Metabolic implications of ammonia production in the ruminant

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NH3 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 NH3 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 NH3 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 NH3 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 NH3, 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 NH3 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 NH3 pool (Cotta & Hespell, 1986; MacKie & White, 1990). More recent studies using 15NH3 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 NH3 pool (Hristov & Broderick, 1994).

Our understanding of the extent of the flux of NH3 within the rumen is based on
The relationship between rumen ammonia irreversible-loss rate (g N/d) and N intake (g N/d). Each point represents animal means from individual experiments. The equation of the regression line is $y = 3.829 + 0.507x$ ($r^2 = 0.853$). 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. (1985b) and Kennedy et al. (1986).

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...
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 NH₃ 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 NH₃ 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 NH₃ concentration result in inefficient use of N for microbial protein synthesis and loss of NH₃ from the rumen by absorption across the gut wall. In addition there may be periods during which rumen NH₃ 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 NH₃ cycling.

**MECHANISM OF INTESTINAL ABSORPTION OF AMMONIA**

The mechanisms involved in the bi-directional movement of NH₃ 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 NH₃ in rumen fluid favour the flux of NH₃ into the bloodstream, but in small intestinal tissues there may be considerable movement of NH₃ back into the intestinal lumen. Free NH₃ diffuses readily across biological membranes because of its lipid solubility and lack of charge, in contrast to NH₄⁺ 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 NH₃ and NH₄⁺ is approximately 9.1, thus under normal physiological conditions most of the NH₃ present in the gut lumen (pH range 2–6) will be in the ionized form. Diffusion of free NH₃ across the rumen wall in the undissociated form has been demonstrated in vivo (Hogan, 1961; Siddons et al. 1985a; Bodeker et al. 1990; Rémond et al. 1993b) and in vitro (Mooney & O’Donovan, 1970). Net NH₃ flux across the rumen...
wall has been shown to be linearly correlated to both free NH₃ (Siddons et al. 1985a) and to total NH₃ concentrations (Rémont et al. 1993a) in rumen fluid, although it is thought that the free NH₃ levels are more significant. Using the isolated rumen technique, however, Bödeker et al. (1990) showed that at a constant rumen NH₃ concentration, NH₃ absorption did not reflect the concentration ratio for undissociated:free NH₃ in the artificial rumen fluid. These results suggest either a flux of NH₃ molecules across the rumen wall or titration of NH₄⁺ at the absorptive surface. Further experiments by Bödeker et al. (1992b) have implicated volatile fatty acids (VFA) in this latter process. NH₃ 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 NH₃ into the ruminal vein of sheep have been reported by Rémont et al. (1993b). The mechanism involved is unclear, although the exchange of protons between the VFA and NH₃ 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 NH₃ absorption from the caecum was increased (Réméry & 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 NH₃ flux across the caecal wall.

The importance of bicarbonate in stimulating colonic NH₃ 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. (1992a), 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 NH₃ flux across the rumen wall. In this experiment addition of VFA to the mucosal incubation solution restored NH₃ flux to control levels suggesting that bicarbonate and VFA may play similar roles in mediating NH₃ uptake. Increasing HCO₃⁻ levels in rumen fluid by bubbling CO₂ into the rumen also caused an increase in NH₃ flux in sheep (Rémont et al. 1993b) 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 NH₃ across the gut wall a significant proportion of dietary N intake can be measured as NH₃ 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 NH₃ flux can represent as much as 0.65 of N intake and in many circumstances exceed net α-NH₂-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 NH₃ in portal blood (Malmlöf, 1987; van Berlo et al. 1988; Yen & Pond, 1990; Yen & Nienaber, 1993). Detoxification of absorbed NH₃ by urea cycle activity in the liver is common to all species and calculation of portal NH₃ flux on a metabolic-weight (W₀.75) basis provides a mechanism of ‘scaling’ the effects of
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 NH₃ 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 NH₃ absorption. Studies of digesta flow in sheep (MacRae & Ulyatt, 1974) showed that some 0.15 of N intake was absorbed as NH₃ in the small intestine and application of ¹⁵N techniques provided a dynamic model of NH₃-N movement across different sections of the tract (Siddons et al. 1985a). This latter study indicated that in sheep fed on a silage diet 0.25 of total NH₃ 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 NH₃ 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 NH₃ 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 NH₃ 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 1. Net portal ammonia absorption in different species

