J Sci Food Agric 1998, **77**, 87–95

The *In Vitro* Gas Coupled with Ammonia Measurement for Evaluation of Nitrogen Degradability in Low Quality Roughages using Incubation Medium of Different Buffering Capacity

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(Received 6 January 1997; revised version received 4 August 1997; accepted 4 September 1997)

Abstract: Owing to low N content in low quality roughages, the estimation of *in vitro* rumen degradable nitrogen (IVRDN) by the method of Raab *et al* (*Br J Nutr* 1983 **50** 569–582) is expected to have a high standard deviation. Incubation of larger amounts of sample will increase the amount of N in the system and decrease analytical errors in the determination of IVRDN. The increase in the amount of sample necessitated an increase in the amount of buffer in the medium. In this study the effect of 30 ml (as is in original method) and 40 ml buffered rumen fluid (containing double the amount of hydrogen carbonate buffer as in the original method) on rumen degradation of N from low quality roughages was evaluated. N degradability of seven cereal straws (barley, millet, oat, rice, sorghum, triticale and wheat) and one grass hay was calculated from the linear regressions of NH_3-N concentration vs *in vitro* gas production. The strength of association (r^2) for gas production and NH_3-N concentration between 30 and 40 ml system was significantly ($P < 0{\cdot}05$) different for grass hay and rice straw but not significant for other feeds. Using both systems, the IVRDN for triticale straw was virtually nil, and for others the values obtained using 40 ml system were either similar (oat straw, rice straw, sorghum stover and wheat straw) or higher (barley straw, grass hay and millet stover) than those obtained using 30 ml system. Although no significant difference was found in the standard deviation by increasing the amount of sample, the larger sample has an advantage in that it allows concomitant determination of *in vitro* apparent and true digestibility. © 1998 SCI.

J Sci Food Agric **77**, 87–95 (1998)

Key words: gas production; *in vitro* rumen degradability of nitrogen; low quality roughage

INTRODUCTION

Protein and organic matter digestibility are critical factors in nitrogen and energy economies of ruminant animals (Hanley *et al* 1992). Protein which is available for digestion and absorption post-rumen includes undegraded feed protein and microbial protein. The process of rumen degradation of protein in low quality roughages serves to up-grade dietary protein of low biological value into microbial protein of good biological value. Therefore, assessing the degradability of protein in the rumen is of great importance as it dictates both the supply of nitrogen for rumen microbes as well as protein available for digestion in the small intestine.

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 J Sci Food Agric 0022–5142/98/$17.50. Printed in Great Britain

Low quality roughages, particularly straws, are important as a ruminant feed, but the relatively slow rate of fermentation and the lack of sufficient available nutrients restrict their utilisation both by rumen microorganisms and consequently by the host animal (Silva and Ørskov 1988).

In vivo methods, in addition to being tedious, are limited by inaccurate differentiation between undegraded feed proteins and microbial protein (Poos-Floyd *et al* 1985; Broderick 1987). Various methods have been developed to evaluate protein degradability in protein-rich feeds (Raab *et al* 1983; Broderick 1987; Broderick *et al* 1988). At present the most widely used method for assessing rumen degradability of dietary protein in protein-rich feeds is the *in sacco* method (Ørskov *et al* 1980). Dietary nitrogen (N) degraded is calculated as the difference between the amount of N contained within the bag prior to incubation in the rumen and the N remaining after incubation. There are a number of factors which limit the use of this method particularly for low protein roughages. Firstly, the undegraded feed particles within the bags may be contaminated with microbial N (Negi *et al* 1988a) and failure to correct for this will lead to an underestimation of protein degradability. Secondly, in the nylon bag technique it is assumed that N leaving the bag at 0 h incubation is soluble and immediately degradable, although this may not always be the case (Chen *et al* 1987). Loss of this material will lead to an overestimation of degradability. Negi *et al* (1988a) reported negative degradability of N from wheat straw, rice straw and grass hay using the nylon bag technique and they attributed this to attachment of microbes to the sample. Therefore, there is a need to develop other relatively simple but reliable laboratory methods for evaluation of rumen degradability of protein in low quality roughage.

