Purine Derivatives Excreted in Urine as an Indicator Estimating Microbial Yield from the Rumen: A-Review

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ABSTRACT : The paper presented here is aimed at increasing knowledge on purine metabolism in ruminants and hence the quantification of microbial cells entering the small intestine from urinary excretion of purine derivatives. Nucleic acid metabolisms of micro-organisms in the rumen, digestion and absorption of nucleic acids entering the intestines, metabolisms of absorbed and endogenous purines involving *de novo* synthesis of nucleic acids in the ruminants host, and the relationship between absorbed and excreted purines are reviewed. Principal concerns about an amount of purine derivatives excreted in urine in relation to a change in purine-N: total-N ratios in rumen microbes that leave the rumen are discussed. The use of urinary excretion of purine derivatives as an indicator of the amount of microbial biomass leaving the rumen has to be done with some caution since it may be impossible to get a representative sample of microbes entering the intestine and thus yield estimates are relative rather than absolute.

(Key Words: Purine Derivatives, Purine-N: total-N, Nucleic Acids, Rumen)

INTRODUCTION

Quantifying net microbial cell synthesis in the rumen is important for ruminant nutrition as it determines particularly the contribution of microbial protein to the host's protein. Measurement of net microbial cell synthesis in the rumen or outflow of microbial cells into the lower digestive tract by using markers and cannulated animals at the intestine is time consuming and often of low precision. A number of microbial markers have been studied by Ling and Buttery (1978) to differentiate microbial protein which enters the intestinal digesta of sheep. The ratio of RNA to total-N in the digesta appears to be an appropriate marker of microbial-N in digesta-N entering the duodenum. From microbial markers used most widely (purines, RNA, DNA, diaminopimelic acid, and isotopic markers), however, Broderick and Merchen (1992) have showed that there is no marker being proved completely satisfactory and the techniques involved are time consuming and animals need to be surgically modified. Alternatively, there appears now that urinary excretion of purine derivatives may prove to be a sounder indicator and a noninvasive method for predicting microbial biomass that leave the rumen and are digested in the intestines of ruminants (Topps and Elliott, 1965;

Antoniewicz et al., 1980; Fujihara et al., 1987; Lindberg, 1989; Verbic et al., 1990; Chen et al., 1990b; Balcells et al., 1991; Puchala and Kulasek, 1992).

Hypoxanthine, xanthine, uric acid and allantoin excreted in urine are metabolic derivatives of both endogenous and exogenous purines (adenine and guanine) that are degraded in the body. The quantities of uric acid and allantoin excreted in the urine have been found to be highly related to the pool of microbial nucleic acids in the rumen (Topps and Elliott, 1965) and to those in the small intestine as well as to the amounts of the purine derivatives in the blood (McAllan, 1980). Thus the utilisation of urinary excretion of purine derivatives as an indicator of the quantities of net microbial cell synthesis in the rumen requires an understanding of purine metabolisms in ruminants.

DISCUSSION

Purine metabolisms in the rumen

There is evidence that purine derivatives excreted in urine originate mostly from microbial nucleic acids which are absorbed in the intestines and further metabolised to their derivatives (McAllan, 1982). Urinary excretion of purine derivatives increases linearly with incremental duodenal input of microbial nucleic acids (Fujihara et al., 1987; Chen et al., 1990b), and of microbial RNA (Antoniewicz et al., 1980; Balcells et al., 1991). The amounts

of nucleic acids in the duodenum digesta are similar to those in the rumen (Smith and McAllan, 1971) but different to those added to the feed (Razzaque and Topps, 1972). Ratios of RNA:DNA of rumen bacteria are not different from those of ruminal fluid but markedly different to those of the natural diets (Smith and McAllan, 1970). Nucleic acids of the diets (McAllan and Smith, 1973) and added nucleic acids (Smith and McAllan, 1970) in the rumen are rapidly degraded into nucleotides, nucleosides and free bases within the rumen (McAllan and Smith, 1973). These substrates can be utilised as a source of carbon or nitrogen for microbial synthesis in the rumen (Belasco, 1954; Jurtshuk et al., 1958) and they may be incorporated as a precursor of nucleic acids by rumen microbes (Smith and Mathur, 1973).

