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Metabolism of nitrogenous compounds in the large gut

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The extent and complexity of nitrogen metabolism in the mammalian large gut (hind-gut) varies considerably between species and is influenced by the physiological age of the animal. It is most highly developed in herbivores and omnivores whose diets, post-weaning, ensure a fairly constant supply of fermentable materials to the lower tract. The present paper reviews some of these topics, with particular emphasis on recent findings in the sheep and pig.

N supply to the large gut

In both species of animal the amount (3–18 g/d) and composition of the ileal N passing to the hind-gut is related to body size, but the nature and quantity of the feed is also important. In the sheep, for example, this supply is influenced to an appreciable extent ($r\ 0\cdot74$) by the duodenal N flow, while in the pig, the flow of N and amino acids is affected by dietary N composition, the type of dietary starch and the passage of fibre (Mason *et al.* 1976; Sauer *et al.* 1977*a*; Taverner *et al.* 1981). With both classes of animal about half this N is water-soluble, one-quarter to one-third is soluble in trichloroacetic acid (Ben-Ghedalia *et al.* 1974; Borgmann *et al.* 1979; Low, 1979*a*) and a substantial part (40–80%) is present in proteinaceous materials of dietary, endogenous and bacterial origin. The contribution of urea-N is low in the sheep ($<2\%$) because of urease activity in the terminal ileum (Clarke *et al.* 1966; Hecker, 1971*b*), but ammonia-N may constitute up to 17% of this fraction (Clarke *et al.* 1966; Coehlo da Silva *et al.* 1972*a,b*; Hogan, 1973; Ben-Ghedalia *et al.* 1974). Nucleic acids (Smith & McAllan, 1971; Coehlo da Silva *et al.* 1972*a,b*) and hexosamines (Badawy & Mackie, 1964; Combe *et al.* 1980) make up $2\cdot5$–$5\cdot0$ and $4\cdot4$–$7\cdot8\%$ of this N respectively in the sheep and calf.

The wall of the large gut supplies N in the form of urea, mucins and sloughed epithelial cells. In one study (Nolan *et al.* 1976) urea, which crossed the wall close to or within the caecum of the sheep, provided about 27% ($1\cdot3$ g/d) of the NH_3-N produced in its lumen, but only 41% ($2\cdot4$ g/d) of the urea degraded in the digestive tract was metabolized in the rumen and caecum. Presumably much of the remainder ($3\cdot4$ g/d) was degraded by bacteria living in close association with the hind-gut wall, in regions other than the caecum. In the goat, urea influx into the colon increased proportionally with increase in plasma urea concentrations for a particular diet (Engelhardt & Hinderer, 1976) and, in contrast to the situation in the rumen, the permeability of the colon wall to this compound appears to be only slightly modified by diet (Engelhardt *et al.* 1978). Little information is currently

available for the pig, but tentative estimates suggest that almost one-quarter of the N entering the hind-gut lumen is secreted by the wall in one or other form (Low, 1982). High-fibre diets stimulate the faecal excretion of water-soluble and endogenous N in the sheep (Mason, 1981) and pig (Bergner *et al.* 1980; Mason *et al.* 1982), but it is not clear whether this reflects increased secretion or decreased efficiency of re-absorption. In man the rate of colon-cell replacement is increased considerably by feeding (Stragand & Hagemann, 1977).

Catabolic processes

In almost all recorded cases, the ileal flow of N in the sheep and pig exceeds the corresponding faecal N excretion, exceptions to this rule occurring only occasionally (Thornton *et al.* 1970; Low, 1979*a*). The daily net absorption of N from the lumen is, however, only 0·4–5·8 and 1·0–6·9 g respectively, suggesting that the processes of degradation and absorption are largely counteracted by those of anabolism and the secretion and sloughing of N.

Considerable proteolytic, deaminative, decarboxylative and ureolytic activity has been observed in the caecum of the sheep and pig (Fauconneau & Michel, 1970; Hecker, 1971*a*; Henderickx & Decuypere, 1973; Erbersdobler, 1971; Chalmers *et al.* 1976; Nolan *et al.* 1976) and in the sheep most hind-gut digestion occurs in the caecum and proximal colon, with only small net changes in N flow beyond this point (Dixon & Nolan, 1982). Animal enzymes probably contribute little to these reactions since there is currently no evidence to suggest the secretion of digestive enzymes by the mucosa of the large gut. Furthermore, the activity of trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1) and carboxypeptidase A (EC 3.4.17.1) is markedly reduced in the terminal ileum of the sheep (Ben-Ghedalia *et al.* 1974) and in the rat trypsin and chymotrypsin are rapidly destroyed by the hind-gut flora (Borgström *et al.* 1959; Genell *et al.* 1976).

