PURINE NITROGEN INDEX, POTENTIALLY A NEW PARAMETER FOR RAPID FEED EVALUATION IN RUMINANTS

X. B. CHEN[1], D. B. SUBBA[2], E.R. ØRSKOV[1], M.C.N. JAYASURIYA[3]

[1] Rowett Research Institute,
Bucksburn,
Aberdeen, United Kingdom

[2] Pakhribas Agricultural Centre,
Dhankuta,
Kathmandu, Nepal

[3] Animal Production & Health Section,
International Atomic Energy Agency,
Vienna, Austria

Abstract

PURINE NITROGEN INDEX, POTENTIALLY A NEW PARAMETER FOR RAPID FEED EVALUATION IN RUMINANTS.

The concept of a new parameter 'Purine Nitrogen Index (PNI)' for feed evaluation in ruminants is discussed. PNI refers to the ratio of purine derivative (PD) nitrogen to total nitrogen in urine. It is suggested that PNI can potentially be used as an indicator of the efficiency with which degradable dietary nitrogen is converted to microbial protein in the rumen. The excretion of PD in the urine provides an estimation of the intestinal flow of microbial protein, and therefore, PNI effectively corresponds to the amount of microbial protein produced in the rumen relative to the nitrogen loss in the urine. If a diet or a dietary regime has a high conversion efficiency, proportionally more rumen degradable nitrogen is converted to microbial protein and less nitrogen is excreted in the urine, resulting in a high PNI. Conversely, if a diet has a poor conversion efficiency, proportionally less dietary nitrogen is converted to microbial protein and more is excreted in the urine, resulting in a low PNI. Preliminary data from six experiments involving 34 sheep confirmed a positive correlation between PNI and the nitrogen conversion efficiency, and suggested that a dietary regime with a PNI lower than 0.08 for sheep appeared to be a less efficient in the production of microbial protein and have a greater loss of nitrogen in the urine. PNI can theoretically be determined in spot urine samples, and has the potential to serve as a 'dipstick' method for the rapid evaluation of ruminant feeds. However, more research with a mathematical modelling approach is required to evaluate and develop the concept further.

1. INTRODUCTION

During microbial fermentation, part of the dietary nitrogen is converted to NH_3, a proportion of which is captured by rumen micro-organisms for the synthesis of microbial protein. The remaining NH_3 is absorbed from the rumen and is finally excreted, as a source of nitrogen, in the urine. Microbial protein is an important source of protein for ruminants. The efficiency with which rumen degradable nitrogen (RDN) in the diet is converted to microbial protein determines the overall utilisation efficiency of ruminant diets on one hand and the loss of nitrogen in the urine on the other. A technique which provides a rapid indication of this efficiency is desirable and will benefit future feeding systems with an orientation for improved biological efficiency and reduced waste secretion to the environment.

If a diet or a dietary regime has a high nitrogen conversion efficiency (NCE), more microbial protein is produced and less nitrogen is excreted in the urine. Therefore, the ratio of intestinal flow of microbial protein nitrogen to urinary nitrogen excretion (MN:UN ratio) will

be higher and *vice versa*. With the assumption that other factors (e.g. protein degradability, intake of undegradable dietary protein) remain unchanged, it is expected that the MN:UN ratio is positively correlated with the NCE. This ratio, however, is difficult to measure.

Urinary PD refer to the sum of allantoin, uric acid, xanthine and hypoxanthine excreted in the urine. The excretion of PD provides an indirect measurement of the intestinal flow of microbial protein [1]. Results of recent work [2-4] showed that the estimates of microbial protein nitrogen based on PD excretion were in close agreement with the direct measurements using microbial markers. Therefore, by replacing the term 'microbial protein nitrogen' (MN) in the expression 'MN:UN' with PD nitrogen (PDN), the ratio of PDN:UN should also be correlated with NCE. This ratio is hereafter referred to as "Purine Nitrogen Index" (PNI). Therefore, PNI refers to the proportion of total urinary nitrogen that is present in the form of PD.

PNI can be easily determined. Moreover, since it is a ratio of two chemical components in the urine, it can theoretically be determined from spot urine samples provided that there is little diurnal variation, a feature required for applications under farm conditions. If the relationship between PNI and NCE is established, determination of PNI from urine samples may provide a rapid indication of the efficiency with which rumen RDN in the diet is converted to microbial protein.

The objectives of this work were to: examine the relationship between the PNI and NCE based on data generated from a range of experiments and assess the diurnal variability of PNI measurements based on spot urine samples to evaluate the feasibility for application under farm conditions when complete urine collection may not be feasible.

2. MATERIALS AND METHODS

Three experiments (Experiments I, II and III) were specifically conducted for obtaining information on PNI. Data were also collated from three experiments (Experiments IV, V and VI) previously conducted in our laboratory. Full details are provided for Experiments I, II and III but only a brief description of the treatments are provided for Experiments IV, V and VI since they have already been published elsewhere [1].

