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Variations with energy nutrition in the concentrations of amino acids of the blood plasma in the dairy cow

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1. The plasma levels of individual amino acids were studied in dairy cows under different conditions of production and energy nutrition.

2. A preliminary experiment was conducted which established that there was no regular change in amino acid levels with time of sampling in animals offered food twice daily at milking.

3. For animals in the 8th month of pregnancy plasma concentrations of lysine, valine, serine and isoleucine were higher, and of threonine lower, in Jersey than in Friesian cows. Lactation was accompanied in most cows by a fall in the plasma concentrations of lysine, arginine, threonine, histidine, glutamic acid, leucine and alanine and a rise in the level of glycine.

4. In lactating cows an improvement in the plane of energy nutrition was associated on average with an increase in the plasma concentrations of 'non-essential' amino acids and a decrease in the concentrations of 'essential' amino acids.

5. Intraruminal infusion of propionic acid in the lactating cow increased the concentrations of certain 'non-essential' amino acids, glutamic acid in particular, and decreased those of most other 'essential' and 'non-essential' amino acids.

6. These observations are discussed in terms of the possibility that an increased output of amino acids in milk proteins results in a depression in the concentrations of the 'essential' and certain of the 'non-essential' animo acids in the plasma. The hypothesis is put forward that the plasma supply of the other 'non-essential' amino acids, glutamic acid and proline in particular, may limit synthesis of milk proteins.

A rise in the plane of energy nutrition of the lactating cow results in a specific increase in the protein content of the milk (Rook & Line, 1961), and this effect has been shown to be associated with an increase in the uptake of propionic acid from the rumen (Rook & Balch, 1961). Rook (1959) suggested that the supply of propionate absorbed from the rumen may, through controlling the overall degradation and synthesis of amino acids in the liver, influence the concentrations of amino acids in the blood plasma, and these in turn may affect the rate of protein synthesis within the mammary gland.

The main purpose of the experiments described here was to determine the effect of the plane of energy nutrition and of intraruminal infusion of propionic acid on the concentrations of amino acids of the blood plasma in the lactating cow. Preliminary experiments were undertaken, one to determine the extent of variations in the concentrations of individual amino acids throughout a day and from day to day (in order to determine the frequency of sampling required in subsequent experiments), and another to establish whether these concentrations were affected by the considerable drain of amino acids from the plasma associated with lactation.

EXPERIMENTAL

Design of experiments

All the animals were selected from the University herds of Friesian and Jersey cows.

Expt 1. One Friesian and one Jersey cow in their 3rd month of lactation were offered a diet of hay and proprietary concentrates for a period of 3 weeks, providing the standards of Woodman (1957) for maintenance and production. The daily diet was given in two equal amounts at milking. On 2 successive days at the end of the 3rd week, samples of jugular venous blood were taken from each animal at 08.30, 10.30, 12.30, 14.30, 16.30 and 18.30 h.

Expt 2. Three Friesian and three Jersey cows in their 7th–8th month of pregnancy were selected. Throughout the experiment, which continued until the end of the 6th week of lactation, they were offered diets of ordinary farm foods calculated to meet the requirements (Woodman, 1957) for maintenance and production at peak yield (based on the results of the previous lactation) and therefore in excess of the requirements in late pregnancy. The daily diet was offered in two equal amounts at milking. On 2 successive days towards the end of the 8th month of pregnancy, and again on 2 successive days in the 6th week of lactation, samples of jugular venous blood were taken at 10.00, 13.00, 16.00 and 19.00 h.

Expt 3. Four Jersey cows at the end of their 1st month of lactation were selected. They were subjected alternately to two planes of energy nutrition over four successive 3-week periods. In the first period the diet of two cows represented a low plane and that of two cows a high plane of energy nutrition, while in the second period each pair was changed to the other diet, and so on alternately. The daily diets at the beginning of the experiment were as follows.

