Metabolic implications of ammonia production in the ruminant

BY D. S. PARKER[1], M. A. LOMAX[2], C. J. SEAL[1] AND J. C. WILTON[3]

[1]*Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

[2]*School of Animal and Microbial Sciences, University of Reading, Whiteknights, Reading RG6 2AJ*

[3]*School of Biochemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT*

NH_3 is generated in the gut of all animal species as a result of two main processes: (1) microbial degradation of nitrogenous compounds such as proteins, peptides, amino acids and nucleic acids within the gut lumen and (2) microbial hydrolysis of urea passing across the gut wall from the blood and interstitial fluids. Whereas in most species the production of NH_3 and its incorporation into microbial protein in the hindgut is considered of little nutritional benefit to the host (apart from coprophagic species), the pathway of N assimilation into microbial protein in the reticulo-rumen is an essential component of protein flow to the small intestine of ruminant animals. As such it has been demonstrated that ruminants can sustain a modest level of productivity when provided only with non-protein-N in the diet (Virtanen, 1969). Protein rationing for this group of livestock is based on provision of rumen-degradable N for microbial protein synthesis in addition to an undegradable component calculated to support required levels of output (Agricultural Research Council, 1980, 1984; Agricultural and Food Research Council, 1993). It is not the purpose of the present review, however, to evaluate rumen N transactions but to identify the pathways by which NH_3 is generated within the gut and factors which affect its absorption and detoxification in the liver. There are a number of excellent reviews which discuss the broader aspects of N metabolism in the ruminant (MacRae & Reeds, 1980; Chalupa, 1984; Egan *et al.* 1986; Lobley, 1991).

RUMEN AMMONIA TURNOVER

The primary sources of NH_3 within the rumen are the protein components of the diet. Protein degradability is dependent on a number of factors including solubility, susceptibility to microbial proteases and residence time in the rumen (Taminga, 1983). These factors combine to produce a pattern of release of peptides, amino acids and NH_3, all of which provide a source of N for microbial protein synthesis. The extent to which the range of microbial species within the rumen are able to utilize these different sources is a matter of debate. Although early work (Bryant & Robinson, 1962) indicated that about 90% of bacterial species isolated from the rumen could utilize NH_3 as the main source of N for growth, further studies have demonstrated a potential for free amino acids and peptides to become incorporated into microbial protein without passing through the rumen NH_3 pool (Cotta & Hespell, 1986; MacKie & White, 1990). More recent studies using $^{15}NH_3$ to quantify uptake of protein degradation products for microbial protein synthesis have also shown that for a range of feed protein sources a maximum of 0·4–0·68 of microbial N was derived from the rumen NH_3 pool (Hristov & Broderick, 1994).

Our understanding of the extent of the flux of NH_3 within the rumen is based on

https://doi.org/10.1079/PNS19950023 Published online by Cambridge University Press

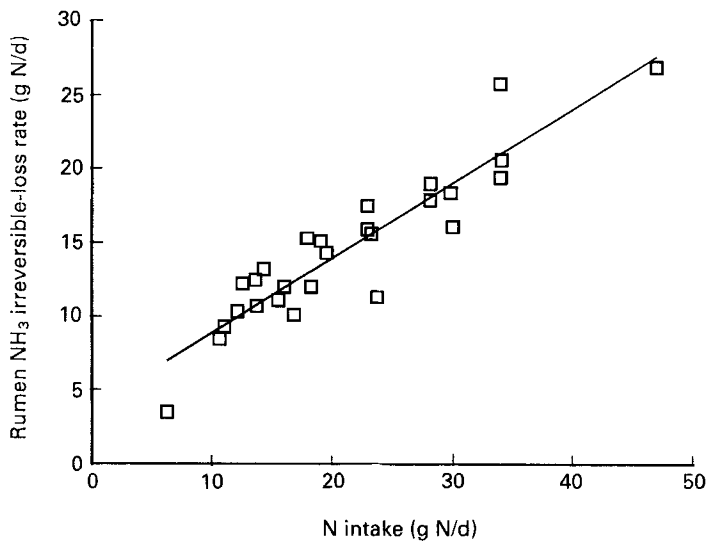

Fig. 1. The relationship between rumen ammonia irreversible-loss rate (gN/d) and N intake (gN/d). Each point represents animal means from individual experiments. The equation of the regression line is $y = 3{\cdot}829 + 0{\cdot}507x$ (r^2 0·853). Data are from the experiments of Pilgrim *et al.* (1970); Mathison & Milligan (1971); Nolan *et al.* (1976); Nolan & MacRae (1976); Kennedy & Milligan (1978); Nolan & Strachin (1979); Siddons *et al.* (1985*b*) and Kennedy *et al.* (1986).

extensive use of ^{15}N-tracer methodology developed to study N transactions within the gut, and between the gut and peripheral blood in sheep (Mathison & Milligan, 1971; Nolan, 1975; Nolan *et al.* 1976; Siddons *et al.* 1985*a*; Kennedy *et al.* 1986). These turnover studies have identified the relationship between N intake in the diet and NH_3 irreversible-loss rates over a wide range of N source and level in the diet (Fig. 1). These findings indicate that 0·5 of dietary N entering the rumen passes directly through the rumen NH_3 pool which is somewhat higher than previously calculated (0·3; Nolan, 1975). The difference reflects the availability of data from an increased number of trials carried out over a wider range of N intakes. Production of NH_3 from endogenous sources within the rumen, calculated from the intercept of the regression line, has also changed from 4·4 g N/d in the original study to 3·8 g N/d when all the available data are included. This value represents primarily the flow of urea into the rumen via saliva and direct transfer across the rumen wall. Apart from incorporation into microbial protein, loss from this small (0·5–1·5 g N) but highly labile pool is either by absorption across the rumen wall or by passage out of the rumen in digesta flowing to the small intestine. Duodenal NH_3 flow measured in cows (Firkins *et al.* 1987; Song & Kennelly, 1990) and in sheep (MacRae *et al.* 1972; MacRae & Ulyatt, 1974) represented 0·02 and 0·09 of N intake respectively. Of this, isotope-exchange studies suggest that about half (0·4–0·6; Siddons *et al.* 1985*b*) represents NH_3 derived directly from the rumen NH_3 pool. It is apparent, therefore, that the primary pathway of NH_3 loss from the rumen is via absorption across the gut wall.

