Review of Literature
II. REVIEW OF LITERATURE

2.1. NPN utilization by ruminants

The feed proteins and N containing compounds ingested by ruminants are subjected to microbial degradation in the rumen prior to digestion by the host enzymatic system. Amino acids, peptides and N, along with the ATP are utilized by the microbes for their growth and in turn offer themselves as microbial proteins to the host animal. Since the microbes are able to utilize N for their growth, NPN substances can be substituted for proteins, as cheaper alternatives in the diet. A schematic model describing the metabolism of N with reference to the utilization and recycling of urea and NPN in ruminants is depicted in Fig. 2.1.

Urea is broken down into ammonia and carbon dioxide by microbial urease. Ammonia is used with keto acids from the fermentation of carbohydrates for the synthesis of amino acids by rumen microbes. These amino acids are then incorporated into microbial protein. Microbial protein is then broken down in the small intestine of the host into free amino acids (NRC, 1989).

Bloomfield et al. (1960) indicated that urea hydrolysis occurred four times faster than uptake of the liberated ammonia and thereby resulted in an eventual loss of available nitrogen for microbial protein synthesis. Rapid release of ammonia is one of the major problems for efficient utilization of urea.

Edwards et al. (2009) reported that the dietary urea is rapidly hydrolyzed upon entry into the rumen, results in a rapid peak in rumen ammonia concentrations within the
first hour after consumption. Much of the excess ammonia is absorbed in the animal's bloodstream, and then is converted back to urea in the liver and excreted. The lost ammonia is not utilized for protein synthesis, which results in a less efficient utilization of available nitrogen. Ruminal carbohydrate degradation and subsequent microbial growth is a much slower process. A synchrony of these processes would improve the efficiency of NPN incorporation into microbial protein and thereby improve the overall efficiency of N use.

An alternate solution therefore could be to modify urea to control its rate of release so that ammonia release more closely parallels carbohydrate digestion. The development of products that slow down the ruminal release of ammonia without limiting the extent of urea degradation in the rumen has been challenging.

2.2. Development of slow release nitrogen products

Dietary urea has been used for decades as a cheaper source of N for ruminant feeding. Economics is primarily responsible for the increased use of urea and other NPN products. One kilogram of urea provides as much nitrogen as 5 to 6 kg of vegetable protein supplement. Urea has a nitrogen equivalent of 281 per cent crude protein; this compares to 44 per cent for cottonseed meal and 50 per cent for soybean meal.

A potential way to minimize excess ammonia accumulating in rumen reaching the liver is to increase microbial utilization of ammonia by modulating its appearance in the rumen. To achieve this goal, some researchers have used microbial urease inhibitors, with mixed results (Whitelaw et al., 1991; Ludden et al., 2000). An alternate approach was to develop slow-release urea (SRU) compounds such as biuret (Waite and Wilson 1968;
Löest et al. (2001), urea formaldehyde (Prokop and Klopfenstein, 1977), urea phosphate, or urea bound to substrates like calcium chloride (Oltjen et al., 1968; Huntington et al., 2006), Starea (Bartley and Deyoe, 1975), urea-corn-carboxy-resin (Huston et al. 1974), Uromol (Malik et al., 1978), urea coated with linseed oil and talc (Forero et al., 1980), lactosylurea (Forman et al., 1982), urea-polyvinyl-alcohol (Sommer et al., 1985), urea-lignocellulose complex (Chahil et al., 1986; Caneque et al., 1998), Uromalt (Virk et al., 1989) and isobutyraldehyde monourea (Mathison et al., 1994). Although these methods may be useful in avoiding ammonia toxicity, they rarely improve utilization of dietary nitrogen or improve animal performance compared with standard feed sources (Owens and Zinn, 1988). More recently, slow-release properties have been achieved by using coatings based on oil (Garrett et al., 2005) or polymers (Tedeschi et al., 2002; Galo et al., 2003) to control the release rate of ammonia from urea. The development of products that slow down the ruminal release of ammonia without limiting the extent of urea degradation in the rumen has been challenging (Males et al., 1979).

Oltjen et al. (1969) reported that biuret was better utilized than urea when steers were fed a high roughage diet twice daily. Biuret is a nitrogenous compound that is formed during the manufacturing process of urea. The N content of biuret is 40.77. Scientists have given considerable attention to biuret as a NPN source for ruminants because the slow enzymatic hydrolysis of biuret in the rumen retards ammonia production.

Huston et al. (1974) developed a slow release urea pelleted product using a mixture of urea, corn starch and carboxy resin. In vitro experiments were carried out to
determine the effects of level of resin and starch on the release rate of urea from the pellets. As the resin content of the pellet increased, the rate of urea release decreased. The preparation that contained the highest content of resin had the slowest release rate, although it was not greatly different from that of the preparation containing only 1 per cent resin.

