PREDICTION OF RUMEN MICROBIAL OUTFLOW BASED ON URINARY EXCRETION OF PURINE DERIVATIVES

XA9951060

J.V. NOLAN
Division of Animal Science,
University of New England,
Armidale, New South Wales,
Australia

Abstract

PREDICTION OF RUMEN MICROBIAL OUTFLOW BASED ON URINARY EXCRETION OF PURINE DERIVATIVES.

The method for predicting microbial protein outflow from the rumen based on the excretion of purine derivatives (PD) in the urine is being increasingly used by nutritionists. In contrast to methods that depend on estimates of digesta flow, the PD method does not require animals to be fitted surgically with cannulae into the gut, and studies can be performed with minimal disturbance to the experimental animals. Methods of analysis of PD have been improved and standardized. Certain assumptions, however, are required that could lead to errors when this method is used to predict microbial protein outflow from the rumen. The need for further investigation of these assumptions by means of isotopic tracers and other techniques is examined.

1. INTRODUCTION

Estimation of the flow of digesta from the rumen (or of any component of the digesta) has always been a difficult problem for ruminant nutritionists. The majority of estimates of digesta outflow from the rumen have been made using surgically modified animals. Several such methods have been used. Commonly, digesta leaving the rumen are either quantitatively collected, or representative samples obtained and their contribution to the total outflow is estimated by reference to a non-absorbable gut marker that passed through the gut at a known rate. These methods involve surgical intervention and the animals are frequently disturbed while digesta samples are collected.

Researchers usually want to estimate the rate of microbial or 'escape' protein outflow rather than total digesta outflow. Determination of microbial outflow (or outflow of a microbial component such as microbial crude protein) require obtaining accurate quantitative information on both digesta and microbial marker flow. Digesta consist of a heterogeneous mixture of dietary, microbial and endogenous materials. Markers that are specific for microorganisms are required and errors associated with these markers further reduce the accuracy of the final estimates of the rate of microbial outflow. External markers such as ^{15}N and ^{35}S, and internal markers such as L-alanine, diaminopimelic acid have been used. Purine compounds have been evaluated and are the subject of the discussion below.

The idea of using microbial purine compounds as a specific marker for the rumen microbial biomass was suggested by McDonald in 1954 [1] and by others [2-5]. Purines are heterocyclic ring structures (nitrogenous bases) with varying functional groups. The purine bases, adenine and guanine are found in both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The purine nucleotides (adenylate and guanylate) are the building blocks of the nucleic acids, DNA and RNA, and consist of, (a) a purine base which is heterocyclic nitrogenous compound, (b) a sugar (ribose in RNA, deoxyribose in DNA) and (c) a

phosphoryl group ester-linked to the sugar. Adenosine and guanosine are the corresponding nucleosides formed when the phosphate groups are removed.

Micro-organisms have high concentrations of purine-containing compounds (RNA and DNA) relative to concentrations in plant and mammalian cells. Moreover, purines in dietary and endogenous materials are, in general, rapidly degraded by microbial enzymes in the rumen [6]. They are therefore likely to be present in only negligible concentrations in digesta leaving the rumen. The microbial purines, on the other hand, remain intact in living microbial cells and pass via the abomasum to the small intestine. The purines present in digesta entering the small intestine can therefore be expected to be almost totally of microbial origin. Microbial purines are, therefore, specific markers of the microbial fraction. If the concentrations of purines in the whole digesta and in a pure sample of the microbial biomass are expressed as a ratio, the result is an estimate of the fraction of the digesta that is of microbial origin. If digesta flow rate is determined using cannulated animals and non-absorbable gut markers as mentioned above, the flow rate of crude protein or any other microbial component in the digesta can also be determined from its ratio relative to the purine concentration.

The use of purines as microbial markers has special appeal by eliminating the need for estimation of digesta flow rate. When microbial materials containing purines enter the abomasum and small intestine, they are degraded enzymatically to nucleotides and purine bases [7]. These are then absorbed into the body of the animal. Although these purine compounds may be incorporated into tissues, the amount absorbed greatly exceeds tissue requirements and the majority is excreted via the kidney. The rates of purine derivatives (PD) in the urine therefore tend to closely reflect, and can therefore be used to predict, the flows of microbial purines into the intestines [8]. If the concentration of purines in a pure sample of the mixed microbial biomass is known, as well as the predicted flow rate of purines from the rumen, then the flow of microbial biomass can be simply determined.

2. PRINCIPLE OF THE PURINE-BASED ESTIMATION OF RUMEN MICROBIAL OUTFLOW

The methodology of this technique has been greatly advanced and an example of the use of the technique for sheep [9] is as follows.

