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SYMPOSIUM ON 'NITROGEN UTILIZATION BY THE RUMINANT'

Evaluation of foods as sources of nitrogen and amino acids

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In any managed system of animal production the problem is to match the nutrient requirement of the animal with a supply of the nutrient in the feed to achieve the maximum economic return. Any matching system has one major prerequisite: the nutrient requirement and nutrient content of feedstuffs must be expressed in the same units. The manner in which this matching is carried out may differ according to the metabolism of the nutrient. The Agricultural Research Council (1965) calculations with respect to minerals and energy are examples of two different methods.

The current method of expressing protein requirements for ruminants is as digestible crude protein (DCP). The requirement values have been determined by the trial and error approach of feeding increasing levels of DCP and determining the point of maximum response. For some considerable time the imperfections of the method have been apparent. Many experiments have shown that diets with equivalent DCP content do not necessarily sustain the same productivity. Balch (1967) has drawn attention to the very significant interaction that exists between protein and energy in determining the response of the animal. Even under isoenergetic, isonitrogenous conditions it is quite easy to demonstrate differences in animal productivity between different types of protein as supplements to a concentrate (Whitelaw, Preston & Dawson, 1961) or roughage basal diet (El-Shazly, 1958; Little, Burroughs & Woods, 1963). Furthermore the relative values of the supplements are not necessarily the same under conditions where either the supply of amino acids to the small intestine or the supply of nitrogen to the rumen micro-organisms is the limiting factor.

The need for a new system of protein evaluation of animal feeds is becoming more imperative with changes in feed technology. Screw pressing, solvent extraction, drying, heating, grinding and pelleting of feeds have all been shown to affect the solubility of the protein, the rate of passage through the rumen and the site of digestion of the protein (Coelho da Silva, Seeley, Beever, Prescott & Armstrong,

1972). Any new method of feed evaluation should enable these differences to be detected. The growing use and economic advantage of using non-protein nitrogen (NPN) compounds in ruminant diets requires that any new system should be capable of predicting their value in a variety of situations. Current developments in the protection of proteins by reaction with aldehydes or tannins (Ferguson, Hemsley & Reis, 1967; Zelter, Leroy & Tissier, 1970; Faichney & Lloyd Davies, 1972) and the encapsulation of amino acids (Neudoerffer, Duncan & Horney, 1971) require precise evaluation, not only of the products themselves but also of accompanying feedstuffs, if their potential value is to be fully exploited.

At present there are insufficient results on a wide variety of feedstuffs to launch a new system. The following skeleton scheme is proposed to stimulate the necessary research to fill the gaps in our knowledge. The principle on which the scheme is based is the need to separate the requirements of rumen micro-organisms for N from the requirements of the host ruminant for amino acids.

Scheme for evaluation of feedstuffs

(a) Determine the requirements of the rumen micro-organisms for fermentable N needed to sustain maximum microbial growth. Express as g N/MJ of metabolizable energy (ME).

(b) Determine, using the factorial method, the requirement of the animal for amino acid N (BV 100) and for individual essential amino acids. Express as:

(i) g N/MJ ME

(ii) g amino acid/MJ ME for each required level of productivity.

(c) Determine for each feedstuff:

(i) the proportion of ME fermented in the rumen

(ii) the proportion of dietary N degraded in the rumen

(iii) the proportion of dietary N escaping rumen degradation and being absorbed from the small intestine

(iv) the pattern of dietary amino acids absorbed from the small intestine.

(d) (i) Calculate the amount of microbial N and microbial amino acids absorbed from the small intestine from the relationship with the amount of energy fermented; sum the values for contributions of dietary and microbial origin.

(ii) Express results as:

(1) g fermentable N/MJ ME

(2) g absorbed amino acid N/MJ ME

(3) g absorbed amino acid/MJ ME for each essential amino acid.

(e) To determine the value of any particular dietary combination sum the contributions of the individual feeds and express the value for the complete diet in terms of MJ ME.

(i) Compare fermentable N of the diet (g/MJ ME) with the requirement under (a) above. If this is inadequate then microbial growth may be limited with resultant poor digestion, particularly of roughage, and voluntary intake may be depressed. The potential for response to NPN may be assessed.

(ii) Correct the total dietary contribution of absorbed amino acid N (g/MJ ME) for efficiency of utilization either by an average BV or a chemical score based on the actual amino acid pattern. Compare this value with the requirement under (b) (i) above. Any deficiency will indicate that the potential productivity of the animal is limited by amino acid N and will enable the potential for response to protein supplements, particularly naturally or chemically protected proteins, to be assessed.

