

"BYPASS" PROTEIN - A REVIEW

A.R. EGAN*

A review of the subject of ruminally undegraded dietary protein ("bypass" protein, for want of a more acceptable, short snappy title) is in reality a review of the ruminant nutritionists' thrusts towards an adequate evaluation of the amino acid requirements of ruminants, and towards prediction of the ability of pastures, conserved feeds and supplements to meet these.

A number of protein requirements systems for ruminants based on equations for computation of the amounts of microbial protein and of undegraded dietary protein digested in the intestine have emerged since 1975 (Burroughs 1975, Roffler and Satter 1975, Kaufmann and Hagemeister 1975, Roy *et al.* 1977, Jarrige *et al.* 1978) and more complex and dynamic approaches are being developed out of the "modelling club" of interacting workers in Australia, New Zealand, U.K. & U.S.A.

The essential elements of all systems are:

1) Part of the food N is protein which resists breakdown by rumen micro-organisms and passes unchanged to the intestines. How this is measured or calculated from empirical measurements or more complex modelling exercises is a matter of argument.

2) Part of the food N will be metabolised by rumen micro-organisms and may be converted into microbial N if sufficient energy and essential nutrients are simultaneously available for the micro-organisms. Some computer based model systems provide for an input of N recycled from the body N pools where this is appropriate. Variability in protein synthetic efficiency and in fractional outflow rate of microbial protein from the reticulo-rumen influence rate of supply of amino acid from this source.

3) A proportion of the undegraded food protein and of the microbial protein is digested in the small intestine. These sources are combined and assigned a common digestibility constant in most systems, though variability is apparent in estimates provided in the literature,

4) The absorbed amino acids are used in synthesis of tissue proteins, replacing losses through catabolism of amino acids from the pool to which body protein turnover and new absorbed amino acids contribute; and in synthesis of product protein (growth, wool production, milk). The extent of use of amino acids for maintenance, growth or production is determined by metabolisable energy intake and amounts and proportionalities of amino acids. That efficiency of use of absorbed amino acids will vary with the composition of the protein source and possibly with the composition of other absorbed products of fermentation and digestion is suggested by comparison with other species. The magnitude of the variation is uncertain, and amino acid utilization factors (Armstrong and Annison 1973; Egan and Walker 1975) remain to be determined, preferably through a dynamic approach responding to such variables as physiological state, nature of energy and dietary protein sources.

*Waite Agricultural Research Institute, Glen Osmond, S.A. 5064.

There are three aspects which typify this drawing together of knowledge of processes of N use in the ruminant, and these represent important elements of context for this review.

1) The importance of rumen metabolism of "degradable N", as distinct from "apparent digestibility" of N, is recognised;

2) The relationships between N and energy requirements are represented at two levels - the utilization of degraded dietary N in microbial protein synthesis which is limited by intra-ruminal 'energy supply; and the use of absorbed amino acids by the animal which must be related to the level of absorbed energy, yielding metabolites to which microbial fermentation products contribute the greater part.

3) The amino acid supply to the animal consists of two exogenous components potentially differing in digestibility and composition which together may fall short of, satisfy, or exceed the animal's requirements; and these two sources have different potential for manipulation.

How did we arrive at this position? In particular what is the significance of the dietary protein which is not degraded in the reticulo-rumen, the so-called "bypass" protein? Under what circumstances, if any, 'will animal production responses follow manipulations which by use of either particular properties of the protein or of physiological mechanisms in digesta movement, favour flow of undegraded dietary protein on to the abomasum?