Absorption of purines (X, mmol/d) is assumed to be related to urinary PD excretion (Y, mmol/d) according to the equation:

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

The equation allows for a non-linearity in the relationship that is thought to be due to a urinary component of PD of endogenous origin that is related to live weight (W) and decreases as an animal's plane of nutrition increases from sub-maintenance to maintenance level (Figure 1).

Experimentally determined values for the daily PD excretion (Y) and live weight (W) are entered and the equation is solved to give a prediction of purines absorbed (X) (Alternatively, the values could be determined from the figure). After allowing for net purine digestibility in the small intestine (assumed to be 0.83), a value for purine entry into the intestines is calculated (X/0.83). The purines entering the small intestine (assumed to contain 70 gN/mol) are considered to be derived entirely from rumen microbes with a purine-N: total N ratio of 0.116:1. Thus, a prediction of microbial N outflow from the rumen (gN/d) is given by 70X/(0.116 x 0.83 x 1000), which simplifies to 0.727X.

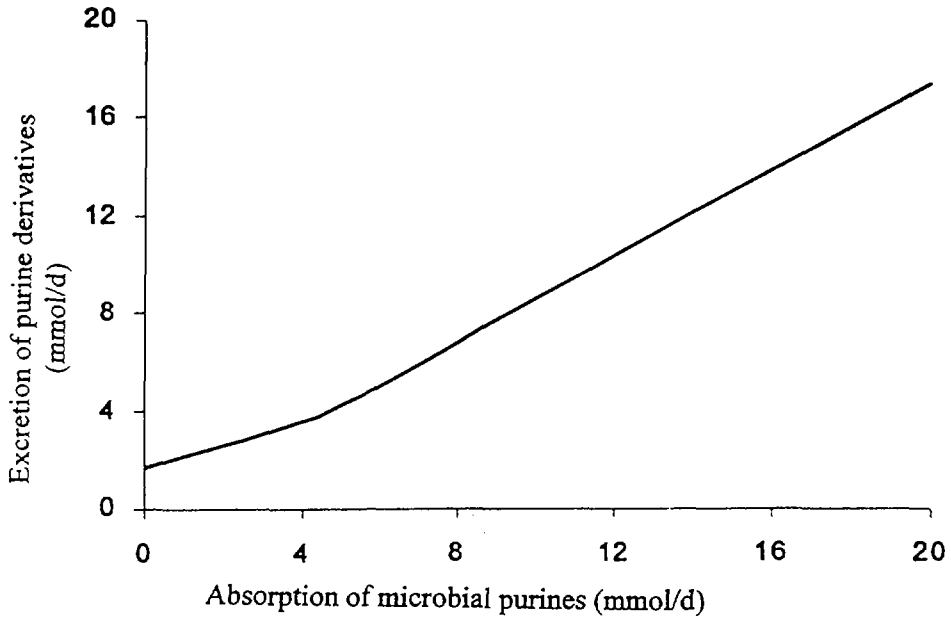

FIG.1. Relationship between excretion of purine derivatives in urine and absorption of dietary purines from the gut of a 35 kg sheep [10].

The method assumes that the relationship between rate of absorbed purines and rate of excretion of PD, intestinal digestibility of purines, and the ratio of purine:N in mixed rumen microorganisms are constants. These assumptions may not be entirely valid under all circumstances and are discussed further below. Alternative models for predicting the rate of absorption of microbial purines and of microbial outflow rate from the rumen from urinary PD excretion rate have been proposed [11-14]. An alternative to predicting values for net microbial synthesis is simply to compare the urinary PD excretion rates between treatments.

3. DEGRADATION OF DIETARY PURINES AND FORMATION OF MICROBIAL PURINES

Nucleic acids are synthesized by bacteria and protozoa and RNA and DNA represent the majority of purine compounds in rumen microorganisms. Of the total N present in rumen bacteria, nucleic acids and proteins comprise 13-19% and 75-85%, respectively. On average, purine-N:total N was 9.6% in bacteria and 4.8% for protozoa [15]. In mixed rumen bacteria from steers, RNA-N:total N ratios were 7.7% (± 0.2 sd) [16]. When predicting rumen microbial outflow from estimates of the rate of flow of purine compounds from the rumen, it would be more convenient if a constant factor was universally applicable. Unfortunately, this is unlikely to be the case. The ratio of RNA:total N increases as bacterial growth rate in the rumen increases [17], with RNA-N:total N varying more than DNA-N:total N [18]. Bates and co-workers [19] found that RNA:protein ratios in mixed rumen bacteria increased with increasing specific growth rate: the ratios were also affected by diet and time after feeding and there was an interaction between diet and free bacteria *vs* particulate bacteria. The total N