For the low plane of energy nutrition the cows were offered 6·4 kg hay, and proprietary concentrates at the rate of 1·6 kg/gal of milk produced less 1·6 kg. For the high plane of energy nutrition, they were offered 4·5 kg hay, 0·6 kg of flaked maize, and proprietary concentrates at the rate of 1·6 kg for each gal of milk produced plus an additional 1·6 kg. At the end of each 3-week period the amounts of food offered were reduced to allow for an assumed lactational decline in milk yield of 2% per week. Samples were taken at morning and afternoon milkings on the last 4 days of each experimental period, and on the last 2 days, samples of jugular venous blood were taken at 08.30, 10.30, 13.30 and 14.30 h.

Expt 4. One Jersey cow and one Friesian cow in their 6th month of lactation and fitted with permanent rumen cannulas were used. They were offered, over a period of 9 weeks, a daily diet of 5.5 kg (Jersey) or 6.4 kg (Friesian) of hay, and proprietary concentrates initially at the rate of 1·6 kg for each gal of milk produced (based on milk yield at the beginning of the experiment). The amount of concentrates was reduced by 0·23 kg/day at the end of every 3rd week. The cows received a continuous intraruminal infusion of 10 gal water/day over weeks 1–3 and 7–9 of the experimental period and of 10 gal water plus 1 kg propionic acid over weeks 4–6. Samples of milk were taken at the morning and afternoon milking on the last 4 days of each 3-week period of infusion, and samples of jugular venous blood at 08.30, 10.30, 12.30, and 14.30 h on the last 2 days of each period.

Sampling and methods of analysis

In Expts 3 and 4, milk samples taken over the last 4 days of each treatment period were used to prepare a weighted composite sample representative of the milk of each animal. These samples were analysed for total N by the Kjeldahl method.

Catheters were inserted into the jugular vein on the day preceding the 2-day sampling periods. Samples of blood were withdrawn into a syringe containing heparin, while care was taken to avoid exciting the animal. The blood was immediately centrifuged and the plasma removed. To allow for possible losses arising from deproteinization or other steps in the procedure for amino acids analysis, norleucine was added to the plasma (5 μmoles/10 ml plasma). Plasma proteins were precipitated by the addition of 5 vol. of 1% (w/v) picric acid. Samples were centrifuged and the supernatant fluid was stored at $-20°$ until required for the next stage in the analysis; there was no loss associated with storage.

Picric acid was removed from the supernatant fluid by a column of Dowex 2 resin (Cl^- form) according to the procedure of Stein & Moore (1954). Free amino acids (lysine, histidine, arginine, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine and methionine) were determined with an automatic amino acid analyser of the type described by Spackman, Stein & Moore (1958) and manufactured by Evans Electroselenium Ltd, using columns of Amberlite I. R. 120 resin.

RESULTS

Variations throughout a day. The variations throughout the course of a day on 2 successive days in the concentrations of individual amino acids in the plasma of each of the two cows are shown in Fig. 1. The mean values for the total of the individual amino acids at each of the six sampling times were 22·3, 21·1, 24·1, 23·4, 23·9, and 18·6 mg/100 ml for the Jersey cow and 16·4, 16·2, 17·4, 15·9, 13·0, and 17·0 mg/100 ml for the Friesian cow. No clear pattern of change over the sampling period emerged either in the total or the individual concentrations. The fall in the concentration of the total of these amino acids in the Jersey cow at the 18.30 (latest) time of sampling reflected mainly a decrease in the concentrations of valine, lysine and serine. There was no similar change in the concentrations of these acids in the Friesian cow. The low concentration of total amino acids in the Friesian cow at 16.30 h arose mainly from a marked drop in the concentrations of lysine, arginine and serine.