The dynamics of rumen N transactions measured using ^{15}N isotope techniques provide important quantitative data relating feed N intake and the transfer of N between gut and body pools. This experimental approach is dependent on the establishment of steady-state conditions within the animal in order to measure nutrient flux in metabolite pools which, ideally, do not alter during the course of the experiment. In practice, however, N dynamics are dominated by cycles of nutrient release which are linked to patterns of intake and the physico-chemical characteristics of the feed. Numerous experiments have reported the effect of feeding either concentrate- or forage-based diets on rumen NH_3 levels and the fluctuations in metabolite concentrations with time period after feeding (Wernli & Wilkins, 1980). Silage feeding is an extreme example of this effect, the soluble nitrogenous components being rapidly degraded in the rumen to result in peaks of NH_3 concentration of 18–20 mM within 1 h after feeding from a basal level of 2–4 mM. These levels can be attenuated by chemical treatment of the forage material before ensilage using acid–formaldehyde to reduce N solubility (Thompson *et al.* 1981) or by provision of a readily-fermentable carbohydrate to provide energy for N capture by the rumen microflora (Rooke *et al.* 1987). Rumen infusion studies in which either pulsed or continuous infusions of N and energy-yielding substrates have been used (Henning *et al.* 1993) demonstrate that providing a constant supply of energy may be a critical factor in improving nutrient utilization. In the absence of such provision, rapid fluctuations in NH_3 concentration result in inefficient use of N for microbial protein synthesis and loss of NH_3 from the rumen by absorption across the gut wall. In addition there may be periods during which rumen NH_3 levels fall below those thought to be optimal for microbial growth; 3·5 mM (Satter & Slyter, 1974) to 6 mM (Kang-Meznarish & Broderick, 1981), thereby reducing both energy and protein supply to the host animal. Synchronization of N and energy release within the rumen in order to maximize nutrient capture by the microbial population has been an objective of ruminant feeding systems. Recent experiments in sheep (Sinclair *et al.* 1993) in which diets were formulated on the basis of either asynchronous or synchronous release of nutrients have shown that manipulation of the pattern of substrate availability in this way can provide a practical method of improving the efficiency of N capture and reducing the magnitude of rumen NH_3 cycling.

MECHANISM OF INTESTINAL ABSORPTION OF AMMONIA

The mechanisms involved in the bi-directional movement of NH_3 across tissues of the gastrointestinal tract between the lumen and blood are not fully understood. Absorption does not appear to be active, but occurs by passive non-ionic diffusion down a concentration gradient. In ruminant animals the high concentrations of NH_3 in rumen fluid favour the flux of NH_3 into the bloodstream, but in small intestinal tissues there may be considerable movement of NH_3 back into the intestinal lumen. Free NH_3 diffuses readily across biological membranes because of its lipid solubility and lack of charge, in contrast to NH_4^+ which as a hydrated, charged molecule has low lipid solubility and cannot diffuse across the cell membrane (Visek, 1969). The pK for the equilibrium between free NH_3 and NH_4^+ is approximately 9·1, thus under normal physiological conditions most of the NH_3 present in the gut lumen (pH range 2–6) will be in the ionized form. Diffusion of free NH_3 across the rumen wall in the undissociated form has been demonstrated *in vivo* (Hogan, 1961; Siddons *et al.* 1985*a*; Bödeker *et al.* 1990; Rémond *et al.* 1993*b*) and *in vitro* (Mooney & O'Donovan, 1970). Net NH_3 flux across the rumen

https://doi.org/10.1079/PNS19950023 Published online by Cambridge University Press

wall has been shown to be linearly correlated to both free NH_3 (Siddons *et al.* 1985*a*) and to total NH_3 concentrations (Rémond *et al.* 1993*a*) in rumen fluid, although it is thought that the free NH_3 levels are more significant. Using the isolated rumen technique, however, Bödeker *et al.* (1990) showed that at a constant rumen NH_3 concentration, NH_3 absorption did not reflect the concentration ratio for undissociated:free NH_3 in the artificial rumen fluid. These results suggest either a flux of NH_3 molecules across the rumen wall or titration of NH_4^+ at the absorptive surface. Further experiments by Bödeker *et al.* (1992*b*) have implicated volatile fatty acids (VFA) in this latter process. NH_3 uptake was stimulated by the presence of VFA in the mucosal buffer solution either individually or as a mixture of acetate, propionate and butyrate. Similar responses to additional butyrate on transfer of NH_3 into the ruminal vein of sheep have been reported by Rémond *et al.* (1993*b*). The mechanism involved is unclear, although the exchange of protons between the VFA and NH_3 either at the cell surface and/or within the mucosa has been suggested. It is interesting that in rats fed on diets containing fermentable carbohydrates NH_3 absorption from the caecum was increased (Rémésy & Demigné, 1989). Although this may in part be due to the increased entry of urea into the caecum and its hydrolysis by the caecal flora, it is possible that the increased concentration of VFA in the caecal digesta also had a more direct effect on NH_3 flux across the caecal wall.

The importance of bicarbonate in stimulating colonic NH_3 absorption has been demonstrated in several studies in single-stomached animals (Wrong, 1978) and it is possible that similar mechanisms occur in the ruminant animal. Bödeker *et al.* (1992*a*), for example, have shown *in vitro* that inhibition of carbonic anhydrase (which would result in a lowering of free bicarbonate ions) caused a reduction in NH_3 flux across the rumen wall. In this experiment addition of VFA to the mucosal incubation solution restored NH_3 flux to control levels suggesting that bicarbonate and VFA may play similar roles in mediating NH_3 uptake. Increasing HCO_3^- levels in rumen fluid by bubbling CO_2 into the rumen also caused an increase in NH_3 flux in sheep (Rémond *et al.* 1993*b*) although it is not clear whether this was a direct effect on transfer across the ruminal epithelium or increasing ruminal vein blood flow.

AMMONIA ABSORPTION INTO PORTAL BLOOD

As a consequence of the extensive turnover of N-containing compounds in the digestive tract of ruminants and the loss of NH_3 across the gut wall a significant proportion of dietary N intake can be measured as NH_3 flux in portal blood. The relationship between these two variables has been examined recently (Seal & Reynolds, 1993) for a wide range of diets and these authors confirm previous observations that portal NH_3 flux can represent as much as 0·65 of N intake and in many circumstances exceed net α-NH_2-N absorption into portal blood. Similar calculations for single-stomached species are hampered by the lack of quantitative information concerning arterio–venous blood concentrations across the gut and measurements of blood flow. There are, however, a number of studies in pigs in which between 0·14 and 0·24 of daily N intake can be accounted for in the measured flux of NH_3 in portal blood (Malmlöf, 1987; van Berlo *et al.* 1988; Yen & Pond, 1990; Yen & Nienaber, 1993). Detoxification of absorbed NH_3 by urea cycle activity in the liver is common to all species and calculation of portal NH_3 flux on a metabolic-weight ($W^{0.75}$) basis provides a mechanism of 'scaling' the effects of

