Establishment of a ruminal protein degradation data base for dairy cattle using the *in situ* polyester bag technique. 2. Energy sources

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The extent of ruminal protein degradation of 11 energy sources was determined in the rumen of cannulated lactating dairy cows, and was calculated at three different fractional outflow rates, using the *in situ* polyester bag technique. The extent of ruminal protein degradation ranged from 45% for bird-resistant sorghum to 91% for oats, when calculated at a fractional outflow rate of 0,08/h, indicating large variation in the resistance of energy sources to protein degradation in the rumen. Among the energy sources, sorghum, maize and maize corn and cob meal were noteworthy in providing larger amounts of undegraded dietary protein (UDP).

Die mate van rumenproteïendegradering van 11 energiebronne is met rumengekannuleerde lakterende melkkoeie bepaal. Die effektiewe rumen proteïendegradering is bereken by verskillende fraksionele uitvloeitempo's deur gebruik te maak van die *in situ*-poliëstersaktegniek. Proteïendegradeerbaarheidswaardes het gevarieer van 45% vir voëlbestande graansorghum tot 91% vir hawer, wanneer bereken by 'n fraksionele uitvloeitempo van 0,08/h. Dit gee 'n aanduiding van die groot variasie wat bestaan tussen energiebronne met betrekking tot weerstandbiedendheid teen rumendegradasie. Wat betref die energiebronne, het graansorghum, mieliemeel en mielieblaarkopmeel redelike hoeveelhede verbyvloeiproteïen (UDP) gelewer.

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During the last decade, a number of new protein requirement systems have been published by research workers in the USA and Europe (ARC, 1984; NKJ/NJF, 1985; NRC, 1985; Rohr, Lebzien, Schafft & Schulz, 1986). The new systems all recognize that protein flowing to the small intestine of the ruminant consists largely of microbial protein synthesized in the rumen (RDP) and dietary protein which has escaped rumen degradation (UDP).

At present, one major factor limiting the widespread application of diet formulation based on RDP and UDP is the absence of reliable data for many feedstuffs (Satter, 1986; Kirkpatrick & Kennelly, 1987). This applies not only to protein feeds, but also to cereals and cereal by-products which comprise a considerable proportion of the ruminant diet (Sampath & Sivaraman, 1986). In diets for high-producing dairy cows, the grain portion may be as high as 40%, which, with maize at 10% crude protein, would contribute about 25% of the total dietary protein.

As the level of feed intake affects both the rate of passage and the *in situ* digestion rate (Erdman, Vandersall, Russek-Cohen & Switalski, 1987), it is imperative that these measurements be made in dairy cows at productive levels of intake. Values for UDP in published tables, e.g. those of NRC (1989), should be used with caution in formulating diets for high-producing dairy cattle. UDP values were obtained using sheep, growing cattle and lactating dairy cows eating less feed than normally consumed by high-producing dairy cattle. Extrapolating the data to high-producing dairy cows consuming large amounts of feed, may provide misleading estimates of the degradation of the same feed protein in the rumen of these animals (Aseltine, 1988). Nocek (1988) recommended that investigators should use the species of animal for which the results will be used, and should feed a diet to meet maximum performance requirements. Feeding at maintenance is useful when comparing results between laboratories.

Protein degradation in feedstuffs has been measured by a number of procedures such as laboratory solubility tests (Poos-Floyd, Klopfenstein & Britton, 1985), *in situ* nylon bag procedures (Mehrez & Ørskov, 1977) and *in vivo* measurements using animals cannulated in the rumen and duodenum (Stern, Rode, Prange, Stauffacher & Satter, 1983). Protein solubility is not suitable for estimating protein degradation across a variety of feedstuffs while *in vivo* estimates are labour intensive, time consuming and subject to considerable error with regards to separation of microbial and dietary protein (Stern & Satter, 1984). The procedure gaining widest application is the *in situ* bag technique whereby the protein under study is contained in synthetic fibre bags suspended within the rumen and the rate of N loss from the bags is used to determine rate and extent of protein degradation (Mehrez & Ørskov, 1977). However, this technique has some limitations as well as advantages. The *in situ* technique measures essentially the rate of disappearance from the bag rather than the actual degradation. An additional problem is that feedstuffs are confined in a bag and are not subjected to chewing and rumination by the animal. Therefore, the data obtained from this technique should be interpreted with due caution and should be compared with those from *in vivo* studies whenever possible (Ha &

Kennelly, 1984). In a recent review, Nocek (1988) examined various methods to estimate the extent of ruminal protein degradation and gave recommended guidelines leading to a standardized *in situ* procedure.

