Control of nitrogen metabolism in the ruminant

By K. HUTTON, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne NE1 7RU* and E. F. ANNISON, *Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford*

The factors which control nitrogen metabolism in the ruminant can be separated into two groups. The first includes all factors other than the digestive tract which exert control over the metabolism of N within body tissues. This group includes hormone and protein interrelationships, hormonal regulation of tissue enzyme levels, and the role of the liver, kidney, heart, skeletal muscle and free amino acid pools in the regulation of protein metabolism. These aspects have been extensively reviewed recently (see Munro & Allison, 1964; Munro, 1970) and will not be referred to subsequently in this discussion. The second group is specific to the digestive tract and includes those factors which affect N metabolism within the tract and thereby control the end-products of N digestion. Since the aim of this review is to consider the regulation of N metabolism in the ruminant animal in relation to the manipulation of rumen fermentation, emphasis has been placed on the N metabolism in the rumen and on the ability of the products to meet the N requirements of ruminant tissues.

N metabolism in the rumen

The supply of essential amino acids to extra ruminal tissues is dependent on the amount, composition and digestibility of dietary protein which escapes ruminal fermentation, and on the extent of microbial protein synthesis in the rumen. Since the availability of N for microbial cell synthesis is influenced by the recycling of ammonia-N and urea-N between the rumen and the tissues, recent quantitative studies on the dynamic aspects of the metabolism of urea and ammonia are also reviewed.

Degradation of dietary protein. The extent of degradation of dietary protein is largely dependent on the solubility of the material in rumen liquor (Henderickx & Martin, 1963) and any process which reduces solubility will result in a decreased breakdown of food protein. Such processes include heating (Chalmers, Cuthbertson & Synge, 1954; Sherrod & Tillman, 1962; Tagari, Henis, Tamir & Volcani, 1965), treatment with vegetable tannins (Zelter, Leroy & Tissier, 1970) and treatment with formaldehyde (Ferguson, Hemsley & Reis, 1967; Reis & Tunks, 1969). Some dietary protein usually escapes rumen breakdown and there is evidence that, as with other components of the diet, the proportion which reaches the duodenum

intact may depend on the level of food intake (Ørskov, Fraser & McDonald, 1971) in addition to the processing involved in preparation of the food (Coelho da Silva, Seeley, Thomson, Beever & Armstrong, 1972). Quantitative results on the degradation of dietary protein in the rumen are limited and mainly confined to certain proteins which can be assayed in the presence of a mixture of proteins of microbial and endogenous origin, which occur in duodenal contents. Two such proteins are casein, which is extremely soluble in rumen liquor, and zein which is largely insoluble; it has been demonstrated that the extent of ruminal digestion of casein (McDonald & Hall, 1957) and zein (McDonald, 1954; Ely, Little, Woolfolk & Mitchell, 1967) was about 95 and 50% respectively. Indirect estimates of the extent of dietary protein breakdown may be obtained by measuring the microbial protein content of the mixture of proteins leaving the rumen and entering the duodenum. Measurement of the contribution of microbial protein has been discussed recently by Hutton, Bailey & Annison (1971) but the general procedure suffers from the limitation that it is not possible to measure simultaneously the contribution which endogenous protein secreted into the omasum–abomasum makes to the total protein entering the duodenum.

Synthesis of microbial protein in the rumen. The peptides, amino acids and ammonia which arise as end-products of protein fermentation are the N sources for the bacteria and protozoa which proliferate in the rumen. These microbial cells, which pass out of the rumen and become available for digestion in the lower gut, constitute a major protein source for the ruminant. The value to the animal of the conversion of dietary protein to microbial protein is largely dependent on the relative biological values of the proteins from these two sources, although the importance of maintaining a viable microbial population to effectively digest roughage must not be overlooked. It is now generally accepted that the essential amino acid profiles of bacterial and protozoal protein and their digestibilities are largely independent of diet, and the available results suggest that microbial protein is a moderately good protein source of relatively constant composition (Purser, 1970).

