CHANGES IN PLASMA AMINO ACID PATTERNS IN SHEEP ASSOCIATED WITH SUPPLEMENTS OF CASEIN AND FORMALDEHYDE-TREATED CASEIN

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Summary

Changes in plasma amino acids were measured in sheep in response to supplements of 80–100 g/day of formaldehyde-treated casein in the diet, untreated casein in the diet, or untreated casein per abomasum.

There was a small increase in the concentration of plasma amino acids when treated casein was fed and the total concentration was approximately doubled when casein was given per abomasum. Both supplements caused large increases in the concentration of the branched-chain amino acids. Treated casein in the diet and casein per abomasum caused similar changes in the proportions of amino acids in plasma; the proportion of glycine was markedly decreased and most essential amino acids were increased in proportion, especially valine, leucine, isoleucine, and phenylalanine. In contrast, untreated casein in the diet did not increase the concentration of amino acids in plasma, and had little effect on the proportions of most essential amino acids. The results suggest that formaldehyde-treated casein in the diet is of similar nutritional value to casein given per abomasum.

The feeding of formaldehyde-treated casein resulted in a sixfold increase in the amount of an unidentified component in plasma, which eluted between lysine and histidine. The component was not increased by the other forms of casein supplementation.

I. Introduction

Casein supplements administered directly into the abomasum of sheep produce substantial increases in wool growth rate and body weight gain (Reis and Schinckel 1964; Reis 1969). In contrast, casein included in the diet is readily degraded in the rumen and is of little value for wool growth (Reis 1969). Treatment of casein with formaldehyde protects it from degradation in the rumen, with the result that inclusion of formaldehyde-treated (treated) casein in the diet leads to stimulation of wool growth (Ferguson, Hemsley, and Reis 1967). It has also been demonstrated that treated casein is equivalent to casein infused into the abomasum for promoting wool growth and nitrogen retention (Reis and Tunks 1969). Treated casein in the diet presumably acts similarly to casein given per abomasum by supplying amino acids for absorption from the intestines.

Hogan, Weston, and Lindsay (1968) have shown that there are substantial increases in the concentration of most essential amino acids in sheep plasma and a decrease in glycine during abomasal supplementation with casein. Consequently, it should be possible to obtain useful evidence regarding the metabolic fate of treated casein by measuring effects on plasma amino acids. Accordingly, changes in plasma amino acid patterns have been measured in response to supplements of treated casein

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in the diet, untreated casein in the diet, and casein given via the abomasum. The plasma samples were collected during the experiments reported by Ferguson, Hemsley, and Reis (1967) and Reis and Tunks (1969).

II. Experimental

(a) *Sheep and Diet*

The experimental sheep were mature Merino or English Leicester × Merino wethers, and were kept indoors in individual pens or metabolism cages. They were fed once daily, between 9 and 10 a.m. During control periods the sheep received 800 g/day of a diet consisting of equal parts of chopped wheaten and lucerne hays. During treatment periods this ration was supplemented with 80–100 g/day of casein (commercial grade, hydrochloric acid-precipitated). There were three types of casein supplement: formaldehyde-treated in the diet, untreated in the diet, and untreated given via the abomasum. Treated casein was prepared as described by Ferguson, Hemsley, and Reis (1967), and casein supplements into the abomasum were given over a period of 8–10 hr each day, commencing when the sheep were fed (Reis and Tunks 1969).

(b) *Experimental Plans*

Full details of the two experiments, from which the blood samples were derived, are given in papers by Ferguson, Hemsley, and Reis (1967) and Reis and Tunks (1969).

In experiment 1 (Reis and Tunks 1969) four sheep received about 100 g/day of casein in three forms, untreated and treated in the diet and untreated per abomasum; the supplements were each provided for 7 weeks. A blood sample was collected from each sheep during the fifth and seventh week of each supplementation period; two control samples were also collected, one before and one 10 weeks after the experiment. A fifth sheep was sampled on the same eight occasions while receiving the control diet only.