(iii) Compare the total dietary contribution of each absorbed essential amino acid (g/MJ ME) with the requirement under (b) (ii) above. Any deficiency will indicate a limiting amino acid and the potential for response to specific proteins or protected amino acid supplements can be assessed.

(f) Develop chemical or in vitro digestion procedures that will enable (c) (i) to (iv) above to be rapidly predicted in individual parcels of feedstuffs.

Some of the progess that has been made under each of these headings is briefly reviewed.

Nitrogen requirements of rumen micro-organisms

Allison (1970), reviewing in vitro work, concluded that the point at which ammonia concentration becomes limiting for growth of rumen bacteria has not been clearly defined. In a continuous culture system, growth of *Bacteroides amylophilus* was limited at ammonia concentrations lower than $4{\cdot}6\times10^{-3}$ M. Two-thirds of the ammonia N became incorporated into bacterial cells (Allison, 1970). We have studied this problem in vivo by supplementing a low-protein–high-energy diet with increments of urea and measuring the maximum non-ammonia N (NAN) flow to the abomasum of lambs, together with the extent to which urea is recycled (Allen & Miller, 1972). When allowance is made for 1 g N/d as abomasal secretions (Phillipson, 1964), the greatest microbial N flow of 13 g N/d was achieved with a dietary intake of 12 g N/d, recycled N of 6 g/d and a rumen ammonia concentration of approximately 17×10^{-3} M. Approximately 70% of the N available was converted to microbial N. It may prove convenient in many situations to equate dietary fermentable N supply and microbial N production, omitting recycled N from calculations but recognizing that it acts as a buffer to the biological inefficiency of the system. Under conditions of severe deficiency of fermentable N the recycled N should be taken into account, but both the amount recycled and the efficiency with which the N is trapped as microbial cells may differ (Allen & Miller, 1972). Assuming a maximum microbial production of 27 g N/kg organic matter fermented or 1·68 g N/MJ (7·0 g N/Mcal) ME (see below), the requirements of the rumen micro-organism would be 2·4 g N/MJ (10·0 g N/Mcal) ME and the dietary supply of fermentable N should be 1·68 g N/MJ (7·0 g N/Mcal) ME.

Determination of the extent of degradation in the rumen of dietary protein

By cannulating animals in the omasum, abomasum or duodenum estimates of N flow from the rumen can be made. When these are combined with cannulation of

the terminal ileum, the N absorbed from the small intestine can be determined. A major problem is to distinguish between bacterial, protozoal, feed and endogenous forms of N. Two approaches to the problem have been made. Firstly the use of markers such as diaminopimelic acid (DAPA) (Hutton, Bailey & Annison, 1971), incorporation of ^{35}S (Roberts & Miller, 1969; Walker & Nader, 1970) and nucleic acids (Smith & McAllan, 1970) enables bacterial N to be identified. The problem of separating feed and endogenous N remains. The second approach is to compare the N flow when a test diet containing the protein supplement is fed with that achieved on a basal diet without the test protein but containing adequate NPN to sustain maximum microbial growth. Microbial N and endogenous N contributions to total N reaching the small intestine are assumed to be constant on all diets (Ørskov, Fraser & McDonald, 1971).

The results of two experiments carried out at Cambridge illustrate the use of these two techniques. In the first trial (Mercer, Allen & Miller, unpublished) isonitrogenous, isoenergetic barley diets with supplements of urea (U), groundnut meal (GNM) or Peruvian fish meal (PFM) were given to cannulated, protozoa-free sheep at the maintenance level of feeding (Table 1). More NAN reached the duodenum on the PFM than on the U diet ($P<0.10$). Subtraction of the bacterial N,

Table 1. *Effect of nitrogen supplements on non-ammonia N flow to the duodenum and the extent to which protein supplements pass undegraded from the rumen of wethers at a maintenance level of feeding*

