

The Role of N Recycling in Improving Efficiency of N Utilization in Dairy Cattle

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Introduction

The growing environmental concerns from the US dairy industry has lead researchers to focus on nutrient management for more efficient utilization of available resources and reduce environmental emissions of greenhouse gases and volatile organic compounds (Place and Mitloehner, 2010). Milk nitrogen (**N**) efficiency, defined as conversion of dietary N into milk N, is typically low (20 to 35%; Chase et al., 2009) in lactating dairy cows. Most of the dietary N is lost in feces and urine and N is considered one of the major pollutants from dairy production systems (Noftsger et al., 2005). The most significant aspect of metabolism that contributes to N inefficiency is massive amount of N losses from gastrointestinal tract (**GIT**) as ammonia (**NH₃**; 46 to 47% of N available in the lumen of gut) and urinary urea excretion (30 to 70% of urea production). In addition, N losses also occur via intense metabolism of GIT where 0.25 to 0.6 of essential amino acids (**AA**) disappear from small intestines and recovered in portal vein. The potential for losses associated with these processes is significant and the magnitude of losses may increase depending on the type of diets fed or physiological stage of animals (Lapierre and Lobley, 2001). Lower N efficiency results in greater excretion of N in manure over milk resulting in reduced farm profitability and greater environmental N excretion (Noftsger et al., 2005; Hristov et al., 2011). The primary goal of ruminant nutritionists to improve N efficiency is to reduce urinary and fecal nitrogen losses and stimulate urea-N entry to rumen and provide ruminal conditions to enhance microbial uptake of recycled urea N. Better understanding of urea N recycling is important for improving N efficiency and reduce environmental impact of N emissions from dairy production systems (Recktenwald et al., 2014).

Urea is considered major end product of **NH₃** and **AA** metabolism in ruminants and it plays important role in N economy for ruminants (Marini and Van Amburgh, 2003). The ability to recycle substantial amounts of urea into the GIT is a physiological mechanism in ruminants for the conservation of N (Lapierre and Lobley, 2001). It is estimated that 40 to 80% of urea synthesized in liver is recycled to different sections of the GIT; however, for recycled urea to contribute to microbial protein synthesis and absorbable microbial protein, urea must be recycled and captured in rumen. Typical values for the partition of urea between urine and GIT are 60:40 to 20:80, depending upon type of diet and level of feed and protein intake (Harmayer and Martens, 1980; Lapierre and Lobley, 2001).

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Urea Synthesis and Transporters

Urea synthesis via ornithine-urea cycle (**Figure 1**) in liver is the only pathway for NH_3 detoxification; however, Oba et al. (2004) showed urea synthesis in rumen epithelium and duodenal mucosal cells. The water-soluble property of urea makes it 10 times less toxic than NH_3 . Ammonia produced in the GIT is transported to liver through portal vein and is converted to urea. The fractional extraction rate of 0.75 to 0.85 suggests that liver is very effective at removing NH_3 (Lobley and Milano, 1997).

We are still lacking mechanisms regulating urea partition to the GIT in ruminants. Recently, the presence of urea transporters in rumen tissues was confirmed suggesting that the process of urea recycling might be regulated and it may involve humoral factors (e.g. hormones, substrate concentration). This suggests that in addition to events occurring within the rumen environment, animal factors may have some control over urea recycling. Urea is transported across ruminal epithelium primarily via urea transporters (**UT**) located on the luminal and basolateral membrane of the epithelium (Lu et al., 2014; Patra and Aschenbach, 2018). In addition, aquaporins, family of membrane spanning proteins involved with movement of water, are also permeable to urea and helps with transporting urea across ruminal epithelium (Walpole et al., 2015). The expression of UT is dependent upon dietary protein and short-chain fatty acid (**SCFA**) concentration. While ruminal NH_3 concentration is a negative regulator of UT-B mRNA and protein expression, SCFA upregulate UT-B expression at low pH (Lu et al., 2015). Both SCFA and NH_3 concentration regulates ruminal influx of urea such that urea influx is adjusted to the consumption capacity of NH_3 and activity of rumen microbiome. Urea influx as proportion of total hepatic urea output ranges from 29 to 99% and N transfer across ruminant GIT can be higher than N intake (Patra and Aschenbach, 2018).