The object of this study was to expand the existing South African protein degradation data base by determining the extent of ruminal protein degradation of the most common energy sources used in the formulation of dairy cattle diets, using the *in situ* technique. Protein degradation values of 20 protein sources have been published (Erasmus, Prinsloo & Meissner, 1988).

Experimental Procedures

The energy sources were obtained commercially and crude protein contents as determined by the Kjeldahl method, are presented in Table 1. The *in situ* technique and the composition of the complete diet fed to the cows have been described by Erasmus *et al.* (1988). Three lactating rumen-cannulated Holstein cows with an average dry-matter intake of 20,8 (± 2,0) kg/d were fed a practical complete dairy cattle diet twice daily for *ad libitum* intake. The basal diet (DM basis) having a roughage:concentrate ratio of 45:55 (15,2% CP, 10,3 MJ ME/kg, 18,2% CF) contained lucerne hay, *Eragrostis curvula* hay, wheat straw, maize meal, urea, fishmeal, sunflower oilcake, soybean oilcake, cottonseed oilcake, minerals and vitamins. The polyester bags (14 × 9 cm; 53 μm pore size) were filled with *ca.* 5 g of test feed (air dry) after being milled in a Wiley mill with 2-mm screen. The bags were tied to a stainless steel disc with 10 evenly spaced small holes drilled through the periphery of the disc. Using the complete exchange method (Paine, Crawshaw & Barber, 1981), one bag per test feed was placed in the rumen of each of three cows for every incubation period (0, 1, 2, 4, 6, 8, 12, 24, and 48 h respectively). The procedure was replicated giving a total of six values for N disappearance of each feed per incubation period. After incubation the bags were rinsed under running tap water (15 s) and washed in a washing machine (cold water) for 10 min followed by drying (65°C, 48 h) and Kjeldahl N-analyses. Cottonseed oilcake was used as a control to monitor day-to-day variation, since it exhibits a linear rate of DM disappearance over extended time intervals (Nocek, 1985). Incubations were repeated when DM disappearance varied more than 10% from established disappearance curves, but differences between days and cows, however, were minimal.

The percentage N disappearance at each incubation time was calculated from the proportion remaining after rumen incubation. The degradation rate was fitted to the equation as suggested by Ørskov & McDonald (1979):

$$p = a + b(1 - e^{-ct})$$

where p = proportion degraded at time t, a = an intercept representing soluble protein, b = the insoluble but potentially degradable fraction, and thus $a + b$ present the maximum extent of degradation or the asymptote of the equation. The degradation rate of the b fraction is described by c, the fractional rate constant/h. Non-linear parameters a, b and c were estimated by an iterative least-square procedure (Du Toit & Herbst, 1981). By introducing the fractional outflow rate, k, the effective protein degradation (P) was calculated as follows (Ørskov & McDonald, 1979):

$$P = a + \frac{bc}{c + k}$$

Fractional outflow rates of 0,02, 0,05 and 0,08 were used in the calculations as rates vary from 0,02/h for animals at maintenance to 0,08/h for high-producing dairy cows (ARC, 1984).

Results and Discussion

The parameters defining the equation for the rate of protein degradation are presented in Table 1. The immediately soluble fraction a, representing the intercept of the degradation curve at time zero, ranged between 16,9% for maize and

Table 1 Crude protein (DM basis) of energy sources and parameters in the equation of Ørskov & McDonald[a] (1979) to describe the rate of protein degradation