Microbial involvement in these processes can be inferred from the large number of viable organisms in hind-gut digesta from the sheep (Mann & Ørskov, 1973; Bauchop, 1975) and pig (Ledinek, 1970; Koch *et al.* 1972) and the ability of individual species to metabolize a wide range of nitrogenous compounds (Draser & Hill, 1974; Wrong *et al.* 1981), many of which animal enzymes cannot digest. Amino acids can be metabolized by decarboxylation to yield the corresponding amine and carbon dioxide, by deamination to give NH_3 and a variety of saturated fatty acids, α-keto acids, α-hydroxyl acids and unsaturated fatty acids and by fission to produce cyclic compounds, other amino acids, α-keto acids and NH_3. Furthermore, bacteria can esterify histamine to *N*-acetyl histamine, convert the diamines putrescine and cadaverine to pyrrolidine and piperidine respectively and open the pyrrolidine ring of proline and the heterocyclic rings of purines and pyrimidines by *N*-dealkylation reactions. Lecithin is degraded to release choline which is further metabolized to trimethylamine, dimethylamine, methylamine and NH_3, dimethylamine being the most abundant secondary amine in human urine. Certain species reduce nitrate to nitrite or NH_3, these reactions being of significance for the possible nitrosation of such secondary amines as piperidine,

pyrrolidine and dimethylamine to the corresponding nitrosamines (Drasar & Hill, 1974; Wrong *et al.* 1981).

NH_3 plays a central role in most catabolic processes of the hind-gut. In the sheep, tracer studies (Nolan *et al.* 1976) showed that about 65% (3·1 g/d) of the total NH_3 flux in the caecum (5·8 g/d) was derived by degradation of bacterial, endogenous and dietary residues from the upper gut and proteinaceous materials from the wall of the caecum itself. The daily ileal flow of N was probably 7–8 g. In other work (Mason *et al.* 1981*a*) infusion of up to 5 g gelatin-N (with or without added antibiotic (Nebacitin))/d into the caecum of sheep greatly increased blood urea concentrations and the urinary excretion of N and urea-N, without significantly altering the corresponding concentrations and amounts of hydroxyproline and other amino acids used as gelatin markers. It was concluded that most of the infused N had been degraded to NH_3 and perhaps amines before absorption.

The significance of bacterial and endogenous residues in the nitrogenous materials degraded in the hind-gut can be deduced from several observations. Greatest proportional losses of amino acids in the large gut of the sheep are usually incurred with 2,6-diaminopimelic acid (DAPA), glutamic acid, valine, alanine, proline, threonine, serine, glycine and cyst(e)ine, in that order (Mason & White, 1971; Coehlo da Silva *et al.* 1972*a,b*; Harrison *et al.* 1973; Hogan, 1973; Ben-Ghedalia *et al.* 1974; MacRae & Ulyatt, 1974; Beever *et al.* 1978), while in the pig the corresponding sequence is found for glycine, glutamic acid, proline, tryptophan, serine, cyst(e)ine and threonine (Holmes *et al.* 1974; Ivan & Farrell, 1976; Mason *et al.* 1976; Sauer *et al.* 1977*b*; Buraczewska *et al.* 1979; Low, 1979*b*). These losses of DAPA, glutamic acid and alanine in the sheep are entirely or partly due to the metabolism of undigested bacterial residues from the upper tract, the latter constituting possibly 25–50% of the ileal N. The cell-wall DAPA and muramic acid of rumen bacteria resists digestion in the small intestine but is extensively degraded in the hind-gut of this animal (Mason & Milne, 1971; Mason & White, 1971). As components of the peptidoglycan of methanol-grown bacteria, DAPA, glutamic acid and alanine accumulate in the terminal ileum of the preruminant calf but suffer considerable losses in the large gut (Guilloteau *et al.* 1980). Relatively small amounts of bacterial N are present in ileal digesta from the pig (Mason *et al.* 1976).