content and the ratio of RNA-N:total N were lower in fluid-phase bacteria than in particle-associated bacteria [20] even though the latter might be expected to grow more slowly than the former. An extensive study of ruminal microorganisms was made with continuous fermenters using a basal feed supplemented with various protein-rich supplements [21]. The ratio of purine N:total N in bacteria averaged 8.3% and was not affected by the type of supplement: however, liquid and solid dilution rates were not varied in this experiment. From the above, it can be seen that some reports indicate that purine-N:total N ratios in mixed bacteria may be lower than the 11.6% used in the previous example. However, it should also be recognised that estimates of the ratio have at times been based on non-specific colorimetric methods for estimating purine concentration and such estimates need to be interpreted with caution.

4. ESCAPE OF DIETARY PURINES TO THE SMALL INTESTINE

In general, it appears that free nucleic acids and derivatives from the diet are rapidly degraded in the rumen. Nucleic acids in hay were also rapidly degraded when incubated in rumen contents [22]. In a study of digestion of feeds in continuous fermenters, dietary purine content (%DM) in a variety of meals ranged from 0.03 in blood meal to 0.08 in fishmeal. Escape of feed purine N averaged 1.7% (± 2.9 sd) of total purine N flow. These and other studies suggest that, because of the relatively low concentrations of purines in most feeds, the escape of feed purine N is generally unlikely to affect predictions of microbial N flow from the rumen based on purine content of digesta. There may, however, be exceptions to this generalisation. One study demonstrated that up to 15% of the RNA entering the small intestine of young steers given a hay/concentrate diet (50/50) was of non-microbial origin [23]. Estimates of the percentage escape of dietary purines in sheep given a mixed diet of vetch-oat hay and concentrate (2:1) by the *in situ* method were 5-17% for lucerne hay, barley, gluten feed, sunflower meal and maize, 11-23% for meat meal and soybean meal, and 20-40% for fishmeal and brewery distillers grains, respectively [24].

5. DIGESTION AND ABSORPTION OF MICROBIAL PURINE COMPOUNDS

There is little change in the flow of nucleic acids in digesta between the rumen and duodenum [25]. Microbial nucleic acids entering the small intestine (of which about 60-70 % is RNA) are extensively degraded to mononucleotides [7], and enzymes capable of removing the phosphate groups from the mononucleotides to form the nucleosides are also present. It is clear that a variety of nucleases and related enzymes occur in the small intestine of ruminants and these degrade nucleic acids to nucleosides. McAllan [18] hypothesised that the rate of removal of the sugar to release the free base was the rate-limiting step for the complete degradation of purine compounds in the intestines.

Pancreatic secretions of ruminants are particularly high in ribonuclease (RNAase) which ensures that microbial nucleic acids are extensively degraded in the small intestine [26]. These enzymes catalyse the hydrolysis of phosphodiester bonds and release poly- or mono-nucleotides [27]. Two phosphodiesterases have been isolated from intestinal mucosa which are essentially exonucleases, the first attacking polyribonucleotides on one end of the nucleic acids and liberating the nucleoside-5-phosphates in a stepwise manner, and the second liberating nucleoside 3-phosphates from the other end. Intestinal 5'-nucleotidase and a non-specific alkaline phosphatase have also been found in ruminants. These nucleosides or their breakdown products, adenine, guanine and ribose are absorbed. Condon *et al.* [28] found that

adenine and guanine were completely absorbed from the small intestine whereas nucleosides were less completely absorbed. Mammalian tissues also have purine nucleoside phosphorylases that would allow nucleosides, if they are absorbed as such, to be further degraded within the body.

Net digestibility coefficients for nucleic acids in the small intestine of ruminants were 80-90% for RNA and 75-85% for DNA [18]. McAllan [27] reported that true digestibility for nucleic acids infused into the duodenum of steers was 97% and Chen and co-workers [10] reported a digestibility of 91% for an infused source of microbial purines. Adenine was also completely absorbed from the small intestine of lambs [28, 29]. A value of 0.83 for net digestibility is used in the example of calculations given previously. However, true digestibility is arguably more appropriate because purines flowing from the ileum are likely to be derived from endogenous sources and should therefore be considered as part of the non-renal excretion of purines.

6. TISSUE PURINE TURNOVER

6.1. Intracellular purine turnover

Studies of recovery in the urine of purine metabolites following the infusion of nucleic acid products into the rumen indicate that the majority of the absorbed purines are eventually excreted. However, Ellis and Bleichner [25] concluded that about 70 % of the nucleosides or bases absorbed from the gut are not immediately excreted in the urine. When ^{14}C-labelled RNA, adenine or guanine were infused into the abomasum, 10% of the radioactivity appeared in tissue nucleic acids [28].