Ruminal degradation and conversion of dietary N. Qualitatively, non-protein N use, hydrolysis of dietary protein in the rumen, and the nutritional significance to the ruminant host of microbial protein and of dietary protein have been themes in theory and practice since the early 1900's. In 1925, Schwarz presented the view that the greatest part of the protein requirement of cattle was met by the digestion of microbial protein derived from fodder protein. Though his evidence was inadequate, as pointed out by Mitchell (1929), this, together with Sym's (1935) observation that proteins were hydrolysed in the rumen, provided enough basis for studies from which Johnson *et al.* (1942, 1944) concluded that the nature of the dietary N was of relatively minor importance in ruminant protein nutrition since most protein 'being digested and absorbed was microbial in origin. However observations made by Lofgren, *et al.* (1947) and Hamilton *et al.* (1948) on comparative biological values of protein sources suggested that the proportions of dietary and microbial protein which actually become 'available to the host might vary considerably on different diets. Loosli *et al.* (1949) were however able to sustain growth of sheep 'on a diet in which virtually all N was supplied as urea. While the growth of micro-organisms in the rumen was becoming accepted as effecting a significant degree of conversion of food N to microbial protein, the extent and the factors affecting this were not clear in data of this period. In fact, this awaited almost 20 years of technique development before it was possible. The variable extent to which dietary proteins may be fermented in the rumen was first clearly established by McDonald (1948, 1954). Soluble proteins were shown to be rapidly fermented and thus led to inefficient protein utilization due to absorption of ammonia from the rumen, with net loss as urea in the urine when the supply of ammonia exceeded the requirement for the microflora. El Shazley (1952) demonstrated that deamination of amino acids occurred in rumen fluid and Annison (1956) made it clear that little if any free

amino acid was absorbed from the reticulo-rumen. Chalmers and Synge (1954) provided heat-treated casein to sheep and, in comparison with untreated casein, showed reduced ruminal NH_3 production and improved performance of the host animal. This implanted the idea of improved nutritional value for proteins which avoid fermentation.

The relationship between protein and NPN as sources of N for microbial protein synthesis became a major focus of attention during the 1950's and early 1960's. From studies indicating an additional advantage if a proportion of the N in the diet was as protein, it became a rule of thumb that urea N could replace no more than 2/3 of the diet crude protein. The idea grew that micro-organisms, or some species in the spectrum, required amino acid substrate, rather than NH_3 ; branched chain amino acids were identified as factors essential for effective urea use (Bryant 1960; Hemsley and Moir 1962; Hume 1970). However good performance of dairy cattle on protein-free urea diets was reported by Virtanen (1966). Bacterial and protozoal growth rates became an area of intense interest (Hungate 1966).

Protein reaching the duodenum. Separately, studies commenced on the measurement of the amount and the composition of digesta flowing from the rumen and into the intestines (Phillipson 1952; Singleton 1953; Hogan and Phillipson 1962). Oyaert and Bouchaert (1961) showed a correlation between mean concentration of NH_3 in the rumen and the loss of dietary N from the digestive tract between mouth and abomasum. Hogan and Phillipson (1962) also had shown gain in digesta N on low N diets, reinforcing the growing view of the nutritional significance of N known to be recycled to the rumen (Haupt 1954; Somers 1960).

Two other sets of observations arose which concentrated attention on the protein which was not degraded in the reticulo-rumen. Reis and Schinkel (1962) showed a wool growth response to casein administered duodenally. Egan and Moir (1965) and Egan (1965) observed an improvement in intake of low quality roughage when casein was infused per duodenum; this was evaluated as a response operating separately from an effect of N in processes in the rumen (Moir and Harris 1962). Very little was known of the absolute and relative amounts of protein and energy absorbed by the ruminant when fed on diets of different composition and Egan (1965b) suggested that alteration of ratio of protein (amino acids) and energy in the absorbed nutrients, or altered potential to retain N initiated an intake response.

Energy limits on microbial growth, From the work of Bauchop and Elsdon (1960) on microbial cell yield in anaerobic fermentation, the concept of energy limitation to microbial protein synthetic capability in the rumen developed and became translated into quantitative relationships. Walker (1965) and Hungate (1966) each presented a stoichiometry for the relationships between organic matter fermented in the rumen, volatile fatty and production, ATP yield and cell yield. Hungate (1965) concluded a discussion of these topics by focusing attention on the importance of "increasing the percent of food synthesized into microbial bodies in the rumen, and finding the means of shunting protein directly to the abomasum".

