Protein degradability of several South African feedstuffs by the artificial fibre bag technique

P.B. Cronje
Animal and Dairy Science Research Institute, Irene

The protein degradability of 13 South African ruminant feed protein sources were determined by the artificial fibre bag technique. Three sheep fed *ad lib.* on a basal diet of lucerne hay were used. Estimates obtained by two methods of calculation were not in good agreement for all the feeds studied. Assuming an arbitrary rate constant for passage of undegraded protein from the rumen of 0,05, the effective percentage nitrogen degradation for the various feedstuffs as calculated by the two methods was found to be as follows: Sunflower oilcake 85,83; groundnut oilcake 93,93; cottonseed oilcake 59,61; lupin 89,89; soyabean 92,92; maize 74,64; maize silage 88,84; lucerne hay 79,72; *Eragrostis curvula* hay 30,27; maize gluten feed 89,87; maize gluten meal 24,25; dried brewers grains (sorghum) 49,38; fishmeal 36,42. Protein solubility in NaOH gave no reliable indication of the rapidly-soluble protein fraction as determined by the artificial fibre bag technique.

S. Afr. J. Anim. Sci. 1983, 13: 225 – 228

Die proteïendegradeerbaarheid van 13 Suid-Afrikaanse herkouer voerproteïenbronne is bepaal deur die kunsveselsak-metode. Die drie skape wat gebruik is het 'n dieet van lusernhooi *ad lib.* ontvang. Bepalings wat deur twee verskillende metodes bereken is, het nie in alle gevalle ooreengestem nie. 'n Arbitrêre tempokonstante van 0,05 vir vloei van ondegradeerde proteïen uit die rumen is aanvaar, en die volgende waardes vir persentasie stikstofafbraak volgens die twee metodes is bereken: Sonneblomoliekoek 85,83; grondboonoliekoek 93,93; katoensaadoliekoek 59,61; lupien 89,89; sojaboon 92,92; mielies 74,64; mieliekuilvoer 88,84; lusernhooi 79,72; *Eragrostis curvula* hooi 30,27; mieliegluten voer 89,87; mieliegluten meel 24,25; gedroogde brouersgraan (sorghum) 49,38; vismeel 36,42, Proteïen oplosbaar in NaOH het geen aanduiding verskaf van die vinnige oplosbare proteïen gedeelte soos bepaal deur die kunsveselsak-metode.

S.-Afr. Tydsk. Veek. 1983, 13: 225 – 228

Keywords: Nitrogen, protein, degradability, solubility, artificial fibre bag

P.B. Cronje*
Animal and Dairy Science Research Institute, Private Bag X2, Irene, Republic of South Africa

Received 2 November 1982

*To be submitted as partial requirement for the degree M.Sc. (Agric.) Dept. of Sheep and Wool Science, University of Stellenbosch

Introduction

Evidence of serious shortcomings in the crude- and digestible-protein systems has been accumulating over the past decade. These inadequacies have been discussed by Roy, Balch, Miller, Ørskov & Smith (1977). As a result, recent research has been directed at the development of alternative systems, culminating in the inception of various new systems in Britain (Roy *et al,* 1977), France (Journet & Vèrity, 1977), Germany (Kaufmann, 1977) and the U.S.A. (Satter & Roffler, 1975; Fox, Sniffen, Van Soest & Robinson, 1979). Although these systems are still evolving, there is sufficient animal response validation (Stock, Merchen, Klopfenstein & Poos, 1981) to indicate that considerable savings of protein may be effected by the practical application of these concepts.

The traditional crude- and digestible-protein systems presently employed for ruminant diet formulation in South Africa, are fast becoming obsolete, while little, if any, research has been directed towards concepts currently being evaluated overseas. With an estimated protein deficit of 226 000 tons by the year 2000 A.D. (Cloete, 1981), it is obvious that research in this direction is urgently needed.

Central to the recently proposed systems is the concept of protein degradability. There is at present a paucity of data pertaining to the rumen degradability of South African feed protein sources. As factors such as processing methods (Mehrez, Ørskov & Opstvedt, 1980) may influence protein degradability, it was considered important to obtain local estimates. A method which is currently being evaluated for this purpose is the artificial fibre bag technique, (Schoeman, De Wet & Burger, 1972) the results of which are reported here.

Procedure

A wide range of commonly used feed protein sources including oil cakes, byproducts, grains, and hays was chosen for this study. The feeds and their chemical composition are presented in Table 1.