Fates of Urea Entering GIT

Urea has potential to enter all compartment of GIT through GIT secretions and diffusion from blood because of its water soluble property (Kennedy and Milligan, 1980). Ruminants enhances transfer of urea to rumen via rumen wall and saliva so that urea-N can be captured by rumen microbes for synthesis of microbial protein. However, urea transfer to rumen is variable and depend upon ruminal environment resulting from the diets fed to animals. Nevertheless, urea that diffuses across the rumen wall is converted to NH_3 and carbon dioxide (**CO_2**) in presence of urease activity from bacteria in vicinity of rumen wall. Ammonia is either utilized the synthesis of microbial protein (~50%), reabsorbed as ammonia (~40%) or the remaining gets excreted in the feces (~10%; **Figure 2**). The NH_3 reabsorbed from GIT is removed by liver and again converted to urea. The amino acids of microbial origin and synthesized from recycled urea-N can also be reabsorbed and catabolized in liver to yield urea (Sarraseca et al., 1998). The fate of recycled urea-N depends upon dietary factors such as protein content, diet fermentability, forage-to-concentrate ratio etc. and ruminal condition including rumen pH, $\text{NH}_3/\text{NH}_4^+$, fermentation energy etc.

Factors Affecting Urea Recycling

Dietary Protein Content, Intake, and Solubility

Urea N recycling and microbial uptake of recycled urea is affected by dietary and ruminal factors with intake of crude protein (**CP**) and digestible organic matter being the major ones (Harmeyer and Martens, 1980; Kennedy and Milligan, 1980). Lapierre and Lobley (2001) reported correlation (R^2) of 0.78 between N intake and hepatic urea production in cattle at low N intake and close of zero N balance; however, the correlation was reduced to 0.45 in cattle with high N intake. The correlation between portal drained viscera NH_3 absorption and hepatic urea production was 0.84 across all cattle (Lapierre and Lobley, 2001). Based on these observations, it can be assumed that in animals with low N requirements, more consistent proportion of N intake is directed towards urea synthesis; however, in high producing animals, N is absorbed as AA and utilized for anabolism rather than deaminated to NH_3 . However, the proportion of urea-N entering the GIT (60 to 70%) and the proportion used for anabolic purposes (45 to 50%) is not affected in sheep fed high levels of feed intake with improved diet quality (Sarraseca et al., 1998; Lobley et al., 2000). In ruminants fed low quality hay or low protein diet, urea recycling to the GIT is 80 to 90% of urea entry rate and greater proportion of absorbed N is retained (Marini and Van Amburgh, 2003).

Inverse relationship was observed between dietary protein intake and urea-N entry into the rumen (Kennedy and Milligan, 1980) suggesting that low protein intake combined with low quality roughage diet will result in decreased hepatic urea synthesis, decreased urinary urea N excretion and lower transfer of urea to post stomach tissues (Marini and Van Amburgh, 2003). Archibeque et al. (2001) determined the effects of two forage species differing in N levels on urea kinetics and N metabolism and observed greater N efficiency at low N intakes. Recently, Batista et al. (2017) conducted meta-analysis compiling 25 studies with ruminants (beef, dairy cows, sheep) for evaluating urea kinetics and microbial uptake of recycled urea N. Based on this meta-analysis, hepatic synthesis of urea-N and gut entry rate (GER; N recycled to gut) linearly increased with increase in N intake; however, the ratio between GER and hepatic synthesis of urea-N decreased with increasing dietary CP concentration.

The major effects of changing CP and rumen degradable protein (**RDP**) on ruminal fermentation is observed on ruminal NH_3 -N concentration. However, we are still lacking enough studies investigating the impacts of altering RDP concentration on urea-N transfer in rumen. Wickersham et al. (2008) observed greater urea-N transfer to the GIT with increasing dietary RDP; however, RDP had no impact on urea-N transfer to GIT was observed when it was expressed as proportion of endogenous urea-N production. We are still lacking studies showing T interactive effects of dietary CP and RDP concentrations on urea-N transfer and microbial protein production in high producing dairy cows with high requirement for metabolizable protein. Chibisa and Mutsvangwa (2013) observed lower ruminal NH_3 -N as RDP was reduced; however, increases in urea entry rate was observed with lower CP and RDP diets resulting in

maintenance of microbial protein supply as observed by utilization for anabolic purposes.