In experiment 2 (Ferguson, Hemsley, and Reis 1967) two groups of sheep received 80 g/day of treated or untreated casein in the diet. Blood samples from the four sheep in each group were bulked for analysis. Two blood samples were taken from one group, approximately 1 week apart, at the end of an 18-week supplementation period with 80 g/day treated casein in the diet; a third sample was taken several months later on the control diet. Three blood samples were taken similarly from a second group receiving 80 g/day untreated casein in the diet for 9 weeks. Samples were also collected, at the same times, from a third group receiving the control diet only.

(c) *Amino Acid Analysis*

Blood samples were collected from the jugular vein approximately 5 hr after the sheep were fed. The collection of blood and the preparation of plasma for analysis was carried out as described by Hogan, Weston, and Lindsay (1968). 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 a gradient elution system based on that of Efron (1966), modified to give adequate resolution in 16 hr. With this system tryptophan was not analysed and proline did not separate consistently. Amino acid concentrations were calculated by reference to norleucine, used as an internal standard. Methionine concentrations include methionine sulphoxide and sulphone, when present.

III. Results

(a) *Changes in the Concentration of Plasma Amino Acids in Response to Casein Supplements*

Changes in the concentration of 17 amino acids in plasma, in response to various casein supplements given during experiment 1, are shown in Table 1. Each value is the mean of eight analyses, comprised of two samples from each of four sheep. Untreated casein in the diet did not alter total plasma amino acids, whereas there was a moderate increase with treated casein in the diet and a large increase with casein given per abomasum. Proline was only estimated satisfactorily in about half the samples and the values are not included in the table. However, the mean values obtained (μmoles/100 ml) indicated large effects with casein per abomasum (81) and treated casein in the diet (47), compared with untreated casein in the diet (15) and control values (18). Plasma samples taken on eight occasions from a sheep receiving only the control diet gave results similar to the control values listed in Table 1; total amino acids were 165 (S.E. ±6) μmoles/100 ml.

Table 1

EFFECT OF CASEIN SUPPLEMENTS ON CONCENTRATION OF AMINO ACIDS IN PLASMA

Plasma samples are derived from experiment 1 (Reis and Tunks 1969). Casein supplements provided approx. 100 g protein per day. Casein treatment with formaldehyde was as described by Ferguson, Hemsley, and Reis (1967). Values are μ moles/100 ml plasma. Samples were taken from each of four sheep on two separate occasions, and each value is a mean of eight determinations ± S.E., representing within- and between-sheep variances. All sheep received 800 g/day of a diet of equal parts chopped wheaten and lucerne hays

Amino Acid	Form of Casein Supplementation			
	Nil	Untreated in Diet	Treated in Diet	Untreated per Abomasum
Glycine	44·9± 4·2	28·2±2·7	28·5±1·2	42·1± 1·8
Serine	17·1± 3·7	14·6±1·8	13·3±0·9	25·2± 2·0
Alanine	15·8± 1·2	13·1±0·5	14·6±0·7	18·4± 1·2
Ornithine	11·0± 1·2	8·2±0·6	11·5±0·8	20·6± 1·2
Glutamic acid	7·7± 1·6	6·2±0·4	5·4±0·3	9·0± 0·6
Tyrosine	4·4± 0·6	6·0±0·5	8·6±0·7	19·6± 1·9
Aspartic acid	4·7± 1·0	3·0±0·2	4·1±0·3	5·0± 0·4
Threonine	16·9± 3·4	27·1±2·0	24·4±2·0	37·0± 3·4
Lysine	7·5± 0·8	7·9±0·4	10·2±0·4	24·8± 2·0
Arginine	6·8± 0·6	9·3±0·5	8·7±0·4	12·9± 1·2
Histidine	5·0± 0·4	5·6±0·2	7·2±0·3	8·9± 0·6
Valine	11·5± 1·2	16·6±0·8	29·1±1·4	50·0± 3·3
Leucine	6·1± 0·6	7·0±0·3	16·5±0·8	36·9± 3·5
Isoleucine	4·9± 0·5	5·6±0·3	9·4±0·4	19·0± 1·0
Phenylalanine	2·1± 0·5	3·3±0·3	5·3±0·3	13·6± 1·4
Cystine (half)	2·2± 0·4	2·6±0·3	4·2±0·3	5·8± 0·4
Methionine	1·3± 0·2	2·3±0·3	3·0±0·4	8·6± 1·1
Total	170±18	167±8	204±8	358±17

