1. Introduction

1.1 Difinition of Urea:

Urea or **carbamide** is an organic compound with the chemical formula $CO(NH_2)_2$. The molecule has two —NH_2 groups joined by a carbonyl (C=O) functional group.

Urea serves an important role in the metabolism of nitrogen-containing compounds by animals and is the main nitrogen-containing substance in the urine of mammals. It is a colorless, odorless solid, highly soluble in water and practically non-toxic (LD_{50} is 15 g/kg for rat). Dissolved in water, it is neither acidic nor alkaline. The body uses it in many processes, the most notable one being nitrogen excretion. Urea is widely used in fertilizers as a convenient source of nitrogen. Urea is also an important raw material for the chemical industry.

The discovery by Friedrich Wöhler in 1828 that urea can be produced from inorganic starting materials was an important conceptual milestone in chemistry, as it showed for the first time that a substance previously known only as a byproduct of life could be synthesized in the laboratory without any biological starting materials, contradicting the widely held doctrine of vitalism.

1.2 Related compounds:-

The terms urea is also used for a *class* of chemical compounds sharing the same functional group RR'N—CO—NRR', namely a carbonyl group attached to two organic amine residues. Examples include carbamide peroxide, allantoin, and hydantoin. Ureas are closely related to biurets and

related in structure to amides, carbamates, carbodiimides, and thiocarbamides.

1.3 History:-

Urea was first discovered in urine in 1727 by the Dutch scientist Herman Boerhaave,[3] though this discovery is often attributed to the French chemist Hilaire Rouelle.

In 1828, the German chemist Friedrich Wöhler obtained urea artificially by treating silver cyanate with ammonium chloride.

- $AgNCO + NH_4Cl \rightarrow (NH_2)_2CO + AgCl$

This was the first time an organic compound was artificially synthesized from inorganic starting materials, without the involvement of living organisms. The results of this experiment implicitly discredited vitalism — the theory that the chemicals of living organisms are fundamentally different from those of inanimate matter. This insight was important for the development of organic chemistry. His discovery prompted Wöhler to write triumphantly to Berzelius: "I must tell you that I can make urea without the use of kidneys, either man or dog. Ammonium cyanate is urea." For this discovery, Wöhler is considered by many the father of organic chemistry.

1.4 Physiology:-

Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia. In this cycle, amino groups donated by ammonia and L-aspartate are converted to urea, while L-ornithine, citrulline, L-argininosuccinate, and L-arginine act as intermediates. Urea production occurs in the liver and is regulated by N-acetylglutamate. Urea is then dissolved into the blood (in the reference range of 2.5 to 6.7 mmol/liter) and further transported and excreted by the kidney as a component of urine. In addition, a small amount of urea is excreted (along with sodium chloride and water) in sweat.

Amino acids from ingested food that are not used for the synthesis of proteins and other biological substances are oxidized by the body, yielding urea and carbon dioxide, as an alternative source of energy.[8] The oxidation pathway starts with the removal of the amino group by a transaminase; the amino group is then fed into the urea cycle.

Ammonia (NH_3) is another common byproduct of the metabolism of nitrogenous compounds. Ammonia is smaller, more volatile and more mobile than urea. If allowed to accumulate, ammonia would raise the pH in cells to toxic levels. Therefore many organisms convert ammonia to urea, even though this synthesis has a net energy cost. Being practically neutral and highly soluble in water, urea is a safe vehicle for the body to transport and excrete excess nitrogen.

In water, the amine groups undergo slow displacement by water molecules, producing ammonia, ammonium ion, and bicarbonate ion. For this reason, old, stale urine has a stronger odor than fresh urine.

1.5 In humans:-

The handling of urea by the kidneys is a vital part of mammalian metabolism. Besides its role as carrier of waste nitrogen, urea also plays a role in the countercurrent exchange system of the nephrons, that allows for re-absorption of water and critical ions from the excreted urine. Urea is reabsorbed in the inner medullary collecting ducts of the nephrons, thus raising the osmolarity in the medullary interstitium surrounding the thin ascending limb of the loop of Henle, which in turn causes water to be reabsorbed. By action of the urea transporter 2, some of this reabsorbed urea will eventually flow back into the thin ascending limb of the tubule, through the collecting ducts, and into the excreted urine. This mechanism, which is controlled by the antidiuretic hormone, allows the body to create hyperosmotic urine, that has a higher concentration of dissolved substances than the blood plasma. This mechanism is important to prevent the loss of water, to maintain blood pressure, and to maintain a suitable concentration of sodium ions in the blood plasmas.

