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Nitrogen Metabolism and Recycling in Ruminant Animals: A Review

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Abstract: This review describes the quantitative aspects of nitrogen metabolism in ruminants and it relates the metabolic or economic costs of that metabolism to practical feeding situations. All animals require amino acids (AA) which are the building blocks of proteins required for optimal growth, reproduction, lactation and maintenance. In ruminants, proteins and AA are first subject to microbial degradation in the rumen making it difficult to predict the quality and quantity of AA that are absorbed by the animal. In ruminants, absorbed AA comes from microbial protein synthesis in the rumen and from dietary amino acid sources that are under graded in the rumen. The bacteria utilize the ammonia as a nitrogen source, producing amino acids and peptides necessary for growth. Interestingly, these microbial products can be reabsorbed back into the host mammalian circulation and used for synthetic processes; because of this reason, urea has commonly become an accepted ingredient in the diets of ruminants. Therefore, this review focuses on urea metabolism and recycling in ruminants. In conclusion, Urea is one of the major non-protein nitrogen feeds for ruminants and the optimal utilization of urea in feed can alleviate to some extent the cost of dietary protein. Urea is hydrolyzed quickly by ureolytic bacteria in the rumen.

Key words: Ruminants · Urea · Ammonia · Nitrogen · Metabolism · Recycling

INTRODUCTION

Both ruminants and non-ruminants, including omnivores, have a mechanism in which urea produced by the liver can enter the intestinal tract and where it is used for microbial protein production or urea production. However, the amount of urea recycled in ruminants is in much larger proportions compared to non-ruminants, which emphasizes the importance of urea recycling in ruminants [1]. Since the discovery of the Haber-Bosch process, N has played a pivotal role in agricultural production and is a key nutrient influencing crop yields. Globally, demands for N fertilizer are growing (in parallel with the growing human population) and were expected to reach 119.4 million ton in 2018, FAO [2]. The successful symbiotic relationship between humans and ruminant livestock developed largely because ruminants did not compete for food with humans. For a very long time in history, grass, straw and other fibrous plant materials that are not suitable for human food have been the main source of food for ruminants. Ruminants in return provide humans with high quality proteins in the form of milk and meat. Furthermore, there is evidence that animal proteins, especially milk proteins, have higher biological value for humans than human-edible plant proteins [3].

Recent meta analyses of experimental data from lactating cows have concluded that the major factor determining total N excretion as manure (feces plus urine) in lactating dairy cows is total dietary N intake [4, 5]. However, there is some variation in the excretion measured at a given level of N intake, which may arise due to effects of experimental variation, animal variation (genetic differences in digestive or metabolic efficiency), or dietary and other environmental variables. Thus, there is opportunity for reducing N excretion by reducing the amount fed but also through other dietary management and breeding strategies. According to Archibeque *et al.* [6] studied the effects of N intake in grass hay diets on N metabolism of steers. Although N digestibility increased as N intake increased, there was no difference in N

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balance between the two levels of N intake. These data indicate that some limitation in post absorptive metabolism may have decreased the ef?ciency of N retention of the steers.

Human edible plant proteins are food proteins that are typically used directly for human consumption and have nutritional value for humans, such as grains and legume seeds. Today the importance of quality food production is as high as ever. In 2014, over 326 million beef animals and 813 million dairy animals (buffalo, cattle, sheep and goats) provided over 68 million tons of meat and over 801 million tons of milk for humans on a global scale [7]. Beef and sheep meat make up 26% of the total amount of meat produced in the world from livestock [7].

As for every other living organism, protein is an essential feed component for ruminants. Proteins are needed for cell growth and production. With intensive farming practices and growing awareness of the need for environmental sustainability, official demand for efficient utilization of feed proteins and reduced nitrogen (N) excretion from dairy farming is growing. The over production of milk and meat in Europe and the USA in recent years as described by Delgado-Elorduy [8]. Also, USDA [9] shows that it is not necessary to reach maximum production, but rather to improve the efficiency. From a farmer's point of view, efficient use of N is important for profits, since. Total and human-edible protein conversion ratio (input per unit of output) [10]. Protein is the most expensive feed component and usually has a high impact on production.

This review is intended to provide an in-depth analysis of the methods currently used to study N metabolism in ruminants and the fate of manure N.

Objectives of the Review: To clarify nitrogen metabolism in ruminant, to understand the function of nitrogen for ruminant animals and understand method of nitrogen recycling for ruminant animals.

Nitrogen Metabolism in Ruminants

Nitrogen Metabolism in the Rumen: Degradation activity of these proteolytic microbes depends on the chemistry and structure of dietary proteins, as well as ruminal pH and predominant species of microbes present in the rumen [11]. The RDP is comprised of true protein and NPN. True protein is degraded to peptides, AA and NH3-N, whereas NPN is comprised of N present in nucleic acids, NH_3-N, AA, small peptides, amides and amines [12]. Microbial protein synthesized in the rumen, along with RUDP and endogenous N, are the major sources of AA available at the small intestine.

Nitrogen Metabolism in the Small Intestine: Ingested dietary proteins and endogenous proteins are mixed in the small intestine lumen. The endogenous luminal proteins are originating from various sources including gastric and pancreatic secretory products, desquamated intestinal epithelial cells and mucous proteins. These proteins are digested in the small intestinal lumen by proteases and peptidases originating from the exocrine pancreas.

