Ann. Zootech. (1989) 38, 269-283

Original article

The evaluation of PDI concentrations in some ruminant feedstuffs: a comparison of *in situ* and *in vitro* protein degradability

P. Susmel, B. Stefanon, C.R. Mills and M. Colitti

Istituto di Produzione Animale, via S. Mauro 2, 33010 Pagnacco, Italy

(Received 11 April 1989; accepted 19 October 1989)

Summary — The effective rumen degradability (Dg) of dry matter (DM), nitrogen (N) and non-protein organic matter (NPOM) of 16 commonly used ruminant feedstuffs was measured *in situ*. The PDIA, PDIE and PDIN contents of the feedstuffs were calculated either using the DgN and DgNPOM *in situ* values or using the DgN and fermentable organic matter (MOF) data derived from INRA tables. The regressions between values from the 2 systems had high r^2 values, but only agreed closely for PDIN values. The difference between PDIA values calculated using the 2 systems was small, but PDIE values differed considerably. The *in vitro* enzymatic degradability of the same 16 feeds was also measured, using protease extracted from *Streptomyces griseus*. The 1-h enzymatic degradability of the feeds was higher than the value *in situ*, except for the maizemeal and forages. However, protease degradability after 24 h was lower than the effective degradability *in situ*. Only the animal proteins deviated from this rule. Significant correlations were found between the coefficient *a in situ* and *as* and *bs in vitro*, and between *bs in vitro* and *b in situ*. The effective degradability *in situ* was highly correlated with the enzymatic degradability.

***in situ* degradability — PDI — *in vitro* degradability**

Résumé — Teneur en *PDI* des aliments pour les ruminants. Comparaison entre la dégradabilité de l'azote *in sacco* et *in vitro*. La dégradabilité théorique dans le rumen (Dg) de la matière sèche (MS), l'azote (N) et la fraction non azotée de la matière organique (NPOM) de 16 aliments classiquement utilisés dans les rations des ruminants a été mesurée *in sacco*. Les teneurs en PDIA, PDIE et PDIN des aliments ont été calculées en utilisant soit les valeurs de DgN et de DgNPOM obtenues *in sacco*, à travers le système proposé par Susmel et Stefanon (1987), soit les valeurs de DgN et la matière organique fermentescible (MOF) proposées dans le système INRA (1988). Une bonne correspondance a été trouvée entre les valeurs de PDIN calculées en utilisant soit la dgN calculée dans cette étude et la dgNPOM, soit la dgN donnée par l'INRA (1988) et la MOF, tandis qu'une différence non négligeable a été trouvée pour les teneurs en PDIE. La dégradabilité enzymatique *in vitro* des mêmes aliments a été mesurée avec une méthode enzymatique utilisant une protéase extraite de *Streptomyces Griseus*, en incubant un échantillon de chaque aliment pendant 1 (*as*) et 24 (*bs*) h. La dégradabilité enzymatique de l'N des aliments *as* a été plus élevée que les valeurs mesurées *in sacco* (*a*), excepté pour le maïs et les fourrages, et la dégradabilité enzymatique *as* + *bs* a été tout le temps inférieure à la valeur *a* + *b* mesurée *in sacco*, sauf pour les farines de viande et de poisson. Une corrélation significative a été observée entre la dégradabilité a mesurée *in sacco* et la dégradabilité enzymatique *as* et *bs*. Une bonne corrélation a été aussi observée entre les valeurs *b in sacco* et *bs in vitro*.

***dégradabilité* in sacco — *PDI* — *degradabilité* in vitro**

INTRODUCTION

The *in situ* technique using the methodology of Ørskov and McDonald (1979) is the method most frequently used for the measurement of rumen N degradation. The values obtained were incorporated in the new protein systems (ARC, 1984; INRA, 1988; Madsen, 1985; NRC, 1985; Susmel & Stefanon, 1987). The use of this technique permits the simultaneous estimation of nitrogen (N), dry matter (DM) and non-protein organic matter (NPOM) degradability, and can therefore be used to estimate the quantity of energy available for bacterial growth (Susmel & Stefanon, 1987). The effective degradability of N and NPOM can subsequently be used to estimate the protein value of feedstuffs (Susmel & Stefanon, 1987).

A large number of factors influence the *in situ* degradability of feedstuffs, the most important of which appears to be diet formulation (Weakley *et al.*, 1983; Susmel *et al.*, 1989). Preliminary results obtained in a United Kingdom ring test (Oldham, 1987) showed that there is poor reproducibility between laboratories measuring *in situ* N degradability. *In situ* degradability measurements are also expensive and time consuming.

The evaluation of degradability *in vitro* allows, at least from a theoretical point of view, better control over the factors responsible for the variation, and hence improved repeatability and reproducibility in the degradability values obtained. *In vitro* techniques also avoid the use of animals and are thus generally less expensive both in terms of time and money.

Techniques based upon N solubility in buffers generally do not show high correlations with degradability values obtained *in vivo* (Crawford *et al.*, 1978; Crooker *et al.*, 1978; Krishnamoorthy *et al.*, 1982; Madsen & Hvelplund, 1985; Stern & Satter, 1984). However, high correlation coefficients (with r^2 values ranging from 0.61 to 0.90) have been obtained comparing *in vitro* degradability using proteolityc enzymes and *in vivo* values (Krishnamoorthy *et al.*, 1983; Poos-Floyd *et al.*, 1985; Sauvant *et al.*, 1987; Broderick *et al.*, 1988).

The enzymatic technique proposed by Aufrere and Cartailler (1988) was applied to some ruminant feedstuffs to test its capability to predict *in situ* protein degradability. The PDI concentrations obtained from *in situ* degradability using the Susmel and Stefanon (1987) system were compared with those obtained using reference French methods and values (Vérité *et al.*, 1987).

