

Development of Urea Products as Rumen Slow-Release Feed for Ruminant Production: A Review

Anusorn Cherdthong and Metha Wanapat

Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract: Dietary protein plays an important role in the nutrition of ruminants, besides providing amino acids; it is also a source of nitrogen for the synthesis of microbial protein. Ruminants have the ability to utilize non-protein nitrogen compounds as N sources for rumen microbial protein synthesis. The use of urea as a protein replacement is attractive in ruminant diets because of its low cost compared with other NPN sources and protein feeds such as soybean meal with high rumen degradability. However, the amount of urea can be used in diets is rather limited due to its rapid hydrolysis to ammonia-nitrogen in the rumen by microbial enzymes, resulting in accumulation and escape of ammonia-nitrogen from the rumen. Slowly ruminal released urea compounds, as a replacement for urea in ruminant rations, have a long history in ruminant feeding. This interest in slowly rumen released nitrogen compounds primarily stems from their potential to slow ammonia release post-feeding, thereby decreasing peak ammonia concentrations in the rumen that lead to its inefficient utilization by ruminal microorganisms, and increased absorption from the rumen. This would also decrease the metabolic cost associated with converting ammonia to urea in the liver, while providing a steady supply of ammonia to rumen bacteria between meals. This review describes the utilization of urea and development of slow-release urea products in ruminants. Recent studies of supplementation of slow-release urea products on rumen fermentation, microbial protein synthesis, and milk production in ruminants are also summarized.

Key words: urea, slow-release urea, rumen fermentation, microbial protein synthesis, ruminant production

INTRODUCTION

Feeds and foods are not equal in their capacity to support the animal functions of maintenance, growth, reproduction and lactation (Van Soest, 1994). They supply energy and essential nutrients in the form of protein, vitamins and minerals. Energy and protein are often the most limiting factors for ruminants and have received the most attention in evaluation systems (Mapato *et al.*, 2010). In the formulation of diets for ruminants, it is important to optimize the balance between the energy and protein contents of the feed, so that balanced rumen fermentation occurs and maximum voluntary intake and feed utilization can achieve.

Dietary protein plays an important role in the nutrition of ruminants, since besides providing amino acids; it is also a source of nitrogen for the synthesis of microbial protein (Nocek and Russell, 1988). Therefore, it is considered the most important nutrient and also the most expensive, which must be efficiently used. Strategies to reduce the feed cost without interfering negatively in production have been constantly researched. The substitution of traditional feeds in the diets of ruminants is common as economic condition changes (Ærskov, 1999; Devendra, 2007). Soybean meal (SBM) has long been used as a prominent source of crude protein for ruminants, however, with its increasing price, the use results in ultimately higher cost of production (Chalupa, 2007). Therefore, the use of urea as a protein (non-protein N, NPN) replacement is attractive in ruminant diets because of its low cost compared with other protein feeds such as SBM with high rumen degradability (Wanapat, 2009; Xin *et al.*, 2010).

Urea is converted via ruminal ammonia (NH_3) into microbial protein, thereby supplying additional protein to the host animals (Nocek and Russell, 1988; Calsamiglia *et al.*, 2008). However, the amount of urea can be used in diets is rather limited due to their rapid hydrolysis to NH_3 in the rumen by microbial enzymes

Corresponding Author: Dr. Metha Wanapat, Tropical Feed Resources Research and Development Center (TROFREC)
Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
Tel. /Fax. (+66) 43-202368, Email: metha@kku.ac.th

(Golombeski *et al.*, 2006; Highstreet *et al.*, 2010). This rapid breakdown to NH_3 can occur at a much faster rate than NH_3 utilization by the rumen bacteria, resulting in accumulation and escape of NH_3 from the rumen.

Attempting to achieve slow NH_3 release from urea to control its rate of release so that NH_3 release more closely parallels to carbohydrate digestion (Pinos-Rodríguez *et al.*, 2010). Slow-release urea compounds, which have been fed to ruminants, include biuret, starea, urea phosphate, coatings based on oil, formaldehyde treated urea and polymer-coated urea (Taylor-Edwards *et al.*, 2009). More recently, slow-release properties have been achieved by using urea bounding to substrates like calcium chloride to control the release rate of NH_3 from urea (Huntington *et al.*, 2006; Golombeski *et al.*, 2006). In an earlier *in vitro* experiment, urea-calcium sulphate mixture products have been also demonstrated to reduce ruminal NH_3 concentrations as well as improve microbial population as compared with feed grade urea (Cherdthong *et al.*, 2010). Therefore, the objective of this article is to review briefly the principles of urea utilization, and development of urea products as rumen slow-release urea on ruminants production.

Utilization of Urea by Ruminants:

Since the early demonstration by Krebs (1937), Hart *et al.* (1939), Reid (1953), Virtanen (1966) of its potential value when fed to ruminants, urea has become widely used as a substitute for preformed protein in ruminant diets. Urea is an important source of N entering the gut; that portion transferred directly across the gut wall is equivalent to 10 to 42% of N intake (Huntington *et al.*, 2006). The presently accepted mechanism of urea action in ruminant nutrition is the hydrolysis of urea by rumen urease to ammonia plus carbon dioxide, carbohydrate fermentation to volatile fatty acids, amination of keto acids to give amino acids, incorporation of the amino acids into microbial protein, and digestion of the microbial cells in the small intestine with subsequent absorption of the resulting amino acids (Nocek and Russell, 1988; Calsamiglia *et al.*, 2008).

