PROTEIN EVALUATION - AN ALTERNATIVE APPROACH

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INTRODUCTION

A system which is suitable for evaluating protein requirements or the efficiency of utilization of protein in animals must consider (i) the requirements of the animal's tissues for nitrogenous substances, (ii) the amount andnature of nitrogen absorbed in relation to the intake and composition of the diet and (iii) the'efficiency with which absorbed nitrogen is used for various body functions. The system must also include aspects of energy utilization because both the tissue needs for nitrogen and, in ruminants, microbial activity in the rumen (hence nitrogen absorption) are affected by energy availability. Several systems, varying in complexity, have recently'been proposed for evaluating protein utilization in ruminants (Burroughs *et al.* 1975; Satter and Roffler 1977; Jarrige *et al.* 1978; C.A.B. 1980; Corbett 1980). The suitability of each for solving agricultural problems depends upon the details of the particular problem to be investigated.

The primary purpose of the European systems is to formulate rations to achieve specified rates of production in hand-fed stock. These systems are based on descriptions of the observed outcome of experiments conducted in situations similar to those in which they are to be applied in practice. Although they describe the outcome of' processes operating within the animal, they represent these as fixed factors and cannot account for the wide variation known to occur. However, they are relatively simple and can be applied satisfactorily to problems which are within the range of experimental observations. These systems are generally unsuitable for the extensive conditions of ruminant production in Australia. Here, animal performance depends on a complex interaction between the animal, pasture and weather, in conditions often well outside the range of experiments upon which the European systems are based. In addition, the problems to which a protein evaluation system would be applied in Australia are generally not associated with formulating rations to achieve chosen rates of production. Rather they are concerned with identifying limitations to production from pasture and predicting likely benefits from various management strategies, including hand-feeding. We believe that a more complex system, which attempts to predict the outcome of protein utilization from a knowledge of the processes operating in the animal, is essential if the wider needs of Australian agriculture are to be met. With the advent of computers, almost any degree of complexity can now be incorporated into a system without hindering its use by people in industry. Current concepts of protein utilization in ruminants have been incorporated into computer programs (Graham *et al.1976;* Black *et al.* 1980-81) and this approach has been recommended as an appropriate basis for protein evaluation in the current development of nationally uniform feeding standards for livestock in Australia (Corbett 1980). This paper describes 'the way in which the processes influencing the utilization of energy and protein in the body tissue of sheep and determining rumen function and the flow of nitrogenous materials from the stomach can be integrated to develop a dynamic protein evaluation system.

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COMPONENTS OF A PROTEIN EVALUATION' SYSTEM

Tissue requirements for nitrogen

Animal tissues have a requirement for nitrogen which varies with body weight, energy intake, physiological state and genotype. Part of this requirement must be supplied by each essential amino acid, but the rest can, in general, be supplied from any nitrogen source. With the aromatic amino acids, tyrosine and phenylalanine, and the sulphur containing amino acids, cystine and methionine, a minimum amount of the total nitrogen must be supplied by the pair, with a certain proportion coming from each respective essential amino acid. The total amount of nitrogen required by the tissues and the proportion which must come from each essential amino acid, depends upon the amounts of specific proteins synthesized in the particular conditions to which the animal is exposed. The first step therefore is to calculate the total amount of nitrogen required for each body function.

Metabolizable energy available to an animal is determined from information about the intake and composition of the diet. The growth of conceptus and udder, and the production of wool and milk that can be sustained by the intake of metabolizable energy given the particular physiological state and genotype of the animal is then calculated. The total nitrogen required for each product is then derived from information about the respective protein content. The deposition of nitrogen in the remaining body tissues is also calculated in relation to the intake of metabolizable energy and the breed and sex of animal. Endogenous nitrogen losses in urine, faeces and scurf are then added to the amount required for the other body functions to provide an estimate of total nitrogen requirement.

The methods used to calculate nitrogen requirement of the animal's tissues vary little from those described by the Agricultural Research. Council (A.R.C.) (C.A.B. 1980), except that the amount of each product formed is not assumed, but is established in relation to the intake of metabolizable energy, physiological state and-genotype of the particular animal. In contrast to the A.R.C. system, the body composition of the animal varies with energy intake (Black 1974) as well as with genotype because of the form of the relationship betweennitrogen deposition and metabolizable energy intake (Black and Griffiths 1975).