MATERIAL AND METHODS

In situ trial

The rumen degradability of dry matter (DM), nitrogen (N) and non-protein organic matter (NPOM) was measured for the following feedstuffs: wheat, maize, barley, whole soyabean meal, dried molassed sugar beet pulp, dried brewers' grains (60 °C), maize gluten meal, sunflower meal, cottonseed cake, linseed cake, extracted soyabean meal, meat meal, fishmeal, maize silage (dough stage), lucerne hay (second cut, full bloom stage) and lucerne silage (autumn third cut, midbloom stage, direct cut with microbial additive; Derasyl, Ceva, France). The proximate analysis of the feeds is shown in Table I.

The lucerne hay, lucerne silage and maize silage were incubated "fresh" after having been chopped to give an average particle length of less than 20 mm. The other protein concentrates and by-products were hammermilled through a 2–mm screen.

The *in situ* rumen degradability was measured using the nylon bag technique (Ørskov &

Table I. Proximate analyses of feeds (g/kg DM).

	DM	*Crude Protein*	*Ash*	*Ether Extract*	*Crude Fibre*	*N-free Extract*
Wheat	864	143	29	13	33	782
Maize meal	864	85	16	31	31	837
Barley	879	106	35	15	72	772
Whole soyabean meal	955	418	57	139	150	236
Dried mol. sugarbeet pulp	898	103	60	5	208	624
Dried brewers' grains	938	268	42	83	176	431
Maize gluten meal	896	602	54	19	19	306
Sunflower meal	904	353	76	18	235	318
Cottonseed cake	943	300	54	67	234	345
Linseed cake	917	345	71	50	127	407
Soyabean meal sol. ext.	886	524	70	8	37	361
Meat meal	970	589	293	101	13	4
Fishmeal	911	672	185	76	5	62
Lucerne hay	898	187	90	20	274	429
Ensiled lucerne	220	169	181	42	261	347
Maize silage	362	72	34	29	198	667

McDonald, 1979). Details of the method have been published previously (Susmel & Stefanon, 1987). A minimum of 4 rumen fistulated cows are required and are fed a basal diet composed of 50% (dry matter basis) concentrate, 25% meadow hay and 25% maize silage. The concentrate is formulated to contain at least 3 protein sources, at least 1 of which is an animal protein; the protein content is sufficient to maintain a dietary crude protein concentration of 130 g/kg DM and a positively balanced PDIE–PDIN ratio. The basal diet is offered in 2 equal meals (at 07.30 and 16.30) and rationed so that the dry matter intake is approximately 15 g DM/kg liveweight. Concentrates and hay are dried at 65 °C and sieved through 40– and 2000–μm sieves before incubation. Polyester bags with a pore size of approximately 40 μm are filled with a quantity of feed sample to give a dry matter weight: free bag surface area of 12.5 mg DM/cm^2. Silages are incubated as fed, after having been cut into small pieces (between 0.5 and 2.0 cm); a quantity equivalent to 12.5 mg DM/cm^2 bag area is incubated in each bag. Duplicate bags are introduced into the rumen immediately after the morning meal, attached to a nylon rope of a length corresponding to the distance between the cannula lid and the bottom of the ventral sac of the cow's rumen; the rope is kept under tension with a 1-kg iron weight. "Zero time" bags are allowed to soak in rumen liquid *in situ* for about 3 min. After incubation, bags are rinsed briefly in a bucket of cold water and then put through the cold rinse cycle of an automatic washing machine prior to drying at 65 °C.

In the present experiment, the concentrates and by-products were incubated for 0, 2, 6, 12, 24 and 48 h; the forages were also incubated for 72 h.

Due to the large number of feeds to be incubated, the trial was conducted in 3 consecutive periods. 8 rumen fistulated *Simmental* cows (average liveweight 630 ± 12 kg), were used. For each experimental period, the cows were

subdivided into 2 groups of 4 animals each; in the first period, the maize silage, maize and dried molassed sugarbeet pulp were incubated in group 1, whilst the maize gluten meal, lucerne hay and whole soyabean meal were tested in group 2. In the second period, the cottonseed cake, meat meal and wheat were incubated in group 1 cows and linseed meal, barley and sunflower meal in group 2 animals. In the third period, the extracted soyabean meal, dried brewers' grains, ensiled lucerne and fishmeal were incubated in group 1 animals.

The basal diet (CP content 135 g/kg DM, PDIE–PDIN balance + 60 g) was composed of 25% (DM basis) maize silage (DM, 377 g/kg; CP, 76 g/kg; CF, 206 g/kg), 25% permanent pasture hay (DM, 905 g/kg; CP, 91 g/kg; CF, 376 g/kg) and 50% concentrate (DM, 874 g/kg; CP, 191 g/kg; CF, 57 g/kg) containing 69% maize, 12% soyabean meal, 9% sunflower meal, 7% meat meal and 3% mineral–vitamin supplement. Before each incubation period, the animals ate the basal diet for at least 21 days. In total, the trial lasted 3 months.

In vitro *trial*

The *in vitro* enzymatic degradability of the same 16 feeds used in the *in situ* trial was measured using the technique of Aufrere and Cartailler (1988). This method requires that the feeds be incubated for 1 and 24 h in a phosphoborate buffer containing protease extracted from *Streptomyces griseus*.

The enzyme solution was obtained by mixing 2 g of *S. griseus* protease (type XIV, Sigma no. P–5147; 4.7 titratable units/mg) with 1000 ml of phosphoborate buffer (pH 8.0, PBB), prepared by disolving 12.20 g Na_2HPO_4 and 8.91 g $Na_2B_4O_710H_2O$ in distilled water, adjusting the pH to 8 with 1 N NaOH and making up to 1000 ml. Each sample (0.5 g) was incubated in an 80–ml pyrex tube, with 0.5 ml enzyme solution and 0.5 ml tetracycline solution (Sigma no. T-3258, 100 mg/ l PBB). At the beginning of the incubation period, 0.5 mg nistatin (Sigma no N-3503) and 50 ml PBB was added to each tube.