Table 1. *Net portal ammonia absorption in different species*

Species	Portal NH_3 (μM)	Arterial NH_3 (μM)	Net absorption (μmol/min per $kgW^{0.75}$)	Reference
Rat	193	72	2·8	Hartman & Prior (1992)
Pig	285	142	14·3	Malmlöf (1987)
	165	44	17·9	van Berlo *et al.* (1988)
	205	39	8·2	Yen & Pond (1990)
Sheep: Fed	487	210	33·2	Gross *et al.* (1990)
Intragastric infusion	377	200	19·5	Gross *et al.* (1990)
Steers	560	220	35·2	Harmon *et al.* (1988)
Cows (lactating)	650	350	71·5	Huntington (1984)
Steers (2 × daily fed): Min	356	122	44·2	Wilton (1989)
Max	525	112	82·1	Wilton (1989)

differences in the digestion of dietary N between ruminant and non-ruminant species. The results of this calculation are shown in Table 1. It is apparent that for both rats and pigs the mean value of 11 μmol/min per $kgW^{0.75}$ is significantly lower than that for ruminants of between 20 and 75 μmol/min per $kgW^{0.75}$ dependent on the diet and pattern of feeding. Where measurements have been made over a period of time it is also apparent that flux rates are reasonably constant in pigs relative to the time interval after feeding (Yen & Nienaber, 1993), whereas in ruminants fed twice daily, rather than continuously as in most of the nutrient absorption studies, portal flux rates can virtually double between prefeeding and 90 min post-feeding (44–82 μmol/min per $kgW^{0.75}$; Wilton, 1989).

Although portal absorption rates provide an overall measure of NH_3 flux into the blood pool, a number of different techniques have been used to study the relative contribution of different sections of the digestive tract to total NH_3 absorption. Studies of digesta flow in sheep (MacRae & Ulyatt, 1974) showed that some 0·15 of N intake was absorbed as NH_3 in the small intestine and application of ^{15}N techniques provided a dynamic model of NH_3-N movement across different sections of the tract (Siddons *et al.* 1985*a*). This latter study indicated that in sheep fed on a silage diet 0·25 of total NH_3 absorption occurred across small intestinal tissues. This proportion was increased to 0·37 when the sheep were fed on a diet consisting of dried grass. These values confirm measurements made in chronically-catheterized animals in which NH_3 flux into blood vessels draining the small intestine (mesenteric vein) has been compared with total uptake into the portal vein which includes absorption from both the rumen–reticulum–omasum and the large intestine. These studies are summarized in Table 2 and show that between 0·25 and 0·41 of portal NH_3 flux is attributable to absorption from the small intestine. The importance of this section of the digestive tract in the cycling of N in this way is underlined by the experiments of Gross *et al.* (1990) in which they maintained sheep by intragastric infusion of nutrients but with the protein component of the diet infused directly into the abomasum. Portal NH_3 flux in these animals was 20 μmol/min per $kgW^{0.75}$ during the infusion periods compared with 33 μmol/min per $kgW^{0.75}$ when the animals were fed on lucerne (*Medicago sativa*).

Table 2. *Contribution of ammonia absorption from the small intestine to total net uptake into the portal vein in steers*

Diet	Net NH_3 uptake (μmol/min per kg$W^{0\cdot75}$) Mesenteric vein	Portal vein	Mesenteric absorption as proportion of total	Reference
Grass nuts	15·8	42·4	0·37	Seal *et al.* (1992)
Grass nuts–flaked maize (70:30 w/w)	10·58	25·6	0·41	
Grass nuts–intraruminal propionate	6·7	23·15	0·29	Seal & Parker (unpublished results)
Lucerne (*Medicago sativa*):				
Time-fed	13·12	46·0	0·28	Reynolds & Huntington (1988)
Meal-fed	17·62	64·68	0·27	
Concentrates (meal-fed)	14·31	28·25	0·51	

$W^{0\cdot75}$, metabolic weight.

HEPATIC DETOXIFICATION OF AMMONIA

Under normal physiological and nutritional conditions, NH_3 absorbed into the portal vein is efficiently extracted by the liver and detoxified by conversion to urea or glutamine. Over a wide range of portal NH_3 concentrations on a variety of different diets, the liver is able to extract 70–95% of portal NH_3 with the result that hepatic NH_3 removal is on average very slightly higher (4%) than portal absorption (Table 3). This results in arterial NH_3 concentrations remaining constant even when portal NH_3 absorption varies threefold. NH_3 is extremely toxic in non-hepatic tissues and causes changes in cerebral metabolism which result in tetany and death when circulating NH_3 concentrations exceed 0·7 mM (Symonds *et al.* 1981). Ruminants are susceptible to diet-induced NH_3 toxicity particularly when non-protein-N is rapidly converted to NH_3 in the rumen and absorbed into the portal vein (Visek, 1984). Most investigators have reported arterial NH_3 concentrations in the 0·1 mM range using a specific enzyme assay which follows the reaction with glutamate dehydrogenase (*EC* 1.4.1.2; Bergmeyer & Beutler, 1985). A number of estimates of circulating NH_3 concentration, however, have employed the Berthelot reaction (McCullough, 1967) which gives values of 300–400 μM (Huntington, 1989; Reynolds *et al.* 1991; see also Table 1) due to non-specific reactions, although these overestimated values do not appear to affect the values for net NH_3 exchange across tissues (L. A. Crompton and C. K. Reynolds, personal communication).

A functional heterogeneity of metabolism, particularly of carbohydrates and N has been established in rat liver parenchymal cells (Jungermann, 1986). This ensures that any NH_3 which escapes conversion to urea in periportal hepatocytes is converted to glutamine in perivenous hepatocytes. The amide-N of glutamine is then removed and metabolized to urea by periportal hepatocytes during subsequent passages through the liver and may also provide a mechanism to prevent a decrease in extracellular pH (Haussinger *et al.* 1992). In ruminants, there is net hepatic uptake of glutamine and

Table 3. *Nitrogen intake (g/d), portal ammonia absorption, hepatic NH_3-N uptake and urea-N output (mmol/min) in cattle fed on a range of diets*