Nitrogenous compounds absorbed

Due to microbial activity in the rumen, the amount and quality of protein available to the ruminant for digestion and absorption may bear little relation to that eaten. The amount of protein flowing from the stomach depends upon the extent to which dietary protein is degraded in the rumen and on the growth and outflow of micro-organisms. These factors are highly variable and interdependent because of the complex interactions that occur in the rumen. Accordingly, it is necessary in many situations to consider the determinants of rumen function. The proposed system thereforeincludes a dynamic representation of the major processes occurring in the rumen as outlined in Fig. 1.

The amount of dietary material which escapes the rumen intact depends upon the relative rates of two competing processes; degradation and flow from the rumen. The rate of degradation is governed mainly by

characteristics of each dietary constituent. Although there is variation within each class, soluble carbohydrates are degraded about 30 times faster than storage carbohydrates which, in turn, are degraded almost 5 times faster than structural carbohydrates. The rate of deqradation is also influenced by the number and type of microbes present in the rumen. With structural carbohydrates and some proteins, a portion of the material may never be degraded, even after prolonged retention in the rumen. It is classed as undegradable and can only be removed by passage to the lower gut. The rate of flow of material from the rumen is affected by feed intake, factors which influence the size of particles in the rumen, osmolarity'of rumen contents, physiological state and weight of the animal and cold exposure (Faichney and Black 1979). Growth of micro-organisms in the rumen depends primarily on the availability of energy, in the form of ATP, which is governed by the amount and type of substrate degraded. Microbial growth may be also limited by the lack of ammonia, specific amino acids or sulphur.