Before incubation, the maize and lucerne silages were dried at 60 °C for 48 h. The hay, the concentrate feeds and the by-products were milled to pass a 1–mm screen. Each sample was incubated in duplicate in 2 batches in a waterbath at 40 °C for 1 and 24 h. In each batch, the 1– and 24–h degradability of extracted soyabean meal was used as an internal standard; duplicate blank tubes were present in each batch. The 24-h tubes were swirled manually after 1, 2, 3, 4, 5, 6, 7 and 12 h; all tubes were cooled and centrifuged for 5 min at 3000 rpm after incubation.

A 10-ml aliquot of supernatant was taken from each tube for nitrogen analysis. The quantity of N rendered soluble was calculated as the fraction of that present before incubation, after adjustments for the relative blanks and the average change in 1– and 24–h solubility of the soya standards between the 2 batches. The quantity of N rendered soluble (degraded) after 1 and 24 h will be refered to as *as* and *as* + *bs* respectively (Ørskov & MacDonald, 1979) (model (i) below).

The repeatability of the *in vitro* experiment was tested using the ISO (1981) method, incubations were repeated when the coefficient of variation (CV) exceeded 2%.

Chemical analyses

The chemical composition and dry matter content of the feeds were determined using standard AOAC (1975) methodology. The N content of each feedstuff before incubation and each supernatant was determined using the micro Kjeldhal technique with samples weighing approximately 200 mg.

Statistical analysis

The *in situ* degradability kinetics for nitrogen, dry matter and non-protein dry matter (calculat-ed as the difference between dry matter and crude protein weights in the bag residues) were studied using the 2-component exponential model proposed by Ørskov & McDonald (1979) using the Marquardt algorithm for non-linear regression analysis (SPSSx, 1985):

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

where Dg(t) = degradability at time t; a = intercept of the degradability curve on the y-axis (solubility); b = curve asymptote at infinite time; c = instantaneous rate of change of degradability. The following boundaries were used: $100 > a > 0$; $100 > b > 0$; $a + b < 100$; $1 > c > 0$.

The effective degradability (Dg, corresponding to the *dégradabilité théorique* in the French protein system proposed by Vérité *et al.*, 1987) was calculated, weighted to account for rumen outflow rate, using the equation of Ørskov and McDonald (1979) :

$$Dg = a + (bc) / (c + k) \qquad (2)$$

where a, b and c are as in eq. (1) and k is the rumen outflow rate.

A rumen outflow rate of 0.07/h was used for the concentrate feeds, this value having been observed previously with basal diets of similar composition and intake level (Stefanon & Ovan, 1988), whilst a rate of 0.04/h was used for the forages in an attempt to account for differences in composition and particle size (Colucci *et al.*, 1982).

Calculation of PDIA and PDIE

Susmel and Stefanon (1987) have assumed that the effective degradability of non-protein organic matter (DgNPOM, %) is equal to the effective degradability of non-protein dry matter multiplied by the feed concentrations of crude fibre and nitrogen-free extracts. This has recently been supported by the regression of the effective degradability of organic matter (DgOM) and dry matter (DgDM) to give the following equation (Susmel & Stefanon, unpublished data):

$$DgSO = 1.049 \times DgDM - 0.044$$

$$r^2 = 0.923; \text{ RSE} = 0.022; \text{ DF} = 21$$

Total fermented carbohydrates (C, g) can then be considered as:

$$C = DgNPOM \times (CF + NFE)/100$$

with CF and NFE in g/kg.

The PDIA , PDIE and PDIN contents of the feedstuffs were calculated as follows:

(a) using the DgN and DgNPOM *in situ* values with the system proposed by Susmel and Stefanon (1987);

(b) Using the DgN, the undegraded amino acid true digestibility in the small intestine (*dr*) and fermentable organic matter data derived from INRA tables and the system proposed by Vérité *et al.* (1987).

The protein system proposed by Susmel & Stefanon (1987) has not previously been published in English, so a brief summary of the equations used is presented below.

(i) PDIA = g N x 6.25 x (1 – DgN%/100) x dr x 0.85

where PDIA = feed protein digestible in the small intestine; dr = true protein digestibility.

$$dr = [(1 - Dg\text{N\%}/100) \times N \times 6.25 - (0.1 \times N \times 6.25)] / [(1 - DgN\%/100) \times N \times 6.25)]$$

where 0.85 = fraction of amino acids in the undegraded protein; DgN% = % effective protein degradability.

(ii) PDIME = ((0.030 x C) + (0.015 x P) x 14.0 x 0.53 x 0.80 x 0.85

where PDIME = potential bacterial protein synthesis as a function of energy when nitrogen supply is not limiting;

P = g degraded protein; C = g fermented carbohydrate (NPOM); 0.030 = ATP yield (moles) per g fermented carbohydrate in the rumen; 0.015 = ATP yield (mol) per g fermented protein in the rumen; 14.0 = microbial DM synthesised/mol ATP; 0.53 = fraction of protein in microbial DM; 0.80 = fraction of aminoacid in microbial protein; 0.85 = microbial protein digestibility.

(iii) PDIMN = N x 6.25 x (DgN%/100) x 0.80 x 0.85

where PDIMN = potential bacterial protein synthesis as a function of nitrogen when energy is not limiting; 0.80 = fraction of amino acid in the microbial protein; 0.85 = microbial protein digestibility.

(iv) PDI = PDIE (if PDIE < PDIN)

PDI = PDIN (if PDIE > PDIN)

RESULTS

RSE values for the N degradability kinetics (Table II) were generally high (*i.e.*, more than 1); goodness of fit with the model was

variable between feeds, with r^2 values between 0.74 and 0.97. Values for maize meal, maize gluten meal, cottonseed cake and the 2 silages were found to fit the model least well (Table II).

