Nitrogen metabolism in calves

Effect of giving different amounts of dietary casein with and without formaldehyde treatment

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1. Calves were given a basal diet of straw and flaked maize (12 g nitrogen/kg dry matter (DM)) or diets with some flaked maize replaced by untreated (UT) casein or formaldehyde-treated (FT) casein to give 19, 26 or 34 g N/kg DM.

2. At all intakes rumen ammonia concentrations were lower and amounts of total-N, non-ammonia-N and amino acid-N entering the duodenum were higher when FT-rather than UT-casein supplements were given.

3. Direct measurement of casein entering the duodenum indicated that giving FT rather than UT casein led to much greater amounts of dietary casein escaping degradation in the rumen (70–90% compared to 10–20%). Calculated values for fermentable N indicated that with this low degradability diets containing FT-casein would have provided inadequate N for maximum microbial synthesis in the rumen, and this probably accounted for the marked reduction in amounts of non-casein-N entering the duodenum when FT rather than UT casein was given.

4. Amino acid patterns in duodenal digesta samples after giving the basal diet or diets containing UT-casein were similar. Giving diets containing FT-casein led to changes in this pattern which could sometimes, although not always, be accounted for by estimated differences in proportions of dietary and microbial proteins.

5. At the highest level of N intake FT-casein-supplemented diets led to significantly higher concentrations of most essential amino acids and lower concentrations of most non-essential amino acids in plasma than did UT-casein-supplemented diets. Plasma urea concentrations increased with increasing N intake but were not significantly different for UT- and FT-casein-supplemented diets.

The over-all effect on the nitrogen nutrition of the ruminant of treating dietary protein to protect it from degradation in the rumen is made up of a number of factors. These include changes in the amounts of ammonia absorbed from the alimentary tract, changes in the absolute or relative amounts, or both, of dietary and microbial amino acids (bound or free) entering the duodenum, and changes in the digestibility of these amino acids in the small intestine. Quantitative information on these changes and on factors influencing them is scanty, and even for casein, which has been studied most frequently as a model protein, current views are based to an appreciable extent upon plausible supposition rather than upon experimentally determined facts. What is certain is that amounts of non-ammonia-N and amino acid-N entering the ruminant small intestine may be increased by treating dietary casein with formaldehyde (Offer, Evans & Axford, 1971; MacRae, Ulyatt, Pearce & Hendtlass, 1972; Hagemeister & Pfeffer, 1973; Faichney, 1974*a*; Sharma, Ingalls & Parker, 1974) but the exact mechanisms responsible for these increases are far from clear. The low concentrations of ammonia in the rumen found with formaldehyde-treated(FT)- compared to untreated (UT)-casein diets (Barry, 1972; Hagemeister & Pfeffer, 1973) implies that FT

casein is more slowly degraded than UT casein in that organ. This indicates that the greater flow of non-ammonia-N into the duodenum with the former supplement may be due, in part, to greater amounts of dietary casein reaching this site. Such an effect has, however, rarely been found directly and there is little information on the extent to which other factors may be involved. Hagemeister & Pfeffer (1973) reported a greater increase in non-bacterial-N flow (estimated by difference after assessing bacterial-N flow by measuring α,ϵ-diaminopimelic acid (DAP) in the distal duodenum (i.e. below the points of entry of the pancreatic and bile ducts) in two cows given FT casein rather than UT casein. Sharma *et al.* (1974) found a similar effect with calves fistulated in the abomasum. However, the use of DAP as a microbial marker in these studies meant that any protozoal contribution to the digesta was ignored. This, in conjunction with the facts that some non-casein protein was also present in the diets, and that for the site studied by Hagemeister & Pfeffer (1973) undetermined and probably considerable amounts of endogenous secretions were present, meant that quantitative estimation of residual casein-N by these methods was not possible. Even greater uncertainty exists about the ways in which protection of casein influences its digestibility and the availability of its constituent amino acids. Thus although it has often been found that protection of dietary casein with formaldehyde may lead to increased wool growth in sheep (e.g. Ferguson, Hemsley & Reis, 1967; Reis & Tunks, 1969), presumably by leading to an increase in amino acid (particularly sulphur amino acid) supply, the ways in which this increase is mediated are unclear. In addition, as will be discussed later, the few published reports on the effects of protein protection on plasma amino acid (PAA) and plasma urea (PU) concentrations do not present a consistent picture.