The hind-gut losses of cyst(e)ine, glutamic acid, glycine, proline, serine and threonine in both species indicate the metabolism of endogenous materials, these amino acids occurring in relatively large proportions in endogenous mucoproteins (Bella & Kim, 1972; Degand *et al.* 1972), pancreas secretions (Corring, 1975) and/or bile acids (Low, 1979*b*) and in ileal digesta collected from pigs given protein-free diets (Holmes *et al.* 1974; Sauer *et al.* 1977*a*; Taverner *et al.* 1981). In vitro studies show that hind-gut microbes metabolize mucosal compounds in the sheep (Hecker, 1973) and non-ruminant (Juhr & Haas, 1976) and hexosamines are more extensively degraded in conventional than in germ-free animals (Combe *et al.* 1980). Possibly 15–40 and 30–60% of the ileal N is contained in endogenous residues in the sheep and pig respectively.

Metabolism of undigested dietary residues from the upper tract is poorly documented for the sheep and pig, but some of the plant cell-wall N which disappears between the duodenum and anus of the sheep (Mason, 1981) is probably digested in this region. Maillard complexes formed during the processing and storage of feeds reduce N digestibility in the small intestine (Finot, 1973; Erbersdobler, 1976), but in the rat the simpler amino acid-sugar products are readily attacked by intestinal microbes (Horn *et al.* 1968; Erbersdobler *et al.* 1970; Tanaka *et al.* 1975; Finot *et al.* 1977). The same is true for isopeptides formed when proteins are heated, appreciable quantities of aspartyl-lysine and glutamyl-lysine disappearing in the large gut in this species (Hurrell *et al.* 1976). In humans suffering from malabsorption of neutral (Hartnup disease; Asatoor *et al.* 1970; Milne & Asatoor, 1975) and basic (cystinuria; Hellier *et al.* 1972; Milne & Asatoor, 1975) amino acids, hind-gut activity leads to the production of amines and other compounds, many of which are recovered in the urine.

Anabolic processes

The hind-gut flora probably synthesize their nitrogenous cell components and secretions from simpler molecules such as NH_3, amino acids and peptides (Payne, 1975; Nolan *et al.* 1976), using carbohydrates as the principle energy source. The quantitative significance of these processes is indicated by the presence of 50–78 and 62–76% bacterial N in the faecal N of the sheep (Mason, 1969, 1979) and pig (Mason *et al.* 1976, 1982; Low *et al.* 1978) and the excretion of 2·4–5·9 and 3·1–6·5 g bacterial N/kg dry matter intake respectively. In contrast to earlier opinion (Mason, 1969) it now seems that in the sheep this N is largely assimilated in the hind-gut from materials released by the catabolism of bacterial ghost cells and endogenous and dietary residues. This would explain the similar amino acid composition of faecal bacteria in these species and the net appearance of methionine and other amino acids in digesta during passage through the hind-gut of the sheep (Coehlo da Silva *et al.* 1972a) and pig (Holmes *et al.* 1974; Mason *et al.* 1976; Sauer *et al.* 1977a,b; Zebrowska *et al.* 1977a; Just *et al.* 1981b).

The importance of energy availability for N assimilation in the hind-gut of the ruminant is illustrated by several studies. Carbohydrate infusion into the caecum of sheep (Ørskov & Foot, 1968; Thornton *et al.* 1970) and cattle (Boekholt, 1976) increased faecal N excretion, mainly through bacterial cell proliferation (Ørskov *et al.* 1970; Mason *et al.* 1981b); urinary N showing a corresponding decrease (Mason *et al.* 1981b). In the sheep, caecal infusions of starch and pectin produced a greater effect than a more slowly fermented cellulose, but the response to increasing energy supply was curvilinear with no quantitative changes in the pathways of N excretion when starch infusion exceeded 110 g/d (Mason *et al.* 1981b). Raising the level of forage intake increased bacterial N excretion per unit dry matter intake (Mason, 1981). In the pig, replacement of 250 g maize starch with 250 g uncooked potato starch/kg in semi-synthetic diets gave increases of 23 and 8% in the faecal excretion of N and bacterial N per kg dry matter intake (Mason *et al.* 1976), whereas substitution of one-third of the cereal in a high-barley

diet with grass meal stimulated corresponding increases of 60 and 63% respectively (Mason *et al.* 1982). In contrast, infusion studies show that although N excretion in the faeces and urine of pigs can be modified by carbohydrate fermentation in the hind-gut (Zebrowska *et al.* 1980; Gargallo & Zimmerman, 1981; Misir & Sauer, 1982) the effects are usually small (Just *et al.* 1981*a*; V. C. Mason and co-workers, unpublished results).