Feedstuff	%CP	a	b	c	r^2
Barley	11,0	25,8	74,7	0,118	0,99
Hominy chop	11,3	45,0	49,0	0,069	0,97
Maize	9,5	16,9	82,5	0,063	0,98
Maize (high-lysine)	10,1	55,1	39,6	0,090	0,99
Maize corn and cob meal	8,3	30,0	68,3	0,045	0,99
Oats	12,3	82,2	11,5	0,394	0,92
Sorghum	10,8	23,4	74,9	0,046	0,99
Sorghum (bird-resistant)	12,5	32,1	76,6	0,025	0,98
Wheat	14,3	38,6	60,8	0,289	0,98
Wheaten bran	17,3	50,6	42,5	0,319	0,99
Triticale	14,5	51,3	45,9	0,434	0,99

[a] $p = a + b(1 - e^{-ct})$, where a, b, and c are constants, p is the proportion degraded at time t, a is the intercept of the degradation curve at time zero, representing the soluble N fraction, b is the insoluble but potentially degradable fraction and degradation rate of the b fraction is described by c, the fractional rate constant/h.

82,2% for oats. In general, the less extensively degraded cereal grains such as sorghum and maize exhibited small *a* fractions while the highly degradable cereal grains such as oats, wheaten bran and triticale exhibited relatively large rapidly degraded fractions. However, the feedstuffs with large *a* fractions did not necessarily exhibit rapid rates of N disappearance. Hominy chop for example, with an *a* fraction of 45,0, had a *c* value of only 0,07. One possible explanation is that a rapid efflux of soluble degradable protein from the bags leaves a residual protein that is more slowly degraded than the protein as a whole (Broderick, Wallace, Ørskov & Hansen, 1988). Fibre may also have an effect, as Ganev, Ørskov & Smart (1979) have suggested that the fibre in vegetable proteins may afford some degree of protection to protein from rumen bacterial degradation. Valentine & Bartsch (1988) found the fibre in barley grain to be poorly degraded and suggested that this may account for the lower rate of degradation of the protein in barley compared to lupin grain. Hyslop, Weir, Offer, Reid & Wilcock (1989) have shown the *a* value for barley always to be lower than the corresponding value for wheat, perhaps because of the absence of a fibrous seed coat in the case of the latter grain.

A major limitation associated with the *in situ* technique is the inability to characterize ruminal availability of soluble and/or filterable material that may be degraded to a greater or lesser extent than the insoluble digestible material (Nocek, 1985). This rapid efflux of protein not yet degraded may lead to overestimation of the degraded fraction (fraction *a*). The validity of the *in situ* method depends on the assumption that net protein loss from the bags occurs only due to degradation (Broderick *et al.*, 1988).

The ruminal protein degradation extents for the 11 energy sources based on three different outflow rates, are given in Table 2. The results once again confirm that feedstuffs vary considerably in protein degradation in the rumen, and that they may vary differentially with fractional outflow rate.

Table 2 Extent of ruminal protein degradation (%) from energy feedstuffs calculated at three different fractional outflow rates

	Extent of ruminal protein degradation calculated at different fractional outflow rates (*k*)			
Feedstuff	0,02	0,05	0,08	*SE*[a]
Barley	90	78	71	0,7
Hominy chop	83	73	68	1,7
Maize	79	63	53	2,6
Maize (high-lysine)	88	81	76	1,7
Maize corn and cob meal	77	62	54	1,2
Oats	93	92	91	0,8
Sorghum	76	60	51	2,1
Sorghum (bird-resistant)	69	52	45	1,0
Wheat	95	91	86	1,5
Wheaten bran	91	87	84	1,2
Triticale	95	92	90	1,2

[a] Standard error of the mean, n = 6.

From Table 2 it appears that the energy sources can be categorized as extensively degraded (oats, wheat, wheaten bran, triticale), partially degraded (barley, hominy chop) and relatively undegraded (maize, maize corn and cob meal, sorghum). Because maize and sorghum are the primary cereal grains used for dairy cattle nutrition in South Africa, it is important to use correct degradation values, as a high UDP content make them important contributors to the UDP content of the total diet.

Protein degradation is not a positive or negative characteristic of feedstuffs, as in some feeding situations a high, and in other a lower protein degradation is necessary for optimum production (Hvelplund, 1985). It is equally important to know something about protein quality, including amino acid composition and availability of the undegraded protein (Satter, 1986).