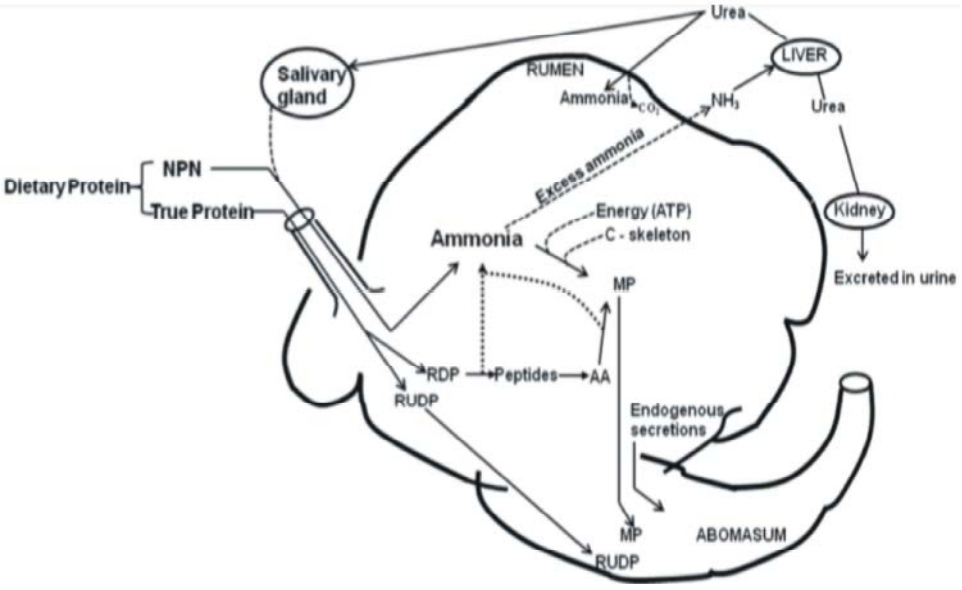

Fig. 1: Overview of nitrogen metabolism in the rumen

The resulting peptides can then undergo the process of final digestion through the catalytic activities of numerous peptidases present in enterocytes. Then, oligopeptides and amino acids are transported from the lumen to the portal bloodstream through a variety of transporters present in the brush border and baso-lateral membranes of the enterocytes [13]. In addition, the enterocytes use several amino acids (glutamine, glutamate and aspartate) [14] as fuels in the context of a high energy requirement for the cell renewal in the epithelial layer and for nutrient absorption. The first step of glutamine utilization by enterocytes is through the mitochondrial glutaminase which converts glutamine into ammonia and l-glutamate. Some data suggest that the intestinal glutamine utilization is responsible for a significant part of the overall ammonia production in the body [15].

Nitrogen Metabolism in the Large Intestine: Alimentary protein digestion followed by amino acid and peptide absorption through the small intestinal epithelium can be considered as an efficient process [16, 17]. Nevertheless, substantial amounts of nitrogenous compounds from both alimentary and endogenous origins can enter the large intestine through the ileocaecal junction. Indeed, even highly digestible proteins may partly escape digestion in the small intestinal lumen and substantial quantities of nitrogenous material are transferred from small intestine to large intestine lumen. Surprisingly proteins and peptides breakdown by colonicmicrobiota has been scarcely studied. Yet bacteria commonly utilize proteins as nitrogen, carbon and energy sources. Little is known about the eco-physiological significance of these phenomena in gut even though they are favored in colonic conditions by the rarefaction of readily fermentable substrates (carbohydrates) from the proximal colon [18].

Tissue Metabolism of Amino Acids: Amino acids exert their action in various tissues and organs and in particular they are able to regulate muscle development. The regulation of protein metabolism by amino acids has been studied extensively in skeletal muscle in order to improve muscle growth and meat quality in animal production and to reduce muscle wasting in some physiological (e.g. early lactation) and physiopathological situations. Indeed, due to the differences between the amino acid composition of acute-phase proteins synthesized during catabolic states and that of muscle proteins, a considerable amount of muscle protein has to be degraded to provide the amino acids used in the acute-phase response. Several reviews have reported that amino acids are signal mediators acting on the same intracellular protein kinases as certain hormones (e.g. insulin) [19- 22].

Urea-N Entry into the Rumen via Salivary Secretions: As outlined in the review by Lapierre and Lobley [1], earlier studies in sheep have shown that contributions from salivary flow to urea-N entry to the rumen can vary between 15 to 100% depending on the type of the diet. Salivary urea-N entry to the rumen calculated as difference between total splanchnic flux and urinary excretions rate as a percent of total hepatic urea-N production represented 72% in steers fed high forage diets as compared to 21% in those fed high concentrate diet High roughage diets stimulate rumination, thus increasing the flow of salivary secretions to the rumen. Reports from other studies also show that salivary flow of urea-N into the rumen as a percent of total urea-N entry to the GIT was 36% in forage-fed.