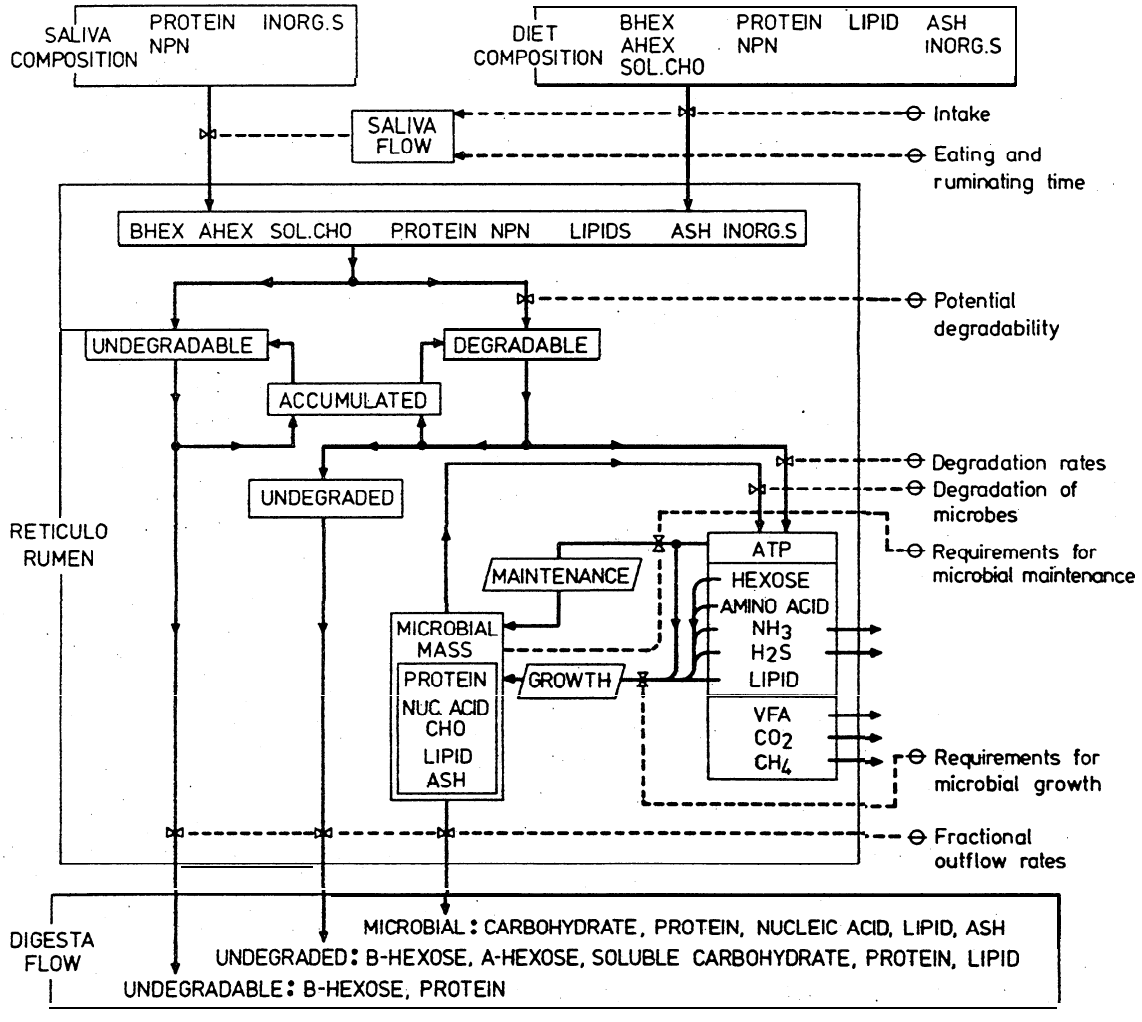

Fig. 1 Flow diagram to illustrate the determinants of rumen function . encompassed by the system. BHEX = structural carbohydrate, AHEX = storage carbohydrate, SOL.CHO = soluble carbohydrate, NPN = non-protein nitrogen, INORG.S = inorganic sulphur, NUC. ACID = nucleic acid.

To calculate protein flow from the stomach, information is required on feed intake, on chemical composition of the diet in terms of structural, storage and soluble carbohydrate, true protein, non-protein nitrogen, lipids, soluble ash and inorganic sulphur, and on the potential degradability of structural carbohydrate and true protein.' The inflow of protein, non-protein nitrogen and inorganic sulphur from saliva or across the rumen wall is then calculated and the amounts of all substrates 'entering the rumen which are broken down, flow from or accumulate in the rumen are determined. From the stoichiometry of the degradation of each substrate, the potential production of ATP is calculated and compared with that required to maintain the existing microbial population. If the potentially available ATP is insufficient for this purpose, a portion of the microbial population is degraded. Conversely, if the calculated ATP supply exceeds the requirements for maintenance, microbial growth can occur and some of the degraded substrates are incorporated directly into microbial protoplasm. The possibility of microbial growth being limited by availability of amino acids, ammonia, total nitrogen or sulphur is tested, and predicted microbial growth determined by the most limiting nutrient., Changes in the microbial mass present in the rumen are calculated from the difference between new growth and that portion of both the initial mass and the- newly grown microbes flowing from the rumen. Finally, VFA and me'thane production are calculated, using stoichiometric equations, from the amount of each substrate actually fermented. The yield of microbial mass per unit of ATP is assessed and the flows of microbial protein, dietary protein, non-ammonia nitrogen and other dietary and microbial components from the rumen are calculated.

This calculation of protein flow from the stomach differs considerably from the A.R.C. approach, although both encompass the same area. The A.R.C. uses a static representation of rumen function in which the outcome of several successive processesaredescribed by constant factors. Most of these factors vary widely (Faichney *et al.* 1980) and inaccuracies can arise particularly through the use of constant values for the amount of protein from each dietary source degraded in the rumen, the availability of energy for microbial growth expressed as a fixed proportion of organic matter digestion occurring in the rumen and a fixed value for the amount of microbial growth per unit of organic matter digested in the rumen. The degree to which errors are introduced by these assumptions depends upon the particular situation being investigated. Likewise, there are times when the dynamic representation of rumen function is unnecessarily complex. This applies particularly when computer programs are used to investigate management options affected primarily by the energy status of the animal as, for example, in determining the influence of shearing or lambing dates on pasture requirements (Black and Bottomley 1980). Therefore, the empirical equation of Hogan and Weston (1981), which relates the flow of non-ammonia nitrogen from the stomach to the intake of nitrogen and digestible organic matter, is available as an option in the computer program.

The digestibility of true protein flowing from the stomach currently is assumed to be 0.7. Like the A.R.C. system, this is an average single factor describing the outcome of the digestion process which can be highly variable. Because the proposed system separates protein flow into that derived from microbes, from potentially degradable dietary protein which was not degraded, and from undegradable protein, it has the capacity to calculate a range in protein digestibility if

satisfactory ways of estimating the digestibility of each component can be developed. Nitrogen released during involution of the uterus and udder is added to that absorbed to obtain an estimate of total nitrogen available for metabolism.

