Protein digestion in ruminants

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Although the protein requirement of domestic ruminants may be calculated from a simple one-compartment model, this approach ignores factors such as microbial fermentation in the rumen and the non-equality of feed proteins. By examining digestion in each of the functional compartments of the digestive tract of the ruminant, a model may be constructed of the complete process. In this review, we propose to show the essential role played by the development of suitable animal preparations in which protein digestion may be studied in detail, and the application of this data to the development of theoretical models. In order to do this, we intend to examine protein digestion in terms of the whole animal, fermentation in the rumen, hydrolysis and absorption from the small intestine. Finally we propose a new technique for examining the interaction between the small and large intestines.

Alhoewel die proteïenbehoefte van plaasherkouers d.m.v. 'n eenvoudige enkelkompartementmodel bereken kan word, word faktore soos mikrobiese fermentasie in die rumen en die onvergelykbaarheid van verskillende voerproteïene deur hierdie benadering uitgesluit. 'n Beskrywende model van die verteringsproses in die herkouer kan opgestel word deur vertering in elke funksionele deel van die spysverteringskanaal afsonderlik te ondersoek en daarna die inligting te integreer. In hierdie oorsig beoog ons om op die belangrikheid van gepaste voorbereidings van diere te wys vir gedetailleerde proteïenverteringstudies, asook die toepassing van sulke data by die ontwikkeling van teoretiese modelle. Om dit te doen sal proteïenvertering ondersoek word in terme van die dier in sy geheel, fermentasie in die rumen, hidrolise en absorpsie in die dunderm. Ten laaste word 'n nuwe tegniek voorgestel om die interaksie tussen die dun- en dikderm te ondersoek.

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Introduction

Proteins play a crucial role in virtually all biological processes such as enzymatic catalysis, transport, storage, motion, mechanical support, immunology, as well as in the control of metabolism. All proteins in the body are in a state of constant flux, the size of the pool depending on a balance between synthesis and hydrolysis. Aside from the first few days of life, when globulins are absorbed intact from the small intestine (Deutsch & Smith, 1957), proteins are hydrolysed to peptides and amino acids in the digestive tract prior to being absorbed. Proteins that provide adequate amounts of the so-called essential amino acids are said to have a high biological value. However, the actual biological value of any feed protein depends on the amount and composition of those amino acids absorbed into the circulation of the animal. Ideally, therefore, the biological value of a feed protein should be determined from the amount and type of amino acid appearing in the portal circulation of the animal, and not simply the dissappearance of amino acids from the tract.

Ruminant digestion may be more easily analysed and understood by subdividing the digestive tract into functional units and examining the role of each by a process of modelling. Since no model can totally represent a real system in every detail, there must always be some degree of simplification. Models that are purely conceptual are generally lacking in rigour, and will not allow the precise interpretation associated with a mathematical structure. Because one of the objectives of biological research is to obtain sufficient information about a complex system so as to manipulate it, the ideal model should be based on sound mathematical principles.

In this review, we propose to show the essential role played by the development of suitable animal preparations in which protein digestion may be studied in detail, and the application of this data to the development of theoretical models. To do this, we intend to examine protein digestion in terms of the whole animal, fermentation in the rumen, hydrolysis and absorption from the small intestine, and recycling in the large intestine. In conclusion, we propose a new technique for examining the interaction between the small and large intestines.

Protein digestion by the whole animal

While a simple one-compartment model may be used to calculate the protein requirement of a ruminant, i.e. protein retained by the animal equals protein taken in (crude protein in the feed) minus protein lost (crude protein in the faeces), such a model will be of limited value for the following reasons. Processes that take place

within the animal, such as microbial synthesis from non-protein nitrogen (NPN) in the rumen, the effect of digestible energy on the rate and degree of protein digestibility, or the contribution of endogenous protein to the indigestible feed protein in the faeces (metabolic faecal nitrogen or MFN), are not accomodated by the model and therefore compromise the validity of the conclusions.

Nitrogen metabolism in the rumen

The rumen is the largest of the three compartments cranial to the stomach, and serves as a continuous culture vat supporting at least three classes of micro-organisms in vast numbers. Of these, bacteria play a dominant role with a population density of $>10^{10}$ per g of digesta (>20 species $>10^7$ per g digesta, Bauchop, 1977). Although protozoa occur in smaller numbers (10^6 per g), they may be divided into at least 15 genera which together account for >50% of the biomass of the rumen (Clarke, 1977b). Little is known about the various fungi found in the rumen (Clarke 1977b), and even less about their outflow to the lower digestive tract (Buttery & Foulds, 1985).

Since the rumen plays such a pre-eminent role in the digestive process of the ruminant, it has attracted a considerable amount of research effort. As a result, the many methods developed to examine the function of the rumen has helped to contribute to our understanding of its function.