Bags (16 × 9 cm) were made from polyester material (pore size 53 μm) using double seams and rounded corners which were sealed with a contact adhesive. The bags were closed by means of a draw-string. A 5 g (air dry) feed sample which had been ground in a Wiley Laboratory Mill to pass through a 5 mm screen was weighed into each bag. Six bags per feed type were placed in the rumen of each of three sheep at 08h00 and 22h00, and withdrawn after 0,5, 1, 2, 4, 6, 8 and 10, 12, 14, 16, 18, 24 hours respectively. This procedure was repeated once, giving a total of six observations for each variable studied. The sheep were fitted with large (8 cm internal

Table 1 Nitrogen (N), crude protein (CP), and dry matter (DM) content of the protein sources examined

Feedstuff	% N	% CP	% DM
Sunflower Oilcake	6,9	42,8	95,3
Lupin	4,1	25,4	91,7
Fishmeal	10,3	64,3	90,2
Cottonseed oilcake	6,6	41,5	94,1
Corn gluten feed	3,0	19,0	88,6
Corn gluten meal	10,0	62,6	92,6
Maize	1,2	7,6	88,8
Groundnut oilcake	7,4	46,2	93,4
Soyabean	5,6	35,0	92,3
Dried brewer's grains (sorghum)	3,6	22,3	95,8
Lucerne hay	2,2	13,6	86,3
Maize silage	0,9	5,8	35,0
Eragrostis Curvula hay	0,7	4,4	93,7

diameter) rumen cannulas which facilitated manual placement of the bags in the ventral portion of the rumen. The sheep (mature wethers) were fed lucerne hay once daily (07h00) at an *ad lib.* intake level. Vitamins A, D and E and a broad-spectrum antihelminthic were administered regularly. The bags were attached to a swivel clip mounted on the inside of the cannula cork by means of a 25 cm length of nylon line. Upon removal from the rumen, the bags were washed in tap water until the water squeezed from the bags was clear, and dried to constant weight at 60 °C in a forced-draught oven. Residual nitrogen was then determined by the macro-kjeldahl method. Results were computed using two methods: Ørskov & McDonald (1979), and Miller (1980).

Nitrogen solubility was determined in 0,02 N NaOH (Craig & Broderick, 1981). Feed samples containing 0,02 g nitrogen were placed in 50 mℓ screw-capped centrifuge tubes containing 20 mℓ 0,02 N NaOH. The tubes were warmed in a water-bath at 39 °C for 30 minutes and then shaken at 39 °C for a further 60 minutes. Samples were then filtered through Whatman no.1 filter paper and a 10 mℓ aliquot of filtrate was taken for nitrogen (N) analysis (Technicon auto-analyzer). All determinations were conducted in triplicate.

Results and Discussion

The artificial fibre bag technique entails placing a weighed sample of feed in a bag which is then suspended in the rumen. At intervals a bag is withdrawn and the contents subjected to further analysis. In this way an estimate of protein degradation over a certain time span may be obtained.

The choice of material porosity of the bags remains, at best, a compromise. The presence of pores in the material is necessary to allow rumen micro-organisms access to the feed. Excessively fine material would definitely prevent entry of certain of the larger protozoa and possibly cause clogging of the pores owing to micro-colony formation on the material; while excessively large pores might cause loss of undigested sample material or permit entry of the surrounding ingesta into the bag.

A review of the technique (Cronjé, 1982) indicated that evidence pertaining to the influence of pore size on sample loss from bags and influx of surrounding ingesta into bags is conflicting. The extent and consequences of the exclusion of protozoa from bags is largely unknown.

In the light of the uncertainty as to the optimum pore size, the 53 μm pore size material selected for this study was considered an acceptable compromise. The method, as detailed above, was designed to comply with the general guidelines set out by Ørskov, Hughes-Jones & McDonald (1981).

The percentage protein disappearance from the bags at various time intervals was determined, and curves (Figures 1 – 4) fitted to the data by an iterative least squares procedure where the rate of nitrogen disappearance is described by an exponential equation of the form:

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

(Ørskov & McDonald, 1979), where p is degradation at time t. This equation has the advantage of providing constants (a, b and c) which have biological relevance (Ørskov, 1980). The a value may be interpreted as a measure of the rapidly-soluble nitrogen fraction. The b value represents that fraction which

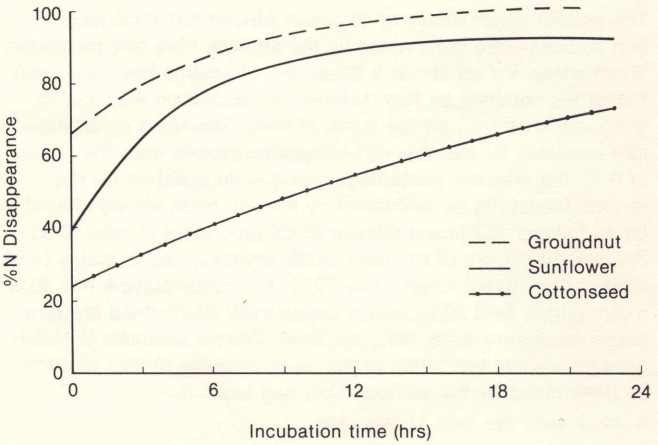

Figure 1 Nitrogen disappearance — oilcakes.