Casein infused into the abomasum resulted in large increases in the concentration of the branched-chain amino acids (valine, leucine, isoleucine) in plasma; treated casein in the diet followed this pattern but untreated casein in the diet had only a small effect. The total concentration of essential amino acids (the last 10 amino acids in Table 1) in plasma (μmoles/100 ml) was 64 with no casein supplement, 87 with untreated casein in the diet, 118 with treated casein in the diet, and 218 with casein given per abomasum.

Similar results were obtained in experiment 2 when treated casein was added to the diet of a group of sheep; total plasma amino acids were increased from 176 to

201 μmoles/100 ml. However, untreated casein in the diet lowered total plasma amino acids from 176 to 130 μmoles/100 ml.

(*b*) *Changes in the Proportions of Plasma Amino Acids in Response to Casein Supplements*

Figure 1(*a*) shows each amino acid as a percentage of the total plasma amino acids for sheep receiving the control diet alone or supplemented with casein, either per abomasum or treated in the diet. The figure is derived from data presented in Table 1. Despite the much higher concentrations of amino acids when casein was given per abomasum (Table 1), the patterns of amino acids obtained in plasma with the two forms of casein supplementation were similar [Fig. 1(*a*)]. The casein supplements caused a substantial decrease in the proportion of glycine and slightly lower proportions of other non-essential amino acids, except tyrosine. All essential amino acids were increased in proportion, except arginine and histidine; the largest increases were with valine, leucine, isoleucine, and phenylalanine. Similar changes in the proportions of plasma amino acids were observed with supplements of treated casein in experiment 2 [Fig. 1(*b*)].

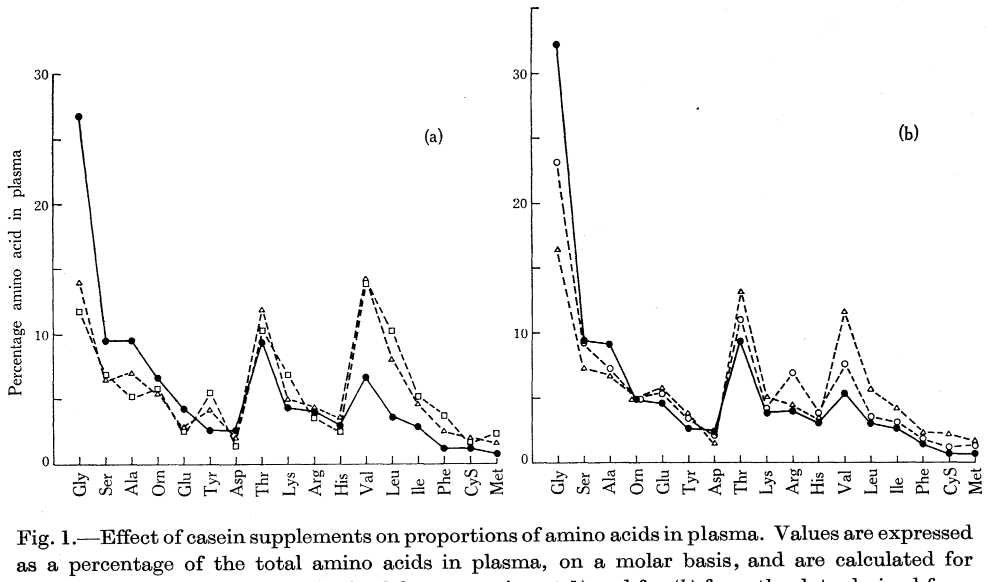

Fig. 1.—Effect of casein supplements on proportions of amino acids in plasma. Values are expressed as a percentage of the total amino acids in plasma, on a molar basis, and are calculated for (*a*) from the data in Table 1 (obtained from experiment 1) and for (*b*) from the data derived from experiment 2. Casein supplements shown as: ● nil; △ treated in the diet; □ untreated per abomasum [(*a*) only]; ○ untreated in the diet [(*b*) only].