The equivalent nitrogen content (in gram) of urea (in mmol) can be estimated by the conversion factor 0.028 g/mmol. Furthermore, 1 gram of nitrogen is roughly equivalent to 6.25 grams of protein, and 1 gram of protein is roughly equivalent to 5 grams of muscle tissue. In situations such as muscle wasting, 1 mmol of excessive urea in the urine (as measured by

urine volume in litres multiplied by urea concentration in mmol/l) roughly corresponds to a muscle loss of 0.67 gram.

1.6 In other species:-

In aquatic organisms the most common form of nitrogen waste is ammonia, whereas land-dwelling organisms convert the toxic ammonia to either urea or uric acid. Urea is found in the urine of mammals and amphibians, as well as some fish. Birds and saurian reptiles have a different form of nitrogen metabolism that requires less water, and leads to nitrogen excretion in the form of uric acid. It is noteworthy that tadpoles excrete ammonia but shift to urea production during metamorphosis. Despite the generalization above, the urea pathway has been documented not only in mammals and amphibians but in many other organisms as well, including birds, invertebrates, insects, plants, yeast, fungi, and even microorganisms.

1.7 Production:-

Urea is produced on an industrial scale: In 2012, worldwide production capacity was approximately 184 million tonnes.[11]

1.7.1 Industrial methods:-

For use in industry, urea is produced from synthetic ammonia and carbon dioxide. As large quantities of carbon dioxide are produced during the ammonia manufacturing process as a byproduct from hydrocarbons (predominantly natural gas, less often petroleum derivatives), or occasionally from coal, urea production plants are almost always located adjacent to the site where the ammonia is manufactured. Although natural

gas is both the most economical and the most widely available ammonia plant feedstock, plants using it do not produce quite as much carbon dioxide from the process as is needed to convert their entire ammonia output into urea. In recent years new technologies such as the KM-CDR process have been developed to recover supplementary carbon dioxide from the combustion exhaust gases produced in the fired reforming furnace of the ammonia synthesis gas plant, allowing operators of stand-alone nitrogen fertilizer complexes to avoid the need to handle and market ammonia as a separate product and also to reduce their 'greenhouse gas' emissions to the atmosphere.

1.7.2 Synthesis:-

The basic process, developed in 1922, is also called the Bosch–Meiser urea process after its discoverers. The various commercial urea processes are characterized by the conditions under which urea formation takes place and the way in which unconverted reactants are further processed. The process consists of two main equilibrium reactions, with incomplete conversion of the reactants. The first is **carbamate formation**: the fast exothermic reaction of liquid ammonia with gaseous carbon dioxide (CO_2) at high temperature and pressure to form ammonium carbamate ($H_2N\text{-}COONH_4$)

$$2NH_3 + CO_2 \quad H_2N\text{-}COONH_4$$

The second is **urea conversion**: the slower endothermic decomposition of ammonium carbamate into urea and water:

$$H_2N\text{-}COONH_4 \quad (NH_2)_2CO + H_2O$$

The overall conversion of NH_3 and CO_2 to urea is exothermic,

the reaction heat from the first reaction driving the second. Like all chemical equilibria, these reactions behave according to Le Chatelier's principle, and the conditions that most favour carbamate formation have an unfavourable effect on the urea conversion equilibrium. The process conditions are, therefore, a compromise: the ill-effect on the first reaction of the high temperature (around 190□□°C) needed for the second is compensated for by conducting the process under high pressure (140–175 bar), which favours the first reaction. Although it is necessary to compress gaseous carbon dioxide to this pressure, the ammonia is available from the ammonia plant in liquid form, which can be pumped into the system much more economically. To allow the slow urea formation reaction time to reach equilibrium a large reaction space is needed, so the synthesis reactor in a large urea plant tends to be a massive pressure vessel.

Because the urea conversion is incomplete, the product has to be separated from unchanged ammonium carbamate. In early "straight-through" urea plants this was done by letting down the system pressure to atmospheric so as to allow the carbamate to decompose back to ammonia and carbon dioxide. Originally, because it was not economic to recompress the ammonia and carbon dioxide for recycle, the ammonia at least would be used for the manufacture of other products, for example ammonium nitrate or sulfate. (The carbon dioxide would be wasted, as likely as not.) Later process schemes were developed to allow recycling of the unused ammonia and carbon dioxide. This was accomplished by depressurizing the reaction solution in stages (first to 18–25 bar and then to 2–5 bar) and passing it at each stage through a steam-heated "carbamate decomposer", then

recombining the resultant carbon dioxide and ammonia in a falling-film "carbamate condenser" and pumping the carbamate solution into the previous stage

1.8 Chemical properties:-

Molecular and crystal structure:-

The nitrogen is pyramidal in the gas-phase minimum-energy structure. In solid urea, the oxygen center is engaged in two N-H-O hydrogen bonds. The resulting dense and energetically favorable hydrogen-bond network is probably established at the cost of efficient molecular packing: The structure is quite open, the ribbons forming tunnels with square cross-section. The carbon in urea is described as sp^2 hybridized, the C-N bonds have significant double bond character, and the carbonyl oxygen is basic compared to, say, formaldehyde. Urea's high aqueous solubility reflects its ability to engage in extensive hydrogen bonding with water.