Surgical preparation of animals

An oesophageal fistula allows samples to be taken of ingested feed as well as mixed saliva (van Dyne & Torell, 1964), while a rumen fistula and cannula (Jarret, 1948) allows access to ruminal contents. Suitable cannulae placed in the abomasum or even in the duodenum (single type, Zinn, Bull, Hemken, Button, Enlow & Tucker, 1980 or reentrant type, Taylor, 1960, Ash, 1962; Harrison, 1962), permits the collection of digesta leaving the reticulo-rumen complex. Owing to the difficulty of maintaining an omasal cannula, digesta is normally sampled from the abomasum or proximal duodenum (Hecker, 1974).

Experimental techniques

Although no current method will adequately measure all aspects of protein degradation in the rumen, due to wide variations in both protein quality and animal differences, many techniques provide reasonable estimates of the degree of digestibility. These may be divided into *in vitro* and *in vivo* techniques.

In vitro

Solubility of a protein may be used as an index of digestibility (Wohlt, Sniffen, Hoover, Johnson & Walker, 1976; Stern & Satter, 1982, the French PDI system proposed by Verite, Journet & Jarrige, 1979). Many important exceptions to this generalization severely limit the application of this technique (Nugent & Mangan, 1978, Mahadevan, Erfle & Sauer, 1980). For example, the nature of the solvent may affect the degree of solubility (Crooker, Sniffen, Hoover & Johnson, 1978), while structural modification of the protein by the attachment of side groups such as sialic acid and n-acetyl galactosamine may inhibit hydrolysis, e.g. bovine submaxillary mucoprotein which, although soluble, is degraded extremely slowly in the rumen (Wallace, 1983).

The accuracy of an alternative approach, based on the production of ammonia from the incubation of a test feed sample with rumen fluid (Broderick, 1978), may be compromised by factors such as sequestration of ammonia by bacteria in the rumen fluid for protein synthesis, and the accumulation of fermentation end-products (leading to changes in the pH of the medium).

In vivo

Flux of digesta through the tract may be estimated by using radioactive isotopes to mark the liquid and solid components of digesta (Warner, 1981). Digesta markers currently used, however, cannot be regarded as 'ideal', as they do not always fully reflect the solid or liquid phase that they are intended to represent (Faichney, 1980).

The flux of nitrogen into and out of the various pools in the rumen may be followed by using the stable isotope ^{15}N (Pilgrim, Gray, Weller & Belling, 1970; Nolan & Leng, 1974), combined with data obtained from digesta flow studies using sheep fitted with reentrant duodenal cannulae.

Certain of the fundamental assumptions — that the animal is in nitrogen equilibrium, the balance between the various nitrogen pools is in steady state and turnover rates remain constant for the duration of the study — are difficult if not impossible to attain in practice. Furthermore, these experiments are time consuming and labour intensive, and therefore not practical for large-scale screening of different protein sources.

Total protein leaving the reticulorumen may be conveniently divided into undegraded feed protein (bypass), microbial protein and endogenous protein (salivary mucoproteins plus epithelial cells).

Microbial protein may be estimated from the presence of endogenous markers such as diaminopimelic acid (DAPA) in bacteria (Weller, Gray & Pilgrim, 1958) and amino-ethylphosphonic acid (AEP) in protozoa (Abou Akkada, Messmer, Fina & Bartley, 1968). Such markers, however, are subject to variation, may enter the rumen via microbial contamination of the feed, or may be confined to a specific pool, e.g. DAPA measures only bacterial protein (Harrison & McAllen, 1980). Alternatively, radioisotopes such as ^{35}S, ^{32}P or ^{15}N may be used to follow the synthesis of this protein fraction (Clarke, 1977a).

The quantity of mucoproteins may be calculated from the flow of saliva to the rumen, provided the concentration of mucoprotein in saliva is known (Phillipson, 1964). Rate of desquamation may be estimated either indirectly from factors such as the mitotic index of epithelial cells (Macrae & Reeds, 1979),

or directly in ruminants that are maintained by means of intragastric infusion (Ørskov & Macleod,1982).

Bypass protein may therefore be calculated as the difference between the total and the sum of the microbial and endogenous protein fractions. Alternatively, Stern & Satter (1984) suggested a method whereby the increased protein outflow to the small intestine, resulting from the incremental addition of dietary protein to the ration, may be used to directly estimate this fraction. This incremental technique assumes that the protein content of the diet will not influence the balance between that which is degraded and that which will bypass, an assumption that may lead to errors (Zinn, *et al.* 1980).