It was recently demonstrated that it is possible to manipulate the quantity and quality of amino acids flowing to the duodenum by the careful selection of feedstuffs (King, Huber, Sadik, Bergen, Grant & King, 1988). Studies on the UDP digestibility and amino acid profile of UDP from various feedstuffs are at present being conducted at the Animal and Dairy Science Research Institute (ADSRI) at Irene.

A comparison between *in situ* values (at 0,08/h) obtained in this study and mean literature values based on *in situ* and *in vivo* measurements by various investigators, is given in Table 3. Most of the literature values in Table 3 are from studies done with dairy cattle and there is generally close agreement with the degradation values from this study and the literature values. Although the *in situ* technique is considered the best substitute for *in vivo* measurements for all feeds in most countries (Miller & Ørskov, 1986; Madsen, 1987), it is difficult to obtain absolute degradation values for protein sources; hence it is more realistic to determine relative RDP and UDP values. These values rank protein sources relative to one another under specific feeding conditions (Erdman *et al.*, 1987; Kirkpatrick & Kennelly, 1987). This overlap of values is especially true for by-product feeds such as hominy chop, because it consist of varying amounts of corn bran, corn germ and finer siftings of the starchy portion of the maize.

No published protein degradation values could be found for bird-resistant sorghum and high-lysine maize. Only two published values were found for hominy chop and one for triticale and maize corn and cob meal, respectively. This supports the view of Owens (1987) that the primary bottleneck of application of the NRC system for formulating diets is the lack of degradation values for feedstuffs.

Of particular interest is the difference in protein degradation between regular and high-lysine maize as well as between regular and bird-resistant sorghum. Cereal grains and protein supplements contain four different protein types, viz. albumins, globulins, prolamins and glutelins. Albumins and globulins are low molecular weight proteins that are soluble in rumen fluid. Prolamins and glutelins are higher molecular weight proteins containing disulphide bonds, which make them less soluble in rumen fluid. Proteins with low solubility in rumen fluid and which contain extensive cross-linking, such as those provided by disulphide bonds,

Table 3 Comparison between protein degradability values from this study and mean literature values

Feedstuff	Protein degradability (%)		
	This study (0,08/h)	Literature values	Reference[a]
Barley	71	75; 70; 79	1; 2; 3
Hominy chop	68	62; 35	4; 3
Maize	53	50; 40; 50; 57; 35	5; 7; 6; 1; 3
Maize corn and cob meal	54	45	7
Oats	91	86; 84; 80	1; 2; 7
Sorghum	51	50; 52; 48	6; 7; 5
Wheat	86	82; 80; 80	2; 6; 3
Wheaten bran	84	77; 80	9; 8
Triticale	91	75	7

[a] 1 – Erdman *et al.*, 1987; 2 – Madsen & Hvelplund, 1985; 3 – Sniffen & Chase, 1987; 4 – Van Horn, 1985; 5 – Owens, 1987; 6 – Satter, 1986; 7 – Preston, 1988; 8 – Sniffen & Chase, 1986; 9 – Sampath & Sivaraman, 1986.

are less accessible to proteolytic enzymes and are relatively resistant to degradation. Albumins and globulins are therefore more rapidly degraded in the rumen than prolamins and glutelins. This is unfortunate, since albumins and globulins often have a much better amino acid profile and higher biological value than prolamins and glutelins (Clark, Murphy & Crooker, 1987). Herein lies the explanation for the higher degradability of high-lysine maize. In the high-lysine maize endosperm, zein protein, one of the low degradable prolamins, is reduced by as much as 50—75%, and this is accompanied by an increase in the more rapidly degraded protein fractions. This can also explain why the nutritional value of high-lysine maize for dairy cattle is not superior to that of regular maize (Andrew, Clark & Davis, 1979). In their study, presumably, the advantage of a better quality protein was cancelled by the higher extent of protein degradation of high-lysine maize.

The protein composition of sorghum and maize endosperm is very similar, but important differences exist (Wall & Paulis, 1978). Intermolecular cross links, called cross-linked kafirins, are found in some sorghum prolamines. The cross links decrease the digestibility of both the protein and starch granules enmeshed in it. Endosperm starch and protein appear to adhere more tightly in sorghum than maize. Some types of cooking have been found to strengthen this starch–protein interaction in sorghum, thereby reducing the rate of strach digestion. Maize endosperm proteins have not been reported to behave similarly (Rooney & Pflugfelder, 1986).