Diet Fermentability

Increasing the fermentable carbohydrate fraction of the diet has potential to increase urea recycling to the rumen (Huntington, 1989) by decreasing urea transfer to post-gastric tissues. Improving fermentability of diets by adding grains, starch, and sucrose as energy source increase ruminal urea degradation probably due to lower NH_3 concentration or greater availability of energy due to greater rate of fermentation of dietary organic matter (Kennedy and Milligan, 1980). Previous studies have shown the potential of greater N utilization when starch is substituted with sugars and these effects may be attributed to increased urea recycling as greater carbohydrate digestion in rumen increase urea entry to rumen from blood and microbial protein synthesis (Huntington, 1989; Theurer et al., 2002). Seal and Parker (1996) observed greater propionate production along with lower ruminal NH_3 and plasma urea concentration and greater urea recycling to the GIT with intraruminal infusion of sucrose. Greater transfer of urea into the rumen with sugars may be attributed to lower ruminal NH_3 concentration. Lu et al. (2014) observed dose-dependent inhibitory effect of ammonia on urea transfer across rumen epithelia. Replacing starch with sugars may increase ruminal ATP supply (Russell et al., 1992) and lower rumen NH_3 concentration (Broderick et al., 2008) creating favorable conditions for urea entry into the rumen. Broderick and Radloff (2004) observed linear decrease in urinary excretion of urea with increasing sugar content of the diet with molasses in lactating dairy cows. Similarly, urinary excretion of urea-N was linearly reduced as sugars replaced corn starch in the diets (Broderick et al., 2008). These findings suggest repartitioning of urea toward recycling in GIT; however, we are still lacking direct evidence of greater urea recycling to GIT with sugars in the diets of lactating dairy cows. Recently, De Seram et al. (2019) observed effects of replacing barley starch with lactose and observed linear decrease in ruminal NH_3 -N concentration; however, no changes were observed on anabolic utilization of recycled urea-N, or urea-N recycled to GIT as total sugar content increased in the diets of lactating dairy cows.

Nitrogen Gradient Across the Rumen Wall

Ruminal NH_3 -N concentration regulate urea-N secretion into the rumen by permeability of ruminal epithelium thereby generating N gradient across rumen wall (Marini et al., 2005). Egan et al. (1986) proposed lower permeability of ruminal epithelium for urea-N with increased NH_3 -N concentration (**Figure 3**). Greater NH_3 -N concentration may limit ruminal entry of urea-N by inhibiting bacterial urease activity (Remond et al., 1996). Ammonia occurs in two forms as NH_3 or NH_4^+ ; however, NH_4^+ is the predominant form at ruminal pH of 6.4. Lu et al. (2014) speculated that luminal NH_4^+ enters the ruminal epithelium via a cation channel driven by potential difference of the apical membrane. A potential difference driven NH_4^+ transport into the cell alters urea transport in a dose dependent manner. Lu et al. (2014) demonstrated inhibitory effects

of NH_3 on urea transfer across ruminal epithelia using chamber and observed that inhibitory effects of NH_3 are concentration dependent with saturation at 5 mM/L.

Feed Processing

Feed processing can affect N efficiency, and transfer of urea-N either by modifying fermentability of diet or ruminal N status. Feed processing may synchronize ruminal starch and N supply thereby reducing N absorption and increasing N retention (Huntington, 1997). Theurer et al. (2002) observed greater urea-N recycling to the portal drained viscera along with lower urinary urea-N in growing beef steers fed steam-flaked sorghum compared to dry rolled sorghum. The effects on urea-N recycling might be attributed to greater starch and CP digestibility with steam-flaking compared to steam-rolling sorghum. Similarly, feeding steam-flaked corn compared to steam-rolled corn increased urea recycling to portal drained viscera by 140% (Delgado-Elorduy et al., 2002) while feeding dry rolled barley compared to pelleted barley to lactating dairy cows increased urea-N entry to GIT by 35% (Gozho et al., 2008). While various studies have shown greater urea-N recycling, no effects were observed on microbial yield suggesting inefficient microbial uptake of recycled urea-N (Gozho et al., 2008; Marini and Van Amburgh, 2003). Hence, such changes in feed characteristics should coincide with greater microbial N utilization.