Urea-N Entry into the Small Intestine: The final protein supply to the small intestine is formed by dietary protein (rumen undegraded protein) and microbial protein [23]. The small intestine contributes to the anabolic salvage of N. It has been found in sheep that 37 and 48% of the total GER of urea entered the small intestine in case of grass silage and dried grass, respectively. However, the quantities of anabolic N formed may by small, e.g. because ammonia production seems to exceed urea entry across the small intestine, although this depends on the type of feed ingested [1]. In ruminants, up to 70% of the total portal-drained viscera flux of urea can enter post stomach compartments [1] of which up to 90% of total portal-drained viscera flux of urea is to the mesenteric-drained viscera in animals fed high fiber diets as compared to only 19% in animals fed high concentrate diets. However, most of the urea-N that enters post-stomach compartments is returned back to the ornithine cycle as NH3 for re-synthesis of urea [1].

Ammonia Absorption Across the Ruminal Epithelium: The quantity of NH3-N absorbed across the ruminal wall is mainly determined by dietary as well as ruminal factors, with the most important factors being dietary protein that is degraded in the rumen, contributions of endogenous sources (e.g., urea) to the ruminal NH_3-N pool and dietary ruminally-available energy [24]. Under a wide variety of dietary and physiological conditions in growing and lactating cattle, Firkins and Reynolds [25] concluded that NH_3-N absorption across the GIT accounts for about 42% of dietary N intake. Sequestration of ruminal NH_3-N into

the bacterial protein in the rumen is energy dependent and, hence, providing adequate ruminally-available energy is associated with lower ruminal NH_3-N concentration and, consequently, reduced NH_3-N absorption into portal blood. Using the arterio-venous difference technique, Delgado-Elorduy (2002) demonstrated that feeding steam flaked sorghum grain to increase ruminal degradable starch decreased net NH3-N absorption across the portal-drained viscera (PDV) as compared to feeding dry-rolled sorghum grain. As Lee *et al.* [26] also suggested that the absorption of NH_4+ may occur through some transport proteins and the movement of NH_4+ across the ruminal epithelium is probably regulated by both chemical and electrical gradients. Absorption of both forms of ammonia across the ruminal wall increases with the increase in ruminal pH and total NH_3-N concentrations.

Rumen Microbiology: Bacteria account for about half of the microbial mass in the rumen but they do most of the fermentation work and contribute the majority of microbial protein flowing to the duodenum [27]. The number of different bacteria species present in the rumen is large and new species are found all the time. Protozoa are the second largest group of microorganisms and form about 20-50% of microbial biomass. Despite being such a large group, their contribution to total microbial protein flow from the rumen is relatively small, due to relatively long retention time in the rumen. The contribution of protozoa to microbial N flow was on average 23% when determined using different techniques, according to a review by Henderson *et al*. [28]. The main role of protozoa in influencing digestion is the engulfment and digestion of bacterial and fungal cells [29]. This increases microbial lysis and is considered a wasteful cycle of bacterial protein breakdown. Removal of the protozoa (defaunation) by chemical therapy (e.g. monensin) or freezing the rumen contents (requires rumen evacuation) usually results in decreased diet digestibility and therefore any benefits from increased microbial protein flow can be lost [29, 30].

The role of fungi in degradation mainly involves fiber break-down by very highly active cellulolytic enzymes [31]. Most studies conclude that fungi have some proteolytic activity, but that their contribution is low compared with that of other microbial communities [29].

About 0.3-3.3% of rumen microbial is archaea which are found free in the rumen fluid, attached to particulate material and rumen protozoa, associated as endosymbiosis within rumen protozoa and attached to the rumen epithelium [32]. The ruminal archaea are strictly anaerobic methanogens using H_2 for growth and often format for energy. Therefore their role in rumen metabolism is more important as secondary fermenters and do not contribute to direct degradation of feed [33]. Since archaea contribute to CH_4 production in ruminants the interest in them is grown in connection to mitigation strategies [34].

Proteolysis: All ruminal microbes contribute to protein breakdown to some extent [35]. Peptides are broken down in steps from oligopeptides to tri- and dipeptides and then further to AA, which are rapidly delaminated by microbes to ammonia. The concentration of free AA increases rapidly within 2 h after feeding, but then decreases rapidly within 2 hours thereafter [36]. Soluble non-ammonia N (SNAN) entering the omasum mainly consists of peptides, suggesting that the rate of peptide breakdown is the main rate-limiting step in protein degradation [37, 38]. The final step in breakdown of dietary proteins is the breakdown of AA to ammonia. Ammonia N is the major source of N for microbial protein synthesis.

Microbial Synthesis: The end-products of protein degradation (peptides, AA and ammonia) meet the N requirements of rumen microbes [39], while excess ammonia N is a product that the animal needs to excrete or recycle. With sufficient energy available, ammonia alone is an adequate source of N for protein synthesis.

Rumen-Undegradable Crude Protein: Rumen-undegradable CP contains potentially digestible fractions only digestible post-ruminally, but also some completely indigestible CP. Some of the proteins escaping ruminal degradation are digested and absorbed in the small intestine, but the amount depends strongly upon the source of RUP. Forage proteins that escape microbial degradation are often poorly digested, but processed grains and protein feeds can have higher value when digested in the intestine than in the rumen. Edmunds *et al*. [40] compared various forages and estimated that about 10-20% of forage CP is RUP. Predation on bacteria by ciliate protozoa leads to higher microbial protein turnover in the rumen. Increased passage rate leaves less time for predation and decreases ruminal recycling of bacterial protein [29]. Diet quality and composition influence microbial synthesis in many ways. It is often claimed that ensiling forages has a negative influence on ruminal microbial protein synthesis. However, some of the few studies comparing hay and silage seem to suggest higher microbial N flow to the duodenum with silage [41]. Rumen-undegradable CP consists mainly of insoluble N

fractions [42], but more recent studies have also shown significant escape of soluble fractions from microbial degradation in the rumen [37, 38].