In the present work a method for determining residual casein in digesta directly (McDonald & Hall, 1957) has been used in a study of the origin, amounts and composition of the nitrogenous compounds reaching the duodenum of the ruminating calf given different dietary supplements of UT or FT casein. Effects of these supplements on rumen ammonia, PAA and PU concentrations have also been studied. Preliminary reports of part of the work have been published (Williams, McAllan & Smith, 1973; Williams & Smith, 1975).

EXPERIMENTAL

Animals and feeding

Five castrated male Friesian calves, which had been weaned at 5–8 weeks onto a normal calf-rearing mixture and hay, were used in these experiments. Operations were performed at 8–15 weeks of age, when the calves were fitted with a rumen cannula i.d. 38 mm) made of Corosite (W. H. Uhlhorn & Co. Ltd, Featherstone St., London EC1) and a single T-piece cannula (i.d. 11 mm) made of Kematal (ICI Plastics Division Ltd, Welwyn Garden City, Herts.) in the proximal duodenum. Periods of at least 3 weeks after the operation, and at least 10 weeks after weaning were allowed before the experiments were begun. During the experimental periods the calves were given a basal diet (diet A) of straw and flaked maize containing 12 g N/kg dry matter (DM) and providing an energy intake for a growth rate of about 0·4 kg/d, or diets with

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

	Diets						
Component	A	B	C	D	E	F	G
Straw	1·30	1·30	1·30	1·30	1·30	1·30	1·30
Flaked maize	1·56	1·45	1·45	1·45	1·45	1·45	1·45
Casein*	—	0·15	—	0·30	—	0·54	—
Formaldehyde-treated casein†	—	—	0·15	—	0·30	—	0·54

*Lactic acid–casein (Glaxo Laboratories Ltd, Greenford, Middx.).
† Prepared by the low-volume method of Hemsley, Reis & Downes (1973) at a level of 10 g formaldehyde/kg protein.

some flaked maize replaced by either UT or FT casein (lactic acid–casein; Glaxo Laboratories Ltd, Greenford, Middx.) to give different N contents (19, 26 or 34 g N/kg DM) (diets B, C, D, E, F and G); details of these diets are given in Table 1. Diets were given in the order A, B, C, D, E, F, G, G, F except for two calves in which they were given in reverse order. Concentrates, which were given in two equal amounts at 10.00 and 17.00 hours, also contained a vitamin and mineral supplement. The straw was given at 17.00 hours only. At least 12 d were allowed between changing a diet and taking samples. Shredded paper impregnated with chromic oxide (kindly supplied by Dr J. F. D. Greenhalgh, Rowett Research Institute, Aberdeen) was added to the concentrates, as a non-absorbable marker, to give a daily intake of Cr_2O_3 of 1·9 g/kg DM intake. The FT casein used in these experiments was prepared by the low-volume procedure of Hemsley, Reis & Downes (1973) at a level (10 g formaldehyde/kg protein) which had been found to markedly reduce ammonia production in the rumen without seriously affecting the nutritive value of the casein (Williams *et al.* 1973).

Sampling of digesta and blood

Digesta samples were taken on days 12 and 14 of each dietary period. Rumen digesta samples were taken 4 h after feeding by inserting a tube (i.d. 15.0 mm) into the rumen and withdrawing about 50 ml digesta by pump. The tube was moved to a new position, another 50 ml taken and the process repeated until about 500 ml digesta had been collected. Coarse food particles were removed by straining through surgical gauze before subsampling. Digesta samples from the duodenum were taken before the morning feed and at 2, 4 and 6 h after feeding, and were obtained by unstoppering the cannulas and waiting for the gushes of digesta. About 200 ml digesta were generally obtained within 20 min. The samples were collected in vessels surrounded by ice and were homogenized in an Atomix blender (Measuring & Scientific Instruments Ltd, 25–28 Buckingham Gate, London SW1) before subsampling and storing at −20° until analysed. Blood samples were taken from the jugular vein 4 h after the morning feed on day 13 of each treatment and were treated as described by Williams & Smith (1974).