Conclusions

The ability to recycle urea is a physiological mechanism in ruminants to ensure high rates of microbial protein synthesis, enhance N efficiency, and to supply high quality protein including meat, milk, and wool. Better understanding of urea N recycling is important for improving N efficiency; however, mechanisms regulating urea recycling and factors affecting urea partitioning in different sections of GIT still remains limited. Specifically, we are lacking information on ideal ruminal conditions that favor urea transfer and uptake by rumen microbes. With better understanding of the ruminal conditions and mechanisms underlying urea partition to the GIT, future nutrient requirement models will be more capable of predicting for maximum N efficiency.

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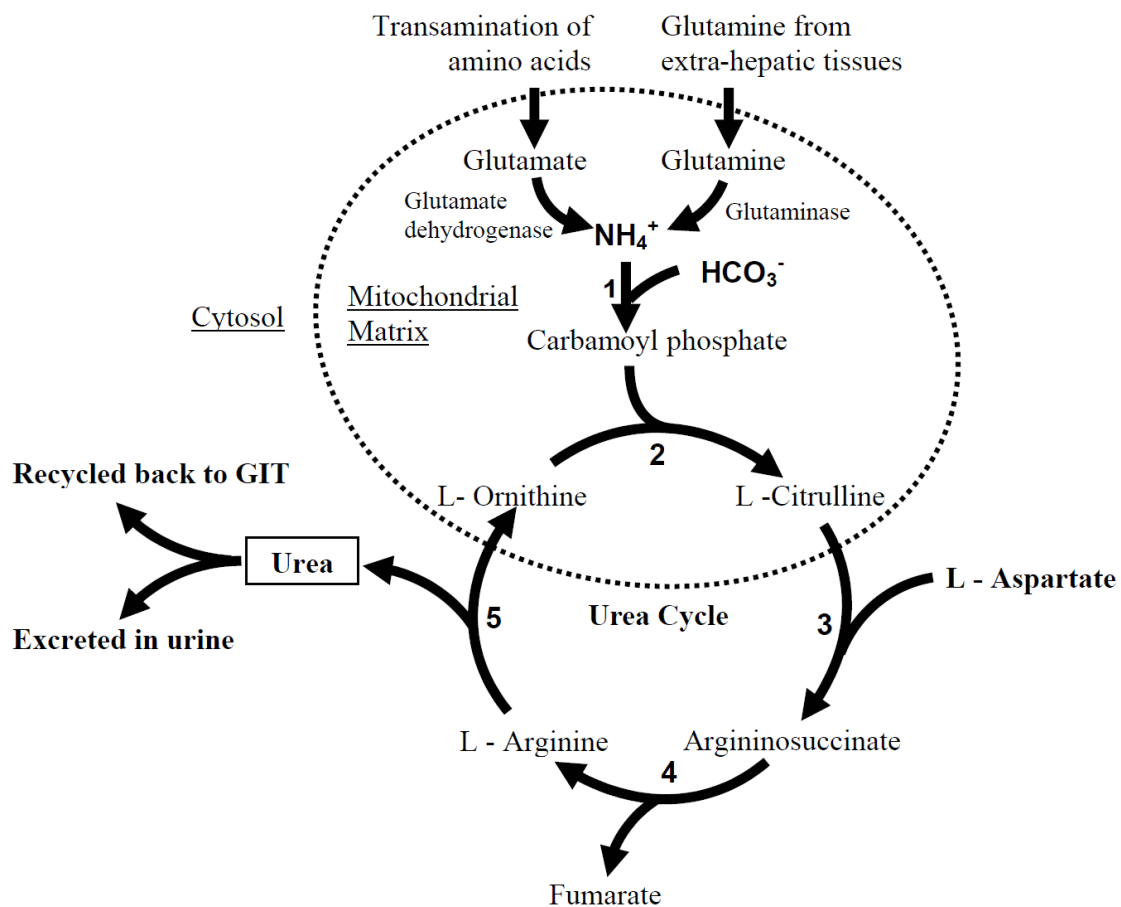


Figure 1. Reactions and Intermediates of urea biosynthesis. Mitochondrial NH_4^+ and cytosolic aspartate provide the two N atoms for urea synthesis. Five enzymes are involved in urea cycle. 1. Carbamoyl phosphate synthase I, 2. Ornithine transcarbamoylase, 3. Argininosuccinate synthase, 4. Argininosuccinate lyase, 5. Arginase. (Adapted from Sunny, 2004).