Smith and co-workers [17] injected *E. coli* or mixed cultures of rumen bacteria, labelled their adenine and guanine by growing them on 8-^{14}C-adenine, and found that the label became incorporated into tissue nucleic acids. They found that only 3-5% of the radioactivity was excreted in faeces and only 15% in the urine in the first 2 days. They concluded that at least 5% of the purine compounds from the bacteria were incorporated into the liver, kidney and spleen and argued that muscle would contain a further 20%. Other workers [29] have also provided evidence for the incorporation of absorbed purines into tissues. They injected rumen bacteria labelled with ^{14}C-adenine into the rumen of lambs and found about 48% of the radioactivity was present in tissues 48 h later. Incorporation of ^{14}C-adenine by such reactions was also reported by Kahn and Nolan [30]. These studies indicate that some of the absorbed purines are usually incorporated into tissues rather than being immediately oxidised and it is clear that absorbed purines are not simply degraded in the body and then excreted via the kidneys. Rather they take part in synthetic and catabolic reactions that constitute the intracellular turnover of nucleic acid components in tissues. With this in mind, it is not surprising that, when purine compounds were infused into the small intestine of intragastrically maintained sheep, the responses in blood and urine purine concentrations were delayed for 2-3 hours [31]. These response lags may be due to biochemical regulation of tissue purine turnover reactions. Lags may also be due to physical sequestering of PD in tissues because intravenously administered ^{14}C-allantoin has been retained in tissue pools for extended periods [32].

Within cells, DNA is strongly conserved and has a slow turnover rate. In contrast, some types of RNA (e.g. messenger RNA) are more rapidly turned over. During catabolism, DNA and RNA are hydrolysed by nucleases and diesterases to oligonucleotides and then to mononucleotides and nucleosides. Nucleosides can be salvaged or further degraded by cleavage of the ribose moiety to yield the free bases. Catabolic pathways predominate in the

metabolism of purines in ruminants. However, adenine and guanine can be incorporated into purine nucleotides, catalyzed by phosphoribosyl transferases. The latter has been found in the liver of cattle [33]. Nucleoside phosphorylases that catalyse the formation of nucleosides from purine bases and ribose-1-phosphate also occur [33].

Purine nucleotides can be synthesized in three ways: by *de novo* synthesis, by reconstruction from purine bases by addition of ribose phosphate (*salvage*) or by phosphorylation of purine nucleosides to the corresponding nucleotides [34]. The *de novo* and *salvage* pathways are considered to be more important quantitatively in mammals and both involve phosphoribosylpyrophosphate (PRPP) as an essential precursor. The latter is formed from ribose-5-phosphate and ATP by the action of ribose-5-phosphate pyrophosphokinase. The first complete purine nucleotide, inosine monophosphate (IMP) is formed in a pathway starting from PRPP during which formate, glutamine, glycine and aspartate are involved. IMP does not accumulate in the cell but is converted to AMP and GMP, and then to ADP and ATP and GDP and GTP, respectively. PRPP is also involved in the salvage pathway by which mononucleotides are formed.

Purine base + PRPP → ribonucleoside-5'-phosphate + PP_i

The PP_i released is rapidly hydrolysed to inorganic phosphate in a coupled reaction catalyzed by inorganic pyrophosphate so that the formation of ribonucleosides by the salvage pathway is irreversible.

Nucleotides are catabolised within cells by several types of intracellular nucleotidases that are under strict regulatory control. Another type of nucleotidase is attached to the outer surface of the outer membrane of many types of cells and dephosphorylates purine ribonucleoside monophosphates to the corresponding nucleosides that are then transported into the cell on specific transporters.

The general pathways for degradation of purine compounds are well known and uric acid and allantoin are the principal end-products in most ruminant species. The purine nucleosides, inosine and guanosine are readily cleaved by purine nucleoside phosphorylase which is found in many tissues. Inosine is phosphorolyzed to yield hypoxanthine and guanosine to guanine. Hypoxanthine and guanine are then converted to xanthine by xanthine oxidase and amino hydrolysase, respectively. Xanthine is then oxidised to uric acid, again by the action of xanthine oxidase. Finally, uric acid is oxidised by the liver enzyme, urate oxidase or uricase, which is a copper protein, to allantoin. Even though it is considered to be a non-salvageable end-product, allantoin is not quantitatively recovered in the urine after being injected intravenously in sheep [31].