Microbial cell synthesis requires energy and the anaerobic nature of ruminal fermentation inevitably limits the extent of conversion of digestible organic matter (OM) to microbial cell material (Walker, 1965; Hungate, 1966). Several groups of workers have attempted to measure the extent of microbial growth under anaerobic conditions both in vitro and in vivo. Bauchop & Elsden (1960) attempted to relate in vitro microbial cell yield to ATP produced during fermentation by pure cultures of bacteria; the value varies with different micro-organisms, with different substrates and, in continuous culture, with variations in dilution rate (see Hogan & Weston, 1970). Other workers have related cell yield to substrate fermented in vitro (see Hungate, 1966; Walker & Nader, 1968; Walker & Nader, 1970), to OM digested in the rumen (Hogan & Weston, 1967; Hume, 1970; Hume, Moir & Somers, 1970) or to OM apparently digested in the rumen (Hogan & Weston, 1970); the term 'OM digested' is OM apparently digested, corrected for microbial cells and endogenous secretions (OM digested=OM apparently digested+microbial cells+an allowance for endogenous secretions). Care has been taken to distinguish between

the values for microbial protein expressed in some instances as total N×6·25 and in other instances as tungstic acid-precipitated N ×6·25; the former includes nucleic acids which may be as much as 20% of the total N in micro-organisms (Smith, 1969), whereas the latter is normally taken as a true protein figure. A further complication when comparing results lies in the fact that different workers have used different values for the level of N in bacteria. In an attempt to compare the results from different laboratories on a uniform basis it has been assumed that (1) the micro-organisms contain 10·5% N (see Hungate, 1966) of which 85% is true protein N (see Smith, 1969) and (2) the fermentation of 100 g carbohydrate is equivalent to the fermentation of 100 g OM in the rumen and results in the production of 1·2 mol of volatile fatty acids (VFA) and 2·4 mol of ATP (see Walker & Nader, 1970).

Table 1. *Growth of micro-organisms in relation to their energy supply*

g crude protein/mol ATP	g crude protein/100 g carbohydrate	g crude protein/mol VFA	Reference
2·7–5·5	6·5–13·1	5·4–11·0	See Hungate (1966)
5·4–8·3	13·0–19·9	10·8–16·6	Bauchop & Elsden (1960)
7·7–8·7	18·5–20·9	15·4–17·3	Walker & Nader (1968)
6·2	14·4	12·3	Walker & Nader (1970)
7·4	17·7	14·8	Hogan & Weston (1967)
4·3–6·3	10·3–15·0	8·5–12·5	Hume, Moir & Somers (1970)
8·0–10·6	19·2–25·3	16·0–21·1	Hume (1970)

VFA, volatile fatty acids.

It is assumed that 2·4 mol ATP≡1·2 mol VFA≡100 g organic matter≡100 g carbohydrate; and that the N content of bacteria = 10·5% of which 85% is true protein N.

The results, calculated from results from several laboratories, are shown in Table 1. Pure cultures of micro-organisms (Bauchop & Elsden, 1960; Hungate, 1966) yielded less protein than rumen micro-organisms in vitro (Walker & Nader, 1968, 1970). Furthermore, yields were higher in vivo (Hogan & Weston, 1970; Hume, 1970; Hume *et al.* 1970) than in vitro, and a value of about 20 g of bacterial crude protein/100 g OM digested in the rumen would seem a reasonable figure, although it should be noted that the value obtained by Hogan & Weston (1970) applies to bacteria whereas those of Hume *et al.* (1970) and Hume (1970) apply to total microbial protein.

Dynamic aspects of the metabolism of urea and ammonia. Peptides and amino acids arise in the rumen as a result of breakdown of food protein but their half-life in the rumen is short and their concentration low as they are rapidly broken down to ammonia and VFA or utilized for microbial cell synthesis (see Allison, 1970). Rumen ammonia may also arise from dietary non-protein N or from urea entering the rumen in saliva (Somers, 1961) or direct across the rumen wall (Houpt, 1970). Endogenous urea results from the deamination of absorbed amino acids, from tissue protein catabolism or from absorbed ammonia which is converted to urea in the liver and recycled. Several attempts have been made to look at quantitative aspects of N

metabolism in ruminants: rumen ammonia production and absorption (Pilgrim, Gray & Belling, 1969), urea metabolism (Cocimano & Leng, 1967), synthesis of microbial protein from ammonia in the rumen (Pilgrim, Gray, Weller & Belling, 1970) and aspects of amino acid metabolism in the rumen (Lewis, 1955; Lewis & Emery, 1962; Portugal, 1963; Portugal & Sutherland, 1966) have all been investigated. The potential use of labelled materials for investigating the metabolism of N-containing compounds has long been appreciated and recently three groups of workers have contributed further quantitative results using either ^{15}N or a combination of ^{15}N and ^{14}C (Pilgrim *et al.* 1970; Mathison & Milligan, 1971; Nolan & Leng, 1972).

