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

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DECLARATION

This is to declare that this thesis has been composed by myself and has not been accepted in any previous application for a degree. It is a true record of work carried out by myself unless otherwise stated. All sources of information have been duly acknowledged by means of references.

……………………

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DEDICATION

In memory of my late father *Bhagiman Limbu* whose blessings are with me forever. May his departed soul rest in peace.

(Born 1979 BS-Departed 2049 BS)

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SUMMARY

Efficacy of the Purine Nitrogen Index (PNI) as a possible tool to measure efficiency of conversion of dietary nitrogen into microbial protein was investigated. Experiment 1 and 2 were conducted in 12 sheep, each in a completely randomised experimental design. An adaptation period of 10 days was followed by an experimental period of 7 days (5d for daily and 2d for 2 hourly spot urine collections).

Experiment 3 was conducted in 4 sheep in a 3x4 Multiple Latin Square Design. The total collection was 12 days for each period with 3 experimental periods in total with 10 days adaptation between each period.
No spot urine collection was made.

Diets were formulated with differing rumen degradable nitrogen at 2 or 3 levels of intake. Urea was supplemented at two levels (U1, U2) and barley (B1) in Experiment 1. In Experiment 2, the treatment diets were fish meal at two levels (FM1 and FM2) and Barley (B2). Grass cubes were the control (basal diet) in both experiments. Intake of the control diet was 1kg/sheep/d. The supplements were 8g, 16g, 50g, 100g, 200g and 400g per day for U1, U2, FM1, FM2, B1 and B2 diets.

The treatment diet in the 3rd experiment was GP (general purpose diet) at 3 levels of intake (1, 2 and 3) which were 800, 1200 and 1600g/sheep/day respectively.

The PNI was calculated from the Purine Derivative Nitrogen (PDN) and the total urine nitrogen (UN) as the proportion of PDN to the total UN. Nitrogen Capture Efficiency (NCE) was calculated from the Microbial Protein Supply (MN) and the Rumen Degradable Nitrogen (RDN) as the proportion of MN to the RDN. The MN was estimated based on the purine derivative method.

Relating, PNI with NCE in the daily urine samples, it was observed that the distribution of data from individual experiments were not well spread and there was no clear difference between the data from each treatment. However, when the 3 experiments were combined, there was a clear difference between the efficient and inefficient diet. The PNI of the combined data sets were found to be positively correlated (r^2 = 0.50) with NCE. In terms of efficiency of conversion, GP was better than the grass cube based diets. The NCE was 74% and 20 – 44% in the GP and grass cube based diets respectively.

No evidence of the relationship between the PNI and the Efficiency of Microbial Protein Supply was found.

No significant difference ($P>0.05$) was seen between the treatment means of the

daily collection with the total PNI per day of the spot collections, when a comparison was made between the same group of sheep in the same treatment diets. The coefficient of variation of PNI in the spot urine sample was found to be as high as 29 %.

Comparison was made between the PNI of the total daily collections with the spot collections. It was seen that the spot samples taken between 3 to 5 hrs post feeding gave as a good relationship as when they were combined. However, the relationship was not strong ($r^2 = 0.25$). Therefore, to derive a better estimate of PNI of a day, further work collecting samples at the same time over a number of days is suggested.

LIST OF ABBREVIATIONS

Abbreviation	Full term
DM	Dry matter (%)
DOMI	Digestible Organic Matter Intake (kg/d)
DOMR	Digestible Organic Matter in Rumen (g/d)
EMPS	Efficiency of Microbial Protein Supply (g N/kg DOMR)
GP	General Purpose Diet
MN	Microbial Protein Supply (g N/d)
N	Nitrogen (%)
NCE	Nitrogen Capture Efficiency (fraction)
OM	Organic Matter (%)
PD	Total Purine Derivatives (mmol/d)
PDN	Purine Derivative Nitrogen (mg/l)
PNI	Purine Nitrogen Index (fraction)
UN	Total Urine Nitrogen (mg/l)

CHAPTER I

INTRODUCTION

It is reported that only about 30% of the total protein consumed by ruminant animals in the UK is converted into meat and milk (Anon, 1995) showing that the ruminant production system is inefficient, particularly in an intensive animal production system. The 'inefficient' utilisation indicates wastage of a large quantity of nutrients, particularly nitrogen, adding to the cost of production and ultimately leading to environmental pollution. Effective synergism between nutrients to produce the desired level of animal production will not only be welcomed in terms of cost effectiveness, it will also keep the process within the current global thoughts of the protection and management of environmental pollution. Considering these factors, animal feed resources need to be utilised at maximum efficiency with minimum or no wastage of nutrients, nitrogen in particular.

The excretion of urinary and faecal nitrogen varies depending upon the dietary nitrogen supply. Therefore, nitrogen over feeding should be reduced and the high animal production needs to be targeted with minimum or no wastage of nutrients. Recent economic studies have also shown interest in increasing the productivity per animal and thus reducing the number of animals (Doreau *et al.*, 1991). In addition, the product quality is now given greater consideration, as consumers are encouraged to eat less fat. Such an approach could only be possible if the dietary

nutrients are efficiently utilised for a desired level of out-put.

Therefore, to maximise the efficiency of animal production, requires the knowledge of the requirement of different nutrients and availability of these nutrients to the animal. Thus, investigation of protein requirement has become one of the important areas of study, which necessitates the estimation of the amount of protein that is digested and absorbed from the intestine.

In order to establish the amounts and ratios of nutrients necessary for optimal microbial and animal response one must first adequately predict the degree to which nutrients are made available in the rumen from a variety of ingredient sources (Nocek, 1988).

In ruminant animals the protein is supplied from the microbial protein and the dietary protein escaping degradation in the rumen. However, the microbial protein forms a major source of protein to the host animals under normal feeding condition. To effectively understand the process of ruminal demand and supply of nutrients to the host animal, there is a need to quantify the microbial protein production. Hence, to quantify microbial protein production and to understand the nutrient needs of animals to maximise the efficiency of animal production, there is a need of development of simple and rapid evaluation methods.

Several new systems of protein evaluation including metabolisable protein system

(AFRC, 1992) have been employed and some are still being developed. Most of the existing methods that are used to measure microbial protein supply are microbial markers. This technique requires ruminally and post ruminally fistulated animals. Furthermore, the processes of utilising markers are complex, tedious and difficult to practice extensive *in vivo* studies.

Chen, *et al.,* (1990d) have greatly simplified the use of urinary purine derivatives as an estimate of intestinal flow of microbial protein. Recently, various workers (Verbic, *et al.*, 1990; Susmel *et al.*, 1994 and Djouvinov and Todorov, 1994) have suggested that the purine derivatives which includes xanthine, hypoxanthine, allantoin and uric acid in urine could serve a simple method of quantifying the intestinal flow of microbial protein to the animal. More recently, Samaniego (1996) showed a good correlation (r^2=0.97) between the purine derivatives and RNA marker method. This has indicated that the measurement of purine derivatives in urine can be a reliable method to estimate microbial supply in ruminant animals. The recent studies (XB Chen, unpublished data) suggested that 'Purine Nitrogen Index' which measures the purine derivative nitrogen as a proportion of total nitrogen in the urine, could indicate the Nitrogen Capture Efficiency (NCE) in the rumen. The amount of purine derivative nitrogen reflects the amount of microbial protein produced in the rumen and supplied to the animal. The NCE, which is expressed as the production of microbial nitrogen as a proportion of the dietary rumen degradable nitrogen, determines the overall efficiency of utilisation of ruminant diets.

In the present work, the efficiency of purine nitrogen index as a method to evaluate the ruminant feed has been studied to provide information for the extension of the use of the purine derivatives method to field situations.

CHAPTER II

LITERATURE REVIEW

2.1 Fate of dietary protein and its inter-conversion to microbial protein

Protein is the most abundant source of nitrogenous compounds, and substantial amounts of nitrate and ammonia may be present depending on the diet. Other nitrogenous additions to the rumen fermentation could be non-protein nitrogen (NPN) in the form of urea.

In the rumen, dietary protein undergoes hydrolysis by rumen microbial enzymes releasing oligo-peptides which are then broken down into smaller peptides and, finally to amino acids. Whether or not peptides accumulate in rumen fluid depends on the nature of the protein (Wallace *et al.*, 1990). Ammonia, simple peptides and free amino acids are utilised by the micro-organisms to synthesise microbial protein. In the small intestine the microbial cells are broken down to amino acids which are absorbed in the intestinal walls and utilised by the host animals as source of amino acids.

Ammonia, in the rumen, is the key intermediate in the microbial degradation and also the main substrate for microbial protein synthesis. The inter-conversion of dietary protein to microbial protein that occurs in the rumen is by microbial enzymes. The endogenous urea and salivary mucoprotein will only be quantitative

significance when dietary protein supply is low. If protein degradation exceeds nitrogen capture by rumen micro-organisms, ammonia will accumulate in the rumen. The absorbed ammonia is then carried to the liver where it is converted to urea. Some of this urea may be returned to the rumen via saliva and also directly by diffusion across the rumen wall. However, the greater part of it is excreted in the urine. Therefore, in these situations, the dietary protein nitrogen is thus used inefficiently.

Dietary protein is usually hydrolysed rapidly in the rumen, the precise rate and extent of break down depends on a number of factors, which ultimately determine the nutritive value of the protein. Attempts can then be made to increase nutrient availability for production by increasing feed intake, optimising ruminal fermentation, and supplementing nutrients to the diet that will escape ruminal degradation.

2.2 Factors affecting microbial protein production

Protein is synthesised in the gut in the form of micro-organisms. Microbial protein, which is subjected to extensive digestion within the intestine of ruminants, is the principal source of amino acids for ruminants. According to AFRC (1992) the digestibility of microbial protein is 75% and the proportion of microbial protein as true protein is 85%. The necessary energy is derived from plant polysaccharides such as cellulose and the nitrogen is derived from ammonia

in the rumen. The greater part of the protein reaching the small intestine is the microbial protein. It is estimated that between 40 to 80% of the total flow of the protein reaching to intestine are from microbial protein (McDonald *et al.*, 1995), the remainder being undegradable and also some endogenous proteins.

The efficiency of protein utilisation in the ruminants depends on the catabolic and synthetic activities of the rumen micro-organisms. For high efficiency the break down products of dietary protein and other nitrogenous compounds must be rapidly synthesised into microbial protein in the rumen.

The conversion of ammonia to microbial protein as a proportion of rumen degradable dietary nitrogen is measured by nitrogen capture efficiency, which determines the efficiency of utilisation of ruminant diets. The efficiency of microbial protein production is expressed as 'g' of microbial nitrogen per kg digestible organic matter fermented in the rumen (DOMR). Based on the literature, the production of microbial protein varies by about four fold, the values ranging from about 14 to 60 g/kg DOMR. This large variation is probably due to the influence of various factors relating to the diet or the rumen environment.

Any increase in the efficiency of microbial growth in the rumen in protein deficient animals is therefore highly desirable as it will reduce the requirement for dietary protein and increase the efficiency of feed utilisation. Furthermore, any manipulation of rumen fermentation that increases the supply of dietary or microbial protein to the intestine has the potential to increase efficiency of feed

utilisation by ruminants and thereby to increase production.

Fermentation or microbial protein production in the rumen is influenced by pH and turn over rate of rumen contents (Hoover and Stokes, 1991). Microbial protein production is therefore, affected by chemical, physiological and nutritional characteristics and the interactions between the nutrients.

Early workers have suggested that protein breakdown in the rumen is proportional to the solubility, but subsequent research has shown that other properties are also important, such as the variation in ruminal availability of nutrients which can have a major impact on the efficiency of microbial protein synthesis in the rumen (Wallace *et al.,* 1990).

In order to achieve a greater amount of production of microbial protein, it is necessary to create a condition where microbial protein synthesis takes place at maximum. Factors that may effect microbial protein production are:

a) Carbohydrate or energy source

Stokes *et al.,* (1991) in a study in cows indicated that non-structural carbohydrate (>24%) and rumen degradable protein (>9%) of DM increase microbial protein flow. Chamberlain *et al.,* (1993) on their study on the effects of purified carbohydrate, found sucrose superior to starch as an energy source for the fixation of nitrogen in the rumen. Similarly, according to Gomes *et al.,* (1994), the microbial protein production will be increased on the use of rapidly fermentable

carbohydrate. The carbohydrate portion of the diet increases the ratio of propionic acid to acetic acid, which is associated with increased protein synthesis (Meyer *et al.,* (1986). However, Voigt *et al.,* (1993) were of the view that the net synthesis of microbial protein in the rumen is not only the result of substrate fermentation but the passage of non-apparently fermented organic matter from the rumen significantly affects energy efficiency of microbial nitrogen synthesis and duodenal supply of amino acids. Also, some workers (Birds *et al.,* 1990) have found an inverse relationship between microbial cell growth and VFA production in the rumen. Topps and Elliot (1965) indicated that both excretion of purine derivatives by sheep and the level of nucleic acids in the rumen were highest when they were given the high energy diets. These diets, which were rich in readily fermentable carbohydrate, would encourage multiplication of micro-organisms in the rumen, which in turn would cause high levels of ruminal nucleic acids.

Carbohydrates provide carbon and energy as ATP for ruminal synthesis of protein. Primarily the amount and rate of hydrolysis of carbohydrate regulate microbial metabolism in the rumen. However, the hydrolysis of which will depend on the physical and chemical form of carbohydrate. Chen *et al.,* (1992) recently showed no difference between the carbohydrate sources, at a restricted intake levels in the efficiency of microbial protein synthesis in sheep.

Dietary fat is energy dense but does not provide fermentable energy for microbial growth in the rumen (Stern *et al.*, 1994). Addition of fat to the diet can affect microbial protein flow out of the rumen by altering the quantity of carbohydrate in

the diet, the fermentability of carbohydrates, and possibly the amount of nitrogen incorporated into microbial protein per unit of fermented carbohydrate. Diet containing increase levels of fermentable carbohydrate reduces the rumen pH and the reduction in rumen pH level has profound effect on the rumen microbial ecology (Hungate, 1966).

The efficiency with which nitrogen is captured by the rumen micro-organisms depends not only upon the speed and extent of breakdown but also provision of utilisable source of energy for microbial protein synthesis (McDonald *et al.,* 1995). As earlier explained, the excess ammonia is absorbed and excreted as urea in urine.

