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Quantitative Comparison of Diversity and Conformity in Nitrogen Recycling of Ruminants*

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ABSTRACT : Domestic ruminant animals are reared in diverse production systems, ranging from extensive systems under semi-arid and tropical conditions with poor feed resources to intensive systems in temperate and cold areas with high quality feed. Nitrogen (N) recycling between the body and gut of ruminants plays a key role in the adaptation to such diverse nutritional conditions. Ammonia and microbial protein produced in the gut and urea synthesized in the liver are major players in N-recycling transactions. In this review, we focus on the physiological factors affecting urea production and recycling. Sheep and buffalo probably have higher abilities to reabsorb urea from the kidney compared with cattle. This affects the degree of urea-N recycling between the body and gut at both low and high N intakes. The synthesis and gut entry of urea also differs between cattle bred for either dairy or beef production. Lactating dairy cows show a higher gut entry of urea compared with growing cattle. The synthesis and recycling of urea dramatically increases after weaning, so that the functional development of the rumen exerts an essential role in N transactions. Furthermore, high ambient temperature increases urea production but reduces urea gut entry. An increase in total urea flux, caused by the return to the ornithine cycle from the gut entry, is considered to serve as a labile N pool in the whole body to permit metabolic plasticity under a variety of physiological, environmental and nutritional conditions. (**Key Words :** Urea, Liver, Gastrointestinal Tract, Nitrogen)

INTRODUCTION

In ruminants, microbial protein synthesized in the rumen, dietary protein that escapes ruminal degradation and endogenous protein all enter the small intestine. These nitrogen (N) sources are digested and absorbed mainly as amino acids (AA). Beside AA absorption from the small intestine, a substantial amount of ammonia is absorbed across the whole digestive tract. Endogenous N sources include urea via the blood and saliva, sloughed epithelial cells and secreted proteins that enter the lumen of the gastrointestinal tract. Some of these N sources are re-absorbed as AA or ammonia. Thus, in some case, the sum of AA-N and ammonia-N recovered from the hepatic portal vein could be greater than the intake of apparent digestible N. In the liver, most absorbed ammonia and considerable part of AA-N are converted to urea-N that is released to the blood circulation, and then excreted into urine via the kidney or re-enters the digestive tract via saliva or directly across the gut wall. These various fates of urea-N in ruminants are collectively termed as "urea recycling" or "urea nitrogen salvage" (Stewart and Smith, 2005).

Since McDonald (1948) and Houpt (1959) demonstrate the pathway of urea recycling in ruminants, considerable research describing and quantifying the site and rates of urea metabolism has been reported (see reviews of Kennedy and Milligan, 1980; Egan et al., 1986; Huntington, 1986; Obara et al., 1991). The main conclusion is that urea production, excretion and entry to the gut are linked to diet composition, intake and the productive priorities of animal. From the practical aspects of urea metabolism, there are three important priorities: maximizing microbial function in the rumen; optimizing amino acid supply to the host ruminant; and minimizing negative environmental effects of cycling N through ruminant production systems (Huntington and Archibeque, 1999).

In order to maximize ruminal microbial production, both the ratio of available protein to energy sources for rumen microbes and synchronizing energy and protein