The *in vitro* gas measurement technique (Menke *et al* 1979) is being widely used for evaluating the nutritive value of feeds and for obtaining a better insight into the rumen fermentation processes. Raab *et al* (1983) reported linearity between *in vitro* gas production and level of starch added and a corresponding linear decrease in NH_3-N in rumen fluid. Linearity was lost when gas production was above 90 ml per 24 h probably due to exhaustion of buffer in the medium. Because gas production in Raab's method is restricted (<90 ml per 24 h), the IVRDN values obtained for low N feeds are expected to have high standard deviations, due to low evolution of NH_3-N in rumen fluid and hence larger experimental error in its quantification. Therefore, when low N feeds are used, it is necessary to incubate larger amounts of feed to increase the quantity of N incubated and improve the accuracy of measurement of IVRDN. Another advantage of increasing sample size is that it allows reliable determination of apparent and true digestibility of feeds from the same incubated material. The increase in feed sample size necessitates an increase in buffered rumen fluid. The objectives of this study were to assess the *in vitro* gas method for measuring rumen degradability of N from low quality roughages and to compare the effect of 30 (2·3 mmol hydrogen carbonate) and 40 ml (4·6 mmol hydrogen carbonate) buffered rumen fluid IVRDN for low quality roughages.

MATERIAL AND METHODS

Standardisation of gas method for IVRDN

Different carbohydrate sources (cellulose, cellobiose, glucose and starch) were incubated alone and in combination with grass hay to determine the maximum concentration of these substrates which still produced linearity between *in vitro* gas production and the concentration of carbohydrate (ie to determine the buffering capacity of the incubation medium) and to assess the pattern of fermentation of carbohydrate sources with the aim of selecting a substrate which is degraded slowly and uniformly over the period of incubation.

IVRDN

Seven straws and one grass hay sample were ground to pass through a 1 mm screen and used for the IVRDN by the method of Raab *et al* (1983) which is based on the method of Menke *et al* (1979) and uses 30 ml buffered rumen fluid (BRF). The samples were weighed, placed at the bottom of 100 ml graduated syringes and the greased piston was inserted into the syringe. Rumen fluid was taken before morning feeding from a rumen-cannulated dairy cow receiving approximately 3 kg hay and wheat straw *ad libitum*. Rumen fluid was filtered through two layers of cheesecloth into a warm flask filled with CO_2. All handling was carried out under continuous flushing with CO_2. Details of the 30 and 40 ml buffered rumen fluid preparation have been given in Menke *et al* (1979) and Makkar *et al* (1995), respectively. The 30 and 40 ml buffered rumen fluid have 2·34 mmol and 4·67 mmol hydrogen carbonate, respectively. The sample weight for incubation in the syringes was decided on the basis that the total volume of gas from the sample should not exceed the buffering capacity of the medium (see Table 2 for quantity of sample used). For determination of IVRDN, the samples were incubated in triplicate with and without cellulose. The amount of cellulose added was 62·5 and 125 mg in 30 and 40 ml systems, respectively.

Incubation was conducted in a thermostatically controlled water bath (39°C) in which the syringes were hand shaken every h for the first 8 h and thereafter at the time of push-back of the syringe-piston. The push-back of the syringe-piston was necessary (maximum three times over the period of 24 h) as the volume of gas production was higher than the capacity of the syringes.

When gas production exceeded 80 ml, the piston was pushed back to the initial position. At the end of the incubation period (24 h) gas production was recorded and NH_3-N was determined in the medium using distillation without digestion of the medium (Makkar and Becker 1996). Nitrogen degradability was calculated from the linear regressions of NH_3-N concentration (y; mg) vs gas production (x; ml) observed on incubation of feed with and without exogenous energy source (cellulose), as described in Raab *et al* (1983). The intercept at Y axis (b_0) represents the amount of NH_3-N which is released when no fermentable carbohydrate is added and hence no synthesis of bacterial protein takes place. The difference between the Y intercept (b_0) and NH_3-N content in the blank indicated the amount of NH_3-N liberated from protein and other nitrogen containing compounds of the feedstuff incubated. The IVDN at 24 h was calculated as a proportion of total nitrogen incubated by the equation:

$$\text{IVDN} = \frac{\text{NH}_3\text{-N at zero gas production } (b_0) - \text{NH}_3\text{-N of blank (at 24 h)}}{\text{total N of feedstuff incubated}}$$

