NITROGEN METABOLISM IN THE RUMINANT: A REVIEW

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SUMMARY

Current systems for the evaluation of protein-feeds for ruminants take account of two principal nitrogen (N) requirements. One is to satisfy the needs of the rumen micro-organisms in order to maximize microbial protein yield - this N can largely be provided in any form that will give rise to enough ammonia in rumen fluid. The other N requirement is for a dietary source of true protein that will pass out of the rumen unfermented (a bypass protein) and augment the microbial amino acid supply in the small intestine when the latter are inadequate for a specific productive function (maintenance, growth, wool growth, lactation). The level of productivity (up to the limit set by the genetic potential) is set by the digestible organic matter intake (DOMI) which is therefore a determinant of tissue N demand.

Various simple models provide predictions of total supply of amino acids to the intestines from knowledge of DOMI. These use constants for factors such as feed "degradability" in the rumen which are known to be variables. Other more complex models are therefore being developed,

Availability of amino acids to the liver and peripheral tissues is determined by their true digestibility in the small intestine and by their metabolism in, and secretion by, gut tissues. Methionine, threonine and lysine are most likely to be first limiting but in practice it is usually only feasible to increase total supply of amino acids for tissue use - either by improving the yield of microbial protein or, more probably, by including a suitable source of bypass protein in the diet.

INTRODUCTION

Two important characteristics set ruminants apart from most other domestic livestock: their ability to synthesize digestible true protein (essential amino acids) from non-protein nitrogen (NPN) sources such as urea and ammonia, and their ability to utilize the energy in cellulosic materials - both characteristics being dependent on the activities of rumen micro-organisms.

The future for ruminants will depend increasingly onexploitation of rumen function to make maximum use of inexpensive feed sources - grasslands, agro-industrial by-products - and to minimize the use of the more expensive protein-rich feeds as sources of additional amino acid supply to the small intestine. Clearly, this involves maximizing the synthesis in, and outflow of microbial protein from the forestomachs (energy being derived from the basal diet) and using expensive protein sources such as fish meal and cottonseed meal only when the microbial amino acid supply is insufficient to meet the animal's current requirements. These will vary according to its physiological status (e.g. pregnant v. non-pregnant; lactating v. non-lactating). Protein supplements when

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needed should be used only in the quantities necessary to produce optimum economic productivity. To achieve these goals it will be necessary to understand more completely the role of protein supplements in the animal and to evaluate the available protein sources using simple routine procedures.

Attaining a practical system for evaluating protein feeds is difficult enough for simple-stomached animals, where all the ingested protein becomes available in the small intestine for digestion and absorption. A knowledge of the amount and amino acid composition of the ingested protein allows accurate assessment of whether that protein will meet the current needs of the animal. The situation is more complex with ruminants. Dietary protein is first subjected to microbial fermentation in the rumen which alters the amount and composition of the proteins that pass into the small intestine. Empirical evaluations such as the digestible crude protein (DCP) system or the ARC (1965) methods of protein evaluation do not take full account of the effect of ruminal fermentation nor of the close relationship between energy availability and N requirements, and cannot predict with sufficient accuracy the true availability of protein for processes in the body. Newer systems have' recently been proposed which take account of ruminal fermentation as well as digestion, absorption and metabolism of amino acids (Verite et al. 1979; ARC 1981). An ideal system should be able to predict the capacity of the diet to provide those individual essential amino acids that are potentially limiting for animal production.

The protein value of a feedstuff is not simply a characteristic of the feedstuff itself. It also depends on the nature of the ruminal. 'fermentation which is influenced by associative effects of other components in the diet, and by the processes for which the protein is used in the animal (the latter depending upon the species and physiological state of the animal). In particular, the feeding value of a protein supplement may depend on whether it stimulates intake of the basal diet.

A feed-protein evaluation system for ruminants can be considered under three separate headings: (i) factors govering the supply of dietary and microbial amino acids to the small intestine, (ii) factors that affect availability of individual amino acids from the small intestine, and (iii) factors that affect the efficiency of amino acid utilization in body tissues - in particular, the relationship with and stimulation of ME supply and whether the mixture of available amino acids is similar to that of the proteins synthesized in the body. The required amino acid mixture will differ according to whether the needs are for maintenance or meat, milk or wool production.