2.1. Animal experiments

2.1.1. Experiment I

A total of 12 female Finn/Dorset × Dorset sheep, approximately one year old and of average body weight of 51 ± 6 kg, were used. The sheep were randomly allocated into 4 groups of 3 each. The four groups of animals were fed with the following four diets: i) basal diet of grass cubes, primarily of rye grass, ii) basal diet supplemented with 8 g urea/d, iii) basal diet supplemented with 16 g urea/d and vi) basal diet supplemented with 173 g rolled barley DM/d. The intake of the grass cubes was identical in all 4 groups at 1000 g/d air dry weight (963 g DM/d). With the urea-supplemented diets, the required amount of urea was dissolved in minimum quantity (24 ml water) of water and sprayed on the grass cubes and well mixed, prior to feeding. The feed was offered in two equal meals twice daily, at 0800 and 1600 h, respectively. The grass cubes contained 909 g DM/kg air dry weight and 28.5 g N and 897 g OM, per kg DM. The estimated RDN contents of the four treatments were: 42.4, 51.0, 9.5 and 37.5 g RDN/kg digestible organic matter apparently fermented in the rumen (DOMR).

The animals were allowed an adaptation period of 10 (two groups) or 17 (other two groups) days before a 7-day measurement period. During the latter period, the animals were housed in metabolism cages for collection of urine and faeces. Total urine was collected at 24

h intervals between Day 1 and 5, and at 2 h intervals between days 6 and 7. The daily urine production was collected into plastic containers containing approximately 200 ml of 10% H_2SO_4, diluted to 5.5 litres with water and sub-sampled. The 2-hourly collection of urine was made with the aid of a fraction collector. Urine excreted was immediately delivered by a continuously running pump into bottles containing 20 ml 10% H_2SO_4 situated on the collector. The 2-h urine can be regarded as a spot urine sample. Sub-samples of both daily and spot urine were stored at -20 °C. Total faecal samples were collected for seven days. A 10% portion of the daily faecal output was kept and bulked at the end of period for each animal. The faecal samples were freeze-dried and stored until analysis.

2.1.2. Experiment II

The same 12 sheep used in Experiment I were used. At end of Experiment I, the sheep were randomly re-grouped into 4 groups of 3 each. They were allocated to one of the following 4 diets: i) basal diet of grass cubes (as in Experiment I), ii) basal diet supplemented with 82 g DM/d of pre-washed fishmeal, iii) basal diet supplemented with 164 g DM/d of pre-washed fishmeal and vi) basal diet supplemented with 346 g DM/d of rolled barley as used in Experiment I. The estimated RDN contents of the four treatments were: 42.4, 45.3, 48.0 and 34.7 g RDN/kg DOMR. The length of adaptation and measurement periods and procedures for urine collection and faecal sampling and processing were as in Experiment I.

2.1.3. Experiment III

Four female sheep (Suffolk cross) of average body weight 46 ± 1.4 kg were used. The animals were fed with a mixed diet containing 50% hay, 30% rolled barley, 10% molasses, 9% fishmeal and 1% minerals and vitamins (hereafter referred to as 'GP' diet). The diet contained 920 g DM/kg air dry weight and 20.2 g N and 925.9 g OM, per kg DM. The estimated RDN content was 23.3 g RDN/kg DOMR. The diet was offered at 3 levels, 800, 1200 and 1600 g/d (air dry weight) to 4 sheep, allocated according to a 3 × 4 design. Each period representing an intake level consisted of 10 days adaptation and 12 days collection. The feeding and housing conditions were similar to Experiment I and II. Total urine and faeces were collected at 24 h intervals. Procedures for urine collection (total only) and faecal sampling and processing were as in Experiment I.

2.1.4. Experiment IV

Nineteen crossbred Suffolk wether sheep with body weights ranging from 22 to 73 kg were all offered 820 g DM/d of the GP diet (same composition as in Experiment III but from a different batch of ingredients). The rumen digesta outflow rates in those animals varied due to different levels of feed intake relative to body weight. Experimental details have been previously presented [1].

2.1.5. Experiment V

Four Blackface × Suffolk wether sheep of average body weight 41 ± 1.5 kg were fed with ammonia-treated barley straw alone or supplemented with either sugar beet pulp or barley each at 20 or 40%. Experimental details have been previously presented [5].

2.1.6. Experiment VI

Five wether sheep of average body weight 58 ± 14.2 kg were given hay supplemented with urea plus either molasses or three levels of a rice polishings. Experimental details have been previously presented [6].

2.2. Measurements and calculations

In all six experiments, RDN intake, digestible organic matter intake (DOMI), and daily excretions of total urinary nitrogen and PD were measured. The microbial nitrogen production (i.e. intestinal flow of microbial protein nitrogen) was estimated based on daily output of PD.

The NCE was calculated as "microbial nitrogen production (g/d) expressed as a proportion of RDN intake (g/d)". The value may be greater than 1 when the RDN intake was low relative to that of DOMI, due to a net flow of urea-N from plasma to the rumen for conversion to microbial protein.

The efficiency of microbial protein supply (EMPS) was expressed as "microbial nitrogen production (g) per kg DOMR". DOMR was taken as 0.65 of the measured digestible organic matter intake. While NCE reflects how efficient RDN is used, EMPS reflects how efficient organic matter is used for synthesis of microbial protein.