Effect of lactation. Mean values for the concentrations of individual amino acids in the plasma of each of the three Friesian and three Jersey cows over 2 successive days in the 8th month of pregnancy are given in Table 1. Values for lysine, valine, serine and isoleucine were consistently higher in the cows of the Jersey than of the Friesian breed, and for lysine, valine and serine the differences were statistically significant ($P < 0·05$). Mean values for threonine were consistently lower in the Jersey than in the Friesian cows, but not significantly so ($P > 0·05$).

The percentage changes with lactation in the mean values for the concentrations of individual amino acids are reported in Table 2. In all the cows, the plasma concentrations of lysine, arginine and threonine were decreased, as were the concentrations of histidine, glutamic acid, leucine and alanine in all but one of the cows. The concentration of glycine increased in all but one animal. The concentration of valine

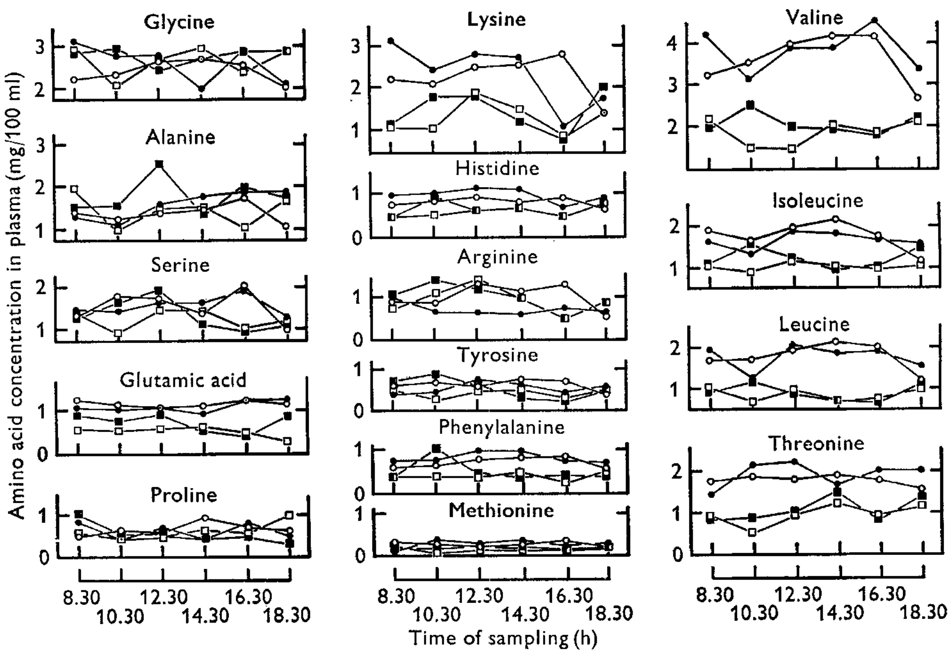

Fig. 1. Expt. 1. Variations throughout a day on 2 successive days in the concentrations of individual amino acids in the blood plasma of a Friesian and of a Jersey cow. Friesian: 1st day, □; 2nd day, ■. Jersey: 1st day, ○; 2nd day, ●.

increased with lactation in the Friesian cows and decreased in the Jersey cows, reflecting the difference observed between breeds while the animals were pregnant.

Effect of plane of energy nutrition. Values for the concentrations of individual amino acids in the plasma of four cows at the two planes of energy nutrition are given in Table 3. Values for the two individual periods of feeding at each plane of energy nutrition did not differ significantly ($P > 0.05$) from each other, and a pooled value is given for each cow. For only one amino acid, glycine, which increased in concentration at the high plane of energy nutrition, was there a consistent change in all the cows. The concentrations of serine and proline were higher and those of isoleucine and phenylalanine lower at the high plane of energy nutrition in three of the four cows.

However, the average effect for all four cows (Fig. 2) was a fall in the concentrations of valine, isoleucine, phenylalanine, tyrosine, leucine, lysine, and methionine, and an increase in the concentrations of alanine, arginine, glutamic acid, glycine, histidine, proline, serine and threonine, a grouping which, with the exception of threonine, conforms to the conventional distinction between 'essential' and 'non-essential' amino acids.