SUPPLY OF MICROBIAL AND DIETARY AMINO ACIDS TO THE SMALL INTESTINE

Fermentation of dietary protein

Dietary proteins are extensively fermented in the rumen and replaced to a variable extent by the proteins of the micro-organisms. The breakdown of, dietary protein is related to two important factors; its solubility, and the level of feed intake. The breakdown may also be partly dependent on the source of substrate, When starch supplied 35% of the digestible energy in a diet as compared to hay and cottonseed hulls, the bacterial species with most proteolytic activity increased their numbers lo-fold, and higher concentrations of ammonia and free amino

acids were present in rumen fluid (Tagari et al. 1964). Dietary proteins that are extensively fermented have little direct feeding value. For example if 100 g amino acids are fermented to VFA and ammonia the production of ATP (1.5 moles) is probably about half that provided by fermentation of a similar amount of carbohydrate. If 12 g microbial cells are produced per mole ATP produced (YATP = 12), 100 g of dietary amino acid will give rise to only 1.8 g dry cells or about 9 g microbial amino acids (see Leng 1981). Proteins that are less extensively degraded may have direct effects by the augmenting of the total supply of amino acid in digesta passing into the small intestine and perhaps also by altering the composition of these amino acids. The proteins which escape fermentation have been termed undegraded dietary protein (UDP) by the ARC (1981) or "bypass" protein (see Kempton et' al. 1977).

Dietary proteins that are "protected" from ruminal fermentation but are intestinally-digestible are often referred to loosely as "by-pass protein sources". The latter include protein-rich feedstuffs such as good-quality dried-grass hays and meals of vegetable or animal origin. 'These are often in high demand and therefore expensive because they are also important sources of protein in the diets of pigs and poultry. Inclusion of by-pass protein sources in the diet of ruminants offers a practical means of providing extra protein to the small intestine when the microbial protein supply is inadequate to meet the animal's needs. The effect of a small quantity of by-pass protein in the diet has been shown, under some circumstances, to markedly improve productivity through stimulating appetite and feed intake (Egan and Moir 1965; Hennessy et al. 1981; see for review, Kempton et al. 1977).

Degradability

The "degradability" of a protein-rich feed in the rumen needs to be known in order to predict its nutritional value for ruminants (ARC 1980). However, degradability is not a precisely definable property of a given feedstuff and strictly speaking a single value should not be ascribed to a given protein source. The value is influenced, for example, by the physical form in which it is fed and the level of intake; these factors in turn may affect the time for which the protein source is present in the rumen and subject to microbial attack (see Kempton and Nolan 1980). Any degradability value is therefore a function of a number of *factors which interact. This has led Black et al. (1980) to suggest that the only satisfactory way of assessing the nutritional value of a protein source, or of a mixed diet, is to consider all relevant factors concurrently by using a dynamic model, and this approach has been adopted as a long-term objective by the Australian Working Party on Feeding Standards (Nolan and Corbett 1980).

Degradability values for proteins in diets for sheep. and cattle have been obtained in vivo by estimating the quantities of undegraded dietary materials in abomasal or duodenal digesta. Even thoughthere have been numbers of studies dependent on this experimental approach, there are major methodological difficulties which confound the results of these measurements. Usually different naturally-occurring or isotopic markers are used to identify the microbial and non-microbial fractions in the digesta and these often given very different values (Ling and Buttery 1978; Siddons et al. 1981). The undegraded dietary fraction is then calculated by subtracting an amount representing endogenous N from the non-microbial fraction. The latter includes undegraded

endogenous inputs to the, rumen and omasum and endogenous secretions into the abomasum, and is also not easily measured. It is usually assumed to be 1-2 g N/d in sheep (Hogan and Weston 1967) but can vary between 0.5-2.6 g N/d (Harrop 1974). The true value is probably dependent on diet and level of intake and the use of a single value proba'bly compounds the errors already present in estimates of the UDP fraction in digesta.

In vivo degradability estimates are an essential part of research on N utilization in ruminants but the time and expense required to obtain results, and the methodological errors that can arise, make the procedures inappropriate for routine classification of feedstuffs. Simpler techniques are needed to allow screening of potential protein . sources. Various workers have suggested that measurement of solubility (Wohlt et al. 1973; Craig and Broderick 1981) or extent of degradation of feedstuffs in vitro (Mehrez and Ørskov 1977; Mathers et al. 1977) or in nylon bags placed in the rumen (Quin et al. 1938; Kempton 1980; Ørskov 1980) might provide predictions of the potential degradability of protein sources when used for feeding ruminants. These methods which are more fully discussed in this symposium elsewhere are useful evaluation techniques but suffer the limitation that they cannot exactly duplicate conditions within the rumen, and in particular provide no information on how long the test material will remain in the rumen of the fed animal.