The efficiency of production of microbial protein is therefore related to the energy of the diet stated in terms of digestible organic matter, digestible organic matter digested in the rumen, Total digestible nutrient (TDN), metabolisable energy (ME), fermentable organic matter (FOM) and fermentable metabolisable energy (FME). FOM and FME do not use energy from fat and volatile fatty acids (AFRC, 1992), as they do not supply adenosine tri-phosphate (ATP) to the rumen microbes.

b) Protein Source

Microbial protein synthesis in the rumen requires specific nutrients such as sulphur, branched chain fatty acids and trace nutrients. However, under most dietary conditions, nutrient supply to the microbes is considered largely in terms

of ruminal availability of nitrogen and carbohydrate that can be fermented in the rumen to provide carbon and energy in the form of ATP for microbial protein synthesis.

Cecava *et al.*, (1991) found that the inclusion of ruminally degradable protein in the diet to increase efficiency of microbial protein synthesis and microbial protein flow to the small intestine. Merry *et al.*, (1990) in a study on *in-vitro* continuos culture studies on the effect of nitrogen source on rumen microbial growth and fibre digestion found that the efficiency of microbial protein synthesis was unaffected by the type of rumen degradable nitrogen source. Cecava and Parker (1993) were of the view that the source of supplemental crude protein had a greater effect on the quantity than on the profile of absorbable amino acid supplied to the duodenum.

Ruminal degradation of protein from dietary feed ingredients is one of the important factors influencing the supply of nitrogen for the synthesis of microbial protein. The degradation of which determines the availability of NH_3, amino acids, peptides and branched chain volatile fatty acids that influence microbial growth rates in the rumen. Stern *et al.,* (1994) reported that the rate and extent of ruminal degradation not only affect microbial protein synthesis but also the quantity and quality of undegraded dietary protein that reach the duodenum. The extent to which protein is degraded in the rumen depends primarily upon microbial access to the protein and ruminal retention time of the protein. Other factors influencing protein degradation include protein solubility and ruminal pH.

Supplementation of low quality diet with protein sources like urea-N to the animals is useful at certain levels only to optimise the rumen function, however excretion of urea-N will increase in response to further levels of nitrogen supplementation (Table 1).

Table 1. Daily urinary excretion of N compounds and purine derivatives (mg/kg $W^{0.75}$/day) in sheep fed on NaOH treated : straw supplemented by continuous infusion of urea (mean values for 5 sheep).

	Level of urea-N supplementation (g/d)				
	0	3	6	9	12
N – total (mg/kg $W^{0.75}$)	74	114	221	310	486
Urea (mg/kg $W^{0.75}$)	6.3	14.2	82.2	144	307
NH_3 (mg/kg $W^{0.75}$)	4.5	9.5	27.1	31.7	28.9
Purine derivatives					
Allantoin (mg N/kg $W^{0.75}$)	26.9	44.1	58.3	57.4	66.4
Uric acid (mg N/kg $W^{0.75}$)	6.3	7.6	7.1	7.4	7.4
Hypoxanthine (mg N/kg $W^{0.75}$)	4.9	5.6	5.1	5.8	5.2

Source: Balcells *et al.,* (1993)

Supplements containing intestinally digestible protein that escapes rumen degradation have frequently been shown to increase animal production (Bird *et al.*, 1990). However, the responses to protein supplements are not always consistent, probably due to the protein quality and its degradability in the rumen. Also, this may be due to the variable supply of microbial protein from the rumen. The variability may also be associated with the physiological state of the animal and the thermal environment (Bird *et al.,* 1990).

There are also reports of increased production of microbial protein when rumen microbes were supplied with some preformed amino acids as compared to the supply of non-protein nitrogen (NPN) only (Argyle and Baldwin, 1989 and Maeng *et al.*, 1976). However, Chikunya *et al.,* (1996) indicated that rumen microbial growth responds to pre-formed amino acids only when the energy source is fermented rapidly.

c) Energy and nitrogen synchronisation

Energy and Nitrogen are the major nutrients supporting microbial growth, the imbalance of which will limit microbial growth and ultimately affect animal production. Carbohydrate is the major factor controlling the supply of energy available for microbial growth whereas proteins affect both total fermentation and production of microbial dry matter per unit of carbohydrate fermented (Hoover and Stokes, 1991).

Therefore, it is also important to understand and identify combinations of feed ingredients that synchronise availability of energy and nitrogen for optimising ruminal digestion, microbial production, nutrient flow to the small intestine and a desired level of animal production. The ruminal fermentation and flow of microbial and dietary protein to the small intestine is affected by feed intake and by the amount and source of energy and protein in the diet (Clark *et al.*, 1992 and Stern *et al.*,1994). In an experiment, Puchala and Kulasek (1992) observed the highest rate of rumen microbial protein synthesis in the high protein and high energy diet fed groups of sheep and conversely, lowest was observed in the sheep fed with low protein and low energy diet groups. They also observed increased efficiency of microbial protein synthesis in sheep when fed the diet in which protein and energy ratio was balanced (Table 2). Efficient utilisation of degraded dietary nitrogen requires that the energy from the fermentation of dietary organic matter must be supplied at a rate which matches the synthetic abilities of the rumen microbes. Stern and Hoover (1979) observed that the readily available carbohydrates are more effective than other carbohydrates in increasing utilisation of degraded dietary nitrogen and/or increasing microbial growth.

Stern *et al.,* (1994) found that the efficiency of microbial protein synthesis in the rumen and microbial protein flow to the small intestine were greatest when a combination of barley (as carbohydrate source) and cottonseed meal (as protein source) were fed to cows. This has indicated that the synchronisation of rapid fermentation stimulates ruminal protein production.

Table 2. Rumen microbial protein synthesis and efficiency estimated using microbial nucleic acid reaching the duodenum (Puchala and Kulasek, 1992).

	Diet					
	LPLE	HPLE	MPME	LPHE	HPHE	SEM
Microbial N (g/d)	3.34a	7.00b	9.44c	4.47a	13.44d	0.40
Efficiency (g N/kg DOMR)	30.1b	22.7a	36.8b	18.4a	34.6b	1.93

LPLE = low protein, low energy; HPLE = high protein, low energy; MPME = maintenance for protein and energy; HPHE = high protein, high energy, DOMR = Digestible Organic Matter in the Rumen

Values in the same row with different letters differ significantly ($p<0.01$)

In studies by Sinclair *et al*., (1995) and Sinclair *et al*., (1993) to see the effect of synchronising the rate of dietary energy and nitrogen release in rumen fermentation and microbial protein synthesis in sheep, found a 27% greater production of microbial nitrogen (g N/kg DM intake) with the synchronous diet and, on average, an improvement in the microbial protein efficiency (g/kg OM truly degraded) of 13% over an asynchronous diet in sheep. They were of the view that synchronising the rate of supply of nitrogen and energy yielding substrates to the rumen micro-organisms, based upon ingredients, could improve microbial protein flow at the duodenum and the efficiency of microbial protein synthesis. Sinclair *et al.,* (1995) also showed evidence that synchronising hourly supply of energy and protein to rumen microbes can improve the efficiency of microbial protein synthesis in the rumen. In a similar experiment Witt *et al*., (1997) observed an increased in growth rates of lambs when they were fed synchronous diets. The increase growth rate may be attributable to an increase in the efficiency of microbial protein production and energy use in the rumen.

However, in the continuous culture system, Mansfield *et al.,* (1994) did not find any improvement of ruminal fermentation by synchronisation of energy and nitrogen release. They suggested that either energy or nitrogen alone was the likely limitations.

d) Rumen dilution or outflow rate

Outflow rate or rumen dilution rate is the fraction of the total liquid or solid

material leaving the rumen per unit time (hr) (Harrison, *et al*., 1975 and McDonald *et al*., 1995). Colucci *et al.,* (1984) and Elimam and Ørskov (1984) have reported higher digesta out flow rates or shorter retention time of digesta as a result of increased intake. Chen (1990) and Shriver *et al.*, (1986) reported similar results which showed a linear relationship between the efficiency of microbial nitrogen supply and the solid or liquid dilution rate. Chen *et al.*, (1992) have also demonstrated that efficiency of microbial protein supply increased with rumen increase in the rumen digesta fractional out flow rate. Gomes *et al*., (1994) have also shown a positive correlation between the efficiency of microbial protein production and the outflow rate. They have found both solid and liquid fractional outflow rates and efficiency of microbial protein supply to have increased significantly as a result of starch supplementation and/or feed intake. Dilution rates are higher with roughage diets than with those containing concentrates, and they increase as intake increases. Increasing the dilution rate often reduces cellulolysis and increases the proportion of propionate; it may also increase the quantity of microbial protein synthesized per unit of organic matter (McDonald *et al.,* 1995) fermented.

Foods with highly lignified cell walls have long rumen retention times whereas more readily digested foods e.g. concentrates, have short retention times. Rapid rates of passage tend to transfer digestion from the rumen to the intestine; for example, they reduce the breakdown of protein in the rumen and thereby increase the protein available for post-ruminal digestion.

Mathers and Millers (1981), did not obtain a clear relationship between microbial yields and the outflow rates. However, the majority of workers have shown that the efficiency of microbial protein production can be improved by increasing ruminal solid or liquid dilution rates. The increase in rumen dilution rate increases the out flow rate of microbial cells synthesised in the rumen, and the amount of metabolisable energy for the microbial population in the rumen. This may be due to the decrease in the out flow rate resulting in the production of ammonia from the breakdown of microbial protein in the rumen. If the ammonia from the microbial protein breakdown is not re-utilised by rumen micro-organisms, it will be lost in the urine. Therefore, rumen dilution rate/out flow rates affects the efficiency at which nitrogen is converted to microbial protein available to the host.

e) Level of feeding

The turn over rate of rumen contents can be altered by level of intake. Alteration of the rumen turn over rate can effect both nutrient digestion and microbial efficiency (Hoover and Stokes, 1991). An increase in the quantity of a food eaten by an animal generally causes faster rate of passage of digestion. Djouvinov and Todorov (1994) in their study to examine the level of feeding on the efficiency of microbial protein synthesis, found that the efficiency of microbial protein synthesis in the rumen was increased with increase in the level of feeding. They have shown that the microbial protein passing to the duodenum was increased proportionally to the dry matter intake. They have also noted that increasing dry matter intake altered the rumen volume and liquid/solid dilution rates. This has reduced the retention of organic matter in the rumen and the time for its digestion.

There was also a shift of organic matter digestion from the rumen to the intestine. The study, therefore demonstrated that the level of dry matter intake or rate of passage could influence (i.e. increase) the supply of protein to the small intestine of ruminants. This is because that a larger amount of microbial protein reaches the intestine with high dry matter intake and also the amount of undegraded feed protein passing to the duodenum increases. Similarly, Chen *et al.,* (1995a) indicated that the efficiency of microbial nitrogen supply was greater with a higher dry matter intake and body weight ratios. In another experiment reported by Chen *et al.,* (1992) a linear increase in efficiency of microbial protein supply (g N/kg DOMR) with increasing DM intakes was observed in sheep. It appears that the energy and nitrogen availability interact positively with intake level in determining the overall efficiency of microbial synthesis as reported by AFRC (1992).

Hence, keeping in view these various dietary interrelationships, which relates to the increase in supply of microbial protein, need to be considered when planning strategies for optimum and efficient production levels.

f) Modification of rumen fermentation

Attempts have been made to alter the patterns of digestion in ways that would improve the nutrition of ruminants. The contribution of protozoa to rumen digestion and hence to the nutrition and productivity of ruminants, has long been a matter of controversy. There were attempts to modify microbial populations in order to suppress undesirable process or stimulate desirable processes e.g. microbial protein synthesis, and other approaches such as to protect nutrients from

rumen fermentation so that they would be digested post ruminally. In an experiment by Ushida *et al.*, (1990) using defaunated animals (which had only bacteria and fungi in the rumen), found an increase in microbial protein flow and an increase in the efficiency of microbial protein synthesis. The increase in protein efficiency may be as a result of the removal of the reduced protozoan population and an increased bacterial population in rumen. Similar was the view of Bird *et al* ., (1990), according to them the bacterial populations increase in number when protozoa are absent and there is an increase in the microbial protein outflow rate from the rumen.

Therefore, removal of ciliate protozoa (or defaunation) would be expected to improve certain production aspects of rumen fermentation. Although, defaunation reduces the digestion of polysaccharides, it increases the quantity of microbial protein reaching the duodenum by about 25% (McDonald *et al.,* 1995). The current view of rumen protozoa is that with low-protein forage diets their presence is detrimental to the host, and defaunation can therefore increase animal productivity. With concentrate based diets, which are better supplied with protein, the presence of protozoa is beneficial. However, it is difficult to keep ruminants free of protozoa when they are on a forage diet and difficult to maintain on a concentrate diet. Removal of the ciliate protozoa or defaunation would be expected to improve certain production aspects of rumen fermentation (Anon, 1995). However, defaunation of ruminants is difficult to achieve and has only been achieved as an experimental tool in few laboratories around the world and the investigation for suitable means are currently underway (Anon, 1995).

2.3 Nucleic acid and the purines

Nucleic acids are high molecular weight compounds the hydrolysis of which yields a mixture of purine and pyrimidine. The principal purine bases present in nucleic acids are adenine and guanine. The linkage of pentose sugar with any one of the purine bases is known as nucleoside and esterification of nucleosides with phosphoric acid will form nucleotides (McDonald, 1995).

Nucleic acids are present in most animal feeds, varying from 1 to 50 g/kg DM. In grass and hay they amount to 5.2 to 9.3 % of the total nitrogen (Wallace and Cotta, 1988). Although, in insignificant amount, the sloughed epithelium cells may also contribute to the non-microbial nucleic acid in the rumen.

In the rumen DNA and RNA are rapidly hydrolysed and transient products formed are the mixtures of nucleotides, nucleosides and bases. In the breakdown of nucleic acids in rumen fluid *in vitro*, the purine nucleotides are hypoxanthine and xanthine while pyrimidine nucleotides are uracil and thymine, cytosine is deaminated to uracil (MacAllan and Smith, 1973). The bacterial species convert the purine bases adenine and guanine into hypoxanthine and xanthine. Therefore, the nucleic acids present in the rumen are mainly of microbial origin (Chen 1989; Chen *et al.*, 1995a). Although, some of the dietary nucleic acids present in the undegraded fraction of the feed that escape ruminal degradation, they may contribute to the duodenal nucleic acid content. However, their quantitative

contribution as feed nucleic acid is negligible.

a) Purine synthesis

In the process of tissue nucleic acid turn over, purine is lost from the body every day. To replace this loss, they are synthesised by *de novo* and salvage processes. In *de novo*, the synthesis of purine may take place in the body by incorporation of glycine, serine, aspartate, glutamate, formate and carbon dioxide requiring 5 moles of ATP per mole of purine synthesised.

The purine formed as a result of degradation of exogenous purines or from tissue nucleotides, could be utilised by salvage for the synthesis of new tissue nucleotides by the specific catalytic enzymes involved. Xanthine could be salvaged to a little extent, but is mostly degraded. Uric acid and allantoin once converted, cannot be re-utilised to form tissue nucleic acids.