Neutral and acid detergent fibre (NDF, ADF), acid detergent fibre bound nitrogen (ADF-N) and pepsin-soluble N were determined by the methods of Van Soest *et al* (1991). Enzymatic degradation of N was measured by treatment with protease enzyme from *Streptomyces griseus*, type XIV (5·6 units mg^{-1} solid, Sigma Chemical Co, St Louis, MO, USA) in a borate-phosphate buffer at pH 8·0 for 24 h (Krishnamoorthy *et al* 1983). Borate-phosphate buffer (50 ml) was added to the flasks containing about 1 g sample followed by addition of 2 ml enzyme buffer solution (5·9 mg protease per 100 ml borate phosphate buffer) and the flasks were placed in a shaking water bath (200 rpm min^{-1}) at 39°C for 24 h. The buffer solubility of N was determined by incubating the samples (1 g) in the borate-phosphate buffer at room temperature for 2 h with occasional stirring (to ensure complete wetting of the sample) and then samples were filtered through filter paper. N was determined using the Kjeldahl method.

The IVRDN values calculated from at least three different *in vitro* runs were analysed using the General linear model (GLM) of SAS (1988). Differences in IVRDN and the strength of association (r^2) for gas production and NH_3-N concentration between 30 and 40 ml systems were tested using a t-test.

RESULTS AND DISCUSSION

Fermentation pattern of different carbohydrates and buffering capacity of the 40 ml buffered medium

Four substrates (cellulose, cellobiose, glucose and starch) tested differed in gas production pattern (Fig 1).

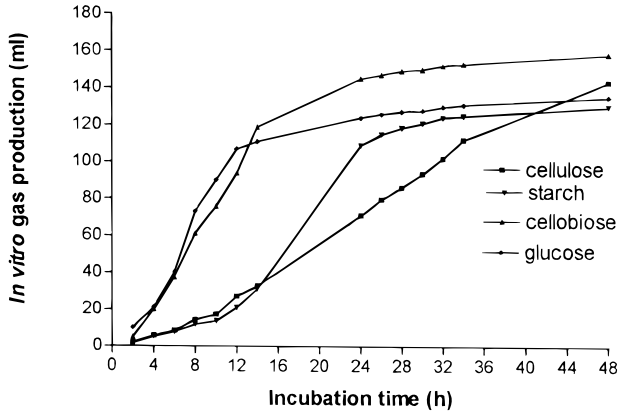

Fig 1. Gas production from incubation of different carbohydrates (300 mg each) in *in vitro* gas system.

Glucose and cellobiose fermented much faster than cellulose and starch. At 24 h, gas production from glucose and cellobiose was about 92% of the 48 h gas value whereas for cellulose and starch these values were 50 and 83%, respectively.

When different levels of starch were incubated, gas production was linear until the amount of starch reached 400 mg, and thereafter the rate of increase in gas production tended to decrease (data not shown). The rate of gas production per mg starch incubated (0·35 ml mg^{-1}) was in close agreement with the value obtained by Beuvink and Spoelstra (1992).

The results from the incubation of grass hay (400 mg) in combination with different levels of carbohydrates (cellulose, cellobiose and starch) indicated a linear relationship between gas production and the level of carbohydrate added (Fig 2). At 24 h, linearity was observed up to gas levels of 140, 178 and 177 ml at 200, 300 and 300 mg cellulose, starch and cellobiose, respectively. Incubation of higher levels of cellobiose resulted in a decrease in pH from 6·67 at 100 mg to 4·47 at 900 mg cellobiose. Only a slight decrease in pH was observed at higher levels of cellulose and starch (increase of cellulose from 100 to 800 mg resulted in a pH drop from 6·75 to 6·34 and increase of starch from 100 to 400 mg resulted in a pH drop from 6·73 to 6·41). For determination of IVRDN, cellulose was selected as a source of carbohydrate because of its relatively slow and uniform fermentability over a period of 24 h (Fig 1).