The nylon bag (in sacco or in situ) technique can give useful and repeatable measurements in routine use and allows the time-course of' ruminal degradation to be recorded (see Kempton 1980; Wilson and Strachan 1980). Ørskov and McDonald (1979) have suggested that the nylon bag results can be combined mathematically with rumen clearance rate estimates made on the same material, in order to give better estimates of probable degradability' in the rumen of an animal fed the test material.

In a study of the degradability of various silages, hays, grasses and other 'on-farm' feeds in the U.K., Wilson and Strachan (1980) produced equations for predicting degradability from (the square root of) their total N content, and concluded that their prediction of the degradability of silages and hays "was better than assigning a simple average degradability of 0.8 as suggested by the ARC (1980), as . . . . . both silages and hays vary widely in degradability." However, these prediction equations could not be expected to provide accurate information on degradability of chemically-treated protein sources or of meals. Whilst these or similar equations might be derived for Australian conditions and used in the ARC (1980) system to obtain estimates of total protein flow to the intestines, it seems likely that alternative models already available (see below section) for making this prediction might at present be equally usef'ul.

Sources of N for growth of ruminal microorganisms

The N requirements can largely be met by ammonia if sufficient concentrations are present in rumen fluid as more than 80% of a cross-section of 89 ruminal species can grow with ammonia as the main source of N (Bryant and Robinson 1962). However, bacteria in the rumen of sheep and cattle on a variety of diets apparently obtain a significant percentage (20-50%) of their total N from peptides and amino acids (Portugal 1973; Nolan 1975; Wright and Hungate 1967; Cottle 1980). This is in accord with the observation that heterotrophic bacteria tend to utilize increasing amounts of peptides and amino acids where these

substrates are available (Warner 1956). There are persistent suggestions that the higher efficiencies of use of dietary OM for microbial protein synthesis will be achieved only if peptides and amino acids are present in sufficient concentrations in rumen fluid (Hume 1970; Maeng et al. 1976). The mechanisms of synthesis of amino acids in ruminal micro-organisms are not well understood and the reasons why peptides and amino acids should be important are also not clear. Stouthamer (1979) suggests that theoretical growth yields are not likely to be much affected by N source in organisms that can grow with'either amino acids or ammonia. Even so, protozoa and some species of bacteria have an obligatory requirement for peptides or amino acids. (Coleman 1967) and are well-adapted to obtaining their peptides and amino acids despite the low concentrations that often exist in rumen fluid. In mixed cultures some peptides and amino 'acids of microbial origin would normally be present, Nevertheless some apparently do not survive if some peptides and amino acids are not provided in the diet, and protozoa were absent 'from the rumen fluid of animals given diets in which urea or ammonia provided the sole source of dietary N while bacterial numbers increased markedly (Virtanan 1966).

Bacteria require ammonia in higher concentrations than peptides and amino acids. Levels of 20-50 mg N/litre are apparently required to maintain normal rumen function with forage diets (Allison 1970; Satter and Slyter 1974) and up to 200 mg N/litre with concentrate diets (see Hogan 1981). When N is limiting, the species most likely to be affected adversely are those that grow more slowly, such as the cellulolytic bacteria and those that use the end-products of fermentation of other species, and whose growth must lag behind these, e.g. methane-producers and lactic acid utilizers' (Schwartz and Gilchrist 1975).

Concentrations of ammonia in rumen fluid are affected by the relative rates of entry and loss'from the rumen fluid pool. Sources of ammonia are (i) deamination of amino acids from the diet or secreted by rumen microbes during growth, or from lysed microbial cells, or cells from'the rumen epithelium and (ii) degradation of 'other non-protein N compounds in the diet or from dietary or recycled urea. Routes of loss of ammonia are (i) incorporation into cells (ii) absorption through the reticulo-rumen wall and (iii) outflow in rumen fluid.

Over the last decade our knowledge of rumen ammonia kinetics has been much improved through the use of tracer dilution studies with ^{15}N (forreview see Nolan 1975). These techniques have clarified a number of apparent misconceptions about the fates of ruminal ammonia. The amount of ammonia N that can be retained in the rumen as a re'sult of 'increases in the size of the ammonia pool is relatively small - in sheep about 0.5 gN for each 100 mg N/litre increase in concentration (5 litre rumen fluid). 'Also the amount which can flow out in the rumen fluid, the product of fluid outflow (l/d) and rumen fluid ammonia concentration (mg N/l), is also relatively small - 0.1 to 5.0 gN/d. It follows that ammonia that is produced in the rumen in excess of the requirements is largely absorbed through the wall of the forestomachs. Absorption occurs in the unionized (NH_3) form, according to the concentration gradient which depends almost entirely on the NH_3 concentration in rumen fluid (see Fig. 1) The ruminal NH_3 concentration varies according to the pH of the rumen fluid but is usually less than 2% of the total ammonia concentration. The same NH_3 concentrations can occur at a variety of different total ammonia concentrations, according to the pH of rumen fluid. pH is therefore a major factor determining the total ammonia concentration by affecting the balance between flows into and out of the ammonia pool.