Analytical procedures

Food components and digesta. Duodenal digesta samples were freeze-dried before analysis. Total N was determined by a micro-Kjeldahl method (Smith & McAllan, 1970). DM content was estimated after heating at 105° for 24 h. Amino acids and chromium were determined as described previously (Williams & Smith, 1974). Ornithine and ε-N-methyllysine, which were found in the plasma of sheep given FT casein by Reis & Tunks (1970) and Fraser & Haden (1970) were not resolved from lysine under our conditions and may therefore have led to over-estimation of this amino acid. Ammonia, which included un-ionized ammonia and ammonium ions, was determined by the procedure of Conway (1957) using boric acid in the central well and titrating with hydrochloric acid. Casein-N in duodenal digesta was estimated by a modification of the method of McDonald & Hall (1957) which is based on casein being a phosphoprotein. In this modification the entire sample, after dialysis, was hydrolysed with 1·4 M-sodium hydroxide–0·3 M-sodium chloride for 24 h at 37°. This modification was introduced because both the UT and FT casein used in the present experiments, were much less soluble in the liquid phase of the duodenal digesta than the light, white soluble casein (British Drug Houses Ltd, Poole, Dorset) used by McDonald & Hall (1957), and were therefore associated with the solid phase of the digesta. To compensate for the presence in the solid phase of substances, other than casein, which might yield inorganic phosphate on alkaline hydrolysis, samples of duodenal digesta from calves given diet A (basal) were treated in the same way. After hydrolysis the samples were filtered, neutralized and the inorganic phosphate content estimated using an AutoAnalyzer (Technicon Instruments Co. Ltd, Basingstoke, Hants), and using the method of Technicon Instruments Corporation (1967). A dialyser was incorporated into the AutoAnalyzer so that it was unnecessary to deproteinize the sample chemically as described by McDonald & Hall (1957). UT and FT casein added to duodenal digesta were quantitatively recovered.

Estimation of the amounts of constituents flowing past the duodenum. These were calculated from the constituent: Cr_2O_3 values in the samples of duodenal digesta and the total intakes of Cr_2O_3, using mean values for samples taken 0, 2, 4 and 6 h after feeding. It is recognized that Cr_2O_3 and some digesta components may move at different rates from the rumen to the abomasum and intestines. Nevertheless it has been found in comparable experiments, for calves fed similarly, that results for total-N and non-ammonia-N recoveries calculated from samples taken at 2 h intervals over periods of 6 h are closely similar to values based on samples taken at 2 h intervals over 24 h (A. B. McAllan, personal communication).

Statistical analysis. Within each level of feeding the mean effect of treatment was tested against a standard error based on the variation in the treatment effect in individual calves.

RESULTS

Effect of formaldehyde treatment of casein on the concentrations of ammonia in the rumen. Ammonia concentrations in the rumen 4 h after feeding are given in Table 2.

Table 2. *Mean quantities (g/24 h) of various nitrogenous constituents present in the food and entering the small intestines of calves given the basal diet (diet A) or diets containing increasing amounts of either untreated (UT) (diets B, D and F) or formaldehyde-treated (FT) casein (diets C, E and G), and mean concentrations (g/l) of ammonia-nitrogen in samples of rumen fluid taken 4 h after the morning feed*

(Mean values with their standard errors for amounts leaving the duodenum were calculated from nitrogenous constituent: chromic oxide values in samples of duodenal digesta taken 0, 2, 4 and 6 h after feeding, and total intakes of Cr_2O_3. Values are for five calves given diet A and for three calves given diets B, C, D, E, F or G. For diets B, C, D, E, F and G mean values with standard errors of the differences between the mean are given)