Dacron bag

Recently refined by Mehrez & Ørskov (1977), this technique combines features from both the *in vitro* and *in vivo* methods. Although the test sample is incubated inside the rumen (*in vivo*), the sample is physically enclosed in a dacron bag for easy recovery. Bags may be incubated for a set period of time or serially removed in order to obtain an estimate of the rate of degradation (Ørskov & McDonald, 1979, Mathers & Miller, 1981, Stern & Satter, 1984). The basic assumption that the environment, and thus the microbial ecology inside the dacron bag, will resemble the surrounding ruminal content was questioned by Meyer & Mackie (1986) who demonstrated that factors such as pore size affect, *inter alia*, the loss of soluble protein and the entry of protozoa as well as that of certain bacteria.

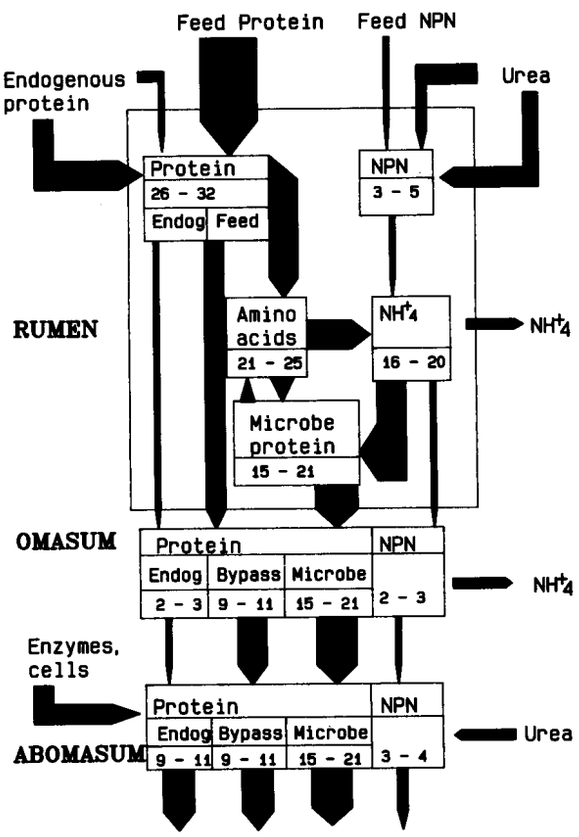

Figure 1 Summary of nitrogen flux (g/day) in the reticulorumen complex. Width of the arrows are proportional to the flux rate. Data selected from references in the text. Abbreviations used are: NPN = non-protein nitrogen; Endog = endogenous

Rumen model

By combining data obtained from diverse experimental approaches, an integrated model describing the function of the reticulorumen may be derived. The information depicted in Figure 1 summarizes the concepts and data given in the following sections.

Protein degradation

Protein entering the rumen may be degraded by both bacteria and protozoa (Tamminga, 1979) which produce proteolytic enzymes, although proteolytic activity is mostly associated with bacterial cells (Wallace, 1983; Wallace & Kopechny, 1983). The expression of this activity is not influenced by the presence of proteins or their lysis products (Cotta & Hespell, 1986), and is therefore not greatly affected by changes in the composition of the diet (Blackburn & Hobson, 1962; Allison, 1970).

Nugent & Milligan (1981) and Russell, Sniffen & van Soest (1983) suggest that proteolysis may be the rate-limiting step, since the concentration of free amino acids in rumen fluid is normally very low (Annison, Lewis & Lindsay, 1959, Lewis, 1962). Free amino acids appearing in rumen fluid are rapidly deaminated to keto-acids and ammonia (El-Shazly, 1958, Lewis, 1955, Cotta & Hespell, 1986), rather than taken up by bacteria, despite an obligatory requirement for amino acids by many species (Hungate, 1966).

Non-protein nitrogen is rapidly degraded (Krishnamoorty, Sniffen, Stern & van Soest, 1983), largely to ammonia, which may then be taken up by bacteria for protein synthesis. Depending on diet, 60 – 90% of the daily nitrogen intake of the ruminant may be converted to ammonia and from 50 – 70% of bacterial-N may be derived from ammonia (Leng & Nolan, 1984).

While the rate of protein degradation is a function of the rate of proteolysis as well as retention time in the rumen, retention time appears to be less important than proteolysis. However, the time a protein is exposed to enzymic action does influence the degree of degradation (Harrison, Beever, Thompson & Osbourn, 1975; Hemsley, 1975). Any factor, such as inter-animal variation, species differences, particle size and quantity of feed that affects retention time will affect the degree of protein degradation (Warner, 1981).