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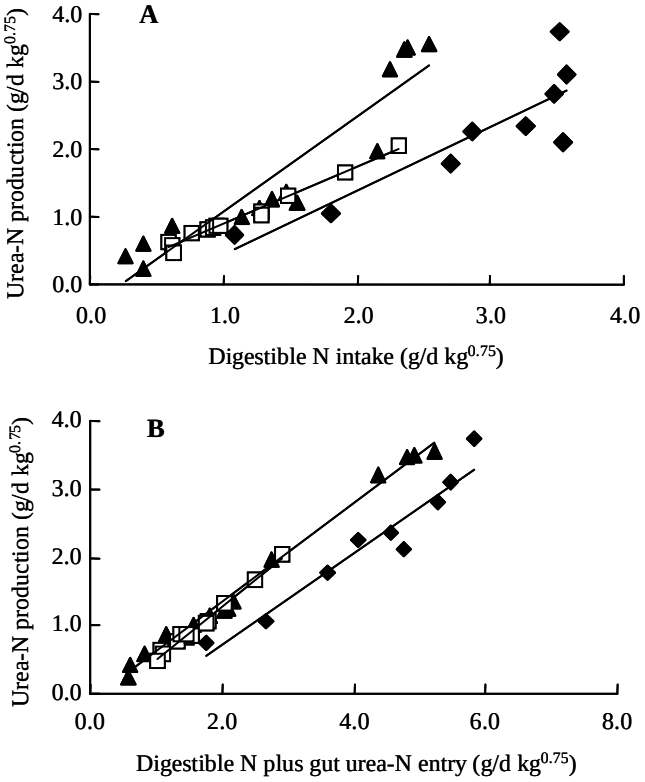

Figure 1. Daily urea-N production relative to (A) digestible N intake or (B) sum of digestible N plus gut urea-N entry for sheep (triangles, n = 14), growing cattle (squares, n = 13) and dairy cows (diamonds, n = 9). Data from: for sheep: Lobley et al., 2000; Marini et al., 2004; Kiran and Mutsvangwa, 2007; Sunny et al., 2007; Tameoka et al., unpublished; for growing cattle: Archibeque et al., 2001, 2002; Marini and Van Amburgh 2003; for dairy cows: Ruiz et al., 2002; Gozho et al., 2008; Obitsu et al., unpublished. Regression equations of (A) urea-N production (y) to digestible N intake (x): for sheep: y = 1.41x-0.32, r^2 = 0.87; for growing cattle: y = 0.84x+0.05, r^2 = 0.98; for dairy cows: y = 0.94x-0.49, r^2 = 0.78. Regression equations of (B) urea-N production (y) to digestible N plus gut urea entry (x): for sheep: y = 0.73x-0.11, r^2 = 0.99; for growing cattle: y = 0.79x-0.30, r^2 = 0.98; for dairy cows: y = 0.67x-0.63, r^2 = 0.93.

supply in the rumen are important (Bach et al., 2005). Because ingestion of forage diets containing high non-protein N but less fermentable carbohydrate causes an imbalance of N and energy for ruminal microbes, a large proportion of dietary N is absorbed as ammonia and converted to urea in the liver. When ruminants are fed forage diets supplemented with readily fermentable carbohydrate sources, urea-N produced by the liver shifts from urinary excretion to gut entry (Obara et al., 1991). Plant cereals and roots are major sources of such readily fermentable carbohydrate but, at present, their use for animal production competes with needs as human foods and biofuel sources. The recent dramatic rise in prices of these feedstuffs causes serious problems for many farmers, especially in the countries depending on imported feedstuffs. In another global context, interests in the impacts of N emissions from manure on the environment have also increased. Thus, improvement of the efficiency of N use by ruminants fed forage diets by nutritional management with alternative carbohydrate sources or feeding strategies is an important subject for the animal scientist. Understanding the regulating factors of urea recycling may provide a key to resolve the problem.

Recently, new techniques based on both veno-arterial (VA) measurements across the splanchnic tissues and isotopomer analysis by the infusion of stable isotopes contributed to provide further quantitative information on the fate of urea-N (Reynolds and Kristensen, 2008). However, the exact mechanisms regulating urea transfer to the gut lumen are still unclear.

The rate of urea production and recycling may depend not only on dietary factors but also on physiological conditions and/or productive states of animals (Brun-Bellu, 1996; Lapierre and Lobley, 2001). The comparison of urea kinetics among various animal species, productive states and environmental conditions may provide novel information on the regulatory mechanisms of urea metabolism. In light of these considerations, our objective was to review and compare the flux of urea-N as well as ammonia-N, AA-N and total N in various ruminant species under various physiological conditions.