Ørskov et al. (1974) developed a method of including urea in whole grains. Impregnation is another way in which better utilization of urea can be achieved. It is a process that causes whole cereal grains to absorb urea. A 50 per cent solution of urea was sprayed onto the whole grain while it was being mixed in a vertical feed mixer to give a 2 per cent inclusion of urea. No crystals of urea reformed and the urea appeared to be completely absorbed into the grains.

Bartley and Deyoe (1975) developed Starea to improve the utilization of urea N by ruminants. Starea is produced by mixing finely ground grains (corn, barley, wheat, sorghum, etc) or other economic starch sources with a NPN source such as urea. Starea makes energy available to the rumen microorganisms at a rate similar to that at which urea releases ammonia so the microorganisms are simultaneously provided with the main components for microbial protein synthesis.

Fishwick (1978) prepared a product by coating prilled urea with sulfur and wax and studied the N utilization by ruminants. Two sulfur coated urea products were used. The first product contained 324 g of N per kg and was coated with 277 g of Sulfur per kg. The second product contained 342 g N per Kg and was coated with 236 g of Sulfur per kg. Both products were coated with 20 g of wax per kg.
Males et al. (1979) studied the effect of feeding a liquid molasses slow release product using dairy cows. Results showed that the slow release urea molasses reduced rumen ammonia levels over that of urea plus molasses. Nitrogen digestibility was lower for the slow release urea product indicating that the urea was bound too tight; therefore, not all the urea was released in the rumen.

Galo et al. (2003) prepared a product, Optigen 1200 (CPG Nutrients, Syracuse, NY) is a polymer-coated, controlled-release urea product (PCU) that, compared with feed-grade urea, is believed to possess a reduced rate of ammonia release to rumen microbes.

Edwards et al. (2009) demonstrated Polymer-coated urea (Optigen 1200) to reduce ruminal ammonia concentrations compared with feed grade urea.

Cherdthong et al. (2011) in an in vitro experiment shown, urea calcium sulphate mixtures reduced ruminal NH₃ concentrations, as well as increased the cellulolytic bacterial population, when compared with urea. Slow urea release properties have been achieved by binding urea to substrates such as calcium chloride (Huntington et al., 2006; Golombeski et al., 2006).

2.3. Effect of Slow-release Urea on growth and feed efficiency

Literature available on the performance of growing heifer calves fed slow release urea / nitrogen products have been limited. Studies conducted on the growth performance of steers, lambs and kids supplemented with slow release nitrogen products have been reviewed in this section.
Duff et al. (2000) evaluated the effect of a slow-release urea product (Ruma Pro) on performance and carcass characteristics of beef steers. In Experiment 1, using 180 crossbred steers evaluated a 90 per cent concentrate diet with Ruma Pro as the supplemental CP source vs. a 90 per cent concentrate diet (Control) with soybean meal (SBM) plus urea (1.21 per cent on DM basis) as the supplemental CP sources. No differences were noted for average daily gain (ADG) for the overall feeding period. Steers fed Ruma Pro consumed 3 per cent less feed than Control steers. They reported that gain:feed ratio improved (P < 0.01) for steers fed Ruma Pro vs. Control for the overall feeding period. In another experiment they used 226 crossbred steers to evaluate the effects of graded levels of Ruma Pro in a 90 per cent concentrate diet on performance and carcass characteristics. No differences were noted among treatments for ADG or daily dry matter intake (DMI) for the overall feeding period, but gain:feed improved (P < 0.05) with Ruma Pro supplementation.

Tedeschi et al. (2002) conducted two growth trials to study the performance of Angus Crossbred steers supplemented with a slow-release urea product (Optigen® 1200, O) and urea (U). In trial one 40 animals (272 ± 4 kg body weight) were individually fed with the base diets and six treatments, corn silage alone and cracked corn supplemented with U or O to supply 50 or 100 per cent of the ruminal N deficiency (U,50; O,50; U,100; O,100 and U25,O25). In another trial, they fed 120 animals (241 ± 7 kg BW) with base diets and four combinations of U and O (U,100 and O,0; U, 66 and O,34; U,34 and O,66; and U,0 and O,100), which were designed to supply 100 per cent of the ruminal N. In trial 1 they observed no differences in performance between the U,100 and O,100 treatments, but animals in the U,50 treatment had a greater ADG (P < 0.05).
and feed conversion (P < 0.05) than animals on O,50 treatment. In trial 2, combinations of U and O did not affect animal performance. They observed no improvement in animal performance when urea was substituted by a slow-release urea / NH₃ product at levels normally found in feedlot cattle diets.