The urea molecule is planar in the crystal structure, but the geometry aroundBy virtue of its tendency to form a porous frameworks, urea has the ability to trap many organic compounds. In these so-called clathrates, the organic "guest" molecules are held in channels formed by interpenetrating helices composed of hydrogen-bonded urea molecules. This behavior can be used to separate mixtures, e.g. in the production of aviation fuel and lubricating oils, and in the separation of hydrocarbons.

As the helices are interconnected, all helices in a crystal must have the same molecular handedness. This is determined when the crystal is nucleated and

can thus be forced by seeding. The resulting crystals have been used to separate racemic mixtures.

1.9 Uses:-

1.9.1 Agriculture

More than 90% of world industrial production of urea is destined for use as a nitrogen-release fertilizer.] Urea has the highest nitrogen content of all solid nitrogenous fertilizers in common use. Therefore, it has the lowest transportation costs per unit of nitrogen nutrient. The standard crop-nutrient rating (NPK rating) of urea is 46-0-0.

Many soil bacteria possess the enzyme urease, which catalyzes the conversion of the urea to ammonia or ammonium ion and bicarbonate ion, thus urea fertilizers are very rapidly transformed to the ammonium form in soils. Among soil bacteria known to carry urease, some ammonia-oxidizing bacteria (AOB), such as species of *Nitrosomonas*, are also able to assimilate the carbon dioxide released by the reaction to make biomass via the Calvin Cycle, and harvest energy by oxidizing ammonia (the other product of urease) to nitrite, a process termed nitrification.[39] Nitrite-oxidizing bacteria, especially *Nitrobacter*, oxidize nitrite to nitrate, which is extremely mobile in soils because of its negative charge and is a major cause of water pollution from agriculture. Ammonium and nitrate are readily absorbed by plants, and are the dominant sources of nitrogen for plant growth. Urea is also used in many multi-component solid fertilizer formulations. Urea is highly soluble in water and is, therefore, also very suitable for use in fertilizer solutions (in combination with ammonium nitrate: UAN), e.g., in

'foliar feed' fertilizers. For fertilizer use, granules are preferred over prills because of their narrower particle size distribution, which is an advantage for mechanical application.

The most common impurity of synthetic urea is biuret, which impairs plant growth.

Urea is usually spread at rates of between 40 and 300 kg/ha but rates vary. Smaller applications incur lower losses due to leaching. During summer, urea is often spread just before or during rain to minimize losses from volatilization (process wherein nitrogen is lost to the atmosphere as ammonia gas). Urea is not compatible with other fertilizers.

Because of the high nitrogen concentration in urea, it is very important to achieve an even spread. The application equipment must be correctly calibrated and properly used. Drilling must not occur on contact with or close to seed, due to the risk of germination damage. Urea dissolves in water for application as a spray or through irrigation systems.

In grain and cotton crops, urea is often applied at the time of the last cultivation before planting. In high rainfall areas and on sandy soils (where nitrogen can be lost through leaching) and where good in-season rainfall is expected, urea can be side- or top-dressed during the growing season. Top-dressing is also popular on pasture and forage crops. In cultivating sugarcane, urea is side-dressed after planting, and applied to each ratoon crop.

In irrigated crops, urea can be applied dry to the soil, or dissolved and applied through the irrigation water. Urea will dissolve in its own weight in

water, but it becomes increasingly difficult to dissolve as the concentration increases. Dissolving urea in water is endothermic, causing the temperature of the solution to fall when urea dissolves.

As a practical guide, when preparing urea solutions for fustigation (injection into irrigation lines), dissolve no more than 3 g urea per 1 L water.

In foliar sprays, urea concentrations of 0.5% – 2.0% are often used in horticultural crops. Low-biuret grades of urea are often indicated.

Urea absorbs moisture from the atmosphere and therefore is typically stored either in closed/sealed bags on pallets or, if stored in bulk, under cover with a tarpaulin. As with most solid fertilizers, storage in a cool, dry, well-ventilated area is recommended.

1.9.2 Chemical industry:-

Urea is a raw material for the manufacture of two main classes of materials: urea-formaldehyde resins and urea-melamine-formaldehyde used in marine plywood.