Utilization of absorbed amino acids

Unless there is a perfect match between the relative proportions of essential amino acids available to an animal and those required for all body functions, available nitrogen will be used with an efficiency less than 1.0. In the original computer program (Graham et *al.* 1976), the efficiency of utilization of amino acid nitrogen (Biological Value, BV) was assumed to be 0.72 for milk fed lambs and 0.7 for ruminating animals. However, BV of absorbed nitrogen can vary substantially depending upon the proteins synthesized and the relative proportions of amino acids absorbed. For example, the BV of cow's milk protein when given to young lambs receiving an energy intake about 3 times maintenance was 0.72 (Black and Griffiths 1975), but when similar diets were given by abomasal infusion to adult sheep at a maintenance level of energy, the BV of absorbed protein was found to be 0.45 (Black *et al.* 1973). Thus, if the purpose of a protein evaluation system is to formulate diets which are adequate in protein, the use of a low, constant BV could be justified, accepting that in some situations, animals would be absorbing excess protein. Alternatively, if the system is to be used to assess whether the protein available to an animal is likely to be limiting and, if so, to determine the relative deleterious effect on each body function, the BV used is critical (Black and Colebrook 1976).

Biological value is the measured outcome of protein utilization within an animal. Therefore, when developing a protein evaluation system which will have general applicability, it may be desirable to estimate BV from its determinants rather than use a single factor. An approximation of BV can be obtained by comparing the molar proportion of each available essential amino acid with the sum of that required for the net synthesis of all proteins. The smallest ratio of the available mole fraction to the required mole fraction defines the amount of protein that can be manufactured from one mole of available protein. An estimate of BV can then be made from information about the nitrogen content of the available and synthesized proteins. Values obtained by this procedure for sheep absorbing amino acids from various protein sources and synthesizing differing amounts of animal proteins are given in Table 1. For these calculations all essential amino acids, except tryptophan, were considered separately'as well as the combinations of tyrosine-phenylalanine and cystine-methionine. Few measurements of the tryptophan content of proteins are available. When various combinations of proteins synthesized were considered, endogenous nitrogen loss was assumed to have the same amino acid composition as muscle.

The estimated BV of available protein varied widely both with its source and with the nature of the proteins synthesized. The range of combinations of synthesized protein given in Table 1 is probably close to the extremes observed in practice. When wool synthesis was a major part of the proteins manufactured, BV was low. However, when muscle or milk synthesis were high, the estimated range in BV was substantially reduced. Nevertheless, these analyses suggest that adoption of a single value for the efficiency of utilization of absorbed amino acids will cause substantial errors in prediction.

TABLE 1 Estimates of the Biological Value of absorbed amino acid nitrogen obtained from comparisons of the mole fractions of essential amino acids in absorbed and synthesized proteins.

Proteins synthesized	Source and absorbed protein			
	Microbial[a] : ryegrass[b] 2 : 1	Barley-soybean[c]	Cow's milk[b]	
Single product				
Ewe's milk[b]	0.80 (Leu)	0.80 (Leu)	0.91 (Val)	
Muscle[d]	0.82 (Cys)	0.76 (Lys)	0.84 (Cys)	
Wool[e]	0.35 (Cys)	0.32 (Cys)	0.23 (Cys)	
Multiple products				
Milk N : Body N + Endogenous N : Wool N				
0 : 1 : 1	0.50 (Cys)	0.34 (Cys)	0.36 (Cys)	
0 : 2.5 : 1	0.67 (Cys)	0.46 (Cys)	0.47 (Cys)	
0 : 4 : 1	0.77 (Cys)	0.53 (Cys)	0.54 (Cys)	
0 : 8 : 1	0.82 (Lys)	0.63 (Cys)	0.65 (Cys)	
4 : 1 : 1	0.88 (Leu)	0.62 (Cys)	0.64 (Cys)	
6 : 2 : 1	0.86 (Leu)	0.70 (Cys)	0.72 (Cys)	
10 : 1 : 1	0.82 (Leu)	0.77 (Cys)	0.79 (Cys)	
10 : 2.5 : 1	0.84 (Leu)	0.78 (Cys)	0.80 (Cys)	

The limiting amino acid is indicated in parenthesis : cys is the cystine-methionine pair.