The purine, which is not salvaged, is converted into purine derivatives and excreted primarily in the urine. The end product of purine catabolism varies between animal species, mainly due to difference in the level of activity of xanthine oxidase (Chen *et al.*, 1990c). Unlike cattle, sheep have low xanthine oxidase activity in the intestinal mucosa; hence they enter the liver in the form that they are available for utilisation (Chen and Gomes, 1992).

b) Purine catabolism and its derivatives

The rumen micro-organisms extensively destroy the dietary nucleic acids entering

the rumen (McAllan and Smith, 1973). In the rumen, dietary nucleic acids are degraded to a mixture of nucleotides, nucleosides almost as readily as the purine nucleic acids. Therefore, the product of the nucleic acid degradation namely purine and pyrimidines could be further degraded. The purine further breaks down into ammonia, carbon dioxide and acetic acid.

The microbial nucleic acids undergo digestion in the small intestine where they are hydrolysed to form purine nucleotides. The nucleotides are further hydrolysed to purine nucleosides and free bases and are absorbed from the lumen of the intestine. In sheep, the absorbed purines can enter to the liver in salvageable form which can be available for incorporation into tissue nucleic acid as and when necessary. The purines, which have not been incorporated into tissue nucleic acids, are also degraded into purine derivatives and excreted in urine.

2.4 Methods to measure microbial protein

Microbial protein contributes a significant part of the total protein flux into the small intestine in ruminants (AFRC, 1992). Various methods have been developed and being used to differentiate microbial and dietary protein in digesta leaving the rumen and entering the duodenum. Most of these methods are based on the use of a microbial marker. The markers are either naturally present in the microbial cell (internal markers) or they are introduced into the microbial cell during their growth in the labelled substrates. The internal markers in use are Diaminopimelic acid (DAPA), Aminoethylphosphonic acid (AEPA) and nucleic acids (RNA,

DNA) whereas the isotopic markers are ^{35}S, ^{15}N and ^{32}P.

a) Principle of the methods based on digesta flow measurement

The principle of these methods is broadly similar, based on the measurement of the proportion of microbial nitrogen in duodenal digesta.

The proportion microbial nitrogen in duodenal digesta (A) is calculated as follows, by comparing the proportion of marker in rumen microbes and the same ratio in the duodenal digestion (Chen,1989).

$$A = \frac{(Microbial : N)\, in\, rumen\, microbes}{(Mar\mathrm{ker} : N)\, in\, duodenal\, digesta}$$

Disadvantages of the methods: These methods require ruminally and post ruminally cannulated animals. Also, sampling of digesta from the sites and analysis of markers makes the process complicated, tedious and costly. They require prolong steady intraruminal infusion of isotopes and separation of ruminal microbes. In addition, surgical invasion causes some changes in the physiology of digestion, reduces feed intake and limits the number of experimental animals than can be used (McRae and Wilson, 1977; Wenham, 1979).

Accurate methods of measurements of microbial protein synthesis are essential in the evaluation of supply of nutrients to the intestine for absorption and utilisation. Most commonly used microbial markers are DAPA, ^{15}N and Purines. Recently, ^{15}N and purines have been recommended for use as microbial markers (Broderick

and Merchen 1992; and Stern *et al*., 1994). Stern *et al*., (1994) indicated that DAPA overestimates microbial protein production. Masson *et al.*, (1991) and Djouvinov and Todnov, (1994) showed that the extensive metabolism of DAPA by micro-organisms, as a result of this, micro-organisms can have a profound effect upon the accuracy of DAPA as a marker of microbial protein. The $^{15}NH_3$ or ^{15}N is a stable isotope and the biggest advantage is that its use does not require expensive removal of hazardous isotope wastes. However, laboratory preparation time and sample analysis takes longer and is more costly. The purine method is less complicated to perform and less costly. For this reason purines are recommended for use as the microbial marker for determining microbial protein synthesis in the rumen.

b) Method based on purine derivatives

A number of systems have been proposed for the evaluation of ruminant diets, in terms of the amount of undegraded dietary protein and the microbial protein flowing to the intestine. The use of urinary purine derivatives as a metabolic marker of microbial synthesis in ruminants was brought to the attention when Topps and Elliot (1965) reported a highly significant correlation between the rumen concentration of nucleic acids and the excretion of purine derivatives by sheep. As the purine derivatives are primarily the degradation products of the absorbed microbial nucleic acids, Chen *et al.,* 1990a showed the use of the purine derivatives could be a potential method to measure the microbial protein

production in ruminants.

Infusion of microbial nucleic acids into the abomasum of sheep and cattle and measurement of the recovery of purine derivatives have shown rapid responses of purine derivatives to the changes in the supply of nucleic acid (Verbic *et al.*, 1990 and Chen *et al*., 1990d). Likewise, Giesecke *et al.,* (1984) also suggested the use of purine derivatives as an effective measure for rumen microbial growth, when they found a significant relationship between the amount of nucleic acids passing out of the rumen and the amount of purine metabolites excreted in urine in sheep, maintained by intragastric infusions. Fujihara *et al.,* (1988) also obtained similar relationship. Chen *et al.,* (1990b); Verbic *et al*., (1990) and Chen *et al*., (1990d) in their series of experiments have shown that purine derivatives (allantoin, xanthine, hypoxanthine and uric acid) could be used to estimate the supply of microbial protein from the rumen to the intestine. Chen *et al.,* (1990a) and Chen *et al*., (1990b) and Chen, (1989) have indicated that the measurement of the total purine derivatives rather than allantoin alone is more appropriate method of measurement of microbial protein flow.

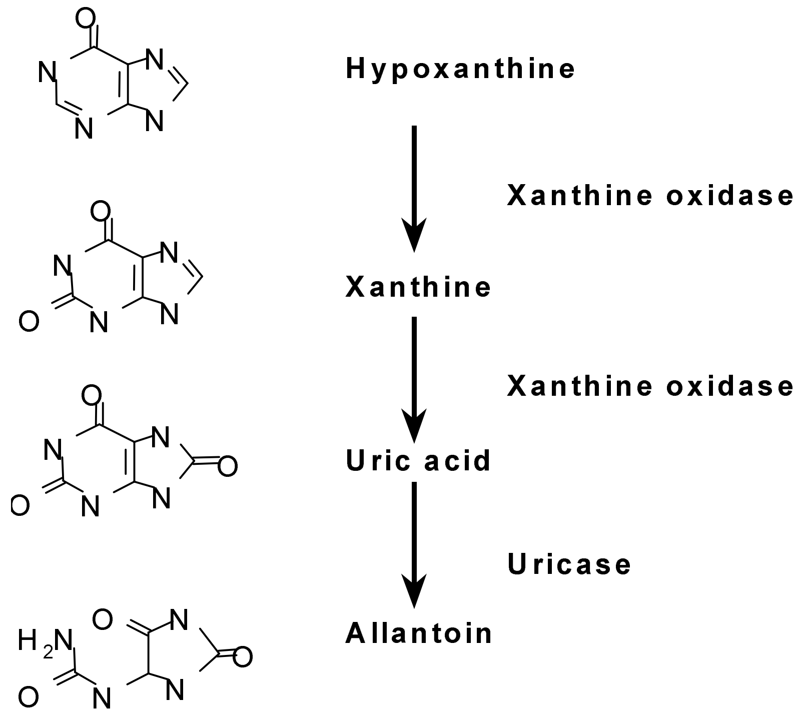
Use of purine derivatives

The methods involve the analysis of the metabolites of the absorbed purines in the form of allantoin, uric acid, xanthine and hypoxanthine in the urine. The methods require collection of urine samples at given time intervals.

The principle of the purine derivative method

Dietary feed is low in nucleic acid and those entering the small intestine are essentially of microbial protein origin. The absorbed nucleic acids are degraded releasing nucleosides and free bases in the small intestine. Salvageable purine nucleosides are utilised for the synthesis of tissue nucleic acids or to replace the endogenous purine loss. The unutilised nucleic acid purines are metabolised into their derivatives (hypoxanthine, xanthine, uric acid and allantoin) and excreted in the urine. The purine derivatives and the sequence of enzymatic break down are as shown in the Figure 1.

Figure 1. Chemical structures and the enzymes involved in the conversion of the derivatives.



The endogenous purine derivatives coming from tissue nucleic acid turn-over, may also contribute to the total excretion in urine for which correction is required. As the excretion of the purine derivatives are directly related to the purine absorption, from the knowledge of Nitrogen; total-Nitrogen ratios in microbial bio-mass; and from the amount of purine excreted, the amount of purine absorbed can be estimated from the purine derivatives excreted in the urine. Chen, *et al* (1990d) proposed a model describing a quantitative relationship between excretion of purine derivatives and absorption of exogenous purines in sheep, the equation is:

$Y = 0.84X + (0.150\ W^{0.75}\ e^{-0.25x})$ ....(1)

Where

W = Metabolic body weight of the animal.

0.84 = proportion of purine derivatives that are excreted in urine.

X = absorbed exogenous purine (mmol/d).

Y = urinary excretion of total purine derivatives of both endogenous and exogenous (mmol/d).
0.150 = endogenous purine derivative excretion (mmol/kg $W^{0.75}$ day) measured when the animals have no supply of exogenous purine.
0.25 = a constant defining the rate of replacement of *de novo* synthesis of exogenous purines.

The within parenthesis component represent the net endogenous contribution of purine derivatives to total excretion after correction for the utilisation of microbial purines by the animals.

Estimation of microbial protein supply: Based on the above equation by Chen *et al* (1990d), the absorption of the microbial purines (X mmol/d) can be estimated from the purine derivative (Y mmol/d). Therefore, microbial nitrogen/protein supply can then be calculated by the following relation:

$$Microbial\ Protein\ Supply\,(g/d) = \frac{X \times 70}{0.83 \times 0.116 \times 1000} \quad ....(2)$$

$$= 0.727 \text{ x } X$$

Where,
0.83 = digestibility of microbial purine
70 = nitrogen content of purine
0.116 = ratio of purine N: total-N in mixed rumen microbes (11.6:100). Assuming that this ratio remains unchanged by dietary treatments)

The microbial protein supply can be expressed as 'g' of microbial N per day or 'g' of microbial N per kg of digestible organic matter intake (DOMI). The efficiency can also be expressed as 'g' microbial nitrogen per kg digestible organic matter apparently fermented in the rumen (DOMR) to facilitate comparison with the

reports in the literature. The DOMR can be assumed to be 0.65 of the DOMI (ARC, 1984).

Though the purine derivative method has already been established to estimate microbial protein in cattle and sheep, improvements are still being made in order to make it more practicable under field situations. The simplicity and non-invasive nature of the method makes it suitable for extensive studies on influences of factors on microbial protein synthesis, so that decision can be made to maximise microbial protein supply to the animal using that knowledge.

2.5 Purine nitrogen index

a) Background

The purine derivative technique is now a well established and a useful indirect method of estimating microbial protein supply to ruminant animals in an experimental condition. However, the application of the technique under farm condition appears to be limited by the difficulty of carrying out a 24 hours collection of urine samples. Alternative methods therefore, need to be sought so that this can be applied to the animals in an outdoor situation.

In the past, the possibility of using spot urine test as an alternative method was investigated by Chen *et al.,* (1995 *a, b*). In this study Chen *et al.,* (1995 *a, b*) did not find any significance difference between the sampling times when they measured the ratio of purine derivative : creatinine in the hourly urine samples.

The ratio, they obtained was linearly correlated with the daily purine derivative excretion (r = 0.92). Based on this relationship, they have suggested that the purine derivatives in spot urine may provide a practical indicator of microbial protein supply status in sheep when fed *ad libitum*. Chen *et al.,* (1992) found that the molar ratio of purine derivative concentration to creatinine concentration in spot urine samples was correlated with daily purine derivative excretion and they concluded that if creatinine excretion is constant, the ratio of purine derivative to creatinine in spot urine samples (corrected for metabolic body weight) could possibly be used as an estimate of microbial protein supply. Chen *et al.,* (1992) observed only a small variation in plasma concentration or purine derivative creatinine ratio in the spot urine, when they fed restricted level of feed to the steers. Chen *et al.,* (1995a) have also found little diurnal variation in spot plasma and urine samples in animals with *ad libitum* feeding once they have corrected for glomerular filtration rate and urine volume respectively.

There is an urgency of a need for a simple tool which will enable one to estimate or evaluate ruminant feed utilisation efficiency rapidly and more effectively at different dietary and management systems. Purine nitrogen index provides an indication of the efficiency of conversion of dietary nitrogen to microbial protein.

b) Definition of the purine nitrogen index

There exists a strong positive correlation between the direct (using microbial markers) and the indirect method (using purine derivative method) on the measurement of microbial protein supply (Samaniego, 1996). It can therefore be

anticipated that the purine derivative nitrogen (PDN) and UN will also correlate strongly with NCE. Purine nitrogen index (PNI) is defined as purine derivative nitrogen expressed as a proportion of the total urinary nitrogen in the urine, i.e. PNI = PDN/UN.

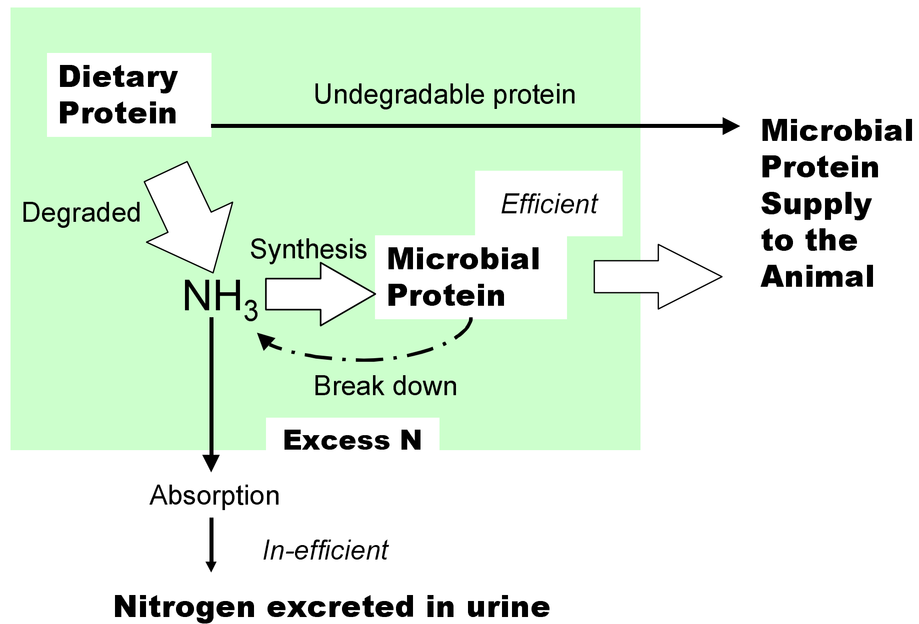
c) Principle behind the concept

As earlier described, during rumen digestion, the dietary nitrogen is converted to ammonia, a proportion of which will be utilised by the rumen micro-organisms for the synthesis of microbial protein. The excess ammonia will be absorbed from the rumen and excreted in the urine (Figure 2). If proportionally more nitrogen is converted to microbial protein, then less nitrogen will be excreted in the urine. An illustration explaining the transformation of dietary nitrogen into microbial protein in the rumen is given in the Figure 2.