The Digestive Tract and its Function for Nitrogen Metabolism: The rumen is the largest and the most complex compartment of the ruminant digestion system, where the feed is degraded by microorganisms that live in symbiosis with the ruminant animal. Microbial digestion of structural carbohydrates is what gives ruminants the ability to utilize low quality feeds more efficiently than mono-gastric animals. However, the marginal efficiency of utilization of supplementary protein is low in dairy cows fed typical Nordic high-quality diets. For example, it has been shown that only 10% of incremental crude protein (CP = N × 6.25) intake from soybean concentrate is converted to milk protein [43]. In growing cattle, marginal protein efficiency is even lower [44].

Urea that enters the gut by means of saliva or flowing through the gut wall can be used for anabolic purposes or is transformed into ammonia and returned to the liver [1]. Much of the NH3 in the GI tract is reabsorbed and used in the liver for the synthesis of glutamate and glutamine and then a variety of other nitrogenous compounds [45].

According to NRC [42], up to 23% CP in feed is theoretically needed to maximize milk production by dairy cattle. However, feeding such large protein quantities would lead to reduced milk N use efficiency [46]. Meeting the N requirements of rumen microbes with minimal ammonia losses from the rumen and meeting the amino acid (AA) requirements of the host animal with a minimal amount of rumen-undegradable protein (RUP) is optimum from a biological point of view [39].

Urea or Nitrogen Recycling in Cattle Fed Low-Quality Forage: Ruminants are capable of recycling N from blood to the gastrointestinal tract (GIT). The gastrointestinal tract entry rate (GER) through blood and saliva contributes substantially to N availability in the GIT and may amount to as much as half of the daily dietary uptake [1]. Recycling of urea-N through the rumen wall does not occur against a concentration gradient; it appears to be strongly regulated by expression of urea transporter proteins in kidney and GIT tissues [47, 48]. Hepatic urea-N synthesis has two fates i.e., it is either excreted in the urine or is recycled back to the GIT via salivary secretions or by the direct transfer across the epithelial tissues of the digestive tract [24]. In general, the percentage of dietary N that is recycled to the gut declines as dietary N intake increases, but the total amount of urea recycled to the rumen increases. For cattle fed prairie hay (approximately 5% crude protein) without supplemental protein, more than 95% of the body's urea production was recycled to the gut [49].

The regulatory mechanisms of urea recycling can also be studied by other techniques. Direct measurement of mRNA abundance of urea transporter proteins in rumen epithelial tissue is possible. However, Lobley *et al.* [47] and Rémond *et al.* [50] did not establish an apparent relationship between mRNA abundance for these transporter proteins and the N supply or urea GER. It must be acknowledged, however, that such measurements basically determine the concentration of mRNA coding for these transporter proteins instead of the actual presence of these proteins. Moreover, such measurements are incapable of determining total tissue mass and transporter protein capacity actually present [51].

Absorption of Amino Acids and Ammonia: Urea is the mammalian end-product of the amino acid metabolism. In the rumen, proteins are degraded into amino acids and finally into ammonia (NH_3) by means of rumen fermentation [39]. Then, absorption of both amino acids and NH_3 through the rumen wall and entrance into the portal circulation to the liver can take place.

Impact of Animal Factors on Urea Recycling: Urea recycling is significantly related to NH_3 production and absorption in the gastrointestinal tract (GIT) of ruminants. All NH_3 absorbed from the rumen epithelium, small intestinal mucosa and large intestinal mucosa travels via the portal vein to the liver; body tissue NH_3 also enters the liver. Liver metabolism has a central role in the integration of body N metabolism. Ammonia in the liver is detoxified by conversion to urea, urea can then be recycled directly into the rumen, small intestine, or large intestine; it can enter the rumen in saliva, be excreted by the kidney, or be secreted in milk or sweat [37].

Production of urea is largely a substrate-driven process. Thus, animal productivity can impact urea recycling by impacting how much N is available for urea synthesis. When cattle use more N for productive purposes (i.e., growth or lactation), less N are available for urea synthesis and therefore less urea are recycled to the gut. Bailey [51] compared urea recycling in forage-fed steers weighing 208 and 391 kg; the larger steers were physiologically more mature and deposited less N in tissue proteins than the younger steers. The more mature cattle had greater urea synthesis and greater urea recycling than the younger cattle that deposited more tissue protein. Thus, body protein utilization impacts urea recycling.