Role of Ammonia in Rumen Fermentation:

The sources of NH_3 -N in the rumen include proteins, peptides and amino acids, and other soluble-N materials (Leng and Nolan, 1984). Urea, uric acid and nitrate are rapidly converted to NH_3 -N in the rumen. Nucleic acids in rumen fluid are probably also degraded extensively to NH_3 -N. Figure 1 indicates possible sources of the NH_3 -N pool. The NH_3 -N pool is a focus for studies of metabolism of N in the rumen, and much knowledge has been gained from measuring fluxes of N through this pool. The NH_3 -N pool in the rumen is relatively small and turns over rapidly. The amount of NH_3 -N entering the pool varies over a wide range according to quantity and degradability of protein in the diet and with the extent and method of supplementation of urea. Concentrations of NH_3 -N in the pool can be expected to change rapidly even when animals have continuous access to food (Leng and Nolan, 1984).

It has been suggested that maximum microbial synthesis rate occurs at NH_3 -N concentrations between 5 and 8 mg N/100 ml (Satter and Slyter 1974). Different optima have been found by other researchers, suggesting that diet influences the optimum NH_3 -N level. Recent studies suggest the value may be as high as 15-20 mg N/100 ml depending on diet (Leng and Nolan, 1984). The high NH_3 -N concentration needed for maximum cell growth suggests that the rumen micro-organisms probably have similar mechanisms for incorporation of NH_3 -N to those in soil microbes, which assimilate NH_3 -N via glutamate dehydrogenase. However bacteria grown under low NH_3 -N concentrations fix NH_3 in a two-step process involving glutamine synthetase and glutamate synthase. These reactions involve conversion of glutamate to glutamine and then a reductive transfer of the amide- N of glutamine to 2-oxoglutarate and this step requires ATP (Figure 2).

Metabolism of Urea in the Liver of Ruminants:

The structure and function of the liver attests to the importance of removing potentially toxic NH_3 from blood of ruminants as well as other mammals. The enzymes of the ornithine cycle and enzymes catalyzing transamination reactions are structurally oriented in mitochondria and cytosol of periportal and perivenous hepatic cells to form urea from NH_3 absorbed from the gut and to use glutamine synthesis as another pathway to remove essentially all NH_3 from hepatic portal blood (Figure 3). Periportal cells remove NH_3 from hepatic portal blood and use their enzymatic machinery to synthesize urea. The specialty of the perivenous cells is production of glutamine through glutamine synthetase, thereby providing another opportunity to remove NH_3 from circulation before blood enters the hepatic veins and subsequently general circulation. This two-stage NH_3 removal system integrates with other systems, including gluconeogenesis, regulation of acid-base balance, and interorgan N shuttles to derive the best metabolic control of substrate and product balances, nutrient supplies, and nutrient needs of the organism (Leng and Nolan, 1984).

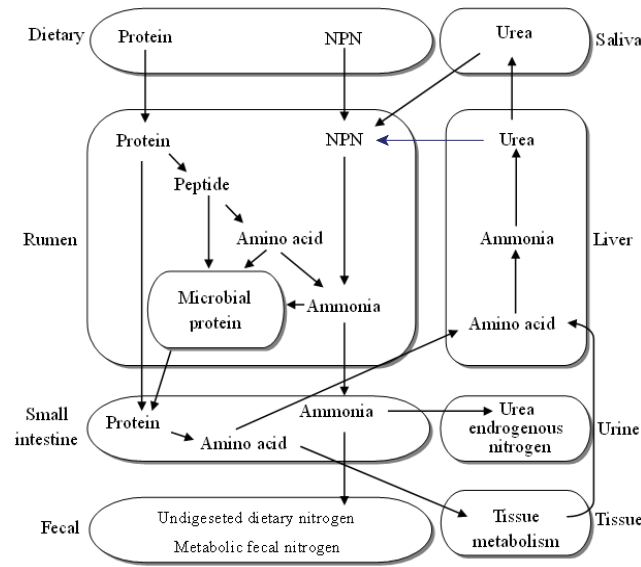


Fig. 1: A model of the metabolism of nitrogen in the rumen (Leng and Nolan, 1984; Modified by Wanapat, 1999).

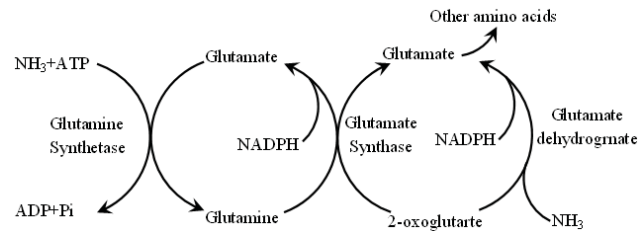


Fig. 2: Two-step process by which ammonia is assimilated by bacteria (Leng and Nolan 1984; Modified by Wanapat, 1999).

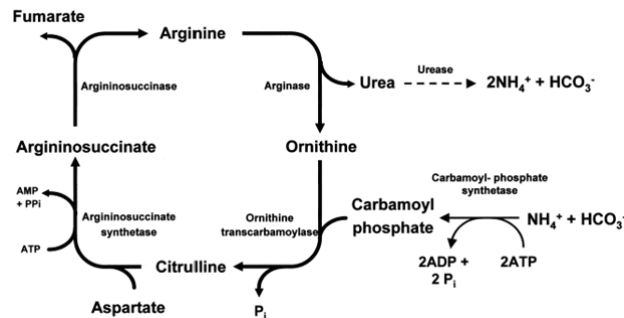


Fig. 3: Urea cycle in the liver of ruminants (Wanapat, 1999)

Disadvantages of Urea Utilization for Ruminants:

The amount of urea that can be used in diets is rather limited due to their rapid hydrolysis to $\text{NH}_3\text{-N}$ in the rumen by microbial enzymes (Golombeski *et al.*, 2006; Highstreet *et al.*, 2010). This rapid breakdown to $\text{NH}_3\text{-N}$ can occur at a much faster rate than $\text{NH}_3\text{-N}$ utilization by the rumen bacteria, resulting in accumulation and escape of $\text{NH}_3\text{-N}$ from the rumen. The net result is that a potentially large part of the N from NPN sources is excreted in the urine and can contribute to environmental pollution (Broderick *et al.*, 2009; Inostroza *et al.*, 2010).

