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Ruminal protein degradation and estimation of rumen microbial protein production

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ABSTRACT

Animal agricultural production systems are major sources of nonpoint pollution affecting quality of water sources. Nitrogen has been identified as the foremost source of nonpoint water pollution and the potential negative impacts of N have become an area of public concern. protein degradation from feed ingredients is an important factor influencing AA supply to the duodenum. Ruminal proteolysis determines the amount of ammonia, AA, peptides, and branched-chain VFA available for microbial growth and proliferation. Carbohydrates are the main components in the dairy ration, comprising roughly 60 – 80% of total dry matter (DM) and could supply to 70% net energy lactation (NEL) for high yielding dairy cows. External markers such as 15N or 35S as well as internal markers such as nucleic acids have been used to determine ruminal microbial protein production.

Key words: Microbial Protein, Metabolism Protein, Purine Derivatives

INTRODUCTION

Animal agricultural production systems are major sources of nonpoint pollution affecting quality of water sources (Williams, 1995). The major nutrients that are considered pollutants from agricultural systems are nitrogen (N), phosphorus, and methane (Kohn et al., 1997). Nitrogen has been identified as the foremost source of nonpoint water pollution (Thomann et al., 1994) and the potential negative impacts of N have become an area of public concern. Much research has gone into understanding the requirement for rumen degraded protein (RDP) of dairy cows, and the effects of various sources of RDP on digestion and lactation performance have been evaluated. Various groups of rumen bacteria respond differently to the type of protein they receive. However, limited research has evaluated the effects of non-protein nitrogen (NPN) and/or amino acid-N (AA-N) on microbial protein yield, lactation performance and N metabolism in lactating dairy cows. Additionally, there has been considerable research investigating the use of microbial-derived purines to predict microbial protein flow out 3 of the rumen as microbial protein is essential to dairy cows; however, the effect of NPN and/or AA-N on purine excretion has not been evaluated. Recent efforts to enhance productive performance of ruminants through synchronization of

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carbohydrate and N to improve fermentation in the rumen did not result in detectable benefits for the animals (Richardson et al., 2003). Furthermore, the introduction of a 12- or 24-h imbalance betweenNand energy supplies in the rumen did not negatively influence microbial protein synthesis nor N use in sheep (Ludden et al., 2002a,b) or growing bulls (Valkeners et al., 2004). Both the ruminal microbes and their host animal possess the means to compensate for variations in kinetics of feed degradation to ensure a relatively continuous supply of nutrients to places of biosynthesis to optimize microbial growth (Dawson, 1999). Synchronization of rumen available protein and energy is one of the conceptual methods to increase the efficiency of utilization of nutrients by the ruminants (Bayati Zadeh and Moradi kor, 2013). Understanding rate and extent of ruminal degradation of various protein sources is important when formulating diets in order to supply enough RDP to optimize rumen microbial growth. Rumen microbial growth is largely dependent on the amount of fermentable carbohydrate and the amount of ammonia present in the rumen (Bryant and Robinson, 1962). Most of the experiments which have evaluated RDP and RUP values of protein feedstuffs have been conducted with roughage diets, and less data is available with grain-based diets. In general, corn-based finishing diets supply the rumen with plenty of readily fermentable carbohydrates. However, the source of N needed to optimize yield and efficiency of MCP along with RUP to provide adequate MP to the host animal has yet to be determined.

Ruminal Metabolism of Protein

Ruminal protein degradation from feed ingredients is an important factor influencing AA supply to the duodenum. Ruminal proteolysis determines the amount of ammonia, AA, peptides, and branched-chain VFA available for microbial growth and proliferation. The rate and extent of ruminal protein degradation not only affects microbial protein synthesis, but also the amount and type of ruminally undegraded feed protein reaching the duodenum for digestion and absorption. Stern and Hoover's (1979) review of the literature showed that approximately 30 g of N were synthesized per kg of organic matter (OM) truly digested, with values ranging from 10 to 50 g of N per kg OM 6 truly digested. The amount of protein that is degraded in the rumen depends on microbial proteolytic activity, protein structure and accessibility of the feedstuff to microbes, ruminal retention time, protein solubility, and ruminal pH (NRC, 1985). Rumen bacterial growth is stimulated by peptides and AA acting as multiplying factors, which ultimately affects the rate and extent of protein degradation in the rumen (Argyle and Baldwin,1989).