Factor Affecting the Efficiency of N Utilization in the Rumen

Dietary Factors: As N intake increases, total endogenous production of urea-N also increases and as percent of N intake, total endogenous urea-N production varied from 77 to 95% [40], clearly indicating the magnitude of the transit of N into the urea pool and the perpetual reliance of ruminants on urea-N recycling to the GIT in order to maintain a positive N balance. Several studies have demonstrated that feeding diets low in dietary N content results in lower total endogenous urea-N production, lower plasma urea-N concentration (PUN) and lower urinary excretions of urea-N, associated with decreased urea-N recycling to the GIT [24]. On the contrary, it is important to note that, in ruminants fed low N diets, the quantity of urea-N recycled to the GIT, as a proportion of total endogenous urea-N production and its utilization for anabolic purposes is greater compared to those fed high N diets; consequently, ruminants can survive for limited period of time under protein deficient situations through the mechanism of urea-N recycling. A study was conducted to examine the effect of two forages (gama grass and switch grass) at two levels of N fertilization application and found an improved N efficiency at low N intakes as a result of higher absolute movement of N across the GIT (by 11.4% units) in steers fed forage fertilized with low N fertilization compared to those fed forages fertilized with high N [6].

Marini *et al.* [41] demonstrated that as dietary N concentration increased, the absolute amount (g N/d) of urea-N that is recycled to GIT also increased; however, as a percent of total dietary N intake and as proportion of total endogenous urea-N production, the quantity of urea-N recycled to the GIT was greater in sheep fed a low N diet compared to those fed a high N diet. In addition, renal urea clearance rates decreased as a result of decreasing dietary N content in sheep (Marini *et al*., 2004) and heifers [42].

Frequency of Dietary Protein Supplementation: Oscillating dietary CP concentrations on a 2-d basis enhanced N retention in ruminants [43], possibly due to an increase in urea-N recycling to the GIT [6], using the venous-arterial difference technique, observed a tendency for a greater net flux of urea-N across the PDV in growing wethers fed oscillating dietary CP concentrations compared to those fed a medium (12.5%) dietary CP concentration. A recent study [43] showed an increase in N retention associated with improved microbial NAN supply to the duodenum in lambs fed oscillating dietary CP compared to those fed medium CP. In cow-calf beef operations, supplementing protein to low quality forages is commonly practiced. Such supplementation strategies are associated with increase in cost of production in terms of labor and machinery. Hence, attempts were made to increase N efficiency by increasing urea-N recycling to the GIT and its capture for microbial protein synthesis in ruminants by altering the frequency of RDP supplementation [49].

Ruminally-Degradable Protein and Protein Solubility: The ruminal NH_3-N concentration is negatively correlated with rate of urea-N transfer across the ruminal wall. Hence, the form of N in the diet, particularly RDP and/or protein solubility, are important and determine how much of the dietary protein is directed towards ruminal NH_3-N [1]. In ruminants fed high RDP level, an increase in ruminal NH_3-N concentration is associated with a decrease in ruminal urease activity, thus a decrease in urea-N transfer from blood into the rumen. Recently, Marini and Van Amburgh [48] showed that in steers fed low dietary N, increasing the amounts of RDP by infusing casein into the rumen linearly increased the quantity of urea-N that was recycled to the GIT (in absolute amounts) and the amount of recycled urea-N that was sequestered into microbial protein (using ^{15}N isotope) ranged from 55.9 to 64.0%.

Archibeque *et al*. [6] showed that urea-N entry rate was greater in steers fed gama-grass and switch grass as compared to those fed tall fescue, due to higher slowly RDP (i.e., B2 fraction of protein) in gama grass and switch grass as compared to tall fescue. Processing of legume seeds especially extrusion is commonly practiced to decrease RDP and increase RUDP. Recently, Lobley *et al*. [47] using arterio-venous difference technique reported that the urea N transfer across the ruminal epithelium (in absolute amounts) was not altered in sheep fed either extruded or raw pea.

Ruminal Factors: Recently, Marini and Van Amburgh [48] reported a linear decrease in the ruminal bacterial urease activity as a result of increasing dietary N levels. Though ruminal NH_3-N concentrations were not measured in that study [48], it is plausible that higher N intakes would have led to increased degradation of dietary N in the rumen, thus increasing ruminal NH_3-N concentrations resulting in decreased bacterial urease activity. In order to maximize urea-N transfer into the rumen and its subsequent utilization for microbial protein synthesis, ruminal NH_3-N

levels have to be reduced by means of altering dietary protein degraded in the rumen. One possible method to decrease RDP in feed ingredients (especially leguminous seeds) is by extrusion. Rémond *et al*. [49] demonstrated that ruminal N loss (as NH_3-N) was lower in sheep fed extruded pea (low RDP) compared to those fed raw pea (high RDP) associated with increased efficiency in urea-N recycling the GIT i.e., greater urea-N flux into the rumen compared to post-stomach compartments in sheep fed extruded pea. As ruminal protozoa are highly proteolytic, eliminating ruminal protozoa (i.e., defaunation) is another possible means to reduce dietary protein degradation in the rumen (and thus reduce ruminal NH_3-N concentrations).