The topic of efficiency of protein use by ruminants has gained attention by environmentalists and government regulators in many parts of the world. Animal feeding practices that reduce the amount of urea in urine have the potential to decrease NH_3 emissions to the environment since urine urea is rapidly converted to NH_3 in fecal/urine slurries due to the action of fecal and environmental ureases. Current dairy research in California, and other parts of the USA and Europe, is focused on decreasing the amount of dietary protein that appears in urine, while maximizing production of milk and its components (Highstreet *et al.*, 2010). Increasing public concern has been focused on ruminant production systems as a major nonpoint source of pollution, which has spurred research aimed to reduce N excretion (Wanapat *et al.*, 2009). Nutrient losses may affect ground and surface water quality; in addition, NH_3 and nitrous oxide emissions can affect air quality, and the latter has been implicated as a significant contributor to global warming, having a 310× more harmful mass-specific effect than CO_2 as a global warming agent (Marini and Van Amburgh, 2005). Therefore, ruminant production systems should support nature conservation, and the environmental load should be low.

Development of Urea Products as Rumen Slow-release Urea:

An alternate solution could be to modify urea to control its rate of release so that NH_3 release more closely parallels carbohydrate digestion (Pinos-Rodríguez *et al.*, 2010). Slow-release urea compounds, which have been fed to ruminants, include biuret, starea, urea phosphate, coatings based on oil, formaldehyde treated urea and polymer-coated urea (Taylor-Edwards *et al.*, 2009). These compounds have not been as advantageous as urea because a substantial part of the NPN in them may leave the rumen without being converted to NH_3 , reducing its incorporation into microbial protein (Galo *et al.*, 2003; Firkins *et al.*, 2007). More recently, slow-release properties have been achieved by using urea bounding to substrates like calcium chloride to control the release rate of NH_3 from urea (Huntington *et al.*, 2006; Golombeski *et al.*, 2006). In an earlier *in vitro* experiment, urea-calcium sulphate mixture products have been also demonstrated to reduce ruminal NH_3 concentrations as well as improve microbial population as compared with feed grade urea (Cherdthong *et al.*, 2010).

Supplementation of Slow-release Urea Products on Ruminant Production:

Effect of Slow-release Urea on Feed Intake and Digestibility:

Digestion balances and feed intake have been a common means of diet evaluation, to the extent that digestibility values are now as much attributes of a feed or diet as compositional values are (Van Soest, 1994). Several studies have been conducted to investigate the influence of feeding slow-release urea on feed intake and nutrient digestibility (Table 1). Previous study from Puga *et al.* (2001) found that the forage to controlled-release urea (CRU) ratios at 70: 30 were significantly increased dry matter intake above the level of the control diet (100% forage: CRU). The higher digestibility of the experiment diets was due to better activity of fiber fermentation in the rumen. It indicates that CRU improves nutrient imbalance for rumen bacteria by increasing availability of energy from simple carbohydrates such as molasses. Similarly, Galina *et al.* (2003) suggested that, supplementation of 1.8 kg dry matter of slow- release urea supplement (SRUS) with sugar cane tops (*Saccharum officinarum*) and maize (*Zea mays*) in 60 Zebu steers, while showing significantly ($P < 0.05$) better improved of digestibility. High fiber forages have been associated with more digestible feeds when NH_3 and urea were added to fibrous hay (Ørskov, 1999). In addition, another polymer-coated SRU (Optigen; CPG Nutrients, Syracuse, NY) has been demonstrated to increase total tract DM and CP digestibilities when fed to lactating dairy cows (Galo *et al.*, 2003). These results were in agreement with the findings from Xin *et al.* (2010), who found that polyurethane coated urea were greater DMI and nutrients digestibilities than those in urea (Table 1).

Effect of Slow-release Urea on Rumen Fermentation Parameters:

The development of products that slow the ruminal release of NH_3 -N without limiting the extent of urea degradation in the rumen has been challenging. Owens *et al.* (1980) reported that ruminal NH_3 -N release was slower for slow release urea product than for uncoated urea, thereby increasing diet acceptability and improving rumen fermentation in ruminants. As reported that, supplementation of sugar cane tops (*Saccharum officinarum*), corn stubble (*Zea mays*) and King grass (*Pennisetum purpureum*) (high fiber diets) with controlled- release urea supplement (CRUS) did improve fermentation in sheep (Puga *et al.*, 2001) (Table 2). Adding 10, 20 or 30% CRUS showed improved NH_3 -N and VFA production. This is strategies to improve the utilization of those feeds, suggesting providing supplements to correct the nutrient imbalances for rumen bacteria (Nocek and Russell, 1988). CRUS could have provided continuous NH_3 -N for microbial growth, superior the minimum of 15-30 mg NH_3 -N/100 ml rumen fluid for maximizing microbial growth previously suggested (Leng and Nolan, 1984).