Table 1. *Expt 1: concentrations (mg/100 ml) of amino acids in the blood plasma of three Friesian and three Jersey cows during the 8th month of pregnancy*

(Values are a mean of twelve values)

	Friesian cows				Jersey cows			
Amino acid	No. 1	No. 2	No. 3	SE	No. 1	No. 2	No. 3	SE
Lysine	1·38	1·27	1·26	0·23	1·77	1·66	1·68	0·33
Histidine	0·70	0·66	0·65	0·10	0·66	0·61	0·77	0·16
Arginine	0·99	0·82	0·78	0·15	0·99	1·04	0·79	0·20
Threonine	0·78	0·74	0·70	0·15	0·62	0·68	0·61	0·10
Serine	0·85	0·87	0·82	0·16	1·48	1·40	1·04	0·32
Glutamic acid	0·66	0·71	0·51	0·12	0·75	0·59	0·64	0·15
Proline	0·58	0·43	0·42	0·12	0·83	0·45	0·56	0·21
Glycine	2·28	2·20	1·77	0·31	1·80	2·45	1·22	0·33
Alanine	1·10	1·21	1·11	0·23	1·28	1·12	1·05	0·22
Valine	1·63	1·43	1·26	0·27	2·30	1·95	1·97	0·32
Isoleucine	1·06	0·98	0·86	0·18	1·21	1·14	1·23	0·20
Leucine	1·16	1·10	0·90	0·19	1·29	1·36	1·10	0·15
Tyrosine	0·40	0·47	0·25	0·10	0·39	0·42	0·28	0·36
Phenylalanine	0·49	0·46	0·29	0·08	0·44	0·47	0·31	0·08

Table 2. *Expt 2: percentage changes with lactation in the concentrations (mg/100 ml) of amino acids in the blood plasma of three Friesian and three Jersey cows*

(Figures reported are the differences between mean values for the 8th month of pregnancy and the 6th week of lactation)

	Friesian cows			Jersey cows			Mean value with SE
Amino acid	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	
Lysine	−37·7*	−18·1	−22·2	−42·4	−17·5	−49·4	−31·2 ± 8·6*
Histidine	−28·6	−36·4	−56·9*	−16·7	+1·6	−49·4	−31·3 ± 12·5
Arginine	−23·8	−23·2	−37·2	−45·5	−26·9	−12·7	−28·3 ± 7·1*
Threonine	−28·2	−33·8	−25·7	−21·0	−26·5	−26·2	−26·9 ± 2·5*
Serine	+10·6	+13·8	+69·5	−33·1	−7·1	+27·9	+17·7 ± 14·1
Glutamic acid	−19·7	−33·8	−17·7	−36·0	−5·1	+1·6	−18·0 ± 7·1
Proline	−5·2	+14·0	−5·0	−27·7	+20·0	−5·4	−1·1 ± 5·8
Glycine	−3·1	+37·7	+10·7	+27·2	+34·7	+136·1	+40·1 ± 29·5
Alanine	−25·5	−13·2	−18·0	−1·6	−19·6	+13·3	−10·8 ± 4·9
Valine	+2·5	+60·1*	+17·5	−41·7*	−4·6	−51·3*	−2·9 ± 15·1
Isoleucine	+11·3	+79·6*	+60·5	−36·4	+9·7	−39·0	+11·1 ± 16·7
Leucine	−27·6	+4·6	−20·0	−37·2	−4·4	−34·6	−19·8 ± 8·8
Tyrosine	−22·5	−31·9	+28·0	−5·1	+4·8	−14·3	−6·8 ± 7·1
Phenylalanine	−10·2	−13·4	+38·0	0·0	+2·1	0·0	+2·2 ± 8·9

* $P < 0·05$.