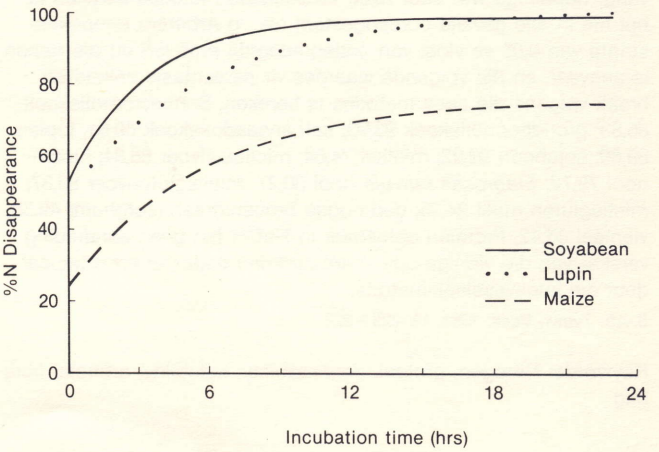

Figure 2 Nitrogen disappearance — grains.

will degrade in time; while c represents the rate at which the b fraction degrades. The factors obtained for the various feeds examined are presented in Table 2. The unsuitability of the model for describing data of certain roughages such as *Eragrostis curvula* hay and maize silage is evident from the low coefficients of determination obtained.

Nitrogen solubility techniques are potentially simple, rapid methods to estimate protein degradation in the rumen (Craig & Broderick, 1981). Results of comparative trials between solubility methods are conflicting (Little, Burroughs & Woods, 1963; Crocker, Sniffen, Hoover & Johnson, 1978; Crawford, Hoover, Sniffen & Crocker, 1978), and there are few direct

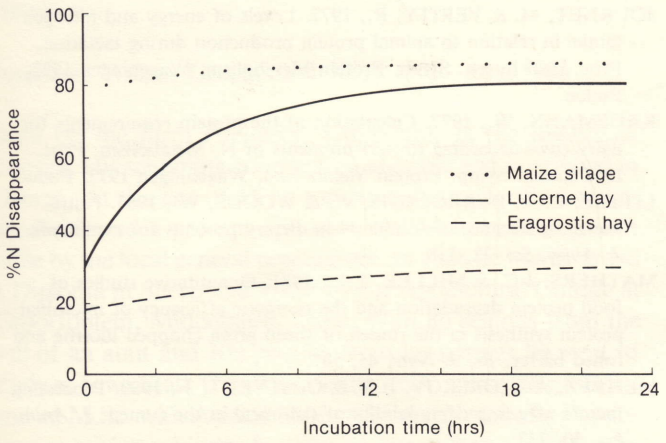

Figure 3 Nitrogen disappearance — roughages.

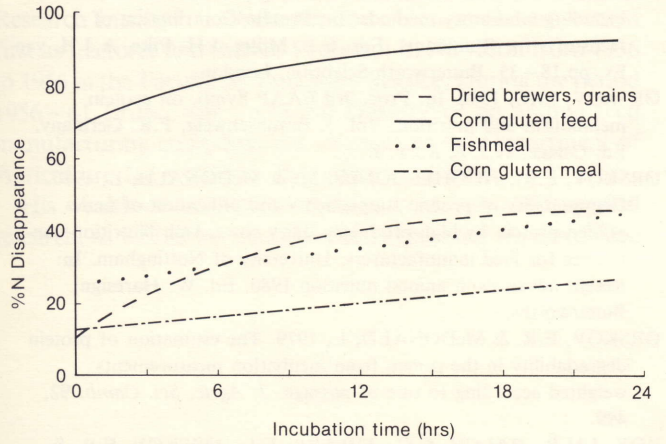

Figure 4 Nitrogen disappearance — byproducts.

comparisons of solubility determinations with *in vivo* or *in situ* studies. To ascertain whether solubility values do equate to the rapidly soluble fraction *in situ,* nitrogen solubility in 0,02 N NaOH was compared to the a value described above (Table 2). It would appear that in most instances solubility in 0,02 N NaOH does not effectively estimate the a fraction.

