Review

The role of bacteria in nitrogen metabolism in the rumen with emphasis of cattle

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Ruminant species supply over half the meat and essentially all of the milk and animal fiber consumed by man, as well as being a significant source of draft power and fuel. The unique digestive and metabolic strategies of these ruminants make them particularly efficient in the conversion of feedstuffs that are of low value to or unfit for human consumption into high-quality food. Two important characteristics set ruminants apart from most other domestic livestock: their ability to synthesize digestible true protein from non-protein nitrogen (NPN) sources such as urea and ammonia, and their ability to utilize the energy in cellulosic materials both characteristics dependent on the activities of rumen micro-organisms. Ammonia is used by bacteria to build their proteins and any excess of it is absorbed through the rumen wall into the blood and then converted to urea in the liver. Bacteria population makes the ruminant animal virtually independent of dietary sources of all vitamins, except for vitamins A and D. The rumen is an exceptional habitat, in providing constant conditions. Rumen bacteria digest cellulose from plant cell walls, digest complex starch, synthesize protein from non-protein nitrogen, and synthesize B vitamins and vitamin K. The microbial population of the rumen is complex and includes members that belong to the three domains of life: bacteria, protozoa and fungi. Bacteria constitute the most significant member of the microbial population based on cell mass (>50%), number (10¹⁰ to 10¹¹/g of contents) and contribution to ruminal fermentation. Species of bacteria may vary from one strain of that species to another and number of bacteria and the relative populations of individual species vary with the animal’s diet. Bacteria inhabiting the rumen have been classified into four groups depending on their environmental habitat. Nitrogen metabolism in the rumen is a result of mainly the metabolic activity of rumen bacteria as the majority of bacteria have proteolytic activity. Bacterial cells and dietary nitrogen that escapes ruminal degradation are the major sources of protein and amino acid requirements of ruminants. The total amount of microbial protein flowing to the small intestine depends on nutrient availability and efficiency of use of these nutrients by ruminal bacteria. The efficiency of bacterial protein synthesis is a major factor affecting the overall amino acid requirement of ruminants. Hence, the aim of this paper is to review the role of bacteria in nitrogen metabolism in the forestomach of ruminants.

Key words: Bacteria, ruminant, protein, nitrogen metabolism

INTRODUCTION

Ruminant species supply over half the meat and essentially all of the milk and animal fiber consumed by man, as well as being a significant source of draft power and fuel. The unique digestive and metabolic strategies of these ruminants make them particularly efficient in the conversion of feedstuffs that are of low value to monogastrics or unfit for human consumption into high-quality food, fiber, and numerous other products that are...
beneficial to man (Fellner, 2005). The host animal supplies the microorganisms in the rumen with plant material, which often is of low nutritional quality, and in return gets access to high-quality microbial protein from microbes.

The rumen is a complex ecosystem in which feeds consumed by the ruminant animal are digested by an active and diverse micro-flora. It is the diversity, adaptability and mutualistic interactions among the ruminal microbes and the host that have given ruminants a competitive edge in their ability to digest and thrive on diets high in fiber but often low in protein (Weimer, 1996). Ruminants along with other mammals (including man) do not possess the enzymes necessary to digest fiber or to efficiently convert non-protein nitrogen (NPN) into protein. The single largest contribution made by rumen microbes is their ability to ferment fibrous materials (Bach et al., 2005).

Ruminants have a more complex protein metabolism than non-ruminants. In monogastrics the amino acid supply for absorption in the small intestine depends on the amount and composition of the feed proteins, whereas in ruminants, the amino acid supply comes from two sources: feed and microorganisms (Hedqvist, 2004). Two important characteristics set ruminants apart from most other domestic livestock: their ability to synthesize digestible true protein (essential amino acids) from non-protein nitrogen (NPN) sources such as urea and ammonia, and their ability to utilize the energy in cellulosic materials both characteristics being dependent on the activities of rumen micro-organisms (Nolan, 1981). The rumen is a complex environment inhabited by different microbial species, each of them with different nutrient requirements and metabolisms. Therefore, this paper reviews and summarizes current knowledge on the role of bacteria on nitrogen metabolism in ruminant animals.