A quantitative model for N pathways in adult sheep has been proposed by Nolan & Leng (1972) from studies of ammonia and urea metabolism involving the use of isotope dilution techniques with [^{15}N]ammonium sulphate, [^{15}N]urea and [^{14}C]urea. In an animal in which the daily N intake was 23·4 g, faecal output of N was 4·7 g and urinary N output was 14·2 g, of which 12·1 g was urea-N. The difference of 4·5 g between input of N in food, and output in faeces and urine contributed largely to the growth of wool. About 40% ($\equiv$9·5 g) of the dietary protein passed through the reticulo-rumen intact and the remainder was digested: of the 12 g of microbial N which left the rumen daily, 11 g was derived from rumen ammonia. The daily contribution of recycled urea to rumen ammonia was only 1·2 g N, and could be accounted for as salivary urea, suggesting that the diffusion of blood urea into the rumen was negligible. Urea-N, 18·4 g/d, was produced in body fluids of which only 2 g arose from rumen ammonia, the remainder resulting from the deamination of amino acids or from ammonia absorbed from the caecum and colon. The major part of the body fluid urea-N was excreted in urine but a considerable amount, about 5·1 g daily, entered the caecum and colon presumably providing a source of N for the fermentation of residual carbohydrate in this area of the digestive tract.

The study of Nolan & Leng (1972) admirably illustrates the advantages to be gained by studying complex biological systems by simulation analysis ('modelling').

Amino acid requirements of ruminants

The extensive breakdown and synthesis of protein in the rumen leads to a completely different quantity and pattern of amino acids at the small intestine, the major site of amino acid absorption, relative to that in the food. Nevertheless, the utilization of large quantities of protein for animal production implies a large tissue requirement for amino acids in certain classes of ruminant livestock. Clearly, in order to determine these requirements a method in which no modifications to the diet are involved is the technique of choice; preferably the chosen method should be proven in monogastric livestock where the results can be compared with dietary requirements determined by giving graded amounts of essential amino acids. Such a method has been applied to pigs, rats and chicks (Williams, Curtin, Abraham, Loosli & Maynard, 1954). We have attempted to extrapolate the results of this work to the young growing steer, and in order to do this a number of assumptions have been made.

1. The gain in carcass N expressed as a percentage of live weight gain is 2·64 for the young growing pig (Woodman & Evans, 1951) and 2·40 for the steer (Agricultural Research Council, 1965).

2. The tissue requirements for essential amino acids for cattle and pigs are the same (Black, Kleiber, Smith & Stewart, 1957; Downes, 1961); this is a reasonable assumption as the amino acid composition of pig meat and of beef are very similar, and muscle is the dominant N-containing tissue in livestock. Furthermore, 95% of the dry matter of muscle comprises amino acids, and the amino acid composition of the whole pig carcass (Williams *et al.* 1954) is very similar to that of pig meat (unpublished observations).

3. The availabilities of essential amino acids in the duodenum of pigs and cattle are the same.

4. There is no significant absorption of essential amino acids prior to the duodenum.

The first step in calculating a tissue requirement for an essential amino acid is to determine the daily N deposition. For a 30 kg pig growing at 0·6 kg/d this would be $2 \times \cdot 64 \times 0 \cdot 610 = 16$ g, and for a growing steer of 200 kg gaining 1 kg/d it would be $2 \cdot 4 \times 1 \cdot 0 \times 10 = 24$ g. The tissue requirement for individual amino acids for the pig and the steer has been determined factorially using the amino acid composition of the pig carcass (Williams *et al.* 1954). The results are shown in Table 2 together with food requirements for the pig (USA) (National Research Council, 1968). The duodenal

Table 2. *Amino acid (g/d) requirements for young growing pigs and cattle*

	Pigs (30 kg; 0·6 kg/d)		Utilization factor for the pig*	Cattle (200 kg steer; 1·0 kg/d)	
	Tissue	Food		Tissue	Duodenal (estimated)†
Nitrogen	16	44	0·36	24	67
Arginine	7·1	3·4	2·09	10·7	5·1
Histidine	2·7	3·1	0·87	4·1	4·7
Isoleucine	3·8	8·5	0·45	5·7	12·7
Leucine	7·1	10·2	0·70	10·7	15·3
Methionine	1·8	8·5	0·21	2·7	12·9
Phenylalanine	3·8	8·5	0·45	5·7	12·7
Threonine	3·8	7·6	0·50	5·7	11·4
Tryptophan	0·7	2·2	0·32	1·1	3·1
Valine	6·0	8·5	0·71	9·0	12·7
Lysine	8·6	11·9	0·72	12·9	17·9

*For explanation see below.