With respect to the degradation of free bases within the rumen, hypoxanthine and xanthine but not adenine and guanine can be transiently accumulated in the rumen digesta (McAllan and Smith, 1973). Hypoxanthine is degraded partly in the rumen but guanine, xanthine and uric acid (Jurtshuk et al., 1958) as well as allantoin (Belasco, 1954) are degraded completely. In the rumen, acetic acid, CO_2 and ammonia are the major end-products of the degradation of purine bases that undergo fermentation by rumen microbes (Jurtshuk et al., 1958).

In regard to the incorporation of nucletides, nucleosides and free bases into nucleic acids, protozoa in the rumen can incorporate nucleotides, nucleosides and free bases (except thymine) into their nucleic acids (Coleman, 1979) possibly via salvage pathways (Jaffe and Gutteridge, 1974). Unlike bacteria in the rumen, the majority of bacterial nucleic acids can be synthesised via the de novo pathway (see McAllan, 1982). However, rumen bacteria are freely permeable to adenine and uracil and these can be incorporated into bacterial nucleic acids (Smith and Mathur, 1973). At present, there appears to be no information available on metabolisms of nucleic acids of fungi in the rumen. The only indirect evidence is the Neocallimastix frontalis zoospores do not show chemotaxis to purines and pyrimidines (Orpin and Bountiff, 1978) and the growth of Neocallimastix patriciarum is not affected by the absence of the nucleotide bases, adenine and guanine in the media (Orpin and Greenwood, 1986).

The nucleic acid (Arambel et al., 1982) or purine (Kanjanapruthipong, 1995) content of bacteria but not that of protozoa in the rumen can vary widely due to different species and types of bacteria, energy sources and the time post-feeding. The nucleic acid-N:total-N of gram-positive bacteria in the rumen appears to be slightly lower than that of gram-negative bacteria (Arambel et al., 1982).

The ratios of nucleic acids:total-N (Craig et al., 1987; Klusmeyer et al., 1991), RNA:total-N (Merry and McAllan, 1983; Bates et al., 1985) and purine:total-N (Kanjanapruthipong, 1995) are all significantly higher in fluid-associated than in particle-associated organisms. The ratio of nucleic acids:total-N decreased markedly within 1 h after feeding and gradually increased over 12 h after feeding (Craig et al., 1987). From the study of Craig and colleague, the feed was offered to the animal only 1 h and the remaining feed was removed. However, when the feed was offered ad libitum, the ratios of RNA:DNA (John, 1984; Baker and Car, 1992) and of RNA:total-N (Susmel et al., 1993) of the fluid bacteria increased sharply after feeding and reached a maximum from 2 to 4 h post-feeding and declined slowly thereafter.

Thus, purines of rumen origin derived predominantly from the microbial purines. These purines are influenced by the time after feeding regardless of diet and types of microbes particularly those associated in the fluid phase of ruminant fed on a concentrate-based diet (see later for further discussion).

Intestinal digestion and absorption of microbial nucleic acids

The majority of purine nucleosides and free bases presented in the small intestine of ruminants (McAllan, 1980) are microbial in origin as mentioned earlier and a small proportion of those (relatively less than 15%) can be contributed from the endogenous purines (see McAllan, 1982) secreted by the small intestine (Berlin and Hawkins, 1968). Microbial nucleic acids entering the small intestine of ruminants are hydrolysed by a series of nuclease enzymes to nucleotides, nucleosides and free bases (see Barnard, 1969; Armstrong and Hutton, 1975; McAllan, 1982). The digestibility between the proximal duodenum and distal ileum of sheep and cattle appeared to be higher for microbial RNA than DNA ranging from 87 to 97% and 75 to 85%, respectively (see Smith and McAllan, 1971; McAllan, 1982; Storm et al., 1983).

When corrected for the endogenous purine, the true digestibility of microbial nucleic acids was 91% (Chen et al., 1990b). The digested nucleosides and free bases (except hypoxanthine) are almost entirely absorbed before reaching the terminal ileum of ruminants (McAllan, 1980; 1982).

Metabolism of the absorbed purines in the ruminant host

Purine derivatives but not nucleosides (guanosine and adenosine) and purines (guanine and adenine) are detected in the plasma samples of sheep and steers

(Balcells et al., 1992). The concentrations of total purine derivatives do not differ between the portal and jugular blood (see Chen et al., 1990b) and between the portal and peripheral blood (Balcells et al., 1992). This may indicate that the absorbed nucleosides and bases are metabolised during passage across the intestinal wall.