Carbohydrate degradation in the rumen

Carbohydrates are the main components in the dairy ration, comprising roughly 60 – 80% of total dry matter (DM) and could supply to 70% net energy lactation (NEL) for high yielding dairy cows. Therefore understanding carbohydrates features and applying their characteristics for the formulation of dairy rations are important for improving milk production and animal health. As the main reservoir of photosynthetic energy in plants, plant carbohydrates composed of 50 – 80% of the DM of forages and cereals (Van Soest, 1994). The carbohydrates in the feed can be divided into two fractions, namely fibre and non-fibre fractions. Fibre fraction, commonly referred to structural carbohydrates (SC) include cellulose, and hemicelluloses. Non structural carbohydrates (NSC) include starch, pectin, and sugars (Van Soest, 1994). In ruminants, nearly all carbohydrate digestion (> 90%) occurs in the rumen (Armstrong and Smithhard 1979), although in some cases, such as at a high rate of passage, a significant portion of NSC digestion can occur in the small intestine (Nocek and Tamminga 1991). The simple sugars found in plant cells are glucose, fructose, and sucrose. They are rapidly degraded in the rumen to yield short chain fatty acids (SCFA), which are absorbed into the blood through the rumen wall. Polysaccharides must be

hydrolysed into simple sugar before being utilized. Starch as a NSC is easily degraded in the rumen while degradation of structural carbohydrates, such as celluloses and hemicelluloses, varies considerably (Baldwin and Allison 1983). Cellulose degradability of forages varies from 30 – 90% while hemicelluloses digestibility varies from 45 – 90% (Van Soest, 1994). This broad range of cellulose and hemicelluloses digestibility from the different feeds is mainly due to lignification. Therefore the rate and extent of digestion of cellulose and hemicelluloses are related to the lignin content (Allen and Mertens 1988; Van Soest, 1994).

Preferred N Source of Rumen Microbes

Rumen microbes are able to convert NPN to high-quality protein for use by dairy cows, but they also degrade high-quality dietary protein to ammonia (Van Soest, 1994). Ammonia is the main source of N for microbial protein synthesis (Nolan, 1975) and 82% of the bacterial strains isolated from one animal grew with NH3 as the sole N source (Bryant and Robinson, 1962). Cows can thrive with urea as the only source of dietary N (Virtanen, 1966); however feeding true protein to cattle typically improves performance (Stock et al., 1986; Rooke and Armstrong, 1989). Both *in vitro* and *in vivo*, the addition of protein or AA-N has also been shown to also increase fiber digestion and microbial protein yield (Maeng and Baldwin, 1976; Cotta and Russell, 1982; Rooke and Armstrong, 1989). Growth of most rumen bacterial strains has been shown to improve when preformed AA are present (Hungate, 1966). Rumen microbes take up amino acids in the form of peptides more rapidly than free amino acids (Wright, 1967).
The AA that make up microbial protein may be synthesized *de novo* using ammonia-N (NH3-N) and C-chains which are derived from a variety of pathways (Wallace et al., 1997). The C-chains result from carbohydrate or amino acid catabolism while NH3-N is derived from AA, NPN, or urea recycling back to the rumen from the blood, deamination of AA and other sources. Amino acids are produced during proteolysis of feed protein, proteolysis of bacterial and protozoal protein released after cells are lysed (intra-ruminal recycling), release of AA from bacteria and protozoa, and from degradation of sloughed rumen epithelial cells (Demeyer and Fievez, 2004). Additionally, peptides and AA may be taken up intact by the rumen microbes and incorporated directly into microbial protein or transaminated prior to incorporation into protein (Bach et al., 2005).

Effects of AAN versus NPN

In pure bacterial cultures, both AA and peptide supplementation increased the maximum specific growth rate of several cellulolytic and amyolytic bacterial strains as 8 compared to supplementation of (NH4)2SO4 (Cruz Soto et al., 1994). However, the degradation of cellulose by three cellulolytic bacterial species (*F. succinogenes, R. albus, R. flavefaciens*) was enhanced when the pure cultures were incubated with (NH4)2SO4 and AA, but not peptide.