Untreated casein in the diet had a less marked effect on the proportions of amino acids in plasma than did treated casein in the diet or casein per abomasum. For simplicity, results are shown only for experiment 2 [Fig. 1(*b*)]. The proportions of essential amino acids were little changed except for increases in valine and threonine; also, in contrast to the other casein supplements, the proportion of arginine was increased with untreated casein.

Changes in the proportions of essential amino acids due to casein supplementation (expressed as a percentage of the total essential amino acids) are shown in Table 2. The patterns of essential amino acids obtained with treated casein and casein per abomasum were similar, but were different from that obtained with the control diet.

Table 2

EFFECT OF CASEIN SUPPLEMENTS ON PROPORTIONS OF ESSENTIAL AMINO ACIDS IN PLASMA

Values are expressed as a percentage of the total essential amino acids in plasma. Essential amino acids are regarded as the 10 listed. The values are calculated from the data in Table 1, where experimental details are given

Amino Acid	Form of Casein Supplementation			
	Nil	Untreated in Diet	Treated in Diet	Untreated per Abomasum
Threonine	26·3	31·0	20·7	17·0
Lysine	11·7	9·0	8·6	11·4
Arginine	10·6	10·6	7·4	5·9
Histidine	7·8	6·4	6·1	4·1
Valine	17·9	19·0	24·7	23·0
Leucine	9·5	8·0	14·0	17·0
Isoleucine	7·6	6·4	8·0	8·7
Phenylalanine	3·3	3·8	4·5	6·2
Cystine (half)	3·4	3·0	3·6	2·7
Methionine	2·0	2·6	2·5	4·0
Total branched-chain amino acids	35·0	33·4	46·6	48·7

In particular, the proportions of branched-chain amino acids were markedly increased whereas threonine was decreased. In contrast, the pattern of essential amino acids obtained with untreated casein in the diet was similar to the control pattern.

Table 3

EFFECT OF CASEIN SUPPLEMENTS ON AMINO ACID RATIOS IN PLASMA

The ratios are calculated from the data in Table 1, where experimental details are given

Casein Supplement	Ratio of Glycine to Valine ±S.E.	Ratio of Glycine to Branched-chain Amino Acids ±S.E.
Nil	3·96±0·20	2·03±0·11
Untreated in diet	1·69±0·14	0·95±0·08
Treated in diet	0·99±0·04	0·54±0·02
Untreated per abomasum	0·86±0·06	0·40±0·01

Because the proportion of glycine was reduced in plasma and that of valine was increased during casein supplementation, the ratio (glycine : valine) was markedly altered. Likewise, the ratio (glycine : branched-chain amino acids) changed during supplementation. There was a four- to fivefold decrease in both ratios due to supplementation with treated casein in the diet or with casein per abomasum; untreated casein in the diet resulted in a smaller decrease in the ratios (Table 3). The similarity of the effects of treated casein and casein per abomasum should be noted.

(c) *Increases in an Unidentified Plasma Component in Response to Formaldehyde-treated Casein*

In all plasma samples analysed a small amount of an unidentified component, which is eluted between lysine and histidine, was consistently found. The normal amount of this component in plasma was $4 \cdot 4 \pm 1 \cdot 0$ (S.E.), and was unaffected by supplements of untreated casein in the diet ($4 \cdot 2 \pm 1 \cdot 0$) or casein per abomasum ($3 \cdot 5 \pm 1 \cdot 0$) in experiment 1, but was increased sixfold (to $26 \cdot 9 \pm 3 \cdot 2$) in plasma from sheep receiving treated casein in the diet, these values being expressed as norleucine equivalents per 100 ml plasma (and probably approximate to micromoles/100 ml of plasma). In experiment 2, a large amount of this component was also found in the plasma from the sheep which received treated casein in the diet. The values, expressed in the same units as above, were: treated casein in diet (27·3), untreated casein in diet (3·2), control (5·5).