Different amounts of grass hay were incubated (0·2–1 g) by a stepwise increase of 0·2 g. The gas production was linear for the amount of hay incubated ($r^2 = 0{\cdot}999$), producing 36 and 179 ml gas when 0·2 and 1 g hay was incubated. When hydrogen carbonate buffer is used, at 24 h about 54% of the total gas generates from buffering of the short-chain fatty acids (SCFA) and the rest (28 and 18% CO_2 and CH_4, respectively) comes from fermentation (Blümmel and Ørskov 1993). About 2·34 and 4·67 mmol of hydrogen carbonate was available in 30 and 40 ml buffered rumen fluid respectively which would release 59 and 119 ml of gas in 30 and 40 ml

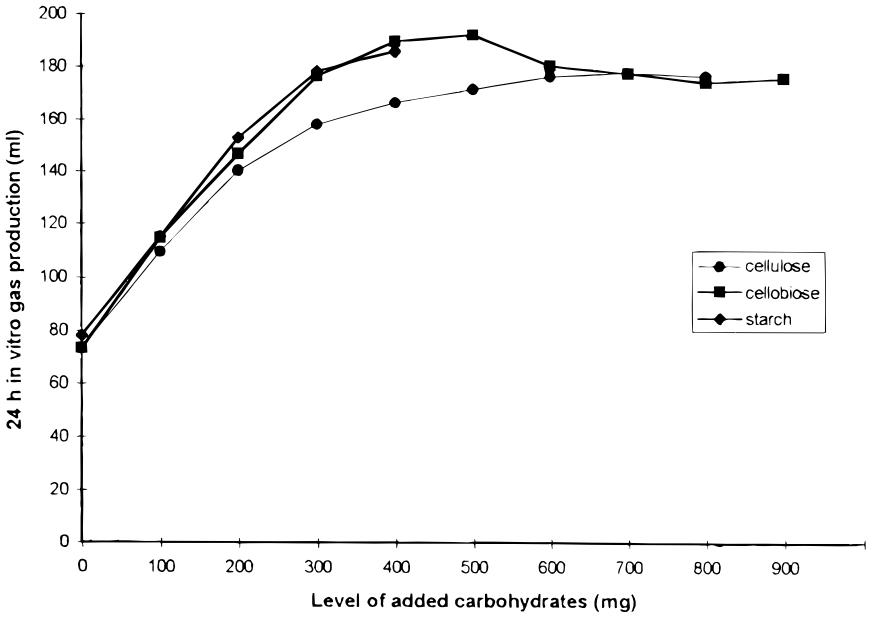

Fig 2. Gas production at 24 h on incubation of different levels of carbohydrates added to 400 mg grass hay.

system, respectively (1 mmol of hydrogen carbonate would buffer 1 mmol of SCFA which releases 25·6 ml gas at 39°C). From the incubations reported in this study, it was evident that the medium had sufficient capacity to buffer the acids evolving from fermentation.

In the 30 ml system, linear relationship between *in vitro* gas production and the amount of substrate added has been reported until the gas production reached 90 ml (Raab *et al* 1983). When 40 ml medium containing 4·67 mmol hydrogen carbonate buffer is used, the medium has a buffering capacity to release gas linearly up to about 180 ml and to maintain pH of approximately 6·5 at this level of gas production.

Chemical composition of the roughages

Chemical composition of low quality roughages used in the experiment is given in Table 1. The crude protein (CP) content ranged from 1·9% (triticale straw) to 10·6% (rice straw). Triticale straw was lowest in CP but it had a high fibre content. Sorghum stover and grass hay were relatively high in CP and low in fibre content as compared to other feeds. Sorghum and grass hay were harvested at green stage while the other straws were harvested after full maturity of the grain.

IVRDN

The relationship between NH_3-N concentration and cumulative gas production (in presence and absence of cellulose) is given in Table 2. For the purpose of comparison, r^2 is considered to be an important parameter as it indicates strength of the relationship between gas production and NH_3-N concentration. Standard deviation of the estimate is also another important indicator as it measures the accuracy of the results of the relation-

TABLE 1
Chemical composition (% DM) of low quality roughages used inthe experiment[a]

Feeds	*Crude protein*	*Neutral detergent fibre*	*Acid detergent fibre*	*Acid detergent fibre-N*	*Ash*
Barley straw	3·1	76·9	57·2	34·5	4·2
Grass hay	6·4	62·1	49·7	39·3	9·9
Millet stover	3·4	75·9	61·8	32·5	7·6
Oat straw	2·5	78·8	66·4	38·1	8·8
Rice straw	10·6	62·7	54·5	39·1	15·8
Sorghum stover	8·2	51·8	40·8	24·3	9·0
Triticale straw	1·9	85·3	70·0	39·7	5·0
Wheat straw	6·1	74·5	65·9	40·1	12·1

[a] Values are the average from two replicates.