As the extent of degradation is influenced by the time a feed remains in the rumen, the fractional outflow rate of undegraded protein from the rumen (kr) must be taken into account in calculating the effective percentage degradation (P). An arbitrary value of 0,05 for kr was used in calculating P according to Ørskov & McDonald (1979);

$$P = a + \frac{bc}{c + kr} \quad (1)$$

An alternative method of calculating the effective degradation is to plot the logarithm of the nitrogen remaining in the bag against time and to determine the rate-constant for nitrogen disappearance (k_d) from a straight line fit (linear regression) over the first 12 hours (Miller, 1980) as shown in Table 3. Effective degradation is represented by the ratio of the respective reaction rates:

$$\text{degradability} = a + (1 - a)\frac{kd}{kr + kd} \quad (2)$$

where a is the proportion of nitrogen disappearing at time = 0. As in the case of the previous model, a low coefficient of determination was observed in the case of *Eragrostis curvula* hay. Estimates for roughages by current simple models must therefore be regarded as poor approximations only.

Table 2 Factors derived from the exponential equation, $p = a + b(1 - e^{-ct})$ and percentage soluble N

Feed	a	b	c	r^2	% Soluble N
Sunflower oilcake	39,3	53,4	0,22	0,98	28,9
Groundnut oilcake	66,2	35,6	0,16	0,97	42,0
Cotton oilcake	23,9	89,0	0,04	0,98	16,5
Lupin	49,7	51,1	0,16	0,99	16,8
Soyabean	52,8	45,7	0,31	0,99	13,0
Maize	24,0	51,6	0,18	0,88	12,0
Maize silage	78,8	6,9	0,21	0,60	–
Lucerne hay	29,8	51,0	0,23	0,98	–
Eragrostis curvula hay	18,1	12,6	0,10	0,73	–
Maize gluten feed	68,6	25,2	0,14	0,99	32,2
Maize gluten meal	12,8	77,0	0,01	0,95	–
Dried brewers grains (sorghum)	10,5	38,2	0,12	0,92	3,6
Fishmeal	25,0	294,4	0,01	0,93	12,7

Table 3 Linear regression of the logarithm of nitrogen remaining in the bag *vs* time (over 12 hours)

Feedstuff	a	kd	r^2
Sunflower oilcake	0,49	−0,122	0,93
Groundnut oilcake	0,58	−0,245	0,95
Cottonseed oilcake	0,24	−0,044	0,97
Lupin	0,45	−0,190	0,98
Soyabean	0,61	−0,207	0,95
Maize	0,24	−0,094	0,97
Maize silage	0,79	−0,036	0,91
Lucerne hay	0,37	−0,097	0,95
Eragrostis curvula hay	0,20	−0,007	0,44
Maize gluten feed	0,69	−0,090	0,99
Maize gluten meal	0,13	−0,007	0,85
Dried brewers grains (sorghum)	0,12	−0,035	0,99
Fishmeal	0,26	−0,008	0,84

Both methods could be criticized; the method of Ørskov & McDonald (1979) requires a relatively sophisticated computer program, while that of Miller (1980) entails a comparatively simple calculation. The former however, takes longer incubation times into account and provides meaningful factors. Comparison between these two methods and with estimates derived from the ^{35}S technique have revealed good agreement for lucerne-barley mixtures (Mathers & Miller, 1981). Although this may be true in some cases, it is clear from Table 4 that this is not always so. Large differences were observed in the case of maize (nine percentage units), lucerne hay (seven percentage units) and dried brewers grains (eleven percentage units). Differences of up to five percentage units have been reported in similar studies (Miller, 1982).

It may be concluded that although factors such as basal diet and rate of passage from the rumen may influence the absolute value of the figures reported, the relative ranking of the more readily available South African feed proteins is reason enough to call for an intensified research effort if the predicted protein deficit is to be averted.

Table 4 The effective percentage degradation of various protein sources calculated according to two methods

Feed	Method		
	Miller (1980)	Ørskov & McDonald (1979)	S.E.[a]
Sunflower oilcake	85,13	83,02	± 1,9
Groundnut oilcake	92,90	93,18	± 0,9
Cotton oilcake	59,38	60,51	± 1,7
Lupin	88,63	88,65	± 0,8
Soyabean	92,46	92,07	± 1,2
Maize	73,75	64,22	± 2,2
Maize silage	87,98	84,39	± 0,7
Lucerne hay	78,65	71,74	± 2,1
Eragrostis curvula hay	29,50	26,61	± 1,5
Maize gluten feed	89,06	86,97	± 0,7
Maize gluten meal	24,17	24,55	± 0,7
Dried brewers grains (sorghum)	48,54	37,76	± 1,5
Fishmeal	36,30	41,64	± 1,0

[a] Standard error of the mean.