Diet constituents	N-intake	Portal NH_3 uptake	Hepatic NH_3 uptake	Hepatic urea-N output	Hepatic NH_3 uptake: hepatic urea output	Reference
Grass nuts–flaked maize (70:30, w/w)	123	0·97	0·92	3·36	0·27	Wilton *et al.* (1988)
Grass nuts–flaked maize (50:50, w/w)	102	1·29	1·68	2·86	0·59	Fitch *et al.* (1989)
Grass nuts	172	3·06	3·18	6·72	0·47	
Maize silage	106	1·51	1·47	1·91	0·79	Maltby *et al.* (1991)
Grass silage–grass nuts (70:30, w/w)	94	2·48	2·50	2·27	1·10	Maltby *et al.* (1993*a*)
Barley–grass nuts (70:30, w/w)	79	1·19	1·28	1·98	0·65	
Lucerne (*Medicago sativa*)	153	4·21	4·31	6·05	0·71	Maltby *et al.* (1993*c*)
Lucerne	162	4·85	4·83	6·10	0·79	Huntington (1989)
Lucerne–cracked maize	95	2·13	2·15	2·65	0·81	
Lucerne–ground maize (25:75, w/w)						
Low intake	98	2·38	2·47	3·92	0·63	Reynolds *et al.* (1991)
High intake	174	4·17	4·33	8·18	0·53	
Lucerne–ground maize (75:25, w/w)						
Low intake	133	3·10	3·23	5·90	0·55	
High intake	209	5·67	5·88	9·88	0·60	

https://doi.org/10.1079/PNS19950023 Published online by Cambridge University Press

output of glutamate (Wolff *et al.* 1972; Reynolds, 1992) which is in agreement with the hepatic intracellular cycle proposed. When urea is added to ruminant diets there is increased hepatic uptake of NH_3 but glutamine uptake is either unchanged or slightly increased whilst net hepatic glutamate output is decreased (Maltby *et al.* 1991, 1993*b*). However, these changes in amino acid flux are small and the increase in hepatic urea synthesis can more than account for NH_3 removal, suggesting that conversion of NH_3 to glutamine or glutamate is not a major detoxification pathway under normal feeding conditions. This has recently been confirmed by an *in vivo* study in sheep which demonstrated that 93·5 and 6% respectively of portal $^{15}NH_4Cl$ is converted to [^{15}N]urea and [^{15}N]glutamine when portal vein NH_3 concentrations are increased to 0·5 mM by intramesenteric vein infusion (Lobley *et al.* 1995). The upper limit to the capacity of ruminant liver to remove NH_3 is 1·2–1·5 μmol/min per g (Linzell *et al.* 1971; Symonds *et al.* 1981; Orzechowski *et al.* 1987), which compares with a range of 0·2–0·8 μmol/min per g over a wide variety of nutritional regimens. Therefore, the capacity of ruminant hepatocytes to detoxify NH_3 directly to urea appears to be well adapted to large changes in portal NH_3 concentration and is only exceeded when NH_3 loads on the liver are abnormal (Symonds *et al.* 1981; Fernandez *et al.* 1990).

CONTRIBUTION OF AMMONIA TO HEPATIC UREA SYNTHESIS

In addition to the relationship between N intake and portal NH_3 absorption (see p. 552), data in Table 3 show that hepatic NH_3 uptake is positively correlated with N intake and accounts for between 16 and 60% of N intake.

$$\text{N intake (g/d)} = 64{\cdot}5 + 22{\cdot}5 \text{ hepatic NH}_3 \text{ uptake (mmol/min)} \quad (r^2\ 0{\cdot}74).$$

The potential contribution of extracted NH_3-N to hepatic urea-N formation ranges from 27 to 110%. This value compares with an estimated contribution of 33% of urea flux from portal NH_3 in humans and rodents (Meijer *et al.* 1990). The reason for the variation in the apparent contribution of NH_3 to hepatic urea production is not clear; the values in Table 3 have been obtained from different laboratories and using various techniques for measuring NH_3 and urea, suggesting that the large range is not an artefact and animal factors such as breed or N quality and intake appear not to be implicated. Dietary differences may be relevant since low values for NH_3 contribution to hepatic urea output have been reported for beef cattle fed on mixtures of dried grass nuts and flaked maize, while high values have been associated with diets based on lucerne hay–maize, grass silage–barley or maize silage (Table 3). There are inconsistencies within this pattern, however, and the results may represent the limitations of the arterio–venous difference techniques to establish stoichiometric relationships across an organ. The proportion of urea-N apparently accounted for by hepatic α-NH_2-N removal ranges from 16 to 30% (Huntington, 1989; Reynolds *et al.* 1991), although these estimates are based on measurement of α-NH_2-N rather than a summation of individual amino acids separated by ion-exchange chromatography. A recent comparison of the two methods suggests that they give approximately similar values (Maltby *et al.* 1993*a*).

IMPACT OF AMMONIA ON HEPATIC AMINO ACID METABOLISM

Wilton *et al.* (1988) imposed an acute (3 h) hepatic NH_3 load in beef cattle by infusion of NH_4Cl into a mesenteric vein and observed that the increase in hepatic urea-N

https://doi.org/10.1079/PNS19950023 Published online by Cambridge University Press

production was three times greater than that in the rate of NH_3-N removed by the liver. The findings suggested that the urea synthesis from non-NH_3 sources had been stimulated during NH_3 load and were supported by a trend for increased free amino acid uptake. The findings of this study agreed with those of a similar experiment in sheep (Orzechowski *et al.* 1987), although in both reports the results could be explained by incomplete recovery in the portal vein of infused NH_3. Lobley *et al.* (1995) have confirmed recently in sheep that a 5 d intramesenteric vein infusion of NH_3 increases hepatic urea production to approximately double that predicted from stoichiometric conversion of NH_3 removed to urea. These findings could help to explain the inefficient retention of absorbed amino acids in forage-fed ruminants, since amino acid deamination may be increased as a result of the increased hepatic uptake of NH_3 observed with forage-based diets (Fitch *et al.* 1989; Huntington, 1989; Reynolds *et al.* 1991). Other studies have altered NH_3 supply to the liver by changing diet composition or level of intake: Fitch *et al.* (1989) and Huntington (1989) compared forage and cereal diets fed to cattle (Table 3) and demonstrated that the forage-based diet doubled both hepatic NH_3 uptake and the proportion of urea-N output that was apparently synthesized from non-NH_3-N sources. Huntington (1989) demonstrated that hepatic α-NH_2-N removal was stimulated twofold on the forage diet with the result that total splanchnic supply of α-NH_2-N to peripheral tissues was decreased by 30%. Regression of hepatic NH_3 uptake *v.* urea production using the values in Table 3 yields a significant positive correlation.

$$\text{Hepatic } NH_3 \text{ uptake (mmol/min)} = 0{\cdot}43 + 0{\cdot}53 \text{ hepatic urea-N output (mmol/min)} \quad (r^2\ 0{\cdot}80).$$

The slope of this line suggests that an increase in hepatic NH_3 uptake is associated with twice as much urea production as that predicted from direct NH_3 detoxification. When N intake is regressed *v.* the values for hepatic urea-N output in Table 3, the equation predicts that as N intake increases there is a proportionately greater increase in hepatic urea-N synthesis.

$$\text{N intake (g/d)} = 60{\cdot}2 + 14{\cdot}8 \text{ hepatic urea-N output (mmol/min)} \quad (r^2\ 0{\cdot}92).$$

Whilst it is possible that these relationships reflect the increasing supply of amino acids to the liver, they support the suggestion that an increased NH_3 load on the liver has a cost of detoxification in relation to amino acid catabolism which would be of considerable significance to growth (muscle deposition) in silage-fed animals.