TABLE 2

Amount of sample (DM) incubated and regression equations showing the relationship between *in vitro* gas production (y, ml) and rumen ammonia-nitrogen (x, mg) when incubated for 24 h in presence and absence of cellulose[a,b]

Feeds	*Sample incubated (mg) in*		*Regression (30 ml BRF)*	r^2	*Regression (40 ml BRF)*	r^2	*Level of Significance (r^2) between 30 and 40 system*	*n*
	30 ml BRF	*40 ml BRF*						
Barley straw	395	790	$y = 5{\cdot}18–0{\cdot}0569\ (0{\cdot}0062)x$	0·98	$y = 9{\cdot}66–0{\cdot}0620\ (0{\cdot}0074)x$	0·98	NS	3
Grass hay	337	674	$y = 6{\cdot}69–0{\cdot}0588\ (0{\cdot}0050)x$	0·95	$y = 13{\cdot}45–0{\cdot}0676\ (0{\cdot}0056)x$	0·99	**	5
Millet stover	468	936	$y = 5{\cdot}50–0{\cdot}0540\ (0{\cdot}0019)x$	0·97	$y = 10{\cdot}53–0{\cdot}0698\ (0{\cdot}0068)x$	0·93	NS	4
Oat straw	464	928	$y = 5{\cdot}23–0{\cdot}0507\ (0{\cdot}0008)x$	0·97	$y = 8{\cdot}83–0{\cdot}0560\ (0{\cdot}0053)x$	0·97	NS	4
Rice straw	432	864	$y = 8{\cdot}02–0{\cdot}0669\ (0{\cdot}0080)x$	0·99	$y = 13{\cdot}36–0{\cdot}0656\ (0{\cdot}0071)x$	0·90	*	4
Sorghum stover	280	560	$y = 7{\cdot}85–0{\cdot}0608\ (0{\cdot}0118)x$	0·96	$y = 14{\cdot}90–0{\cdot}0696\ (0{\cdot}0022)x$	0·98	NS	5
Triticale straw	467	934	$y = 3{\cdot}91–0{\cdot}0406\ (0{\cdot}0037)x$	0·98	$y = 7{\cdot}39–0{\cdot}0492\ (0{\cdot}0078)x$	0·92	NS	5
Wheat straw	600	1·200	$y = 6{\cdot}38–0{\cdot}0550\ (0{\cdot}0049)x$	0·98	$y = 12{\cdot}29–0{\cdot}0551\ (0{\cdot}0096)x$	0·99	NS	4

[a] Values in parentheses are standard deviations of the regression coefficients; n, number of *in vitro* runs for each BRF system.

[b] 62·5 mg for 30 ml system and 125 mg for 40 ml system.

* Significant at $P < 0{\cdot}05$, ** $P < 0{\cdot}01$; BRF, buffered rumen fluid.

ship. The strength of association (r^2) for gas production and NH_3-N concentration between 30 and 40 ml system was significantly different ($P < 0{\cdot}05$) for grass hay and rice straw but was not significant for other feeds.

The standard deviation of the estimates of IVRDN in both systems were not significantly different ($P > 0{\cdot}05$). Raab *et al* (1983) suggested that the amount of N per syringe should be at least 20 mg so as to avoid high standard deviation in estimation of IVRDN. In the present study, incubating higher N per syringe did not improve the standard deviation of the IVRDN estimates. Higher gas production in 40 ml system necessitated frequent push back of the syringe-piston which might have reduced, to some extent the accuracy of gas recording. However, these deviations were within the range reported from nylon bag technique (von Keyserlingk *et al* 1996).

The relationship between the amount of N per syringe and standard deviation of IVRDN was not significant ($P > 0{\cdot}05$) in both systems. The IVRDN values ranged from 24 to 76% in 30 ml system and 24 to 87% in 40 ml system, respectively (Table 3). The IVRDN of grass hay using 40 ml BRF reported in this study was similar to the value for spear grass (Krishnamoorthy *et al* 1995). The IVRDN values in the 30 ml system appear to be underestimated since the IVRDN in the 40 ml system were in close agreement with other findings (Negi *et al* 1988a; von Keyserlingk *et al* 1996). The reason for observed negative IVRDN in triticale straw by the method is not clear, but the results do suggest that the IVRDN for triticale straw is virtually nil.