The proportion of bypass protein that leaves the rumen may also be manipulated by reducing proteolysis of feed protein. Feed processing methods, such as pelleting, steam rolling or flaking, tend to denature the feed protein due to the generation of heat, thereby 'protecting' the protein from lysis in the rumen (Beever & Thompson, 1981). A similar degree of protection may be obtained from the chemical treatment of feed protein using compounds such as formaldehyde (Ferguson, Hemsley & Reis, 1967), tannins (Driedger & Hatfield,

1972), propanol and ethanol (van der Aar, Berger & Fahey, 1982) and inhibitors of amino acid deamination (Chalupa & Scott, 1976). Unfortunately, protein that is protected against degradation in the rumen is often resistant to proteolysis further down the digestive tract (Ferguson, 1975). Despite these dangers, careful treatment of dietary protein to prevent proteolysis in the rumen will increase the amount of bypass protein that arrives at the duodenum (van Bruchem, Rouwers, Bangma, Lammer-Weinhoven & van Adrichem, 1985).

Passage of nitrogen from the rumen

Between 60 and 85% of the total amino acid-nitrogen entering the small intestine is microbial protein. Despite wide variation in the composition of feed protein, level of intake, and experimental design, the amino acid composition of bacterial and protozoal protein remains remarkably constant (Purser & Beuchler, 1966, Bergen, Purser & Kline, 1968, van't Klooster & Boekholt, 1972, Harrison, Beever, Thompson & Osbourn, 1973), although protozoa would appear to have more lysine and glutamic acid, while bacteria tend to have more histidine, threonine, serine, cystine and methionine (Ibrahim & Ingalls, 1972). Since the bypass fraction is only about 15–40% of the total protein entering the duodenum, the composition of this fraction will have to be markedly different from that of the microbial protein to significantly affect the overall composition of the total protein. Despite this, any protein that supplies significant amounts of 'limiting' amino acids will have a disproportionate effect on the nitrogen retention of the ruminant (Macrae & Lobley, 1986) since microbial protein is deficient in certain amino acids such as methionine, lysine, histidine and arginine (Storm & Ørskov, 1984).

Protein is not materially affected by passage through the omasum, although both urea and ammonia may be absorbed (0,9 g/d nitrogen, 1975), together with considerable amounts (Gray, Pilgrim & Weller, 1958) of water (33–64% of that entering) and volatile fatty acids (40–69% of that entering). Most of this water (about 5 l/day, Masson & Phillipson, 1952) is returned to the abomasum together with 1,2 – 2,0 g/day nitrogen in the form of digestive enzymes, urea and ammonia (Egan, Boda & Varady, 1986).

Nitrogen recycling

A positive gain in nitrogen across the reticulo-rumen when ruminants are fed low-nitrogen diets suggests that recycled nitrogen may be returned to the system (Hogan & Weston, 1970). Some of this nitrogen may come from endogenous protein which enters the rumen via saliva (1,0–1,3 g/d) or desquamation (4–12 g/d). The rest of the recycled nitrogen is added to the rumen in the form of small nitrogen-containing molecules, of which urea is the most important (Varady, Havassy, Kosta & Fejes, 1976), either with the saliva (0,5–2,0 g urea nitrogen/kg dry matter consumed — Norton, Murray, Entwhistle, Nolan, Ball & Leng, 1978) or directly by diffusion (1–13 g urea nitrogen/d, depending on diet — Nolan & Macrae, 1976; Kennedy & Milligan,1978).

In addition to nitrogen recycled between the rumen and the endogenous metabolism of the animal, a considerable amount appears to be recycled within the rumen. While 50–75% of microbial protein may be synthesized from the ruminal ammonia pool, about 30–50% of this protein may be hydrolyzed within the rumen and returned to the ammonia pool (Leng & Nolan, 1984), primarily as a result of the lysis of micro-organisms and secretions of amino acids and ammonia by bacteria and protozoa. This degradation of microbial protein within the rumen may significantly reduce the efficiency of microbial growth and hence of protein synthesis.

Nitrogen metabolism in the small intestine

Despite the fact that the small intestine is the major site of amino acid absorption in the ruminant (Tagari & Bergman, 1978), little has been done (in comparison to the effort expended on investigating the function of the rumen) to elucidate the process of protein digestion in this segment of the tract, partly due to the difficulty of obtaining suitable animal preparations.

Surgical preparation of the animals

Protein digestion in the small intestine may be investigated by dividing the gut of slaughtered animals into segments, quantitatively recovering the digesta from each segment and determining the concentration of nitrogenous compounds in each fraction. When used in conjunction with fluid or solid phase markers, the uptake of nitrogen from the various regions of the small intestine may be quantitatively calculated (Church, 1979). In order to carry out long-term studies, animals may be fitted with reentrant duodenal and ileal cannulae. These cannulae may affect the normal physiological function of the digestive tract (MacRae, 1975). Alternatively, simple 'T' cannulae in the duodenum and ileum, which are less disruptive than reentrant cannulae, may be used in conjunction with solid- and liquid-phase markers. However, in such studies, large numbers of animals are required to reduce the inherently large coefficient of variation for the measurement of digesta flow (Miller, 1982).