MECHANISM FOR THE INTERRELATIONSHIP BETWEEN AMMONIA AND HEPATIC AMINO ACID METABOLISM

The synthesis of urea involves the assimilation of two N atoms, one from NH_3 via mitochondrial carbamoylphosphate synthesis and the other from cytoplasmic aspartate. Mitochondrial and cytosolic aspartate–glutamate transamination pools are thought to be in equilibrium (Cooper *et al.* 1991), with the result that N from NH_3 or amino acids can contribute both N atoms of the urea molecule via the reversible action of glutamate dehydrogenase (Meijer *et al.* 1990). The *in vivo* studies discussed previously have led us to suggest, however, that under conditions of high urea flux, the mitochondrial supply of NH_3 may not be able to supply both N moieties of urea, with the result that amino acid-N is transferred to urea. This would effectively induce an amino acid deamination 'cost' for

NH_3 detoxification. Strong evidence in support of this proposal has recently been obtained by the use of isotopomer analysis to examine the contribution of NH_3 to the two N moieties of urea by measuring the flux of $^{15}NH_4Cl$ into [^{14}N,^{15}N]- and [^{15}N,^{15}N]urea: after 5 d of an infusion of NH_3 into the mesenteric vein of sheep, at least 97% of the [^{15}N]urea molecules formed were as [^{14}N,^{15}N]urea and the amounts of [^{15}N,^{15}N]urea were close to the detection limits (Lobley *et al.* 1995). In the same study an increase in leucine oxidation was noted, supporting the concept of increased requirement of α-NH_2-N for urea synthesis during NH_3 detoxification. This evidence, that there is a 'NH_3 detoxification cost' in terms of hepatic amino acid deamination, may not be exclusive to ruminants since ingestion of $^{15}NH_4Cl$ by fed and fasted humans is followed by the appearance of the majority of the label in [^{14}N,^{15}N]urea (Weijs *et al.* 1995).

The specific intracellular mechanism responsible for the proposed interaction between NH_3 and amino acid deamination is still unclear. Recent studies in fasted sheep hepatocytes (Luo *et al.* 1995) have demonstrated that when $^{15}NH_4Cl$ is the only N source in the incubation, [^{15}N,^{15}N]urea is the predominant form of urea suggesting that pathways of NH_3 conversion to urea in ruminants are similar to those of rodents. Addition of a physiological mixture of unlabelled amino acids to hepatocyte incubations increased the rate of [$^{14}N^{15}N$]urea appearance, but this did not increase with higher rates of NH_3 detoxification as would be predicted from the *in vivo* results of Lobley *et al.* (1995).

Krebs *et al.* (1979) suggested that increased amino acid utilization with increased ureagenesis may be the result of competition between gluconeogenic and ureagenic pathways for cytoplasmic oxaloacetate. Gluconeogenesis is a major synthetic pathway in fed ruminant liver and NH_3 has been demonstrated to inhibit glucose synthesis by ovine hepatocytes (Weekes *et al.* 1978; Aiello & Armentano, 1987; Demigné *et al.* 1991) and urea feeding to calves has been shown to decrease glucose disposal rates (Spires & Clark, 1979). The experiments of Wilton *et al.* (1988) and Maltby *et al.* (1991, 1993*a*), however, have failed to confirm this effect *in vivo* since net hepatic glucose release is not altered either by mesenteric vein NH_3 infusion or by feeding urea to calves. Furthermore, altering the media concentration of propionate, the major gluconeogenic substrate in ruminants, does not alter the appearance of [^{14}N,^{15}N]urea from $^{15}NH_4Cl$ in sheep hepatocyte incubations (Lou *et al.* 1995).

Nissim *et al.* (1992) reported the synthesis of both [^{14}N,^{15}N] and [^{15}N,^{15}N]urea from ^{15}N-labelled amino acids in rat hepatocytes, but suggested that a lag in the increase in isotopic enrichment of [^{15}N,^{15}N]urea was due to flux through glutamate dehydrogenase. It is possible that during NH_3 detoxification by ruminant liver *in vivo*, the equilibrium of glutamate dehydrogenase is also against the amination of 2-oxoglutarate and, thus, inhibits NH_3 contribution to both N moities of the urea molecule. The equilibrium of this step will be determined in part by the mitochondrial redox state, and in sheep liver the concentrations of NADH and NADPH are lower on diets likely to increase NH_3 supply to the liver (Prior *et al.* 1970). In rat mitochondrial preparations it has been proposed that NH_3 inhibits the activity of citric acid cycle enzymes by causing a decrease in pyridine nucleotide levels (Katanuma *et al.* 1966), but the relevance of these results is unclear since an increase in hepatic O_2 uptake has been reported during conditions of increased NH_3 arrival at the liver (Wilton, 1989; Maltby *et al.* 1991). Calculations based on the maximum number of four ATP molecules hydrolysed for every mole of urea synthesized reveals that only 13% of the increased O_2 uptake can be accounted for by urea synthesis, a value similar to that obtained by Reynolds *et al.* (1991). If the proposal that NH_3 detoxification causes an increase in amino acid deamination is correct, then it would appear that amino acid-C skeletons are oxidized rather than used for gluconeogenesis.

CONCLUSION

The ability of the liver to detoxify NH_3 to urea appears to be similar in ruminant and non-ruminant species, the principal difference being that the production of NH_3 by foregut fermentation in ruminants is extremely variable and dependent on feed sources, whilst in non-ruminants, NH_3 is produced in the hindgut and, therefore, absorption into the portal vein is affected far less by diurnal feed cycles. Despite the high rates of uptake from the gut which result from rapid fermentation of soluble N in forage diets, the ruminant liver is extremely adept at detoxifying NH_3 to urea. However, there is evidence to suggest that NH_3 detoxification to urea imposes a metabolic 'cost' in terms of amino acid deamination. This could explain observations of poor N retention in forage-fed ruminants, although further specific metabolic studies are required to identify a mechanism which could explain this interaction of NH_3 with amino acid metabolism.