The protein content of the milk (%) was increased significantly ($P < 0·05$) in all the cows at the high plane of energy nutrition. The mean value, with standard error, for all four cows for the two periods at a low plane of energy nutrition was $3·2 \pm 0·06$, and for the two periods at a high plane of energy nutrition $3·5 \pm 0·03$. The average yield of protein at the high plane of energy nutrition was 484 ± 10 g/24 h and at the low plane 346 ± 8 g/24 h.

Effect of intraruminal infusion of propionic acid. Values for the concentrations of

Table 3. *Expt 3: mean values, with their standard errors, for the concentrations (mg/100 ml) of amino acids in the blood plasma of four Jersey cows at two planes of energy nutrition*

Amino acid	Cow 1		Cow 2		Cow 3		Cow 4		SE	Mean percentage change for all the cows $\left(\frac{H-L}{L}\times 100\right)$
	L	H	L	H	L	H	L	H		
Lysine	2·21	1·96	1·75	1·71	1·62	1·95	1·85	1·45	0·18	−3·7
Histidine	0·62	0·76	0·47	0·59	0·49	0·58	0·64	0·45	0·08	+9·2
Arginine	1·07	0·97	0·94	0·86	0·91	1·28	0·92	0·89	0·12	+4·9
Threonine	1·07	1·17	1·12	0·90	1·25	1·22	0·92	1·21	0·13	+4·7
Serine	2·03	1·71	1·57	1·84	1·41	1·97	1·68	2·13	0·14	+17·0
Glutamic acid	0·79	0·47	0·53	0·69	0·47	0·76	0·62	0·47	0·10	+6·8
Proline	0·52	0·78	0·60	0·65	0·66	1·02	0·76	0·64	0·08	+24·3
Glycine	3·73	4·06	3·63	3·73	3·44	4·90	4·44	5·15	0·27	+28·5
Alanine	2·35	1·98	1·86	1·96	2·30	2·35	1·53	2·43	0·18	+9·8
Valine	3·05	2·32	2·21	2·34	1·98	2·44	3·48	2·68	0·20	−4·5
Isoleucine	1·80	1·24	1·26	1·23	1·26	1·71	2·61	1·72	0·11	−8·0
Leucine	1·69	1·16	1·11	1·23	1·17	1·52	1·73	1·26	0·09	−4·5
Tyrosine	0·61	0·41	0·51	0·48	0·52	0·70	0·50	0·45	0·06	−3·5
Phenylalanine	0·72	0·51	0·62	0·57	0·72	0·73	0·78	0·61	0·06	−14·4
Methionine	0·26	0·19	0·24	0·19	0·26	0·28	0·26	0·20	0·03	−15·8

L, low plane of energy nutrition, H, high plane of energy nutrition.

* $P < 0·05$.

individual amino acids in the plasma of each of the two cows during the two periods of water infusion, and during the single period in which propionic acid was infused, are given in Table 4. The concentrations of lysine, histidine, arginine, glycine, valine, isoleucine and leucine were decreased in both cows during the infusion of propionic