6.2. Metabolism of absorbed purines

The intestinal mucosa is the first possible site for the degradation of absorbed purines. The mucosal cells of buffaloes and cattle are rich in xanthine oxidase (EC 1.2.3.2) which increases the potential for oxidation of absorbed purines before they enter the bloodstream, and this reduces the potential for salvage. Sheep mucosa in contrast has only trace amounts of this enzyme [35] so that a higher blood concentrations of xanthine and hypoxanthine can be expected in sheep than in cattle or buffaloes. Purines entering the blood are again subject to oxidation by xanthine oxidase and uricase. Xanthine oxidase is present in buffalo and cattle plasma but not in sheep plasma [36], whereas uricase is not present in cattle blood but is present in sheep blood, although with relatively low activity [37]. In sheep, the liver has quite high activities of both enzymes, and this is the principal site of purine catabolism in this species.

7. RENAL AND NON-RENAL REMOVAL OF PURINE COMPOUNDS FROM THE BLOOD

If purine absorption, and eventually microbial purine outflow from the rumen, are to be predicted from PD excretion then, ideally, the absorbed purines should be excreted quantitatively via the kidney. However, the results of various studies indicate that purines administered into the gut or into the bloodstream, though often variable, are not quantitatively recovered in the urine. There are several reasons why urinary recovery may be incomplete.

- Some PD may be salvaged, resulting in increases in storage of purines in tissues.
- Some PD may be secreted via saliva into the gut.
- Some PD may enter the gut by non-salivary routes.
- Some purines may be excreted in milk.

Chen and co-workers [38] analyzed saliva of sheep by a colorimetric method and, assuming a daily secretion of saliva of 10 L/d, estimated that the secretion of PD in saliva was about 10% of the daily excretion in urine. Surra and others [39] on the other hand, analyzed sheep saliva by a reverse-phase HPLC method that was probably less prone to non-specific reactions and found allantoin and total PD in much lower concentrations. These workers later concluded that salivary excretion of PD accounted for only 0.3% of urinary excretion [40]. When Kahn and Nolan [30] injected ^{14}C-allantoin intravenously into sheep only negligible amounts of radioactivity were found in saliva, also indicating that allantoin excretion via this route was low. The non-recovery of absorbed purines is not as yet fully explained. However, transfer of purines into the gut does occur by several routes. Xanthine, hypoxanthine and uric acid are transported through intestinal tissues of hamsters [41] and uric acid is transferred into the gut of humans [42]. Allantoin has been found in the bile of the dog [43] and non-renal loss of purines was closely related to plasma purine pool size [44], indicating that the non-renal excretion is a concentration-dependent process. Various workers have investigated the excretion of PD in milk [45]. Excretion is generally less than 5% of the urinary excretion and does not appear to be reliably related to urinary excretion [46].

The fractional loss of PD via the kidneys suggested in the example for sheep given above (0.81) may not be applicable under all conditions. Work with tropical cattle and buffaloes [36, 47] suggests that lower values may be applicable to these species. The aspects discussed above are shown in Figure 2.

8. RELATIONSHIPS BETWEEN PURINE ABSORPTION AND EXCRETION IN URINE

If relationships between purine absorption and urinary purine excretion are to be used for prediction purposes, any variability occurring under different conditions also needs to be predictable. These relationships are curvi-linear [35, 48], probably reflecting the degree of biochemical feedback on *de novo* synthesis which is a source of endogenous purines and the balance between the utilisation of absorbed purines by tissues, and the degree of salvage of PD released within tissue cells. These factors are likely to be most affected when absorption of purines is relatively low. The distribution in gut, liver and other tissues of enzymes such as xanthine oxidase that are responsible for degrading potentially salvagable purines will also be a major factor, diverting purines produced from tissue nucleotide turnover into the oxidation pathway.