DIFFERENCE IN ANIMALS

Animal species

Ruminant animals have evolved diversely and with the flexibility to adapt to various plant feedstuffs. Hofmann (1989) classified ruminants into three categories, concentrate selector, intermediate and grass/roughage eaters, according to their feeding behavior and anatomical characteristics of the digestive tract. The difference in anatomical characteristics and feeding behavior may affect the inflow of urea-N into the digestive tract. Cattle and sheep are grass/roughage eaters and have a larger rumen and smaller hindgut compared with animals in concentrate selector and intermediate groups. Furthermore, compared with sheep, cattle have a relatively greater omasum and a smaller large intestine (McLeod and Baldwin, 2000; Hata et al., 2005).

Lapierre and Lobley (2001) summarized available published data quantifying trans-hepatic VA differences and concluded that urea production rate is positively correlated with digestible N intake. The relative hepatic urea-N production to apparent digestible N in the total tract ranged from 49 to 178% (mean 93%), 43 to 123% (mean 88%) and 49 to 230% (mean 161%) for growing steers, dairy cows and sheep, respectively. In the same data summary, the

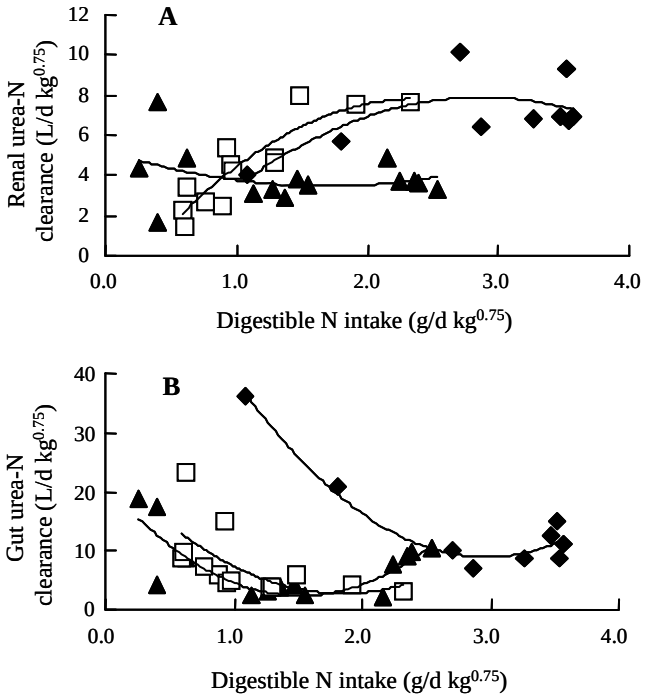

Figure 2. Plasma urea-N clearance rate in (A) the kidney or (B) gastrointestinal tract versus digestible N intake for sheep (triangles, n = 14), growing cattle (squares, n = 13) and dairy cows (diamonds, n = 9). Data were from the dataset as detailed in Figure 1. Regression equations of (A) renal clearance rate(y) to digestible N(x): for sheep: $y = 0.62x^2-2.11x+5.25$, $r^2 = 0.10$; for growing cattle: $y = -1.80x^2+8.53x-2.31$, $r^2 = 0.81$; for dairy cows: $y = -1.27x^2+7.32x-2.62$, $r^2 = 0.50$. Regression equations of (B) gut clearance rate (y) to digestible N (x): for sheep: $y = 8.27x^2-25.1x+21.5$, $r^2 = 0.66$; for growing cattle: $y = 6.46x^2-23.80x+24.7$, $r^2 = 0.39$; for dairy cows: $y = 7.90x^2-46.9x+78.4$, $r^2 = 0.95$.

proportion of urea-N removed by the PDV to the apparently digestible N, representing the fraction of urea-N entering the gut through the gut wall averaged 34, 38, and 77% for growing steers, dairy cows and sheep, respectively. These figures indicate that sheep produce and recycle more urea-N than cattle.