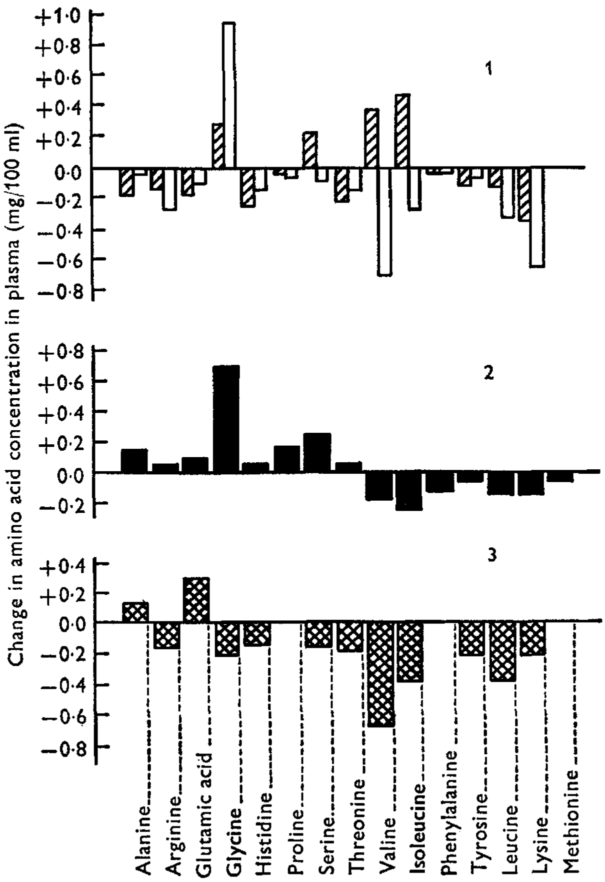

Fig. 2. Expts 2–4. Mean changes in the concentrations of individual amino acids in the blood plasma of cows associated with (1) lactation, (2) an increase in the plane of energy nutrition and (3) intraruminal infusion of propionic acid. □, mean of values for three Jersey cows; ▨, mean of values for three Friesian cows; ■, mean of values for four Jersey cows; ▩, mean of values for one Friesian and one Jersey cow.

acid, and the effects on valine, isoleucine and leucine were significant ($P < 0.05$) in both cows. The levels of tyrosine, serine and threonine were depressed significantly ($P < 0.05$) in one cow only and there was an opposite change or no change in the other animal. Glutamic acid increased markedly ($P < 0.05$) in both cows and there was also a small but not significant ($P > 0.05$) increase in alanine. The changes in proline, phenylalanine and methionine were small and variable.

Infusion of propionic acid was associated with an increase in the yield of protein in milk, of 21% in the Jersey cow and 6% in the Friesian cow.

Table 4. *Expt 4: mean values, with standards errors, for the concentrations (mg/100 ml) of amino acids in the blood plasma of a Friesian and a Jersey cow during the intraruminal infusion of* (1) *water or* (2) *propionic acid*

	Friesian cow			Jersey cow		
Amino acid	(1)	(2)	(2)−(1) as a percentage of (1)	(1)	(2)	(2)−(1) as a percentage of (1)
Lysine	1·38±0·10	1·17±0·05	−15·2	2·24±0·14	1·94±0·37	−13·4
Histidine	0·65±0·04	0·58±0·04	−10·8	0·88±0·04	0·69±0·09	−21·6
Arginine	0·97±0·08	0·86±0·06	−11·3	0·90±0·06	0·69±0·11	−23·3
Threonine	0·93±0·07	0·58±0·04	−37·6*	1·77±0·16	1·94±0·20	+9·6
Serine	1·28±0·08	1·27±0·06	−0·01	1·62±0·09	1·35±0·10	−16·7*
Glutamic acid	0·60±0·05	0·90±0·07	+50·0*	1·00±0·05	1·31±0·06	+31·0*
Proline	0·56±0·06	0·58±0·12	+3·6	0·70±0·06	0·69±0·12	−1·4
Glycine	2·96±0·11	2·75±0·06	−7·1	2·80±0·12	2·52±0·14	−10·0
Alanine	1·63±0·10	1·70±0·13	+4·3	1·55±0·07	1·71±0·08	+10·3
Valine	1·95±0·05	1·58±0·09	−19·0*	3·42±0·16	2·41±0·16	−29·5*
Isoleucine	1·18±0·04	0·89±0·06	−24·6*	1·65±0·06	1·16±0·08	−29·7*
Leucine	0·84±0·04	0·68±0·06	−19·0*	1·58±0·08	0·98±0·10	−38·0*
Tyrosine	0·52±0·05	0·61±0·05	+17·3	0·59±0·04	0·28±0·03	−52·5*
Phenylalanine	0·49±0·06	0·51±0·04	+4·1	0·81±0·05	0·79±0·06	−2·5
Methionine	0·17±0·01	0·18±0·02	+5·9	0·22±0·02	0·20±0·02	−9·1

* $P<0·05$.