Diets†	A		B	C	Treatment difference (C−B)	SE of (C−B)	D	E	Treatment difference (E−D)	SE of (E−D)	F	G	Treatment difference (G−F)	SE of (G−F)
Casein supplement ...	—		UT	FT			UT	FT			UT	FT		
	Mean	SE												
N in food	27·7	—	46·2	46·2	—	—	70·1	70·1	—	—	101·9	101·9	—	—
N at duodenum	25·4	0·4	41·6	44·7	3·1 NS	1·5	58·2	61·6	3·4 NS	1·5	62·2	80·9	18·7 NS	8·5
Non-ammonia-N at duodenum	21·4	0·3	30·7	37·5	6·8*	0·7	42·7	48·1	5·4 NS	1·3	49·4	70·2	20·8*	4·7
Amino acid-N in food	19·5	—	35·2	35·2	—	—	54·5	54·5	—	—	80·8	80·8	—	—
Amino acid-N at duodenum:														
Essential	9·4	0·2	13·8	16·2	2·4**	0·1	18·6	23·4	4·8*	0·6	21·3	33·4	12·1*	2·2
Non-essential	8·3	0·2	12·8	15·2	2·4**	0·1	17·2	22·4	5·2*	0·9	19·0	30·1	11·1 NS	3·1
Casein-N in food	—	—	19·1	19·1	—	—	41·2	41·2	—	—	72·7	72·7	—	—
Casein-N at duodenum	—	—	3·8	17·2	13·4**	0·6	5·7	28·7	22·9*	2·6	7·3	53·8	46·5*	9·7
Non-casein, non-ammonia-N at duodenum	—	—	26·9	20·3	−6·7**	0·5	37·0	19·4	−17·6*	2·4	42·1	16·4	−25·7 NS	6·0
Ammonia-N in rumen fluid	6·1	1·4	15·9	4·6	−11·3 NS	9·4	91·1	8·9	−82·2 NS	38·1	208·2	11·1	−197·1**	19·6

NS, not significant.
* $P < 0.05$, ** $P < 0.01$.
† For details, see Table 1.

Table 3. *Concentrations (g/kg total amino acids) of amino acids in casein, bacterial protein and duodenal contents of three calves given diets containing either untreated casein (diet D) or formaldehyde-treated casein (diet E) to give 26 g N/kg dry matter*

Amino acids	Casein	Bacterial protein§	Duodenal contents: Diet D†	Duodenal contents: Diet E†	Duodenal contents: Treatment difference (E−D)	Duodenal contents: SE of (E−D)
Threonine	40	53	48·6	48·0	−0·6*	0·1
Valine	64	68	49·9	55·9	5·9*	1·3
Methionine	28	21	19·1	16·4	−2·7*	0·5
Cystine	7	9	16·0	11·0	−4·9**	0·3
Isoleucine	50	66	50·0	47·9	−1·1 NS	1·2
Leucine	90	75	93·9	92·9	−1·0 NS	1·3
Phenylalanine	49	53	57·9	50·3	−7·6*	0·8
Lysine	79	82	62·8	59·9	−2·9 NS	0·9
Histidine	28	17	24·0	28·7	4·7**	0·4
Arginine	34	49	44·8	40·4	−4·4*	0·8
Total essential amino acids	469	493	465·9	451·3	−14·6 NS	7·3
Aspartic acid	66	108	101·5	88·7	−12·8**	0·7
Serine	52	47	49·5	54·8	5·3**	0·2
Glutamic acid	203	137	145·7	185·7	40·0*	9·0
Proline	112	46	61·4	78·0	16·6**	0·9
Glycine	17	52	53·8	41·0	−12·8**	0·4
Tyrosine	53	39	50·0	42·7	−7·2*	0·7
Alanine	28	78	72·3	57·8	−14·6**	1·0
Total non-essential amino acids	531	507	534·1	548·7	14·6 NS	7·3

NS, not significant. * $P \leqslant 0{\cdot}05$, ** $P \leqslant 0{\cdot}01$.
† For details, see Table 1.
§ Williams & Dinusson (1973).