From data based on isotopomer analysis with [$^{15}N_2$]urea as a tracer, sheep also have a relatively higher rate of production and gut entry of urea-N (Figure 1A). The mean regression coefficient between urea-N production and digestible N intake for sheep (1.41) is greater than those for growing cattle (0.84) and dairy cows (0.94). However, the regression between urea-N production and the sum of digestible N and the gut entry of urea-N gives similar slopes among both species with a lower intercept for dairy cows (Figure 1B). This implies that greater absorption of ammonia-N derived from the greater gut entry of urea-N possibly contributes to the higher urea production in sheep.

Based on plasma concentration, urinary excretion and gut entry of urea-N from the same data summary as Figure 1, clearances of plasma urea-N by the kidney and gut were calculated (Figure 2A and B). Compared with cattle, the renal clearance of plasma urea-N of sheep remains lower when digestible N increases (Figure 2A), whereas the change in the gut clearance of plasma urea-N in sheep is similar to that for growing cattle (Figure 2B). These differences indicate a higher ability for the renal re-absorption of urea-N in sheep at greater N load compared with cattle. The higher re-absorption in the kidney and greater concentration of plasma urea-N (Thornton, 1970) may lead to more gut entry of urea-N with subsequent higher absorption of ammonia and re-entry of urea-derived N to the ornithine cycle in sheep. Based on the data summary mentioned above, the averaged proportions of the re-entry of the urea-derived N into the ornithine cycle to gut urea entry were 32, 49 and 52% for growing cattle, dairy cows and sheep, respectively.

Facilitative urea transporters, UT-A and UT-B, involving urea movement across the plasma membranes have been detected in ruminants. UT-B was expressed in the rumen, duodenum, ileum, cecum, liver and kidney and UT-A was only present in the kidney, liver and duodenum in sheep (Marini et al., 2004). The observation that an increase in protein intake did not affect UT-A and UT-B expression in sheep kidney (Marini et al., 2004) supports the observations of a constant renal urea-N clearance at high protein intake observed in the ovine. Higher expression of rumen UT-B at low protein intake was observed in growing cattle but not in sheep (Marini and Van Amburgh, 2003; Marini et al., 2004), even though similar responses of the gut clearance of plasma urea-N relative to protein intake were detected. These discrepancies suggest that there are different mechanisms regulating urea transfer across the gut and kidney between sheep and cattle.

Water buffalo is a multi-purpose animal that provides labor, meat and milk in the Asia-Pacific region (Wanapat, 1989). Although the ability to utilize low quality diets appears higher for buffalo than cattle (Wanapat, 1989), this probably depends, in part, on the type of diet (Kennedy et al., 1992). In an early study comparing urea metabolism between buffalo and cattle, Norton et al. (1979) reported that the plasma concentration, production and gut entry of urea-N in buffalo were greater than those in cross-breeds of *Bos indicus* and *Bos taurus* fed the same diet. In that study, the greater entry of urea-N into the post-ruminal tract accounted for the difference in the total gut entry between buffalo and cattle. Other found that buffalo compared with cattle had higher urea production (Kennedy et al., 1992) and greater post-ruminal entry of urea (Dhiman and Arora, 1987; Abudullah et al., 1992), as well as higher ammonia concentration in the rumen (Abudullah et al., 1992) and less urinary urea excretion (Norton et al., 1979; Dhiman and Arora, 1987).

These lower urinary urea-N excretion and higher plasma

urea-N concentration in buffalo seems to be associated with higher ability for renal re-absorption (Norton et al., 1979; Chen et al., 1996). The higher post-ruminal entry of urea-N may enhance ammonia absorption and re-entry to the ornithine cycle of urea-N that previously entered the gut. This recycling would increase total urea production. In addition, different endocrine responses may provide further reasons for the differences in urea-N production between buffalo and cattle (Ban-Tokuda et al., 2007).