The absorbed nucleosides are split by nucleoside phosphorylases to deliver their free bases. Guanine is then deaminated by guanase to xanthine (see Zöllner, 1982). Unlike guanine, there appears to be no adenase activity in the small intestine (Berlin and Hawkins, 1968). Adenosine is deaminated by adenosine deaminase to form inosine (see Berlin and Hawkins, 1968) where it is catabolyzed by nucleoside phosphorylase to hypoxanthine (Zöllner, 1982). Hypoxanthine and xanthine can be further oxidised or salvaged depending upon the existence of xanthine oxidase in tissues particularly in the mucosal epithelium of the small intestine as well as in the blood.

In sheep hypoxanthine, xanthine, uric acid and allantoin are consistently detected in the plasma and the concentrations of hypoxanthine and xanthine but not uric acid and allantoin are significantly higher in the portal than peripheral blood (Balcells et al., 1992). In steers, uric acid and allantoin but not hypoxanthine and xanthine are consistently found in the plasma and the concentration of uric acid is higher in the portal than peripheral blood (Balcells et al., 1992). The differences in concentrations of purine derivatives between the portal and peripheral blood as well as between sheep and cattle can be explained by the differences of the activities of xanthine oxidase in different organs and species. The activities of xanthine oxidase in cattle are abundant in the tissues of all 20 organs tested (except the pancreas) including blood and serum whereas those in sheep are rich only in the liver (Al-kalidi and Chaglassian, 1965). Therefore, in sheep, hypoxanthine and xanthine are available to be reutilised or salvaged by other organs before reaching the liver (Chen et al., 1990b) but, in cattle, the purine derivatives might be salvaged only in tissues of the small intestine where xanthine oxidase can be detected (Al-kalidi and Chaglassian, 1965).

Tissue nucleic acids in ruminants may originate from the de novo synthesis of purines and from absorbed purines that have been salvaged. There is evidence that the absorbed purines are used by various organs in ruminants. Microbial adenine-8-^{14}C (Condon et al., 1970; Smith et al., 1974; Razzaque et al., 1981) and RNA-^{14}C-UL (Condon et al., 1970) absorbed from the small intestine of lambs were substantially incorporated into the body tissues of lambs. On the other hand, glycine-^{14}-C-UL which is a precursor for the de nove synthesis of

nucleotides is not significantly incorporated into the tissues of lambs (Condon et al., 1970). Condon et al. (1970) concluded that salvage of the absorbed purines is preferable to de novo synthesis of purines for the animal to replace nucleic acids losses from tissue turnover. An incremental increase in the amounts of purines absorbed in the small intestine appeared to be associated with a gradual increase in the salvage of the absorbed purines but with a progressive decrease in the de nove synthesis of purines in sheep (Chen et al., 1990b). This may be a benefit in reduced amounts of ATP that are required for nucleotides formation. In general, the energetic cost of mononucleotide formation via the salvage pathway is 3.5-4 times less than that via the de novo pathway (see Mathews and Van Holde, 1990). Pathways of purine metabolisms in the body involving enzymes of purine biosynthesis and salvage are well described by Murray (1971) and Zöllner (1982).

The relationship between absorbed and excreted purines

Purine derivatives in the plasma of ruminants can be derived from the turnover of tissue nucleic acids (endogenous purine derivatives) and from absorbed purines which have not been incorporated into tissues (exogenous purine derivatives). Although the plasma purine derivatives can be excreted via the renal route and secreted via non-renal routes i.e via the salivary (Chen et al., 1989), milk (Giesecke et al., 1994), and gut secretion (Berlin and Hawkins, 1968), the concentrations of purine derivatives in the plasma are highly correlated with those in the urine (McAllan, 1980). Therefore purine derivatives excreted in urine arise from both the endogenous and exogenous purine derivatives.

The endogenous purine can be measured in ruminants nourished by intragastric infusion of VFA and casein and by abomasal infusion of different amounts of RNA (Giesecke et al., 1984; Fujihara et al., 1987) and of nucleic acids (Chen et al., 1990b). It can be also examined by full replacement of duodenal digesta followed by the administration of different amounts of purines (Balcells et al., 1991). Then, the amounts of purines excreted in urine administrated with zero RNA or nucleic acids or purines is a value of the endogenous purine excretion. In addition, it can be observed with preruminants fed on a N-free liquid diet (Lindberg, 1989).