Table 1: Effects of slow-release urea products on dry matter intake and nutrient digestibility

Source	Type of SRU	Suppl., % diet	Animal	DMI kg/d	Digestibility, %			
					DM	CP	NDF	OM
Puga <i>et al.</i> (2001)	Urea	0	Sheep	5.9	58.6	-	67.8	57.6
	Control release	30*		8.2	64.8	-	74.0	63.2
Galina <i>et al.</i> (2003)	SRU	0	Beef	5.8	58.8	-	57.1	48.4
	SRU	1.8		8.2	68.7	-	75.1	59.7
Highstreet <i>et al.</i> (2010)	Urea	1.8	Cows	28.2	-	70.9	50.9	-
	Encapsulated urea	1.7		28.6	-	70.8	50.0	-
Xin <i>et al.</i> (2010)	Urea	0.6	Cows	20.2	46.3	43.5	13.9	46.7
	Polyurethane coated urea	0.6		22.8	51.0	44.6	18.5	51.2

*Supplementation of 30% control release in forages

Table 2: Effects of slow-release urea products on rumen fermentation parameters

Source	Type of SRU	Suppl., % diet	Animal	NH ₃ -N, mg%	Total VFA, (mM/L)	VFA, %		
						C2	C3	C4
Galina <i>et al.</i> (2003)	SRU	0	Beef	6.8	-	78.2	14.4	7.4
	SRU	1.8		12.3	-	72.2	16.0	11.8
Golombeski <i>et al.</i> (2006)	Ruma Pro	0	Cows	5.4	54.0	62.9	21.2	11.4
	Ruma Pro	0.61		6.0	50.0	63.2	21.5	11.1
Taylor-Edwards <i>et al.</i> (2009)	Urea	1.6	Steers	14.1	99.7	62.7	19.7	14.0
	SRU	1.6		8.9	103.2	63.6	20.3	13.8
Pinos-Rodríguez <i>et al.</i> , 2010	Optigen®	0.6	Steers	-	97.6	52.0	34.9	13.0
	Optigen®	1.1		-	94.8	52.3	35.2	12.5
Xin <i>et al.</i> (2010)	Urea	0.6	Cows	2.0	64.08	56.8	33.3	5.3
	Polyurethane coated urea	0.6		1.4	66.08	56.3	34.4	5.3

A recent study by Taylor-Edwards *et al.* (2009) who conducted the effects of slow-release urea (SRU) versus feed-grade urea on ruminal NH₃-N in beef steers. Multi-catheterized steers were used to determine effects of intraruminal dosing (5 kg of BW) SRU or urea on PDV nutrient flux and blood variables for 10 h after dosing. Intraruminal dosing of SRU prevented the rapid increase in ruminal NH₃-N concentrations that occurred with urea dosing. Urea undergoes rapid hydrolysis in the rumen to NH₃-N. Mean ruminal NH₃-N concentrations were 263% greater for steers dosed intraruminally with urea than steers dosed with SRU primarily because ruminal NH₃-N concentrations for urea treatment rose markedly within 0.5 h of dosing. This rapid rise in NH₃-N concentrations for urea treatment was substantial enough to increase ruminal pH by over 0.5 units within 0.5 h of dosing. Indeed, ruminal pH and ruminal NH₃-N concentrations were positively related, an effect that has been observed previously (Puga *et al.*, 2001). Additionally, ruminal NH₃-N concentrations remained greater for steers dosed with urea than those dosed with SRU until 8 to 10 h after dosing. These results demonstrate that *in vivo* SRU does indeed have a slower release rate of NH₃-N than urea and can effectively modulate ruminal NH₃-N concentrations when substituted for urea (Huntington *et al.*, 2006; Golombeski *et al.*, 2006; Taylor-Edwards *et al.*, 2009; Cherdthong *et al.*, 2010; Highstreet *et al.*, 2010; Inostroza *et al.*, 2010; Pinos-Rodríguez *et al.*, 2010; Xin *et al.*, 2010).

Nitrogen utilization by rumen microorganisms can be reflected by ruminal NH₃-N concentration. In the study by Xin *et al.* (2010), the NH₃-N concentrations of all the diets increased within 1 h, and then declined gradually. However, the polyurethane coated urea (PCU) diet resulted in the lowest concentrations of NH₃-N at all time points. During 8 h *in vitro* fermentation, the PCU diet decreased NH₃-N concentration by 8.2-20.6% as compared with the FGU diet. This agrees with the result of Prokop and Klopfenstein (1977), who found that slow-release urea (combination of urea and formaldehyde) could decrease ruminal NH₃-N concentration by 25.3% compared to urea. No significant differences were found between PCU and soybean meal (SBM) diets on ruminal NH₃-N release. A similar result was found in the report of Galo (2003), in which urea release from a polymer-coated urea was 83% as extensive as uncoated urea after 1 h incubation with distilled water. Other products, such as a urea-calcium combination, have had similar effects. Cass and Richardson (1994) made a comparison in an *in vitro* study and observed that a urea-calcium combination produced slower NH₃-N release rate than regular urea. Ammonia- N concentrations began to increase at 8 h for the FGU diet, which indicates that bacterial autolysis may occur. However, NH₃-N concentrations with PCU and SBM diets still declined. Based on this result, it could be inferred that slow-release urea diets prolong microbial utilization of additional N sources during ruminal fermentation. Therefore, the synchronization between ruminal NH₃-N release and carbohydrate availability might be improved, consequently resulting in greater microbial protein synthesis.

For more possible reason, slow-release urea product reduced NH₃-N concentration through the inhibition of the hyper-ammonia-producing bacteria, a small group of ruminal bacteria that are responsible for the

production of most of the $\text{NH}_3\text{-N}$. Ferme *et al.* (2004) also reported that the inhibition of major ammonia-producing bacteria (such as *Prevotella ruminantium* and *Prevotella bryantii*) resulted in a reduction in $\text{NH}_3\text{-N}$ concentration in continuous culture fermenters of ruminal microbes. Continuous culture fermenters have low numbers of protozoa; however, in vivo, protozoa play a major role in protein degradation. The most important aspect of protozoa is their ability to engulf large molecules, protein, CHO, or even ruminal bacteria (Van Soest, 1994). In addition, protozoa play a role in regulating bacterial N turnover in the rumen, and they supply soluble protein to sustain microbial growth. Because protozoa are not able to use $\text{NH}_3\text{-N}$, a fraction of previously engulfed insoluble protein is later returned to the rumen fluid in the form of soluble protein (Dijkstra, 1994). This is one of the main reasons why defaunation decreases $\text{NH}_3\text{-N}$ concentration in the rumen.