The reasons for these different responses to energy supply are probably associated with differences produced in the balance between fermentable nitrogenous compounds and carbohydrates in hind-gut digesta. The relatively high value for N:readily-fermentable energy sources in ileal digesta from the forage-fed sheep would be expected to encourage a microbial response to extra energy, especially since more N is provided from the wall of the large gut itself. In the pig, however, this ratio is usually lower because the contribution of bacterial residues to the ileal N supply is less and much of the undigested carbohydrate at the terminal ileum is in the form of readily-fermentable starch (Keys & De Barthe, 1974). When the supply of N from the ileum is low, provision of N from the wall of the caecum and colon (the main fermentation sites) could be crucial and possibly inadequate for optimal microbial growth. It seems likely that much of the urea-N metabolized by microbes living in close association with the hind-gut wall is rapidly re-absorbed as NH_3 and is therefore unavailable to organisms located on feed particles. Furthermore, the influx of urea and other secretions further down the large intestine would be of little benefit to microbes if there were a local deficiency in readily-fermentable energy sources.

Nutritional significance of N metabolism in the large gut

It appears that N metabolism in the large gut of the sheep and pig confers relatively little benefit on the N status of the host. Amino acids may be absorbed from this region but there is little evidence to suggest that the quantities involved are nutritionally very meaningful once microbial activity is established, though active transport of methionine across the wall of the proximal colon of the newborn pig does occur (James & Smith, 1976). Direct proof that specific amino acids are not absorbed from this organ in significant quantities in the sheep has been obtained using synthetic DAPA (Mason & White, 1971) and ^{35}S-labelled bacterial cyst(e)ine (Judson *et al.* 1975; Elliott & Little, 1977). In the pig, infusion of enzymically-hydrolysed casein (Zebrowska, 1975), unhydrolysed heated or unheated casein (Zebrowska *et al.* 1977*b*; Gargallo & Zimmerman, 1981), isolated soya-bean protein or lysine hydrochloride (Sauer, 1976) into the terminal ileum of animals given protein-free or low- to medium-protein diets, gave almost complete digestion in the large intestine but poor utilization within the body, as measured by N balance. When mixed protein, lysine or methionine were infused into the caecum of pigs given lysine- and methionine-deficient diets the effect on N balance was positive but very small (Just *et al.* 1981*a*) and at high rates of infusion much of the infused lysine was excreted in the faeces. B and K vitamins are assimilated and

excreted in bacterial cells, but little is known of the proportion absorbed in the soluble form (Baker *et al.* 1950; Henderickx *et al.* 1964).

Most of the N is probably absorbed as ammonia (McDonald, 1948; Erbersdobler, 1971), amines being absorbed in smaller quantities (Henderickx & Decuypere, 1973). These materials can serve as N sources for the synthesis of non-essential amino acids and other compounds in the gut wall and liver; in the ruminant animal, recycled N may help to support microbial growth in the reticulorumen (Nolan *et al.* 1976). The latter may have survival value for animals consuming N-deficient diets, but with high-N feeds absorption of excess NH_3 requires the expenditure of energy to convert it to urea for excretion.

An important aspect of N metabolism in the hind-gut is that it sustains a microbial population actively engaged in the conversion of hygroscopic or osmotically-active materials to absorbable molecules. A feature of digestion in herbivores and omnivores is that passage of the more slowly digested dietary constituents through the small intestine encourages the movement of large amounts of water into the hind-gut (Partridge, 1978), the water containing appreciable quantities of organic compounds and minerals. Metabolism of organic materials assists the absorption of water, sodium and other essential minerals and at the same time provides the host animal with an extra supply of energy in the form of volatile fatty acids (Mason, 1980).

REFERENCES

Asatoor, A. M., Cheng, B., Edwards, K. D. G., Lant, A. F., Matthews, D. M., Milne, M. D., Navab, F. & Richards, A. J. (1970). *Gut* 11, 380–387.

Badaway, A. M. & Mackie, W. S. (1964). *Quarterly Journal of Experimental Physiology* 49, 356–364.

Baker, F., Nasr, H., Morrice, F. & Bruce, J. (1950). *Journal of Pathology and Bacteriology* 62, 617–638.