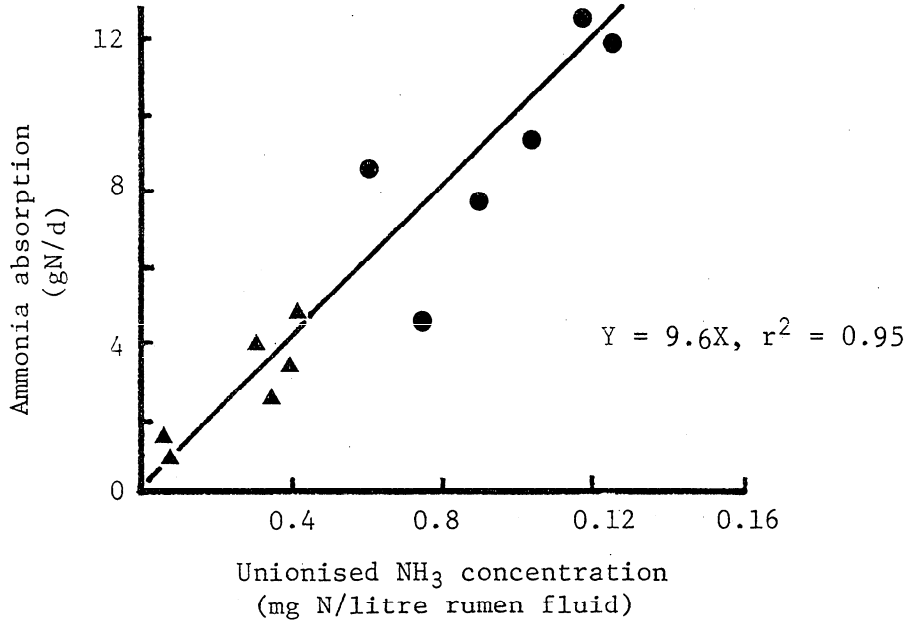


Fig. 1 Relationship between ammonia-absorption from the reticulo-rumen and concentration of un-ionized ammonia in rumen fluid of sheep given silage (●) or dried grass (▲) (Nolan et al. 1981).

Un-ionized ammonia concentration is a function of total ammonia concentration and pH which ranged between 30-180 mgN/l and 5.8-7.2 respectively, i.e. fraction of total ammonia un-ionized

$$= 1 - \frac{1}{1 + \text{antilog}\,[pH - pK'_a]} \quad \text{where } pK'_a = 9.02$$

If comparisons between ruminal bacteria and E. coli are valid, it appears that ammonia may be transported into rumen microorganisms in the NH_4^+ form by an energy-dependent process that is under metabolic control (Stevenson and Silver 1977). If this is so, a tendency for pH to rise towards 7 or above would greatly facilitate ammonia absorption through the rumen wall and thereby decrease not only ammonia availability in the rumen, but also lower the total ammonia and NH_4^+ concentrations. The effect of these changes could be to reduce the ability of bacterial cells to obtain enough NH_4^+ to meet their requirements.

An important consideration when using dietary protein sources with low solubility is whether sufficient ammonia is available to support the potential microbial yield. Failure to ensure that there is always adequate rumen degradable N (RDN) may be one reason for conflicting responses to the use of protein supplements with sheep and cattle. With protein sources of higher solubility which make available large quantities of ammonia, ammonia may still be in short supply for part of the feeding cycle because of the relatively low capacity for storage in the ammonia pool, as discussed above. Some workers have therefore evaluated various "slow-release" ammonia forms as a means of making ammonia continuously available, e.g. Starea, isobutyl diurea, dicyanodiamide (Bartley and Deyoe 1977).

Microbial protein yield

The nitrogen (N) requirements for microbial growth can be determined if the potential rate of microbial growth (which is determined by the ATP made available during fermentation of dietary substrates) is also known. In practice, microbial N requirements are usually assessed in relation to digestible organic matter intake (DOMI), metabolizable energy intake of the diet (MEI) or the amounts of OM apparently or truly fermented in the rumen itself. With many publications it is difficult to distinguish whether true or apparent digestibility coefficients are being reported.