2.3. Chemical analysis

Details of chemical analysis for Experiments I-III are described as follows, but those for Experiments IV-VI were as in the cited original publications [1, 5, 6]. Dry matter and ash contents of the diets and faecal samples were determined according to AOAC [7]. Nitrogen in the urine was determined using the method described by Davidson *et al.* [8]. Rumen degradability of OM and nitrogen was determined by incubating the feed samples in the rumen of three separate sheep according to the Nylon bag technique [9], and the effective degradability was calculated from the measured potential degradability assuming a rumen digesta outflow rate of 5%/h. Urinary PD were measured as the sum of allantoin, uric acid, xanthine and hypoxanthine. Allantoin was determined using a HPLC [10], and the other components using an Auto Analyzer [11]. Creatinine in urine was determined using the method of Larsen [12].

2.4. Statistical analysis

Analysis of variance was carried out to examine the effects of urea, barley and fishmeal supplementation (Experiments I and II) and the effect of intake levels on PNI and NCE. The possible relationship between the PNI and NCE was examined by regression analysis. The statistical work was aided with the computer program GENSTAT 5.

3. RESULTS

3.1. PNI, NCE and EMPS

3.1.1 Experiment I

Results are shown in Table I. There were significant differences ($P < 0.05$) in PD excretion and thus the estimated microbial nitrogen supply. NCE (ranging from 0.209 to 0.437) decreased significantly with urea supplementation. PNI ranged from 0.027 to 0.048. Urea supplementation at the higher level had a significantly lower PNI than the other treatments. Barley supplementation did not show a significant effect on either NCE or PNI. The EMPS decreased with high level of urea supplementation.

TABLE I. TOTAL PD, PNI, MN, EMPS AND NCE IN 12 SHEEP FED BARLEY AND UREA SUPPLEMENTS WITH GRASS CUBES AS BASAL DIET (MEAN OF 3 SHEEP IN A 5-DAY PERIOD)

Diet	DOMI (kg/d)	Total PD (mmol/d)	N-excretion (g/d)	Microbial protein nitrogen		PNI (fraction)	NCE (fraction)
				MN (g N/d)	EMPS (g N/kg DOMR)		
B1	0.620	8.73	10.18	7.6	19.5	0.048	0.367
C1	0.510	9.69	13.86	8.4	25.0	0.039	0.437
U1	0.470	8.15	12.99	7.1	22.5	0.040	0.292
U2	0.560	6.67	13.62	5.8	14.7	0.027	0.209
SED	0.046	1.68	1.23	1.58	5.69	0.010	0.078
F-test	P <0.001	NS	P <0.001	NS	P <0.05	P <0.05	P <0.001

3.1.2. Experiment II

Results are shown in Table II. NCE ranged from 0.291 to 0.373 and PNI from 0.042 to 0.051. Fishmeal or barley supplementation did not have significant effects on either NCE, PNI or EMPS.

TABLE II. TOTAL PD, PNI, MN, EMPS AND NCE IN 12 SHEEP FED BARLEY AND FISHMEAL SUPPLEMENTS WITH GRASS CUBES AS BASAL DIET (MEAN OF 3 SHEEP IN A 5-DAY PERIOD)

Diet	DOMI (kg/d)	Total PD (mmol/d)	N excretion (g/d)	Microbial protein nitrogen		PNI (fraction)	NCE (fraction)
				MN (g N/d)	EMPS (g N/kg DOMR)		
B2	0.760	9.69	11.59	8.2	18.0	0.047	0.373
C2	0.510	7.87	9.43	6.4	19.3	0.046	0.344
FM 1	0.480	7.47	8.48	6.1	19.4	0.051	0.317
FM 2	0.540	8.05	10.85	6.7	18.8	0.042	0.291
SED	0.031	1.67	1.02	1.40	4.08	0.007	0.062
F-test	P<0.001	NS	0.001	P<0.05	NS	NS	NS

3.1.3. Experiment III

Results are shown in Table III. PD excretion and thus the estimated microbial N supply increased significantly with level of intake. However, EMPS tended to be higher with the highest level of feed intake, but the difference was not significant. NCE ranged from 0.616 to 0.738 and PNI from 0.07 to 0.083.

TABLE III. TOTAL PD, PNI, MN AND NCE IN 4 SHEEP FED GP DIET AT 3 LEVELS OF INTAKE (MEAN OF 4 SHEEP AT 7 DAYS COLLECTION PERIOD)

Intake level	Treatment	DOMI	Total PD	N-excretion	Microbial protein nitrogen		PNI	NCE
		(kg/d)	(mmol/d)	(g/d)	MN (g N/d)	EMPS (g N/kg DOMR)	(fraction)	(fraction)
1	GP1	0.575	9.06	7.58	7.6	20.6	0.070	0.738
2	GP2	0.780	11.17	8.65	9.6	18.7	0.083	0.616
3	GP3	0.897	15.58	10.61	13.4	23.0	0.082	0.651
	SED	0.035	0.808	1.36	0.704	1.3	0.0108	0.031
	F-test	P<0.01	P <0.01	P <0.001	P <0.01	NS	NS	P <0.05

3.1.4. Experimentts VI-VI

The ranges of PNI, NCE and EMPS are listed in Table IV. All three variables had a larger spread within individual experiments than in Experiments I-III.