1.9.3 Explosive:-

Urea can be used to make urea nitrate, a high explosive that is used industrially and as part of some improvised explosive devices. It is a stabilizer in nitrocellulose explosives.

1.9.4 Automobile systems:-

Urea is used in SNCR and SCR reactions to reduce the NO_x pollutants in exhaust gases from combustion from Diesel, dual fuel, and lean-burn natural gas engines. The BlueTec system, for example, injects water-based urea solution into the exhaust system. The ammonia produced by the hydrolysis of the urea reacts with the nitrogen oxide emissions and is converted into nitrogen and water within the catalytic converter.

1.9.5 Niche:-

a. A component of animal feed, providing a relatively cheap source of nitrogen to promote growth
b. A non-corroding alternative to rock salt for road de-icing, and the hardening of ski-resort terrain park take-offs and landings
c. A flavor-enhancing additive for cigarettes
d. A main ingredient in hair removers such as Nair and Veet
e. A browning agent in factory-produced pretzels
f. An ingredient in some skin cream,[40] moisturizers, hair conditioners
g. A reactant in some ready-to-use cold compresses for first-aid use, due to the endothermic reaction it creates when mixed with water
h. A cloud seeding agent, along with other salts
i. A flame-proofing agent, commonly used in dry chemical fire extinguisher charges such as the urea-potassium bicarbonate mixture
j. An ingredient in many tooth whitening products
k. An ingredient in dish soap
l. Along with ammonium phosphate, as a yeast nutrient, for fermentation of sugars into ethanol

m. A nutrient used by plankton in ocean nourishment experiments for geoengineering purposes

n. As an additive to extend the working temperature and open time of hide glue

o. As a solubility-enhancing and moisture-retaining additive to dye baths for textile dyeing or printing

1.9.6 Laboratory uses:-

Urea in concentrations up to 10 M is a powerful protein denaturant as it disrupts the no covalent bonds in the proteins. This property can be exploited to increase the solubility of some proteins. A mixture of urea and choline chloride is used as a deep eutectic solvent, a type of ionic liquid.

Urea can in principle serve as a hydrogen source for subsequent power generation in fuel cells. Urea present in urine/wastewater can be used directly (though bacteria normally quickly degrade urea.) Producing hydrogen by electrolysis of urea solution occurs at a lower voltage (0.37V) and thus consumes less energy than the electrolysis of water (1.2V).[41]

Urea in concentrations up to 8 M can be used to make fixed brain tissue transparent to visible light while still preserving fluorescent signals from labeled cells. This allows for much deeper imaging of neuronal processes then previously obtainable using conventional one photon or two photon confocal microscopes.[42]

1.9.7 Medical use:-

Urea-containing creams are used as topical dermatological products to promote rehydration of the skin. Urea 40% is indicated for psoriasis, xerosis, onychomycosis, ichthyosis, eczema, keratosis, keratoderma, corns, and calluses. If covered by an occlusive dressing, 40% urea preparations may also be used for nonsurgical debridement of nails. Urea 40% "dissolves the intercellular matrix"[43] of the nail plate. Only diseased or dystrophic nails are removed, as there is no effect on healthy portions of the nail.[*citation needed*] This drug is also used as an earwax removal aid.[*citation needed*]

Urea can also be used as a diuretic. It was first used as a diuretic by a Dr. W. Friedrich in 1892. [44] In a 2010 study of ICU patients in Belgium, urea was used as a diuretic to treat euvolemic hyponatremia and was found to be a safe, inexpensive and simple treatment. [45]

Certain types of instant cold packs (or ice packs) contain water and separated urea crystals. Rupturing the internal water bag starts an endothermic reaction and allows the pack to be used to reduce swelling.[*citation needed*]

Like saline, urea injection is used to perform abortion.[*citation needed*]

Urea is the main component of an alternative medicinal treatment referred to as urine therapy.[*citation needed*]

The blood urea nitrogen (BUN) test is a measure of the amount of nitrogen in the blood that comes from urea. It is used as a marker of renal function,

though it is inferior to other markers such as creatinine because blood urea levels are influenced by other factors such as diet and dehydration.[46]

Urea labeled with carbon-14 or carbon-13 is used in the urea breath test, which is used to detect the presence of the bacteria *Helicobacter pylori* (*H. pylori*) in the stomach and duodenum of humans, associated with peptic ulcers. The test detects the characteristic enzyme urease, produced by *H. pylori*, by a reaction that produces ammonia from urea. This increases the pH (reduces acidity) of the stomach environment around the bacteria. Similar bacteria species to *H. pylori* can be identified by the same test in animals such as apes, dogs, and cats (including big cats).