A theoretical estimate of the minimum N requirement for microbial growth can be obtained from the N content of mixed ruminal micro-organisms (58-125 gN/kg dry cells; Smith 1975) and the yield of micro-organisms (0.15-0.20 kg' dry cells/kg apparently digested OM;Church 1979) i.e. 9-25 gN/kg apparently digested dietary OM. However, the range of values in the literature for both factors is so large that this estimate of N requirement cannot be used with any confidence. Further uncertainty is introduced by the need to make allowances for the inevitable losses of NH_3 from the rumen which may not be balanced by equal gains of N as a result of endogenous recycling. The ARC (1980), after summarizing a large number of trials, adopted a higher average value for microbial N requirement which they equated with microbial yield, i.e. 30 gN/kg apparently digested dietary OM (rumen), or 1.25 gN (7.8 g crude protein)/megajoule ME. This is equivalent to approximately 23 gN/kg dietary OM truly fermented in the rumen (after correction for rumen organisms of average composition). The derivation also assumes there is no gain or loss of N in the forestomachs as a result of NH_3 absorption and endogenous recycling even though net gains do occur in animals given low protein diets (Hogan and Phillipson 1962).

This ARC (1980) prediction employs a number of simplifying assumptions involving the use of constants to describe processes that are known to vary and to be highly interactive (Faichney et al. 1980). Literature values for the efficiency of microbial protein synthesis vary from about 50-150% of the value used above. Much of the variation may be due to errors in the methods used for measuring-microbial yield (see Siddons et al. 1979) but some variation will be real, reflecting differences in fractional outflow rates, energy costs of maintenance of different microbial systems, the presence of protozoa and other factors (see Leng 1981).

Models for estimating total protein yield from the forestomachs

Some- research workers have reported relationships that predict protein metabolism in the rumen and outflow to the small intestine from intakes of dietary crude protein (CPI) and digestible organic matter. (DOMI). For example, Vérité et al. (1979) summarized data from 158 diets (including mixtures of forages and concentrates) as follows:

Crude protein entering the intestines (g/d)
= 0.41 (CPI,g/d) + 0.124 (DOMI,g/d)

Hogan and Weston (1981) have derived a similar equation to summarize results relating to forage-based diets:

. Crude protein *entering* the intestines (g/d)
= 0.36 CPI(g/d) + 0.160 DOMI (g/d) + 6 g/d endogenous CP

These equations imply that a constant fraction (respectively 0.41 or 0.36) of the dietary CPI escapes fermentation in the forestomachs and that microbial CP yield is a constant fraction (0.124 or 0.160) of the DOMI. Hogan (1981) points out that the equations also indicate . that for diets containing 500 g CP/kg DOM (immature 'forage) and 100 g CP/kg DOM (mature forage), approximately 0.5 and 0.75 of the CP in abomasal digesta is of microbial origin.

Similar prediction equations are being developed for grazing sheep (Corbett and Pickering 1981) and comparisons of their results with the models above suggest that degradabilities are higher, and the UDP fraction (approx. 0.13 CPI) is correspondingly lower for sheep grazing lucerne, phalaris or native pastures. Their data suggest that further improvement in predictions for grazing animals may be possible if factors such as season of the year and various types of pasture are also accounted for.

These relatively simple empirical models reflect the gulf between our understanding of factors that ought, hypothetically, to affect dietary protein degradability and microbial protein yield and our knowledge of the qualitative importance of those factors. The development of computer simulation models incorporating the "hypothetical" factors (Black et al. 1980) and use of sensitivity analysis and validation against feeding trials maybe the next step in development of feed protein evaluation. However any model is only as good as the information on which it is based. Computer models allow a large number of interactive factors to be reviewed simultaneously, but will not necessarily produce better predictions of total protein yield to the intestines unless our knowledge of the metabolic processes is adequate and all primary data needed to 'feed' the model can be correctly supplied;

Digestion in the small intestine

About 80% of the crude protein in duodenal digesta is true protein or amino acids (Hogan 1981); 13-19% of the NPN is microbial'nucleic acids (Smith 1975). There is some variation in the amino acid composition of mixed digesta, partly as a result of variation in the composition of the bacterial fraction, particularly in the content of methionine and lysine (Purser and Beuchler 1966) which are both potentially-limiting amino acids. Appreciable quantities of UDP rich 'in particular amino acids also could be expected to alter the amino acid composition of mixed digesta. However, MacRae (1980) examined results of experiments with ruminants fed protected protein supplements, all of which led to improved weight gains and N retention, and concluded that the only amino acid with significantly increased concentration in duodenal digesta of supplemented animals as compared with unsupplemented control animals was methionine; responses were therefore attributable'to an increased total supply of amino acids to the small intestine rather than to differences in amino acid content of digesta.