Sorghum cultivars vary considerably in processing properties and feeding values. Brown bird-resistant sorghums have significantly lower digestibilities and often yield poorer animal performance than other sorghums (Maxson, Shirley, Bertrand & Palmer, 1973; Hahn, Rooney & Earp, 1984). The tannins present in bird-resistant sorghums bind proteins, inhibit some enzyme systems and may reduce starch digestion (Hahn *et al.*, 1984). The results from our study confirm the suggestion by Meissner, van Staden, Janse van Rensburg & Slabbert (1982) that tannins influence extent of protein degradation and that feed protein in the presence of tannins is less extensively degraded in the rumen. However, the UDP fraction would not be as readily digested in the small intestine either (Meissner *et al.*, 1982). Currently, bird-resistant sorghum varieties comprise no more than 10% of the total sorghum crop in South Africa (personal communication: P. Skinner, Grain Sorghum Board, Arcadia, Pretoria, 1988).

In evaluation of the data from this study, one should consider that effects attributed to chemical composition may be confounded with particle size, as small particles may have been leached out of the bags, rather than being degraded. In bags with fine pores (10 μm), the effect of particle size can be seen at long (24 h) but not at short (2—6 h) incubation times (Lindberg, 1981). If, on the other hand, the pore size is increased (40—50 μm), the effect of particle size is most pronounced at short incubation times (Freer & Dove, 1984). It appears that, in general, the negative effect on the rate of degradation of increasing the sample particle size is less pronounced with larger pore sizes, such as being used in this study (Lindberg, 1987). The effect of particle size can be minimized by sieving milled samples across a 45-μm sieve, thereby removing fine particles. This is included in the standard method for *in situ* measurement of nitrogen disappearance as proposed by the ARC Interdepartmental Protein Working Party (Cottrill & Evans, 1984).

Conclusions

Results from this study indicate that energy sources normally used in dairy cattle diet formulation differ markedly in extent of ruminal protein degradation, ranging from 45—91% (at a passage rate of 0,08/h). Maize and sorghum provide more UDP than the other feedstuffs evaluated. Although high-lysine maize has an improved amino acid profile, this advantage may not be available to the dairy cow because of the high ruminal degradation of its protein.

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References

ANDREW, S.M., CLARK, J.H. & DAVIS, C.L., 1979. Feeding value of Opaque-2 corn grain and corn silage for lactating dairy cows. *J. Dairy Sci.* 62, 1619.

ARC, 1984. The nutrient requirements of ruminant livestock, Suppl. No. 1. Report of the protein group of the ARC working party. Commonwealth Agricultural Bureau, Farnham Royal, UK.

ASELTINE, M.S., 1988. New NRC requirements reflect scientific knowledge. *Feedstuffs*, September 26, p. 16.

BRODERICK, G.A., WALLACE, R.J., ØRSKOV, E.R. & HANSEN, LISBETH, 1988. Comparison of estimates of ruminal protein degradation by *in vitro* and *in situ* methods. *J. Anim. Sci.* 66, 1739.

CLARK, J.H., MURPHY, M.R. & CROOKER, B.A., 1987. Supplying the protein needs of dairy cattle from by-product feeds. *J. Dairy Sci.* 70, 1092.

COTTRILL, B.R. & EVANS, P.J., 1984. A standard method for *in situ* measurement of nitrogen disappearance from dacron bags suspended in the rumen. ARC Technical Review. Interdepartmental Protein Working Party.

DU TOIT, S.H.C. & HERBST, A., 1981. Die interaktiewe rekenaarprogram ST-kromme vir die ontleding van groeikrommes. S. Afr. Instituut vir Statistiese Navorsing. RGN, Pretoria.

ERASMUS, L.J., PRINSLOO, J. & MEISSNER, H.H., 1988. The establishment of a protein degradability data base for dairy cattle using the nylon bag technique. 1. Protein sources. *S. Afr. J. Anim. Sci.* 18, 23.