RUMINANT ANIMALS

Ruminant animals (e.g. cattle, sheep, goats, deer, etc.) do not synthesize fiber digesting enzymes, but they formed a symbiotic relationship with ruminal microorganisms that can. The ruminant provides the microorganisms with a habitat for their growth, the rumen, and microorganisms supply the animal with fermentation acids, microbial protein and vitamins (Hungate, 1966). Unlike non-ruminants, formulating for precise nutrient requirements in ruminant rations is a challenge. Both ruminants and non-ruminants utilize nutrients in tissues but ruminants have another metabolic system, bacterial metabolism in the rumen.

Feed proteins are degraded by microorganisms in the rumen via amino acids into ammonia. Ammonia is used by bacteria to build their proteins and any excess of it is absorbed through the rumen wall into the blood and then converted to urea in the liver (Altuntas, 2008). When a diet is low in nitrogen, large amounts of urea return to the rumen where it can be used by the microbes. In non-ruminants, urea is always entirely lost in the urine. If ammonia levels in the rumen are too low there will be a nitrogen shortage for bacteria and feed digestibility will be reduced. Too much ammonia in the rumen leads to wastage, ammonia toxicity, and in extreme cases, death of the animal (Leibholz, 1972).

The rumen

The rumen is an exceptional habitat, first, in providing constant conditions of moisture, pH, temperature, an aerobiosis, and food. Second, in being an open system in which no stringently restrictive factors such as humoral defense mechanisms limit to a few the number of kinds of organisms which can survive. Some restrictions are inevitable in any habitat maintaining fairly constant conditions; in the rumen the type of food and the lack of oxygen prevent the growth of many types found in other habitats (Hungate, 1968). The rumen is sometimes called the “paunch.” It is lined with papillae for nutrient absorption and divided by muscular pillars into the dorsal, ventral, caudodorsal, and caudoventral sacs. The rumen acts as a fermentation vat by hosting microbial fermentation. About 50 to 65 percent of starch and soluble sugar consumed is digested in the rumen. Rumen microorganisms (primarily bacteria) digest cellulosic material from plant cell walls, digest complex starch, synthesize protein from non protein nitrogen, and synthesize B vitamins and vitamin K. Rumen pH typically ranges from 6.5 to 6.8 (Jane et al., 2009)

The rumen environment

The conditions in the rumen are not only complex but they are intermittent. The rate at which feed enters the rumen will be very different during grazing or meal feeding. Salivary flow rate is not steady and rumination activity that is not continuous will depend on the type of diet. The flow of substances into and out of the rumen may involve more than one pathway. Volatile fatty acids can leave the rumen via passage into the lower tract or they can be absorbed and partly metabolized in the epithelium (Bach et al., 2005). Urea may enter the rumen via saliva or directly from blood through the rumen epithelium. Despite all these variations there are certain generalizations that Fellner, (2005) could make regarding the rumen:

i. Temperature is usually maintained within the range of 38-41°C with 39°C used as a common mean temperature.

ii. Rumen pH can range from around 7.0 on forage diets.
Bacteria make up about half of the living organisms present in the rumen. However, they do more than half of the work in the rumen (Hungate, 1966). The bacteria work together. Some breakdown certain carbohydrates and proteins which are then used by others some require certain growth factors, such as B-vitamins, which are made by others. Some bacteria help to clean up the rumen of others’ end products, such as hydrogen ions, which could otherwise accumulate and become toxic to other organisms. This is called “cross-feeding”. Species of bacteria may vary from one strain of that species to another and number of bacteria and the relative populations of individual species vary with the animal’s diet; for example, diets rich in concentrate foods promote high total counts and encourage the proliferation of lactobacilli (Leibholz, 1972).

Rumen bacteria are predominantly strict anaerobes (no tolerance to oxygen) although a few facultative anaerobes exist, performing a key role in removing oxygen quickly from the rumen environment. The bacterial population is diverse ranging from those who digest carbohydrates (cellulose, hemicelluloses, pectin, starch, sugars) to those who use acids or hydrogen as energy sources (Russell, 2002). The bacteria are highly dependent on B vitamins, ammonia (NH₃), carbon dioxide and VFAs. Given that the digestion of fiber (cellulose and hemicelluloses) is commonly thought of as the primary role of the rumen, it is the fiber digesting bacteria which receive the most press. Lignin, the third component of fiber, remains undigested (Brazier, 2010).