Bauchop, T. (1975). Quoted by Ulyatt, M. J., Dellow, D. W., Reid, C. S. W. & Bauchop, T. (1975). In *Proceedings of the 4th International Symposium on Ruminant Physiology*, Sydney, pp. 119–133 [I. W. McDonald and A. C. I. Warner, editors]. Armidale, NSW, Australia: University of New England Publishing Unit.

Beever, D. E., Terry, R. A., Cammell, S. B. & Wallace, A. S. (1978). *Journal of Agricultural Science, Cambridge* 90, 463–470.

Bella, A. & Kim, Y. S. (1972). *Archives of Biochemistry and Biophysics* 150, 679–689.

Ben-Ghedalia, D., Tagari, H. & Bondi, A. (1974). *British Journal of Nutrition* 31, 125–142.

Bergner, H., Simon, O. & Bergner, U. (1980). In *Proceedings of the 3rd EAAP Symposium on Protein Metabolism and Nutrition*, Braunschweig, vol. I, pp. 198–206 [H. J. Oslage and K. Rohr, editors]. European Association of Animal Production.

Boekholt, H. A. (1976). Nitrogen metabolism of the lactating cow and the role of gluconeogenesis from amino acids. Doctorate Thesis, Mededelingen Landbouwhogschool, Wageningen. Wageningen: H. Veemann & B. V. Zonen.

Borgmann, E., Kreienbring, F., Meinl, M. & Wünsche, J. (1979). *Archiv für Tierernährung* 29, 419–435.

Borgström, B., Dahlqvist, A., Gustafsson, B. E., Lundh, G. & Malmquist, J. (1959). *Proceedings of the Society of Experimental Biological Medicine* 102, 154–155.

Buraczewska, L., Zebrowska, T., Wünsche, J., Hennig, U., Krawielitzki, K., Kreienbring, F., Meinl, M., Borgmann, E. & Bock, H. D. (1979). *Archiv für Tierernährung* 29, 437–460.

Chalmers, M. I., Grant, I. & White, F. (1976). In *Proceedings of the 1st International Symposium on Protein Metabolism and Nutrition, EAAP,* Nottingham, pp. 159–179 [D. J. A. Cole, K. N. Boorman, P. J. Buttery, D. Lewis, R. J. Neale and H. Swan, editors]. London: Butterworths.

Clarke, E. M. W., Ellinger, G. M. & Phillipson, A. T. (1966). *Proceedings of the Royal Society* B**166,** 63–79.

Coehlo da Silva, J. F., Seeley, R. C., Beever, D. E., Prescott, J. H. D. & Armstrong, D. G. (1972*a*). *British Journal of Nutrition* **28,** 357–371.

Coehlo da Silva, J. F., Seeley, R. C., Thomson, D. J., Beever, D. E. & Armstrong, D. G. (1972*b*). *British Journal of Nutrition* **28,** 43–61.

Combe, E., Patureau-Mirand, P., Bayle, G. & Pion, R. (1980). *Reproduction, Nutrition et Développement* **20,** 1707–1715.

Corring, T. (1975). *Annales de Biologie Animale, Biochemie et Biophysique* **15,** 115–118.

Degand, P., Gaveriaux, M. & Havez, R. (1972). *Compte Rendu des Séances de la Societe de Biologie, Paris* 622–627.

Dixon, R. M. & Nolan, J. V. (1982). *British Journal of Nutrition* **47,** 289–300.

Drasar, B. S. & Hill, M. J. (1974). *Human Intestinal Flora,* pp. 72–102. London and New York: Academic Press.

Elliott, R. & Little, D. A. (1977). *Australian Journal of Biological Sciences* **30,** 203–206.

Engelhardt, W. v. & Hinderer, S. (1976). In *Tracer Studies on Non-protein Nitrogen for Ruminants,* vol. III, pp. 57–58. Vienna: International Atomic Energy Authority.

Engelhardt, W. v., Hinderer, S. & Wipper, E. (1978). In *Ruminant Digestion and Feed Evaluation,* pp. 4.1–4.12. Hurley: Grassland Research Institute.

Erbersdobler, H. (1971). *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **27,** 217–218.

Erbersdobler, H. (1976). In *Proceedings of the 1st International Symposium on Protein Metabolism and Nutrition, EAAP,* Nottingham, pp. 139–158 [D. J. A. Cole, K. N. Boorman, P. J. Buttery, D. Lewis, R. J. Neale and H. Swan, editors]. London: Butterworths.

Erbersdobler, H., Gunsser, I. & Weber, G. (1970). *Zentralblatt für Veterinärmedzin* A**17,** 573–575.