Galina et al. (2004a) evaluated the effect of a slow-intake urea supplementation (SIUS) using 162 growing Alpine kids (16 kg BW) fed corn stubble (CS) or alfalfa hay (AH) with a balanced concentrate for 150 days. The DMI, organic matter intake, apparent digestibility and BW gains were similar for 150 days for the two experimental diets fed goats. Growth in 150 days averaged 112±23 g gain per day for CS / SIUS group compared to 86±21 g per day for the AH balanced diet group without SIUS (P < 0.05). Digestibility and intake improved in kids supplemented with SIUS, and they had higher weight gains over the AH based diet kids. They suggested that improvement was apparently due to elevation of rumen pH and augmentation of fiber fermentation by ruminal bacteria through offering of non-protein N, essential amino acids, extra sulphur, phosphorus and continuous ammonia supply in the rumen.

Galina et al. (2004b) fed 80 Rambouillet lambs (15.9 ± 0.35 kg BW) with 1000 g corn stubble (CS) per day, 200 g alfalfa hay (AH) per day, and 200 g of a slow-intake urea supplement (SIUS) per day, and another group of 80 Rambouillet lambs with 800 g AH per day and 600 g with 18 per cent CP balanced concentrate (BC) per day for 90 days. They observed that body weight gain was 351 g per day (±46) for SIUS compared to 315 g per day (±58) for the AH/BC diet (P < 0.05). Results showed that a non-protein
N (NPN) slow-intake supplementation could improve ruminal conditions with high fiber forages utilization.

Edwards *et al.* (2008) conducted an experiment using 180 Angus-cross steers (330 ± 2.3 kg) fed corn silage-based diets supplemented with urea or SRU for 56 days to evaluate the effects on feed intake, body weight gain, and gain : feed ratio. They fed no supplemental urea (control) or urea or SRU at 0.4, 0.8, 1.2, or 1.6 per cent of DM as treatments. They found that Slow-release urea did not affect ADG, DMI, or gain : feed ratio when supplemented at 0.8 or 1.2 per cent of DMI, but decreased (0.4 per cent) or increased (1.6 per cent) concentrations of supplementation of SRU reduced ADG and gain : feed ratio without substantial changes in DMI. These interactions of concentration and source of urea were not observed during the first 28 days of the feeding trial but did become significant during days 29 to 56 and they concluded that SRU can provide equal performance to urea supplements without the potential hazards associated with feed grade urea.

Rodriguez *et al.* (2010) evaluated the effects of a slow-release polymer-coated urea product (CU), fed at 1 per cent of the DM of diet and studied the growth performance using 20 beef steers (330±20 kg) for a period of 48 days duration. They found that daily gain, feed efficiency and carcass dressing were not affected by CU and concluded that CU can replace soybean meal in diets for beef steers without any negative effect on growth performance and further they suggested that N from the CU diet could be degraded faster than N from control diet with soybean meal, but probably slower than common urea.
2.4. Effect of SRNP on the Yield and Composition of milk.

Van Horn and Mudd (1971) showed no differences in milk yields, milk fat content or feed intakes in cows fed dry or liquid supplements (liquid supplement contained urea, minerals and molasses) containing urea.

Shiehzadeh and Harbers (1974) reported that utilization of urea in high roughage rations improved when urea is properly extruded with starch over that of prilled urea. Results showed that cows receiving either soybean meal or Starea as the protein supplement in their grain ration consumed more grain and produced more milk than those receiving urea.

Tedeschi et al. (2002) fed a polymer-coated slow urea (Optigen 1200) and found an improvement in feed efficiency and this would be expected because cows consumed less DM and there was no drop in daily milk yield for cows fed SU. Optigen 1200 also had a beneficial effect on the fat yield of milk and also protein yield of milk was enhanced. However, the addition of SU had no overall impact on milk component composition.

Galo et al. (2003) reported that feeding lactating dairy cows with a diet including 0.77 per cent polymer-coated urea had no impact on milk production. The diets did not affect milk fat percentage or milk true protein percentage. Fat content of the milk approximated 3.7 per cent across diets and is an indicator of good rumen fermentation and fiber digestion.
Golombeski et al. (2006) compared two diets containing nitrogen sources either as slow urea diet or no slow urea diet, which partially replaced soybean meal. Dietary treatment had no effect on energy-corrected milk, milk fat yield, milk protein percentage, or milk urea N. Replacement of soybean meal with slow release urea did not alter true protein percentage or yield demonstrating that slow urea can be an alternative source of N in dairy cow diets without causing inefficient use of N.

Santos and Huber (2008) reported that milk yield was unaffected when SBM was partially replaced by Optigen and when uncoated prilled urea plus RUP sources were partially replaced by a polymer coated prilled urea product.

Inostroza et al. (2010) carried out an experiment to determine the effect of Optigen, as a source of NPN on milk yield, milk composition, and milk component yields. There have been reports of increased milk yield when Optigen partially replaced either uncoated prilled urea (Tikofsky and Harrison, 2007) or an oilseed meal mixture in dairy cattle diets. Yields of milk fat, milk protein, and milk protein percentage were unaffected by treatment.