Classification of rumen bacteria

Bacteria inhabiting the rumen have been classified into four groups depending on their environmental habitat: free-living bacteria associated with the liquid phase in the rumen; bacteria associated with feed particles; bacteria associated with rumen epithelium; and bacteria attached to the surface of protozoa (Czerkawski and Cheng, 1988; McAllister et al., 1994). Microbial populations associated with feed particles are estimated to be responsible for 88-91% of ruminal endoglucanase and xylanase activity (Williams and Strachan, 1984; Minato et al., 1993). Bacterial roles are particularly important because bacterial populations associated with feed particles are predominant numerically, accounting for up to 75% of the total microbial population (Minato et al., 1993). These indicate that fiber-associated bacterial populations are pivotal for ruminal fiber digestion. Because attachment is an essential step for fibrolytic bacteria to initiate digestion of plant fiber in the rumen.

The role of rumen bacteria in nitrogen metabolism

Before entering the intestine, feedstuffs consumed by ruminants are all initially exposed to fermentative activity in the rumen. Fermentation of feedstuffs in the

Microbial ecology

The rumen contains one of the most diverse and dense microbial ecologies known in nature. A possible explanation for the diversity is the complex nature of the feed, which contains carbohydrate, proteins, fats, other organic compounds and minerals. In order to utilize these compounds organisms are either highly specialized to compete for a few of the feeds or become widely adapted and are capable of using many nutrients (Fellner, 2005). The contents in the rumen are heterogeneous and consist primarily of a microbial suspension in free liquid, a solid mass of digesta, and a gas phase. In a fully functioning rumen, there is a dynamic equilibrium, as ruminal microbes adhering to and detaching from feed particles are constantly leaving or re-entering the fluid compartment. The microbial population of the rumen is complex and includes members that belong to the three domains of life: bacteria, protozoa and fungi. Bacteria constitute the most significant member of the microbial population based on cell mass (>50%), number (10¹⁰ to 10¹¹/g of contents) and contribution to ruminal fermentation (Nagaraja and Bauchop, 1977). Moreover, products of the metabolism of some species of microorganisms are sources of energy for the other species. These interactions regulate in a large part the concentrations and activities of individual species as well as the nature of the fermentation products.

Ruminal bacteria

Bacteria make up about half of the living organisms inside of the rumen. However, they do more than half of
Nitrogen sources in the rumen are commonly divided into two categories; degradable crude protein (RDP) and non-protein nitrogen (NPN). Both RDP and NPN are hydrolyzed and utilized by rumen microbes (Figure 1). Dietary protein is rapidly degraded into peptides and amino acids. Peptides can then be converted to amino acids or converted directly to microbial protein. Amino acids can be used directly by microorganisms for protein synthesis or can be further broken down through deamination to produce carbon skeletons and NPN compounds, such as ammonia or urea (Namkim, 2010).

In addition to meeting the needs of the ruminal microbiome, rumen microorganisms provide enough protein to meet the protein requirements of the cattle. This is true even when the ruminant is subjected to a protein free diet (Namkim, 2010). The ruminal bacteria play the most significant role in protein breakdown; the bacterial fraction exhibits 6 to 10 times higher specific proteinase activity than the protozoal fraction (Brock et al., 1982). Rumen protein degradation follows the scheme: proteins $\rightarrow$ oligopeptides $\rightarrow$ dipeptides $\rightarrow$ amino acids or converted directly to microbial protein. Amino acids can be used directly by microorganisms for protein synthesis or can be further broken down through deamination to produce carbon skeletons and NPN compounds, such as ammonia or urea (Namkim, 2010).
Figure 1. Protein degradation process. Source: (Publ, 2004).

Acids → ammonia (Wallace, et al., 1995; Cotta and Russell, 1996). The degradation of amino acids to ammonia is an intracellular process, but the degradation of protein to amino acids is an extracellular, but cell-associated, process (Cotta and Russell, 1996). According to Wallace (1995), the rate-limiting step in protein degradation is the degradation of oligopeptides to dipeptides from the N terminal.