In some studies, Xin *et al.* (2010) who evaluated the effects of polyurethane coated urea on ruminal VFA concentration of Holstein dairy cows fed a steam-flaked corn-based diet. Three treatment diets with isonitrogenous contents (13.0% CP) were prepared: i) feedgrade urea (FGU) diet; ii) polyurethane coated urea (PCU) diet; and iii) isolated soy protein (ISP) diet. There were no significant differences in total VFA concentration among the three dietary treatments. Because ruminal VFAs are derived mainly from dietary carbohydrate fermentation (Firkins *et al.*, 2007), the similar total ruminal VFA concentrations reflected no adverse fermentation by addition of FGU or PCU to the diet. Molar percentages of individual VFAs were significantly altered ($p < 0.05$) by the dietary treatment. Urea-based diets resulted in a higher proportion of acetate and less propionate than the ISP diet, which caused a significantly higher ratio of acetate to propionate ($p < 0.01$). The isobutyrate molar percentage on the ISP diet was several fold higher than the other two urea treatment diets. This observation is in agreement with the report that isobutyrate concentration increased linearly with increasing level of peptides in continuous culture. Isobutyrate is considered to be a product of valine catabolism during ruminal fermentation, so the lower concentration of isobutyrate with FGU or PCU diets is presumably a result of lower dietary valine content. The lower molar percentage of butyrate on PCU and FGU diets might be attributed to inter conversion between acetate and butyrate in the rumen. Less acetate was used to produce butyrate with urea based supplementation in this study. The significance of valerate accumulation with the ISP diet in the present study was not clear, but the absolute values on all three diets were slightly higher than those noted by other researchers (Griswold *et al.*, 2003) when urea was included in buffer solution in continuous culture.

Effect of Slow-release Urea on Rumen Microbes and Microbial Protein Synthesis:

The ultimate goal of proper rumen nutrition is to maximize microbial growth and the amount of RDP that is captured into rumen microbial cells. Maximizing the capture of degradable N not only improves the supply of AA to the small intestine, but also decreases N losses. Knowledge of the N compounds required for growth of ruminal bacteria is important in understanding the protein nutrition of ruminants and factors affecting ruminal fermentation, particularly fiber digestion. There is a long-held belief that cellulolytic ruminal bacteria use $\text{NH}_3\text{-N}$ as their sole source of N. Some recently published results are not consistent with this conclusion, however. Bryant (1973), in summarizing the nutrient requirements of ruminal bacteria, concluded that cellulolytic bacteria used only NH_3 as an N source for growth. They were unable to grow on other N sources in the absence of NH_3 (Russell *et al.*, 2009). The stimulation of cellulolytic species by precursors of various N sources also suggests a quantitative dependence on $\text{NH}_3\text{-N}$ -release rate for optimum growth. Furthermore, there is experimental evidence that preformed slow-release $\text{NH}_3\text{-N}$ stimulate microbial growth and increase fiber digestion.

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. 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. 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 (Russell *et al.*, 2009). The NRC (2001) assumes that rumen-degradable protein (RDP) from NPN sources such as urea are as effective as RDP from true protein for microbial protein formation. Slow release urea that is more slowly hydrolyzed to $\text{NH}_3\text{-N}$ than unprotected urea could potentially be used more efficiently by rumen microorganisms.

A recent study by Xin *et al.* (2010) who found that supplementation of feed grade urea (FGU) diet had the lowest microbial efficiency (11.3 g N/kg OMTD) and the isolated soy protein (ISP) diet (14.7 g N/kg OMTD) had the greatest ($p = 0.05$), with the polyurethane coated urea (PCU) diet (13.0 g N/kg OMTD) being intermediate. The higher microbial efficiency with the ISP diet might be explained by use of peptide or amino acid N to form true proteins to enhance microbial growth. However, according to NRC (2001), the microbial efficiency should be in the range of 12 to 54 g N/kg OMTD. The absolute values of microbial efficiency of all the diets in their study were slightly lower. This might reflect a limited N supply or lack of available N sources (peptide or amino acid) for ruminal microbial growth in the fermenters during incubation. Although

all dietary treatments were under the same condition of limited N source which may constrain rumen microbial protein synthesis, the PCU diet had 15.6% greater microbial efficiency as compared to the FGU diet, which matched results of daily microbial N production.

In contrast, Galo *et al.* (2003) reported that feeding polymer-coated urea (Optigen 1200 Controlled Release N; CPG Nutrients, Inc., Syracuse, NY) in dairy cows were not alter rumen microbial crude protein (MCP) production. NRC (2001) predicts MCP yields of 150 to 225 g MCP per kilogram of DOM with ruminal N balances of +20 and -20%, respectively. In a study by Timmermans *et al.* (2000), testing the effects of several dietary factors, MCP flow to the duodenum ranged from 765 to 1925 g/d, DMI ranged from 15.5 to 26 kg/d, and N intakes ranged from 428 to 832 g/d. Klusmeyer *et al.* (1990) fed cows two concentrations of N, 390 g/d (11% CP) and 500 g/d (14% CP) and found no changes in MCP flow from the rumen (2110 g MCP per day). Stokes *et al.* (1991) fed different levels of NSC and RDP to Holstein cows and found no differences in microbial efficiencies in terms of MCP/DOM; the average was 150 g MCP per kilogram of DOM. These authors did see a reduction (-700 g/d) in MCP flow from the rumen for cows eating a diet low in NSC (24%) and low in RDP (9%).