The attention of research workers in this area can now be seen to have been directed primarily into these two directions over the next ten years. Efforts were made in a number of laboratories to measure the amount of protein reaching, and being digested in, the small intestine

(Gray et al. 1958; Hogan 1964, 1965; Clarke et al. 1966) on a wide range of diets', and to distinguish between dietary and microbial protein flowing to the duodenum (reviewed by Hogan and Weston 1970; Egan and Walker 1975; ARC, 1980). Some concentrated on microbial protein synthesis, its magnitude and variability (Hume 1970; Walker and Nader 1970; Beever et al. 1974; McAllan and Smith 1970; Walker et al. 1975). Separation of intestinal digesta protein into microbial or undegraded dietary components using markers or tracers became possible (Weller et al. 1958; Hutton et al. 1971; Smith, R. H. 1975; Harrison and McAllan 1980) and this yielded more information on the relationships *in vivo* between microbial protein synthesis and organic matter fermented in the rumen (Hogan and Weston 1970; Beever et al. 1974; Egan and Walker 1975).

Relating protein supply and requirements. As more data became available, calculations were commenced to relate the new empirical knowledge of the amounts of intestinally digestible proteins or estimated amino acid absorption to the animal's requirements for maintenance and production (Hogan 1970; Hogan and Weston 1970; Purser 1970; Armstrong and Annison 1973; Miller 1973; Egan 1974; Egan and Walker 1975). The calculations lacked sophistication, being based on "static" and averaged data to which "best guess" estimates for digestibility and utilization were applied. However, they suggested that microbial protein, synthesized at rates determined by ruminal organic matter fermentation and hence broadly related to digestible (or metabolizable) energy, would be insufficient to allow for rapid growth or lactation, or for high rates of wool growth in sheep (Hogan 1970; Armstrong and Annison 1973; Miller 1973; Egan and Walker 1975). Making broad allowances for variation in efficiency of microbial growth, survival, and outflow rate from the rumen, it was generally considered that there was a need for undegraded dietary protein to reach and be digested in the small intestine to meet many of these needs. It was clear that, while on many diets undegraded dietary protein passed to intestines (Hogan and Weston 1967; MacRae and Ulyatt 1974; Hume and Purser 1974; Walker et al. 1975), this was not always sufficient to meet the animal's estimated needs over and above that provided by microbial protein. Chalupa (1975) suggested that 20-60% of dietary protein is not degraded in the rumen; the animal is normally supplied with a mixture of microbial and undegraded dietary proteins, providing a wide range in terms of total digestible protein per MJME. The total protein provision at the small intestine may in almost all instances be adequate for finishing animals (Preston 1972; Young et al. 1973) but responses to increased quantities of abomasal supplements of protein and amino acids suggests that genetic potential of cattle and sheep during growth and early fattening periods, of sheep on low energy intakes kept for wool production, and of lactating dairy cows is not reached because of amino acid inadequacies.

Manipulation of protein supply. Parallel with this line of development, attempts were being made to find ways of providing dietary protein or later, amino acids, in a form or in a way which would avoid substantial degradation through fermentation. Fergusson (1975) has reviewed this subject.

Rumen degradation of protein is reduced when naturally insoluble proteins are fed, or when soluble proteins are treated to decrease solubilities, by heat denaturation (Chalmers and Synge 1954) or by formaldehyde binding (Nitschman et al. 1943; Fergusson et al. 1967) or tannin binding (Leroy and Zelter 1970). Even with soluble proteins, rumen

degradation may be significantly reduced at high intake and high flow rates (Potter *et al.* 1972; Miller 1973; Orskov *et al.* 1973; Thompson *et al.* 1978; Egan and Ulyatt 1980). An alternative approach, capitalising on the reflex closure of the oesophageal groove during sucking (Orskov and Benzie 1969) allows true "by-pass" of protein to the abomasum. As experimental tools, these approaches have allowed tests of potential for response to increased flow of protein to the duodenum for digestion. Responses in wool growth (Reis and Tunks 1969; Barry *et al.* 1969; Fergusson 1975), nitrogen retention (Black, 1970; Faichney, 1971; Orskov *et al.* 1972; Barry 1972) liveweight gain (Chalupa 1975; Kempton *et al.* 1979) appetite (Egan 1965b; Weston 1971; Orskov *et al.* 1970; Preston 1976; Kempton *et al.* 1979) milk production (Clark 1975) have been reported. However these need to be viewed in relation to the characteristics of the basal diet, the genotype, age and physiological state of the animal, and whether or not feed intake was increased with the additional intestinally digested protein. Protection from ruminal degradation allows more amino acids to reach the intestine than would otherwise be the case, provided that microbial protein synthesis is not depressed. Positive responses can be expected if the animal needs or can use more amino acids for maintenance and production. If amino acid supplies are adequate, or if intake of energy or of other essential nutrients limits protein synthetic rate, additional quantities should produce no response. Certainly there is good evidence that extra intestinally digested protein does not always improve performance (eg. Redman *et al.* 1980).