TABLE IV. THE RANGE OF NCE, PNI AND EMPS FOR DATA COLLATED FROM EXPTERIMENTS IV-VI

	Number of observations	NCE (fraction)	PNI (fraction)	EMPS (g N/kg DOMR)
Experiment IV	19	0.194 - 0.964	0.024 - 0.160	7.0 - 35.8
Experiment V	5	0.309 - 0.515	0.034 - 0.064	19.1 - 22.6
Experiment VI	4	0.854 - 1.034	0.095 - 0.118	16.2 - 18.6

The pooled data from all six experiments showed that PNI was positively correlated with NCE (Figure 1). Linear effect was significant (P <0.001), but quadratic effect was not.

$$PNI = -0.002\ (0.006\ se) + 0.130\ (0.010\ se)\ NCE\ (n = 39,\ R^2 = 0.811)$$

There was a trend for PNI to increase with the EMPS (Figure 2), but the data points were more scattered ($R^2 = 0.435$) than in Figure 1. Most of the data points had a EMPS values of 20-25 g microbial N/kg DOMR.

$$PNI = -0.0059\ (0.0137\ se) + 0.0037\ (0.0007\ se)\ EMPS\ (n = 39,\ R^2 = 0.435)$$

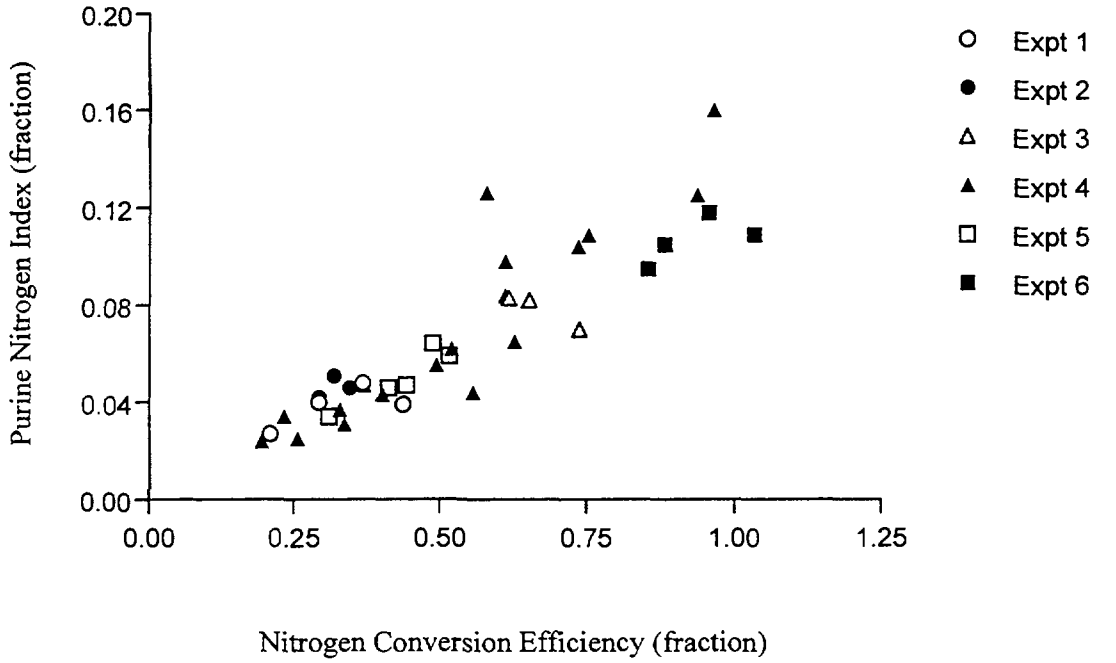

FIG.1. Pooled data from all six experiments showing the relationship of PNI to NCE.