1.10 Urea and nonprotein nitrogen (NPN) compounds for Cattle and Sheep:

1.10.1 Quick Facts...

a. Urea can be fed to ruminants as an economical replacement for a part of the protein in a ration.

b. The amount of urea a ruminant animal can use depends on the digestible energy or total digestible nutrients (TDN) content of the ration.

c. No more than 0.1 to 0.25 pound urea per head per day should be fed to feedlot cattle consuming a high concentrate ration.

d. Toxicity should not be a problem if urea is fed according to recommendations.

e. Vinegar is a helpful emergency treatment for urea poisoning if the animal is treated before tetany develops.

Many years ago, researchers recognized that nonprotein nitrogen (NPN) compounds are used by bacteria in the rumen of cattle and sheep. Since that time, studies show that these compounds are broken down to ammonia during the normal fermentation process in the rumen. Microorganisms in the rumen combine the ammonia with products of carbohydrate metabolism to form amino acids and hence, proteins. The proteins formed in this manner (from NPN compounds) are similar in amino acid content to the proteins available to the animal when the principal source of dietary nitrogen is intact protein.

The bacteria and protozoa, and the protein they contain, are digested by the animal farther on in the digestive tract. In this manner, the ruminant animal

makes use of certain NPN compounds even though it does not possess enzymes of its own for their breakdown. Animals with simple stomachs (pigs and chickens) cannot make use of large concentrations of NPN compounds because of a lack of enzymes and bacteria to break down the NPN to ammonia and synthesize it into protein.

Many common feedstuffs fed to livestock contain some NPN. Forages generally are higher in NPN than are concentrates. Corn silage may contain as much as 50 percent of its total nitrogen as NPN. Alfalfa hay may contain 10 to 20 percent of the nitrogen in this form. Since many feeds contain some NPN, it is not a foreign substance in ruminant rations.

1.11 Commercial Sources of NPN:-

The most common NPN source used in ruminant feeding is urea. Many other products have been used experimentally and commercially, but most of them do not compare favorably to urea, because of greater toxicity, higher cost or lower palatability.

1.11.1 Ammoniated products:-

Many low-protein feeds and by-products of the milling industry have been ammoniated and fed as sources of nitrogen for ruminants. Examples are ammoniated molasses, ammoniated condensed distillers' molasses solubles, ammoniated citrus pulp, ammoniated beet pulp and ammoniated furfural (bran-type) residue. These products generally have been found to be less satisfactory than urea as a protein substitute. In some instances they have been more toxic and less palatable than urea. They cannot be stored for a

great length of time, especially under moist conditions, because much of the ammonia will be lost as a gas.

1.11.2 Ammonium salts:-

Diammonium phosphate (DAP) and monoammonium phosphate (MAP) are two ammonium salts that show promise as sources of NPN and phosphorus. Research conducted at the Oklahoma Agricultural Experiment Station indicated that DAP was a satisfactory source of phosphorus, but its nitrogen was not retained as well by sheep as that supplied by urea. Rations containing DAP also were less palatable than those containing urea because of ammonia losses when the feed came in contact with water or saliva. Monoammonium phosphate is more stable and palatable than DAP and is a good source of both nitrogen and phosphorus.

1.11.3 Urea:-

Urea is a simple compound that contains 46.7 percent nitrogen. It is found in many plants and is a normal end product of protein metabolism in mammals. A part of the urea produced in the animal's body is returned to the digestive tract in the saliva and by absorption through the rumen wall. The remainder of the urea is passed off in the urine as waste.

One pound of pure urea furnishes as much nitrogen as 2.92 pounds of protein (protein equivalent of 292 percent). The feed grade of urea has other ingredients, such as kaolin, wheat middlings, rice hulls or limestone, added to it to prevent caking and lumping. This material lowers the protein

equivalent to 281, 283, 287 or 262 percent, respectively, depending on the amount added. The 281 urea is the most common.

1.12 Urea Is a Protein Replacement:-

Urea is not necessary in the diet of ruminant animals; it is fed as a replacement for a part of the protein in a ration. Whether it is used is a matter of the cost of urea in relation to high protein feeds. When plant protein feeds, such as soybean meal, are high priced, it is economical to use urea as a protein supplement in ruminant rations. If sufficient protein is furnished by homegrown feeds, feed costs will not be lowered if urea is added.

Using the protein equivalent of 281 percent, 13.5 pounds of urea and 86.5 pounds of corn or similar grain are equal in protein and energy value to 100 pounds of 44-percent protein soybean meal or similar protein supplement for ruminant animals. The cost of the urea-corn mixture normally would be less than the cost of soybean meal, and the use of urea obviously would reduce protein supplement costs.