Rumen ammonia concentrations increased markedly with increasing amounts of UT casein, but remained as low as when diet A (basal) was given when increasing amounts of FT casein were given. This indicated considerable protection of the casein against rumen degradation by the formaldehyde treatment although differences were only significant at the highest level of supplementation.

Effect of formaldehyde treatment of casein on the amounts, composition and origin of nitrogenous constituents entering the small intestines. The quantities of nitrogenous constituents entering the small intestines (Table 2) were estimated from mean values for constituent: Cr_2O_3 in samples of duodenal digesta taken before and at 2, 4 and 6 h after the morning feed. There were no marked variations in total amino acid: Cr_2O_3 values obtained over the four sampling periods; for example for the five calves given diet A mean values (± SE) for methionine: Cr_2O_3 at 0, 2, 4 and 6 h after feeding were 0·56 ± 0·03, 0·55 ± 0·02, 0·58 ± 0·05 and 0·57 ± 0·04 respectively. It is believed therefore that at least for comparative purposes the present estimates are valid.

Accepting this, then with diet A the results indicated that about the same amounts of total N entered the duodenum as were eaten, but with increasing levels of N intake

Table 4. *Concentrations (μmol/l) of plasma amino acids (PAA) and urea in the jugular blood from three calves 4 h after a meal of the basal diet (diet A), or diets containing either untreated casein (diet F) or formaldehyde-treated casein (diet G) to give 34 g N/kg dry matter*

Diets† ...	A		F	G	Treatment difference (G−F)	SE of (G−F)
	Mean	SE				
Threonine	106	4·4	157	170	14 NS	9
Valine	225	11·8	233	455	222**	9
Methionine	31	1·5	41	66	25*	3
Isoleucine	71	10·4	80	156	76*	10
Leucine	118	1·5	125	255	130**	7
Phenylalanine	60	2·1	58	80	21**	1
Lysine‡	190	12·0	211	424	213**	10
Histidine	120	13·6	91	130	39**	2
Arginine	96	3·2	131	177	46*	5
Total essential PAA	1015	46·3	1126	1912	786**	27
Aspartic acid	15	0·9	14	12	−2*	0
Serine§	157	6·3	160	141	−19**	1
Glutamic acid	200	15·3	212	179	−33 NS	18
Glycine	513	18·6	483	219	−264*	21
Tyrosine	53	2·9	60	68	8 NS	2
Alanine	227	12·5	261	179	−82***	1
Total non-essential PAA	1166	48·3	1190	798	−392**	38
Total PAA	2180	94·2	2316	2710	394**	17
Plasma urea	989	48·1	4894	5212	319 NS	91

NS, not significant.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.
† For details, see Table 1.
‡ Includes ornithine and ε-*N*-methyllysine.
§ Includes glutamine and asparagine.

increasing amounts of N disappeared before reaching the duodenum. This disappearance was considerably less, although not significantly so, with FT than with UT casein particularly at the highest level of intake. Similar disappearances were found for non-ammonia-N, and for essential and non-essential amino acid-N, and for these compounds differences between FT- and UT-casein diets were generally significant. Expressed as proportions of intake the amounts of dietary UT casein-N that escaped degradation in the rumen were 0·20, 0·14 and 0·10, for diets containing 19, 26 and 34 g N/kg DM respectively. Corresponding values for FT casein were 0·90, 0·69 and 0·73. It appeared that formaldehyde treatment of casein significantly increased the amounts of casein-N that escaped degradation in the rumen. The results also indicated that amounts of non-casein, non-ammonia-N (mainly microbial and endogenous N) entering the duodenum were greater for UT casein than for corresponding levels of FT casein. These changes also led to differences in amino acid composition in digesta entering the duodenum and, for example, the concentrations of glutamic acid, proline and histidine were higher while concentrations of aspartic acid, glycine, alanine and arginine were lower when FT rather than UT casein was given. Results are given for

