrogen intake but the maximum level, 87 μmole/100 ml, was substantially lower than the 152 μmole/100 ml observed with hay and casein. With the ryegrass diets, there was a general tendency for the plasma levels of all essential amino acids except lysine and histidine to rise as digesta nitrogen increased; lysine showed no consistent changes and histidine fell. The maximum values observed with the ryegrass diets were

Table 7

EFFECT OF DIFFERENT DIETS ON THE LEVELS OF NON-ESSENTIAL AMINO ACIDS IN THE PERIPHERAL PLASMA

Experimental details as for Table 6. Amino acids are expressed as concentrations (μmoles/100 ml) and as relative amounts ($10^2\times$ratio of non-essential to total essential amino acids)

Amino Acid	Amount in Peripheral Plasma of Sheep fed: Wheaten Hay	Ryegrass Diet 1	Ryegrass Diet 2	Ryegrass Diet 3	Ryegrass Diet 5	Ryegrass Diet 4	Hay+ Casein
Aspartate							
Concn.	2·3	1·7	1·3	2·1	1·9	2·0	2·8
Rel. amount	4·1	2·9	2·9	2·8	2·6	2·3	2
Glutamate							
Concn.	8·9	6·0	3·6	17·0	9·7	7·7	10·4
Rel. amount	16·2	10·4	7·9	23·0	13·3	8·9	7
Glycine							
Concn.	78·1	82·0	57·8	80·4	44·9	43·7	46·0
Rel. amount	143·1	141·9	127·2	108·8	61·4	50·5	30
Alanine							
Concn.	21·3	11·6	8·7	15·7	10·7	11·2	14·7
Rel. amount	38·7	20·1	19·1	21·2	14·7	12·9	10
Tyrosine							
Concn.	4·1	4·3	4·0	6·8	7·7	12·2	9·7
Rel. amount	7·5	7·4	8·8	9·2	10·5	14·1	6

generally lower than those observed with hay and casein. However, observations were made with only one sheep with each level of intake of the ryegrass diets and it is not easy to assess the significance of differences between these and other data.

The release of increasing amounts of essential amino acids in the digesta with the ryegrass diets caused rises in the proportions (relative to total essential amino acids) of valine, cysteine, methionine, isoleucine, and leucine in the plasma. The relative proportions of lysine and histidine declined, whereas arginine and phenylalanine showed no consistent change.

Of the five non-essential amino acids studied in the plasma, the level of tyrosine alone rose consistently with increasing amino acid nitrogen in the digesta. Glutamate showed a variable response, aspartate little change, and glycine and alanine substantial falls. Considered in relation to total essential amino acids, glycine showed the most noticeable response; alanine also declined at the higher levels of nitrogen intake, aspartate showed little change, and the glutamate response was quite variable. Tyrosine alone showed a consistent increase in concentration relative to the essential amino acids.

IV. Discussion

The present studies confirm previous work showing that changes in the intake of dietary protein may markedly affect the levels of amino acids in peripheral plasma (e.g. Frame 1958; Schelling, Hinds, and Hatfield 1967). As might be expected, the changes were generally greater with the essential than with the non-essential amino acids, and in addition, wide variations between amino acids were observed in the nature of the changes, with the levels of some amino acids rising and some falling in response to increased protein digestion. However, because of substantial differences between sheep, the absolute levels of these amino acids in plasma are of little general value in indicating the amount of protein digested in the intestines.

A more consistent picture emerges from both the casein infusion experiment and the ryegrass diets when the individual amino acids are considered in relation to total essential amino acids. Thus, with increasing protein digestion there was a relative increase in the levels of valine and leucine and a relative decline in the levels of lysine, histidine, and glycine. Similar trends may also be calculated from data from studies with man (Stein, Bearn, and Moore 1954; Frame 1958), rats (Swendseid, Villalobos, and Friendrich 1963), and sheep (Schelling, Hinds, and Hatfield 1967). These relationships are worthy of further study to determine their value for predicting the quantities of protein digested in the intestines of sheep.

The amino acids in plasma are presumably in equilibrium with those in the whole extracellular fluid and hence changes in plasma amino acids reflect changes in the balance between the rates of entry and removal of amino acids from extracellular fluid. Amino acids enter this fluid from endogenous sources and from the diet; the relative contributions from the two sources will be determined by the dietary intake of protein and energy, the contribution from endogenous sources being greater at low levels of intake. This alteration in contribution from the two sources may possibly explain some of the changes observed in the levels of individual amino acids with increasing protein and energy intakes. In addition, however, the rates of removal of individual amino acids will also be affected by the protein and energy reaching the tissues because the nutrient supply will determine the proportion of amino acids oxidized to supply energy and the proportion incorporated into larger molecules.

Estimations of the amino acids in the extracellular fluid can be made assuming that the concentration of amino acids in peripheral plasma represents that in the whole extracellular fluid, and that extracellular fluid occupied 18 l, the urea space estimated for similar sheep fed the wheaten hay diet (Weston and Hogan 1967*b*). Hence, with the basal diet and the three casein infusions, the essential amino acids in the extracellular fluid would not have exceeded 10, 13, 24, and 27 m-mole respectively, equivalent to about 10, 5, 6, and 5% of the essential amino acids digested in the intestines each day. The rate of removal of essential amino acids from plasma is thus very rapid and it is not surprising that the concentrations of plasma amino acids soon returned to the basal level when the casein infusions were stopped.

In view of the similarity between casein and digesta in the proportions of the essential amino acids, it is not surprising that increased casein digestion should affect plasma amino acids in the same way as increased protein digestion in the animals consuming forage diets. The main difference between the casein infusions and the ryegrass diets was in the plasma levels of phenylalanine observed with the higher casein infusions. Although the sheep probably received much the same amount of phenylalanine from the 20–22 g amino acid nitrogen derived from ryegrass as from casein, phenylalanine represented only about 6% of plasma essential amino acids with the ryegrass diet compared with 22% of plasma essential amino acids with the casein infusion. The reverse tendency for tyrosine to be equivalent to 14% of plasma essential amino acids with ryegrass diet 4 but 6% of plasma essential amino acids with the highest casein infusion (Table 7) suggests that some limitation to the rate of conversion of phenylalanine to tyrosine may have existed with the casein diets.

V. References

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