Highstreet et al. (2010) tested a slowly rumen released encapsulated urea product (Nitroshure) which is 0.9 unit urea and 0.1 unit fat according to the manufacturer. Feeding a slowly rumen released urea increased milk fat, protein and energy output in early lactation high producing dairy cows fed a diet high in soluble N, versus feeding an equivalent amount of urea on an N basis, but that it had little impact in mid-lactation cows.
Xin et al. (2010) found no effect of dietary treatment on milk fat, lactose content, milk yield and energy-corrected milk yield. With respect to the content of milk protein, significant difference among the dietary treatments was detected (P<0.04). Cows consuming polymer coated urea and soybean meal diets had similar milk protein concentration, both being greater than those fed the feed grade urea diet. Milk protein percentage and yield were higher for cows receiving the polymer coated urea diet than the feed grade urea diet but were similar to the SBM diet.

2.5. Pattern of ammonia release from slow release nitrogen compound

Davis and Stallcup (1967) observed an increased alkaline rumen pH when Urea was fed due to the high concentration of ammonia and the low VFA production. SBM and Starea were associated with high VFA production and with more acid rumen pH.

Oltjen et al. (1968) evaluated Urea, Biuret, Urea Phosphate and Uric acid as NPN sources for Cattle. They reported that ruminal ammonia concentration 4 hours after feeding was lower when steers consumed the biuret diet compared with the urea phosphate (P<0.01), uric acid (P<0.01) and urea (P<0.05) diets.

Williams et al. (1968) reported a greater difference in ammonia production between urea and starea supplements at 60 and 120 min after feeding but found that concentrations from both regressed at a nearly equal rate thereafter.

Stiles et al. (1970) studied the effects of Starea on ammonia toxicity in cattle. Starea was compared with grain that had been cracked, finely ground and pelleted or expanded. All diets contained 57 per cent urea. Two sets of rumen fistulated twins were
used. The rumen ammonia concentration of the Starea-fed animals was lower than the controls.

Thompson et al. (1972) found a peak rumen \( \text{NH}_3 \)-N for Starea rations after 90 min of feeding. Although rumen \( \text{NH}_3 \)-N values for Urea and Starea were not significantly different at either 1 or 2 h, the decline in \( \text{NH}_3 \)-N from 1 to 2 h was 12.8 mg per cent for Urea and 6.4 mg per cent for Starea, suggesting a slower hydrolysis for Starea than Urea rations, which is in agreement with Stiles et al. (1970) and Schmidt et al. (1973).

Schmidt et al. (1973) in a study consisting of 36 Hereford steers fed finger millet treated urea and reported that at 1.5 h sampling time, ruminal ammonia levels were not different for animals fed urea or Starea, however, at 3 h the ammonia concentrations in animals fed starea were higher than that of the urea fed group, perhaps indicating that Starea was hydrolyzed slower than urea.

Tiwari et al. (1973) conducted \textit{in vitro} and \textit{in vivo} experiments to determine if solubility or biuretolysis rate limits ammonia release. Biuretolysis rate and insolubility, of nitrogen was found to be responsible for slow degradation of biuret in the rumen.

Huston et al. (1974) developed a slow release urea pelleted product using a mixture of urea, corn starch and carboxy resin. An \textit{in vivo} experiment was carried out to determine the slow release properties of this mixture. Unlike prilled urea, which was totally dissolved within a few minutes, urea from these slow-release pellets was 50 per cent dissolved after 2 h, 80 per cent after 4 h and near 100 per cent after 8 h in the rumen.
The lambs fed slow release urea retained 55 per cent of the N compared to 38 per cent for urea.

Ørskov et al. (1974) conducted a metabolism trial to determine the effect of impregnation of whole barley with urea on the rate of rumen ammonia release and voluntary intake of growing lambs. Treatment 1 was barley alone; treatment 2 was barley with 2 per cent absorbed urea. Treatment 3 was barley with 2 per cent urea crystals adhered to the surface of the grain with the aid of 0.25 per cent liquid molasses. The ammonia concentration was greater with the whole barley diet when urea was adhered to the grain than when urea was absorbed into the grain.

Fishwick (1978) reported on the effects of coating prill urea with sulfur and wax on N utilization by ruminants. Two metabolism trials using cattle and sheep were conducted to evaluate two sulfur coated urea products, one with 324 g of N per kg coated with 277 g of S per kg and the other with 342 g N per Kg coated with 236 g of S per kg. Ruminal ammonia concentrations were higher in cattle fed urea than for cattle fed either of the sulfur coated products. Examination of the rumen contents of the cows given these two sulfur coated products indicated that intact and partially intact sulfur coated urea particles were still present after 24 h.