The nitrogen capture efficiency (NCE) expressed as the production of microbial protein nitrogen as a proportion of the dietary rumen degradable nitrogen (RDN) determines the overall efficiency of ruminal diets. The principle behind the concept is that a diet with a higher NCE will produce more microbial protein and proportionally less N will be excreted in the urine. Therefore in an efficient diet the ratio of microbial protein nitrogen to the urinary nitrogen will be higher. However, a diet with a lower NCE will produce less microbial protein and excrete higher nitrogen in urine. This will result to a lower rate of microbial protein to urine nitrogen. Hence, based on these relationships, it can be expected that microbial protein supply (MN) and total urine nitrogen (UN) ratio (MN:UN) is

positively correlated to NCE.

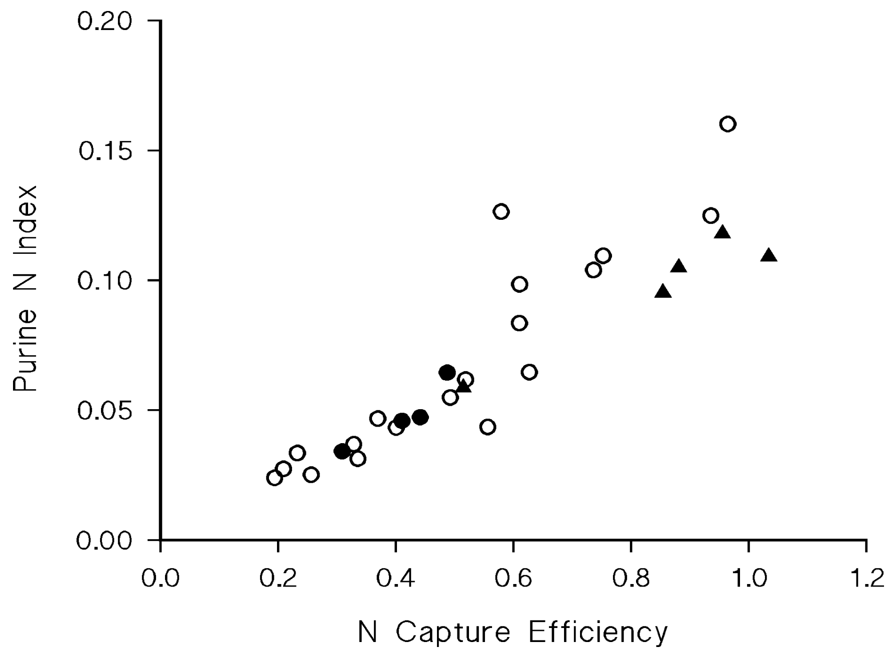
Figure 2. Inter-conversion of dietary nitrogen into microbial protein.



d) Preliminary study

The preliminary study (X B Chen, Unpublished data) suggests that the PNI could provide a rapid indication of the NCE at the rumen level. The preliminary data showed a curvilinear correlation between PNI and the NCE (Figure 3).

Figure 3. Relationship between the Purine Nitrogen Index and the Nitrogen Capture Efficiency in 29 sheep under different dietary regimes.



The measurement was taken from 29 sheep under different dietary regimes. The data points have shown that the PNI of 0.08 which corresponded to a NCE of about 0.65 and the efficiency of microbial protein supply of 25g N/kg DOMR. This relationship has indicated that the PNI of less than 0.08 for sheep would seem less satisfactory. The figure has also explained that the diets with lower PNI values gave higher nitrogen excretion in the urine.

CHAPTER III

OBJECTIVES

The study was undertaken with the following main objectives:

1. To evaluate the use of PNI as an indicator of efficiency of the conversion of dietary nitrogen to microbial protein in sheep. A range of diets with different levels of RDN content were formulated so as to simulate different extent at which RDN was converted to microbial protein nitrogen.

2. To examine the variability of PNI in the spot urine samples so as to explore the potentiality of its application under farm condition.

CHAPTER IV

MATERIALS AND METHODS

4.1 Animal experimentation

With the facilities and animals available, three experiments were carried out. The purpose of which were to evaluate the relationship between the PNI and the efficiency of microbial protein supply or NCE in the rumen. In order to achieve this, a range of diets differing in rumen degradable nitrogen (Experiments 1 and 2) or the same diet at different levels of intake (Experiment 3) was considered. The idea was to derive a varied and well spread set of data so that a distinct relationship between the PNI and NCE or efficiency of microbial protein supply can be established.

a) Experiment 1

Treatment design

Sheep were fed with a basal diet alone, or the basal diet supplemented with urea or barley. The basal diet was grass cubes, primarily of rye grass. Urea was supplemented at two levels (8 and 16 g/d respectively), referred to as U1 and U2. The rolled barley was offered at 200 g/d (treatment B1).

A total of 12 female Finn/Dorset x Dorset sheep, approximately one year old with an average body weight of 51±6 kg, were used. The sheep were randomly put into

4 groups so as to fit into a completely randomised experimental design. Each group thus consisted of 3 animals.

The treatment design, the animal numbers and their respective treatment diets in the Experiment 1 was as shown in Table 3.

Table 3. Allocation of animals into 4 treatment groups.

Control diet (Grass cube)	Urea1 (8g/d)	Urea2 (16g/d)	Barley (200g/d)
Sheep 143	Sheep 142	Sheep 140	Sheep 145
Sheep 149	Sheep 146	Sheep 139	Sheep 147
Sheep 141	Sheep 148	Sheep 144	Sheep 150

Preparation of treatment diets

The U1 and U2 diets were prepared by dissolving 4g and 8g of commercial urea in water. The solution was sprayed on the grass cubes at least a few hours prior to feeding, taking into account that the cubes were thoroughly mixed. To avoid loss of solution, the addition of the amount of water was kept as minimum as possible (12ml and 24ml of water was added in 4g and 8g of urea respectively).

b) Experiment 2

Treatment design

In this experiment, sheep were fed with a basal diet alone, or the basal diet supplemented with pre-washed fish meal or barley. The basal diet was grass cubes,

the same as in Experiment 1. Fish meal was supplemented at two levels (50 and 100 g/d respectively), referred to as FM1 and FM2. The rolled barley was offered at 400 g/d (treatment B2).

After the completion of Experiment 1, the same animals were used in the 2nd experiment . However, the animals were regrouped into four to fit into the similar experimental design as Experiment 1.

The treatment design, the animal numbers and their respective treatment diets in Experiment 3 was as shown in the following table.

Table 4. Allocation of animals into 4 treatment groups.

Control (Grass cube)	Fish meal1 (50g/d)	Fish meal 2 (16g/d)	Barley (400g/d)
Sheep 140	Sheep 141	Sheep 149	Sheep 146
Sheep 147	Sheep 150	Sheep 143	Sheep 142
Sheep 148	Sheep 139	Sheep 145	Sheep 144

c) Adaptation period in Experiments 1 and 2

Prior to each experiment, the randomly selected animals of each group were allowed to undergo a 10 days adaptation period, in order to accustom the animals with the diet and to clear from the tract the residues of previous foods. The sheep were individually penned during the adaptation period, thereafter transferred to the metabolism cages for the experimental period. During the adaptation period the animals received their respective treatment diets on top of the basal diet. The

control group received grass cubes only during this period.

Since there were only 6 metabolism cages available, the collection of samples was made at two groups (6 animals at a time) i.e. when the two groups were in the cages, the other groups (6 animals) were in the pen still on their adaptation diet. So, the second two groups of each experiment had longer i.e. 17 days adaptation period as compared to the first two groups of each experiment.

d) Feeding

The animals in both experiments were fed equal meals twice daily, once at 08:00 and another at 16:00 hours. All the sheep received grass nuts as the basal diet. They had access to drinking water at all times. No supplementary vitamins and minerals were offered.

f) Experiment 3

The objective of the experiment was to compare the PNI and NCE or efficiency of microbial protein supply in sheep receiving three levels of the same diet.

Treatment design

A mixed diet (referred to as GP diet) was given to sheep at three levels of intake (800, 1200 and 1600g/day). The GP contained 50% hay, 30% rolled barley, 10% molasses, 9% fish meal and the rest minerals and vitamins. The total collection period of sample was 12 days for each period and there were 3 experimental periods in total and a 10 days adaptation period between each period. The

experimental design was based on 3x4 multiple Latin squares design, taking 4 female sheep. The sheep in this experiment were ranged between 40 to 45 kg. The feeding, housing and management conditions were similar to Experiments 1 and 2 above.

The method of urine collection, analysis of the purine derivatives and nitrogen are the same as Experiments 1 and 2 which are described in the following pages.

4.2 Composition of the major nutrients of the treatment diets

The approximate composition of the treatment diets are as shown in Table 5 and the calculation of the Rumen Degradable Nitrogen (RDN) and Digestible Organic Matter in Rumen (DOMR) is presented in the Table 6.

Table 5. Dry matter and the major nutrients of grass nuts, fish meal, GP diet and rolled barley grains. Nitrogen and OM are expressed on dry matter basis.

Ingredients	DM (%)	N (%)	OM (%)
Barley (rolled)	86.55	1.96	97.52
Fish meal	82.14	11.89	78.05
GP diet*	96.52	2.02	92.59
Grass nuts	96.32	2.78	89.53

DM =Dry matter, N = Nitrogen, OM = Organic matter, GP = General purpose diet
* used in Experiment 3.

Table 6. Nitrogen, RDN and DOMR supplied by the treatment diets.

Treatment diets	Total N supplied (g/d)	DOMR (g/d)	RDN (g/d)	RDN (g/kg DOMR)
Control diet	25.93	435.91	18.49	42.42
U1 + basal diet	29.66	435.91	22.22	50.97
U2 + basal diet	33.39	435.91	25.95	59.52
B1 + basal diet	29.33	552.32	20.83	37.52
B2 + basal diet	32.71	668.73	23.17	34.65
FM 1 + basal diet	30.82	446.44	20.20	45.25
FM 2 + basal diet	35.70	456.98	21.91	47.95
GP diet 800	16.16	443.26	10.34	23.33
GP diet 1200	24.24	664.90	15.51	23.33
GP diet 1600	32.32	886.53	20.68	23.33

Basal diet = 1000 g/day, U1 = 8 g/day, U2 = 16 g/day, B1 = 200 g/day, Barely 2 = 400 g/day, FM 1 = 50 g/day, FM 2 = 100 g/day. The GP diet was used in the Experiment 3.

4.3 Calculation and formulation of supplementary diets

The amounts of total nitrogen and RDN g/kg DOMR provided by the treatment diets are given in Table 6. The Nylon bag technique was used to measure the degradability of the treatment diets. The degradability at 5% out flow rate was taken as the degradability of the diets.

The degradability of the fish meal appeared to be linear ($Y = 0.7285X + 23.02$, $r^2 = 0.99$), based on this equation the degradability was calculated at 20 hours mean retention time (MRT). The MRT was calculated ($MRT = 1/k$) from the fractional outflow rate (k) of 0.05 /h giving a mean retention time of 20 hours.

4.4 Collection of urine and faecal samples

Two days prior to the collection of samples, animals were placed in their individual cages and the daily output of faeces and urine were recorded. In Experiments 1 and 2, the urine and faecal samples were collected for 7 days. Daily urine samples were collected from day 1 to day 5 and spot urine samples (every two hourly) at day 6 and 7. Total faecal samples were however, collected for seven days. In Experiment 3, urine and faeces were collected in the 12 days of collection period.

a) Collection of urine

Daily collection

The metabolic cages were constructed with a facility to collect urine and faecal samples, separately. To ensure clean separation of faeces and urine, the separator and the screen were cleaned daily and also the angle and the position of the collector was suitably adjusted.

The daily urine was collected into clean plastic containers containing approximately 200ml of 10% H_2SO_4. The acid was added as preservative ensuring that the pH level be maintained below 3. At the time of sampling, the pH of the urine was again checked and adjusted accordingly. During preparation of urine samples, the collected urine samples were filtered using glass wool. The samples were then diluted to 5.5 litres with water and an aliquot taken for analysis. Undiluted samples were also retained as an emergency stock. The labelled diluted

and undiluted urine samples in plastic vials were stored at -20°C until analysis.

Spot urine collection

Spot urine collections were made in Experiment 1 and 2 only. At day 6 and 7, the urine samples were collected every two hours using fraction collector. The urine excreted by the sheep immediately pumped into one of the 12 bottles (each contain 20ml of 10% H_2S0_4) during 24 hours periods. To ensure clean separation of urine and faecal samples the separators and containers were cleaned prior to each sampling time. The samples were labelled and stored in freezer at -20°C until analysis.

Prior to the analysis of total nitrogen, creatinine and purine derivatives, the urine samples in the vials were thoroughly mixed and placed in an ultrasonic bath for 20 minutes to break down any particles.

b) Faecal samples

Sub-samples (about 10%) of the daily faecal out-put were collected in polythene bags and stored at 4 ^{0}C. At the end of the 10 day collection, the faecal samples of the individual sheep were pooled and mixed using a mechanical mixer, sampled, labelled, and stored at 4 ^{0}C until analysis. The sub-samples of faeces of individual sheep on the different treatment diets were taken for the analysis of dry matter and organic matter. A sub-sample from the bulk faecal sample of each sheep during the period, was freeze dried for 48hours, then ground using a coffee grinder. The

freeze-dried faecal samples were used for the analysis of total nitrogen.

4.5 Chemical analysis

a) Feed and faecal samples

Dry matter : The dry matter of the treatment diets and faecal samples was determined by drying the samples in a hot air oven at 100 °C for 48 hours (AOAC 1980).

Organic matter : The total ash of the faecal samples was determined by ashing the samples overnight in a muffle furnace at 550°C. The percentage of organic matter was calculated by the difference of the total ash from 100 (AOAC 1980).

Total urine nitrogen : Total nitrogen in the urine was determined using the method described by Davidson *et al.,* (1970). The principle of the method is that the nitrogen is first converted to ammonium sulphate by boiling with concentrated sulphuric acid using potassium and mercuric oxide as catalyst. The samples are treated with 35% NaOH to release the ammonia. The ammonia released then reacts with alkaline sodium phenate and sodium hypochlorite to produce an indophenol blue, a complex which absorbs light at 625 nm.