Microbial protein synthesis in the rumen provides the majority of protein supplied to the small intestine of ruminants, accounting for 50 to 80% of total absorbable protein (Storm and Orskov, 1983). The total amount of microbial protein flowing to the small intestine depends on nutrient availability and efficiency of use of these nutrients by ruminal bacteria. According to the National Research Council (NRC, 1994), microbial protein synthesis in ruminant is important for the demand of the protein in small intestine. Therefore, N metabolism in the rumen can be divided into 2 distinct events: protein degradation, which provides N sources for bacteria, and microbial protein synthesis (Clark et al., 1992). The key to optimizing microbial protein production is to supply the rumen with fermentable carbohydrates, which stimulate microbial growth, along with N sources that meet microbial N requirements. Two primary groups of bacteria ferment feed in the rumen: those that ferment sugars and starches and those that ferment fiber. Microbes that ferment sugars and starches prefer peptides and AAs as their N source, and adequate concentrations of ruminally degradable dietary protein act as a growth stimulant to this group. Fiber-fermenting microbes rely solely on NH₃ as their N source; the NH₃ comes primarily from non protein N sources in forages and urea, as well as from the degradation of feed protein. An imbalance of protein or feed N sources in the diet can cause excess ruminal NH₃ that is absorbed through the rumen wall and excreted in urine and milk as urea (NRC, 1994).

The efficiency of bacterial protein synthesis is a major factor affecting the overall amino acid requirement of ruminants, and is influenced by a number of factors including: 1) energy source, 2) supply of nutrients such as nitrogen, sulfur, branched chain fatty acids, and 3) ruminal environmental characteristics such as dilution rate, pH and microbial species present in the rumen (Hespell and Bryant, 1979). An average efficiency of microbial synthesis of 17 grams of microbial protein per 100 grams of digestible organic matter was determined for many diets, although values were generally higher for sheep compared with cattle, and forage-based diets compared with grain-based diets (Bergen et al., 1982).

Nitrogen (N) utilization in ruminants

Improving the efficiency of nitrogen (N) utilization in ruminant animals is an important factor in reducing feed costs and mitigating the negative environmental impact of intensive livestock operations. Ruminants are relatively inefficient at utilizing dietary N. For example, in beef cattle approximately 25% of dietary N is retained in tissue, with the remainder being excreted in the faeces (29%) and urine (39%) (Gayle, 1996). In dairy cows, 25 to 30% of dietary N is deposited in milk protein, with 70 to 75% excreted in the feces and urine (Tamminga, 1992). The excretion of excess dietary N, particularly as urinary N, can have a negative impact on the
environment. Understanding microbial and whole animal metabolic N utilization can aid in formulating cattle rations for optimum efficiency. Such a ration would provide N in a way that meets animal requirements and reduces N loss, ultimately mitigating both economical and ecological problems associated with N lost to the environment via feces and urine (Figure 2).

**Urea-N recycling**

Urea is a highly concentrated source of CP that is commonly used to provide degradable intake protein (DIP) to ruminants. Urea is rapidly broken down to ammonia in the rumen by the action of bacterial urease (Satter and Slyter, 1974). Ammonia is used by rumen microbes to produce microbial proteins and is required by many rumen bacteria including cellulose degraders (Hungate, 1966). Sources recycled within the body derived from sloughed cells and urea that re-enters the rumen across the ruminal epithelium or in saliva (Huntington and Archibeque, 1999). Ruminants have the ability to recycle N in the rumen, which reduces the amount of DIP that needs to be fed to meet the bacteria’s requirement for N for growth. However, N recycling differs greatly between diets (Sultan et al., 1992). Nitrogen recycling to the rumen is a characteristic unique to ruminant animals that serves to augment low N diets. Nitrogen is conserved by decreasing urinary excretion of cleared urea (Schmidt-Nielsen, 1977).

Understanding or appreciating the level of urea recycling and accounting for this and the microbial utilization of the recycled N improves our ability to formulate more N efficient diets. All ruminants are obligate recyclers of N and the amount of urea N recycled is a function of N intake, the rate of degradation of the carbohydrate and protein and the associated microbial uptake of feed. There are other factors impacting urea N recycling, but N intake and the total pool size of N within the animal will have the largest impact (Recktenwald, 2010). Urea production ranges from approximately 40 to 70% of total N intake per day and this hepatic function does not require a significant amount of energy.