Males et al. (1979) tested a liquid molasses slow release product and studied the rate of ammonia nitrogen release from corn urea and molasses urea, two gelatinised starch urea products one with 64.5 per cent CP ; 40 to 50 per cent starch damage, and the other with 66 per cent CP and 98 to 100 per cent starch damage, and a commercial slow release molasses liquid supplement with 32 per cent CP. The slow release molasses
liquid supplement had an initial rate of NH$_3$-N release that was faster than molasses urea; however only 40 per cent of the total N was released as NH$_3$ from the slow release molasses liquid supplement resulting in less NH$_3$ accumulation. Rumen fluid pH did not vary drastically.

Owens et al. (1980) compared a "slow- release" urea supplement with prilled feed grade urea. The data indicated that the ammonia concentration in cattle rumen was 53 mg per dl with prilled urea, and 32 mg per dl with the "slow- release" urea. This represented a 40 per cent reduction of rumen ammonia. Ammonia concentration at 3 hours of incubation was reduced by 68 per cent when a slow release urea product (Optigen 1200) replaced urea as the NPN supplement.

Owens et al. (1980) evaluated a slow release urea (prilled urea coated with a tung oil-linseed oil talc-catalyst mixture) product for ammonia nitrogen release rate. They reported that ruminal ammonia release was slower for an oil coated prilled urea product than for uncoated prilled urea, thereby increasing diet acceptability and reducing urea toxicity in steers. Totality of release was evaluated by measuring ruminal ammonia of steers fed prilled urea or slow release urea at 2 h intervals and both produced virtually equal ruminal ammonia concentration. This observation suggests that an equal amount of ammonia nitrogen was eventually released from both SRU and prilled urea. Alternatively, SRU degradation or ammonia absorption and/or utilization may differ.

Cass et al. (1994) made a comparison in an in vitro study and observed that a urea-calcium combination produced slower ammonia nitrogen release rate than regular urea. It was inferred that slow-release urea diets prolong microbial utilization of
additional nitrogen sources during ruminal fermentation. Therefore, the synchronization between ruminal ammonia release and carbohydrate availability might be improved, consequently resulting in greater microbial protein synthesis.

Recently, Galo et al. (2003) fed a polymer-coated urea to dairy cows and observed coated urea is more slowly hydrolyzed to ammonia than unprotected urea and could potentially be used more efficiently by rumen microorganisms. The report in which urea release from a polymer-coated urea was 83 per cent as extensive as uncoated urea after 1h incubation with distilled water. Other products, such as a urea-calcium combination had similar effects.

Golombeski et al. (2006) compared two diets containing nitrogen sources either as slow urea diet or no slow urea diet, which partially replaced soybean meal. Ruminal ammonia N did not differ across diets, suggesting that slow urea (0.61 per cent Ruma Pro, XF Enterprises, Hereford, TX), did exhibit slow-release properties as demonstrated by Huntington et al. (2006).

More recently, Huntington et al. (2006) concluded that urea-calcium was effective in mitigating rapid ammonia release in the rumen and subsequent effects on ammonia metabolism. Slow-release urea and other similar products attempt to achieve a slower rate of N release in the rumen and allow time to use N more efficiently while preventing ammonia toxicity.

Gonzalez et al. (2007) reported lower ruminal and plasma ammonia concentrations for steers fed Optigen than for steers fed uncoated prilled urea; the in situ
rate of nitrogen disappearance for Optigen was 0.237 per cent per hour. These authors concluded that Optigen is a ruminally protected source of NPN with controlled-release characteristics in the rumen, which is in agreement with the report by Stewart et al. (2008).

Edwards et al. (2008) conducted an experiment to evaluate the effects of slow-release urea versus feed-grade urea on ruminal metabolite characteristics and concluded that slow release urea reduces the rapidity of ammonia release in the rumen without affecting other ruminal fermentation metabolites. After 35 d of feeding there was no evidence of microbial adaptation to the slow release urea product, suggesting that the slow release urea will continue to maintain slow release properties for long term feeding regimes. These experiments demonstrate that slow release urea can be utilized as N supplement to modulate the appearance of N in the rumen and can provide equal performance to urea supplements without the potential hazards associated with feed-grade urea.