Digestibility (apparent) : The apparent digestibility of dry matter or organic matter was calculated from the difference of the quantity of the dry matter or

organic matter consumed and excreted by the individual animal (average of 7 days).

Rumen degradability : The digestibility of dry matter and nitrogen of the diets was measured in 3 sheep using the Nylon bag technique (Ørskov *et al.,* 1980). Throughout the measurement, the sheep were maintained at hay and grass cube diet.

Feed and faecal nitrogen: Both feed and faecal nitrogen were determined using an automated Dumas system, manufactured by Foss and Heraeus (Foss electric, UK, Ltd) as per the method described by Ebeling (1968). The principle of the method is that the sample undergoes oxidative combustion at above 1000°C. This allows the conversion of sample nitrogen into gaseous nitrogen. Thus the amount of nitrogen converted into gaseous N_2 can be quantitatively measured by thermal conductivity.

b) Urine samples

Measurement of purine derivatives

The purine derivatives were measured as the sum of allantoin, uric acid, xanthine and hypoxanthine using an auto-analyser as described by Chen *et al.*, (1990e).

Allantoin : Allantoin was measured using the High Performance Liquid Chromatography (HPLC) and pre-column derivatization as described by Chen *et*

al., (1993). The principle of the method is that the allantoin is hydrolysed at alkaline condition at 85ºC to allantoic acid. The allantoic acid thus formed is further degraded in acidic condition to urea and glyoxylic acid. The glyoxylic acid is reacted with phenyl hydrazine to form phenyl hydrazone. This is then measured by reversed phase C_{18} column at 360nm wave length.

Uric acid, xanthine and hypoxanthine : The uric acid in urine was measured using the phosphotungstic acid method as described by Chen *et al.,* (1990e). The xanthine plus hypoxanthine were determined as uric acid after treatment with xanthine oxidase. The principle of the method is that the xanthine oxidase converts xanthine and hypoxanthine into uric acid and the xanthine and hypoxanthine are measured as uric acid.

Estimation of microbial protein supply : The estimation of microbial protein supply was based on the purine derivative method. The total purine derivative was calculated as the sum of allantoin, uric acid, xanthine and hypoxanthine. This has been described in the equations 1 and 2 in the earlier sections.

Calculation of PNI and NCE

The PNI was calculated as the proportion of Purine derivative nitrogen (PDN (mg/l) to UN (mg/l). Similarly, NCE was calculated as the proportion of microbial protein supply (MN) (g/d) to the RDN (g/d) intake.

4.6 Statistical analysis

Comparison of the difference between the means of the treatments was analysed by the analysis of variance using both Genstat and MINITAB software computer programmes. The difference between the two means was compared by paired t-Test (two-tail) using the Microsoft excel computer programme.

CHAPTER V

RESULTS

5.1 Daily excretion of the purine derivatives and microbial protein supply

a) Experiment 1

Total purine derivatives (PD), PNI, the microbial protein supply (MN), efficiency of microbial protein supply (EMPS) and the NCE measured in 12 sheep on diets with barley and/or urea supplemented diets are presented in Table 7. The total PD excretion was lower in both the urea supplemented diets (U1 and U2), lowest being in the U2. However, the differences between the treatment means were not significant.

The EMPS of urea-supplemented diets was found to be significantly lower ($P<0.05$) compared with the control and barley supplemented diets.

Table 7. Total PD, PNI, MN, EMPS and NCE in 12 sheep at barley and urea diets with grass cubes as basal diet. Result is the average of 3 sheep in a 5-day period (Details in the apperndix 1)

Diet	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)
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	<0.001

DOMI = Digestible Organic Matter Intake, PD = Purine Derivatives, PNI = Purine Nitrogen Index, PDN = Purine Derivative Nitrogen, UN= Total Urine Nitrogen, NCE = Nitrogen Capture Efficiency, MN = Microbial Protein Supply, EMPS = Efficiency of Microbial Protein Supply, RDN= Rumen Degradable Nitrogen

b) Experiment 2

The results of the total PD, PNI, MN, EMPS and the NCE in the fish meal and barley supplemented diets, measured in 12 sheep, are given in Table 8). It can be seen from the table that total PD and EMPS were not significantly different between the means of treatment diets. The MN was however, significantly higher ($p<0.05$) in the B2 as compared to the control and the FM1 and FM2. It therefore, showed that the additional supply of fish meal protein to the diet of animal has no effect on excretion of PD, and the supply of microbial protein nitrogen in the rumen.

Table 8. Total PD, PNI, MN, EMPS and NCE in 12 sheep at barley and fish meal diets with grass cubes as basal diet. Result is the average of 3 sheep in a 5-day period. (Details in the appendix 2).

Diet	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)
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	4.078	0.007	0.062
F-test	$P<0.001$	NS	0.001	$P<0.05$	NS	NS	NS

DOMI = Digestible Organic Matter Intake, PD = Purine Derivatives, PNI = Purine Nitrogen Index, PDN = Purine Derivative Nitrogen, UN= Total Urine Nitrogen, NCE = Nitrogen Capture Efficiency, MN = Microbial Protein Supply, EMPS = Efficiency of Microbial Protein Supply, RDN= Rumen Degradable Nitrogen

c) Experiment 3

The result of total PD, PNI, MN, EMPS and NCE at three levels of GP diet are presented in Table 9. The effect of the level of diets in the MN and the total PD excretion was significant ($p<0.01$) i.e. the total PD excretion and MN was higher in the higher intake level groups of animals. There was no significant difference in the EMPS between levels of intake.

The trend of variation in the PNI, efficiency of microbial protein supply and NCE is not clear. However, the values were generally higher in intake level 2 compared to the other two GP diet intake levels.

5.2 PNI and NCE measured in daily urine

a) Experiment 1

It can be seen from Table 7 that the U2 diet showed a significantly lower ($P<0.05$) level of PNI values compared to the control and the barley supplemented diet. Likewise, a general trend showed that when there was more RDN i.e. urea, there was less NCE. The lowest NCE was observed in U2 compared with the U1, C1 and B1. Difference between these treatment means was highly significant ($p<0.001$).

Table 9. Total PD, PNI, MN and NCE in 12 sheep at GP diet at 3 levels. Result is the average of 4 sheep at 7 days collection period (details in appendix 3)

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	1.30	0.0108	0.031
	F-test	P<0.01	P<0.01	P<0.001	P<0.01	NS	NS	P<0.05

DOMI = Digestible Organic Matter Intake, PD = Purine Derivatives, PNI = Purine Nitrogen Index, PDN = Purine Derivative Nitrogen, UN= Total Urine Nitrogen, NCE = Nitrogen Capture Efficiency, MN = Microbial Protein Supply, EMPS = Efficiency of Microbial Protein Supply, RDN= Rumen Degradable Nitrogen

The variation of the PNI of individual animals during each period was not significantly different. However, the level of NCE was found to be significantly different (P< 0.001) between the animals within the same group. The coefficient of variation of NCE in this experiment ranged from 12 to 22%.

b) Experiment 2

The PNI and NCE were found not to be significantly different between treatments in this experiment (Table 8). No general trend of variation due to the effect of supplementation of rumen undegradable nitrogen source i.e. fish meal was observed in the levels of PNI and NCE.

The variation of the PNI and NCE of individual animals during each period was not found to be significantly different. However, there was a considerable variation between the animals in their levels of NCE (P<0.001) and PNI (P<0.01). The coefficient of variation in NCE in this experiment was as high as 66% in the C2 followed by the FM2 (37.7%). The variability of PNI was higher with an increase in fish meal supplement (see appendix 2).

c) Experiment 3

Table 9 shows that the level of intake had no significant effect on PNI. There was a significant variation between the diets in the levels of NCE. The NCE level was higher in feeding level 1 and lower in levels 2 and 3 where as PNI level was lower in feeding level 1 than in levels 2 and 3. However, the trend of the variation in

these parameters was not consistent and is not clear.

5.3 Relationship between PNI and NCE and between PNI and EMPS

A relationship between the PNI and NCE or the EMPS of the combined sets of data from the experiments 1 and 2 showed that the PNI was lower in U1 and U2 followed by the B1 and B2. The FM1 and FM2 showed higher PNI levels whereas the C1 and C2 and B1 and B2 showed higher NCE levels. However, PNI was found to be poorly correlated with the EMPS (Experiment 1, r^2 = 0.163 and Experiment 2, r^2 = 0.119). The relationship between PNI with NCE was slightly better correlated (r^2 = 0.350) in Experiment 1 compared to Experiment 2 (r^2 = 0.171).

Similarly, the relationship between the PNI with EMPS or NCE at 3 levels of GP diet was poorly correlated. This showed that the increase levels of intake did not influence the changes in PNI.

A relationship between PNI and the EMPS of the 3 experiments (pooled data) showed that there was very little or no relationship (r^2 = 0.081, Y = 0.0003X + 0.049, Y = PNI, X = EMPS) between the PNI and the EMPS. It appears therefore that the PNI is not relative to the digestible organic matter intake.

The relationship between PNI and NCE from the pooled data of the 3 Experiments showed that the PNI is better correlated with NCE (r^2 = 0.5019, Y = 0.0727X + 0.0239, Y = PNI, X = NCE).

As predicted the PNI and NCE were found to be lower in the case of urea supplemented diet. The GP diet showed the highest PNI and NCE.

5.4 Diurnal variation of PNI in spot urine

The diurnal variability in the PNI levels plotted from the data of Experiments 1 and 2 are given in Figure 4 and 5 respectively. A general trend has been seen in the U1 and U2, in which the PNI level has been found to decline after the animal were fed. The level increased again between the two feeding times. No specific trend in the variability of the PNI levels was observed in other treatment diets.

The diurnal variation (in terms of co-efficient of variation) was between 3.6 to 29% and 2 to 16% in experiment 1 and 2 respectively.

Figure 4. Diurnal variation in the PNI measured in 12 sheep fed with grass cubes alone or grass cubes supplemented with barley and urea.

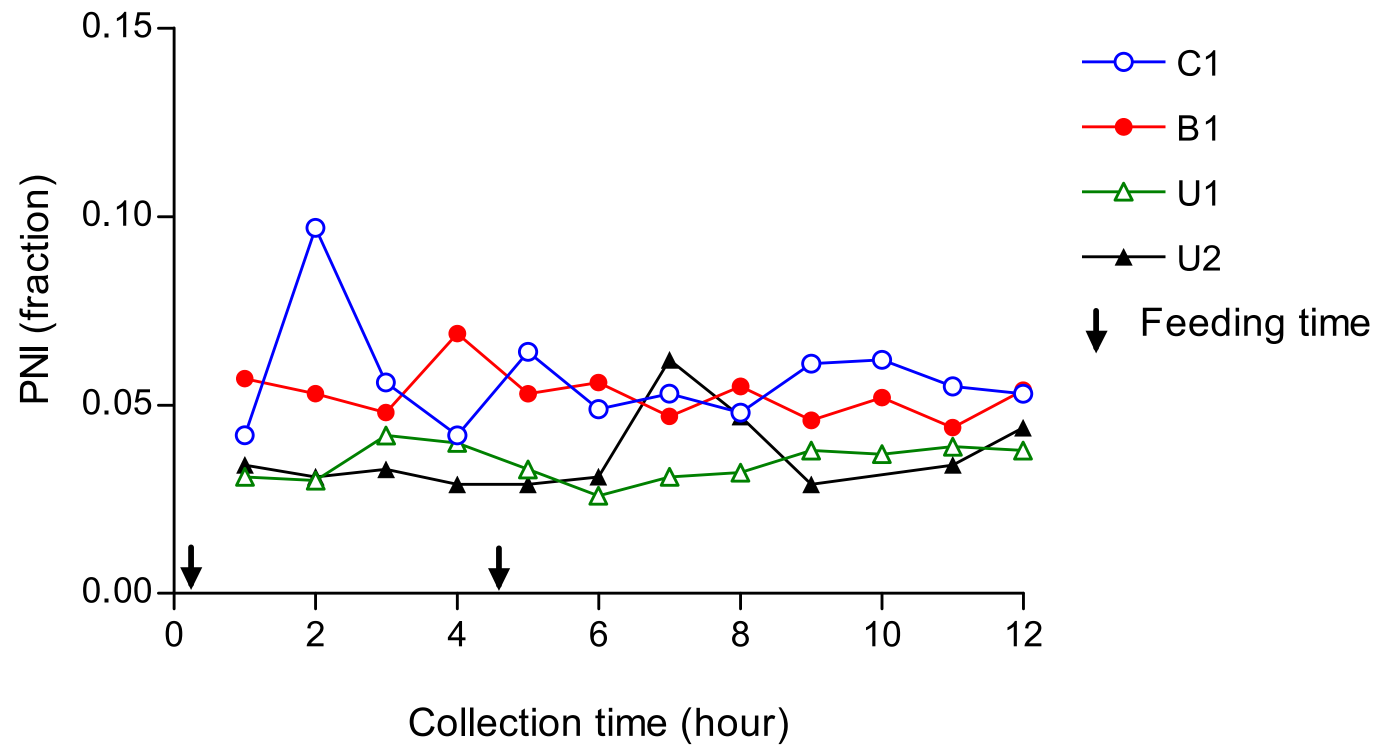
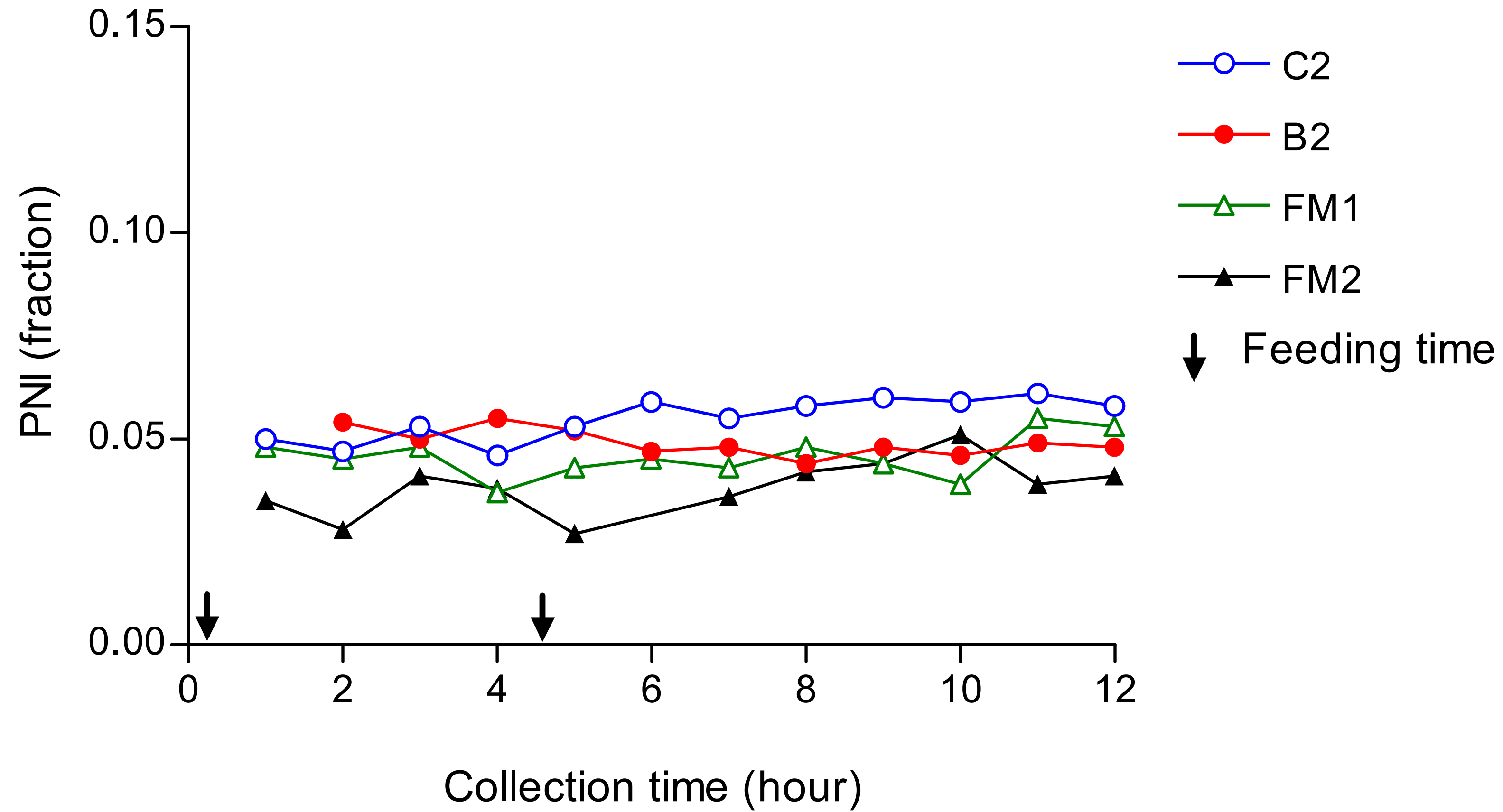