References

CLOETE, J.G., 1981. New protein feeds and strategies for future animal production. *S. Afr. J. Anim. Sci.* 11, 139.

CRAIG, W.M. & BRODERICK, G.A., 1981. Comparison of nitrogen solubility in three solvents to *in vitro* protein degradation of heat treated cottonseed meal. *J. Dairy Sci.* 64, 769.

CRAWFORD, R.J. HOOVER, W.H., SNIFFEN, C.J. & CROCKER, B.A., 1978. Degradation of feedstuff nitrogen in the rumen *vs* nitrogen solubility in three solvents. *J. Anim. Sci.* 46, 1768.

CRONJÉ, P.B., 1982. The *in situ* artificial fibre bag technique — a review. In: Proc. 1st South African Worshop on problems in ruminant protein metabolism, CSIR, May 1982. Ed. H.M. Schwartz. p.152.

CROCKER, B.A., SNIFFEN, C.J., HOOVER, W. & JOHNSON, L., 1978. Solvents for soluble nitrogen measurements in feedstuffs. *J. Dairy Sci.* 61, 437.

FOX, D.G., SNIFFEN, C.J., VAN SOEST, P.J. & ROBINSON, P.H., 1979. A computerized net protein system for formulating rations for beef and dairy cattle using feed analysis data. Proc. Cornell. Nutr. Conf. 57.

JOURNET, M. & VERITY, R., 1977. Levels of energy and nitrogen intake in relation to animal protein production during lactation. Proc. IInd Intern. Symp. Protein Metabolism, Wageningen 1977, Pudoc.

KAUFMANN, W., 1977. Calculation of the protein requirements for dairy cows according to measurements of N. Metabolism. Proc. IInd Intern. Symp. Protein Metabolism, Wageningen 1977, Pudoc.

LITTLE, C.O., BURROUGHS, W. & WOODS, W., 1963. Nutritional significance of soluble N in dietary proteins for ruminants. *J. Anim. Sci.* 22, 358.

MATHERS, J.C. & MILLER, E.L., 1981. Quantitative studies of food protein degradation and the energetic efficiency of microbial protein synthesis in the rumen of sheep given chopped lucerne and rolled barley. *Br. J. Nutr.* 45, 587.

MEHREZ, A.Z.,ØRSKOV, E.R. & OPSTVEDT, J., 1980. Processing factors affecting degradability of fish meal in the rumen. *J. Anim. Sci.* 50, 737.

MILLER, E.L., 1980. Protein value of feedstuffs for ruminants. In: Vicia faba: feeding value, processing and virusses. Ed. D.A. Bond. Martinus Nijhoff publishers, the Hague.

MILLER, E.L., 1982. Methods of assessing proteins for ruminants, including laboratory methods. In: Protein Contribution of feedstuffs for Ruminants. Eds. E.L. Miller, I.H. Pike, A.J.H. van Es. pp.18 – 35. Butterworth Scientific, London.

ØRSKOV, E.R., 1980. In: Proc. 3rd EAAP Symp. on protein metabolism and nutrition. Vol. 3, Braunschweig, F.R. Germany. Ed. Oslage, H.J. & Rohr, K.

ØRSKOV, E.R., HUGHES-JONES, M. & McDONALD, I., 1980. Degradability of protein supplements and utilization of undegraded protein by high-producing dairy cows. 14th Nutrition conference for feed manufacturers, University of Nottingham. In: Recent advances in animal nutrition 1980. Ed. W. Haresign. Butterworths.

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

ROY, J.H.B., BALCH, C.C., MILLER, E.L., ØRSKOV, E.R. & SMITH, R.H., 1977. Calculations of the N-requirement for ruminants from nitrogen metabolism studies. In: Protein metabolism and nutrition. Wageningen, Centre for agricultural publishing and documentation. Ed. S. Tamminga.

SATTER, L.D. & ROFFLER, R.E., 1975. Nitrogen requirement and utilization in dairy cattle. *J. Dairy Sci.* 58, 1219.

SCHOEMAN, E.A., DE WET, P.J. & BURGER, W., 1972. The evaluation of the digestibility of treated proteins. *Agroanimalia* 4, 35.

STOCK, R., MERCHEN, N., KLOPFENSTEIN, T. & POOS, M., 1981. Feeding value of slowly degraded proteins. *J. Anim. Sci.* 53, 1109.