Experimental techniques

Protein digestibility in the small intestine may be expressed in terms of net disappearance or true uptake, in which case the values are corrected for the addition of endogenous protein. These values may differ by as much as 20% (Tamminga, 1980). Depending on the aim of the study, either of these values may be used to determine the disappearance of protein from the small intestine. However, the digestibility of the microbial and feed components should be separately determined.

Partial digestion studies may be carried out in sheep fitted with suitable cannulae in the duodenum and ileum (Waldo, 1973; Armstrong & Beever, 1969), although such work is laborious and time consuming. For these

reasons, considerable effort has been expended in developing alternative techniques.

The classical technique may be partially simplified by infusing a test meal into the abomasum for 3 days, and collecting samples of digesta from the ileum over the last 24–48 h, although cannulated sheep are still required. Digestion of the feed nitrogen may be calculated from the difference between the amount of nitrogen infused and that amount of nitrogen in the ileal digesta that is surplus to control (Hvelplund & Moller, 1976).

Alternatively, a specially modified nylon bag may be used to carry the test feed protein down the small intestine, after which it may be recovered from the faeces and the contents analysed for loss of protein (mobile nylon bag technique; Sauer, Jorgensen & Berzins, 1983). Although attractive in its simplicity, the latter method has several inherent problems. The feed in the bag may not be adequately exposed to the digestive process, while nitrogen may be added in the form of microbial protein in passage through the hindgut (Hvelplund, 1985).

Several regression techniques allow the flow of microbial and/or bypass protein to the duodenum to be indirectly estimated from other parameters, (INRA, 1978; ARC, 1984; Satter & Roffler, 1975; Burroughs, Nelson & Mertens, 1975; Waldo & Goering, 1979; Mehrez, Ørskov & McDonald, 1976). Although values calculated in this way appear to correspond well with those determined by direct methods (Mathers & Miller,1981; Zinn, Bull & Hemken, 1981; Madsen, 1983; Madsen & Hvelplund, 1985), more experimental observations are required to obtain the required precision.

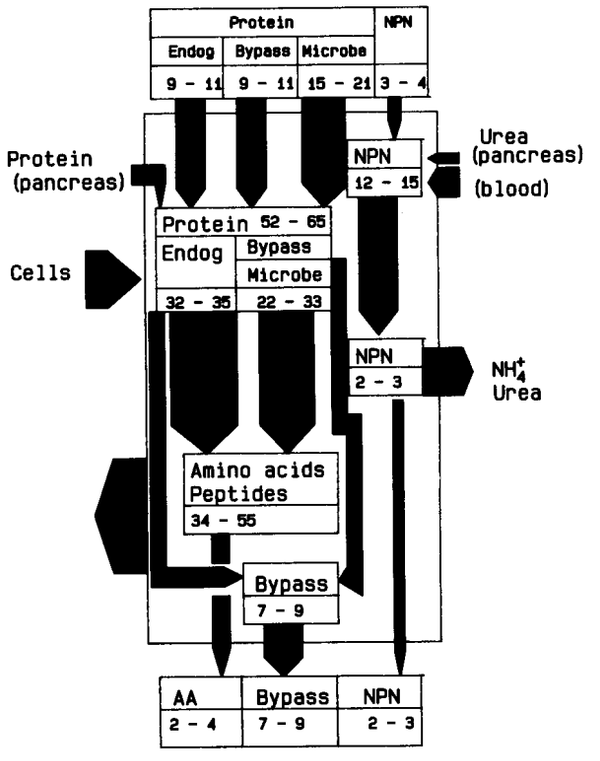

Figure 2 Summary of nitrogen flux (g/day) in the small intestine. The width of the arrows are proportional to the magnitude of the flux rate. Data selected from the references in the text. Abbreviations used are: NPN = non-protein nitrogen; Endog = endogenous; AA = amino acids

Small intestine model

By combining data obtained from diverse experimental approaches, an integrated model describing the function of the small intestine may be derived. The information given in Figure 2 is derived from the following sections.

Nitrogen input

Most (65%) of the nitrogen reaching the duodenum consists of microbial, bypass and endogenous protein (Smith, 1975). The rest of the nitrogen (Oldham & Tamminga, 1980) may be divided between nucleic acids (11%), ammonia (6%) and amides (4%).