Figure 5. Diurnal variation in the PNI in 12 sheep fed with grass cubes alone or grass cubes supplemented with barley and fish meal.



5.5 Correlation between PNI in spot urine with that of daily urine

In order to validate the PNI level is spot urine, a comparison was made with the PNI in the daily urine samples. The comparison was made between the same treatment groups of animals in Experiments 1 and 2. It was observed that the PNI measured based on daily urine was not significantly different from the averaged PNI of the spot urine samples (Table 10).

Table 10. Comparison between the means of PNI in daily urine and that of spot urine samples

			Means		P (two-tail t-test)
			Daily PNI	Spot PNI	
Experiment 1	Diet	n			
	B1	3	0.048	0.052	0.617
	C1	3	0.042	0.054	0.191
	U1	3	0.040	0.042	0.861
	U2	3	0.032	0.041	0.506
Experiment 2	C2	3	0.050	0.057	0.041
	B2	3	0.050	0.049	0.868
	FM1	3	0.055	0.048	0.435
	FM2	3	0.044	0.041	0.347

The individual variability of the PNI in both the experiments is given in the Table 11. The maximum variation was observed in the urea diet (29 %CV) in the first experiment. In the 2nd experiment the greatest variability was observed in the fish meal diets, the coefficient of variation being 16%.

Table 11. The Mean, SD and % CV of PNI measured 2 hourly in 12 sheep in experiments 1 and 2, (quarterly mean values of 12 collections)

	Purine Nitrogen Index Collection Time (hour)								
	Sheep	Diet	09-15 hours	15-21 hours	21-03 hours	03-09 hours	Mean	SD	CV (%)
Experiment 1	145	B1	0.0578	0.0798	0.0531	0.0471	0.059	0.014	23.98
	147	B1	0.0517	0.0402	0.0618	0.0538	0.052	0.009	17.22
	150	B1	0.0483	0.0483	0.0385	0.0460	0.045	0.005	10.22
	141	C1	0.0393	0.0420	0.0481	0.0692	0.050	0.014	27.24
	143	C1	0.0739	0.0680	0.0549	0.0647	0.065	0.008	12.17
	149	C1	0.0436	0.0393	0.0405	0.0394	0.041	0.002	4.91
	142	U1	0.0233	0.0250	0.0298	0.0356	0.028	0.006	19.42
	146	U1	0.0377	0.0379	0.0373	0.0403	0.038	0.001	3.56
	148	U1	0.0274	NA	NA	0.0380	0.033	0.007	22.85
	139	U2	0.0473	0.0307	0.0633	0.0441	0.046	0.013	28.88
	140	U2	0.0322	0.0288	0.0303	0.0344	0.031	0.002	7.77
	144	U2	0.0304	0.0294	0.0287	0.0321	0.030	0.001	4.90
Experiment 2	140	C2	0.0568	0.0466	0.0593	0.0596	0.056	0.006	11.03
	147	C2	0.0490	0.0583	0.0571	0.0664	0.058	0.007	12.37
	148	C2	0.0520	0.0487	0.0551	0.0539	0.052	0.003	5.28
	139	FM1	0.0435	0.0428	0.0578	0.0468	0.048	0.007	14.58
	141	FM1	0.0556	0.0437	NA	0.0607	0.053	0.009	16.32
	150	FM1	0.0447	0.0353	0.0469	0.0445	0.043	0.005	12.09
	143	FM2	0.0359	0.0412	0.0448	0.0423	0.041	0.004	9.14
	145	FM2	0.0380	0.0290	0.0373	0.0413	0.036	0.005	14.40
	149	FM2	NA	0.0423	0.0439	0.0425	0.043	0.001	1.97
	142	B2	0.0496	0.0485	0.0418	0.0422	0.046	0.004	9.02
	144	B2	0.0465	0.0498	0.0496	0.0488	0.049	0.002	3.16
	146	B2	0.0585	0.0539	0.0478	0.0555	0.054	0.004	8.31

NA: Not available

In order to explore the best fit for the equation, the samples from 12 hour collections were pooled to give 4 groups as shown in Table 11 and 12. Among the four groups, the first group (i.e. the first three collections post feeding) gave a better relationship with the daily PNI compared to the rest of the pooled groups.

The three spot samples were further compared individually with the daily results (Table 13). The comparison was made between the same group of sheep on the same treatments.

It was found that the 2nd spot sample i.e. obtained between 3 to 5 hours post feeding gave as good correlation (r^2 = 0.25) as it was from the combined spot samples (r^2 = 0.26) of the first group.

Table 12. Comparison between the daily and the spot PNI when 12 collections of 24 hours were pooled into 4 groups

Time	Equation	R^2
09-15 hours	Y = 0.66X + 0.02	0.26
15-21 hours	Y = 0.58X + 0.02	0.15
21-03 hours	Y = 0.35X + 0.03	0.08
03-09 hours	Y = 0.57X + 0.02	0.24

Y=spot PNI, X = daily PNI

Table 13. Comparison between the daily and the spot PNI of the first 2 hourly collections.

Time	Equation	R^2
09-11 hours	Y = 0.44X + 0.024	0.09
11-13 hours	Y = 1.14X-0.004	0.25
13-15 hours	Y = 0.55X + 0.02	0.02

Y=spot PNI, X = daily PNI

CHAPTER VI

DISCUSSION

6.1 Relationship between Purine Nitrogen Index and Nitrogen Capture Efficiency

Experiment 1 which consisted of Urea and Barley as supplements was designed with the expectation that the PNI level will be reduced with an increase in supplementation of the urea in the grass cube diet. Similarly, Experiment 2 was designed with an expectation that the PNI level in the fish meal supplemented diet would remain unchanged, or a slight increase may occur when the level of the fish meal is increased. Likewise, the expectation from the supply of barley at two levels (level 1 in Experiment 1, 200g/d and level 2 in Experiment 2, 400g/d) was that the increased barley grain supplement will increase the PNI. A similar expectation i.e. increase in the PNI with an increase in dietary intake was assumed in the 3rd Experiment, in which GP diet at 3 levels of intake was given.

The overall expectation was that there would be a good correlation between the PNI and NCE i.e. the changes in PNI would influence changes in the level of NCE.

As expected, Experiment. 1 revealed that the NCE was reduced in proportion to the intake of RDN (g/kg DOMR). The NCE was 0.367, 0.437, 0.292 and 0.209 respectively for B1, C1, U1 and U2 diets. The dietary supplements of RDN g/kg DOMR were 37.52, 42.42, 50.97 and 59.52 in this order for B1, C1, U1 and U2 diets. The difference between the treatment means was highly significant $P<0.001$.

In the case of PNI, a similar trend of variation i.e. decrease in PNI with an increase in the dietary supply of RDN (g/kg DOMR) was obtained. The difference was significant at $P<0.05$. However, the C1 showed slightly lower PNI levels than it was expected. Although not statistically significant, as expected, the trend of the levels MN was found to decline with an increase in the levels of RDN (g N/kg DOMR) supplemented in the diets. As the PNI is derived from MN and the total urine nitrogen, the difference i.e. lower PNI value of C1 as compared to the U1 could be due to larger amount of excretion of nitrogen in the urine of the C1 group.

Experiment 2, which included fish meal (at two levels), barley (400g/d) and a similar control diet (C2) as in the Experiment 1 shows that the trend of the levels of NCE and MN in these diets were similar to my expectation. Although statistically insignificant there was a clear trend that NCE was higher with the increase in the level of intake. The treatments C2, FM1 and FM2 which included more or less similar DOMI (digestible organic matter intake, kg/d) showed lower NCE compared to the B2 diet which included a higher DOMI (kg/d). This agreed with the expectation.

However, in the case of PNI, a correlation between PNI and NCE can be seen in all treatments except FM1. It was expected that PNI from FM1 should be similar to FM2 or at least less than the PNI level of B2. The higher level of PNI in FM1 could possibly be due to lower level of total nitrogen excretion in urine.

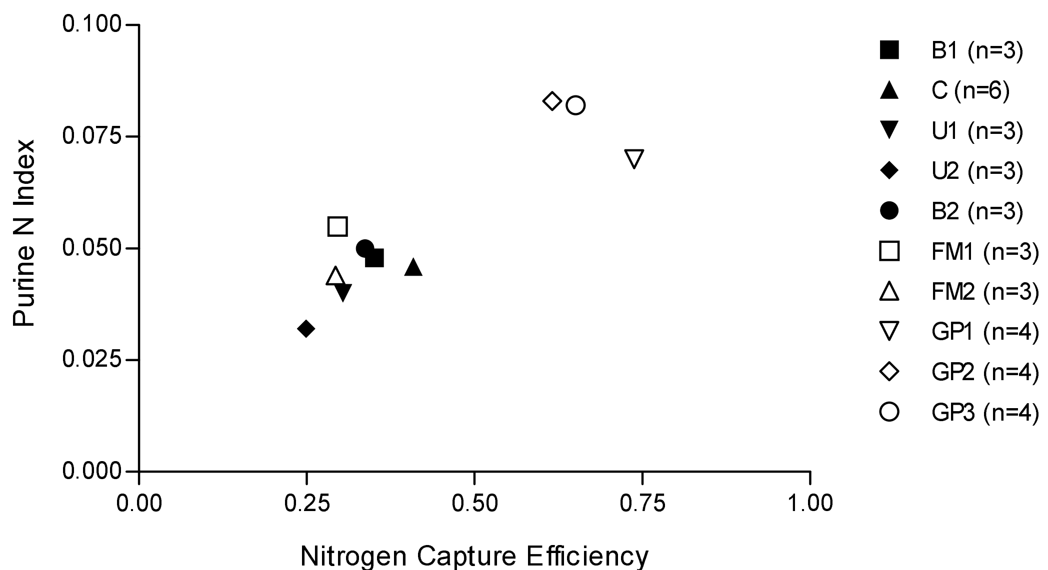
Comparing the NCE and PNI levels between B1 (Experiment 1) and B2 (Experiment 2), as expected the B2 diet showed slightly higher levels of NCE. However, the difference in PNI between B1 and B2 was almost similar. Three levels of the GP diet in Experiment3, shows a trend similar to my expectation. It shows an increase in PNI with an increase in DOMI (kg/d), proportionally there was an increase in MN. However, the level of NCE among the treatments was not consistent. No any specific trend in the levels of NCE in relation to DOMI or

RDN was seen.

Looking at the results of the three experiments individually, it has generally been observed a narrow distribution of data i.e. the data were closer and not well spread or separated from one data point of one treatment diet to another. Although, the general trend to my expectation was evident but not to a significant level.

However, when all the three experiments are combined together, a difference among the data points have been clearly visible. The GP diet (Experiment 3) had a higher NCE than the grass cubes based diets (Experiments 1 and 2). The difference was clearly reflected in the PNI (Figure 6). PNI of the combined sets of data was positively correlated with NCE (r^2 = 0.50). The PNI (average of each treatment) ranged from 0.027 to 0.083, and the corresponding NCE from 0.022 to 0.74.

Figure 6. Relationship between PNI and NCE at Urea, Barley, Fish meal and GP diet.



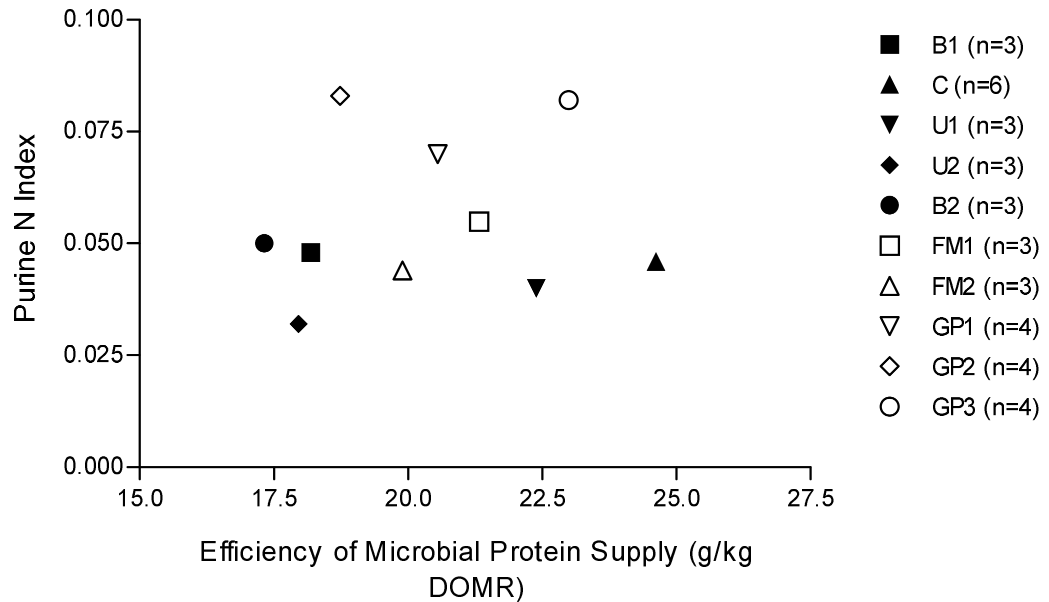
It appears PNI does reflect the magnitude of the NCE, thus providing an indication of how well dietary nitrogen is converted into microbial protein. However, it may

not be able to distinguish diets when the differences are too small.