Effect of Slow-release Urea on Milk Production:

Supplementation of slow-release urea to the diets of ruminants fed high levels of rapidly fermentable carbohydrates could improve the ability of microbial protein synthesis, these improving its efficiency of conversion into milk (Galo *et al.*, 2003; Broderick *et al.*, 2009) (Table 3). Previous study from Inostroza *et al.* (2010) who determine the effect of a controlled-release urea product (CRU; Optigen, Alltech Inc., Lexington, KY) on milk production in commercial Wisconsin dairy herd diets. Sixteen trial herds were randomly assigned to a treatment sequence, control to CRU to control, in a crossover design with two 30-d periods. The control diet for each herd was formulated by the herd nutritionist based on the level of milk production, and the CRU diets contained 114 g/d per cow of CRU, replacing an equivalent amount of supplemental CP, primarily from soybean meal. The results shown that milk yield was 0.5 kg/d per cow greater for CRU than for control. Similarly, Tikofsky and Harrison (2007) reported trends for increased milk yield when diets containing Optigen were fed to dairy cows. However, Galo *et al.* (2003) and dos Santos *et al.* (2008) reported that milk yield was unaffected when SBM was partially replaced by CRU and when uncoated prilled urea plus RUP sources were partially replaced by a polymercoated prilled urea product, respectively. A greater yield of microbial N for CRU than for uncoated prilled urea in ruminal continuous culture has been reported (Chalupa, 2007; Tikofsky and Harrison, 2007; Harrison *et al.*, 2008), which may partially explain their observed increase in milk yield. In addition, the filling of the diet formulation space created by the use of CRU with DM from either corn silage or corn grain may have improved the rumen-fermentable carbohydrate and energy status, thereby contributing to the response (NRC, 2001).

Table 3: Supplementation of slow-release urea product on milk production in dairy cows

Source	Type of SRU	Suppl., % diet	Animal	Milk, kg/d			Milk composition, %	
				Fat		Protein	Lactose	
Galo <i>et al.</i> (2003)	Urea	0.3	Cows	35.6	3.8	3.1	-	-
	Optigen®	0.8		34.8	3.6	3.1	-	-
Golombeski <i>et al.</i> (2006)	Ruma Pro	0	Cows	26.1	4.2	3.7	4.8	
	Ruma Pro	0.61		26.2	4.4	3.7	4.8	
Inostroza <i>et al.</i> (2010)	Optigen®	0	Cows	35.4	3.7	3.0	-	-
	Optigen®	114*		35.9	3.7	3.0	-	-
Highstreet <i>et al.</i> (2010)	Urea	1.8	Cows	46.9	3.6	2.8	4.7	
	Encapsulatedurea	1.7		47.6	3.7	2.8	4.7	
Xin <i>et al.</i> (2010)	Urea	0.6	Cows	32.48	3.71	2.94	5.09	
	Polyurethane coated urea	0.6		34.53	4.01	3.16	4.99	

*Fed 114 g of Optigen® per head per day

In some studies, Inostroza *et al.* (2010) reported that milk urea N (MUN) was greater for CRU than for control (13.2 vs. 12.4 mg/dL). These MUN values are within the normally expected range of 10 to 14 mg/dL, and thus are probably not of consequence. An increase in MUN from 8.6 mg/dL for the control treatment to 9.8 mg/dL for the CRU treatment was reported by Broderick *et al.* (2009).

Previous study from Xin *et al.* (2010) showed that *Butyrivibrio fibrisolvens* and *Ruminococcus spp.* are two of the primary cellulose digesters with end product fermentation of succinate and acetate, respectively (Russell *et al.*, 2009), reduced peak NH₃-N levels in cows fed the encapsulated urea diet may have shifted microbial species proportions in the rumen to change rumen volatile fatty acid (VFA) profiles and, if this resulted in increased acetate levels, it could have shifted fat synthesis from body to milk. In the absence of an increase in ruminal cellulose fermentation, suggested by similar whole tract aNDFom digestibility between treatments

in cows at both stages of lactation, there is little likelihood that ruminal VFA production increased. This suggests that increased milk fat yield was due to a shift in the profile of VFA produced, perhaps due to a changed proportion of rumen cellulolytic microorganisms. Grummer *et al.* (1984) infused ammonium chloride to the rumen of dairy cows to increase the concentration of $\text{NH}_3\text{-N}$ from 4.8 to 17.3 mg/dl. This also caused an increase in total VFA concentrations, as well as a decrease in the acetate to propionate ratio. Song and Kennelly (1989) infused ammonium chloride to the rumen to increase rumen $\text{NH}_3\text{-N}$ concentrations and, while the total VFA concentration was not influenced by $\text{NH}_3\text{-N}$ concentration, there were trends to decreased acetate and increased propionate proportions in rumen fluid with increasing NH_3 concentration, which resulted in a decreased acetate to propionate ratio. In a similarly designed study, Song and Kennelly (1990) infused varying levels of ammonium bicarbonate to the rumen of Holstein cows and also found no impact on ruminal degradation, but they did observe a proportional increase in mixed bacterial counts and total VFA concentrations. In addition, as the rumen $\text{NH}_3\text{-N}$ levels increased, the acetate to propionate ratio decreased. Thus, under current study by Xin *et al.* (2010), found that increased milk fat synthesis in cows fed the encapsulated urea diet may have been due to lower rumen $\text{NH}_3\text{-N}$ levels, at times of the day that they were the highest, that increased the acetate to propionate ratio in ruminally produced VFA.