†The duodenal requirement has been determined using the estimated tissue requirement for the steer and the utilization factor for the pig.

requirement for the young growing steer has been determined using the estimated tissue requirement for the steer and the utilization factor for the pig. The utilization factor is simply the ratio of calculated tissue requirement to food requirement for the pig and is a reflection of losses of a particular amino acid incurred in digestion and subsequent metabolism. The digestibilities of essential amino acids in the small intestine of sheep are reasonably constant (see Coelho da Silva *et al.* 1972). If the

same is true for the pig then the utilization factors reflect the extent of losses in tissue metabolism. High utilization factors for leucine and valine (Table 2) suggest that their primary function is as protein constituents, but the low value for methionine emphasizes the importance of this amino acid in other roles such as the donation of methyl groups and the synthesis of sulphur-containing compounds. The high value for arginine suggests a considerable synthesis of this amino acid in animal tissues.

A weakness of the factorial method for the assessment of the amino acid requirements of ruminants is immediately apparent: although the endogenous urinary N of pigs and cattle may be regarded as approximately equal to 0·15 g/d per $kg^{0·75}$, the metabolic faecal N outputs are very different, that of the pig being only about 1·5 g/kg dry-matter intake whereas that of the ruminant is much higher at about 5 g/kg dry-matter intake. If essential amino acids contribute significantly to the excretion of endogenous urinary N and metabolic faecal N, this would result in a higher requirement for essential amino acids per unit of N retained by ruminants than shown in Table 2.

We must now consider what proportion of the essential amino acid requirements of a young steer of 200 kg live weight growing at a rate of 1 kg/d can be met by bacterial protein. The animal would require about 5 kg dry-matter intake daily (USA) (National Research Council, 1970) and if it is assumed that the digestible OM of the ration is 4 kg and that 70% of this is digested in the rumen, the extent of microbial protein synthesis can be estimated. The synthesis of 20 g of bacterial crude protein (85% true protein) per 100 g OM digested in the rumen (see earlier) is equivalent to a daily synthesis of 76 g true protein N in the steer. The daily synthesis of bacterial amino acids has been calculated using the bacterial protein amino acid composition results of Purser & Beuchler (1966) and a value of 1·6 g tryptophan/16 g true protein N (Hutton, unpublished observation). The values obtained have been tabulated together with the estimates of amino acid requirements in Table 3 and it is clear that microbial synthesis is adequate to meet the requirement of the steer for protein and most of the essential amino acids. However the quantity of methio-

Table 3. *Comparison of bacterial essential amino acids entering the duodenum of a 200 kg steer with daily calculated requirements (g/d)*

	Requirement	Synthesized	Ratio, requirement: synthesized
True protein N	67	76	0·87
True protein	420	475	0·87
Arginine	5·1	25·7	0·19
Histidine	4·7	10·9	0·43
Isoleucine	12·7	30·4	0·42
Leucine	15·3	34·6	0·44
Methionine	12·9	12·4	1·04
Phenylalanine	12·7	24·3	0·52
Threonine	11·4	26·3	0·44
Tryptophan	3·1	7·6	0·41
Valine	12·7	31·5	0·43
Lysine	17·9	44·5	0·43

nine synthesized is barely adequate for the needs of the animal and clearly methionine is the first limiting amino acid in this instance.

In conclusion, the method which we have used to predict the requirement of ruminant tissues for amino acids and the ability of rumen bacteria to supply these suggests that some dietary protein must escape fermentation in the rumen for maximal growth of ruminants, since methionine synthesis is barely adequate. Furthermore it is likely that in sheep, where wool growth is dependent on the supply of sulphur-containing amino acids, and in high-yielding dairy cows there is a definite need for large quantities of food protein to escape fermentation in the rumen. It is with these two classes of livestock that the protection from ruminal attack of dietary proteins, or preferably of specific essential amino acids, should prove to be of the greatest benefit.

REFERENCES

Agricultural Research Council (1965). *The Nutrient Requirements of Farm Livestock: Ruminants.* London: Agricultural Research Council.

Allison, M. J. (1970). In *Physiology of Digestion and Metabolism in the Ruminant* p. 456 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press Ltd.

Bauchop, T. & Elsden, S. R. (1960). *J. gen. Microbiol.* **23**, 457.

Black, A. L., Kleiber, M., Smith, A. H. & Stewart, D. N. (1957). *Biochim. biophys. Acta* **23**, 54.

Chalmers, M. I., Cuthbertson, D. P. & Synge, R. L. M. (1954). *J. agric. Sci., Camb.* **44**, 254.

Cocimano, M. R. & Leng, R. A. (1967). *Br. J. Nutr.* **21**, 353.

Coelho da Silva, J. F., Seeley, R. C., Thomson, D. J., Beever, D. E. & Armstrong, D. G. (1972). *Br. J. Nutr.* **28**, 43.