ERDMAN, R.E., VANDERSALL, J.H., RUSSEK-COHEN, E. & SWITALSKI, G., 1987. Simultaneous measures of rates of ruminal digestion and passage of feeds for prediction of ruminal nitrogen and dry matter digestion in lactating dairy cows. *J. Anim. Sci.* 64, 565.

FREER, M. & DOVE, H., 1984. Rumen degradation of protein in sunflower meal, rapeseed meal and lupin seed placed in nylon bags. *Anim. Feed. Sci. Technol.* 11, 87.

GANEV, G., ØRSKOV, E.R. & SMART, R., 1979. The effect of roughage or concentrate feeding and rumen retention time on total degradation of protein in the rumen. *J. agric. Sci., Camb.* 93, 651.

HA, J.K. & KENNELLY, J.J., 1984. *In situ* dry matter and protein degradation of various protein sources in dairy cattle. *Can. J. Anim. Sci.* 64, 443.

HAHN, D.H., ROONEY, L.W. & EARP, C.F., 1984. Tannins and phenols of sorghum. *Cereal Foods World* 29, 776.

HVELPLUND, T., 1985. Digestibility of rumen microbial protein and undegraded dietary protein estimated in the small intestine of sheep and by *in sacco* procedure. *Acta Agric. Scand.* Suppl. 25, 132.

HYSLOP, J.A., WEIR, J., OFFER, N.W., REID, S.J. & WILCOCK, J.M., 1989. Effect of formaldehyde treatment on the rumen digestion of barley and wheat grain and its influence on hay intake and digesta by sheep. *Anim. Feed Sci. Technol.* 25, 55.

KING, K.J., HUBER, J.T., SADIK, M.S., BERGEN, W.G., GRANT, A.L. & KING, V.L., 1988. Influence of protein sources fed lactating cows on the amino acids (AA) available for digestion and metabolism. *J. Dairy Sci.* 71 (Suppl. 1), 159.

KIRKPATRICK, B.K. & KENNELLY, J.J., 1987. *In situ* degradability of protein and dry matter from single protein sources and from a total diet. *J. Anim. Sci.* 65, 567.

LINDBERG, J.E., 1981. The effect of sample size and sample structure on the degradation of dry matter, nitrogen and cell walls in nylon bags. *Swedish J. Agric. Res.* 11, 71.

LINDBERG, J.E., 1987. Measurement of feed protein digestibility by the *in sacco* and other methods. In: Feed evaluation and protein requirement systems for ruminants. Eds. Jarrige, R. & Alderman, G., Commission of the European Communities, Luxembourg. p. 85.

MADSEN, J., 1987. Present situation of the modern protein systems: Nordic countries. In: Feed evaluation and protein requirement systems for ruminants. Eds. Jarrige, R. & Alderman, G., Commission of the European Communities, Luxembourg. p. 29.

MADSEN, J. & HVELPLUND, T., 1985. Protein degradation in the ruminant. A comparison between *in vivo*, nylon bag, *in vitro* and buffer measurements. *Acta Agric. Scand.* Suppl. 25, 103.

MAXSON, W.E., SHIRLEY, R.L., BERTRAND, J.E. & PALMER, A.Z., 1973. Energy values of corn, bird-resistant and nonbird-resistant sorghum grain in rations fed to steers. *J. Anim. Sci.* 37, 1451.

MEHREZ, A.Z. & ØRSKOV, E.R., 1977. A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. *J. agric. Sci., Camb.* 88, 645.

MEISSNER, H.H., VAN STADEN, J.H., JANSE VAN RENSBURG, N. & SLABBERT, HEIDI, 1982. Sorghum grain as substitute for maize in fattening diets for beef steers. *S. Afr. J. Anim. Sci.* 12, 129.

MILLER, E.L. & ØRSKOV, E.R., 1986. Degradability of protein and its prediction. *Bull. Internat. Dairy Fed.*, No. 196, Brussels, Belgium.

NKJ/NJF, 1985. Protein evaluation for ruminants. *Acta Agric. Scand.* Suppl. 25, 220 pp.

NOCEK, J.E., 1985. Evaluation of specific variables affecting *in situ* estimates of ruminal dry matter and protein digestion. *J. Anim. Sci.* 60, 1347.