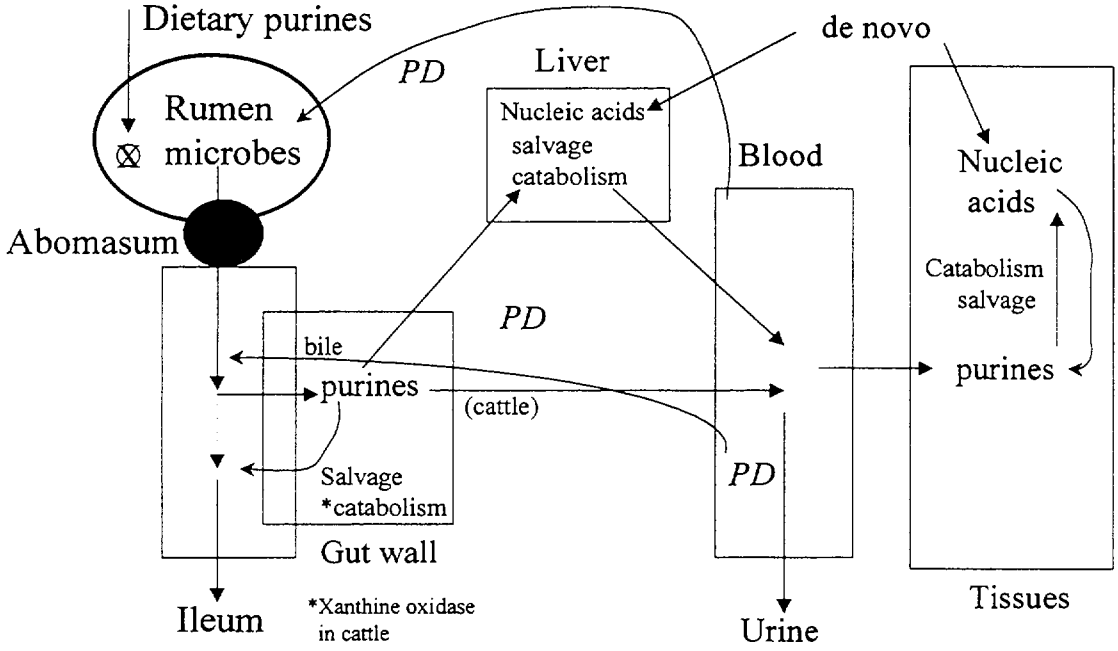

FIG.2. A representation of the fluxes of purine compounds from the rumen into the intestines and turnover of absorbed purines and excretion of oxidation end-products in ruminants.

Estimates of the extent of endogenous release of purines have been made using the technique of maintaining animals by intragastric infusion of nutrients, or by replacing normal digesta entering the intestines with digesta devoid of purines. The endogenous PD excretion is higher in cattle, per unit of metabolic weight, than in buffaloes and sheep, and is not inhibited as purine absorption increases. Sheep, goats, pigs and humans are similar. A consequence of these factors is that endogenous secretion of purines is always present in cattle, and the relationship between purine absorption and urinary PD excretion is more nearly linear [11, 34]. A further consequence is that different prediction equations will be required for different species of livestock [15]. Pertinent information has been collected by various groups in recent years, and some of the results are published in this IAEA TECDOC.

9. COMPARISON OF METHODS OF ESTIMATING MICROBIAL OUTFLOW FROM THE RUMEN

A comparison was made of estimates of rumen microbial N outflow from the rumen of duodenally cannulated sheep given a daily ration of 550 g lucerne hay, or the same ration plus either 220, 400 or 550 g rolled barley [49]. Estimates were made from measurements of digesta flow into the duodenum coupled with ^{15}N or purines as markers specific for the microbial fraction, or by using the method based on urinary PD excretion. Appreciable differences were found between the methods used, with predictions based on the urinary PD being 18-29% lower than values based on digesta flow. Currently, there is no method available that can be guaranteed to give a true measure of microbial flow from the rumen. However, the differences between the results in this comparison probably indicate that some of the assumptions used in the PD excretion method were incorrect in the conditions of these experiments. An alternative method of standardization of the PD technique is to compare its predictions with estimates of microbial outflow from the rumen based on feeding standards models that summarize results of many different studies using a variety of methods. An elegant demonstration of the potential of this kind of validation was presented by Susmel [14].

REFERENCES

[1] ANNISON, E.F., LEWIS, D., Metabolism in the Rumen, Methuen & Co Ltd, London (1959).

[2] TOPPS, J.H., ELLIOT, R.C., Nature **205** (1965) 498.

[3] McALLAN, A.B., SMITH, R.H., Proc. Nutr. Soc. **31** (1972) 545

[4] RYS, R., ANTONIEWICZ, A., MACIEJEWICZ, J., "Allantoin in urine as an index of microbial protein production in the rumen", Tracer studies on non-protein nitrogen for ruminants II, Panel Proc. Series, IAEA, Vienna, (1975) 95-98.

[5] ANTONIEWICZ, A.M., HEINEMANN, W.W., HANKS, E.M., The effect of changes in the intestinal flow of nucleic acids on allantoin excretion in the urine of sheep, J. Agric. Sci., Cambridge **95** (1980) 395-400.

[6] SMITH, R.H., McALLAN, A.B., Nucleic acid metabolism in the ruminant. 2. Formation of microbial nucleic acids in the rumen in relation to the digestion of food nitrogen, and the fate of dietary nucleic acids, Br.J. Nutr. **24** (1970) 545-56.

[7] ARMSTRONG, D.G., HUTTON, K., "Fate of nitrogenous compounds entering the small intestine,", Digestion and Metabolism in the Ruminant (Proc. IV Int. Symp. Sydney, 1974), (MCDONALD, I.W., WARNER, A.C.I., Ed.), The University of New England Publishing Unit, Armidale (1975) 432-447.