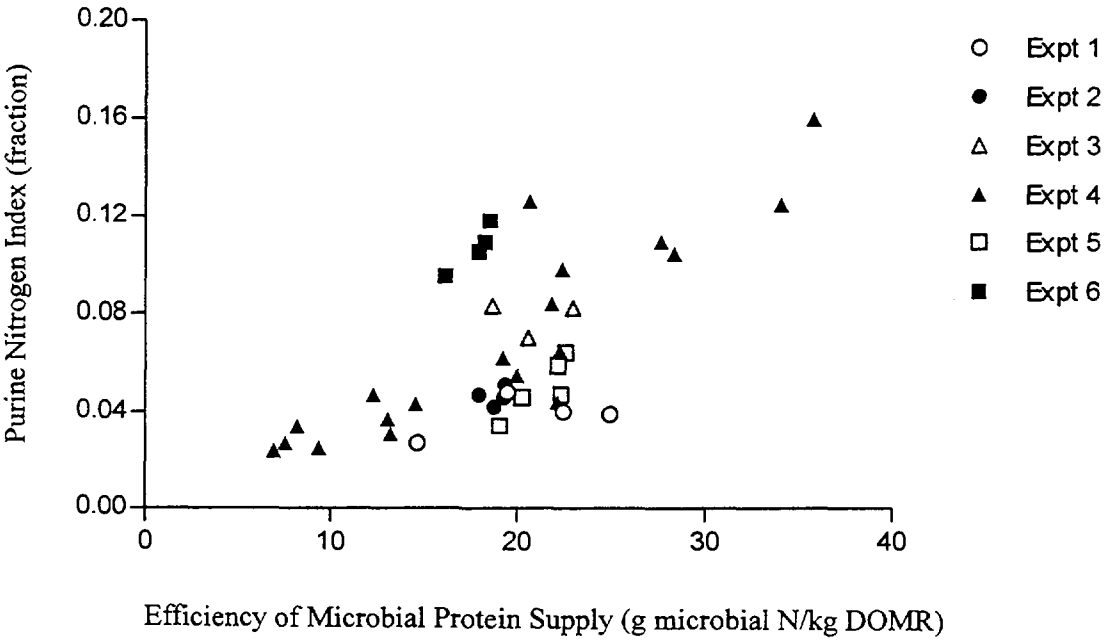

FIG.2. Pooled data from all six experiments showing the relationship of PNI to EMPS.

3.2. Diurnal variation in PNI

From the data (12 animals, each measured during two periods of 2-h urine collection for 24 hours) in Experiment I and II, there was no clear pattern of diurnal variation in PNI, although with urea and fishmeal supplemented diets, PNI decreased after feeding. However, the variability (in terms of coefficient of variation (CV) among the 12, 2-h measurements) was relatively large; CV averaged 19.7% (± 11.5) (n = 24). The ratio of PD:creatinine (mmol/mmol) was also measured. Its variability was much smaller (CV = 10.4% ± 4.0) than PNI (ratio of PD:N), indicating that N output in the urine was more variable than PD output.

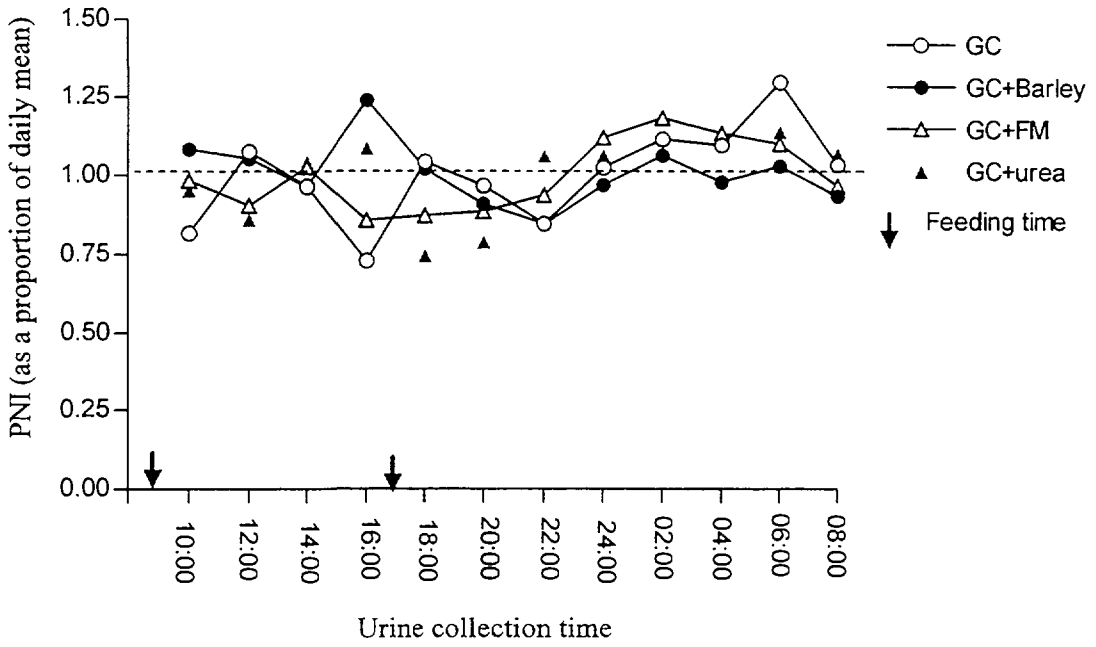

FIG.3. Diurnal variation in PNI as affected by dietary supplements.

4. DISCUSSION

4.1. Assumptions made in this work

In the six experiments reported here, microbial protein N production was not measured directly but estimated based on PD excretion. It can thus be criticised that the relationship between NCE and PNI has some element of auto-correlation since PD excretion as a variable is present in both terms. However, microbial protein N flow is an independent variable that can be measured by other methods, such as those based on ^{35}S or RNA as microbial markers. The concept of using PNI to indicate microbial N production relative to RDN intake should therefore still be valid.

The NCE values in this work should not be taken as absolute but as relative, again since microbial N production was not measured directly. Results of several studies have shown that there was a close agreement, and a linear relationship, between microbial protein nitrogen estimated by PD excretion and direct measurements based on isotopic and microbial markers [3, 4]. Therefore, the NCE thus calculated should be rather close to, or linearly correlated

with, the true values. Further experiments should be conducted in the future in which direct measurements of microbial N production are made to calculate NCE.

4.2. PNI and NCE relationship and other factors affecting PNI

As expected, over the NCE range of 0.19-1.03, PNI, which ranged from 0.024-0.160, was positively correlated with the NCE based on the pooled data of six experiments. Within Experiments 1, 2, 3 and 6 individually, this relationship could not be revealed since the ranges of either NCE or PNI were too small relative to the error of the regression.