NOCEK, J.E., 1988. *In situ* and other methods to estimate ruminal protein and energy digestibility: A review. *J. Dairy Sci.* 71, 2051.

NRC, 1985. Ruminant nitrogen usage. U.S. National Academy of Science, Washington, DC.

NRC, 1989. Nutrient requirements of dairy cattle (6th revised edn., update, 1989). National Academy Press, Washington, DC.

ØRSKOV, E.R. & McDONALD, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. *J. agric. Sci., Camb.* 92, 499.

OWENS, F.N., 1987. The US–NRC approach to ruminant nitrogen usage. In: Feed evaluation and protein requirement systems for ruminants. Eds. Jarrige, R. & Alderman, G., Commission of the European Communities, Luxembourgh. p. 55.

PAINE, C.A., CRAWSHAW, R. & BARBER, W.P., 1981. A complete exchange method for the *in sacco* estimation of rumen degradability on a routine basis. In: Forage protein in ruminant animal production. Eds. Thompson, D.J., Beever, D.E., & Gunn, R.G. BSAP Occasional publication No. 6, D&J Croal Ltd., Haddington. p. 177.

POOS-FLOYD, M., KLOPFENSTEIN, T. & BRITTON, R.A., 1985. Evaluation of laboratory techniques for predicting ruminal protein degradation. *J. Dairy Sci.* 68, 829.

PRESTON, R.L., 1988. Typical composition of feed for cattle and sheep, 1988—89. *Feedstuffs* October 17, p. 18.

ROHR, K., LEBZIEN, P., SCHAFFT, H. & SCHULZ, E., 1986. Prediction of duodenal flow of non-ammonia nitrogen and amino acid nitrogen in dairy cows. *Livest. Prod. Sci.* 14, 29.

ROONEY, L.W. & PFLUGFELDER, R.L., 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. *J. Anim. Sci.* 63, 1607.

SAMPATH, K.T. & SIVARAMAN, E., 1986. *In situ* dry matter disappearance and protein degradability of yellow maize, wheaten bran and guinea grass (*Panicum maximum*) in the rumen of cattle. *Ind. J. Anim. Sci.* 56, 1241.

SATTER, L.D., 1986. Protein supply from undegraded dietary protein. *J. Dairy Sci.* 69, 2734.

SNIFFEN, C.J. & CHASE, L.E., 1986. Field application of the degradable protein system. In: Proc. 1986 feed dealer seminars, Nov. 3—7. Animal Science Mimeograph Series No. 93, Cooperative Extension, Dept. Animal Science, Cornell University, Ithaca, NY.

SNIFFEN, C.J. & CHASE, L.E., 1987. Field application of the degradable protein system. In: Proc. Advanced dairy nutrition seminar for agribusiness, June 1—4. Animal Science Mimeograph Series No. 102, Cooperative Extension, Dept. Animal Science, Cornell University, Ithaca, NY.

STERN, M.D., RODE, L.M., PRANGE, R.W., STAUFFACHER, R.H. & SATTER, L.D., 1983. Ruminal protein degradation of corn gluten meal in lactating dairy cattle fitted with duodenal type cannulae. *J. Anim. Sci.* 56, 194.

STERN, M.D. & SATTER, L.D., 1984. Evaluation of nitrogen solubility and the dacron bag technique as methods for estimating protein degradation in the rumen. *J. Anim. Sci.* 58, 714.

VALENTINE, S.C. & BARTSCH, B.D., 1988. Degradation of dry matter, crude protein, fat, crude fibre and nitrogen-free extract in milled barley and lupin grains incubated in nylon bags in the rumen of dairy cows. *J. agric. Sci., Camb.* 110, 395.

VAN HORN, H.H., 1985. Dairy nutrition notes. Publication DS 85—87. Dairy Science Dept., Univ. of Florida, Gainesville, Florida.

WALL, J.S. & PAULIS, L.W., 1978. Corn and sorghum grain proteins. In: Advances in cereal science and technology II. Ed. Pomeranz, Y. Am. Assoc. Cereal Chem., St. Paul, MN. p. 135.