Conclusions and recommendation:

The use of urea as a protein replacement is attractive in ruminant diets because of its low cost compared with other protein feeds such as SBM with high rumen degradability. Moreover, slow release urea products resulted in more efficiency than urea on rumen fermentation, microbial protein synthesis, and milk yield in ruminants. Based on this review it could be concluded that slow release urea products can be effective product for ruminants and should be applied further in practical ruminant feeding in the tropics.

ACKNOWLEDGEMENTS

The paper was supported by the Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand Research Fund (TRF) through the Royal Golden Jubilee PhD Program.

REFERENCES

- Broderick, G.A., M.J. Stevenson and R.A. Patton, 2009. Effect of dietary protein concentration and degradability on response to rumen-protected methionine in lactating dairy cows. *J. Dairy Sci.*, 92: 2719–2728.
- Bryant, M. P., 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. *Fed. Proc.*, 32: 1809–1813.
- Calsamiglia, S., P. W. Cardozo, A. Ferret and A. Bach, 2008. Changes in rumen microbial fermentation are due to a combined effect of type of diet and pH. *J. Anim. Sci.*, 86: 702–711.
- Cass, J.L. and C.R. Richardson, 1994. *In vitro* ammonia release from urea/calcium compounds as compared to urea and cottonseed meal. Texas Tech. Univ. Agr. Sci. Natl. Res. Tech. Rpt. No. T-5-342. Texas Tech University, Lubbock, TX, USA.
- Chalupa, W., 2007. Precision feeding of nitrogen to lactating dairy cows: A role for Optigen II. In *Nutritional Biotechnology in the Feed and Food Industries*. Proc. Alltech's 23rd Annu. Symp. T. P. Lyons, K. A. Jacques, and J. M. Hower, ed. Alltech Inc., Lexington, KY, USA. pp: 221.
- Cherdthong, A., M. Wanapat, C. Wachirapakorn and M. E. Van Amburgh, 2010. Evaluation of urea-calcium mixtures (UCM) as slow-release: I. Fermentation characteristics using *in vitro* gas technique. In: *Proceedings of the Agriculture Conference 11th. January 25-26, 2010*. Kawee Jutikul Auditorium, Khon Kaen University, Khon Kaen, Thailand, pp: 138-141.
- Devendra, C., 2007. Perspectives on animal production systems in Asia. *Livest. Sci.*, 106: 1 – 18.
- Ferne, D., M. Banjac, S. Calsamiglia, M. Busquet, C. Kamel and G. Avgustin, 2004. The effects of plant extracts on microbial community structure in a rumen-simulating continuous-culture system as revealed by molecular profiling. *Folia. Microbiol.*, 49: 151–155
- Firkins, J.L., Z. Yuand M. Morrison, 2007. Ruminal nitrogen metabolism: perspectives for Integration of microbiology and nutrition for dairy. *J. Dairy Sci.*, 90 (E. Suppl.): E1–E16.
- Galina, M.A., F. Perez-Gil, R.M.A. Ortiz, J.D. Hummel and R.E. Ørskov, 2003. Effect of slow release urea supplementation on fattening of steers fed sugar cane tops (*Saccharum officinarum*) and maize (*Zea mays*): ruminal fermentation, feed intake and digestibility. *Livest. Prod. Sci.*, 83: 1–11.
- Galo, E., S. M. Emanuele, C. J. Sniffen, J. H. White and J. R. Knapp, 2003. Effects of a polymer-coated urea product on nitrogen metabolism in lactating Holstein dairy cattle. *J. Dairy Sci.* 86: 2154–2162.

Golombeski, G.L., K.F. Kalscheur, A.R. Hippen and D.J. Schingoethe, 2006. Slow-release urea and highly fermentable sugars in diets fed to lactating dairy cows. *J. Dairy Sci.*, 89: 4395–4403.

Griswold, K.E., G.A. Apgar, J. Bouton and J.L. Firkins, 2003. Effect of urea infusion and ruminal degradable protein concentration on microbial growth, digestibility, and fermentation in continuous culture. *J. Anim. Sci.*, 81: 329-336.

Grummer, R.R., J.H. Clark, C.L. Davis and M.R. Murphy, 1984. Effect of ruminal ammonia-nitrogen concentration on protein degradation *in situ*. *J. Dairy Sci.*, 67: 2294-2301.

Harrison, G.A., M.D. Meyer and K.A. Dawson, 2008. Effect of Optigen and dietary neutral detergent fiber level on fermentation, digestion, and N flow in rumen-simulating fermenters. *J. Dairy Sci.*, 91(Suppl. 1): 489. (Abstr.).

Hart, E.B., G. Bohstedt, H.J. Deobald and M.I. Wegner, 1939. The utilization of sample nitrogenous compounds such as urea and ammonium bicarbonate by growing calves. *J. Dairy Sci.*, 22: 785–798.

Highstreet, A., P.H. Robinson, J. Robison and J.G. Garrett, 2010. Response of Holstein cows to replacing urea with a slowly rumen released urea in a diet high in soluble crude protein. *Livest. Sci.*, 129: 179–185.

Huntington, G.B., D.L. Harmon, N.B. Kristensen, K.C. Hanson and J.W. Spears, 2006. Effects of a slow-release urea source on absorption of ammonia and endogenous production of urea by cattle. *Anim. Feed Sci. Tech.*, 130: 225–241.

Inostroza, J.F., R.D. Shaver, V.E. Cabrera and J.M. Tricárico, 2010. Effect of diets containing a controlled-release urea product on milk yield, milk composition, and milk component yields in commercial Wisconsin dairy herds and economic implications. *Professional Animal Scientist*, 26: 175-180.