The authors gratefully acknowledge the help of Dr G. E. Lobley in the preparation of this review.

REFERENCES

Agricultural and Food Research Council (1993). *Energy and Protein Requirements of Ruminants.* Wallingford: CAB International.

Agricultural Research Council (1980). *The Nutrient Requirements of Farm Livestock* no. 2. *Ruminants.* Farnham Common: Commonwealth Agricultural Bureaux.

Agricultural Research Council (1984). *Report of the Protein Group of the Agricultural Research Council Working Party on the Nutrient Requirements of Ruminants – Supplementary Report to Chapter 4 (ARC, 1980).* Farnham Common: Commonwealth Agricultural Bureaux.

Aiello, R. J. & Armentano, L. E. (1987). Gluconeogenesis in goat hepatocytes is affected by calcium, ammonia and other key metabolites but not primarily through redox state. *Comparative Biochemistry and Physiology* **88,** 193–201.

Bergmeyer, H. U. & Beutler, H. O. (1985). Ammonia. In *Methods of Enzymatic Analysis*, vol. 8, pp. 454–461 [H. U. Bergmeyer, editor-in-chief]. Basel: Weinheim.

Bödeker, D., Oppelland, G. & Höller, H. (1992*a*). Involvement of carbonic anhydrase in ammonia flux across rumen mucosa *in vitro. Experimental Physiology* **77,** 517–519.

Bödeker, D., Shen, Y., Kemkowski, J. & Höller, H. (1992*b*). Influence of short-chain fatty acids on ammonia absorption across the rumen wall in sheep. *Experimental Physiology* **77,** 369–376.

Bödeker, D., Winkler, A. & Höller, H. (1990). Ammonia absorption from the isolated reticulo-rumen in sheep. *Experimental Physiology* **75,** 587–595.

Bryant, M. P. & Robinson, I. M. (1962). Some nutritional characteristics of predominant culturable ruminal bacteria. *Journal of Bacteriology* **84,** 605–614.

Chalupa, W. (1984). Discussion on protein symposium. *Journal of Dairy Science* **67,** 1134–1146.

Cooper, A. J. L., Nieves, E., Coleman, A. E., Filc-DeRicco, S. & Gelbard, A. S. (1987). Short-term metabolic fate of [^{13}N]ammonia in rat liver *in vivo. Journal of Biological Chemistry* **262,** 1073–1080.

Cotta, M. A. & Hespell, R. B. (1986). Protein and amino acid metabolism of rumen bacteria. In *Control of Digestion and Metabolism in Ruminants*, pp. 122–136 [L. P. Milligan, W. L. Grovum and A. Dobson, editors]. Washington, DC: Butterworths.

Demigné, C., Yacoub, C., Morand, C. & Rémésy, C. (1991). Interactions between propionate and amino acid metabolism in isolated sheep hepatocytes. *British Journal of Nutrition* **65,** 301–317.

Egan, A. R., Boda, K. & Vardy, J. (1986). Regulation of nitrogen metabolism and recycling. In *Control of Digestion and Metabolism in Ruminants*, pp. 386–402 [L. P. Milligan, W. L. Grovum and A. Dobson, editors]. Washington, DC: Butterworths.

Fernandez, J. M., Croon, W. J., Tate, L. P. & Johnson, A. D. (1990). Subclinical ammonia toxicity in steers: Effects on hepatic and portal-drained visceral flux of metabolites and regulatory hormones. *Journal of Animal Science* **68,** 1726–1742.

Firkins, J. L., Lewis, S. M., Montgomery, L., Berger, L. L., Merchen, N. R. & Fahey, G. C. (1987). Effects of feed intake and dietary urea concentration on ruminal dilution rate and efficiency of bacterial growth in steers. *Journal of Dairy Science* **70,** 2312–2321.

Fitch, N. A., Gill, M., Lomax, M. A. & Beever, D. E. (1989). Nitrogen and glucose metabolism by the liver of forage- and forage-concentrate fed cattle. *Proceedings of the Nutrition Society* **48,** 76A.

Gross, K. L., Harmon, D. L. & Avery, T. B. (1990). Portal-drained visceral flux of nutrients in lambs fed Alfalfa or maintained by total intragastric infusion. *Journal of Animal Science* **68,** 214–221.

Harmon, D. L., Avery, T. B., Huntington, G. B. & Reynolds, P. J. (1988). Influence of ionophore addition to roughage and high-concentrate diets on portal blood flow and net nutrient flux in cattle. *Canadian Journal of Animal Science* **68,** 419–429.

Hartman, W. J. & Prior, R. L. (1992). Dietary arginine deficiency alters flux of glutamine and urea cycle intermediates across the portal-drained viscera and liver of rats. *Journal of Nutrition* **122,** 1472–1482.

Haussinger, D., Lamers, W. H. & Moorman, A. F. M. (1992). Hepatocyte heterogeneity in the metabolism of amino acids and ammonia. *Enzyme* **46,** 72–93.

Henning, P. H., Steyn, D. G. & Meissner, H. H. (1993). Effect of synchronization of energy and nitrogen supply on ruminal characteristics and microbial growth. *Journal of Animal Science* **71,** 2516–2528.

Hogan, J. P. (1961). The absorption of ammonia through the rumen of the sheep. *Australian Journal of Biological Science* **14,** 448–460.

Hristov, M. & Broderick, G. A. (1994). *In vitro* determinations of rumination protein degradability using [^{15}N]ammonia to correct for microbial nitrogen uptake. *Journal of Animal Science* **72,** 1344–1354.

Huntington, G. B. (1984). Net absorption of glucose and nitrogenous compounds in lactating Holstein cows. *Journal of Dairy Science* **67,** 1919–1927.

Huntington, G. B. (1989). Hepatic urea synthesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a high concentrate diet. *Canadian Journal of Animal Science* **69,** 215–223.

Jungermann, K. (1986). Functional heterogeneity of periportal and perivenous hepatocytes. *Enzyme* **35,** 161–180.

Kang-Meznarich, J. H. & Broderick, G. A. (1981). Effects of incremental urea supplementation on ruminal ammonia concentration and bacterial protein formation. *Journal of Animal Science* **51,** 422–431.

Katunuma, N., Okada, M. & Nishi, Y. (1966). Regulation of the urea cycle and TCA cycle by ammonia. *Advances in Enzyme Regulation* **4,** 317–336.

Kennedy, P. M., Early, R. J., Chrispherson, R. J. & Milligan, L. P. (1986). Nitrogen transformations and duodenal amino acid content in sheep given four forage diets and exposed to warm and cold ambient temperatures. *Canadian Journal of Animal Science* **66,** 951–957.