[a] Purser and Beuchler (1966); [b] F.A.O. (1970);
[c] Faichney and White (1979), diet 2U; [d] Munro and Fleck (1969);
[e] Marshall and Gillespie (1977).

Simulation of the experiment of Black *et al.* (1973), in which cow's milk proteins were infused into the abomasum of adult sheep, produced an estimated BV by the procedure outlined above of 0.48, which is similar to that observed (0.45). Distribution of the limiting amino acid (cystine + methionine) between competing body processes by direct proportionality in relation to total demand and supply, produced a predicted increase of 0.13 g nitrogen in wool for each g of milk nitrogen absorbed. This is the same as the value obtained from a series of experiments (Hogan *et al.* 1979) and suggests that the procedure may satisfactorily predict the synthesis of various proteins when amino acid supply is limiting.

These analyses indicate that, in a protein evaluation system, an option to vary BV in relation to the relative supply and requirement of essential amino acids should be included. The A.R.C. currently adopts a constant value of 0.75 for the efficiency of utilization of absorbed amino acid nitrogen.

Integration to predict animal performance

After estimating the amount of protein synthesized in each body function, the rates of growth or production of conceptus, udder, wool and milk are reduced, when necessary, in proportion to the respective deficits by assuming that the composition of the products is unaltered.

Allowances are made then for energy expenditure associated with animal activity and cold exposure so that finally tissue energy balance is calculated and expressed as a gain or loss in body fat. Thus, the system calculates the production of milk and wool, the growth of conceptus and birthweight of lambs, gain in empty body weight and change in body composition. Because the system is incorporated into a computer program, it is possible to increment animal parameters daily so that consequences of change in body weight or physiological state of the animal, in the composition or type of diet and in the weather can be followed readily through time.

APPLICATION OF THE SYSTEM

The system outlined considers simultaneously the effects of dietary, animal and environmental factors on both rumen function and nutrient utilization within the animal. It has the potential to be used either to formulate rations to meet selected production goals, or to predict whether amino acids, non-protein nitrogen or inorganic sulphur are limiting microbial growth in the rumen and whether amino acid availability is limiting animal performance. The extent to which various productive functions are reduced can be estimated. Beever et *al*. (1980-81) demonstrated the ability of the program representing rumen function to predict the effects of variation in the supply of non-protein nitrogen or inorganic sulphuron microbial growth and the flow of total protein from the rumen. Following incorporation of this representation of rumen function into the whole sheep program, the recycling of nitrogen to the rumen across the rumen wall as well as in saliva has been included. In addition, the programs have been used to identify situations where amino acid absorption may limit animal performance (Black *et al*. 1976).

Because the computer programs integrate both energy and protein utilization in sheep, they are not restricted to evaluating just the protein status of the animal. They can also be used to look at the implications of wider management problems such as the effect on pasture requirements of changing shearing or lambing times (Black and Bottomley 1980). In addition, since the current program considers both the potential energy demand of an animal and the factors which affect the accumulation of digesta in the rumen, it has the potential to predict voluntary feed consumption when more is known about the determinants of the upper limit to rumen digesta load (Black et *al*. 1981).

Lack of information about the chemical composition and digestion characteristics of animal feedstuffs currently hinders the widespread application of the program. Few estimates have been made of potential degradability or rate of degradation of structural carbohydrates in the plant materials selected by animals. There is a dearth of information about the potential degradability within the rumen of dietary proteins. Of the 1,600 feedstuffs on file in the Australian Feeds Information Centre, only 20.4% have recordings for cell wall constituents., 9.5% for soluble carbohydrate,' 0.7% for starch and in only 1% of the feedstuffs is nitrogen separated into protein and non-protein nitrogen (Leche and Groenendyk 1978). There is also limited information on the amino acid composition of feedstuffs and either the availability or accuracy of estimates for tryptophan and cystine seriously limit the value of these data. In addition, the predicted outcome of rumen function is highly sensitive to the values used for the fractional outflow rates of water, microbial and dietary constituents. The determinants of these rates are

not well understood, but techniques are now available for measuring them (Faichney 1980).

The approach described provides a framework on which a practical and flexible system of protein evaluation for ruminants can be constructed. Although its application is currently limited by an inadequate description of feedstuffs, considerable effort is being made to obtain this information. The system is less constrained than others by empirical equations and therefore is better able to cope with the extremes of Australian animal production.

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