Protein digestion is initiated by pepsins in the abomasum and is continued in the intestines by pancreatic proteases, (the quantity of the latter being regulated by the quantity of protein in the digesta). Free amino acids are absorbed from the lumen of the small intestine Small peptides are also taken up by a process that does not compete with absorption of free amino acids. These peptides are degraded to free amino acids before entry into the portal blood. Essential amino acids are apparently absorbed more rapidly' than non-essential acids, and amino acids entering from the forestomachs are also, rather intriguingly, more rapidly absorbed than the endogenous proteins of digestive secretions - perhaps because the latter are not subject to gastric pepsins.

Amino acid availability from the small intestine depends not only on the quality and composition of mixed digesta but also on the true absorption coefficients for individual amino acids in the microbial, endogenous and UDP fractions. The true absorption coefficient for microbial NAN in sheep, estimated using ^{15}N, was approximately 0.75 (J.V. Nolan, J.C. MacRae, D.E. Beever, R.C. Siddons, in preparation) and estimated by a regression approach was 0.66 (Lindsay et al 1980). Lower and more variable values (0.49-0.62) have been reported for calves given diets containing urea (Smith 1975)'. A method'for estimating the 'true' absorption of microbial and other proteins (based on their continuous administration in to the abomasum andcollection of ileal residues) has been used by workers' in Germany (Hagemeister et al. 1980). In these studies, the absorption coefficient for bacteria and protozoa was 0.81-0.83. Values for other protein sources were generally in the range 0.83-0.92 (e.g. soya-beanmeal, 0.84; fish meal, 0.90; cotton seed meal 0.93) but some protein sources had lower coefficients (e.g. sunflower meal, 0.75; palm kernel meal 0.54).

MacRae (1980) summarized 21 separate estimates of the efficiency of absorption of amino acids from the small intestine of animals given. diets with a three-fold range of N intakes, some with and some without protein supplements. He concluded that the availability of all essential amino acids from mixed digesta ranged between 0.70-0.80. Cystine was not included in this summary because values for it are seldom reported; however, Armstrong (1979) and Lindsay et al. (1980) suggested values as low as 0.52, which may be of particular importance when considering the availability of sulphur-amino acids for wool growth.

On the basis of these studies it is reasonable to accept an average value for amino acid availability from microbial protein of 0.75; a similar value for most UDP sources will probably suffice for many purposes but it should be recognized that lower values can occur - particularly if the protein source is "over-protected", for example by chemical reactions with tannins or as a result of Maillard-type reactions during processing. Some attempts have been made to estimate the intestinally-indigestible part of the UDP fraction, from its acid detergent fibre N content, and to allow for this fraction when. calculating amino acid availability (Wilson and Strachan 1980).

Fermentation in the large intestine

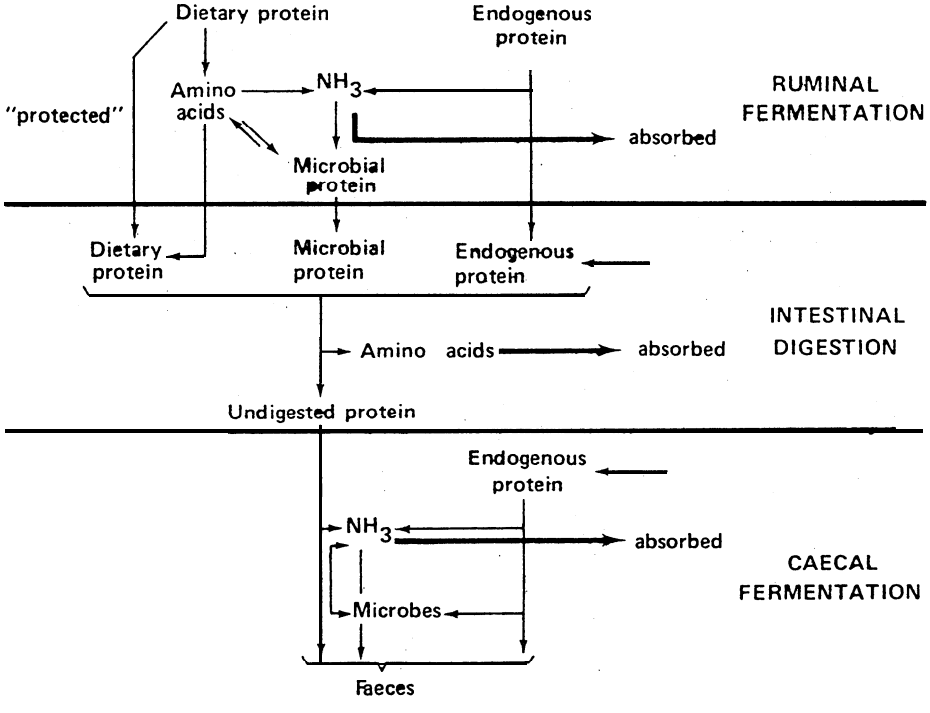
The large intestine, like the rumen, contains a dense population of bacteria capable of fermenting materials entering from the small intestine. Synthesis of bacterial protein occurs, particularly in the caecum and proximal colon, but it appears that amino acids are not absorbed from the large intestine of ruminants. Net absorption of *N*