1.13 Factors Influencing Urea Utilization:-

Source of readily available carbohydrates**.** The single most important factor influencing the amount of urea a ruminant animal can use is the digestible energy or total digestible nutrients (TDN) content of the ration. Rations high in digestible energy (high grain) result in good urea utilization; those that are low in digestible energy (high forage) result in a lowered utilization of urea. The addition to a high forage ration of any feed that will increase TDN will

improve urea utilization. Utilization of urea by animals fed high forage rations will be improved by the addition of grain or molasses. Molasses will ot improve the utilization of urea when high grain rations are fed, however.

1.14 Frequency of feeding urea:-

Feed urea containing supplements at least daily. A constant or continuous intake of urea will improve its utilization over abrupt or periodic intake. This is due to an adjustment by enzyme systems required to use urea.

1.15 Level of urea fed:-

Low levels of urea are utilized more efficiently and with less problems than high levels.

Thorough mixing of urea-containing supplements into the daily feed. If urea-containing supplements are mixed with the entire daily ration, the intake of urea at any one time likely will not be great, and the ability of the microbes to synthesize protein likely will not be exceeded.

Adequate supply of phosphorus, sulfur traces minerals And Substitution of urea for natural protein sharply changes the quality and quantity of minerals available for ruminal bacteria and cattle. Although needed only in small quantities, these elements are necessary building blocks for microbial protein synthesis. Feeding dehydrated alfalfa meal, which is high in trace minerals and sulfur, aids urea utilization. These often are found in many urea-containing supplements.

1.16 Solubility of proteins:-

Natural proteins such as soybean meal and cottonseed meal have different solubility's or rates of hydrolysis in the rumen. The more soluble the protein, the more rapidly it is hydrolyzed to ammonia in the rumen. For this reason, some natural proteins may be more competitive with urea.

1.17 Mechanism of urea utilization:-

When urea from feed sources enters the rumen, it is rapidly dissolved and hydrolyzed to ammonia by bacterial urease. The ammonia can then be utilized by the bacteria for synthesis of amino acids required for their growth. Amino groups are also split from amino acids and from intact proteins and used by bacteria in the same manner. Protein synthesis within the rumen by micro-organisms is very closely associated with the activity of these same organisms in breaking down cellulose and other carbohydrate materials and in the formation of organic acids as by-products of this fermentation process. The solubilities of natural proteins vary greatly and thus the rate at which they are hydrolyzed and utilized by bacteria differs appreciably. There is evidence, however, that a fairly high proportion of the more soluable proteins such as casein will be utilized by bacteria in about the same way as the ammonia from urea. For the less soluble proteins such as zein, the process of ammonia liberation is much less rapid and fairly large proportions of the protein may pass through the rumen to the abomasum without being broken down. When ammonia is produced too rapidly in the rumen or if the concentration becomes too high, appreciable amounts are absorbed directly into the bloodstream, reconverted to urea in the liver, excreted through the kidneys in the urine and thus lost to the animal. There is, however, always a small amount of urea in the bloodstream and other body fluids. This urea finds its way into the saliva and re-enters the rumen. Urea has been shown to pass into the rumen directly through the rumen wall from the circulating blood.

Schmidt-Nielsen *et al.* (1957) observed that camels had an unusual ability to conserve nitrogen under conditions of nutritional stress. On normal rations which supplied ample energy, protein and water, 40 percent of the urea filtered in the glomeruli of the kidneys was excreted in the urine, but on a low-protein ration only 1 to 2 percent of the urea was excreted, the rest being recycled into the rumen. The ability to recycle and utilize urea differs among various species of ruminants. Water intake and the nature of the ration also influence the response.

Houpt (1959) used an isolated rumen procedure, in anesthetized sheep, to demonstrate that urea was secreted from the bloodstream into the rumen in amounts about 15 times greater than by way of saliva. This secretion or recycling appears to occur under normal conditions. It has been proposed that this mechanism will supply nitrogen to preserve the rumen microbial population when the feed supply is limited or of very low nitrogen content.

Watson *et al*. (1949) fed urea labeled with N^{15} to sheep twice daily in gelatin capsules just before the regular feed of a low-protein basal ration. The sheep were slaughtered after being fed the labeled urea for 4 days, and their blood, liver and kidneys were examined for N^{15}-containing protein. Since the protein separated from these tissues contained N^{15} in excess of the amount found in control animals, it was concluded that urea was utilized by ruminants for the formation of body protein.

By feeding to lambs purified diets in which urea was the only source of nitrogen (Loosli *et al*., 1949), it was demonstrated that the 10 amino acids essential for growth were all synthesized in the rumen. The lambs gained in

body weight and produced wool of normal amino-acid composition. They remained in positive nitrogen balance over several months of the test.