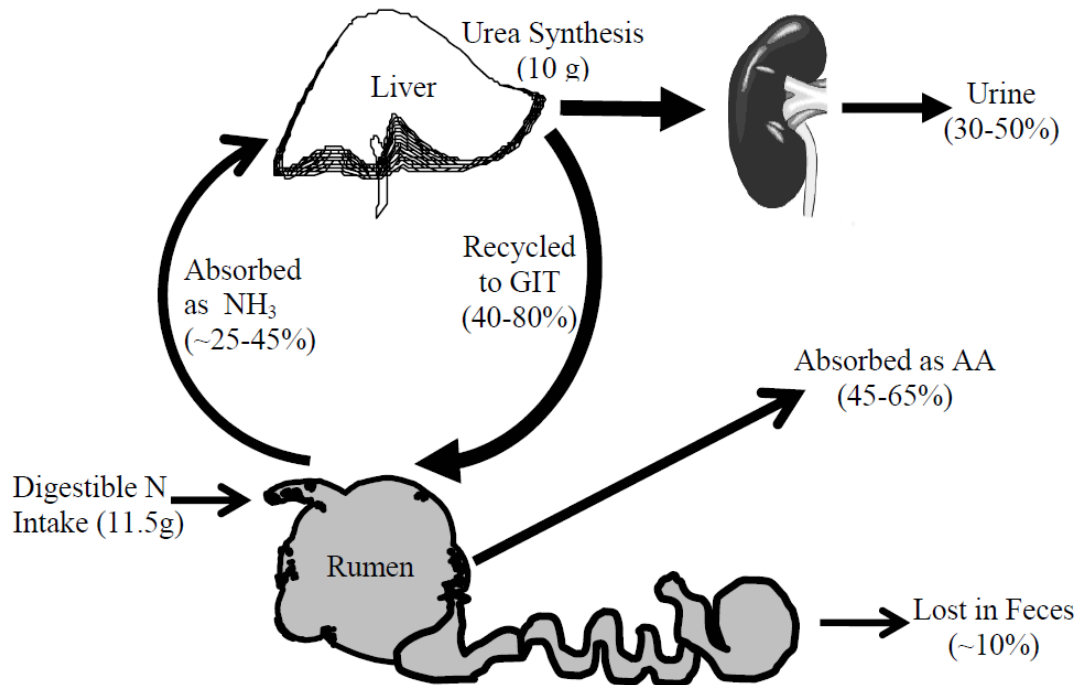
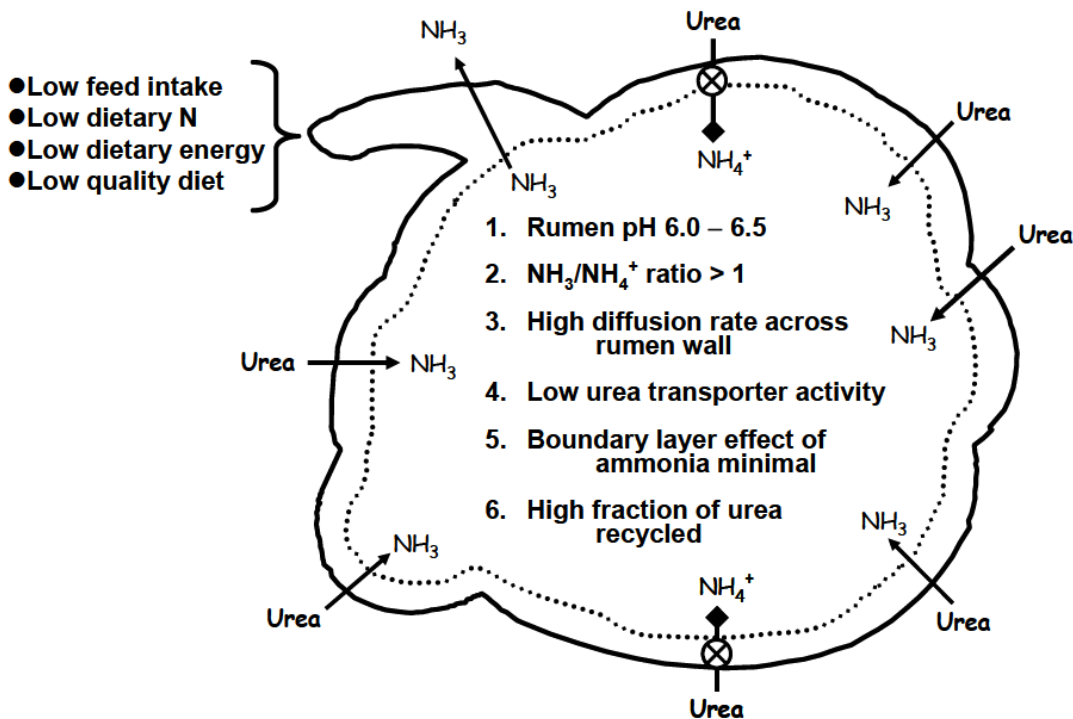