Apart from wool growth and short term N retention responses (Reis and Tunks 1969; Egan 1970), there is usually a close association between any production response and an increase in intake (Preston 1972; Leng *et al.* 1977). Where ruminally undegraded dietary protein supplements have had no effect, this is usually associated with a lack of intake response (eg. Redman *et al.* 1980). Egan (1977) attempted to define the relationship between the ratio of intestinally digested protein to digestible energy (P:E value) and the likelihood of response in intake to additional protein provided at the duodenum. Other factors limiting intake (eg. fractional outflow rate of fibre - Weston 1971; Thornton and Minson 1973) may affect the ability of the animal to express a response to "bypass" protein, but for diets providing about 6g of intestinally digested protein/MJDE or more there was no evidence of any intake response on roughage diets. Below 6g/MJDE, responses were variable. With semi-purified diets and high energy concentration diets intake may be less effectively restricted by digesta outflow characteristics, permitting greater intake responses (Preston and Willis 1970; Kempton and Leng 1979) and provide opportunity for greater production responses.

The responses, where observed, have been assumed to be specifically to improved provision of essential amino acid(s). Studies with individual amino acids (eg. methionine) have also produced increases in intake and N retention (Schelling and Hatfield, 1968; Chalupa *et al.* 1973; Barry 1973; Fennessy 1976). However, in some situations where "protein" reaches the intestine in greater amounts, it may be accompanied by other energy yielding materials, or may itself serve as a source of energy. In this sense the response may be to gluconeogenic substrate, as suggested by Kempton, Smith and Leng (1978). Alternative gluconeogenic precursors or glucose did not increase intake in the early studies of Egan and Moir (1965).

The formalisation of undegraded dietary protein ("bypass" protein) as a requirement. From both lines of approach - one relating measured supply and estimated requirements for intestinally digested protein, the other testing for response to supplemental "bypass" protein - the idea that diets can be improved by manipulation of microbial protein production and, for productive purposes, by provision of additional protein to pass undegraded to the intestines has strongly developed roots. The challenge has been extended into a variety of approaches in gaining improved control over the many processes affecting the two sources of intestinally digested protein; and to predicting, with models of considerable complexity, (Nolan 1975; Black and Faichney 1978) the likely amino acid absorption, or level of animal production with a variety of dietary sources.

It is important to remember, however, that "bypass" protein occurs naturally, that the concept covers a multitude of materials which can be fed in a variety of circumstances. Zero to poor responses to feeding of such protein sources may indicate absence of any real amino acid inadequacy. However a lack of response can also be due to unavailability of the protein in intestinal digestion ("over-protection"), limitations due to inadequacies of other essential nutrients, and possibly even to amino imbalances (Egan and Rogers 1977) with some protein sources (Bloodmeal - Egan and Rogers, unpublished). Inability to increase energy intake may limit the ability to take advantage of the improved amino acid supply. A positive response may be due to something other than essential amino acid availability, or may be affected indirectly (hormonally?) rather than through supplementation with a limiting essential amino acid at the protein synthetic level. In some instances the response may exceed expectations (Kempton et al. 1977) possibly because intake increases and ruminal digesta outflow rates are increased. Because of altered dynamics affecting the intra-ruminal degradation of dietary protein, the balance of substrates for microbial fermentation and protein synthesis attending on the ruminal dilution rates, and on the fractional outflow rates of fluids and particulate material, the evaluation of the complex pattern of effects which underly a response, or the lack of one, to extra inputs of "degradable" or "undegradable" protein is no simple matter. Effective prediction also awaits a better basis for estimation of amino acid requirements and of the effects of interactions between amino acids and energy-yielding substrates in the ruminant host.