[8] COELHO DA SILVA, J.H., SEELEY, R.C., BEEVER, D.E., PRESCOTT, J.H.D., ARMSTRONG, D.G., The effect in sheep of the physical form and stage of growth on the sites of digestion of dried grass, Br. J. Nutr. **28** (1972) 357-371.

[9] INTERNATIONAL ATOMIC ENRGY AGENCY, Estimation of rumen microbial protein production from purine derivative in urine, IAEA-TECDOC-945, Vienna (1996).

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

[11] VERBIC, J., CHEN, X.B., MACLEOD, N.A., ORSKOV, E.R., Excretion of purine derivatives by ruminants: Effect of microbial nucleic acid infusion on purine derivative excretion by steers, J. Agric. Sci., Cambridge **114** (1990) 243-248.

[12] BALCELLS, J., GUADA, J.A., CASTRILLO, C., GASA, J., Urinary excretion of allantoin and allantoin precursors by sheep after different rates of purine infusion into the duodenum, J. Agric. Sci., Cambridge **116** (1991) 309-317.

[13] PUCHALA, R., KULASEK, G.W., Estimation of microbial protein flow from the rumen of sheep using microbial nucleic acid and urinary excretion of purine derivatives, Can. J. Anim. Sci. **72** (1992) 821-830.

[14] SUSMEL, P., STEFANON, B., MILLS, C.R. Use of purine derivatives to predict microbial protein supply in ruminants, personal communication, 1996.

[15] STANGASSINGER, M., CHEN, X.B., LINDBERG, J.E., GIESECKE, D., "Metabolism of purines in relation to microbial production", Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction, (Proc.VIIIth Int. Symp. Stuttgart, 1995) (ENGELHARDT, W.V., LEONHARD-MAREK, S., BREVES, G., GIESECKE, D., Eds.), Ferdinand Enke Verlag., Germany (1995) 387-406.

[16] SMITH, R.H., McALLAN, A.B., Some factors influencing the chemical composition of mixed rumen bacteria, Br. J. Nutr. **31** (1974) 27-34.

[17] SMITH, R.C., MOUSSA, N.M., HAWKINS, G.E., Utilization of the nucleic acids of Escherichia coli and rumen bacteria by sheep, Br. J. Nutr. **32** (1974) 529-537.

[18] McALLAN, A. B., "The fate of nucleic acids in ruminants," Proc. Nutr. Soc. Vol. 41 (1982) 309-317.