The lower entry of urea-N into the rumen of buffalo may be associated with higher ruminal ammonia concentration caused by a longer retention time of feed particles (Bartocci et al., 1997; Terramoccia et al., 2000), higher degradation of feed protein (Terramoccia et al., 2000) and higher microbial activity (Homma, 1986; Puppo et al., 2002) in the rumen. Higher ruminal ammonia concentration reduces ruminal entry of urea-N (Kennedy and Milligan, 1980; Remonds et al., 1993).

Breeds of cattle

Bos indicus and associated cross-breeds have higher urea production and recycling than *Bos taurus*. Norton et al. (1979) reported that cross-breeds from Brahman cattle produced 30% more urea-N and transferred 60% more urea-N into the gut compared with the Shorthorn breed. Higher renal re-absorption of urea-N seemed to account for this higher gut entry in Brahman cattle (Norton et al., 1979). *Bos indicus* crossbred cattle are often utilized in beef production in semi-arid environments due to their ability to adapt to high environmental temperature and low quality feed. In grazing studies in semi-desert rangeland, Brahman cows maintained higher body condition scores and had greater serum concentrations of NEFA and urea-N in early lactation than *Bos taurus* cows (Obeidat et al., 2002). The authors suggested that different mechanisms exist between these breeds for tissue mobilization as energy sources for maintenance and production.

Different breeds of cattle for dairy and beef production also show quantitative variation in urea metabolism. During the growing and fattening stages, Japanese Black and Japanese Brown cattle had greater plasma concentration of urea-N than Holstein cattle under a similar feeding condition (Matsuzaki et al., 1997). In early-weaned calves of different breeds reared at the same body weight gain, Japanese Black calves have higher plasma urea concentration and greater rate of urea production and recycling compared with Holstein calves (Shingu et al., 2007). Although the reasons for these differences between Japanese Black and Holstein calves are not clear, differences in body composition and endocrine status (Matsuzaki et al., 1997) probably affect the variation of body protein turnover in these cattle during growth.

PHYSIOLOGICAL AND PRODUCTIVE STATUS

Rumen development

The rumen of the neonatal ruminant is immature. The consumption of solid feed facilitates the morphological and functional development of the rumen, especially after weaning when dry mater intake dramatically increases. Development of the rumen increases ruminal fermentation and subsequent absorption of the end products including short chain fatty acids and ammonia. After weaning, the major origin of absorbable AA in the small intestine shifts from milk protein to microbial protein. Thus, a quantitative change of urea metabolism during weaning period is expected in young ruminants. Analysis of N utilization in milk-fed calves and weaned growing heifers (Zanton and Heinrichs, 2008) indicates that milk-fed calves excrete relatively more N in urine, whereas weaned heifers excrete relatively more N in feces. The reduced urinary excretion in weaned heifers is consistent with increased contribution of urea recycling to the overall N economy in the body after weaning.

In the early weaning system used for the intensive dairy faming, calves are generally weaned around 6 weeks of age when calves are able to ingest more than 1 kg/d of solid diets. Shingu et al. (2007) reported that weaned calves fed concentrate and hay at 13 weeks of age had a relatively greater rate of urea-N production and recycling compared with calves fed only milk at 4 weeks, although the planes of nutrition at both ages were identical. This increase in urea production after weaning is probably associated with elevated ammonia production in the rumen (Khan et al., 2008) and enhanced expression of hepatic enzymes involving the urea cycle (Takagi et al., 2008).

In a study using isotopomer analysis with [$^{15}N_2$]urea (Obitsu et al., 2003), weaned calves had higher rates of production and gut entry of urea-N, as well as higher rates of anabolic use and recycling of urea-N that entered the gut compared with pre-weaned calves. However, the proportion of the anabolic use of urea-N to the gut entry rate did not differ between pre- and post-weaning calves. These results indicate that the rate of the anabolic use of endogenous urea-N through the microbial conversion in calves depends on the rate of urea-N entry to the gut as affected by rumen development.