The effective degradability of N (DgN, Table III) was always higher than 50% in the cereals and whole soya, the highest value being 74% for wheat. DgN for the by-products was much lower, with the maize gluten meal value very low at 30%. The DgN values for the vegetable and animal proteins were very variable.

The high N degradability in sunflower meal and cottonseed cake was particularly noticeable, this being associated with high initial solubility (*a*). The meatmeal was more degradable than the fishmeal. Of the 3 forages, the lucerne silage had a higher proportion of undegradable protein, higher even than the lucerne hay.

The effective degradability of DM (DgDM, Table III) was low (approximately 40%) in brewers' grains, maize gluten meal, cottonseed cake, linseed meal, fishmeal and meatmeal. It was higher (approximately 60%) in the cereals and soyabean meal.

The effective degradability of NPOM (Table III) was much more variable between feedstuffs than the DgN and DgDM values. Wheat again had the highest value (81%), followed by soyabean meal (68%); low values of DgNPOM (between 7 and 39%) were observed for meatmeal, fishmeal, cottonseed and linseed cake, dried brewer's grains and ensiled lucerne.

The effective degradability of N was only partly correlated with that of DM (r = 0.65), and was independent of NPOM degradability (r = 0.40), whilst, as expected, a high correlation was observed between DgDM and DgNPOM (r = 0.92). In particular, of the cereals, wheat and barley had N and NPOM degradability similar to that of DM, but this was not the case with the maize meal, where the DgN value was lower than DgDM. The DgN value of the oil

Table II. Coefficients of degradability kinetics, RSE and r^2 values of nitrogen.

	a	*b*	*c*	RSE	r^2
Wheat	12.1	86,7	0,1710	1,5	0,91
Maize meal	30.1	69.9	0.0317	1.5	0.79
Barley	16.7	75.7	0.1080	1.1	0.93
Whole soyabean meal	19.4	80.6	0.0492	1.0	0.93
Dried mol. sugarbeet pulp	8.9	91.1	0.0392	1.6	0.88
Dried brewers' grains	13.5	62.7	0.0640	0.5	0.97
Maize gluten meal	7.4	92.6	0.0220	1.6	0.82
Sunflower meal	30.3	65.4	0.1581	0.6	0.97
Cottonseed cake	32.2	55.8	0.0876	1.4	0.81
Linseed cake	5.2	94.8	0.0377	1.3	0.91
Soyabean meal sol. ext.	18.4	81.6	0.0490	1.1	0.90
Meat meal	19.5	48.8	0.1339	1.0	0.87
Fishmeal	23.2	26.2	0.1070	0.4	0.90
Lucerne hay	37.2	55.7	0.0568	0.8	0.94
Ensiled lucerne	40.5	24.7	0.0477	0.6	0.74
Maize silage	63.0	37.0	0.0083	0.5	0.75

Table III. Effective degradability of N (DgN), dry matter (DgDM) and non-protein organic-matter (DgNPOM) and PDI concentrations of feedstuffs.

	DgN	*DgDM*	*DgNPOM*	*PDIA*	*PDIE*	*PDIN*
Wheat	73.6	79.6	80.7	20	127	91
Maize meal	51.9	60.5	61.3	28	111	57
Barley	62.6	60.0	59.7	25	106	69
Whole soyabean meal	52.7	55.6	57.7	133	170	282
Dried mol. sugarbeet pulp	41.6	47.5	48.2	42	110	72
Dried brewers' grains	43.4	33.2	29.7	106	142	185
Maize gluten meal	29.5	34.4	43.4	309	344	430
Sunflower meal	75.6	54.0	42.2	43	98	225
Cottonseed cake	63.2	38.0	27.1	68	106	197
Linseed cake	38.4	34.3	32.7	151	188	241
Soyabean meal sol. ext.	52.0	60.0	68.4	169	231	355
Meat meal	51.6	31.9	6.9	192	215	399
Fishmeal	39.1	32.6	20.7	291	312	469
Lucerne hay	69.9	56.6	53.5	44	100	123
Ensiled lucerne	54.0	41.0	38.8	57	94	115
Maize silage	69.3	55.9	54.9	14	88	47

DgN, Dg DM, DgNPOM = $a + b(1 - e^{(-\alpha)})$.
DgN, Dg DM, DgNPOM = $a + (bc) / (c + k)$, with $k = 0.07$/h for concentrate and by-products and 0.04/h for forages.

seed meals and cakes (with the exception of the extracted soyabean meal), of the animal proteins and the forages were noticeably higher than the DgDM values.

The PDIA, PDIE and PDIN concentrations of the 16 feedstuffs, estimated using the system proposed by Susmel & Stefanon (1987), are reported in Table III. Figures 1, 2 and 3 show the regression equations and the plots of the feed concentrations of PDIA, PDIE and PDIN calculated using the INRA (Vérité *et al.*, 1987) method with values obtained using the Susmel & Stefanon (1987) method.

The regressions, calculated by taking the Italian values as independent variables, show a high degree of correlation. However, the 2 protein systems only agree closely for PDIN values, with the gradient equal to 1.1 ($P < 0.001$). The differences between the PDIA and PDIE values calculated using the 2 systems are consistent, with gradients equal to 1.3 and 1.4 respectively ($P < 0.001$). The constant terms of the regressions for PDIA, PDIE, and PDIN were not found to be significantly different from zero.

Table IV shows the results of the *in vitro* protease degradability experiment. The 16 feeds showed variable degradability values: from 8.1% for maize gluten meal to 62.1% for sunflower meal, and from 41.6% for whole soyabean meal to 84.7% for sunflower meal after 1 and 24 h respectively.