Ruminal VFA and pH: Simmons *et al*. [50] reported a higher but-B2 mRNA and protein expression in steers fed concentrate-based diet compared to those fed silage-based diet. In that study, ruminal butyrate concentration was numerically higher (9.3 vs. 11.7 as % of total VFA) in steers fed concentrate-based compared to silage-based diet and, may play a role in expression of but-B2 thus, increasing urea-N transfer into the rumen. Recently, Abdoun *et al.* [51] demonstrated in vitro using isolated ruminal epithelium in Using chambers that in presence of short-chain fatty acids, reducing ruminal mucosal buffer pH from 7.4 to 5.4 showed a bell-shaped curve for urea transport from serosal to mucosal direction with highest rate of urea transport between pH 6.0 to 6.4. If the ruminal pH is approximately in the range of 6.0 to 6.4, the range which is typically observed under in vivo physiological conditions in the rumen, changing the ruminal factors (e.g., VFA) may have a positive impact on urea-N recycling to the rumen [51].

Efficiency of N Utilization: In both cattle and sheep, the inefficient use of intake-N is associated with large ammonia absorption representing on average 0.46 and 0.47 of N available from the lumen of the gut (digestible N plus urea-N entry across the PDV) [1]. As mentioned earlier, one strategy is to reduce the amount of N directed towards ammonia absorption and hepatic urea genesis, but the situation is more complex than that. The target of reduction of ammonia absorption has to be integrated in a wider context where this decrease would result 1) from a smaller degradation of dietary N into the rumen or 2) from an increased utilization of rumen ammonia for microbial protein synthesis. Lowered N degradation can result from diet manipulation. As Lapierre and Lobley [1] summarized from several studies that cattle fed concentrate-based diets had decreased ammonia absorption, both in absolute amounts and relative to digested N, compared with forage rations. Increased utilization of N for bacterial synthesis can also be influenced by dietary manipulation, particularly provision of additional energy. From several studies, it can be concluded that supplements of rumen fermentable energy sources increase the transfer of urea into the rumen and therefore the capture of dietary N and GER into anabolic products, mainly amino acids. However, there appear to be upper limits to the overall efficiency of the process [1].

The limited data available suggest that a maximum of 50 to 60% of dietary N, or 70 to 90% of apparently digested N, will be converted into amino acids released into the portal vein. Energy sources may also improve utilization of dietary and urea-N by less direct means, e.g. by energy-sparing effects within the cells of the gut tissues rather than alteration of rumen fermentation [1].

CONCLUSION

Amino acids are recognized to be essential in controlling metabolism. Despite the increased understanding of the role of amino acids, many questions still remain unanswered. Identifying the mechanisms by which amino acids regulate metabolism, signaling, gene expression and cell functioning is thus essential to improve the control of nutrient utilization and to optimize dietary amino acid provision for animal production purposes. Urea is one of the major non-protein nitrogen feeds for ruminants and the optimal utilization of urea in feed can alleviate to some extent the cost of dietary protein. Urea is hydrolyzed quickly by ureolytic bacteria in the rumen. Ideally, protein digestion should be measured in the small intestine, which is the major site of protein absorption. This is seldom done, as a result of several factors. Despite access to the small intestine in the ruminant, quantifying the rate of passage of nitrogen through the small intestine remains fraught with practical difficulties. Furthermore, the role of the reticulo-rumen complex in supplying a major source of protein (microbial) to the small intestine tends to obscure the effect of the bypass protein on the overall nitrogen metabolism of the animal, because of imprecise definition of the various protein fractions. Finally, the presence of the large intestine in which feed and microbial protein that escapes digestion in the small intestine is degraded, so alters the composition of the digesta that analysis of fleeces cannot be used to obtain accurate digestibility data.