	Protein supplement			
	Urea	Groundnut meal	Fish meal	SEM
N intake (g/d)	14·3	14·8	15·2	—
Non-ammonia N to duodenum (g/d)	13·5	15·2	15·4	0·58
Bacterial N leaving rumen (g/d)	13·0	13·4	11·0	1·27
Non-bacterial N to duodenum (g/d)	0·5	1·8	4·4	—
Supplement undegraded (%)	Assumed zero	22	69	—

estimated by ^{35}S and DAPA, and comparison with the U diet to obtain an estimate of endogenous secretions and undegraded barley N, gives the estimates for the proportion of groundnut meal and fish meal escaping degradation. More of the fish meal escaped degradation and this was reflected in an increased concentration (g/16 g N) of many of the essential amino acids in the duodenum, particularly lysine ($P<0.005$) and methionine ($P<0.01$). In the second experiment (Mercer & Miller, unpublished) cannulated lactating ewes were given a diet based on barley and barley straw alone (B) or supplemented with urea (B+U) or with Peruvian fish meal (B+PFM) at near *ad lib.* feeding level. The B+PFM diet supplied 19·2 g N/d more than diet B and resulted in an extra 24·7 or 20·2 g N (SE 3·3) reaching the duodenum compared with diet B or B+U respectively. This suggests that all of the fish-meal protein escaped degradation.

Unfortunately the same batch of fish meal was not used in these two experiments but the results suggest that the rate of passage through the rumen could be an important factor in the extent to which dietary protein escapes degradation. A similar

suggestion has recently been made with respect to soya-bean meal (Ørskov & Fraser, 1973). We have investigated this by feeding lambs a basal diet and the basal supplemented with sunflower-seed meal at two levels of feeding, 1·25 and 2·5 times the energy requirement for maintenance (Stedman & Miller, unpublished). The results are shown in Table 2. Feeding twice as much of each diet resulted, within experimental error, in a doubling of NAN flow through the abomasum; there was no

Table 2. *Influence of level of feeding on the non-ammonia nitrogen (NAN) flow from the abomasum of lambs and on the extent to which sunflower-seed meal escapes degradation in the rumen*

	Diet			
	Basal		Basal + sunflower-seed meal	
Level of feeding (× maintenance)	1·25	2·50	1·25	2·50
N intake (g/d)	8·22	16·44	15·18	30·36
N from supplement (g/d)	—	—	12·0	24·0
NAN from abomasum (g/d)	5·80	12·99	9·11	17·52
Additional NAN from abomasum due to supplement (g/d)	—	—	3·31	4·53
Supplement undegraded (%)			28	19

evidence of increased proportion of the sunflower seed escaping degradation at the higher level of feeding, the mean value being 25%. Probably highly soluble proteins are extensively degraded no matter what the feeding level, while the degradation of more resistant proteins is more affected by high flow rates from the rumen associated with high levels of feeding. Consequently the determination of the extent of protein degradation should be made under conditions as close to *ad lib.* feeding as possible. Suggested values for percentage of protein escaping degradation are barley 10, cottonseed (soluble) and groundnut meal 20, sunflower-seed meal 25, soya-bean meal 45, dried grass and white fish meal 50, Peruvian fish meal 70.

Extent of microbial protein production and absorption

The energetic constraints placed on microbial growth by the anaerobic conditions in the rumen have now been recognized. Using a protein-free diet, Hume (1970) found the flow of protein through the omasum corresponded to a yield of 27 g microbial N/kg organic matter fermented. In further experiments a mean value of 30 g/kg was obtained (Hume & Bird, 1970). In our experiments at Cambridge, seven estimates have ranged between 26 and 30 with a mean value of 27 g/kg. Ørskov, Fraser & McDonald (1972), also feeding barley–urea diets to lambs, calculated a mean value of 25 g/kg. In a completely different type of experiment Walker & Nader (1970) estimated the yield as 23 g/kg. In the light of present information the value of 27 g microbial N/kg fermentable organic matter is preferred. Assuming 117 g N/kg bacterial organic matter, 70% of the apparent digestion of food organic matter takes place in the rumen, digested organic matter contains 18 MJ/kg and ME: DE ratio is 0·81, this corresponds to a yield of 1·68 g microbial N/MJ (7·0 g N/Mcal) ME fed. When allowance is made for 19% nucleic acid N (Smith & McAllan, 1970)

and 75% digestion of amino acids in the small intestine (Coelho da Silva *et al.* 1972) the value becomes 1·03 g absorbed microbial amino acid N/MJ (4·3 g/Mcal) ME given.

Assessment of the scheme

Predicted live-weight gains in close agreement with determined values have been obtained when the values given in this paper are applied to published results of feeding trials (e.g. Whitelaw, Preston & MacLeod, 1963).

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