Rodriguez et al. (2010) conducted an experiment with 20 animals to compare a diet containing slow-release coated urea product (Optigen 1200, Alltech Inc. Nicholasville, KY USA) with a Control diet (standard diet with soybean meal as protein source); The coated urea did not affect ruminal fermentation patterns (i.e, pH and AGV) as compared to the control diet, which agree with previous studies using the same coated urea product (Gonzalez et al., 2007; Harrison et al., 2007). Ruminal ammonia N was higher (P<0.05) with coated urea than the control diet.
Xin et al. (2010) studied the ammonia-N concentrations of the three dietary treatments (diets had three N sources including polyurethane-coated urea, feed-grade urea and isolated soy protein) during 8 h fermentation *in vitro*. In this study, the ammonia-N concentrations of all the diets increased within 1 h, and then declined gradually, however, the polymer coated urea diet resulted in the lowest concentrations of ammonia-N at all time points. During 8 h *in vitro* fermentation, the polymer coated urea diet decreased ammonia-N concentration by 8.2 to 20.6 per cent as compared with the feed grade urea diet. This agrees with the result of Prokop and Klopfenstein (1977), in which it is found that slow-release urea (combination of urea and formaldehyde), could decrease ruminal ammonia-N concentration by 25.3 per cent compared to urea. No significant differences were found between polymer coated urea and soy protein diets on ruminal ammonia release.

Cherdthong et al. (2011) conducted an experiment with four ruminally fistulated crossbred (Brahman×native) beef cattle steers. *In vitro* incubations of urea, urea CaCl₂ mixture and urea CaSO₄ mixture showed the slower release rates of urea in both urea CaCl₂ mixture and urea CaSO₄ mixture versus uncoated Urea and the rumen pH was unaffected by the different nitrogen sources.

2.6. Effect of SRNP on feed intake and digestibility

Karr et al. (1965) conducted a series of metabolism experiments, a feeding experiment and a fermentation study to compare urea and biuret as nitrogen sources in a basal ensiled diet using lambs. Addition of biuret to the basal mixture significantly increased nitrogen retention by 1.25 g in two of the three experiments, while the addition
of urea consistently lowers nitrogen retention. There were no differences (both tended to be higher) in DM digestibility co-efficients when either urea or biuret was added to the basal.

Campbell et al. (1963) from their experiment to compare urea and biuret reported similar CF digestibilities by adapted animals fed natural diets containing urea and biuret.

Oltjen et al. (1968) conducted an experiment using eight 214 kg Angus steers fed a standardization diet containing 85 per cent timothy hay supplemented with soybean meal for 21 days. The steers were then abruptly changed either to the urea or biuret diet in a single feeding. The NPN sources contributed about 50 per cent of the total nitrogen to each diet and the steers were fed these diets at the rate of 2 per cent of the body weight of each steer. The apparent digestibility of DM and acid detergent fibre was not influenced by NPN source.

Ward and Cullison (1970) fed prilled urea and ethyl cellulose-coated urea to ewes in a toxicity study showed that coating of urea improved acceptability of diet against urea in the diet. They observed that the coated urea was non toxic when fed at the same level as a toxic amount of uncoated urea.

Stiles et al. (1970) studied the effects of Starea on ammonia toxicity in cattle. Starea was compared with grain that had been cracked, finely ground and pelleted or expanded. All diets contained 57 per cent urea. Two sets of rumen fistulated twins were used. The diet containing Starea was the only diet that was readily consumed. Palatability problems were encountered with other three diets.
Thompson et al. (1972) conducted an experiment to compare the urea, starea, urea with sulphur and starea with sulphur supplemented finishing rations. With Starea supplementation feed conversion was intermediate and palatability of rations containing starea was apparently improved compared to urea rations since steers fed starea adjusted to full feed earlier and consumed slightly more feed throughout the feeding trials and they reported that replacing urea with a slow-release urea source had not affected DMI as stated in past experiments.

Ørskov et al. (1974) developed a method of including urea in whole grains and a metabolism trial was conducted to determine the effect of impregnation of whole barley with urea on the voluntary Intake of growing lambs. Treatment 1 was barley alone; treatment 2 was barley with 2 per cent absorbed urea and treatment 3 was barley with 2 per cent urea crystals adhered to the surface of the grain with the aid of 0.25 per cent liquid molasses. The effect of feeding whole barley with urea absorbed into it resulted in an increased voluntary intake of lambs over feeding whole barley with 2 per cent urea crystals.

Shiehzadeh and Harbers (1974) reported that utilization of urea in high roughage rations improved when urea is properly extruded with starch over that of prill urea. Starea was found to be utilized equally as well as soybean meal in lactating dairy cows. Results showed that cows receiving either soybean meal or Starea as protein supplement in their grain ration consumed more grain and produced more milk than those receiving urea. Grain intakes were lower for the urea supplemented group in all three periods.
Sheihzadeh and Harbers (1974) designed experiments to compare several extruded starch-urea products with soybean meal and urea in high-roughage lamb rations with 25 crossbred lambs and found an increased feed efficiency with extruded starch-urea products. Slightly more feed was consumed by lambs fed extruded supplements than urea, indicating some improvement in palatability.