Virtanen (1966) reported the production of more than 4,000 kilograms of milk in a year by cows fed diets in which urea and ammonium salts were the only nitrogen sources, along with purified carbohydrates as the energy source. The milk protein was normal in amount and composition as were also the water-soluble vitamins. These studies and many others leave no doubt of the effective conversion of urea nitrogen into tissue and milk proteins of ruminants.

A great many *in vitro* studies have been carried out in the past 20 years on the activity of rumen micro flora and the utilization of NPN compounds. The literature relative to the role of specific rumen bacteria in the synthesis of protein has been reviewed by Doetsch and Robinson (1953). Some of the different major types of bacteria found in the rumen have been isolated and their growth requirements defined. While these studies have contributed greatly to the understanding of the reactions that occur in the process of digestion in ruminants, the results are not reviewed in this study because interest is here largely directed toward the practical use of urea in feeding livestock.

1. Urea $\xrightarrow[\text{Urease}]{\text{Microbial}}$ $NH_3 + CO_2$

2. Carbohydrates $\xrightarrow[\text{Enzymes}]{\text{Microbial}}$ Volatile Fatty Acids (VFA) + Keto Acids

3. NH_3 + Keto Acids $\xrightarrow[\text{Enzymes}]{\text{Microbial}}$ Amino Acids

4. Amino Acids $\xrightarrow[\text{Enzymes}]{\text{Microbial}}$ Microbial Protein

5. Microbial Protein $\xrightarrow[\text{Abomasum and Small Intestines}]{\text{Animal Enzymes in the}}$ Free Amino Acids

6. Free amino acids are absorbed from the small intestine and used by the host animal.

1.18 Urea Poisoning in Cattle:

1.18.1 INTRODUCTION:-

Urea poisoning is one of the more commonly suspected toxicities of cattle in the Top End. Urea is used as a source of non-protein nitrogen (NPN) in feed supplements. In ruminants, nitrogen from urea is released in the rumen as ammonia and can be used by rumen microflora to synthesis protein. This protein then becomes available to the animal through the normal processes of digestion and absorption. However, if more urea is consumed than the rumen organisms can metabolize, the ammonia is absorbed from the rumen into the blood. The ammonia is then converted back to urea in the liver, and is then excreted by the kidneys. This pathway can easily be overwhelmed, when excess ammonia and urea circulate in the blood, causing poisoning. Poisoning can occur rapidly from a few minutes to four hours after consumption. Suspect urea poisoning if cattle are found dead close to the supplement.

1.18.2 CAUSES OF UREA POISONING:-

Urea poisoning has been documented many times and is characterized by an over-consumption of urea containing feeds or feeding of urea without a suitable fermentable carbohydrate source. Primary causes include:

1) Poor mixing of feed

2) Errors in ration formulation

3) Inadequate period of adaptation

4) Low intake of water

5) Feeding of urea in conjunction with poor-quality roughages

6) Low feed intake prior to exposure to feed containing urea

7) Rations that promote a high pH in rumen fluid

In either situation, the breakdown of the urea and subsequent release of ammonia into the rumen exceeds the rumen microbe's ability to complex it into bacterial protein and thus the ammonia concentration in the rumen increases. This causes an increase in the rumen pH. This increase in the alkalinity of the rumen contents then facilitates the passage of the ammonia through the rumen wall. When this happens, excessive amounts of ammonia are absorbed into the blood stream. The increased alkalinity appears to be the most important factor contributing to high blood ammonia concentrations and related toxicity in animals consuming urea. This increase in blood ammonia concentration can exceed the liver's capability to process the ammonia back into urea for recycling in the salivary gland or the body's ability to excrete it through the urine. Concentrations of ammonia nitrogen approximating two to four milligrams (mg) per deciliter (dl) of blood are generally associated with urea-induced deaths. However, the chance of toxicity has been found to be high when blood ammonia nitrogen exceeds .8 mg/dl in 60 minutes following urea consumption. As in the rumen, when blood ammonia levels rise so does blood pH. This leads to interference in a number of normal physiological processes including normal cellular energy metabolism, increased uptake of ammonia by the brain and an upset of the central nervous system. Although not well defined, the precise cause of death in ammonia toxicity appears to be respiratory arrest. You will note that it is ammonia toxicity that creates the actual problem, not the urea itself.

*1.18.*3 SIGNS OF UREA POISONING :-

Signs of poisoning can include twitching of ears and facial muscles, grinding of the teeth, frothy salivation, bloat, abdominal pain, frequent urination, forced rapid breathing, weakness, staggering, violent struggling and bellowing, and terminal spasms. Often, animals are found dead near the source of the urea supplement.