The amount of endogenous purine derivatives excreted in urine is 3 times higher in cattle than in sheep. In cattle it ranges from 454 to 514 μmol/kg $BW^{0.75}$ per day (Fujihara et al., 1987; Chen et al., 1990a) and in sheep from 164 to 189 μmol/kg $BW^{0.75}$ per day (Giesecke et al.,

1984; Fujihara et al., 1987; Chen et al., 1990a; Balcells et al., 1991). Similar levels are expected in sheep and goats (Lindberg, 1989) whereas the value between 345 and 391 μmol/kg BW$^{0.75}$ per day is expected in buffalo as the amount of endogenous purine derivatives excreted in urine of buffalo is presumably 76% of that reported in cattle (Vercoe, 1976; Liang et al., 1994). However, as the absorbed purines increase with increasing supplies of microbial nucleic acids, the contribution of endogenous purine derivatives to total purine derivatives that are excreted in urine becomes relatively small (Antoniewicz et al., 1980; Giesecke et al, 1984; Fujihara et al., 1987; Lindberg, 1989; Chen et al., 1990a, b; Balcells et al., 1991).

A linear model has been used by Antoniewicz et al. (1980), Giesecke et al. (1984) and Fujihara et al. (1987) to describes the relationship between absorbed and excreted purines. However, Chen et al. (1990b) and Balcells et al. (1991) have shown that a curvilinear model provides a better explanation. An increase in urinary excretion of purine derivatives (Y; mmol/d) with increasing amounts of absorbed purines (X; mmol/d) is described by Chen and Gomes (1992) using the following equations:

$$Y = bX + (cBW^{0.75} \, e^{-kX}) \quad \cdots\cdots\cdots \text{for sheep}$$
$$Y = bX + (cBW^{0.75}) \quad \cdots\cdots\cdots \text{for cattle}$$

The slope b of 0.84 and 0.85 for sheep and cattle, respectively, represents the recovery of the absorbed purines that are excreted in urine. This implies values of 0.16 and 0.15 in sheep and cattle respectively, for the amount of absorbed purines lost via non-renal routes. The part of the equations within parenthesis represents the contribution of the endogenous purine derivatives excreted in the urine. Where c (0.150 for sheep and 0.385 for cattle) is the endogenous purine derivatives excreted in urine when the absorbed purines are zero. BW$^{0.75}$ is the metabolic body weight (kg) of the animal and k is the rate-constant of -0.25 for the replacement of $de\ novo$ synthesis of purines by salvage of the absorbed purines.

Different equations are used for sheep and cattle to describe the relationship between absorbed and excreted purines. In sheep, the endogenous contribution reduces to zero as exogenous purines are utilized and $de\ novo$ synthesis of purine is almost negligible. In cattle, this is taken as a constant of 0.385 mmol/kg BW$^{0.75}$ per day.

The concentrations of purine derivatives excreted in urine are highly correlated with those in the plasma, in the small intestine and in the rumen. Purine derivatives excreted in urine are derived mostly from microbial

purines in the rumen and hence appear to represent a robust indicator of microbial biomass that leave the rumen and are digested in the small intestine.

Principal concerns in relation to a variation of purine-N:total-N ratios in rumen microbes

An estimated yield from purine derivatives excreted in urine can be confounded with a variation of the RNA-N: total-N or RNA : total-N or purine : total-N ratio in microbes that leave the rumen (see table 1). The purine-N: total-N ratio in rumen microbes isolated from lambs used by Kanjanapruthipong (1995) was 0.0824 which is lower than the value of 0.116 quoted by Chen and Gomes (1992). This difference (41%) highlights the need to know the purine-N:total-N ratio in rumen microbes when estimating the net microbial cell synthesis of the microbial yields entering the intestines from the urinary excretion of purine derivatives. A change in the purine-N: total-N ratio in microbes that leave the rumen can be due mainly to different growth rates of rumen microbes and various pools of microbes flowing from the rumen.