REFERENCES

- Agricultural Research Council (1980). 'The Nutrient Requirements of Farm Livestock' : Ruminants 2nd Edn. ARC, London.
- ANNISON E.F. (1956). Biochem J. 64: 705 .
- ARMSTRONG, D.G. and ANNISON, E.F.(1973).Proc. Nutr. Soc. 32: 107.
- BARRY, T.N.(1972). N.Z. J. Agric. Res. 15:107.
- BARRY, T.N. (1973). N.Z. J. Agric. Res. 16: 191.
- BAUCHOP, T. and ELSDEN, S.R. (1960). J. Gen. Microbiol. 23: 457.

- BEEVER, D.E., HARRISON, D.G., THOMSON, D.J., CAMELLI, S.B. and OSBOURNE, D.F. (1974). Brit. J. Nutr. 32: 99.
- BLACK, J.L. and FAICHNEY, G.J. (1978) in "Physiological and Environmental Limitations to Wool Growth" p.221 (J.L. Black and P.J. Reis, Ed.).
- BRYANT, M.P. (1965) in "Physiology of Digestion in the Ruminant" p. 411 (R.W. Dougherty, Ed.) Butterworths, London.
- BURROUGHS, W., NELSON, D.K. and MERTENS, D.R. (1975). J. Anim. Sci. 41: 933.
- CHALMERS, M.I. and SYNGE, R.L.M. (1954). J. Agric. Sci, Camb. 44: 263.
- CHALUPA, W. (1972). Fed. Proc. 31: 1152.
- CHALUPA, W., CHANDLER, J.E. and BROWN, R.E. (1973). J. Anim. Sci. 37: 339.
- CHALUPA, W. (1975). J. Dairy Sci. 58: 1198, ,
- CLARKE, E.M.W., ELLINGER, G.M. and PHILLIPSON, A.T. (1966). Proc. Roy. Soc, Ser B. 166: 63.
- CLARK, J.H. (1975). J. Dairy Sci. 58: 1178.
- EGAN, A.R. (1965a). Aust. J. Agric. Res. 16: 169.
- EGAN, A.R. (1965'0). Aust. J. Agric. Res. 16: 451.
- EGAN, A.R. (1970). Aust. J. Agric. Res. 21:
- EGAN, A.R. (1974). Aust. J. Agric. Res. 25: 613.
- EGAN, A.R. (1977). Aust. J. Agric. Res. 28: 907.
- EGAN, A.R. and MOIR, R.J. (1965). Aust. J. Agric. Res. 16: 437.
- EGAN, A.R. and WALKER, D.J. (1975) in Proc 3rd World Conf. Amin. Prod, p. 551 (R.L. Reid, Ed.). Sydney Univ. Press, Sydney.
- ELSHAZLEY, K. (1952). Biochem. J. 51: -647'.
- FAICHNEY, G.J. (1971): Aust. J. Agric. Res. 22: 461.
- FENNESSY, P.F. (1976). Ph.D. Thesis, University of Adelaide.
- FERGUSON, K.A. (1975) in "Digestion and Metabolism in the Ruminant, pp. 448 (I.W. McDonald and A.C.I. Warner, Eds.) Univ. New England Publ. Unit, Armidale, N.S.W.
- FERGUSON, K.A., HEMSLEY, J.A. and REIS, P.J. (1967). Aust. J. Sci. 30: 215.
- GRAY, F.V., PILGRIM, A.F. and WELLER, R.A. (1958). Brit. J. Nutr. 12: 41.3.