Summers and Sherrod (1974) conducted two stage *in vitro* experiments on samples of corn and sorghum grains. Four treatments were used: T1 (control corn + 1.3 per cent added urea), T2 (dry urea (1.3 per cent) impregnated corn), T3 (control sorghum + 1.25 per cent added urea) and T4 (dry urea (1.25 per cent) impregnated sorghum). *In vitro* dry matter disappearance for both T2 and T4 were greater than the control grains. The results indicate that there is a definite increase in IVDMD when corn and sorghum grains are impregnated with urea over adding free urea. An increase in IVDMD could be the result of slower ammonia release in the rumen from the impregnated grains.

Owens *et al.* (1980) evaluated a slow release urea product and stated that dry matter digestibility and nitrogen retention values tended to be same between prilled urea and slow release urea supplements. Digestible dry matter intake (DMI) was greatest with slow release urea when compared to urea treatment. Sheep fed *ad libitum* cottonseed hulls were offered a supplement containing 5 per cent or 10 per cent urea from urea or slow release urea product once daily. Intake of slow release urea supplement was 7 and 17 per cent greater indicating slow release urea is quite palatable when compared to urea.

Cass *et al.* (1994) conducted a metabolism experiment with 12 wethers to determine the nutritional value of two urea calcium compounds. The compounds were
composed of 23-0-0-7 and 10-0-0-11 N, P, K and Ca, respectively. These two compounds were compared to feed grade urea and cotton seed meal for DM intake, over all diet digestibilities of DM and crude protein, nitrogen utilization, and calcium utilization. Digestibilities of DM and crude protein were similar across all the treatments though the feed intake and nitrogen intake were lower for 10-0-0-11 treatment compared to urea, 23-0-0-7, and the cotton seed meal treatments. By this it was concluded that compounds that contain a mixture of urea and calcium could be well utilized in ruminant diets as an NPN source.

Cass et al. (1995) conducted a feedlot experiment with growing and finishing crossbred steers to determine the effects of a slow ammonia release combined urea calcium compound compared to isonitrogenous diets containing feed grade urea or cottonseed meal. Both tended to improve feed efficiency, however, the extent of improvement was greater for feed efficiency when combined urea calcium compound was supplemented. Energetic efficiency was also improved with combined urea calcium diet.

Duff et al. (2000) evaluated the effect of a slow-release urea product (Ruma Pro) on performance of animals. Treatments used in the study included a standard finishing diet with slow-release urea (Ruma Pro) replacing a combination of natural protein and urea in standard diet. For the overall feeding period, daily DMI tended to be less by Ruma Pro fed cattle vs. control cattle and also gain: feed was improved for steers fed Ruma Pro vs. Control.
Tedeschi *et al.* (2002) noted an improvement in feed conversion when Optigen 1200 was substituted for urea at levels normally found in feedlot diets. This improvement would be expected because cows consumed less DM and there was no drop in daily milk yield for cows fed slow urea. Replacement of soybean meal with slow urea (Optigen 1200) significantly improved feed efficiency.

Galo *et al.* (2003) formulated diets by including coated urea in the total mixed ration. The three diets T1 (CP18 without coated urea) and T2 (CP18 with coated urea) were formulated to be isonitrogenous, and T3 (CP16 with coated urea) was formulated to contain more forage (55 per cent vs. 50 per cent) and have less nitrogen to test for efficiency of N utilization. Total tract apparent DM and CP digestibilities were higher for T2 when compared to T1. Apparent ADF digestibility was lower for both T2 and T3. Total DMI and apparent digestibilities for other feed components (organic matter, NDF, starch, sugar, and NSC) did not differ across diets.

Golombeski *et al.* (2006) in an experiment with 12 lactating Brown Swiss cows were fed diets with either no slow urea or with slow urea (0.61 per cent Ruma Pro) as a nitrogen source, which partially replaced soybean meal. Feeding slow urea decreased the dry matter intake and increased feed efficiency compared with cows fed no slow urea diet. No studies have reported feed efficiencies for dairy cows fed slow urea. In research using feedlot steers, diets containing calcium chloride-bound slow urea have been shown to improve feed efficiency (*Cass et al.*, 1995; *Duff et al.*, 2000). Not all slow urea products are the same.
Edwards *et al.* (2009) stated that although apparent total tract digestibilities of DM, OM, NDF and ADF were not affected, the treatment with slow release urea increased fecal N excretion and reduced apparent total tract N digestibility. Intake of DM, OM, NDF, and ADF did not differ among treatments and there were no detrimental effects on DM and fiber digestibility associated with feeding a slow release urea.

Highstreet *et al.* (2010) conducted an experiment with an objective to determine if use of a slowly rumen released urea could increase productive performance. Total mixed rations formulated to supply 5 per cent of ration CP from urea or encapsulated urea. Nitroshure, a slowly rumen released encapsulated urea, which is 0.9 unit urea and 0.1 unit fat according to the manufacturer. Fecal digestibility of CP and neutral detergent fiber were unaffected by the treatment with nitroshure.