Figure 2. Fates of urea-N (g/d) synthesized and recycled in sheep fed pelleted barley and grass hay diet. Of the total urea synthesized in the liver (10g), 30-50 % is excreted in the urine and 40-80 % is recycled back to the GIT. Of this portion recycled back to the GIT, 25-45 % is reabsorbed to liver as ammonia where it is reutilized for the synthesis of urea, 45-65 % is absorbed as amino acids which is utilized for productive purposes and around 10 % is excreted in feces (Adapted from Lobley et al., 2000).

A. Conditions in the rumen favoring a high rate of urea recycling



B. Conditions in the rumen causing a reduced rate of urea recycling

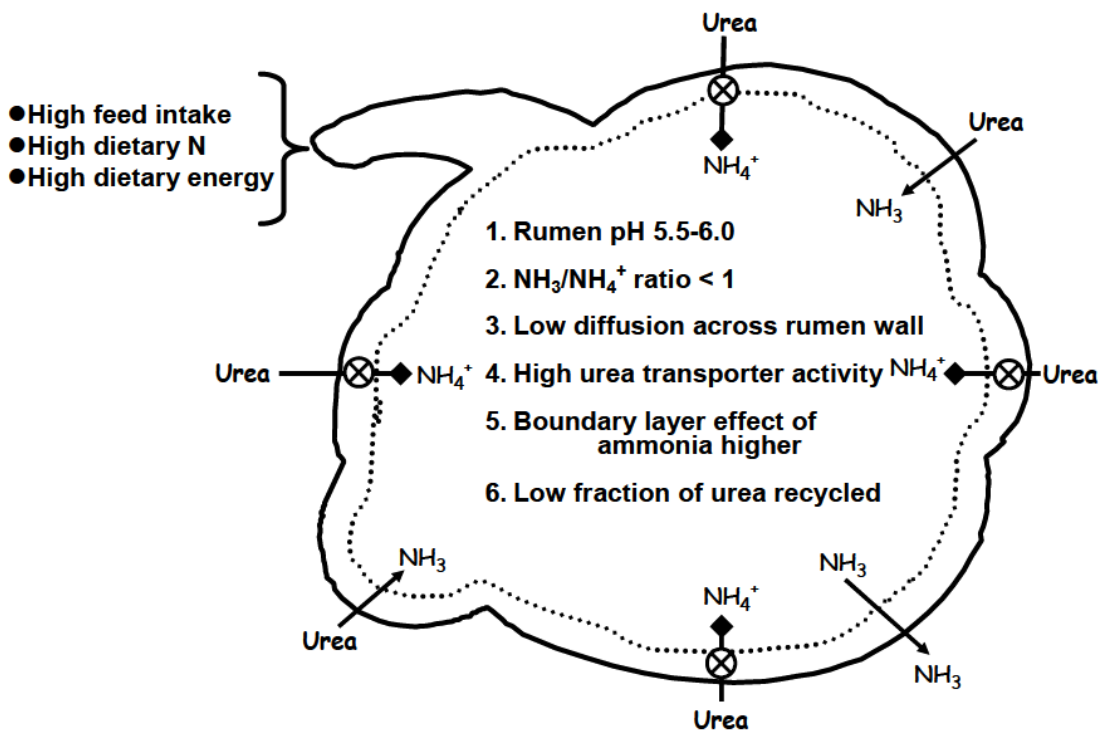


Figure 3. Ruminal conditions to promote or reduce urea recycling in response to diet and composition (Adapted from Bequette and Sunny, 2005).

SESSION NOTES