normally occurs but mainly in the form of ammonia. Much of the N in faeces is of microbial origin, i.e. mostly bacterial N synthesized in the large intestine. Small quantities of rumen microbial residues and some undigested dietary N and residues of mucus and other secretions into the gut are also present.

Animals given a N-free, but otherwise adequate diet continue to lose N in the faeces in the form of bacterial and cell residues and undigested cell debris and secretions from the digestive tract. This N loss is termed the endogenous faecal nitrogen (EFN). It is appreciably higher in ruminant than in non ruminant animals, and this is related, in part, to the influence of the digestion process in the forestomachs, the level of feed intake and the nature of the feed consumed, e.g.

EFN = 5 g N/kg dry matter consumed.

A summary of the events taking place in the'digestive tract of ruminants is given in Fig. 2.



Kempton et al. 1977

Fig. 2 Degradation and digestion of dietary protein in the ruminant

REQUIREMENTS AND UTILIZATION OF ABSORBED AMINO ACIDS

Metabolism in the gut and liver

Considerable metabolism of non-essential amino acids occurs, in the intestinal tissues after absorption and 30-50% do not appear in portal blood. The ratios of essential amino acids carried to the liver in portal vein blood are generally similar to those absorbed (MacRae 1980). However intestinal absorption of essential amino acids may considerably exceed the supply to the liver. This is to be expected if endogenous proteins formed in the gut mucosa enter the digestive tract anterior to the intestines and pass to the intestines along with dietary and microbial protein.

The liver regulates the partition of the absorbed amino acids and it releases -into the blood, plasma proteins and free amino acids, with branched-chain amino acids (leucine, iso-leucine and valine) being predominant. The liver is the exclusive site of'catabolism of the essential amino acids except for the branched chain group which arc degraded mainly in the muscle and kidney. The liverapparently can regulate the extent of catabolism of essential amino acids' such as lysine (and tryptophan) in response to the concentration in systemic blood, and the plasma levels of these acids increase rapidly when the body requirements are exceeded. (The sudden change in concentration of essential amino acids forms the basis of one method of assessing amino acid requirements of ruminants (Wakeling et al. 1970)). Catabolism of amino acids in the liver leads to the production of urea which is either excreted in the urine or recycled to the upper or lower parts of the digestive tract and degraded to ammonia.

Tissue metabolism of amino acids

Amino acids in blood are taken up by body tissue cells by an active ATP-requiring process which may concentrate free amino acids within the cells. These amino acids are not immediately metabolized or incorporated into cell components. The accumulated amino acids thereford provide a limited reserve of cellular amino acids.. During protein synthesis in cells, messenger ribo-nucleic acid (RNA) which is transferred from the nucleus to the cytoplasm is translated by the ribrosomes using transfer-RNA charged with free amino acids within the cell. This is an ATP-. requiring process. Essential amino acids must be largely derived from outside the cell but non-essential acids may be formed by inter-conversions inside the cell itself.

Some synthetic processes in the body have precedence over others, e.g. in early lactation the dairy cow may lose body-weight, suggesting that nutrients are more effectively removed by the mammary gland than by skeletal muscle tissues; wool growth rate is depressed during late pregnancy and lactation when synthesis of protein for other purposes is high. 'The exact mechanisms are not understood although hormone levels presumably play a role.