[19] BATES, D.B., GILLETT, J.A., BAROO, S.R., BERGEN, W.G., The effect of specific growth rate and stage of growth on nucleic-acid protein values of pure cultures and mixed rumen bacteria, J. Anim. Sci. **61** (1985) 713-724.
[20] MERRY, R.J., McALLAN, A.B., A comparison of the chemical composition of mixed bacteria harvested from the liquid and solid fractions of rumen digesta, Br. J. Nutr. **50** (1983) 701-709.
[21] CALSAMIGLIA, S., STERN, M.D., FIRKINS, J.L., Comparison of nitrogen-15 and purines as microbial markers in continuous culture, J. Anim. Sci. **74** (1996) 1375-1381.
[22] McALLAN, A.B., SMITH, R.H., Degradation of nucleic acids in the rumen, Br. J. Nutr. **29** (1973) 331-345.
[23] SMITH, R.H., MCALLAN, A.B., HEWITT, D., LEWIS, P.E., Estimation amounts of microbial and dietary nitrogen compounds entering the duodenum of cattle J. Agric. Sci., Cambridge **90** (1978) 557-565.
[24] PEREZ, J.F., RODRIGUEZ, C.A., GONZALEZ, J., BALCELLS, J., GUADA, J. A., Contribution of dietary purine bases to duodenal digesta in sheep - In situ studies of purine degradability corrected for microbial contamination, Anim. Feed Sci. Technol. **62** (1996) 251-262.
[25] ELLIS, W.C., BLEICHNER, K.C., Apparent utilization of absorbed purines by sheep, J. Anim. Sci. **29** (1969) 157.
[26] DAVIDSON, J.N., Biochemistry of the Nucleic Acids, 7th ed: Chapman and Hall and Science Paperbacks, U.K. (1972).
[27] McALLAN, A. B., The degradation of nucleic acids in, and the removal of breakdown products from the small intestines of steers, Br. J. Nutr. **44** (1980) 99-112.
[28] CONDON, R.J., HALL, G., HATFIELD, E.E., J. Anim. Sci. **31** (1970) 1037.
[29] RAZZAQUE, M.A., TOPPS, J.H., KAY, R.N., BROCKWAY, J.M., Metabolism of the nucleic acids of rumen bacteria by pre-ruminant and ruminant lambs, Br. J. Nutr. **45** (1981) 517-527.
[30] KAHN, L.P., NOLAN, J.V., Prediction of microbial yield from the rumen using urinary excretion of purine derivatives and studies of the kinetics of labelled purines," Development of Feeding Strategies for Improving Ruminant Productivity in Areas of Fluctuating Nutrient Supply, IAEA-TECDOC-691, IAEA, Vienna (1993) 109-121.
[31] CHEN, X.B., FUJIHARA, T., NAKAMURA, K., MAWUENYEGAH, P.O., FRANKLIN, M.F., KYLE, D.J., Response of urinary and plasma purine derivatives to various rates and infusion patterns of purines in sheep nourished by intragastric infusion, J. Agric. Sci. **129** (1997) 343-352.
[32] KHAN, L.P., NOLAN, J.V. (personal communication)
[33] BARMAN, T., Enzyme Handbook, Springer-Verlag, Berlin (1969).
[34] LEHNINGER, A.L., Biochemistry, Worth Publishers (1982)
[35] AL-KHALIDI, U.A.S., CHAGLASSIAN, T.H., The species distribution of xanthine oxidase, Biochem. J. **97** (1965) 318-320.
[36] CHEN, X., SAMARAWEERA, L., KILE, D., ØRSKOV, E., ABEYGUNAWARDENE, H., Urinary excretion of purine derivatives and tissue xanthine oxidase activity in buffaloes, with special reference to differences between buffaloes and *Bos taurus*, Br. J. Nutr. **75** (1996) 397-407.
[37] CHEN, X.B., ORSKOV, E.R., HOVELL, F.D., Excretion of purine derivatives by ruminants: endogenous excretion, differences between cattle and sheep, Br. J. Nutr. **63** (1990) 121-129.
[38] CHEN, X.B., DEB HOVELL, F.D., ORSKOV, E.R., Excretion of purine derivatives by ruminants: recycling of allantoin into the rumen via saliva and its fate in the gut, Br. J. Nutr. **63** (1990) 197-205.

[39] SURRA, J., GUADA, J.A., BALCELLS, J., CASTRILLO, C., Salivary losses of purine derivatives, Br. Soc. Anim. Prod. **56** (1993) 463, Abstract 176A.
[40] SURRA, J.C., GUADA, J.A., BALCELLS, J., CASTRILLO, C., Renal and salivary clearance of purine derivatives in sheep, Anim. Sci. **64** (1997) 83-91.
[41] BERLIN, R.D., HAWKINS, R.A., Secretion of purines by the small intestine: general characteristics, Am. J. Physiol. **215** (1968) 932-941.
[42] SORENSEN, L.B., Extrarenal disposal of uric acid, Uric acid - Handbook of experimental pharmacology, (KELLY, W.N., WEINER, I.M., Eds.), Springer-Verlag, Berlin, (1978) 325-336.
[43] ALTMAN, P.L., DITTMER, D.J., Metabolism, Federation of American Societies for Experimental Biology, USA, 256.
[44] LOFFLER, W, GROBNER, W., MEDINA, R., ZOLLNER, N., Influence of dietary purines on pool size, turnover and excretion of uric acid during balance conditions, Res. Expt. Med. **181** (1982) 113-123.
[45] ROSSKOPF, R., GIESECKE, D., The effect of the energy intake of cows on the rumen metabolism studied using the excretion of allantoin in milk, Zentralbl Veterinarmed [A], **39** (1992) 515-524.
[46] GONDA, H.L., LINDBERG, J.E., Effect of diet on milk allantoin and its relationship with urinary allantoin in dairy cows, J. Dairy Sci. **80** (1997) 364-373.
[47] LIANG, J.B., MATSUMOTO, M., YOUNG, B.A., Purine Derivative Excretion and Ruminal Microbial Yield In Malaysian Cattle and Swamp Buffalo, Anim Feed Sci. Technol. **47** (1994) 189-199.
[48] BALCELLS, J., PARKER, D.S., SEAL, C.J., Purine metabolite concentrations in portal and peripheral blood of steers, sheep and rats, Comparative Biochem. Physiol. **101B** (1992) 633-636.
[49] PEREZ, J.F., BALCELLS, J., GUADA, J.A., CASTRILLO, C., Determination of rumen microbial-nitrogen production in sheep: a comparison of urinary purine excretion with methods using ^{15}N and purine bases as markers of microbial-nitrogen entering the duodenum, Br. J. Nutr. **75** (1996) 699-709.

NEXT PAGE(S) left BLANK