The data from the six experiments showed that PNI was linearly correlated with the NCE without considering other factors. However, it is known that, apart from NCE, endogenous N output, nitrogen intake, protein degradability, digestibility of protein, and inefficiency of absorbed amino acids can all affect the value of PNI. The interrelationship between these factors are shown in Figure 4. In order to understand the intrinsic relationship between PNI and NCE, a mathematical modelling approach is required. Here we make some attempt to derive an equation that relates PNI and NCE.

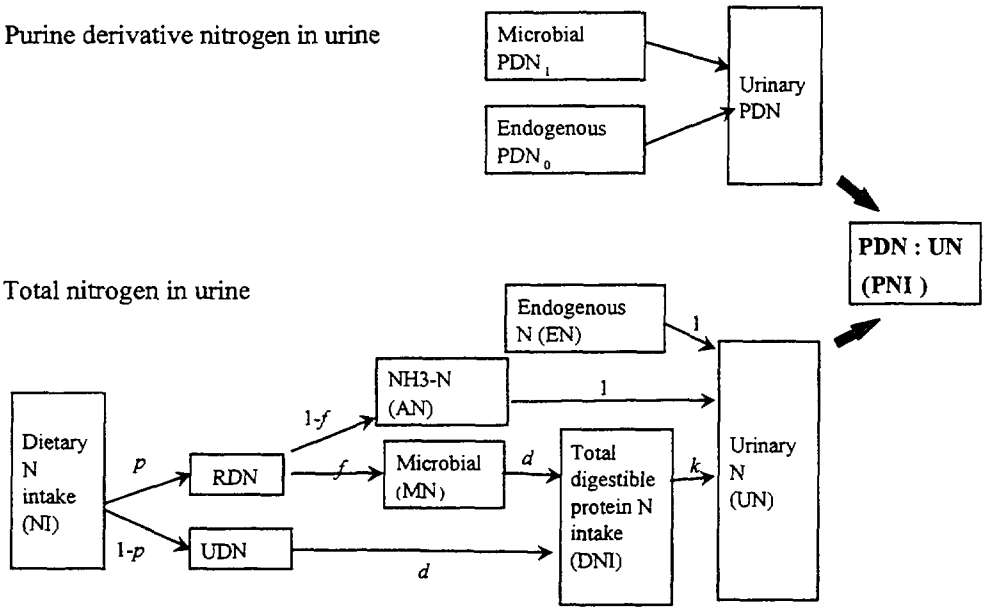

FIG.4. An illustration of factors affecting PNI.

Total nitrogen excretion in the urine (UN) is determined by endogenous nitrogen excretion (EN), intake and digestibility (d) of total protein, i.e. microbial protein (MN) plus un-degraded dietary protein (UDN), the proportion of the absorbed amino acids that is not retained but excreted in the urine (k) and absorbed ammonia nitrogen (AN). Here it is assumed that all of the AN is excreted in the urine and that MN and UDN have the same digestibility.

UN = EN + d k (MN+UDN) + AN .....(1)

Equation (1) can be re-arranged as:

$$1 = \frac{EN}{UN} + dk\frac{MN}{UN} + dk\frac{UDN}{UN} + \frac{AN}{UN}$$

The terms UDN/UN and AN/UN can be replaced by MN/UN, taking into account protein degradability (p), and NCE (f):

$$1 = \frac{EN}{UN} + dk\frac{MN}{UN} + dk\frac{(1-p)MN}{p \times f \times UN} + \frac{(1-f)MN}{f \times UN}$$

The above equation can finally be re-arranged as:

$$\frac{MN}{UN} = \left(1 - \frac{EN}{UN}\right) \times \left[\frac{pf}{(dk + p - dkp) + (dk - 1)pf}\right] \quad \text{.....(2)}$$

The urinary excretion of PD nitrogen is a function of the endogenous purine excretion and absorption of microbial biomass. Derived from the equations of Chen *et al.* [1, 13], PDN (g/d) in sheep can be expressed as a function of microbial N supply (MN, g/d):

$$PDN = 0.0647\ MN + 0.0084\ W^{0.75} e^{-0.34\ MN} \quad \text{.....(3)}$$

$$\text{Thus } PNI = \frac{PDN}{UN} = 0.0647\frac{MN}{UN} + \frac{0.0084W^{0.75}e^{-0.34MN}}{UN}$$

We are unable to derive a simple equation whereby PNI can be calculated. The following equation gives an approximation:

$$PNI \approx \left(1 - \frac{EN}{UN}\right) \times \left[\frac{0.0647pf}{(dk + p - dkp) + (dk - 1)pf}\right] + 0.024 \quad \text{.....(4)}$$

At an extreme situation where the animal does not have any exogenous input of nitrogen or microbial nitrogen,

PDN_0=0.0084 g/kg $W^{0.75}$ per day and

UN_0=EN = 0.350 g/kg $W^{0.75}$ per day [14].

$$\text{Thus } PNI = \frac{0.0084W^{0.75}}{0.350W^{0.75}} = 0.024$$

In Equation (4), the term $\left(1 - \frac{EN}{UN}\right)$ (referred to as A) represents the proportion of total urine nitrogen (UN) that is not endogenous. Its value increases with nitrogen intake. The value can not be determined easily but may be estimated based on the creatinine:N ratio at fasting (R_0) and in the same urine where PNI is calculated as (R_1): A = 1-R_1/R_0. In Equation 4, d, k and p are parameters that can be measured or already published in the literature. Figure 5 shows the simulated values of PNI at different A values and the observed values, both plotted against the observed NCE (f in the equations). The parameters used in the simulation were: d = 0.85 (from Storm *et al.* [15]), p = 0.80, k = 0.25 (calculated as 1-0.75, the later is reported value for the efficiency of utilisation of apparently digested amino acid nitrogen in the small intestine, recommended by ARC [14]).