Klusmeyer, T.H., R.D. McCarthy, Jr., J.H. Clark and D.R. Nelson, 1990. Effects of source and amount of protein on rumen fermentation and passage of nutrients to the small intestine of lactating cows. *J. Dairy Sci.*, 73: 3526–3537.

Krebs, K., 1937. Value of the Amides in the Feeding of Cattle. Historical consideration of the Development of the Amide Question, Critical Evaluation of the State of Our Present Knowledge (Translated title). *Biedermanns Zentr. B. Tierernähr.*, 9: 394-507.

Leng, R.A. and J.V. Nolan, 1984. Nitrogen metabolism in the rumen. *J. Dairy Sci.*, 67: 1072-1089.

Mapato, C., M. Wanapat and A. Cherdthong, 2010. Effects of urea treatment of straw and dietary level of vegetable oil on lactating dairy cows. *Trop. Anim. Health Prod.*, DOI: 10.1007/s11250-010-9613-3.

Marini, J.C. and M.E. Van Amburgh, 2005. Partition of nitrogen excretion in urine and the feces of Holstein replacement heifers. *J. Dairy Sci.*, 88: 1778–1784.

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle. 7th rev. ed. *Natl. Acad. Sci.*, Washington, DC, USA.

Nocek, J.E. and J.B. Russell, 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. *J. Dairy Sci.*, 71: 2070-2107.

Orskov, E.R., 1999. Supplement strategies for ruminants and management of feeding to maximize utilization of roughages. *Prev. Vet. Med.*, 38: 179-185.

Owens, F.N., K.S. Lusby, K. Mizwicki and O. Forero, 1980. Slow ammonia release from urea: Rumen and metabolism studies. *J. Anim. Sci.*, 50: 527-531.

Pinos-Rodríguez, J.M., L.Y. Peña, S.S. González-Muñoz, R. Bárcena and A. Salem, 2010. Effects of a slow-release coated urea product on growth performance and ruminal fermentation in beef steers. *Italian J. Anim. Sci.*, DOI:10.4081/ijas.2010.e4.

Prokop, M.J. and T.J. Klopfenstein, 1977. Slow ammonia release urea. *Nebraska Beef Cattle Report No. EC 77-218 Nebraska*.

Puga, D.C., H.M. Galina, R.F. Perez-Gil, G.L. Sangines, B.A. Aguilera and G.F.W. Haenlein, 2001. Effect of a controlled-release urea supplement on rumen fermentation in sheep fed a diet of sugar cane tops (*Saccharum officinarum*), corn stubble (*Zea mays*) and King grass (*Pennisetum purpureum*). *Small Rumin. Res.*, 39: 269–276.

Reid, J.T., 1953. Urea as a protein replacement for ruminants: a review. *J. Dairy Sci.*, 36: 955–996.

Russell, J.B., R. E. Muck and P. J. Weimer, 2009. Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. *FEMS Microbiol. Ecol.*, 67: 183–197.

Satter, L.D. and L.L. Slyter, 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Brit. J. Nutr.*, 32: 199-208.

Song, M.K. and J.J. Kennelly, 1989. Effect of ammoniated barley silage on ruminal fermentation, nitrogen supply to the small intestine, ruminal and whole tract digestion and milk production of Holstein cows. *J. Dairy Sci.*, 72: 2981-2990.

Stokes, S.R., W.H. Hoover, T.K. Miller and R. Blauweikel, 1991. Rumen digestion and microbial utilization of diets varying in type of carbohydrate and protein. *J. Dairy Sci.*, 74: 871–881.

Taylor-Edwards, C.C., N.A. Elam, S.E. Kitts, K.R. McLeod, D.E. Axe, E.S. Vanzant, N.B. Kristensen and D.L. Harmon, 2009. Influence of slow-release urea on nitrogen balance and portal-drained visceral nutrient flux in beef steers. *J. Anim. Sci.*, 87: 209-221.

Tikofsky, J. and G. A. Harrison, 2007. Optigen II: Improving the efficiency of nitrogen utilization in the dairy cow. In *Nutritional Biotechnology in the Feed and Food Industries*. Proc. Alltech's 23rd Annu. Symp. T. P. Lyons and Jacques, K. A., ed. Alltech Inc., Lexington, KY, USA, pp: 373.

Timmermans, S.J. Jr., L.M. Johnson, J.H. Harrison and D. Davidson, 2000. Estimation of the flow of microbial nitrogen to the duodenum using milk uric acid or allantoin. *J. Dairy Sci.*, 83:1286.

Van Soest, P.J., 1994. *Nutritional ecology of the ruminant*. 2nd ed. Cornell University Press. Ithaca, NY, USA, pp: 476.

Virtanen, A.I., 1966. Milk production of cows on protein-free feed. *Science*, 153: 1603–1614.

Wanapat, M., 1999. *Feeding of ruminants in the tropics based on local feed resources*. Khon Kaen Publishing Company Ltd., Khon Kaen, Thailand., pp: 236.

Wanapat, M., 2009. Potential uses of local feed resources for ruminants. *Trop. Anim. Health. Prod.*, 41: 1035–1049.

Wanapat, M., S. Polyorach, K. Boonnop, C. Mapato and A. Cherdthong, 2009. Effects of treating rice straw with urea or urea and calcium hydroxide upon intake, digestibility, rumen fermentation and milk yield of dairy cows. *Livest. Sci.*, 125: 238–243.

Xin, H.S., D.M. Schaefer, Q.P. Liu, D.E. Axe and Q.X. Meng, 2010. Effects of polyurethane coated urea supplement on *in vitro* ruminal fermentation, ammonia release dynamics and lactating performance of Holstein dairy cows fed a steam-flaked corn-based diet. *Asian-Aust. J. Anim. Sci.*, 23: 491-500.