Metabolism of amino acids by peripheral tissues is indirectly affected by the rate of amino acid (particularly essential amino acid) absorption as well as by level of absorption of other energy-supplying nutrients, and by insulin and other hormones. If absorption of energy nutrients is restricted, N excretion increases due to the breakdown of protein stores for energy. Conversely, if an energy-restricted diet is supplemented with carbohydrate, N-retention mav be improved., Insulin growth hormone and testosterone promote deposition of skeletal muscle proteins. Higher levels of insulin in blood increase the rate of uptake of amino acids and protein synthesis by muscle cells, thereby reducing blood amino acid levels. In contrast, elevated adrenal corticoid and thyroid hormones generally promote mobilization of muscle proteins and transfer of amino acids to the liver where they are metabolized and eliminated. Various aspects of hormonal control of protein metabolism have been reviewed by Manchester (1976).

Protein turnover and obligatory N excretion

Body tissues undergo continual synthesis and breakdown but the process is not completely efficient and there is some obligatory N wastage as a result of formation of materials that cannot be reused, e.g. muscle creatine is converted to creatinine which is secreted in the urine, and some urea is always present in the urine. This wastage decreases in animals provided with a N-free diet containing adequate energy until a minimum rate of N loss is achieved. This is often termed the endogenous urinary nitrogen (EUN) and it represents the smallest N loss in urine which permits the animal's survival. The EUN can be calculated from equations based on data from a variety of studies (see ARC 1980), e.g.

For European cattle, $EUN\ (gN/d) = 5.92\ (\log W) - 6.76$,

For sheep $EUN\ (gN/d) = 0.023\ W + 0.54$,

where W is liveweight (kg).

It seems clear that EUN for Zebu cattle (Bos indicus) is lower., probably by about 20%, than in European cattle.

The sum of EUN and endogenous faecal N (EFN) (discussed earlier) . represents a minimum amount of N which must be supplied to ensure survival of an animal and is therefore an estimate of the minimum maintenance nitrogen requirement of that animal. Some systems for calculating maintenance N requirements assume that N is supplied by the apparently absorbed N. These systems do not include EFN as part of the minimum N requirement.

Amino acid requirements

Amino acids are required to meet the demands for maintenance, protein deposition in body tissues and for milk production and wool growth. The efficiency of protein synthesis is reduced by non-optimal rates of supply of essential amino acids from digesta. This can result because the total supply of essential amino acids is insufficient, or because individual acids are in low concentrations in digesta reaching the small intestine.

The spectrum of amino acids in microbial protein as compared to ruminant tissues suggests that, if microbial protein is not augmented by UDP, methionine, lysine, histidine, threonine and arginine are most likely to limit growth (Chalupa 1976). Methionine was shown, using the plasma concentration inflection point with increasing duodenal supply of methionine, to be the first limiting amino acid for sheep (Wakeling et al. 1970). Threonine has been identified as second-most limiting for sheep and the same two acids may be limiting for calves (Williams and Smith 1974). For wool growth, cyst(e)ine and methionine are first limiting (Reis 1979) and for milk production, methionine and lysine may be marginally-limiting (see Tamminga and Oldham 1980).

When a limiting amino acid is identified, the possible manipulations are to alter the composition of absorbed amino acids or to increase the total supply. With respect to microbial amino acids, because microbial composition is largely constant, manipulation for increased microbial

yield represents the only practical possibility. If supplementary bypass protein is to be used, attention should be given to obtaining optimum yields of intestinally-digestible UDP, preferably rich in sulphur amino acids, threonine and lysine. It should be remembered also, that the effect of the amino acids of UDP on the amino acid spectrum in mixed duodenal digesta may be masked by the presence of endogenous proteins whose quantitative significance is still largely unknown.

Some consideration has been given to the possibility of supplementation with individual limiting amino acids but there are many problems. For example, synthetic methionine analogues administered in the diet of sheep are rapidly absorbed from the rumen and do not give the predicted responses in wool growths (Ferguson 1975). This is probably because, as a result of rapid absorption, increases in methionine concentrations in body amino acid pools are only transitory. Batterham and O'Neill (1978) found that the growth response to free lysine by pigs fed once daily was only 67% of that achieved with frequent feeding.

Estimating amino acid requirements

Estimates of protein requirements are often based on assessment of total N requirements for maintenance (minimal losses in urine, hair, scurf) and for production (milk, tissue proteins). The quantities of amino acids required for synthesis of products (such as milk) will be dependent on the rates of synthesis of those products which will be affected by many factors, the principal one being energy supply (availability of ME and net energy). Clearly, account needs to be taken of the energy component of gain or loss in body weight (ARC 1980).

Examples of calculations which exemplify the close relationship between energy and amino acid supply are given in the following paper (Armstrong and Brooks 1981). In the final analysis, however, all such calculations will be found to be inadequate to some degree'. In these cases, a feeding trial in the production system will provide the most definitive protein evaluation.

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