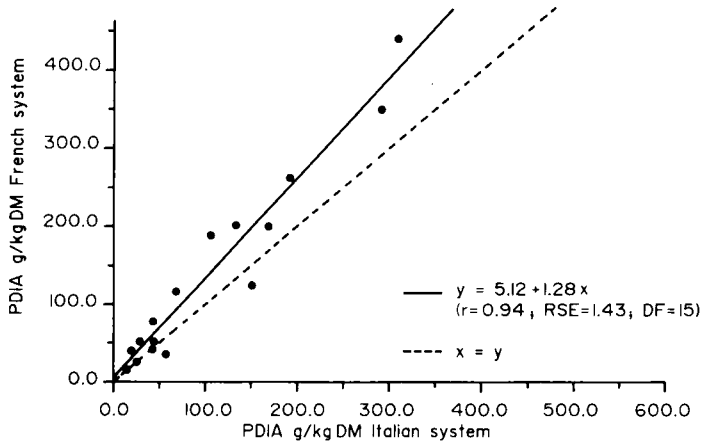

Fig. 1. Regression of PDIA concentrations calculated using the INRA system with data obtained using the Italian system.

Table IV also shows the calculated times (CTs, h) at which the *in situ* degradability equals the 1– or 24–h *in vitro* degradability; these times vary greatly between feeds, as is evident from the standard deviations. The enzymatic degradability *as* (after 1 h; Table III) of the feeds was higher than the *a* value *in situ* (Table II), except for the maize meal and the forages. This is shown by positive CTs in Table IV, with negative values for the maize meal and forages. After 24 h, degradability *in vitro* (*as* + *bs*) was considerably lower for the cereals, whole soyabean meal, sunflower meal, cottonseed cake, lucerne hay and maize silage, with CTs con-

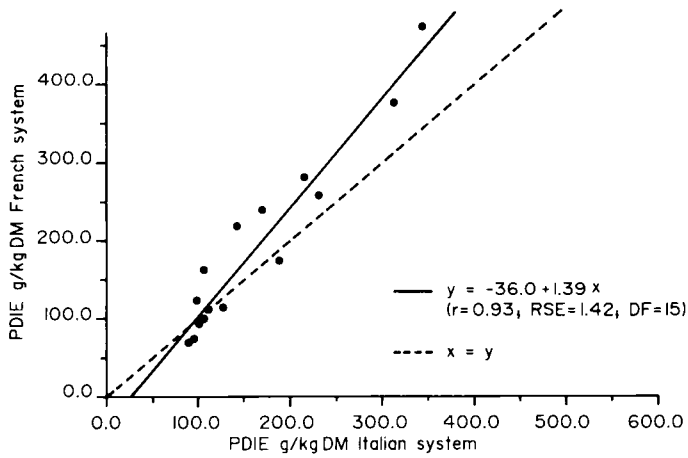

Fig. 2. Regression of PDIE concentrations calculated using the INRA system with data obtained using the Italian system.

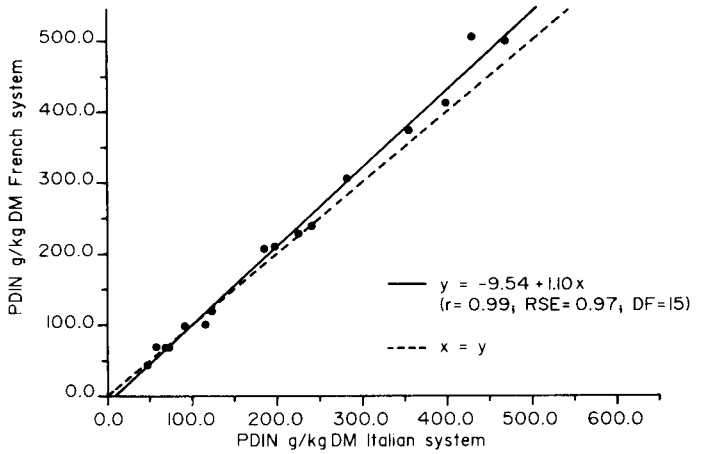

Fig. 3. Regression of PDIN concentrations calculated using the INRA system with data obtained using the Italian system.

siderably less than the 24–values. Only fishmeal had a higher 24–h *in vitro* degradability than the $a + b$ *in situ* value.

Table V shows the correlation coefficients (r) between degradability *in vitro* and degradability *in situ* of the 16 feeds. Significant correlations were found between the coefficient a *in situ* and *as* and *bs in vitro*, and between *bs in vitro* and *b in situ*. Significant correlations were also found between *in situ* effective degradability (DgN) and the *in vitro* degradability values *as*, $as + bs$ and *bs*.

Having considered the results in Table V, the possibility of a relationship between *as* and $as + bs$ values with the effective nitrogen degradability *in situ* was investigated using multiple regression analysis.

The regression equation for all the feeds (group 1) was

$$DgN = 0.78 \times as - 0.10 \times (as + bs) + 33.5$$

$$(r^2 = 0.61; \text{RSE} = 2.32)$$

The coefficient for *as* and the constant term were significant ($P < 0.01$ and $P < 0.05$ respectively).

The plot of standardised residuals with standardised degradability values is shown in Fig. 4. Multiple regression analysis of the feeds within the range of ± 1 standardised residual units (maize, whole soyabean meal, dried molassed sugarbeet pulp, maize gluten meal, brewers' grains, sunflower meal, cottonseed cake, linseed cake, meat meal, ensiled lucerne and maize silage) gave a higher r^2 (0.88) and RSE (1.31) values with the following equation:

$$DgN = 0.92 \times as - 0.30 \times (as + bs) + 40.9$$

with the coefficient for *as* and the constant significantly different from zero ($P < 0.01$).