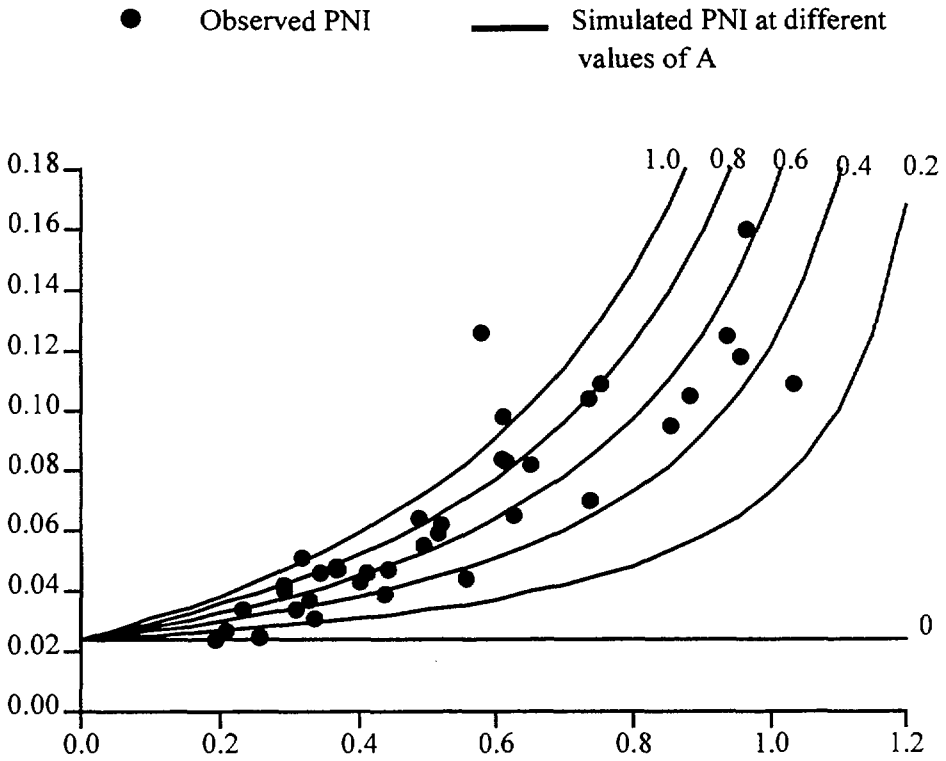

FIG.5. PNI values calculated based on Equation 4.

It should be stated that the mathematical modelling presented here is only tentative and has yet to be improved. However, the modelling exercise indicates that in order for the PNI to provide an indication of the NCE, other parameters such as, dietary protein degradability and dietary nitrogen intake, should also be considered or measured.

4.3. Changes in PNI in response to dietary treatments

The responses of PNI to dietary treatments in Experiments I and II were as expected. Urea supplementation at 16 g/d lead to an increased urinary nitrogen output and thus a lower PNI.

4.4. Use of spot urine samples

The feasibility of using PNI measurements in spot urine samples are subject to two criteria. First, there should be relatively little diurnal variability in the PNI. Second, the PNI measurements made based on spot samples must be correlated with those based on daily urine collection. Results from Experiments I and II can be used to evaluate the first criterion. The CV for the spot measurements was 20%, based on which the least significant difference (LSD) between two treatments can be estimated. If four spot measurements are made (n = 4) from each treatment, the LSD should not be lower 33% in order to reach statistical significance at $P < 0.05$ level. If three or two measurements are made, the LSD needs to be 42

and 64%, respectively ($LSD = t\sqrt{\frac{2}{n}} \cdot CV$, where t = the tabulated t distribution at (2n-1) degree of freedom; n = number of spot measurements from which the mean is derived). Although there is no clear pattern of diurnal variation, the data did indicate that PNI could be affected by time of feeding if the diet contains high content of a highly degradable N source. Taking this into consideration, multiple samples would be required in order to derive a measurement of PNI as representative as possible, and it is recommended to spread the sampling for different times after feeding. There is no appropriate data in this work to evaluate the second criterion. This is because in Experiments I and II when spot urine sampling was made, the ranges of the PNI values based on daily urine collection, and of those based on spot urine collection, were both too small to evaluate the correlation.

4.5. Potential application, limitations and future work

PNI is unique in that it provides an indication of efficiency at which RDN is converted to microbial protein, as well as the potential cost of N waste to the environment that a feeding regime may incur. PNI can be measured readily particularly if urine spot samples can be used.