DISCUSSION

The results of Expt 1 showed that the plasma concentrations of individual amino acids varied in an irregular manner throughout a day, and from day to day, and against this background variation no clear pattern of change emerged which could be associated with time of sampling. These observations contrast with those of Squibb (1966) who found a pronounced diurnal rhythm in the chick. The absence of a distinct pattern in the present investigation may reflect the more protracted passage of dietary and microbial protein to the hind gut in the ruminant. Purser, Klopfenstein & Cline (1966) reported a 12 h cycle of changes, over a period of 24 h during which no food was offered, in sheep accustomed to feeding twice daily. The previous diet had, however, been provided in pelleted form.

Because of the observed irregularities in the concentrations of individual amino acids, in our later experiments samples of blood plasma were taken on 2 successive days at the end of each treatment period at four fixed intervals after feeding.

The plasma level of each of the various amino acids must be controlled by balances between the entry and exit from the plasma, as holds for the blood sugar level. The main factors contributing to this balance will be the uptake of amino acids from the gut and synthesis within the body on the one hand, and on the other the drain on amino acids for the synthesis of tissue or milk proteins and deamination in the liver and kidney. In Expt 2, the level of feeding was kept approximately constant throughout the experimental period, and the most important change in amino acid requirements between the two sampling occasions would be that resulting from the onset of lactation. There is a significant requirement for amino acids by the foetus in late pregnancy, but the requirement for milk secretion at peak lactation has been estimated by Blaxter (1964) as being three to four times as high. With the exception of glycine in animals of both breeds, and of valine, serine and isoleucine in the Friesian cows, lactation was associated with a fall in the average concentration of all the amino acids in the plasma. This suggests that plasma amino acid levels are sensitive to the high requirement of amino acids for milk protein synthesis during lactation. An increased requirement for glucose resulting in gluconeogenesis from amino acids in the liver may also have increased the drain on the glucogenic amino acids (Ballard, Hanson, Kronfeld & Raggi, 1968). The reason for the increase in glycine level during lactation is not clear. At peak lactation, catabolism of body protein for gluconeogenesis may result in a release of glycine in excess of the needs for milk protein formation, since muscle protein is comparatively rich in glycine—observations with the rat have shown that abnormally high levels of glycine in the diet may produce metabolic disturbances (see, for example, Swendseid, Hickson & Friedrich, 1962) and the capacity to dispose of excess glycine may be limited. The elevation of the plasma level of glycine in lambs whose protein nutrition is suboptimal, reported by Schelling, Hinds & Hatfield (1967), may be a related phenomenon. No satisfactory explanation can be offered for the breed differences in the behaviour of valine and isoleucine which arose mainly from differences in their plasma concentrations in the pregnant animal.

The change in plane of energy nutrition in Expt 3 would be expected to alter the uptake of several products of digestion; the uptake of amino acids, of propionic acid from the rumen and of glucose from the hind gut would probably all be increased, as would the balance of glucogenic to ketogenic materials. The average effect of the improvement in the plane of energy nutrition (Fig. 2) was to increase the plasma level of the group of amino acids whose carbon skeletons may be derived from the general pool of 'glucogenic materials' and which on deamination are in turn glucogenic: arginine, alanine, glutamic acid, glycine, histidine, proline and serine. The plasma level of those amino acids whose carbon skeletons cannot be derived from the general metabolic pool, but on deamination give rise to ketogenic (lysine, leucine) or glucogenic (threonine, valine) materials, or both (isoleucine, tyrosine, pheylalanine) was depressed. It is unlikely that these effects are a reflection simply of the change in the balance of glucogenic to ketogenic materials. Whilst such a change could account for the increased levels in the plasma of certain of the glucogenic amino acids owing to a 'sparing' of the acids, or to an increased synthesis of the non-essential amino acids, a reduction in the levels of the ketogenic amino acids would be expected only if their uptake from the gut were reduced. A more probable explanation of the decrease in plasma levels of the second group of amino acids is the increased drain on those amino acids resulting from the increased milk protein synthesis at the higher plane of energy nutrition; the acids which showed the most marked fall are those present in milk protein in highest concentration: valine, isoleucine, leucine and lysine.