<table>
<thead>
<tr>
<th>Species</th>
<th>Portal NH₃ (μM)</th>
<th>Arterial NH₃ (μM)</th>
<th>Net absorption (μmol/min per kgW^{0.75})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>193</td>
<td>72</td>
<td>2.8</td>
<td>Hartman &amp; Prior (1992)</td>
</tr>
<tr>
<td>Pig</td>
<td>285</td>
<td>142</td>
<td>14.3</td>
<td>Malmlöf (1987)</td>
</tr>
<tr>
<td></td>
<td>165</td>
<td>44</td>
<td>17.9</td>
<td>van Berlo et al. (1988)</td>
</tr>
<tr>
<td></td>
<td>205</td>
<td>39</td>
<td>8.2</td>
<td>Yen &amp; Pond (1990)</td>
</tr>
<tr>
<td>Sheep: Fed</td>
<td>487</td>
<td>210</td>
<td>33.2</td>
<td>Gross et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>377</td>
<td>200</td>
<td>19.5</td>
<td>Gross et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>560</td>
<td>220</td>
<td>35.2</td>
<td>Harmon et al. (1988)</td>
</tr>
<tr>
<td>Steers</td>
<td>650</td>
<td>350</td>
<td>71.5</td>
<td>Huntington (1984)</td>
</tr>
<tr>
<td>Cows (lactating)</td>
<td>356</td>
<td>122</td>
<td>44.2</td>
<td>Wilton (1989)</td>
</tr>
<tr>
<td>Steers (2 × daily fed): Min</td>
<td>525</td>
<td>112</td>
<td>82.1</td>
<td>Wilton (1989)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Contribution of ammonia absorption from the small intestine to total net uptake into the portal vein in steers

<table>
<thead>
<tr>
<th>Diet</th>
<th>Net NH₃ uptake (µmol/min per kg W⁰.⁷⁵)</th>
<th>Mesenteric absorption as proportion of total</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mesenteric vein</td>
<td>Portal vein</td>
<td></td>
</tr>
<tr>
<td>Grass nuts</td>
<td>15.8</td>
<td>42.4</td>
<td>0.37</td>
</tr>
<tr>
<td>Grass nuts-flaked maize (70:30 w/w)</td>
<td>10.58</td>
<td>25.6</td>
<td>0.41</td>
</tr>
<tr>
<td>Grass nuts-intraruminal propionate</td>
<td>6.7</td>
<td>23.15</td>
<td>0.29</td>
</tr>
<tr>
<td>Lucerne (Medicago sativa):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time-fed</td>
<td>13.12</td>
<td>46.0</td>
<td>0.28</td>
</tr>
<tr>
<td>Meal-fed</td>
<td>17.62</td>
<td>64.68</td>
<td>0.27</td>
</tr>
<tr>
<td>Concentrates (meal-fed)</td>
<td>14.31</td>
<td>28.25</td>
<td>0.51</td>
</tr>
</tbody>
</table>

W⁰.⁷⁵, metabolic weight.

HEPATIC DETOXIFICATION OF AMMONIA

Under normal physiological and nutritional conditions, NH₃ 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 NH₃ concentrations on a variety of different diets, the liver is able to extract 70-95% of portal NH₃ with the result that hepatic NH₃ removal is on average very slightly higher (4%) than portal absorption (Table 3). This results in arterial NH₃ concentrations remaining constant even when portal NH₃ absorption varies threefold. NH₃ is extremely toxic in non-hepatic tissues and causes changes in cerebral metabolism which result in tetany and death when circulating NH₃ concentrations exceed 0.7 mM (Symonds et al. 1981). Ruminants are susceptible to diet-induced NH₃ toxicity particularly when non-protein-N is rapidly converted to NH₃ in the rumen and absorbed into the portal vein (Visek, 1984). Most investigators have reported arterial NH₃ 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 NH₃ 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 NH₃ 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 NH₃ 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