Table 5. *Estimated quantities of fermentable nitrogen (g/24 h) entering the small intestines of calves given the basal diet (diet A) or diets containing increasing amounts of either untreated (UT) casein (diets B, D, and F) or formaldehyde-treated (FT) casein (diets C, E and G)*

(Mean values for five calves for diet A and for three calves given diets B, C, D, E, F or G)

Diets*	A	B	C	D	E	F	G
Casein supplement ...	—	UT	FT	UT	FT	UT	FT
Metabolizable energy (ME) intake (MJ/24 h)	25·8	27·2	27·2	31·2	31·2	33·4	33·4
Fermentable N required for maximum microbial production (ME × 1·68)	43·3	45·7	45·7	52·4	52·4	56·1	56·1
Fermentable N supplied by:							
Straw and flaked maize†	16·6	16·3	16·3	17·3	17·3	17·5	17·5
Casein‡	—	15·3	1·9	35·5	12·5	65·4	18·9
Total	16·6	31·6	18·2	52·8	29·8	82·9	36·4

* For details, see Table 1.
† Assuming 60% of protein fermented in rumen.
‡ Calculated from results given in Table 2.

diets E and D (26 g N/kg DM) in Table 3. Similar differences were generally found for other levels of intake.

Effects of formaldehyde treatment of casein on PAA and PU concentrations. PAA and PU concentrations in jugular blood 4 h after feeding are given in Table 4. Supplementation of diet A with UT casein at the highest level of intake (diet F) led to only small changes in the concentrations of individual and total PAA, but PU increased markedly with the increase in dietary protein intake. Compared with UT casein the FT casein resulted in significant increases in the concentrations of total PAA and of total and most individual essential PAA. Concentrations of total and most individual non-essential PAA, however, decreased significantly. PU concentrations were not significantly different between FT- or UT-casein diets.

DISCUSSION

The present results for the ruminating calf are in accordance with previous findings for sheep (Offer *et al.* 1971; Barry, 1972; MacRae *et al.* 1972; Faichney, 1974*a*, *b*), adult cattle (Hagemeister & Pfeffer, 1973) and calves (Sharma *et al.* 1974) in indicating that lower concentrations of ammonia develop in the rumen and greater amounts of non-ammonia-N and amino acid-N enter the duodenum when FT rather than UT casein is given as a dietary supplement. In addition, the results provide a direct measure of the amount of casein reaching the duodenum, and indicate that over a wide range of intake about 70–90% of FT casein survived passage through the stomachs. The results of Hagemeister & Pfeffer (1973) for cows and Sharma *et al.* (1974) for calves indicated that considerable amounts of undegraded FT casein passed into the duodenum of these animals, but their results did not permit a quantitative estimate of the amounts. The present values could be underestimates if appreciable quantities

of casein-bound phosphorus were released in the abomasum. However, such release is unlikely since it has been found that the bond between P and the rest of the casein molecule is very resistant to acid-hydrolysis and appears to be only very slowly attacked by enzymes, including pepsin (*EC* 3.4.4.1) (Rimington & Kay, 1926).

The results indicated that feeding FT rather than UT casein led to decreased amounts of N from non-casein sources reaching the duodenum. It is probable that this was due to decreased microbial protein synthesis, an effect also indicated by the finding of Hagemeister & Pfeffer (1973) that DAP flow in the duodenum was less when an FT- rather than UT-casein-supplemented diet was given to cows. If it is assumed that the maximum yield of microbial-N in the rumen is about 1·68 g/MJ metabolizable energy (ME) fed (Miller, 1973) then the requirements of dietary fermentable N in the rumen for maximum microbial growth (ignoring differences due to variation in recycling) can be calculated for the ME intakes given in the present experiments. Comparison of these requirements with estimated amounts supplied (Table 5) indicates that microbial synthesis was N-limited for diet A (basal) and all the FT-casein-supplemented diets, but only for the lowest level of supplemention for the UT-casein-supplemented diets. A further indication that FT-casein-supplemented diets provided inadequate N for the rumen bacteria was provided by the fact that rumen ammonia concentrations for the calves given these diets were considerably lower than the 50 mg ammonia-N/l reported by Satter & Slyter (1974) to be necessary for maximum microbial protein synthesis. It seems likely that an increase in microbial protein synthesis could have been achieved by adding urea to the diet, and it is of interest that in the experiments of Sharma *et al.* (1974), in which this was done, there was no significant difference in estimated bacterial protein flow at the abomasum between FT- and UT-casein-supplemented diets.