Urea-N recycling occurs in both ruminant and non-ruminant animals. However, in ruminants, 40 to 80% of endogenously produced urea-N can be recycled to the gastrointestinal tract (GIT) as compared to 15 to 39% in non-ruminants (Huntington 1989; Russell et al. 2000). In ruminants, urea-N can be recycled to the GIT via transfer from the blood to the lumen of the GIT (Stewart et al., 2005). However, it has been estimated that 23 to 69% of endogenously produced urea-N is recycled to the GIT through the saliva in ruminants (Huntington 1989). The recycling of urea-N to the GIT represents an opportunity for the anabolic use of recycled urea-N, improved overall N efficiency and the opportunity to reduce the excretion of N into the environment.

![Figure 2. Protein utilization by the ruminant. Source: Lee, 2009](image-url)
Factors affecting urea-nitrogen recycling to the rumen

The rate of urea-N recycled to the rumen and its utilization for anabolic purposes can be influenced by a number of both dietary and ruminal factors. Many ruminal and dietary factors are interrelated in terms of affecting urea-N recycling to the rumen. For example, the recycling of urea-N to the rumen has been shown to be negatively correlated with ruminal NH$_3$ concentration (Kennedy and Milligan, 1980). Therefore, dietary factors affecting the rate of dietary N partitioning to ammoniagenesis will influence urea-N recycling to the rumen (Lapierre and Lobley 2001). Furthermore, increasing the supply of ruminally-fermentable carbohydrate increases the incorporation of ruminal NH$_3$-N into microbial protein. This, in turn, reduces ruminal NH$_3$-N concentration leading to an increase in the proportion of urea-N recycled to the rumen (Kennedy and Milligan, 1980).

Nitrogen waste and pollution

Due to increased population and income levels, particularly in developing countries, worldwide production of meat and milk will have to double within 50 years (Dijkstra et al., 2011). This will require a massive increase in the productive output from animals without any appreciable increase in land availability. However, maximizing production from ruminants is often associated with an increase in excretion of waste products that may be harmful to the environment (VandeHaar and St-Pierre, 2006). Rumen fermentation brings some disadvantages. Methane is produced as a natural consequence of the anaerobic fermentation; it is a potent greenhouse gas. Dairy farming is the largest agricultural source of methane. Furthermore, the major environmental concern associated with the animal industry is ammonia volatilization, which increases atmospheric acid deposition because of the impact of nitrogen-rich excreta on the environment (Altuntas, 2008).

Nitrogen is one of the major sources of pollution from ruminant operations, along with phosphorus and methane. Nitrogen is of particular concern in cattle production (Arriaga et al., 2009) and N pollution results in eutrophication of natural water sources, pollution of groundwater with nitrates and atmospheric pollution by de-nitrification and ammonia volatilization (Dijkstra et al., 2011). Volatilized ammonia returns to the land or water via rainfall, dry precipitation, or direct absorption. Volatilized ammonia also can contribute to odor problems. Although ammonia may be beneficial as a fertilizer for agricultural fields, it may not be beneficial in other ecosystems. Manure in the form of slurry when injected into the soil will have minimal losses of ammonia. The higher the N contents of the manure, the greater the risk of ammonia loss. For example, most beef cattle are produced in open feedlots. Ammonia losses can represent as much as 70% of the N excreted by those cattle (Arriaga et al., 2009). Minimizing N pollution is necessary at all stages of production, from crop production to feeding and management practices and manure management (Rotz, 2004).

CONCLUSION

Ruminant animals have unique digestive and metabolic strategies which make them particularly efficient in the conversion of low value of feedstuffs into high-quality food, fiber, and numerous other products. Both ruminants and non-ruminants utilize nutrients in tissues but ruminants have another metabolic system, bacterial metabolism in the rumen. Feed are degraded by microorganisms in the rumen via amino acids into ammonia. Ammonia is used by bacteria to build their proteins and any excess of it is absorbed through the rumen wall into the blood and then converted to urea in the liver. Microbial protein synthesis in the rumen provides the majority of protein supplied to the small intestine of ruminants. The total amount of microbial protein flowing to the small intestine supplied to the small intestine of ruminants. The total amount of microbial protein flowing to the small intestine depends on nutrient availability and efficiency of use of these nutrients by ruminal bacteria. Hence, improving the efficiency of nitrogen utilization in ruminant animals is an important strategy in reducing feed costs and mitigating the negative environmental impact of intensive livestock operations.

REFERENCE

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