Downes, A. M. (1961). *Aust. J. biol. Sci.* **14**, 254.

Ely, D. G., Little, C. O., Woolfolk, P. G. & Mitchell, G. E. Jr (1967). *J. Nutr.* **91**, 314.

Ferguson, K. A., Hemsley, J. A. & Reis, P. J. (1967). *Aust. J. Sci.* **30**, 215.

Henderickx, H. & Martin, J. (1963). *C. r. Rech. Inst. Encour. Rech. scient. Ind. Agric.* **31**, 9.

Hogan, J. P. & Weston, R. H. (1967). *Aust. J. agric. Res.* **18**, 973.

Hogan, J. P. & Weston, R. H. (1970). In *Physiology of Digestion and Metabolism in the Ruminant* p. 474 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press Ltd.

Houpt, T. R. (1970). In *Physiology of Digestion and Metabolism in the Ruminant* p. 119 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press Ltd.

Hume, I. D. (1970). *Aust. J. agric. Res.* **21**, 305.

Hume, I. D., Moir, R. J. & Somers, M. (1970). *Aust. J. agric. Res.* **21**, 283.

Hungate, R. E. (1966). *The Rumen and its Microbes.* New York and London: Academic Press.

Hutton, K., Bailey, F. J. & Annison, E. F. (1971). *Br. J. Nutr.* **25**, 165.

Lewis, D. (1955). *Br. J. Nutr.* **9**, 215.

Lewis, T. R. & Emery, R. S. (1962). *J. Dairy Sci.* **45**, 1487.

McDonald, I. W. (1954). *Biochem. J.* **56**, 120.

McDonald, I. W. & Hall, R. J. (1957). *Biochem J.* **67**, 400.

Mathison, G. W. & Milligan, L. P. (1971). *Br. J. Nutr.* **25**, 351.

Munro, H. N. (editor) (1970). *Mammalian Protein Metabolism* Vol. 4. London: Academic Press.

Munro, H. N. & Allison, J. B. (editors) (1964). *Mammalian Protein Metabolism* Vol. 1. London: Academic Press.

National Research Council (1968). *Publs natn. Res. Coun., Wash.* no. 2, 6th ed.

National Research Council (1970). *Publs natn. Res. Coun., Wash.* no. 4, 4th ed.

Nolan, J. V. & Leng, R. A. (1972). *Br. J. Nutr.* **27**, 177.

Ørskov, E. R., Fraser, C. & McDonald, I. (1971). *Br. J. Nutr.* **25**, 243.

Pilgrim, A. F., Gray, F. V. & Belling, C. B. (1969). *Br. J. Nutr.* **23**, 647.

Pilgrim, A. F., Gray, F. V., Weller, R. A. & Belling, C. B. (1970). *Br. J. Nutr.* **24**, 589.

Portugal, A. V. (1963). Some aspects of amino acid and protein metabolism in the rumen of sheep. PhD Thesis, University of Aberdeen.

Portugal, A. V. & Sutherland, T. M. (1966). *Nature, Lond.* **209**, 510.

Purser, D. B. (1970). *J. Anim. Sci.* **30**, 988.

Purser, D. B. & Beuchler, S. M. (1966). *J. Dairy Sci.* **49**, 81.

Reis, P. J. & Tunks, D. A. (1969). *Aust. J. agric. Res.* **20**, 775.

Sherrod, L. B. & Tillman, A. D. (1962). *J. Anim. Sci.* **21**, 901.
Smith, R. H. (1969). *J. Dairy Res.* **36**, 313.
Somers, M. (1961). *Aust. J. exp. Biol.* **39**, 111.
Tagari, H., Henis, Y., Tamir, M. & Volcani, R. (1965). *Appl. Microbiol.* **13**, 437.
Walker, D. J. (1965). In *Physiology of Digestion in the Ruminant*. p. 296 [R. W. Dougherty, editor]. Washington, DC: Butterworths.
Walker, D. J. & Nader, C. J. (1968). *Appl. Microbiol.* **16**, 1124.
Walker, D. J. & Nader, C. J. (1970). *Aust. J. agric. Res.* **21**, 747.
Williams, H. H., Curtin, L. V., Abraham, J., Loosli, J. K. & Maynard, L. A. (1954). *J. biol. Chem.* **208**, 277.
Woodman, H. E. & Evans, R. E. (1951). *J. agric. Sci., Camb.* **41**, 102.
Zelter, S. Z., Leroy, F. & Tissier, J. D. (1970). *Annls Biol. anim. Biochim. Biophys.* **10**, 111.

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