Estimating Microbial Protein Production – Use of Purine Derivatives

External markers such as 15N or 35S as well as internal markers such as nucleic acids have been used to determine ruminal microbial protein production (Broderick and Merchen, 1992). However, determining digesta flow is necessary when using 10 these markers which require cannulated animals (Broderick and Merchen, 1992). As a result, finding a non-invasive method to estimate microbial protein production in the rumen of cattle would be beneficial in ration formulation. In ruminants, purines are excreted as purine derivatives (PD) in urine and milk as allantoin, uric acid, xanthine and hypoxanthine. Because of the high

xanthine oxidase activity found in the blood of cattle, xanthine and hypoxanthine are converted to uric acid in blood and tissues prior to urinary excretion (Chen et al., 1990). Through the use of nucleic acid infusion, PD were found to originate from the catabolism of purines of both endogenous and exogenous origin (Verbic et al., 1990). Concentrations of nucleic acids in the rumen are used to estimate MN production because nucleic acids from the diet were shown to be degraded in the rumen (Smith and McAllen, 1970). As a result, most of the purines and pyrimidines found in the duodenum are assumed to originate from microbial protein production.

Purine Derivatives in Urine

Balcells et al. (1991) concluded that urinary allantoin may be a useful index to estimate duodenal input of purines when animals are fed close to or above their maintenance requirements. Measuring all PD excreted in urine may provide a more accurate estimate of microbial protein production than allantoin alone (Giesecke et al., 11 1984; Lindberg et al., 1989). However, urinary allantoin excretion more precisely estimated microbial protein synthesis than all PD excreted in urine because allantoin is excreted in greater concentration compared to the other PD and therefore less error is associated with its measurement (Puchala and Kulasek, 1992).

Purine Derivatives in Milk

Only a few studies have evaluated the relationship between MN at the duodenum and allantoin excretion in milk. Allantoin excretion in milk was positively correlated with MN flow ($R2 = 0.28$; $P < 0.0001$) in lactating multiparous Holstein cows; however, this was determined averaging responses across ten different experiments (Timmermans et al., 2000). Shingfield and Offer (1998) and Giesecke et al. (1994) reported a high correlation between milk allantoin excretion and concentration with milk yield in Holstein cows. However, individual cow milk allantoin concentration and excretion were poorly correlated with urinary purine excretion or calculated microbial protein supply (Shingfield and Offer, 1998). Kirchgessner and Kreuzer (1985) reported that though milk urea increased as dietary crude protein increased, milk allantoin concentration was not altered. As DMI increased, milk yield increased causing a subsequent increase in the overall yield of milk allantoin though there was no change in milk allantoin concentration when 13 lactating cows were energy and protein depleted followed by normal or excessive nutrient supply (Kirchgessner and Windisch, 1989). The dietary effects of PD secretion in milk have been reported with variable results. Feeding diets high in energy was shown to increase excretion of milk allantoin in lactating cows (Kirchgessner and Kaufmann, 1987). Dry matter intake was also found to be positively correlated with allantoin excretion in milk (Gonda and Lindberg, 1997). When lactating beef cows were fed increasing amounts of urea, no changes in milk allantoin or uric acid were noted (Susmel et al., 1995). Feed restriction has also been shown to influence milk and urine allantoin and uric acid concentrations (González-Ronquillo et al., 2004).

Correlation of Urine and Milk PD Excretion

Gonda and Lindberg (1997) reported that urinary excretion of allantoin was positively correlated with its excretion in milk in lactating dairy cows. Allantoin concentrations in milk were correlated with urinary excretion of allantoin in Holstein cows (Vagnoni et al., 1997). Additionally, milk allantoin excretion was highly correlated with urinary PD excretion when milk yield was included as a covariate in the model (Shingfield and Offer, 1998). In ewes, allantoin excretion in milk was not correlated with its excretion in

urine although its relationship with urinary purine excretion tended towards significance (Martín-Orúe et al., 1996).

Purine Derivatives in Plasma

Little research has been conducted on the circulating concentrations of PD in plasma as results on the correlation of plasma PD concentration to PD excretion have been variable (Giesecke et al., 1994). Plasma allantoin was found to be correlated with urinary allantoin in lambs (R2 = 0.88; Fukihara et al., 2003) and to milk and urinary allantoin in cows (R2 = 0.84; Giesecke et al., 1994). Additionally, plasma allantoin is correlated with energy intake and milk yield (Giesecke et al., 1994). It would be beneficial; however, to develop a technique utilizing spot samples of plasma since collection of total urine in the field is difficult (Chen et al., 1997).

Conclusion

Synchronization of rumen available protein and energy is one of the conceptual methods to increase the efficiency of utilization of nutrients by the ruminants. Ruminal proteolysis determines the amount of ammonia, AA, peptides, and branched-chain VFA available for microbial growth and proliferation. Concentrations of nucleic acids in the rumen are used to estimate MN production because nucleic acids from the diet were shown to be degraded in the rumen. Allantoin concentrations in milk were correlated with urinary excretion of allantoin in Holstein cows.

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