Endogenous protein is derived from the following sources. Pancreatic fluid and bile may add about 1,5–3,0 g/d of nitrogen as protein , 0,1–0,4 g/d as amino acids, and 0,1–0,4 g/d as urea into the small intestine (Phillipson, 1964; Varady, Boda, Tashenov & Fejes, 1979), the amount increasing with an increase in digesta flow, protein intake or nitrogen retention (Harrison & Hill, 1962; Smith, 1979). In addition, 5–6 g/d of urea may enter via the *succus entericus*, although most of this appears to be reabsorbed as urea by the time the digesta reaches the ileum (Egan, *et al.*, 1986). Endogenous protein from cells and secretions may be added at the rate of 17–20 g nitrogen/d (Smith, 1979), which may increase with increased fibre content in digesta. As a result, the total contribution of endogenous protein to amino acid absorbed from the small intestine of sheep may be 15–29 g nitrogen/day, contributing about 1,5- to four-fold the amount of digesta nitrogen flowing from the abomasum (Smith, 1979).

Protein digestibility

Although digestion in the abomasum and small intestine of ruminants appears to be similar to that of non-ruminants, some of the differences that do exist may influence this process. For example, the concentration of bicarbonate in the pancreatic exocrine secretion of ruminants is lower (Taylor, 1960; Chalupa, 1978), thereby extending proteolysis along the length of the small intestine. As a result, optimum pH for trypsin, chymotrypsin and carboxypeptidase is only attained in mid-jejenum, while that for exopeptidase and dipeptidase is attained in mid-ileum (Ben-Ghedalia, Tagari, Bondi & Tadmor, 1974).

Amino acids appear to be absorbed mainly from mid- to lower-ileum (Johns & Bergen, 1973; Phillips, Webb & Fontenot, 1976), although the highest rate of amino acid absorption may occur in mid-jejenum (Ben-Ghedalia, *et al.*, 1974).

The technique used to measure the digestibility of bypass protein appears to influence the absolute value (Zinn & Owens, 1982; Smith & McAllan, 1974; Tas, Evans & Exford, 1981; Salter & Smith, 1974; Hvelplund, 1985), with values ranging from 64% to 86% for various feed components. However, pretreatment of

feedprotein may decrease this digestibility to as little as 11% depending on the type and degree of processing (Hvelplund, 1985).

True digestibility of microbial protein in the small intestine appears to be remarkably constant (74–85%) when measured *in vivo* using labelled microbial protein, despite wide variation in diet, feeding regime, age and sex (Salter & Smith, 1972; Hvelplund, 1985). Similar values may be obtained (about 87% absorption for microbial amino acids), using a multiple regression technique (Tas, *et al.*, Zinn & Owens, 1982).

Digestibility of endogenous protein is difficult to measure directly, although a considerable quantity of endogenous protein appears to leave the ileum (241 mg nitrogen/kg ^{0,75} in a 60 kg sheep, Schwartings & Kaufmann, 1978). This may not be due to an inherently low digestibility but may be related to the short transit time of endogenous protein originating from the caudal part of the small intestine (Ochoa-Solano & Gitler, 1968).

Gut wall metabolism

Protein metabolism of intestinal tissues may be estimated by comparing the dissappearance of amino acids from the lumen of the small intestine to that appearing in portal blood, since mucosa of the small intestine contains systems for the uptake of free amino acids and peptides (Matthews, 1972; Munck, 1976). Storm, Brown & Ørskov (1983) reported that less cysteine and histidine appeared to be absorbed than the other amino acids in microbial protein (73% and 68% compared to a range of 80–88% respectively). About 67–71% and 55–57% of amino acids absorbed may be metabolized (some for energy production — MacRae, 1978) in passage through the gut wall of sheep fed a high (19,8% CP) or low (15,6% CP) protein diet respectively (Tagari & Bergman, 1978).

Nitrogen metabolism in the large intestine

In the large intestine, the caecum and proximal colon appear to be the major regions of fermentation and absorption of fermentation end-products (Dixon & Nolan, 1982). The role of the large intestine in recycling nitrogen to the rumen has attracted most of the research effort in this region.

Surgical preparation of the animals

To gain access to the caecum, a cannula may be placed into the mid portion of the caecum, and exteriorized through the body wall below the mid-lateral line to prevent loss of function (MacRae, Reid, Dellow & Wyburn, 1973). Owing to the absorption of water, the digesta in the hindgut becomes paste-like, thereby making the use of cannulae further down the large intestine impractical (Hecker, 1974).

Experimental techniques

Protein digestion may be investigated by the isolated segment technique described for the small intestine. When used in conjunction with fluid or solid phase markers, there being little difference between their rates of flow in the hindgut, the uptake of nitrogen from the various compartments of the large intestine may be quantitatively calculated. Alternatively, ^{15}N may be utilized as a tracer (Dixon & Nolan, 1986) to follow the metabolism of nitrogen in the large intestine.