6.2 Relationship between Purine Nitrogen Index and Efficiency of Microbial Protein Supply.

From the observation of the pooled data sets of the 3 combined experiments i.e. urea, barley, fish meal and the GP diet, it can be seen that the PNI is poorly correlated with EMPS (Figure 7). In fact, it is not expected that EMPS is correlated with either NCE nor PNI. The distribution of data also indicated that the PNI is not affected by the digestible organic matter intake.

Figure 7. Relationship between PNI and EMPS at Barley, Urea, Fish meal and GP diet.



The supply of microbial protein into the intestine may be related to the rumen digesta outflow rates. The diets with slow rumen digesta outflow rates may reduce the efficiency of microbial nitrogen production (Chen, 1992). When the outflow of the microbial protein from the rumen is slow, proportionally there will be an

increase in the breakdown of the microbial cells into ammonia. The ammonia can be re-utilised by the animal to form microbial protein but a greater portion will be excreted in urine. Thus the efficiency of supply of microbial protein will decline at low rate of passage of rumen digesta.

6.3 Correlation between PNI in spot urine with that of daily urine

There was not any defined pattern of diurnal variation due to different diets observed in all the experiments. However, in the case of the urea fed groups a tendency that the concentration of PNI was found declining sometimes after each feeding time. This is perhaps due to that the urea is degraded and recycled rapidly and the excess nitrogen is excreted in the urine.

There was a larger variation of spot PNI between the sampling time (3.60 – 29% in Experiment 1 and 2 – 16% in Experiment 2). Digestion and absorption of nutrients are related to the rate of passage and the retention time of the feed in the rumen. The poor correlation between the spot PNI and the daily PNI therefore, could be associated with feeding time i.e. the sheep were fed twice a day. It may be necessary to carry out a comparative study with animals fed continuously.

Other reason of the variability could be due to the frequency of urination by the individual animals i.e. in this experiment some excreted urine through-out the collection period whereas others excreted in long intervals. Therefore, this difference in urinary out-put might have effected in the concentration of PNI in the spot urine sample.

The results showed that it is promising to use PNI based on spot samples to provide a quick indicator of nitrogen utilisation efficiency. Since the PNI is a ratio, it does not require total urine collection.

However, due to the variability of spot PNI, the collection of spot urine sample will be required to give a reasonable representation of the daily collection. To derive a better estimate an approach to collect samples at the same time over a number of days as suggested by Chen (1995b) could be made. The average value of such measurement could then provide better estimate of the PNI for the given diet.

CHAPTER VII

CONCLUSION

7.1 Conclusions from the experiments

1. Based on combined data of the 3 experiments on the relationship between PNI and NCE, the study revealed that PNI could differentiate between the 'efficient and inefficient' diets in terms of the efficiency of conversion of dietary nitrogen into microbial protein. About 74% of dietary nitrogen in the GP diet was converted into microbial protein, however in the grass cube based diets the figure was 20 - 44%. Therefore, in terms of efficiency of conversion, GP was better than the grass cube based diets

2. However, based on the data of the individual experiment, it was also evident that PNI can not distinguish between small differences.

3. The study clearly showed that PNI was not related with the efficiency of microbial protein supply (EMPS). It has thus indicated that the PNI is not effected by digestible organic matter intake. Based on Experiment 3, the EMPS could be related to the level of intake.

4. The variation of PNI in the spot urine sample was as high as 29%. The collection of the spot urine samples between 3 - 5 hours of feeding better represented PNI of the total daily urine collection than other times. An approach to

collect the samples at the same time over a number of days is suggested to get a better daily estimate.

7.2 Limitations

The PNI will not provide an explanation of the cause of poor efficiency of nutrient conversions.

It can not differentiate small differences. It is more suitable for indicating inefficiencies when PNI has reached a critical point.

7.3 Future areas of work

In future there is a need to validate the concept with direct measurement of microbial protein.

Derivation of the PNI factors or reference values for ruminants at various production levels to put the concept into practice.

Confirmation of spot urine collection time so that it will best represent the daily collection.

REFERENCES

AFRC (1992). Technical Committee on response to nutrients. Report no 9, **Nutritive Requirements of Ruminant Animals: Protein**, CAB

AOAC (1980). Official methods of analysis. 13th edition, Association of Official Analytical Chemists, Washington, DC, USA

ARC (1984) Agriculture Research Council, U K

Anon (1995). Annual Report, Rowett Research Institute, Bucksburn, Aberdeen. AB21 9SB

Argyle, J. and Baldwin, R. (1989). Effects of amino acids and peptides on rumen microbial growth yields, Journal of Dairy Science, **72**, 2017

Balcells, J., Gauda, J.A., Castrillo, C and Gasa, C (1993). Rumen digestion and urinary excretion of purine derivatives response to urea supplementation of sodium treated straw fed to sheep. *British Journal of Nutrition*, **69**, 721 - 732

Bird, S. H., Nolan, J. V. and Leng, R. A. (1990). The nutritional significance of rumen protozoa**. In: The Rumen Ecosystem-The Microbial Metabolism and Its Regulation** (ed. Sadao Hoshino, Ryoji Oonodera, Hajime Minato and Hisao Itabashi) Japan Scientific Societies Press, 151-160

Broderick, G. A. and Merchen, N. R. (1992). Markers for quantifying microbial protein synthesis in the rumen, *Journal of Dairy Science*, **75**, 2618

Cecava, M. J. and Parker, J. E. (1993). Intestinal supply of amino acids in steers fed ruminally degradable and undegradable crude protein sources alone and in combination. *Journal of Animal Science*. Champaign iii, American Society of Animal Science, **71 (6),** 1596 - 1605

Cecava, M. J., Merchen, N. R., Beger, L. L., Mackie, R. I., Fahey, G. C. Jr. (1991). Effects of dietary energy level and protein source on nutrient digestion and ruminal nitrogen metabolism in steers, *Journal of Animal Science*, Champaign, iii: American Society of Animal Science, **69 (5)**, 2230 - 2243

Chamberlain, D. G., Robertson, S. and Choung, J. J. (1993). Sugars versus starch as supplements to grass silage: effects on ruminal fermentation and the supply of microbial protein to the small intestine, estimated from the urinary excretion of purine derivatives, in sheep. *Journal of Science Food and Agriculture*, **63**, 189-194

Chen, X. B. (1989). *Excretion of purine derivatives by sheep and cattle and its use for the estimation of absorbed microbial protein*, PhD Thesis, University of Aberdeen

Chen, Y. K. (1990). *The effect of feeding level on rumen dilution rate and efficiency of microbial protein production in sheep*, MSc Thesis, University of Aberdeen

Chen, X. B., Hovell, F. D. D. and Ørskov, E. R. (1990a). Excretion of Purine derivatives by ruminants: recycling of allantoin into the rumen via saliva and its fate in the gut, *British Journal of Nutrition*, **63**, 197 - 205

Chen, X. B., Mathieson, J., Hovell, F. D. D. and Reeds, P. (1990b). Measurement of Purine derivatives in urine of ruminants using automated methods, *Journal of Science and Agriculture*, Great Britain.

Chen, X. B., Ørskov, E. R. and Hovell, F. D. D. (1990c). Excretion of purine derivatives by ruminants: endogenous excretion, differences between cattle and sheep, *British Journal of Nutrition*, **63 (1)**, 121 - 129

Chen, X. B., Hovell, F D DeB., Ørskov, E. R. and Brown, D S (1990d). Excretion of purine derivatives by ruminants : effect of exogenous nucleic acid supply on purine derivative excretion by sheep. *British Journal of Nutrition*, **63**, 131-142

Chen, X B., Mathieson, J., Hovell, F D Deb and Reeds, P J (1990e). Measurement of purine derivatives in urine of ruminants using automated methods. Journal of Science Food and Agriculture, **53**, 23 - 33

Chen, X. B. Kyle., D. J. and Ørskov, E. R. (1993). Measurement of allantoin in urine and plasma by high-performance liquid chromatography with pre-column derivatization. Journal of Chromatography, 617, 241 - 247

Chen, X. B., Chen, Y. K., Franklin, M. F., Ørskov, E. R. and Shand, W. J. (1992). The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep, *Journal of Animal Science*, **70**, 1534 - 1542

Chen, X. B. and Gomes, M. J. (1992), Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives - an over view of the technical details, *Occasional Publication*, Rowette Research Institute, Aberdeen, UK

Chen, X. B., Mejia, A. T., Kyle, D. J. and Ørskov, E. R. (1995a). Evaluation of the use of the purine derivative: creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants: Studies in sheep, *Journal of Agricultural Science*, Cambridge, **125**, 137 – 143

Chen, X. B., Susmel, P., Stefanon, B. and Ørskov, E. R. (1995b). On the use of purine derivatives in spot urine, plasma and milk samples as indicators of microbial protein supply in sheep and cattle. In: Protein metabolism and nutrition, Proceeding *of the 7th International Symposium on Protein Metabolism and Nutrition*, Vale de Santare'm Portugal, 24 - 27 May 1995, EAAP publication no 81

Chikunya, S., Newbold, C. J., Rode, L., Chen, X. B., Wallace, R. J. (1996). Influence of dietary rumen degradable protein on bacterial growth in the rumen of sheep receiving different energy sources. *Animal Feeds Science and Technology*, **63 (1-4)**, 333 - 340

Clark, J. H., Klusmeyer, T. H. and Cameron, M. R. (1992). Symposium : nitrogen metabolism and amino acid nutrition in dairy cattle - microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. *Journal of Dairy Science*, **75**, 2304 - 2323

Colucci, P. E., Macleod, G. K., Grovum, W. and McMillan, I. (1984). Comparative digestion and digesta kinetics in sheep and cattle.*Canadian Journal of Animal Science*, **64**, 173 - 174

Davidson, J., Mathieson, J., and Boyne, A.W. (1970). The use of automation in determining nitrogen by the Kjeldahl method with final calculation by computer. *Analyst*, **95**, 181 - 193

Djouvinov, D. S. and Todorov, N. A. (1994). Influence of dry matter intake and passage rate on microbial protein synthesis in the rumen of sheep and its estimation by cannulation and a non-invasive method. *Animal Feed Science and Technology*, **48**, 289 – 304

Doreau, M., Sauvant, D. and Verite, R (1991). Which diets for tomorrow ? Rumen microbial metabolism and rumen digestion, In: J. P. Jouany (ed.), Rumen microbial metabolism and rumen digestion, INRA editions, 369 – 372, Paris

Ebeling, M. E. (1968). The Dumas method for nitrogen in feeds. Journal of the association of analytical chemists. **51 (4)**

Elimam, M. E. and Ørskov, E. R. (1984). The effect of the frequency of feeding of the basal diet and the addition of sodium chloride on the outflow of chromium-treated protein supplements from the rumen of high yielding cows, *Animal Production*, **36**, 503

Fujihara, T., Chen, X. B., Ørskov, E.R. and Hovell, F. D. D. (1988). The possible use of purine derivatives in urine to estimate rumen microbial protein production, *Proceeding of the 5th International symposium on protein metabolism and nutrition*, European Association for Animal Production, Publication No p. 35, 2.s. 17 - 18 Rostock: Winhelm - Pieck - University

Giesecke, D., Ehrentreich, L., Stangassinger, M. and Ahrens, F. (1994). Mammary and renal excretion of purine metabolites in relation to energy intake and milk yield in dairy cows. *Journal of Dairy Science*, Champaign iii, American dairy science association, **77 (8)**, 2376 - 2381

Giesecke, D., Stangassinger, M. and Tiemeyer, W. (1984). Nucleic acid digestion and urinary purine metabolites in sheep nourished by intragastric infusions. *Canadian Journal of Animal Science*, **64** (Supplement) 144 - 145

Gomes, M. J., Hovell, F. D. D., Chen, X. B., Nengomasha, E. M. and Fikremariam, D. (1994). The effect of starch supplementation of straw on microbial protein supplies in sheep. *Animal Feed Science and Technology*, **49**, 277-286

Harrison, G. D., Beever, D. E., Thompson, D. J. and Osborne, D. F. (1975). Manipulation of rumen fermentation in sheep by increasing the rate of flow of water from the rumen, *Journal of Agricultural Science*, Cambridge, **85**, 93 - 101

Henning, P. H., Steyn, D. G. and Meissner, H. H. (1993). Effect of synchronisation of energy and nitrogen supply on ruminal characteristics and microbial growth. *Journal of Animal Science*, **71**, 2516 - 2528

Herrera-Saldana, R., Gomez-Alarcon, R., Torabi, M. and Huber, J. T. (1990). Influence of synchronising protein and starch degradation in the rumen on nutrient utilisation and microbial protein synthesis. *Journal of Dairy Science*, **73**, 142 - 148

Hoover, W. H. and Stokes, S. R. (1991). Balancing carbohydrate and protein for optimum rumen microbial yield. *Journal of Dairy Science*. **74 (10)**, 3630 - 3644

Huber, J. T. and Herrera-Saldana, R. (1994). Synchrony of protein and energy supply to enhance fermentation. In: J. Malcolm Asplund (ed.), **Principles of Protein Nutrition of Ruminants**, CRC

Hungate, R. E. (1966). **The Rumen and Mcrobes**. Academic press, New York.