It appears then that feeding FT rather than UT casein is likely to lead both to an increase in total amino acid-N flow at the duodenum and to changes in the proportions of dietary and microbial protein contributing to that flow. The latter effects were presumably responsible, at least in part, for changes in amino acid composition in duodenal contents, and in particular for the increases in glutamic acid, proline and histidine concentrations and decreases in aspartic acid, glycine, alanine and arginine concentrations which were found (Table 3). Changes in the proportions of dietary and microbial protein had little consistent effect on the relative proportions of essential amino acids in duodenal digesta, a conclusion which was also apparent from an inspection of results from other studies (MacRae *et al.* 1972; Hagemeister & Pfeffer, 1973; Faichney, 1974*a*; Sharma *et al.* 1974), presumably because of the similarity in essential amino acid composition of casein and microbial protein.

There were, however, some changes in the amino acid composition of digesta reaching the duodenum which were difficult to explain simply in terms of changing proportions of casein and microbial protein. This was true for the methionine and tyrosine results given in Table 3, and the results of other workers include similar apparent anomalies. For example, Faichney, (1974*a*) for sheep, and Sharma *et al.* (1974) for calves reported slightly lower proportions of methionine in the amino acids entering the duodenum when FT rather than UT casein was fed, whereas marked

increases might have been expected. These effects, which may be due in part to unrecognized variations in endogenous protein secretion, to varying contributions from other dietary constituents or to analytical errors, would interfere with the use of amino acid patterns in digesta to assess dietary protein survival as suggested by Evans, Axford & Offer (1975).

Both the results of the present study and those of other workers have indicated that concentrations of PAA may change when FT rather than UT casein is given to ruminants, but it is difficult to relate these variations in detail to changes in the amino acid composition of digesta reaching the duodenum. Our findings that total essential PAA concentrations increased when calves were given FT casein were probably due to an increased total protein supply as there was no apparent increase in the proportion of essential relative to non-essential amino acids at the duodenum. It appeared that our FT casein must have been readily digested in the small intestine to provide this increased supply. Similar changes reported for sheep (Fraser & Haden, 1970; Reis & Tunks, 1970; Faichney, 1974*a*) were smaller, perhaps because FT-casein intakes were sometimes smaller, but also, in the experiments of Reis & Tunks (1970), because protein digestibility or amino acid availability in the small intestine may have been reduced by the high level of formaldehyde treatment (24 g formaldehyde/kg protein) used. It has been reported, for example, that methionine availability, as measured by microbiological assay, is markedly reduced in casein treated with more than about 14 g formaldehyde/kg protein (Williams, 1974), and Reis & Tunks (1970) found much larger increases in essential PAA concentrations when UT casein was infused directly into the abomasum than when a comparable amount of their FT casein was given in the diet.

The significant decreases in non-essential PAA concentrations found in the present study when FT rather than UT casein was given were not found in sheep (Fraser & Haden, 1970; Reis & Tunks, 1970; Faichney, 1974*a*). This may have been due to differences in the proportions of dietary N supplied as FT casein.

PU concentrations increased with increasing amounts of dietary protein, as found by Preston, Schnakenberg & Pfander (1965) and Williams & Smith (1974), but differences between FT- and UT-casein-supplemented diets were not significant. Several workers have reported similar findings with calves (Schmidt, Jorgensen & Benevenga, 1972; Sharma, Ingalls & McKirdy, 1972; Schmidt, Benevenga & Jorgensen, 1974), but others have reported lower PU concentrations for FT-compared to UT casein-supplemented diets in calves (Faichney, 1974*b*; Sharma & Ingalls, 1974 and in sheep (Barry, 1972).

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