With respect to the different growth rates of rumen microbes, the purine: total-N ratio of rumen microbes can be expressed as a function of the nucleic acids: total-N ratio. A change in the purine:total-N ratio is assumed to be due primarily to a change in the RNA:total-N ratio which, in turn, used as an index of the specific growth rate of rumen microbes. The RNA:total-N and the specific growth rate of non-rumen (Koch, 1970) and rumen (Bates et al., 1985) microbes are highly correlated. A high RNA:total-N ratio is associated with a high specific growth rate. The RNA:total-N ratio of bacteria in the fluid phase has been found to vary due to the bacterial species (Arambel et al., 1982), the time post-feeding (John, 1984; Susmel et al., 1993), levels of feed intake (John, 1984) and diets (Bates et al., 1985; Susmel et al., 1993). The RNA:total-N ratio of particle-associated microbes has also been observed to be influenced by diets, being lower in organisms isolated from concentrate-fed animals than similar organisms isolated from roughage fed animals (Bates et al., 1985). Regardless of diet, the RNA:total-N ratio of particle-associated microbes was significantly lower than that of bacteria isolated in the fluid phase (Merry and McAllan, 1983; Bates et al., 1985). Different growth rates between the fluid and particle-associated microbes as well as between bacteria and protozoa (see Broderick and Merchen, 1992) result in different purine-N:total-N ratios in rumen microbes.

In regard to the different pools of microbes flowing from the rumen, the contribution of various microbial

Table 1. RNA-N:total-N, RNA:total-N and purine:total-N ratios in the fluid-associated bacteria (FB) and fluid-associated microbes (FM), the particle-associated bacteria (PAB) and particle-associated microbes (PAM) and protozoa (P) in the rumen of ruminants fed on various diets

Animals	Diets	Microbes	RNA-N: total-N	RNA: total-N	purine: total-N	References
Sheep	All roughage	FB	0.074			Smith and
	55% concentrate	FB	0.076			McAllan
Cows	All roughage	FB	0.080			(1974)
	75% concentrate	FB	0.078			
Calves	50% concentrate	FB	0.077			
Sheep	85% concentrate	FB	0.092			Ling and
		P	0.050			Buttery (1978)
Cattle	15% concentrate	FB	0.054			Arambel et al.
		P	0.038			(1982)
	85% concentrate	FB	0.048			
		P	0.040			
Steers	48% concentrate	FB	0.093			Merry and
		PAB	0.075			McAllan (1983)
Sheep &	–	FB	0.113			Storm and
Cattle		P	0.096			Orskov (1983)
Cows	All roughage	FB	0.065			Legay-Carmier
						and Bauchart
	43% concentrate	FB	0.051			(1989)
Sheep	12% concentrate	FB		1.82		Bates et al.
		PAB		1.42		(1985)
	50% concentrate	FB		1.80		
		PAB		0.92		
	90% concentrate	FB		2.56		
		PAB		1.35		
Steers	All roughage	FB		0.62		Susmel et al.
	43% concentrate	FB		0.89		(1993)
Cows	30% concentrate	FM			0.98*	Craig et al.
		PAM			0.92*	(1987)
Steers	50% concentrate	FB			0.80*	Firkins et al.
		PAB			0.78*	(1987)
		P			0.43*	
Steers	30% concentrate	FB			1.30*	Cecava et al.
		PAB			1.39*	(1990)
	60% concentrate	FB			1.56*	
		PAB			1.33*	
Cows	–	FB			1.32*	Klusmeyer
		P			1.25*	et al. (1991)
Sheep	All roughage	FB			0.184	Kanjanapruthi-
		PAM			0.164	pong (1995)
		P			0.149	

* Purine:total-N ratios are grams of yeast RNA equivalents per gram of N.

pools to net microbial cell movement out of the rumen remains unclear (see Broderick and Merchen, 1992). There is a considerable amount of evidence in the literature indicating a negative correlation between the densities of protozoa and bacteria in ruminal fluid (see Coleman, 1989), a variation of bacterial and protozoal pools (see Schwartz and Gilchrist, 1975), the retention of the majority of protozoa within the rumen (Weller and Pilgrim, 1974; Leng, 1982), and a different pool size of the fluid and particle-associated microbes (see Preston and Leng, 1987; Cheng et al., 1990). In addition, there is different purine:total-N ratios between bacteria and protozoa as well as between the fluid and particle-associated microbes (see table 1). Microbial cells flowing from the rumen represent a mixture of various pools of these microbes and hence different purine-N:total-N ratios.

CONCLUSION

Purine derivatives excreted in urine appear to be a theoretically sound indicator of the quantification of microbial yields that leave the rumen and digest in the intestine. However, a purine-N:total-N ratio in rumen microbes used must be considered with extreme caution as it can be varied and may not be indicative of the true microbial cells that enter the intestine and thus yield estimates from urinary excretion of purine derivatives are relative rather than absolute.

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