Kennedy, P. M. & Milligan, L. P. (1978). Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep. *British Journal of Nutrition* **39,** 105–117.

Krebs, H. A., Lund, P. & Stubbs, M. (1979). Interrelations between gluconeogenesis and urea synthesis. In *Gluconeogenesis: Its Regulation in Mammalian Species*, pp. 269–291 [W. Hanson and M. A. Melhan, editors]. New York: Wiley.

Linzell, J. L., Setchell, B. P. & Lindsay, D. B. (1971). The isolated perfused liver of sheep: assessment of its metabolic, synthetic and secretory functions. *Quarterly Journal of Experimental Physiology* **56,** 53–71.

Lobley, G. E. (1991). Some interactions between protein and energy in ruminant metabolism. In *Proceedings of the 6th International Symposium on Protein Metabolism and Nutrition, Herning, Denmark*, pp. 66–79 [B. O. Eggum, S. Boisen, C. Børsting, A. Danfær and T. Hvelplund, editors]. Foulum: National Institute of Animal Science.

Lobley, G. E., Connell, A., Lomax, M. A., Brown, D. S., Milne, E., Calder, A. G. & Farningham, D. A. H. (1995). Hepatic detoxification of ammonia in the ovine liver: possible consequences for amino acid catabolism. *British Journal of Nutrition* **73,** 667–685.

Luo, Q. J., Maltby, S. A., Lobley, G. E., Calder, A. G. & Lomax, M. A. (1995). The effect of amino acids on the metabolic fate of $^{15}NH_4Cl$ in isolated sheep hepatocytes. *European Journal of Biochemistry* **228,** 912–917.

McCullough, I. (1967). The determination of ammonia in whole blood by a direct colorimetric method. *Clinica Chimica Acta* **17**, 297.

MacKie, R. I. & White, B. A. (1990). Recent advances in rumen microbial ecology and metabolism: potential impact on nutrient impact. *Journal of Dairy Science* **73**, 2971–2995.

MacRae, J. C. & Reeds, P. J. (1980). Prediction of protein deposition in ruminants. In *Protein Deposition in Animals*, pp. 225–249 [P. J. Buttery and D. B. Lindsay, editors]. London: Butterworths.

MacRae, J. C. & Ulyatt, M. J. (1974). Quantitative digestion of fresh herbage in sheep. II. The sites of digestion of some nitrogenous constituents. *Journal of Agricultural Science, Cambridge* **82**, 309–319.

MacRae, J. C., Ulyatt, M. J., Pearce, P. D. & Hendtlass, J. (1972). Quantitative intestinal digestion of nitrogen in sheep given formaldehyde-treated and untreated casein supplements. *British Journal of Nutrition* **27**, 39–50.

Malmlöf, K. (1987). Porto-arterial plasma differences of urea and ammonia-nitrogen in growing pigs given high- and low-fibre diets. *British Journal of Nutrition* **57**, 439–446.

Maltby, S. A., Beever, D. E., Lomax, M. A., Crompton, L. A. & Pippard, C. J. (1993*a*). The influence of diet and increased ammonia supply on energy and nitrogen metabolism across splanchnic tissues in growing cattle. *Animal Production* **56**, 431.

Maltby, S. A., Crompton, L. A., Lomax, M. A., Beever, D. E. & Pippard, C. J. (1993*b*). The effect of increased ammonia supply on post-prandial hepatic metabolism in growing steers fed either forage or cereal-based diets. *Proceedings of the Nutrition Society* **52**, 295A.

Maltby, S. A., Lomax, M. A., Beever, D. E. & Pippard, C. J. (1991). The effect of increased ammonia supply on post-prandial portal-drained viscera and hepatic metabolism in growing steers fed maize silage. In *Energy Metabolism of Farm Animals. European Association of Animal Production Publication* no. 58, pp. 20–23 [C. Wenk and M. Boessinger, editors]. Zurich: ETH.

Maltby, S. A., Reynolds, C. K., Lomax, M. A. & Beever, D. E. (1993*c*). The effect of increased absorption of ammonia and arginine on splanchnic metabolism of beef steers. *Animal Production* **56**, 462–463.

Mathison, G. W. & Milligan, L. P. (1971). Nitrogen metabolism in sheep. *British Journal of Nutrition* **25**, 351–366.

Meijer, A. J., Lamers, W. H. & Chamuleau, R. A. F. M. (1990). Nitrogen metabolism and ornithine cycle function. *Physiological Reviews* **70**, 701–748.

Mooney, P. & O'Donovan, D. J. (1970). The permeability of the rumen to simple nitrogenous compounds. *Biochemical Journal* **119**, 18P–19P.

Nissim, I., Cattano, C., Nissim, I. & Yudkoff, M. (1992). Relative role of the glutaminase, glutamate dehydrogenase and AMP-deaminase pathways in hepatic ureagenesis: studies with ^{15}N. *Archives of Biochemistry and Biophysics* **292**, 393–401.

Nolan, J. V. (1975). Quantitative models of nitrogen metabolism in sheep. In *Digestion and Metabolism in the Ruminant*, pp. 416–431 [I. W. McDonald and A. C. I. Warner, editors]. Armidale: University of New England.

Nolan, J. V. & MacRae, J. C. (1976). Absorption and recycling of nitrogenous compounds in the digestive tract of sheep. *Proceedings of the Nutrition Society* **35**, 110A.

Nolan, J. V., Norton, B. W. & Leng, R. A. (1976). Further studies on the dynamics of nitrogen metabolism in sheep. *British Journal of Nutrition* **35**, 127–147.

Nolan, J. V. & Strachin, S. (1979). Fermentation and nitrogen dynamics in Merino sheep given a low-quality-roughage diet. *British Journal of Nutrition* **42**, 63–80.

Orzechowski, A., Motyl, T., Pierzynowski, G. & Barej, W. (1987). Hepatic capacity for ammonia removal in sheep. *Journal of Veterinary Medicine* **34**, 108–112.

Pilgrim, A. F., Gray, F. V., Weller, R. A. & Belling, C. B. (1970). Synthesis of microbial protein from ammonia in the sheep's rumen and the proportions of dietary nitrogen converted to microbial nitrogen. *British Journal of Nutrition* **24**, 589–598.

Prior, R. L., Clifford, A. J., Hogue, D. E. & Visek, W. J. (1970). Enzymes and metabolites of intermediary metabolism in urea-fed sheep. *Journal of Nutrition* **100**, 438–444.