Fauconneau, G. & Michel, M. C. (1970). In *Mammalian Protein Metabolism,* vol. IV, pp. 481–522 [H. N. Munro, editor]. New York and London: Academic Press.

Finot, P. A. (1973). In *Proteins in Human Nutrition,* pp. 501–514 [J. W. G. Porter and B. A. Rolls, editors]. London and New York: Academic Press.

Finot, P. A., Magnenot, E., Mottu, F. & Bujard, E. (1977). *Proceedings of Alimentation et Travail,* Nancey.

Gargallo, J. & Zimmerman, D. (1981). *Journal of Nutrition* **111,** 1390–1396.

Genell, S., Gustafsson, B. E. & Ohlsson, K. (1976). *Scandinavian Journal of Gastroenterology* **11,** 757–762.

Guilloteau, P., Patureau-Mirand, P., Toullec, R. & Prugnaud, J. (1980). *Reproduction, Nutrition et Développement* **20,** 615–629.

Harrison, D. G., Beever, D. E., Thomson, D. J. & Osbourn, D. F. (1973). *Journal of Agricultural Science, Cambridge* **81,** 391–401.

Hecker, J. F. (1971*a*). *British Journal of Nutrition* **25,** 85–95.

Hecker, J. F. (1971*b*). *British Journal of Nutrition* **26,** 135–145.

Hecker, J. F. (1973). *Journal of Agricultural Science, Cambridge* **80,** 63–69.

Hellier, M. D., Holdsworth, C. D., Perrett, D. & Thirumalai, C. (1972). *Clinical Science* **43,** 659–668.

Henderickx, H. & Decuypere, J. (1973). In *Germfree Research,* pp. 361–368 [J. B. Heneghan, editor]. New York and London: Academic Press.

Henderickx, H. K., Teague, H. S., Redman, D. R. & Grifo, A. (1964). *Journal of Animal Science* **23,** 1036–1038.

Hogan, J. P. (1973). *Australian Journal of Agricultural Research* **24,** 587–598.

Holmes, J. H. G., Bayley, H. S. & Leadbeater, P. A. (1974). *British Journal of Nutrition* **32,** 479–489.

Horn, M. J., Lichtenstein, H. & Womack, M. (1968). *Journal of Agriculture and Food Chemistry* **16,** 741–745.

Hurrell, R. F., Carpenter, K. J., Sinclair, W. J., Otterburn, M. S. & Asquith, R. S. (1976). *British Journal of Nutrition* **35**, 383–395.

Ivan, M. & Farrell, D. J. (1976). *Animal Production* **23**, 111–119.

James, P. S. & Smith, M. W. (1976). *Journal of Physiology* **262**, 151–168.

Judson, G. J., Abdelsamie, R. & Bird, R. B. (1975). *Australian Journal of Agricultural Research* **26**, 743–749.

Juhr, N. C. & Haas, A. (1976). *Zeitschrift für Versuchstierkunde* **18**, 206–215.

Just, A., Jørgensen, H. & Fernández, J. A. (1981*a*). *British Journal of Nutrition* **46**, 209–219.

Just, A., Jørgensen, H. & Fernández, J. A. (1981*b*). *Proceedings of the 6th International Symposium on Amino Acids*, Warsaw.

Keys, J. E. & De Barthe, J. V. (1974). *Journal of Animal Science* **39**, 57–62.

Koch, G., Ledinek, M. & Gieseke, D. (1972). *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **30**, 222–231.

Ledinek, M. (1970). Untersuchungen über die Beteiligung von Mikroorganismen am Verdauungsstoffwechsel des Schweines bei Fütterung roher und gedämpfter Kartoffeln. Dissertation Ludwig-Maximilians-Universität, Munchen, 101 pp.

Low, A. G. (1979*a*). *British Journal of Nutrition* **41**, 137–146.

Low, A. G. (1979*b*). *British Journal of Nutrition* **41**, 147–156.

Low, A. G. (1982). In *Physiologie Digestive Chez le Porc*, pp. 189–198 [J. P. Laplace, T. Corring and A. Rerat, editors]. Paris: INRA.

Low, A. G., Sambrook, I. E. & Yoshimoto, J. T. (1978). *29th EAAP Annual Meeting*, Stockholm.

McDonald, I. W. (1948). *Biochemical Journal* **42**, 584–587.