DISCUSSION

The basal diet was formulated to be as similar as possible to production rations for lactating dairy cows, within the constraints of the experimental design (Susmel & Stef-

Table IV. Protease degradability of nitrogen after 1 and 24 h of incubation and the time this value would occur in situ. (CTs) calculated using the kinetics reported in Table II.

Feed	Degradability		CT[a]	
	1 h *as*	24 h *as + bs*	1 h	24 h
Wheat	49.8	71.3	3.3	6.7
Maize meal	23.7	45.6	−2.8	7.9
Barley	32.7	65.9	2.2	9.7
Whole soyabean meal	19.9	41.6	0.1	6.6
Dried mol. sugarbeet pulp	27.0	65.6	5.6	24.8
Dried brewers' grains	21.8	69.7	2.2	35.5
Maize gluten meal	8.1	46.7	0.3	25.1
Sunflower meal	62.1	84.7	4.2	11.3
Cottonseed cake	39.5	69.2	1.6	12.4
Linseed cake	21.1	68.0	4.8	28.8
Soyabean meal sol. ext.	52.0	78.2	10.8	26.9
Meat meal	38.9	66.8	3.8	26.0
Fishmeal	34.3	57.5	5.1	–
Lucerne hay	35.9	72.3	−0.4	17.5
Ensiled lucerne	38.5	59.6	−1.6	31.0
Maize silage	57.0	65.5	−18.1	8.7
Mean value			1.33	18.60
s.d.			6.13	10.07

[a] The following equation can be derived from Ørskov and McDonald (1979)
CT (1 h) = $(\ln b - \ln(a + b - as))/c$ and solved using values in Table II.
CT (24 h) = $(\ln b - \ln(a + b - (as + bs)))/c$ and solved using values in Table II.

anon, 1987). Michalet-Doreau *et al.*, 1987, also recommended the use of production levels of intake and diet composition when performing *in situ* degradability experiments. The basal diet has been shown to have a considerable effect on degradability kinetics (Gonzales *et al.*, 1987; Susmel *et al.*, 1989), and rumen outflow rate (Eliman & Orskov, 1983; Colucci *et al.*, 1982), and hence on effective degradability (Weakley *et al.*, 1983; Vik-Mo & Lindberg 1985; Ganev *et al.*, 1979; Susmel *et al.*, 1989).

In this paper, the degradability data were interpolated with a 2-component kinetics model. This choice depended upon the methodological approach used. The progress of the degradation of the principal nutrients with time can, in fact, be distinguished from simple, theoretical first-order kinetics (NRC, 1985; Krishnamoorthy *et al.*, 1983; Mathers & Miller, 1981; Robinson *et al.*, 1986) by lag phases and different rates of bacterial attachment and enzymatic digestion. Moreover, using the nylon

Table V. Correlation coefficients (r) between *in situ* degradability of nitrogen and *in vitro* degradability (N = 16).

Degradability *in situ*	Degradability *in vitro*		
	1 h (*as*)	24 h (*as* + *bs*)	24 – 1 h (*bs*)
a	0.552 (*)	0.078	– 0.692 (**)
b	– 0.371	– 0.035	0.486 (*)
a + *b*	– 0.014	0.025	0.048
DgN	0.775 (***)	0.507 (*)	– 0.531 (*)

(*) $P < 0.05$; (**) $P < 0.01$; *** $P < 0.001$.

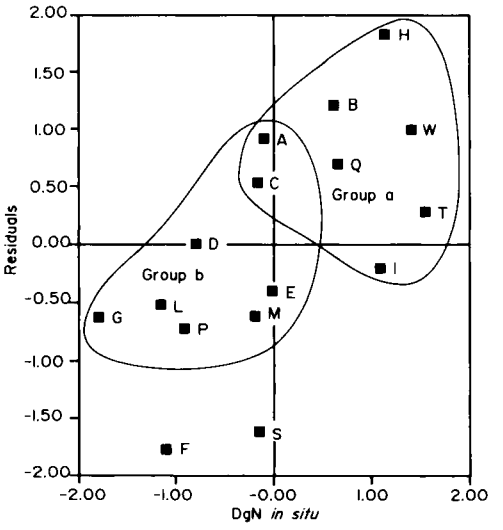

Fig. 4. Plot of the standardised residuals of regression for DgN : A = whole soyabean meal; B = barley; C = maize meal; D = dried brewers' grains; E = ensiled lucerne; F = fishmeal; G = maize gluten meal; H = lucerne hay; I = maize silage; L = linseed cake; M = meat meal; P = dried molassed sugarbeet pulp; Q = cottonseed cake; S = soyabean meal sol. ext.; T = sunflower meal; W = wheat.

bag technique, it is difficult to obtain observations at sufficiently frequent intervals, for example less than 30 min, or every 1/2 h for the first 6 h. Furthermore, bacterial contamination (Varvikko, 1986; Crooker *et al.*, 1978) can affect the degradability kinetics (Siva Bhat *et al.*, 1988). For nearly all the feeds, the r^2 and RSD values for the N kinetics (Table II) confirm the good interpolation obtained by employing the Ørskov and McDonald (1979) 2-component model.

For nearly all feeds, the effective N degradability obtained in this current work (Table III) was lower than the "degradabilité théorique" found by INRA (Vérité *et al.*, 1987). The most noticeable differences were for whole soya, barley, linseed cake, soyabean meal, fishmeal and the silages. Similar values to INRA (Vérité *et al.*, 1987) were observed for wheat, dried brewers' grains and meatmeal. Higher values than those obtained by INRA were found only for maize meal and maize gluten meal. The effective degradability values for dried brewers' grains, soyabean meal, fishmeal and ensiled lucerne agree well with values obtained previously in this laboratory (Susmel *et al.*, 1989).

The differences between our results and those of INRA could be due to the different rumen outflow rate (0.06 for INRA, Vérité *et al.*, 1987) used in the calculations, but are more likely to be due to differences in feed chemical composition and the characteristics of the basal diets.