Increasing ammonia absorption from the gut and subsequent hepatic ammonia removal might decrease amino acid availability for non-splanchnic tissue utilization. Originally, it was proposed that the liver requires an equal N input from both amino acids and ammonia for net urea production (Reynolds, 1992; Parker et al., 1995), although later studies with sheep and cattle (Milano et al., 2000; Maltby et al., 2005) did not support this hypothesis.

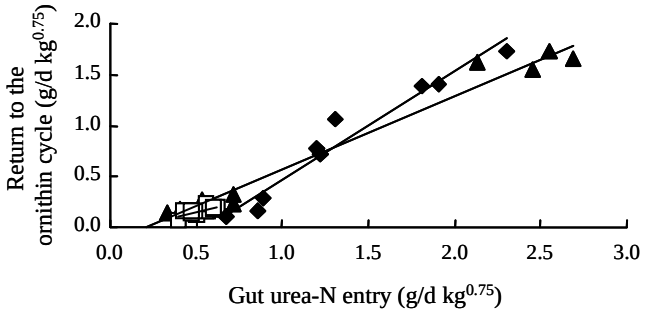

Figure 3. The rate of return to the ornithine cycle of urea-N that entered the gut (y) versus the gut entry rate of urea-N (x) for sheep (triangles, n = 14), growing cattle (squares, n = 13) and dairy cows (diamonds, n = 9). Data were from the dataset as detailed in Figure 1. Regression equations: for sheep: y = 0.72x-0.15, r^2 = 0.98; for growing cattle: y = 0.39x-0.03, r^2 = 0.52; for dairy cows: y = 1.06x-0.58, r^2 = 0.95.

Nonetheless, the higher demand for amino acids to support growth in young calves may influence the balance of hepatic removal of ammonia and amino acid. We observed that the rate of conversion from the guanido-N of plasma arginine to plasma urea-N increased with raised urea production in weaned calves compared with pre-weaned, milk-fed calves (unpublished data).

Pregnancy and lactation

Variations in N balance during pregnancy and lactation in ruminants have been reported. Urinary N excretion was decreased and N retention was increased during pregnancy in goats (Brun-Bellut, 1996). After parturition, N intake and milk N output were increased but urinary N was reduced (Brun-Bellut, 1996). In late lactation, N retention was increased at the same time that milk N decreased in goats (Brun-Bellut, 1996) and dairy cows (Knowlton et al., 2001). These changes in N utilization during pregnancy and lactation reflect altered urea metabolism. In the pregnant ewe, urea production by the maternal liver was only reduced at the end of pregnancy when feed intake was reduced (Freetly and Ferrell, 1998). This reduction of maternal urea production may be counter-acted by an increase of fetal urea synthesis which is estimated as 12% of the maternal synthesis rate in late pregnancy (Faichney and White, 1987). Freety and Ferrell (1998) also reported that neither the stage of pregnancy nor the fetal number affected net urea-N entry to the portal drained viscera (PDV) of ewes. In goats, however, the gut entry of urea-N increased as pregnancy progressed (Brun-Bellut, 1996).

Based on the data summary of $^{15}N_2$ tracer studies, the different intercepts of regression lines between digestible N plus gut entry and urea-N production for dairy cows and growing cattle (Figure 1B) indicates that lactating cows produce relatively less urea-N in the liver compared with growing cattle. Inversely, the rate of both gut urea-N entry and return to the ornithine cycle of urea-N that enter the gut in lactating cows are relatively greater than those in growing cattle (Figure 3). However, there seems to be large variations dependent on the dietary factors as well as physiological factors including stage of lactation, parity and body condition.

After parturition, hepatic urea production and gut urea entry increase with increased nutrient intake and milk production. Reynolds et al. (2003) reported the changes in net splachnic flux of nutrients from late pregnancy to early lactation in dairy cows. In that study, the net PDV absorption of ammonia-N and net PDV removal of urea-N increased gradually until 83 days after parturition, whereas hepatic urea production increased rapidly after parturition. This dramatic increase in hepatic urea production is probably a consequence of increased mobilization of body protein and catabolism of amino acid besides an increase in N intake.