- HAMILTON, T.S., ROBINSON, W.B. and JOHNSON B.C. (1948). J. Anim. Sci. 7: 26.
- HARRISON, D.G., BEEVER, D.E., THOMSON, D.J. and OSBOURNE, D.F. (1975). J. Agric. Sci. Camb. 85: 93.
- HARRISON, D. and McALLAN, A.B. (1980) in "Digestive Physiology and Metabolism in Ruminants" p. 205 (Y. Ruckesbusch and P. Thrend, eds) MTP Press, Lancaster.
- HEMSLEY, J.A. and MOIR, R.J. (1963). Aust. J. Agric. Res. 14:509.
- HOGAN, J.P. (1964). Aust. J. Agric. Res. 15: 384.
- HOGAN, J.P. (1965). Aust. J. Agric. Res. 16: 179.
- HOGAN, J.P. (1970). Proc. Aust. Soc. Anim Prod. 8: 1.
- HOGAN, J.P. and PHILLIPSON, A.T. (1962). Brit. J. Nutr. 14:147.
- HOGAN, J.P. and WESTON, R.H. (1967). Aust. J. Agric. Res. 18: 803.
- HOGAN, J.P. and WESTON, R.H. (1970) in "Physiology of Digestion and Metabolism in the Ruminant" p. 478 (A.T. Phillipson, Ed) Griel Press, N
- HOUP, T.R. (1959). Amer. J. Physiol. 197: 115.
- HUME, I.D. (1970). Aust. J. Agric. Res. 21: 305.
- HUME, I.D. (1974). Aust. J. Agric. Res. 25: 155.
- HUME, I.D. and PURSER, D.B. (1975). Aust. J. Agric. Res. 26: 199.
- HUNGATE, R.E. (1965) in "Physiology of Digestion and Metabolism in the Ruminant" p. 310. Butterworth, London.
- HUNGATE, R.E. (1966). "The Rumen and its Microbes" Academic Press, N.Y.
- HUTTON, K. and ANNISON, E.F. (1972). Proc. Nutr. Soc. 151.
- HUTTON, K., BAILEY, F.J. and ANNISON, E.F. (1971). Brit. J. Nutr. 25: 165.
- JARRIGE, R., JOURNET, M. and VERITE, R. (1978) in INRA Alumentation des Ruminants p. 89 INRA, Versailles
- JOHNSON, B.C., HAMILTON, T.S., MITCHELL, H.H. and ROBINSON. W.B. (1942). J. Anim. Sci. 1: 236.
- JOHNSON, B.C., HAMILTON, T.S., ROBINSON, W.B., GAREY, J.C. (1944) J. Anim. Sci. 3: 287.
- KAUFMAN, W. and HAGEMEISTER, H. (1975). Ubers. Tierernahr 3: 33.
- KEMPTON, T.J. and LENG, R.A. (1979). Brit. J. Nutr. 42: 289.

- KEMPTON, T.J., NOLAN, J.V. and LENG, R.A. (1977). Wld. Anim Rev. 22: 2.
- KEMPTON, T.J., NOLAN, J.V. and LENG, R.A. (1979). Brit. J. Nutr. 42: 303
- KEMPTON, T.J., SMITH, G.H. and LENG, R.A. (1978). Proc. Aust. Soc. Anim. Prod. 12: 139.
- LENG, R.A., KEMPTON, T.J. and NOLAN, J.V. (1977). A.M.R.C. Review 33: 1.
- LeROY, F. and ZELTER, S.Z. (1970). Am. Biol. Anim. Biochim Biophys. 10: 401.
- LOFGREN, G.P., LOOSLI, J.K. and MAYNARD, L.A. (1947). J. Anim. Sci. 6: 343.
- LOOSLI, J.K., WILLIAMS, H. H., THOMAS, W.E., FERRIS, F. H. and MAYNARD, L.A. (1949). Science 110: 321.
- McALLAN, A.B. and SMITH, R. H. (1974). Brit. J. Nutr. 31: 77.
- MacDONALD I.W. (1948). Ph.D. Thesis, University Camb.
- MacDONALD, I.W. (1952). Biochem J. 51: 647.
- MacDONALD, I.W. (1954). Biochem J. 56: 120.
- MacRAE, J.C. and ULYATT, M.J. (1974). J. Agric. Sci., Camb 82: 309.
- MILLER, E.L. (1973). Proc. Nutr. Soc. 32: 79.
- MITCHELL, H.H. and HAMILTON, T.S. (1929). "The Biochemistry of the Amino Acids" Chemical Catalogue Co, N.Y.
- MOIR, R.J. and HARRIS, L.E. (1962). J. Nutr. 77: 285.
- NITSCHMAN, H.S., HARDORN, I-I. and LAUENER, H. (1943). Helv. Chim. Acta. 26: 1069.
- NOLAN, J.V. (1975) in "Digestion and Metabolism in the Ruminant" p. 416 (I.W. McDonald and A.C.I. Warner' eds) Univ. New England Publ. Unit. Armidale N.S.W.
- ORSKOV, E.R. (1977). World Rev. Nutr. Diet. 26: 225.
- ORSKOV, E.R. and BENZIE, D. (1969). Brit. J. Nutr. 23: 413.
- ORSKOV, E.R., FRAZER, C. and CORSE, E.L. (1970). Brit. J. Nutr. 24: 803.
- GYAERT, W. and BOUCHAERT, J.H. (1960). Leutr. Veternaremed 7: 929.
- PHILLIPSON, A.T. (1952). J. Physiol 116: 84
- PRESTON, T.R. (1972) in "Trace Studies on Non-Protein N for Ruminants" p. 1. IAEA Vienna.
- PRESTON, T.R. (1976) in Review in Rural Science, No. 2 (T.M. Sutherland, J.R. McWilliam and R.A. Leng, Eds) Univ. New Engl. Publ. Unit, Armidale, N.S.W.