Throughout Expt 4, the composition of the diet was unaltered and the only major change expected during the experimental period would be an increased uptake of propionic acid, if the possibility of a direct effect of the addition of propionic acid on digestion within the rumen is ignored. Plasma levels of glutamic acid and of alanine showed a consistent increase in response to the infusion of propionic acid, but all the other acids showed little change or a decrease. Again the decrease in concentration of valine, isoleucine, leucine and lysine was especially marked, and this gives further support to the suggestion that the lowered plasma concentration of the second group of amino acids reflects an increased uptake for milk protein secretion. The differences between Expts 3 and 4 in the effects on amino acids of the first group except glutamic acid and alanine are, however, more difficult to explain. It may be that in Expt 3 at the low plane of energy nutrition there was an extensive demand for gluconeogenesis to satisfy the requirements of the whole animal for glucose, and the changes in the plasma levels of the first group of amino acids with improvement in energy nutrition may reflect a reduced use of these amino acids for gluconeogenesis. In Expt 4, on the other hand, the need during the control period for gluconeogenesis from amino acids may have been more limited, and the results may reflect mainly a net synthesis of certain of the amino acids in the presence of a surplus of glucogenic materials. Such an interpretation would, however, imply some restriction on 'interconvertibility' within the first group of amino acids since the level of glutamic acid rose while the level of some of the other acids fell.

Though it is possible to explain certain of the observed changes in plasma amino acid levels in Expts 3 and 4 in terms of an alteration in the requirements for milk protein synthesis, there is no clear indication from the present results as to the cause of the response in milk protein synthesis to the imposed experimental conditions. For milk protein synthesis to proceed, the mammary gland must be supplied by the plasma with all the amino acids in the second group, and with either all of the amino acids in the first group or with a surplus of glucogenic materials and a source of amino nitrogen. Little precise information is available concerning the ability of the gland to interconvert the amino acids of the first group or to form them from glucogenic sources, or indeed about the overall nitrogen balance of the gland, and it is possible that some gluconeogenesis may occur within the gland to satisfy its large requirements for glucose. Some urea is produced by the gland (Mepham & Lindzell, 1966) but this may represent merely disposal of surplus amino groups from basic amino acids taken up in excess and not net gluconeogenesis. The results of Mepham & Linzell (1966) suggest that the uptake of certain of the acids of the first group by the gland does not always match the output in the milk, at least in the goat, and that synthesis *de novo* or interconversion must occur, but this situation could change with an alteration in dietary circumstances. There is other evidence (Rook, Storry & Wheelock, 1965) that synthesis of milk protein, in contrast to that of milk lactose, is not sensitive to plasma glucose concentration. It is tempting, therefore, to suggest that the specific increases in milk protein content which occur in response to an improvement in the plane of energy nutrition or to intraruminal infusion of propionic acid, arise from an increased supply of certain of the amino acids of the first group to the mammary gland, there being in this tissue some restriction upon amino acid formation from glucose and on interconvertibility among the first group of amino acids. The major amino acid of milk proteins is glutamic acid, and the plasma concentration of this acid was specifically increased in response to infusion of propionic acid. However, no consistent increase in plasma glutamic acid level was observed in Expt 3, but there was a significant increase in plasma proline concentration. It may be that only certain of the glucogenic amino acids, glutamic acid and proline in particular, can contribute to the 'glutamic acid' pool within the mammary gland, and that an increase in the concentration of any or all of these in the plasma increases the pool size and the rate of synthesis of milk protein.

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