Rodriguez *et al.* (2010) conducted an experiment with 20 animals randomly assigned to the following treatments 1) Control (standard diet with soybean meal as protein source 2) a slow-release coated urea product (Optigen 1200). The DM, CP, NDF, ADF, ash and NEg were similar for experimental diets. The coated urea diet had a higher protein fraction than control and DMI was also unaffected by the treatment, as compared to the control diet.

Xin *et al.* (2010) in their experiment to compare the polymer coated urea and feed grade urea diets and found similar digestibilities of DM, OM, NDF, ADF and CP. DMI of cows fed the polymer coated urea diet was approximately 12.8 per cent greater (p<0.02) than that of the feed grade urea diet, and was similar to that of the soy protein diet.
Cherdthong et al. (2011) compared different SRU sources (urea CaCl$_2$ mixture; urea CaSO$_4$ mixture) with Urea, using ruminally fistulated animals. Rice straw, concentrate, total DM intake were not influenced by feeding the different N sources, except NDF digestibility was highest with urea CaSO$_4$ mixture supplementation, intermediate with soy protein and urea CaCl$_2$ mixture and lowest with control (P<0.05). Apparent digestibilities of DM, OM, CP and ADF were not affected by the urea calcium treatments. This finding is consistent with other experiments wherein substitution of a slow release urea product for urea did not affect DM, OM and ADF digestibilities (Highstreet et al., 2010). In contrast to these findings, a polymer-coated slow release urea was demonstrated to increase total tract DM and CP digestibilities when fed to lactating dairy cows (Galo et al., 2003).

Ribeiro et al. (2011) designed an experiment with four ruminally cannulated beef steers to compare ruminal infusion effects of different sources of non-protein nitrogen (NPN) on intake, digestibility, ruminal dry matter (DM) and neutral detergent fiber (NDF) degradability, ruminal pH, and ammonia nitrogen (N-NH$_3$) of beef cattle and observed that SRU had a more adequate concentration of N during the fermentation of low-quality hay and also had a proper pH for rumen activity.

2.7. Safety of slow release nitrogen product over urea feeding

Meiske et al. (1955) compared urea and biuret and stated that biuret does not seem to be toxic to the animal even when large amounts are given as a drench as compared to urea in the same level of feeding.
Bartley and Deyoe (1975) reported that a number of animals fed high urea diets developed toxic symptoms but that the cattle fed starea were spared from the same as a result of the reduction in solubility of NPN in that form.

Stiles et al. (1970) studied the effects of Starea on ammonia toxicity in cattle. Starea was compared with grain that had been cracked, finely ground and pelleted or expanded. All diets contained 57 per cent urea. Two sets of rumen fistulated twins were used. The diet containing Starea was the only diet that was readily consumed. Palatability problems were encountered with the other three diets. The rumen ammonia concentration of the Starea-fed animals was lower than the controls. Ammonia toxicity did not occur in animals fed Starea. Animals fed the three control diets did show symptoms of ammonia toxicity.

Ward and Cullison (1970) fed prilled urea and ethyl cellulose-coated urea to ewes in a toxicity study and observed coated urea to be non toxic when fed at the same level as a toxic amount of uncoated urea.

Owens et al. (1980) prepared a slow release urea compound using tung oil. SRU and urea supplements were fed to five fasted steers. Amounts of urea of each form consumed were equal. Toxicity of slow release urea was tested by feeding steers a ration supplemented with 10 per cent prilled urea or slow release urea equal to 10 per cent prilled urea. Muscle tremors were observed in steers fed prilled urea 35 min after feeding. Ruminal ammonia concentrations at evacuation exceeded 120 mg per dl. Steers fed slow release urea exhibited no abnormalities, and rumen ammonia concentrations never
exceeded 35 mg per dl. Extrapolated from observed ammonia concentrations, an intake of 900 g urea in the slow release form would be required for toxicity.

Edwards *et al.* (2009) conducted an experiment to evaluate the effects of slow-release urea versus feed-grade urea on ruminal metabolite characteristics, daily gain and DMI in steers. Slow release urea can be utilized as an N supplement to modulate the appearance of N in the rumen and can provide equal performance to urea supplements without the potential hazards associated with feed-grade urea.

Literature reviewed in this chapter has revealed that numerous products of slow release nitrogen / urea have been developed considering the safety of using urea in the dairy cattle diets and improve the efficiency of N utilization in ruminants. Great deal of work has been done to utilize these in the diet of ruminants to improve rumen fermentation, digestibility and lactational performance. However, not much success has been achieved in terms of improved lactational performance by using slow nitrogen release products. Further studies are required to elucidate the lack of response in lactation performance by dairy cows after the feeding of these slow release nitrogen product.
Fig. 2.1: Schematic model describing the metabolism of dietary protein and NPN in ruminants (Adapted from Leng and Nolan., 1984)