- PRESTON, T.R. and WILLIS, M.B. (1970). "Intensive Beef Production" Pergamon Press, Oxford.
- PURSER, D.B. (1970). J. Amer. Sci. 30: 988.
- REDMAN, R.G., KELLAWAY, R.C. and LEIBHOLZ, J. (1980). Brit. J. Nutr. 44: 343.
- REIS, P.J. and SCHINKEL, P.G. (1961). Aust. J. Agric. Res. 12: 335.
- REIS, P.J. and TUNKS, D.E. (1969). Aust. J. Agric. Res. 20: 775.
- RÖFFLER, R.E. and SATTER, L.D. (1975). J. Dairy Sci. 58: 1889.
- ROY, J.H.B., BALCH, C.C., MILLER, E.L., ORSKOV, E.R. and SMITH, R.H. (1977) in "Protein Metabolism and Nutrition" p.126. EAPP Publ. No. 22. EAPP, Wageningen.
- SCHELLING, G.T. and HATFIELD, E.E. (1968). J. Nutr. 96: 319.
- SCHWARZ, A. (1925). Biochem Z. 156: 130.
- SINGLETON, A.G. (1953). Nature, Lond. 172: 834.
- SOMERS, M. (1961). Aust. J. Exp. Biol. Med. Sci. 39: 123.
- SYM, E.A. (1938). Acta Biol. Exp. Varsovie 12: 192.
- THOMSON, D.J., BEEVER, D.E., LATHAM, M.J., SHARPE, M.E. and TERRY, R.A. (1978). J. Agric. Sci., Camb. 91: 1.
- THORNTON, R.F. and MINSON, D.J. (1973). Aust. J. Agric. Res. 24: 889.
- VIRTANEW, A. L. (1966). Science, N.Y. 153: 1603.
- WALKER, D.J. (1965) in "Physiology of Digestion in the Ruminant" p. 251 (R.W. Dougherty, ed.) Butterworths, London.
- WALKER, D.J., EGAN, A.R., NADER, C.J., ULYATT, M.J. and STORER, G.E. (1975). Aust. J. Agric. Res.
- WALKER, D.J. and NADER, C.J. (1970). Aust. J. Agric. Res. 21: 747.
- WELLER, R.A., GRAY, F.V. and PILGRIM, A.F. (1958). Brit. J. Nutr. 12: 421.
- WESTON, R.H. (1971). Aust. J. Agric. Res. 22: 307.
- WESTON, R.H. (1967). Aust. J. Agric. Res. 18: 983.
- YOUNG, A.W., BOLING, J.W. and BRADLEY, N.W. (1973). J. Anim. Sci. 36: 803.