IV. Discussion

The large increases in the concentration of plasma amino acids, especially of some essential amino acids, during casein supplementation per abomasum confirm the results of Hogan, Weston, and Lindsay (1968). The term essential amino acids is used here to refer to those amino acids usually regarded as essential for the growing animal, as the amino acids essential for wool growth cannot be defined. Cyst(e)ine is included with the essential amino acids because of its special importance for wool growth (Reis and Schinckel 1963; Reis 1967). Arginine is included in accord with Hogan, Weston, and Lindsay (1968), even though it may not be an essential amino acid for sheep (Downes 1961).

Casein supplements per abomasum resulted in much greater increases in the concentration of plasma amino acids than did supplements of treated casein in the diet. However, on the basis of changes in the proportions of amino acids, treated casein in the diet and casein per abomasum had similar effects. Higher concentrations of amino acids would be expected with abomasal supplements as plasma samples were taken about 5 hr after the beginning of an 8-hr infusion period, whereas treated casein would presumably lead to an increased flow of amino acids to the intestines over a more extended period. The apparent equivalence of the two forms of casein supplement supports the hypothesis that treated casein releases amino acids in the abomasum and small intestine (Ferguson, Hemsley, and Reis 1967; Reis and Tunks 1969). The above conclusions apply only to casein that has been effectively treated with formaldehyde; it is possible to produce both under-treated and over-treated casein (Hemsley, Downes and Reis, unpublished data). The much smaller changes, especially in essential amino acids, obtained with untreated casein in the diet is in agreement with its poor utilization for wool growth and nitrogen retention (Ferguson, Hemsley, and Reis 1967; Reis 1969; Reis and Tunks 1969).

Plasma samples were all collected approximately 5 hr after the sheep were fed and after the beginning of abomasal infusions. Diurnal variations have been observed in plasma amino acid concentrations with sheep fed once daily (Leibholz 1965; Theurer, Woods, and Poley 1966), but these are unlikely to influence the comparisons made here as all samples were collected at comparable times.

The high proportion of glycine in plasma agrees with other published values for sheep (Theurer, Woods, and Poley 1966, 1968; Little, Mitchell, and Potter 1968; Hogan, Weston, and Lindsay 1968). A decrease in the proportion of glycine in plasma was also observed by Hogan, Weston, and Lindsay (1968) during the abomasal administration of casein and with high protein intakes from roughage diets. A comparison of the ratios (glycine : valine) or (glycine : branched-chain amino acids) may be a useful index of protein nutritional status because glycine is readily decreased in response to an improved supply of amino acids and the branched-chain amino acids are substantially increased in both concentration and proportion. The large increases in the branched-chain amino acids in plasma during supplementation with casein per abomasum or treated casein in the diet can be explained on the basis of the relatively high content of branched-chain amino acids in casein and the fact that these amino acids are metabolized relatively slowly by the liver (Elwyn 1968; Shoemaker and Elwyn 1969).

The physiological significance of the unidentified component in plasma is not known. However, high concentrations were associated only with the feeding of formaldehyde-treated casein; administration of casein into the abomasum had no effect on the amount in plasma. It has also been observed (the authors, unpublished data) that plasma from sheep receiving formaldehyde-treated cottonseed meal contained three times as much of this component as did plasma from sheep receiving untreated cottonseed meal. Identification of the component has not been undertaken and it is possible that the large increase observed with treated casein is due to a compound, different from that found in control samples, but which elutes in the same position. However, no separation of the peak into more than one component has been observed in any sample.

An increased amount of a plasma component eluting near lysine has also been observed in Romney sheep in New Zealand, receiving formaldehyde-treated casein (Dr. I. E. B. Fraser, personal communication). This component is presumably the one observed in the present study. Recently, Perry, Diamond, and Hansen (1969) have identified ϵ-N-methyl lysine in human plasma, which eluted between lysine and histidine in their system. As the ϵ-amino group of lysine reacts readily with formaldehyde to form methylol groups (Walker 1964), this lysine derivative may be produced following formaldehyde treatment. We have tested ϵ-N-methyl lysine in our system and it elutes in the same position as the unidentified component. Further work is needed to establish the nature, origin, and possible biological importance of this compound.

V. References

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