Multiparous cows are generally larger in body weight and mobilize more body tissues for greater milk production during early stage of lactation compared with primiparous cows. Flis and Wattiaux (2005) reported that multiparous cows efficiently retained the increased digestible N from undegradable feed protein, whereas primiparous cows excreted more urinary N with excess supply of undegradable feed protein. The authors reasoned that the factors contributing to this difference in N utilization with parity might include a greater need to restore body protein reserves mobilized in early lactation and/or a higher urea-N pool in multiparous cows compared with primiparous cows.

ENVIRONMENTAL FACTORS: THERMAL STRESS

High ambient temperature reduces feed intake and milk production in lactating dairy cows (West, 2003). Urinary N excretion is also increased and milk N output is reduced in heat stressed dairy cows (Kamiya et al., 2005). In growing heifers, high ambient temperature also reduces dry matter intake and efficiency of N utilization (Nonaka et al., 2008). The less efficient utilization of N under high ambient temperature is often accompanied by elevation of plasma urea-N concentration and ruminal ammonia concentration (Kamiya et al., 2005; Nonaka et al., 2008), so that quantitative changes in urea metabolism in the hot environment would be expected.

We examined the effect of high ambient temperature on urea metabolism in lactating dairy cows with urea isotopomer analysis (unpublished data). The intakes of dry matter and digestible N were reduced at a high (28°C) compared with a moderate (18°C) temperature, whereas urea-N production was not affected. This implies that, in relative terms, urea-N production to N intake was increased

at high ambient temperature. Furthermore, high ambient temperature reduced urea-N entry to the gut and increased urinary urea-N excretion with unaltered urine volume. However, the proportions of re-entry to the ornithine cycle, anabolic use and fecal excretion of urea-N to the gut entry were not affected by ambient temperature. The relative increase of urea production under the high ambient temperature could be explained by increased catabolism of amino acid accompanied by the lower dry matter intake and/or enhanced body protein degradation driven by the thermal stress. Under the high ambient temperature conditions, plasma concentration of 3-methylhistidine, a biomarker of myofibrillar proteolysis, was increased compared with cows at the same feed intake under the moderate ambient temperature (Kamiya et al., 2006). Furthermore, the longer retention time of digesta in the gut observed at the high ambient temperature (Nonaka et al., 2008) may increase ruminal degradation of feed protein and subsequent ammonia absorption from the rumen. However, McGuire et al. (1989) did not observe an increase in net ammonia absorption by PDV of dairy cows under the higher ambient temperature conditions.

The reduction of urea-N entry to the gut at a high ambient temperature may be caused by a raised ruminal ammonia concentration as a result of increased feed protein degradation. A high ruminal concentration of ammonia restricts urea transfer from blood to the rumen (Kennedy and Milligan, 1980; Remond et al., 1993) as mentioned previously. A change in blood flow caused by thermal stress may also affect gut entry of urea. McGuire et al. (1989) reported that portal blood flow decreases with the reduced dry matter intake caused by the thermal stress. In our study, the gut clearance of plasma urea, an indicator of changed permeability and/or blood flow, was also markedly decreased at the high ambient temperature. These results indicate that thermal stress would increase urinary urea excretion by accelerating hepatic urea production but inhibiting the gut entry of urea.

CONCLUSION

This review shows that urea recycling into the gut varies with ruminant species, the physiological status and the environmental conditions as well as dietary regimes. However, the information on how much urea is recycled to the rumen and utilized for microbial protein synthesis entering the small intestine is currently insufficient for practical production systems. Quantitative data on urea recycling within each ruminant species offered a variety of feedstuffs with a linkage to the amount of ruminal degradable protein and available carbohydrate are needed. Urea recycling serves as a labile N pool to confer metabolic plasticity, and this can be harnessed as a tool for nutritional management in extensive production systems to cope with the seasonal variations in feed quality.

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