Rémésy, C. & Demigné, C. (1989). Specific effects of fermentable carbohydrates on blood urea flux and ammonia absorption in the rat cecum. *Journal of Nutrition* **119**, 560–565.

Rémond, D., Chaise, J. P., Delval, E. & Poncet, C. (1993*a*). Net flux of metabolites across the ruminal wall of sheep fed twice a day with Orchardgrass hay. *Journal of Animal Science* **71**, 2529–2538.

Rémond, D., Chaise, J. P., Delval, E. & Poncet, C. (1993*b*). Net transfer of urea and ammonia across the ruminal wall of sheep. *Journal of Animal Science* **71**, 2785–2792.

Reynolds, C. K. (1992). Metabolism of nitrogenous compounds by ruminant liver. *Journal of Nutrition* **122**, 850–854.

Reynolds, C. K. & Huntington, G. B. (1988). Partition of portal-drained visceral net flux in beef steers. 1. Blood flow and net flux of oxygen, glucose and nitrogenous compounds across stomach and post-stomach tissues. *British Journal of Nutrition* **60,** 539–551.

Reynolds, C. K., Tyrell, H. F. & Reynolds, P. J. (1991). Effect of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: net nutrient metabolism by visceral tissues. *Journal of Nutrition* **121,** 1004–1015.

Rooke, J. A., Lee, N. H. & Armstrong, D. G. (1987). The effects of intraruminal infusions of urea, casein, glucose syrup and a mixture of casein and glucose syrup on nitrogen digestion in the rumen of cattle receiving grass-silage diets. *British Journal of Nutrition* **57,** 89–98.

Satter, L. D. & Slyter, L. L. (1974). Effect of ammonia concentration on rumen microbial protein production *in vitro*. *British Journal of Nutrition* **32,** 199–208.

Seal, C. J., Parker, D. S. & Avery, P. J. (1992). The effect of forage and forage-concentrate diets on rumen fermentation and metabolism of nutrients by the mesenteric- and portal-drained viscera in growing steers. *British Journal of Nutrition* **67,** 355–370.

Seal, C. J. & Reynolds, C. K. (1993). Nutritional implications of gastrointestinal and liver metabolism in ruminants. *Nutrition Research Reviews* **6,** 185–208.

Siddons, R. C., Nolan, J. V., Beever, D. E. & MacRae, J. C. (1985*a*). Nitrogen digestion and metabolism in sheep consuming diets containing contrasting forms and levels of N. *British Journal of Nutrition* **54,** 175–187.

Siddons, R. C., Paradine, J., Gale, D. L. & Evans, R. T. (1985*b*). Estimation of the degradability of dietary protein in the sheep rumen by *in vivo* and *in vitro* procedures. *British Journal of Nutrition* **54,** 545–561.

Sinclair, L. A., Garnsworthy, P. C., Newbold, J. R. & Buttery, P. J. (1993). Effect of synchronising the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. *Journal of Agricultural Science, Cambridge* **120,** 251–263.

Song, M. K. & Kennelly, J. J. (1989). Effects of ammoniated barley silage on ruminal fermentation, nitrogen supply to the small intestine, ruminal and whole tract digestion, and milk production of Holstein cows. *Journal of Dairy Science* **72,** 2981–2990.

Spires, H. R. & Clark, J. H. (1979). Effect of intra-ruminal urea administration on glucose metabolism in dairy steers. *Journal of Nutrition* **109,** 1438–1447.

Symonds H. W., Mather, D. L. & Collis, K. A. (1981). The maximum capacity of the liver of the adult dairy cow to metabolize ammonia. *British Journal of Nutrition* **46,** 481–486.

Taminga, S. (1983). Recent advances in our knowledge on protein digestion and absorption in ruminants. In *Protein Metabolism and Nutrition, Proceedings of the 4th EAAP International Symposium*, pp. 263–287 [M. Arnal, R. Pion and D. Bonin, editors]. Paris: INRA.

Thompson, D. J., Beever, D. E., Lonsdale, C. R., Haines, M. J., Cammell, S. B. & Austin, A. R. (1981). The digestion by cattle of grass silage made with formic acid and formic acid-formaldehyde. *British Journal of Nutrition* **46,** 193–207.

van Berlo, C. L. H., van Leeuwen, P. A. M. & Soeters, P. B. (1988). Porcine intestinal ammonia liberation. Influence of food intake, lactulose and neomycin treatment. *Journal of Hepatology* **7,** 250–257.

Virtanen, A. I. (1969). On nitrogen metabolism in milking cows. *Federation Proceedings* **28,** 232–240.

Visek, W. J. (1969). Some aspects of ammonia toxicity in animal cells. *Journal of Dairy Science* **51,** 286–295.

Visek, W. J. (1984). Ammonia: Its effects on biological systems, metabolic hormones and reproduction. *Journal of Dairy Science* **67,** 481–498.

Weekes, T. E. C., Richardson, R. I. & Geddes, N. (1978). The effect of ammonia on gluconeogenesis by isolated sheep liver cells. *Proceedings of the Nutrition Society* **38,** 3A.

Weijs, P. J. M., Calder, A. G. & Lobley, G. E. (1995). Incorporation of [^{15}N]ammonia into urea and amino acids as influenced by fasting and feeding. *Proceedings of the Nutrition Society* **54** (In the Press).

Wernli, C. G. & Wilkins, R. J. (1980). Nutritional studies with sheep fed conserved ryegrass. 1. Silage and dried grass offered *ad libitum* without supplements. *Quarterly Journal of Experimental Physiology* **60,** 89–94.

Wilton, J. C. (1989). The effect of ammonia upon the metabolism of carbohydrates and amino acids in the liver of growing steers fed silage. PhD Thesis, University of Reading.

Wilton, J. C., Gill, M. & Lomax, M. A. (1988). Uptake of ammonia across the liver of forage-fed cattle. *Proceedings of the Nutrition Society* **47,** 153A.

Wolff, J. E., Bergman, E. N. & Williams, H. H. (1972). Net metabolism of plasma amino acids by liver and portal-drained viscera of fed sheep. *American Journal of Physiology* **223,** 438–446.

Wrong, O. (1978). Nitrogen metabolism in the gut. *American Journal of Clinical Nutrition* **31,** 1587–1593.

Yen, J. T. & Nienaber, J. A. (1993). Effects of high-copper feeding on portal ammonia absorption and on oxygen consumption by portal-drained organs and whole animal in growing pigs. *Journal of Animal Science* **71**, 2157–2163.
Yen, J. T. & Pond, W. G. (1990). Effect of Carbadox on net absorption of ammonia and glucose into hepatic portal vein of growing pigs. *Journal of Animal Science* **68**, 4236–4242.

Printed in Great Britain