Owing to the lack of a suitable method to evaluate IVRDN in low quality roughages, the contribution these feeds make to the total N supply to the animals has been overlooked. The determination of N degradability of these feeds by the nylon bag technique requires correction for microbial contamination of feed residues left in the bag after incubation in the rumen. Negi *et al* (1988a) developed an approach to correct for microbial attachment by incubating N-free materials with the assumption that microbial adherence to N-free material and feed sample was similar. Although not significant, the extent of microbial attachment to different N-free material was variable and this method (Negi *et al* 1988a) for determination of the extent of microbial contamination is tedious for routine use.

The IVRDN reported in this study and by other workers (Negi *et al* 1988a) were higher than generally assumed. The degraded protein is utilised for microbial protein synthesis. However, wastage of feed protein caused by NH_3 overflow occurs when fermentable energy is insufficient to support the microbial growth required to utilise the excess degraded protein (Broderick *et al* 1992). The major limiting factor for ruminant production in the tropics is insufficient extraction of energy by microbes from cell wall-rich crop residues. The rumen degradable N (RDN) and metabolisable energy (ME) requirements for maintenance of a 200 kg cow are 36 g and 29 MJ day^{-1}, respectively (ARC 1980). Based on the present observations the RDN and ME available from barley straw (assuming intake of 3·5 kg DM and 6 MJ ME kg^{-1} DM per day) would be 9·4 g (which is about 30% of the total RDN requirement) and 21 MJ day^{-1}. The corresponding RDN values for sorghum stover, grass hay, rice, wheat, oat and millet straws are 40, 27, 21, 12, 9 and 3 g, respectively. The RDN values reported in this study are from 24 h incubation. The RDN contribution of low quality roughages to the total RDN supply would be much higher than the values mentioned above, as retention time of roughages in the rumen is expected to be about 48 h (Van Soest 1994). As the available RDN in low quality roughages is lower than

TABLE 3
The amount of nitrogen incubated and *in vitro* degradable nitrogen (IVRDN[a]) in 30 and 40 ml rumen fluid buffer mixture[b]

Feeds	*Nitrogen in the sample incubated (mg)*		*n*	*IVRDN*		*Level of significance IVRDN*
	30 ml BRF	*40 ml BRF*		*30 ml BRF*	*40 ml BRF*	
Barley straw	2·1	4·2	3	0·33 (0·20)	0·54 (0·10)	*
Grass hay	3·6	7·2	5	0·56 (0·07)	0·74 (0·06)	**
Millet stover	2·7	5·4	4	0·24 (0·02)	0·47 (0·11)	**
Oat straw	2·0	4·0	4	0·25 (0·08)	0·24 (0·06)	NS
Rice straw	7·9	15·8	4	0·39 (0·06)	0·36 (0·08)	NS
Sorghum stover	4·0	8·0	5	0·76 (0·09)	0·87 (0·06)	NS
Triticale straw	1·6	3·2	5	−0·42 (0·18)	−0·22 (0·18)	**
Wheat straw	6·4	12·8	4	0·27 (0·03)	0·34 (0·04)	NS

[a] Values express the nitrogen fractions as a percentage of total nitrogen after 24 h incubation.
[b] Figures in parentheses indicate standard deviations; BRF, buffered rumen fluid; *n*, number of *in vitro* runs for each BRF system.

the requirement of the animal, the utilisation of these roughages can be improved by supplementation with urea (Campling *et al* 1962; Pal and Negi 1977; Dias-da-Silva and Sundstol 1986) or cultivated forages (Getachew *et al* 1994).

Protease, buffer and pepsin solubility of nitrogen

Enzyme, pepsin and buffer solubility of nitrogen in low quality feeds are given in Table 4. Protease and buffer solubility of N in grass hay and rice straw found in this study were similar to the findings of Krishnamoorthy *et al* (1995). Protease solubility of rice straw varied from 59·6 to 68·7% (Krishnamoorthy *et al* 1995). The correlation between protease solubility and the IVRDN was not significant ($P > 0{\cdot}05$) using IVRDN in 30 and 40 ml BRF medium. Solubility of feed N by protease is considered as rumen degradable N (Krishnamoorthy *et al* 1983), but the insignificant correlation between IVRDN and solubility of N by protease observed in the present study do not support this contention for low quality roughages. ADF-N and pepsin solubility were significantly negatively correlated ($P < 0{\cdot}05$; $r^2 = 0{\cdot}57$). Pepsin solubility of N from low quality roughages ranged from 60 to 72% which were higher than the value obtained by Negi *et al* (1988a) for roughages but within the range with those obtained for cultivated forages (Negi *et al* 1988b). Pepsin solubility was well correlated with the IVRDN values in 30 ml ($P < 0{\cdot}05$; $r^2 = 0{\cdot}71$) and in 40 ml systems ($P < 0{\cdot}05$; $r^2 = 0{\cdot}70$). Procedures involving the use of enzyme solubility offer potential advantage over other techniques, particularly in terms of cost and speed of operation (Cottrill 1983), however the results from the protease technique were not consistent (Laycock *et al* 1985).