MacRae, J. C. & Ulyatt, M. J. (1974). *Journal of Agricultural Science, Cambridge* **82**, 309–319.

Mann, S. O. & Ørskov, E. R. (1973). *Journal of Applied Bacteriology* **36**, 475–484.

Mason, V. C. (1969). *Journal of Agricultural Science, Cambridge* **73**, 99–111.

Mason, V. C. (1979). *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **41**, 140–149.

Mason, V. C. (1980). In *Current Concepts of Digestion and Absorption in Pigs*, pp. 112–129 [A. G. Low and I. G. Partridge, editors]. Technical Bulletin No. 3, NIRD, Reading and HRI, Ayr.

Mason, V. C. (1981). *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **45**, 161–173.

Mason, V. C., Bech-Andersen, S. & Narang, M. P. (1981*a*). *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **46**, 150–164.

Mason, V. C., Just, A. & Bech-Andersen, S. (1976). *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **36**, 310–324.

Mason, V. C., Kessank, P., Ononiwu, J. C. & Narang, M. P. (1981*b*). *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **45**, 174–184.

Mason, V. C., Kragelund, Z. & Eggum, B. O. (1982). *Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkunde* **48**, 241–252.

Mason, V. C. & Milne, G. (1971). *Journal of Agricultural Science, Cambridge* **77**, 99–111.

Mason, V. C. & White, F. (1971). *Journal of Agricultural Science, Cambridge* **77**, 91–98.

Milne, M. D. & Asatoor, A. M. (1975). In *Peptide Transport in Protein Nutrition*, pp. 167–182 [D. M. Matthews and J. W. Payne, editors]. Amsterdam: Association of Scientific Publishers.

Misir, R. & Sauer, W. C. (1982). *Journal of Animal Science* **55**, 599–607.

Nolan, J. V., Norton, B. W. & Leng, R. A. (1976). *British Journal of Nutrition* **35**, 127–147.

Ørskov, E. R. & Foot, M. H. (1968). *Proceedings of the Nutrition Society* **28**, 31A–32A.

Ørskov, E. R., Fraser, C., Mason, V. C. & Mann, S. O. (1970). *British Journal of Nutrition* **24**, 671–682.

Partridge, I. G. (1978). *British Journal of Nutrition* **39**, 539–545.

Payne, J. W. (1975). In *Peptide Transport in Protein Nutrition*, pp. 283–364 [D. M. Matthews and J. W. Payne, editors]. Amsterdam: Association of Scientific Publishers.

Sauer, W. C. (1976). Factors influencing amino acid availability for cereal grains and their components for growing monogastric animals. PhD Thesis, University of Manitoba.

Sauer, W. C., Stothers, S. C. & Parker, R. J. (1977*a*). *Canadian Journal of Animal Science* **57**, 775–784.

Sauer, W. C., Stothers, S. C. & Phillips, G. D. (1977*b*). *Canadian Journal of Animal Science* **57**, 585–597.

Smith, R. H. & McAllan, A. B. (1971). *British Journal of Nutrition* **25,** 181–190.

Stragand, J. J. & Hagemann, R. F. (1977). *American Journal of Physiology* **233,** E208–E211.

Tanaka, M., Lee, T. C. & Chichester, C. O. (1975). *Journal of Nutrition* **105,** 989–994.

Taverner, M. R., Hume, I. D. & Farrell, D. J. (1981). *British Journal of Nutrition* **46,** 149–158.

Thornton, R. F., Bird, P. R., Somers, M. & Moir, R. J. (1970). *Australian Journal of Agricultural Research* **21,** 345–354.

Wrong, O. M., Edmonds, C. J. & Chadwick, V. S. (1981). *The Large Intestine.* Lancaster: MTP Press Ltd.

Zebrowska, T. (1975). *Rocznik Naukrol Niczych* **B97,** 117–123.

Zebrowska, T., Buraczewska, L. & Buraczewski, S. (1977a). *Rocznik Naukrol Niczych* **B99,** 87–98.

Zebrowska, T., Buraczewska, L. & Horaczynski, H. (1977b). *Rocznik Naukrol Niczych* **B99,** 99–105.

Zebrowska, T., Zebrowska, H. & Buraczewska, L. (1980). *Proceedings of the 3rd EAAP Symposium on Protein Metabolism and Nutrition,* Braunschweig, vol. I, pp. 222–226 [H. J. Oslage and K. Rohr, editors]. European Association of Animal Production.

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