PNI could be used as one of the criteria to help formulate ruminant diets that are biologically more efficient and produce less N waste. A practical application would be to set an empirical threshold criteria for a specific group of ruminants, and diets with PNI values lower than this threshold are graded as unsatisfactory with respect to N utilisation. For example, based on the preliminary data from this work, a diet with a PNI lower than 0.08 for sheep would seem unsatisfactory. In this system, PNI is effectively used as a semi-quantitative parameter and its application would not be detrimentally affected by the 20% variability from spot measurement. Therefore, where complete urine collection is not plausible, measurements of PNI may be made based on an incomplete but major fraction of the urine, or multiple spot urine samples. PNI may also be measured in digestion studies, the data of PNI would complement the data of efficiency of microbial N supply per kg organic matter fermented, and provide an indication of the efficiency of utilisation of nitrogen.

Measurement of PNI alone is however not sufficient to assess the scale of NCE as indicated in Equation (4). PNI measurements should therefore be used together with other parameters such as protein degradability and dietary nitrogen intake.

The limitations of the PNI are: i) the index does not offer any explanation as to the cause of poor efficiency, ii) the variations as noted in this work indicate that PNI is not sensitive for detecting small differences. The latter limitation could be overcome by using it in a 'grouping' system.

5. CONCLUSIONS

In this work, we conceptually propose a new parameter, PNI, which could potentially provide a simple and rapid means for assessing the efficiency of conversion of dietary nitrogen to microbial protein. Used in conjunction with other existing parameters, it would be particularly useful in research into improving ruminant feeding efficiency at the rumen level and reducing nitrogen waste to the environment. Some preliminary data are presented in this work, but further experiments with direct measurements of microbial nitrogen production need to be made to validate the concept and to provide information for application. The mathematical model which relates PNI with NCE and other parameters also needs further development.

REFERENCES

[1] CHEN, X.B., CHEN, Y.K., FRANKLIN, M.F., ØRSKOV, E.R. SHAND, W.J., The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep, J. Anim. Sci. **70** (1992) 1534-1542.

[2] OOSTING, S.J., VAN BRUCHEM, J., CHEN, X.B., Intake, digestion and small-intestinal protein availability in sheep in relation to ammoniation of wheat-straw with or without protein supplementation, Br. J. Nutr. **74** (1995) 347-368.

[3] SAMANIEGO, M.A., CHEN, X.B., HOVELL, F.DEB., MACRAE, J.C. MAYES, R.W., Comparison of methods for the measurement of microbial protein supply in sheep, Proc. Br. Soc. Anim. Sci. (1997) 125A.

[4] PÉREZ, J.F., BALCELLS, J., GUADA, J.A., CASTRILLO, C., Determination of rumen microbial-N production in sheep. A comparison of urinary purine excretion with methods using ^{15}N and purine bases as markers of microbial-N entering the duodenum, Br. J. Nutr. **75** (1996) 699-709.

[5] CHEN, X. B., ABDULRAZAK, S.A., SHAND, W.J. ØRSKOV, E.R., The effect of supplementing straw with barley or unmolassed sugar beet pulp on microbial protein supply in sheep estimated from urinary purine derivative excretion, Anim. Prod. **55** (1992) 413-417.

[6] CARDENAS, D., NEWBOLD, C.J., GALBRAITH, H., TOPPS, J.H., CHEN, X.B. ROOKE, J.A., Rice polishing as an alternative to sugar cane molasses as a supplement with urea to low quality forage diets for ruminants, Anim. Prod. **56** (1993) 85-92.

[7] ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, Official methods of analysis. 13th edition, Association of Official Analytical Chemists, Washington, DC, USA (1980).

[8] DAVIDSON, J., MATHIESON, J., BOYNE, A.W., The use of automation in determining nitrogen by the Kjeldahl method with final calculation by computer, Analyst **95** (1970) 181-193.

[9] ØRSKOV, E. R., HOVELL, F. D. D. MOULD, F. L., The use of nylon bag technique for the evaluation of feedstuffs, Trop. Anim. Prod. 5 (1980) 195-213.

[10] CHEN, X.B., KYLE, D.J.. ØRSKOV, E.R., Measurement of allantoin in urine and plasma by high-performance liquid chromatography with pre-column derivatization, J. Chrom. **617** (1993) 241-247.

[11] CHEN, X.B., MATHIESON, J., HOVELL, F.D.DEB., REEDS, P.J., Measurement of purine derivatives in urine of ruminants using automated methods, J. Sci. Food Agric. **53** (1990) 23-33.

[12] LARSEN, K., Creatinine assay by Reaction-kinetic principle, Clinical Chimica Acta **41** (1972) 209-217.

[13] CHEN, X.B., HOVELL, F.D.DEB., ØRSKOV, E.R., BROWN, D.S., Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid supply on purine derivative excretion by sheep, Br. J. Nutr. **63** (1990) 131-142.

[14] AGRICULTURAL RESEARCH COUNCIL, The Nutrient Requirements of Ruminant Livestock, Supplement No 1, Commonwealth Agricultural Bureaux, Slough, UK. (1984).

[15] STORM, E., ØRSKOV, E. R. SMART, R., The nutritive value of rumen micro-organisms in ruminants.2.The apparent digestibility and net utilization of microbial N for growing lambs, Br. J. Nutr. **50** (1983) 471-478.