<table>
<thead>
<tr>
<th>Diet constituents</th>
<th>N-intake</th>
<th>Portal NH$_3$ uptake</th>
<th>Hepatic NH$_3$ uptake</th>
<th>Hepatic urea-N output</th>
<th>Hepatic NH$_3$ uptake: hepatic urea output</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass nuts-flaked maize (70:30, w/w)</td>
<td>123</td>
<td>0.97</td>
<td>0.92</td>
<td>3.36</td>
<td>0.27</td>
<td>Wilton et al. (1988)</td>
</tr>
<tr>
<td>Grass nuts-flaked maize (50:50, w/w)</td>
<td>102</td>
<td>1.29</td>
<td>1.68</td>
<td>2.86</td>
<td>0.59</td>
<td>Fitch et al. (1989)</td>
</tr>
<tr>
<td>Grass nuts</td>
<td>172</td>
<td>3.06</td>
<td>3.18</td>
<td>6.72</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Maize silage</td>
<td>106</td>
<td>1.51</td>
<td>1.47</td>
<td>1.91</td>
<td>0.79</td>
<td>Maltby et al. (1991)</td>
</tr>
<tr>
<td>Grass silage–grass nuts (70:30, w/w)</td>
<td>94</td>
<td>2.48</td>
<td>2.50</td>
<td>2.27</td>
<td>1.10</td>
<td>Maltby et al. (1993a)</td>
</tr>
<tr>
<td>Barley–grass nuts (70:30, w/w)</td>
<td>79</td>
<td>1.19</td>
<td>1.28</td>
<td>1.98</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Lucerne (Medicago sativa)</td>
<td>153</td>
<td>4.21</td>
<td>4.31</td>
<td>6.05</td>
<td>0.71</td>
<td>Maltby et al. (1993c)</td>
</tr>
<tr>
<td>Lucerne</td>
<td>162</td>
<td>4.85</td>
<td>4.83</td>
<td>6.10</td>
<td>0.79</td>
<td>Huntington (1989)</td>
</tr>
<tr>
<td>Lucerne–cracked maize</td>
<td>95</td>
<td>2.13</td>
<td>2.15</td>
<td>2.65</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Lucerne–ground maize (25:75, w/w)</td>
<td>98</td>
<td>2.38</td>
<td>2.47</td>
<td>3.92</td>
<td>0.63</td>
<td>Reynolds et al. (1991)</td>
</tr>
<tr>
<td>Low intake</td>
<td>174</td>
<td>4.17</td>
<td>4.33</td>
<td>8.18</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>High intake</td>
<td>133</td>
<td>3.10</td>
<td>3.23</td>
<td>5.90</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Lucerne–ground maize (75:25, w/w)</td>
<td>209</td>
<td>5.67</td>
<td>5.88</td>
<td>9.88</td>
<td>0.60</td>
<td></td>
</tr>
</tbody>
</table>

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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, 1993b). 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$_4$Cl is converted to $[^{15}\text{N}]$urea and $[^{15}\text{N}]$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 $\mu$mol/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 $\mu$mol/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.

\[ \text{N intake (g/d)} = 64.5 + 22.5 \times \text{hepatic NH}_3 \text{ uptake (mmol/min)} \]  
\[ (r^2 0.74). \]

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 $\alpha$-NH$_2$-N removal ranges from 16 to 30% (Huntington, 1989; Reynolds et al. 1991), although these estimates are based on measurement of $\alpha$-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. 1993a).

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$_4$Cl into a mesenteric vein and observed that the increase in hepatic urea-N
production was three times greater than that in the rate of NH₃-N removed by the liver. The findings suggested that the urea synthesis from non-NH₃ sources had been stimulated during NH₃ 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 NH₃. Lobley et al. (1995) have confirmed recently in sheep that a 5 d intramesenteric vein infusion of NH₃ increases hepatic urea production to approximately double that predicted from stoichiometric conversion of NH₃ 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 NH₃ observed with forage-based diets (Fitch et al. 1989; Huntington, 1989; Reynolds et al. 1991). Other studies have altered NH₃ 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 NH₃ uptake and the proportion of urea-N output that was apparently synthesized from non-NH₃-N sources. Huntington (1989) demonstrated that hepatic α-NH₂-N removal was stimulated twofold on the forage diet with the result that total splanchnic supply of α-NH₂-N to peripheral tissues was decreased by 30%. Regression of hepatic NH₃ uptake v. urea production using the values in Table 3 yields a significant positive correlation.

\[
\text{Hepatic NH}_3\text{ uptake (mmol/min)} = 0.43 + 0.53 \text{ hepatic urea-N output (mmol/min)}
\]

\[
(r^2 0.80).
\]

The slope of this line suggests that an increase in hepatic NH₃ uptake is associated with twice as much urea production as that predicted from direct NH₃ 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.

\[
\text{N intake (g/d)} = 60.2 + 14.8 \text{ hepatic urea-N output (mmol/min)} (r^2 0.92).
\]

Whilst it is possible that these relationships reflect the increasing supply of amino acids to the liver, they suggest the idea that an increased NH₃ 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 NH₃ 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 NH₃ 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 NH₃ 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$_4$Cl 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$_4$Cl 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$_4$Cl 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, 1993a), 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$_4$Cl 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 O₂ uptake can be accounted for by urea synthesis, a value similar to that obtained by Reynolds et al. (1991). If the proposal that NH₃ 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 NH₃ to urea appears to be similar in ruminant and non-ruminant species, the principal difference being that the production of NH₃ by foregut fermentation in ruminants is extremely variable and dependent on feed sources, whilst in non-ruminants, NH₃ 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 NH₃ to urea. However, there is evidence to suggest that NH₃ 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 NH₃ with amino acid metabolism.

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