Buffer solubility of N ranged from 32 to 63%. Buffer solubility of N from grass hay (32%) was in close agreement with value obtained with Timothy grass (Krishnamoorthy *et al* 1982). In phosphate hydrogen carbonate buffer the solubility of N from Guinea grass hay, Timothy hay, tall Fescue hay, rice straw and corn stover were 39, 26, 26, 41 and 43%, respectively (Krishnamoorthy *et al* 1982). The buffer-soluble fraction contains non-protein nitrogen but also protein to a varying degree while the buffer insoluble fraction contains rumen degradable proteins and totally unavailable nitrogen for the rumen microorganisms (Krishnamoorthy *et al* 1982). Stern and Satter (1984) found poor correlation between protein solubility in buffer solution and *in vivo* measurements of protein degradation in the rumen. Cottrill (1993) reported a strong relationship between the solubility of protein in phosphate buffer and its degradability in the rumen only for short term incubation times. A positive relationship ($r = 0{\cdot}79$) was observed between N solubility and N disappearance from nylon bags at 1 h of incubation and as incubation time increased, the correlation progressively decreased (Stern and Satter 1984). This could probably be due to the differences in proportions of N fractions which vary considerably in their rates of degradation. The lack of correlation between N solubility and *in vitro* degradability of N observed in this study and inconsistent relationships reported by other workers (Nocek *et al* 1979; Stern and Satter 1984; Negi *et al* 1988a; Cottrill 1993) indicate that determination of nitrogen solubility as an indicator of degradable nitrogen appears to be of limited potential in predicting rumen degradability of N in low quality feeds.

Determination of rumen degradability of N is of considerable nutritional significance in establishing nitrogen availability to sustain optimal microbial environment, and in turn influencing the amount of nitrogen that potentially could be digested in the lower gut. Rumen degradable protein determination will also be useful in developing sound supplementation strategies and to increase the efficiency of utilisation of low quality roughages. The *in vivo* method, in addition to being tedious, is limited by inaccurate differentiation between proteins of feed and microbial origin (Broderick 1987). In view of the limitation of the nylon

TABLE 4
Solubility of nitrogen in low quality roughages[a]

Feeds	*Protease solubility*[b]	*Buffer solubility*[b]	*Pepsin solubility*[b]
Barley straw	0·64	0·35	0·49
Grass hay	0·72	0·32	0·65
Millet stover	0·64	0·44	0·64
Oat straw	0·69	0·45	0·43
Rice straw	0·66	0·39	0·59
Sorghum stover	0·71	0·63	0·84
Triticale straw	0·66	0·38	0·49
Wheat straw	0·60	0·41	0·52

[a] Values are average from two replicates.
[b] Proportion of the total nitrogen incubated.

bag technique for evaluation of N degradation from low quality feeds, the gas method together with measurement of NH_3-H in the medium can be used for a quick assessment of rumen degradability of N from these feeds on a relative basis and it appears to provide results in reasonable accord with corrected (for microbial contamination) values from nylon bag technique (Negi *et al* 1988a). The method can also be combined with estimation of organic matter digestibility and metabolisable energy estimations (Menke and Steingass 1988), and estimation of *in vitro* apparent and true digestibility (Blümmel and Becker 1996) of feeds and therefore it offers significant time and cost savings over nylon bag technique. Although no difference was found in standard deviation by increasing the amount of sample, larger sample and the use of 40 ml buffered rumen fluid have an advantage over the smaller amount in that it allows concomitant determination of *in vitro* apparent and true digestibility.

ACKNOWLEDGEMENT

One of the authors (GG) is grateful to the DAAD (Deutscher Akademischer Austauschdienst) for the financial assistance during the course of this work.

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