The low correlation between DgN and DgDM values shows that it is not possible to predict the degradability of nitrogen from that of dry matter. The different behaviour of nitrogen and dry matter in the rumen, as shown in this experiment, was also observed by Ha & Kennelly (1984) and Ehle *et al.* (1982). The subdivision of the dry matter into nitrogen and non protein organic matter fractions shows clearly that nitrogen does not necessarily have an effective degradability similar to that of NPOM, as has been already shown by Susmel *et al.* (1989) and Nocek (1985).

French PDIA values (Fig. 1) are generally higher than those of the Italian system (Table III), probably because of the latter's lower digestibility values, with a concomitant effect on degradability. However, the regressions between the French and Italian protein systems (Fig. 3) reveal that PDIN values are approximately equal, with a constant term of 9 g.

The different methods for estimating energy available for bacterial synthesis (fermentable carbohydrates and degraded protein in the rumen for the Italian, and fermentable organic matter for the French system) cause a considerable effect in determining the feed PDIE contents (Fig. 2). The regression between the 2 systems shows, on average, a lower PDIE content using the Italian system, although this difference is not significant.

The protease degradability values at 1 and 24 h, shown in Table IV, reveal, for many of the feeds, substantial differences when compared with those *in situ*. For the silages, this effect may have been due to the different physical form in which the samples were incubated *in vitro*, *i.e.* dried.

Examining the values in Table V, it can be observed that the *in situ* degradability coefficients (i.e. *a*, *b* and *a* + *b*) were generally poorly correlated with the *in vitro* degradability values, with r (DF = 15) values of less than 0.7, even if some of the correlation coefficients were significant. A plot of the data produced widely dispersed points (not shown). A moderate but highly significant ($P < 0.001$) correlation was observed between the *in situ* effective degradability and the *in vitro* degradability value *as*.

The value *b in situ* was higher than that of *bs in vitro* only for fishmeal and meatmeal, and this would appear to show, over the above-mentioned phenomena, that there might be a physical inhibition effect acting on the enzymatic digestion of the other feeds, possibly linked to the presence of cell-wall carbohydrates in the feeds of vegetable origin which limit the purely proteolytic attack on the part of the enzyme.

Other workers have shown a higher correlation between solubility, either in enzyme or buffer solutions, for short incubation times (from 1 to 4 h) with the effective degradability measured with dacron bags (Crawford *et al.*, 1978; Stern and Satter, 1984; Poos-Floyd *et al.*, 1985). In particular, Poos-Floyd *et al.* (1985) reported a correlation coefficient of 0.82 between the quantity of residual nitrogen after 1 h of protease incubation *in vitro* and the undegradable N fraction contained in dacron bags. Krishnamoorthy *et al.* (1983) reported a high rumen nitrogen degradability for mixed diets and a low *in vitro* solubility for the same diets, with a correspondingly low correlation coefficient. Similarly, Poos-Floyd *et al.* (1985) showed that the correlation between *in vitro* and *in situ* degradability decreases with increasing enzyme incubation times. Krishnamoorthy *et al.* (1983)

attributed this different behaviour to the fact that the degradation of protein *in vitro* occurs in a closed system, where there is the distinct possibility of enzyme inhibition by degradation products. The estimation of enzymatic solubility using hydrazine and chloramphenicol as inhibitors (Broderick *et al.*, 1988) gave an average value to the effective nitrogen degradability as 83% of that *in situ.*

Sauvant *et al.* (1987) have reported a high correlation ($r^2 = 0.90$) between the effective nitrogen degradability *in situ* and that obtained with the same enzymatic technique reported in this paper. Unfortunately, Sauvant *et al.* (1987) did not report an equation for the calculation of nitrogen degradability from the 1– and 24–h enzyme degradability. In the absence of a reference model, the correlation between the available *in situ* and *in vitro* data were studied using multiple regression analysis.

The regression equations contained a large constant term ($P < 0.05$), indicating that the *in vitro* data did not correspond to the measured *in situ* solubility (*a*) and potential degradability (*b*). Apart from the groups taken from the residual plot (as described above, see fig. 4), a visual appraisal of the residual plots would suggest the presence of at least 2 additional groups:

Group (a) feeds in the upper-right position of the graph (wheat, barley, maize, whole soyabean meal, sunflower meal, cottonseed cake, lucerne hay and maize silage);

Group (b) feeds in the central zone of the graph (brewers' grains, ensiled lucerne, linseed cake, maize, maize gluten meal, meat meal, whole soyabean meal and dried sugar beet pulp).

Statistical analysis produced higher r^2 values (0.92 and 0.79) and lower RSE values (0.76 and 1.28) for Groups (*a*) and (*b*) respectively, in comparison with the "all-feeds" group. It is surprising that the composition of these additional groups was independent of the nature of the feedstuffs, so that cereals, forages and by-products could be included in the same group. Unfortunately, there were insufficient data to allow a general application of the results, so that further work with additional feeds is required to clarify this issue.

ACKNOWLEDGMENT

This research was carried out with an MPI 40% (U.O.B. Stefanon).

REFERENCES

AOAC (1975) *Official Methods of Analysis.* Association of Official Analytical Chemists, 12 th edn. Washington DC

ARC (1984) *The nutrient Requirements of Ruminant Livestock.* Suppl. 1. Agricultural Research Council, Farnham Royal, Slough, U.K.