Jouany, J. P. (1991). Defaunation of the rumen: Rumen microbial metabolism and ruminant digestion (ed.: J. P. Jouany) INRA, France

Maeng, W. J., Van Nevel, C. J., Baldwin, R. and Morris, J. (1976). Rumen microbial growth rates and yields:effects of amino acids and protein, *Journal of Dairy Science*, 59 – 68

Mansfield, H. R., Endres, M. I. and Stern, M. D. (1994). Influence of non-fibrous carbohydrate and degradable intake protein on fermentation by ruminal micro-organisms in continuous culture. *Journal of Animal Science*, champaign iii : American Society of Animal Science **72 (9)**, 2464 - 2474

Masson, H. A., Denholm, A. M. and Ling, J. R. (1991). *In-vivo* metabolism of 2-2'-diaminopimelic acid from gram-positive and gram-negative bacterial cells by rumianl micro-organisms and ruminants and its use as a marker of bacterial biomass, *Applied Environmental Microbiology*, **57**, 1714

Mathers, J. C. and Miller, E. R. (1981). Quantitative studies of food protein degradation and the energetic efficiency of microbial protein synthesis in the rumen of sheep given chopped Lucerne and rolled barley, *British Journal of Nutrition*, **45**, 587 - 604

McAllan, A. B. and Smith, R. H. (1973). Degradation of nucleic acids in the rumen. *British Journal of Nutrition*, **29**, 331-345

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D. and Morgan, C. A. (1995). **Animal Nutrition**. Fifth edition, Longman scientific and technical, New York.

McRae, J. C. and Wilson, S. (1977). The effects of various forms of gastrointestinal cannulation on digestive measurements in sheep. *British Journal of Nutrition*, **38**, 65-74

Merchen, N. R., Firkins, J. L. and Berger, L. L. (1985). Effect of intake and forage level on ruminal turnover rates, bacterial protein synthesis and duodenal amino acids flows in sheep. *Journal of Animal Science*, **62**, 216 - 225

Merry, R. J., McAllan, A. B. and Smith, R. H. (1990). *In-vitro* continuous culture studies on the effect of nitrogen source on rumen microbial growth and fibre digestion, *Animal Feed Science and Technology*, **31 (1-2)**, 55 - 64

Meyer, J. H. F., Van der Watt, S. I. and Schwartz, H. M. (1986). The influence of diet and protozoal numbers on the breakdown and synthesis of protein in the rumen of sheep. *Journal of Animal Nutrition*, **62**, 509 - 520

Nocek, J. E. (1988). Production research papers: *In situ* and other methods to estimate ruminal protein and energy digestibility-a review. *Journal of Dairy Science*, **71**, 2051 - 2069

Ørskov, E. R., Hovell, F. D. D. and Mould, F. L. (1980). The use of nylon bag technique for the evaluation of feedstuffs. *Tropical Animal Production* **5,** 195-213

Puchala, R. and Kulasek, G. W. (1992). Estimation of microbial protein flow from the rumen of sheep using microbial nucleic acid and urinary excretion of purine derivatives. *Canadian Journal of Animal Science*. **72**, 821-830

Samaniego, M. A. (1996). *Comparison of methods for the measurement of digesta flow and microbial protein supply in sheep*. M. Sc. Thesis, University of Aberdeen

Shriver, B. J., Hoover, W. H., Sargent, J. P., Crawford, R. J. Jr. and Thayne, W. V. (1986). Fermentation of a high concentrate diet as affected by ruminal pH and digesta flow, *Journal of Dairy Science*, **69**, 413

Sinclair, A., Garnsworthy, P. C., Newbold, J. R. and Buttery, P. J. (1995) Effects of synchronising the rate of dietary energy and nitrogen release in diets with a similar carbohydrate composition on rumen fermentation and microbial protein synthesis in sheep. *Journal of Agricultural Science*, Cambridge, **124**, 463 - 472

Sinclair, L. A., Garnsworthy, P. C., Newbold, J. R. and Buttery, P. J. (1993). Effect of synchronising the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep, *Journal of Agricultural Science*, **120 (2)**, 251 - 263

Stern, M. D. and Hoover, W. H. (1979). Methods for determining and factors affecting rumen microbial protein synthesis: a review. *Journal of Animal Science*, **49 (6)**, 1590 - 1601

Stern, M. D., Varga, G. A., Clark, J. H., Firkins, J., Huber, J. T. and *Palmquist*, D. (1994). Symposium: metabolic relationships in supply of nutrients for milk protein synthesis . In: *Journal of Dairy Science*, **77**, 2762-2786.

Stokes, S. R., Hoover, W. H., Miller, T. K., Blauwiekel, R. (1991). Ruminal digestion and microbial utilisation of diets varying in type of carbohydrate and protein. *Journal of Dairy Science*, **74 (3)**, 871-881

Susmel, P., Stefanon, B., Plazzotta, E., Spanghero, M. and Mills, C. R. (1994). The effect of energy and protein intake on the excretion of purine derivatives. *Journal of Agricultural Science*, Cambridge, **123**, 257-265

Topps, J. H. and Elliot, R. C. (1965). Relationship between concentration of ruminal nucleic acids and excretion of purine derivatives by sheep. *Nature*, **205**, 498 - 499

Ushida, K., De smet, S., Kayouli, C. and Jouany, J. P. (1989). Effect of defaunation on nitrogen digestion in sheep fed ammonia treated straw with or without maize. In: **The Role of Protozoa and Fungi in Ruminant Digestion** (eds. J V Nolan; R A Leng and D I Demeyer) Penambul Books, Australia, 309 – 310

Ushida, K., Kayouli, C., Smel, S de. and Jouany, J. P. (1990). Effect of defaunation on protein and fibre digestion in sheep fed on ammonia-treated straw-based diets with or without maize. *British Journal of Nutrition*, Cambridge, **64 (3)**, 765 - 775

Van Nevel, C. J. and Demeyer, D. I. (1988). Manipulation of rumen fermentation. In: **The Rumen Microbial Ecosystem** ed. P N Hobson, Elsvier, 387 - 443

Verbic, J., Chen, X. B., Macleod, N. A. and Ørskov, E. R. (1990). Excretion of purine derivatives by ruminants - effect of microbial nucleic acid infusion on purine derivative excretion by steers. *Journal of Agricultural Science*, **114**, 243 - 248

Voigt, J., Jentsh, W., Schonhusen, U., Beyer, M. and Kreienbring, F. (1993). Influence of starch sources barley, maize, potatoes and their dietary proportions on nutrient digestibility and energy utilisation in ruminants. 4. Nitrogen metabolism in the rumen. **Archives of Animal Nutrition**. **44 (4)**, 369 - 382

Wallace, R. J. and Cotta, M. A. (1988). Metabolism of Nitrogen containing compounds, In: **The Rumen Microbial Ecosystem** (ed. P N Hobson), Elsevier applied science, London and Newyork, 217 - 249

Wallace, R. J., Newbold, C. J. and McKain, N. (1990). Patterns of peptide metabolism by rumen micro-organisms. In: **The Rumen Ecosystem - The Microbial Metabolism and Its Regulation** (eds: Sadao Hoshino, Ryoji Onodera; Hajime Minato and Hisao Itabashi), Japan Scientific Societies Press, 43 - 59

Wenham, G. (1979). Effects of cannulation on intestinal motility. *Ann. Rech. Vet.* **10**,157 - 166

Witt, M. W., Sinclair, L. A., Wilkinson, R. G. and Buttery, P. J. (1997). Effects of synchronising the rate of energy and nitrogen supply to the rumen in diets with two rates of carbohydrates release on growth and metabolism of male lambs. Proceedings of the British Society of Animal Science, BSAS, UK

Appendix 1. Total Purine Derivatives, PNI, Microbial Protein Supply and NCE in 12 sheep at barley1 and urea diets with grass cubes as basal diet (Results are the Mean, SD and %CV of 3 sheep in a 5 days period).

Diet		DOMI	RDN	Excretion of purine derivatives				PDN	N	Total Nitrogen	PNI	Microbial Nitrogen		NCE
				Allantoin	Uric acid	H+X	Total PD							
		kg/d	g/d	mmol/d	mmol/d	mmol/d	mmol/d	mg/l	g/d	mg/l	(fraction)	g N/d	g N/kg DOMR	(fraction)
B1	Mean	0.62	20.83	6.48	1.22	1.03	8.73	88.06	10.18	1832.64	0.048	7.559	19.540	0.367
	SD	0.08		0.84	0.10	0.11	0.89	9.11	1.29	238.92	0.005	0.804	0.938	0.043
	%CV	12.39		12.90	8.48	10.35	10.18	10.34	12.67	13.04	10.058	10.641	4.800	11.739
C1	Mean	0.51	18.49	6.54	1.76	1.39	9.69	97.91	13.86	2501.24	0.039	8.383	24.983	0.437
	SD	0.11		0.59	0.79	0.17	1.21	12.79	1.52	289.63	0.001	1.131	11.623	0.085
	%CV	22.08		9.05	45.11	12.55	12.54	13.06	11.0	11.58	1.778	13.495	46.526	19.358
U1	Mean	0.47	22.22	5.69	1.29	1.18	8.15	82.23	12.99	2341.41	0.040	7.058	22.493	0.292
	SD	0.05		0.94	0.27	0.28	1.21	12.13	0.73	139.04	0.004	1.143	7.234	0.063
	%CV	9.98		16.54	21.32	24.16	14.90	14.75	5.65	5.94	11.161	16.200	32.161	21.609
U2	Mean	0.56	25.95	4.61	0.94	1.13	6.67	66.55	13.62	2422.16	0.027	5.775	14.743	0.209
	SD	0.05		0.51	0.14	0.13	0.73	7.28	0.86	149.85	0.005	0.746	4.152	0.041
	%CV	8.12		11.12	14.88	11.54	10.99	10.94	6.30	6.19	17.110	12.923	28.159	19.387

DOMI = Digestible Organic Matter Intake, RDN = Rumen Degradable Nitrogen, H+X = Hypoxanthine + Xanthine, PDN = Purine Derivative Nitrogen, PNI = Purine Nitrogen Index, NCE = Nitrogen Capture Efficiency, MN = Microbial Protein Supply, EMPS = Efficiency of Microbial Protein Supply

Appendix 2. Total PD, PNI, Microbial Protein Supply and NCE in 12 sheep at barley and fish meal supplemented diets with grass cubes as basal diet (Results are the Mean, SD and %CV of 3 sheep in a 5 days period).

Diet		DOMI	RDN	Excretion of purine derivatives				PDN	N	Total Nitrogen	PNI	Microbial Nitrogen		NCE
				Allantoin	Uric acid	H+X	Total PD							
		kg/d	g/d	mmol/d	mmol/d	mmol/d	mmol/d	mg/l	g/d	mg/l	(fraction)	g N/d	g N/kg DOMR	(fraction)
B2	Mean	0.76	23.17	6.91	1.73	1.06	9.69	95.88	11.59	2050.69	0.047	8.223	17.986	0.373
	SD	0.04		2.31	0.38	0.10	2.21	23.62	1.15	235.75	0.006	2.001	5.202	0.114
	%CV	5.45		33.40	21.98	9.76	22.80	24.64	9.94	11.50	12.389	24.329	28.922	30.554
C2	Mean	0.51	18.49	5.44	1.42	1.02	7.87	78.76	9.43	1686.15	0.046	6.431	19.319	0.344
	SD	0.03		2.44	0.49	0.17	3.05	31.19	2.70	498.69	0.006	2.999	11.991	0.229
	%CV	6.03		44.94	34.55	16.36	38.75	39.60	28.64	29.58	14.214	46.628	62.070	66.599
FM 1	Mean	0.48	20.20	5.05	1.18	1.24	7.47	74.51	8.48	1513.24	0.051	6.151	19.392	0.317
	SD	0.08		0.34	0.03	0.33	0.45	4.05	1.16	220.44	0.010	0.428	2.965	0.004
	%CV	17.08		6.82	2.83	26.58	6.06	5.44	13.68	14.57	20.025	6.956	15.288	1.118
FM 2	Mean	0.54	21.91	5.85	1.18	1.02	8.05	80.02	10.85	1926.60	0.042	6.703	18.755	0.291
	SD	0.03		1.43	0.20	0.31	1.92	18.95	0.11	20.43	0.010	1.794	8.231	0.110
	%CV	6.12		24.39	16.79	29.83	23.80	23.68	1.03	1.06	24.720	26.757	43.884	37.723

DOMI = Digestible Organic Matter Intake, RDN = Rumen Degradable Nitrogen, H+X = Hypoxanthine + Xanthine, PD = Purine Derivatives, PDN = Purine Derivative Nitrogen, PNI = Purine Nitrogen Index, NCE = Nitrogen Capture Efficiency, MN = Microbial Protein Supply, EMPS = Efficiency of Microbial Protein Supply

Appendix 3. Total PD, PNI, Microbial Protein Supply and NCE in 4 sheep at 3 levels of GP diet. (Result is the average 4 sheep at 12 days period)

Diet		DOMI	RDN	Excretion of Purine Derivatives				PDN	N	Total Nitrogen	PNI	Microbial Protein Nitrogen		NCE
				Allantoin	Uric acid	H+X	Total PD					MN	EMPS	
		kg/d	g/d	mmol/d	mmol/d	mmol/d	mmol/d	mg/l	g/d	mg/l	(fraction)	g N/d	g N/kg DOMR	(fraction)
GP1	Mean	0.575	10.340	7.54	1.30	0.21	9.06	169.03	7.58	2475.1	0.070	7.635	20.554	0.738
	SD	0.023		1.406	0.124	0.046	1.541	28.767	1.65	583.6	0.007	1.412	4.502	0.137
	%CV	3.980		18.6	9.5	21.9	17.0	17.0	21.84	23.6	9.7	18.5	21.9	18.5
GP2	Mean	0.780	15.510	9.47	1.48	0.22	11.17	208.53	8.65	2703.8	0.08	9.56	18.74	0.62
	SD	0.063		2.383	0.270	0.057	2.605	48.622	1.93	915.5	0.025	2.327	3.486	0.150
	%CV	8.080		25.2	18.2	26.0	23.3	23.3	22.29	33.9	30.3	24.4	18.6	24.4
GP3	Mean	0.897	20.680	13.57	1.84	0.18	15.58	290.86	10.61	3536.6	0.08	13.45	22.99	0.65
	SD	0.145		3.063	0.367	0.087	3.329	62.146	1.03	343.03	0.011	2.911	2.645	0.141
	%CV	16.206		22.57	19.99	49.26	21.37	21.37	9.70	9.70	13.76	21.64	11.50	21.64

GP1 = Fed at 800 g/d, GP2= Fed at 1200 g/.d and GP3 =Fed at 1600 g/d: H+X = Hypoxanthine + Xanthine, PD = Purine Derivatives, PDN = Purine Derivative Nitrogen, PNI = Purine Nitrogen Index, NCE = Nitrogen Capture Efficiency, RDN = Rumen Degradable Nitrogen, DOMI = Digestible Organic Matter Intake, MN = Microbial Protein Supply, EMPS = Efficiency of Microbial Protein Supply