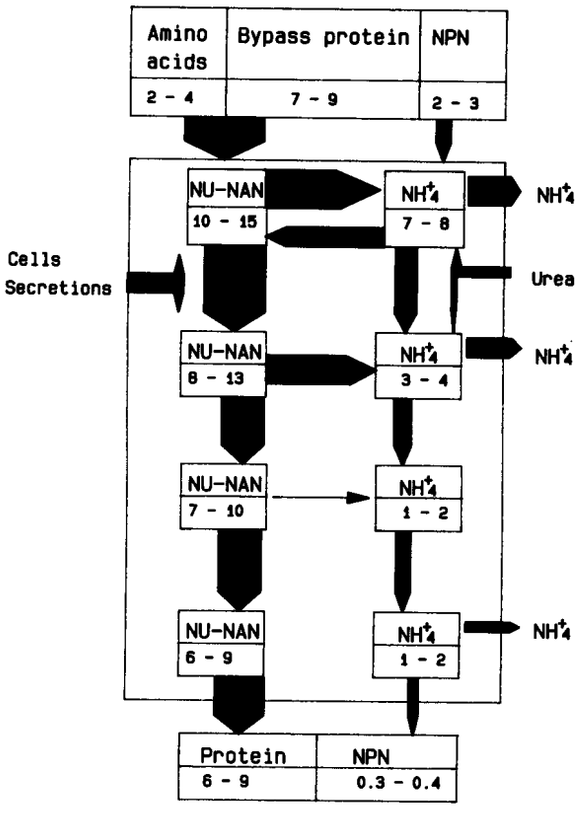

Figure 3 Summary of nitrogen flux (g/day) in the large intestine. Width of the arrows are proportional to the flux rate. Data selected from the references in the text. Abbreviations used are: NPN = non-protein nitrogen; NU-NAN = non-urea, non-ammonia nitrogen

Large intestine model

The information given in the following sections, largely obtained from the studies of Dixon & Nolan (1983), is summarized in Figure 3.

Nitrogen input

Nitrogen enters the caecum in the form of undigested feed residues, ruminal micro-organisms and endogenous material (Hecker, 1973; Nolan, Norton & Leng, 1973). Some bacterial growth does occur in the ileum and may contribute to this flow of nitrogen (Ben-Ghedalia, 1982). A total of about 8–12 g/d nitrogen, subdivided into amino acids (40–60%), nucleic acids (3–4%), ammonia (1–13%), and urea (up to 15%) may pass from the ileum to the caecum (Clarke, Ellinger & Phillipson, 1966; Coelha da Silva, Seeley, Thompson, Beever & Armstrong, 1972; van't Klooster & Boekholt, 1972) of sheep fed lucerne chaff 800 g/day (Thompson, Beever, Harrison, Hill & Osbourn, 1970). About 2 g/d of urea may flow from the blood into the caecum of sheep fed a low-nitrogen diet (Dixon & Milligan, 1984).

Fermentation and protein degradation

In general, degradation of protein in the large intestine appears to be similar to that in the rumen. Caecal contents exhibit cellulase, protease, deaminase and urease activities (Hecker, 1967; Nolan, *et al.*, 1973), and fermentation products such as the volatile fatty acids, ammonia, and microbial protein are present (Williams, 1965). Proteolytic activity in particular, appears to be greater in hindgut digesta than in rumen fluid (Hecker, 1971), leading to extensive degradation of protein in this organ (Ørskov, Frazer, Mason & Mann, 1970).

Hindgut fermentation is energy limited and may be increased by the addition of starch or glucose (Ørskov, Frazer, McDonald & Smart, 1974). Ruminants fed high-fibre diets appear to pass little fermentable energy on to the large intestine (about 4% of total OM in sheep fed forage diets, Ulyatt, Dellow, Reid & Bauchop, 1975), while those fed high-concentrate diets may pass on considerable amounts of fermentable energy (about 37% of total OM in cattle fed a production type diet, Zinn & Owens, 1981). Such an increase in the energy supply to the hindgut may increase faecal nitrogen output while decreasing urine-nitrogen excretion (Thornton, Bird, Sommers & Moir, 1970; Ørskov, *et al.*, 1970; Mason, Narang, Ononimu & Kessank, 1977).

Nitrogen uptake

Under most feeding conditions, more nitrogen enters the large intestine in the digesta than leaves it, suggesting that nitrogen is absorbed in net amounts from this organ (0,5–2,0 g/d for sheep, Clarke, *et al.*, 1966; Hecker, 1971; Ørskov, Frazer & McDonald, 1971 and 0–5 g/d for cattle, van't Klooster & Boekholt, 1972; Zinn & Owens, 1982), mainly in the form of ammonia (McDonald, 1948) from the caecum and colon (Dixon & Nolan, 1983). Although amino acids appear to be actively transported from the colon (Scharrer, 1978) and the caecum (Hoover & Heitmann, 1975; Slade, Bishop, Morris & Robinson, 1971), the amount transferred into the bloodstream would appear to be quantitatively unimportant (Wrong, Edwards & Chadwick, 1981).