REFERENCES

1. Lapierre, H. and G.E. Lobley, 2001. Nitrogen Recycling in the Ruminant: A Review. Journal of Dairy Science, 84: Suppl. E223-E236.
2. FAO (Food and Agriculture Organization of the United Nations), 2015. World fertilizer trends and outlook to 2018. FAO, Rome, Italy.
3. Van Den Borne, J.J., J. Verdonk, J.W. Schrama and W.J.J. Gerrits, 2006. Reviewing the low efficiency of protein utilization in heavy preruminant calves a reductionist approach. Reprod Nutr. Dev., 46: 121-37.
4. Archibeque, S.L., J. Burns and G.B. Huntington, 2000. Nitrogen metabolism of beef steers fed endophyte-free fescue hay: Effects of rumen protected methionine supplementation. J. Anim. Sci., 78 (Suppl. 1): 302.
5. Yan, T., J.P. Frost, R.E. Agnew, R.C. Binnie and C.S. Mayne, 2006. Relationships among manure nitrogen output and dietary and animal factors in lactating dairy cows. J. Dairy Sci., 89: 3981-3991.
6. Archibeque, S.L., J.C. Burns and G.B. Huntington, 2001. Urea flux in beef steers: Effects of forage species and fertilization. J. Anim. Sci., 78(Suppl. 1): 282.
7. FAOSTAT, 2014. Livestock Primary - FAO Global Statistical Yearbook. Available at: http://faostat.fao.org/beta/en/#data/QL [2017-02-24] microbial populations in the rumen. In: Ruminant physiology, digestion, metabolism and impact of nutrition.
8. Delgado-Elorduy, A., C.B. Theurer, J.T. Huber, A. Alio, O. Lozano, M. Sadik, P.D. Cuneo, H.D. Young, I.J. Simas, J.E.P. Santos, L. Nussio, C. Nussio, K.E. Webb and H. Tagari, 2002. Splanchnic and mammary nitrogen metabolism by dairy cows fed dry-rolled or steam-flaked sorghum grain. Journal of Dairy Science, 85: 148-159.
9. USDA, 2017. Dairy data. Available at: https://www.ers.usda.gov/data-products/dairy-data.aspx [2017-02-24].
10. Huntington, G. and S. Archibeque, 2009. Practical aspects of urea and ammonia metabolism in ruminants. J. Anim. Sci., 77: 1-11.
11. Bach, A., S. Calsamiglia, H.M.R. Greathead and C. Kamel, 2005. Effects of a combination of eugenol and cinnamaldehyde on ruminal protein and energy metabolism in lactating dairy cows. Proceedings Rencontre Recherche Ruminants Paris, Francia, 12: 246.
12. Blachier, F., F. Mariotti, J.F. Huneau and D. Tome, 2007. Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. Amino Acids., 33: 547-62.
13. Mariotti, F., M.E. Pueyo, D. Tome, S. Berot, R. Benamouzigand S. Mahe, 2001. The influence of the albumin fraction on the bioavailability and postprandial utilization of pea protein given selectively to humans. J. Nutrition, 131: 1706-1713.
14. Macfarlane, G.T. and S. Macfarlane, 2012. Fermentation in the human large intestine: its physiologic consequences and the potential contribution of prebiotics. Journal of Clinical Gastroenterology, 45(Suppl.): 120-127.
15. Kimball, S.R. and L.S. Jefferson, 2006a. New functions for amino acids: effects on gene transcription and translation. The American Journal of Clinical Nutrition, 83: 500-507.
16. Dann, S.G. and G. Thomas, 2006. The amino acid sensitive TOR pathway from yeast to mammals. FEBS Letters, 580: 2821-2829.
17. Tesseraud, S., M. Abbas, S. Duchene, K. Bigot, P. Vaudin and J. Dupont, 2006. Mechanisms involved in the nutritional regulation of mRNA translation. Features of avian model. Nutrition Research Reviews, 19: 104-116.
18. Métayer, S., I. Seiliez, A. Collin, S. Duchêne, Y. Mercier, P.A. Geraert and S. Tesseraud, 2008. Mechanisms through which sulphur amino acids control protein metabolism and oxidative status. The J. Nutritional Biochemistry, 19(4): 207-215.
19. Caldas Neto, S.F., L.M. Zeoula, I.N. Prado, A.F. Branco, R. Kazama, L.J.V. Geron, E.M. Maeda and F. Fereli, 2008. Proteínadegradável no rúmennadieta de bovinos: digestibilidades total e parcial dos nutrientes e parâmetrosruminais. Revista Brasileira de Zootecnia, 37(6): 1094-1102.
20. Reynolds, C.K. and N.B. Kristensen, 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: an asynchronous symbiosis. J. Anim. Sci., 86: 293-305.
21. Firkins, J.L., A.N. Hristov, M.B. Hall and G.A. Varga, 2005. Integration of ruminal metabolism in dairy cattle. J. Dairy Sci, 89(E-Suppl. 1): 31-51.
22. Sylvester, J.T., S.K. Karnati, C.J. Newbold and J.L. Firkins, 2005. Evaluation of a real-time PCR assay quantifying the ruminal pool size and duodenal flow of protozoal nitrogen. Journal of Dairy Science, 88: 2083-2095.