Aufrere J. & Cartailler D. (1988) Mise au point d'une méthode de laboratoire de prévision de la dégradabilité des protéines alimentaires des aliments concentrée dans le rumen. *Ann. Zootech.* 37, 255-270

Broderick G.A., Wallace R.J., Orskov E.R. & Hansen L. (1988) Comparison of estimates of ruminal protein degradation by *in vitro* and *in situ* methods. *J. Anim. Sci.* 66, 1739-1745

Colucci P.E., Chase L.E. & Van Soest P.J. (1982) Feed intake, apparent diet digestibility and rate of particulate passage in dairy cattle. *J. Dairy Sci.* 65, 1445-1456

Crawford R.J. Jr., Hoover W.H., Sniffen C.J. & Crooker B.A. (1978) Degradation of feedstuff nitrogen in the rumen *versus* nitrogen solubility in three solvents. *J. Anim. Sci.* 46: 1768-1775

Crooker B.A., Sniffen C.J., Hoover W.H. & Johnson L.L. (1978) Solvents for soluble nitrogen

measurements in feedstuffs. *J. Dairy Sci.* 61, 437-447

Ehle F.R., Murphy M.R. & Clark J.H. (1982) *In situ* particle size reduction on degradation of crude protein and dry matter in the rumen of dairy steers. *J. Dairy Sci.* 65, 963-971

Eliman M.E. & Ørskov E.R. (1983) The effect of affecting the outflow of protein supplements from the rumen. 1. Feeding level. *Anim. Prod.* 38, 45-51

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.* 93, 651-656

Gonzales J., Michalet-Doreau B. & Poncet C. (1987) Effet du niveau d'ingestion et du pourcentage de concentré dans la ration sur la dégradabilité de l'azote *in sacco* chez le mouton. *Reprod. Nutr. Dev.* 27, 155-156

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, 443-452

INRA (1988) *Alimentation des bovins, ovins et caprins.* (R. Jarrige, ed) INRA, Paris

ISO (1981) *International standard 5725. Precision of test methods. Determination of repeatability and reproductibility by inter-laboratory test.* Ref. No. 150, 5725-1981

Krishnamoorthy U., Muscato T.V., Sniffen C.J. & Van Soest P.J. (1982) Nitrogen fractions in selected feedstuffs. *J. Dairy Sci.* 65, 217-225

Krishnamoorthy U., Sniffen C.J., Stern M.D. & Van Soest P.J. (1983) Evaluation of a mathematical model of rumen digestion and an *in vitro* simulation of rumen proteolysis to estimate the rumen undegraded nitrogen content of feedstuffs. *Br. J. Nutr.* 50, 555-568

Madsen J. (1985) The basis for the proposed nordic protein system for ruminants. The AAT.PBV system. *Acta Agric. Scand.* 25 (Suppl.), 9-20

Madsen J. & Hvelplund T. (1985) Protein degradation in rumen. A comparison between *in vivo*, nylon bag, *in vitro* and buffer measurements. *Acta Agric. Scand.* 25 (suppl.), 103-124

Mathers I.C. & Miller E.L. (1981) Quantitative studies of feed 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, 587-604

Michalet-Doreau B., Vérité R., Chapoutot P. (1987) Methodologie de mesure de la dégradabilité *in sacco* de l'azote des aliments dans le rumen. *Bull. Tech. CRZV, Theix INRA,* 69, 5-7

Nocek J.E. (1985) Evaluation of specific variables affecting *in situ* estimates of ruminal dry matter and protein digestion. *J. Anim. Sci.* 60, 1347-1358

NRC (1985) Ruminant Nitrogen Usage. National Academy Press, Washington, DC, USA

Oldham J.D. (1987) Testing and implementing the modern systems: UK *Feed Evaluation and Protein Requirement Systems for Ruminants.* Commission of the European Communities, Luxembourg

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, 499-503

Poos-Floyd M., Klopfenstein T. & Britton R.A. (1985) Evaluation of laboratory techniques for predicting ruminal protein degradation. *J. Dairy Sci.* 68, 829-839

Robinson P.H., Fadel J.G. & Tamminga S. (1986) Evaluation of mathematical models to describe neutral detergent residue in terms of its susceptibility to degradation in the rumen. *Anim. Feed Sci. Technol.* 15, 249-271

Sauvant P., Aufrere J., Michalet-Doreau B., Giger S. & Chapoutot P. (1987) Valeur nutritive des aliments concentrés simple : tables et prévision. *Bull. Tech. CRVZ Theix* 70, 75

Siva Bhat, Wallace R.J. & Ørskov E.R. (1988) Study of the relation between straw quality and its colonisation by rumen micro-organisms. *J. Agric. Sci. Camb.* 110, 561-565

SPSSx (1985) *SPSS.X User Guide.* McGraw-Hill, New York

Stefanon B. & Ovan M. (1988) Impiego di bicromato sodico per la valutazione della velocità di transito ruminale degli alimenti. *Zoot. Nutr. Anim.* 15, 431-438

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, 714-724

Susmel P. & Stefanon B. (1987) Sistema per la valutazione delle protein alimentari per i ruminanti. *Zoot. Nutr. Anim.* 13, 567-582

Susmel P., Stefanon B. & Piasentier E. (1989) Effect of forage and concentrate intake level on rumen degradability of protein sources having different *in vitro* rates of N solubilisation. *Anim. Feed Sci. Technol.* 26, 231-249

Varvikko T. (1986) Microbially corrected amino acid composition of rumen-undegraded feed and amino acid degradability in the rumen of feeds enclosed in nylon bags. *Br. J. Nutr.* 56, 131-40

Vérité R., Michalet-Doreau, B. Chapoutot P., Peyraud J.L. & Poncet C. (1987) Révision du système des protéines digestibles dans l'intestin (PDI). *Bull. Tech. CRVZ, Theix, INRA* 70, 19-34

Vik-Mo L. & Lindberg J.F. (1985) *In sacco* degradability of protein (N) and dry matter in samples of individual feeds or combination, tested with diets medium or high in protein. *Acta Agric. Scand.* 35, 117-128

Weakley D.C., Stern M.D. & Satter L.D. (1983) Factors affecting disappearance of feedstuffs from bags suspended in the rumen. *J. Anim. Sci.* 56, 493-507