Nitrogen recycling

Owing to the presence of large amounts of urease, most of the urea entering the large intestine is converted to ammonia, which may be readily absorbed (digesta pH = 7–8) into the bloodstream (Nolan, Norton & Leng, 1976). Some of this ammonia (derived from 20% of all urea entering the large intestine) may be incorporated into microbial protein and lost in the faeces (Ulyatt, *et al.*, 1975). Generally, the amount of urea degraded in the post-ruminal tract, as calculated from differences between tracer estimates of total degradation and ruminal degradation, appears to increase with plasma urea concentration and whole-body urea production rate (Cocimano & Leng, 1967; Norton, *et al.*, 1978; Kennedy & Milligan, 1980).

Although some studies (Nolan & Leng, 1972; Nolan & Stachiw, 1979) suggest that substantial quantities of urea may be degraded in the hindgut, more direct estimates all suggest that the large intestine plays a relatively minor role in this process (Varady, *et al.*, 1976; Dixon & Nolan, 1983; Dixon & Milligan, 1984). Regardless of the actual quantity degraded, urea will only contribute to the nitrogen economy of the animal if the resultant ammonia is returned to the rumen (either as ammonia or as urea) for microbial protein synthesis (Egan, *et al.*, 1986)

Nitrogen appears to flow mainly from the nonammonia-nonurea nitrogen pool (about 3 g/d nitrogen) to the ammonia pool, of which >70% may be absorbed. As a result, between 9–19% of plasma urea and, as a consequence, <3% of ruminal ammonia nitrogen, may be derived from caecal ammonia. However, under conditions where endogenous urea may be transferred to the rumen, ammonia from the large intestine may contribute more substantially to rumen microbial protein synthesis, and hence to the overall nitrogen economy of the ruminant (Kennedy & Milligan, 1980).

Conclusion

Protein degradation in the rumen has been extensively studied, largely due to the ease of creating a rumen fistula, which has a minimal effect on the animal and which is simple to maintain. Despite this easy access, several problems remain unsolved. In the first place, considerable controversy still surrounds the selction of markers which are suitable for quantitating the flux of the different protein pools in the rumen. Secondly, while it is clear that the efficiency of microbial protein synthesis may be improved by reducing the recycling of nitrogen within the rumen, insufficient knowledge of the exact mechanisms involved prevents the practical implementation of such a strategy. Furthermore, the quantitative assessment of the amount of nitrogen returning to the rumen from the large intestine remains unclear, appearing to depend upon the quality of the diet fed to the ruminant.

Ideally, protein digestion should be measured in the small intestine, which is the major site of protein absorption. This is seldom done, as a result of several factors. Despite access to the small intestine in the ruminant, quantifying the rate of passage of nitrogen through the small intestine remains fraught with practical difficulties. Furthermore, the role of the reticulo-rumen complex in supplying a major source of protein (microbial) to the small intestine tends to obscure the effect of the bypass protein on the overall nitrogen metabolism of the animal, because of imprecise definition of the various protein fractions. Finally, the presence of the large intestine, in which feed and microbial protein that escapes digestion in the small intestine is degraded, so alters the composition of the digesta that analysis of faeces cannot be used to obtain accurate digestibility data.

In order to resolve these conflicting conclusions, it would be necessary to eliminate the large intestine, thereby allowing the quantitative collection of ileal digesta, and removing the source of nitrogen that is recycled to the rumen. Although the large intestine may

be temporarily bypassed by means of ileal reentrant cannulae, these are prone to blockage, due mainly to the paste-like consistency of the digesta. Creating a permanent bypass would eliminate these objections, and facilitate the application of many other procedures, such as the direct collection of ileal digesta, the direct recovery of mobile nylon bags, and the direct determination of nutrient uptake from the small intestine into the mesentric vein.

As a result of this perceived need, we are currently investigating an animal model in which the large intestine is permanently bypassed by means of an ileorectal anastomosis. The ileum is transected about 10 cm proximal to the ileocaecal valve, mobilized from the mesentry for a suitable distance, and connected via a T anastomosis to the rectum, about 30 cm proximal to the anus. Cannula(e) may be inserted into the duodenum during the same operation.

Preliminary trials indicate that while the intake of water increases, intake of feed (in terms of dry matter) and crude protein digestibility appears to be unaffected in sheep fed lucerne hay chaff *ad libitum*. Plasma volume, acid-base parameters and electrolytes all appear to fall within the normal range, despite a slight metabolic alkalosis that is fully compensated. While the extra intake of water appears to increase the rate of passage of digesta through the rumen compartment, the other compartments appear to be unaffected. Other aspects currently being investigated include feed intake, influence of protein intake, rate of passage of both the water and particle phases, water balance, blood pH and gases, distribution of bacteria in, and the anatomy and histology of, the digestive tract.

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