23. Hristov, A.N. and J. Jouany, 2005. Factors affecting the efficiency of nitrogen utilization in the rumen. In: Pfeffer, E. and Hristov, A. N. (eds). Nitrogen and phosphorus nutrition of cattle: reducing the environmental impact of cattle operations. CABI Publishing. Wallingford, Oxfordshire, UK, pp: 117-166.
24. Walker, N.D., C.J. Newbold and R.J. Wallace, 2005. Nitrogen metabolism in the rumen. In: Pfeffer, E. and Hristov, A. N. (eds). Nitrogen and phosphorus nutrition of cattle: reducing the environmental impact of cattle operations. CABI Publishing. Wallingford, Oxfordshire, UK, pp: 71-115.
25. Koenig, K.M., C.J. Newbold, F.M. McIntosh and L.M. Rode, 2000. Effects of protozoa on bacterial nitrogen recycling in the rumen. Journal of Animal Science, 78: 2431-2445.
26. Lee, S.S., J.K. Ha and K.J. Cheng, 2000. Relative contributions of bacteria, protozoa and fungi to in vitro degradation of orchard grass cell walls and their interactions. Applied and Environmental Microbiology, 66: 3807-3813.
27. Janssen, P.H. and M. Kirs, 2008. Structure of the archaeal community of the rumen. Applied and Environmental Microbiology, 74: 3619-3625.
28. Henderson, G., F. Cox, S. Ganesh, A. Jonker, W. Young and P.H. Janssen, 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Scientific Reports, 5: 14567.
29. Pfeffer, E. and A.N. Hristov, 2005. Interactions between cattle and the environment: a general introduction. In: Pfeffer, E. and Hristov, A. N. (eds). Nitrogen and phosphorus nutrition of cattle: reducing the environmental impact of cattle operations. CABI Publishing. Wallingford, Oxfordshire UK, pp: 1-13.
30. Brito, A.F. and G.A. Broderick, 2006. Effects of different protein supplements on milk production and nutrient utilization in lactating dairy cows. Journal of Dairy Science, 90: 1816-1827.
31. Choi, C.W., S. Ahvenjärvi, A. Vanhatalo, V. Toivonen and P. Huhtanen, 2002. Quantitation of the flow of soluble non-ammonia nitrogen entering the omasal canal of dairy cows fed grass silage based diets. Animal Feed Science and Technology, 96: 203-220.
32. Reynal, S.M., I.R. Ipharraguerre, M. Lineiro, A.F. Brito, G.A. Broderick and J.H. Clark, 2007. Omasal flow of soluble proteins, peptides and free amino acids in dairy cows fed diets supplemented with proteins of varying ruminal degradabilities. Journal of Dairy Science, 90: 1887-1903.
33. Schwab, C.G., P. Huhtanen, C.W. Hunt and T. Hvelplund, 2005. Nitrogen Requirements of Cattle. In: Pfeffer, E. and Hristov, A. N. (eds). Nitrogen and phosphorus nutrition of cattle: reducing the environmental impact of cattle operations. CABI Publishing. Wallingford, Oxfordshire, UK, pp: 13-70.
34. Edmunds, B., K.H. Südekum, R. Bennett, A. Schröder, H. Spiekers and F.J. Schwarz, 2013. The amino acid composition of rumen-undegradable protein: a comparison between forages. Journal of Dairy Science, 96: 4568-4577.
35. Martineau, R., H. Lapierre, D.R. Ouellet, D. Pellerin and R. Berthiaume, 2007. Effects of the method of conservation of timothy on nitrogen metabolism in lactating dairy cows. Journal of Dairy Science, 90: 2870-2882.
36. NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press. Washington, DC, USA.
37. Huhtanen, P., M. Hetta and C. Swensson, 2011. Evaluation of canola meal as a protein supplement for dairy cows: A review and a meta-analysis. Canadian Journal of Plant Science, 91: 529-543.
38. Huuskonen, A., M. Pesonen and E. Joki-Tokola, 2014. Effects of supplementary concentrate level and separate or total mixed ration feeding on performance of growing dairy bulls. J. Agricultural and Food Science, 24: 257-265.
39. Huhtanen, P. and A.N. Hristov, 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. Journal of Dairy Science, 92: 3222-3232.
40. Rojen, B.A., S.B. Poulsen, P.K. Theil, R.A. Fenton and N.B. Kristensen, 2011. Short communication: Effects of dietary nitrogen concentration on messenger RNA expression and protein abundance of urea transporter-B and aquaporins in ruminal papillae from lactating Holstein cows. J. Dairy Sci., 94: 2587-2591.

41. Spek, J.W., J. Dijkstra, G. Van Duinkerken and A. Bannink, 2013a. A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cattle. J. Agric. Sci., 151: 407-423.
42. Wickersham, T.A., E.C. Titgemeyer, R.C. Cochran, E.E. Wickersham and D.P. Gnad, 2008a. Effect of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci., 86:3079-3088.
43. Røjen, B.A., P. Lund and N.B. Kristensen, 2012. Urea and short-chain fatty acids metabolism in Holstein cows fed alow-nitrogen grass-based diet. Animal 2, 500-513. Sands JM 2003. Mammalian urea transporters. Annual Reviews of Physiology, 65: 543-566.
44. Bannink, A., H.J. Van, J.L. Lingen, Ellis, J. France and J. Dijkstra, 2016. The contribution of mathematical modeling to understanding dynamic aspects of rumen metabolism. Front. Microbiol., 7: 1820.
45. Alio, A., C.B. Theurer, J.T. Lozano, R.S. Huber, A. Swingle, P. Delgado Elorduy, D. Duneo, De Young and J.R. Webb, 2000. Splanchnic nitrogen metabolism by growing beef steers fed diets containing sorghum grain flaked at different densities. J. Anim. Sci., 78: 1355-1363.
46. Bailey, E.A., 2010. Effects of supplemental energy and protein on forage digestion and urea kinetics in beef cattle. MS Thesis, Kansas State University, Manhattan.
47. Lobley, G.E., D.M. Bremner and G. Zuur, 2000. Effects of diet quality on urea fates in sheep as assessed by refined, non-invasive [15N15N] urea kinetics. Br. J. Nutr., 84: 459-468.
48. Marini, J.C. and M.E. Van Amburgh, 2003. Nitrogen metabolism and recycling in Holstein heifers. Journal of Animal Science, 81: 545-552.
49. Wickersham, T.A., E.C. Titgemeyer, R.C. Cochran, E.E. Wickersham and E.S. Moore, 2008b. Effect of frequency and amount of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci., 86: 3089-3099. Wilkinson, J.M. (2011). Re-defining efficiency of feed use by livestock. Animal, 5: 1014-1022.
50. Rémond, D., L. Bernard and C. Poncet, 2009. Free and peptide amino acid net flux across the rumen and the mesenteric- and portal-drained viscera of sheep. J. Anim. Sci., 78: 1960-1972.
51. Abdoun, K., F. Stumpff, I. Rabbani and H. Martens, 2010. Modulation of urea transport across sheep rumen epithelium in vitro by SCFA and CO2. Am. J. Physiol. Gastrointest. Liver Physiol., 298: 190-202.