1.19 DIAGNOSIS OF UREA POISONING

The most useful diagnostic indicators are the history of access to urea and the signs shown by live, affected animals. Laboratory tests of blood samples are not very helpful, and no specific changes are seen at post-mortem examination. The following are general indicators of urea poisoning:

· History of access to urea.

· Laboratory testing of collected blood and rumen fluid immediately after death may indicate urea poisoning.

· Post-mortem – bloat; white foam in airways; ammonia odour when the rumen is opened; rumen pH (7.5-8).

Often a large pool of rumen fluid is seen on the ground at the nose of the beast. The animals usually suffer severe bloat and the fluid build up in gases forces the rumen fluid out through the mouth when the animal dies. Keep rumen and reticulum samples in formalin for subsequent diagnosis

1.19.1 History of access to urea :-

Recent feeding history is important. Cattle become accustomed to metabolising urea, but if they miss out for a couple of days, and then are

allowed sudden access, or if they consume more than normal, then poisoning can occur. Urea is very soluble and dissolves rapidly into puddles of water that can form on blocks after rain. Cattle that lick up these puddles can consume excess urea. Recommended feeding quantities vary according to what other feed is available and whether the cattle are accustomed to urea. Tolerance is decreased by starvation and by a low protein, high fibre diet. About 35 g of urea per day is considered sufficient for a 400 kg cow (i.e. approximately 0.1 g/kg body weight). It is recommended that urea should provide no more than 3% of the concentrate ration, or 1% of the total feed intake, and no more than one third of the total nitrogen intake should be NPN. In cattle, 0.3-0.5 g/kg/day (e.g. 120-200 g for a 400 kg cow) is considered to be toxic and 1-1.5 g/kg/day (e.g. 400-600 g for a 400 kg cow) can be fatal.

1.19.2. Laboratory testing:-

Blood ammonia levels can be measured, but this is only useful in live, sick animals. Proteins in the blood break down rapidly after death and produce ammonia, so testing blood from dead animals is of no value. For the same reason, the handling and storage of blood after collection is very important. Blood must be taken into lithium heparin or EDTA, placed immediately on ice and the plasma separated within 30 minutes of collection. Plasma may be stored for 2 hours at 4°C before testing, or frozen immediately and kept frozen until ready to test. These restrictions on measuring blood ammonia make it impractical as a diagnostic test in field situations. If it is important to measure blood ammonia levels, then collect blood from animals that appear unaffected, as well as from sick animals, and treat all samples the same way. If all samples show elevated ammonia, then it is likely to be a non-specific elevation (i.e. due to storage). Ammonia levels in rumen fluid can also be

measured, but only fluid taken straight after death is likely to be of any value. Again, it must be frozen immediately and kept frozen until tested.

1.19.3. Post mortem examination and histopathology :-

Animals decompose rapidly after death from urea poisoning and there are no specific signs of poisoning. Post-mortem examination immediately after death can show evidence of bloat, generalised congestion of the carcase, excess fluid in the pericardial sac, pulmonary oedema with excess stable white foam in the large airways and haemorrhages on the heart (epicardial and endocardial). There can be a marked ammonia smell when the rumen is opened. The pH of fresh rumen contents is a useful test that can be done in the field. An alkaline rumen (pH greater than 7.5-8) is suggestive of urea poisoning. There is very little in the literature on histopathological signs, but from our experience at Berrimah Veterinary Laboratories, there appear to be inflammatory changes in the rumen, particularly in animals that may survive the initial poisoning but die or are euthanised a day or two later. Inclusion of formalin-fixed sections of rumen and reticulum from animals that die from suspected urea poisoning, will assist diagnosis.

1.20 TREATMENT OF UREA POISONING:-

Treatment is rarely effective. However, if cattle can be handled, a stomach tube can be passed to relieve the bloat and then used to drench the animal with a large volume of cold water: 45 L for an adult cow is suggested, followed by 2-6 L of 5% acetic acid or vinegar. This dilutes rumen contents, reduces rumen temperature and increases rumen acidity, which all help to slow down the production of ammonia. Treatment may need to be repeated

within 24 hours, as relapses can occur. Rumenotomy and removal of rumen contents is suggested for valuable animals.

1.21 Management:-

As with the other compounds we discussed, the best treatment is good management and prevention. By managing cattle and our feeding program carefully, feeding of urea inclusive products is a cost effective practice. Some of the management considerations to keep in mind include:

1) Never provide urea inclusive feeds to excessively hungry cattle where over-consumption might take place.

2) Adapt cattle to feeds containing urea slowly, over a period of one to two weeks if at all possible.

3) Do not “slug feed” feeds containing urea. In situations where cattle are only provided supplement once or twice per week (a practice I strongly discourage for a number of reasons), this feed should not contain urea.

3) Provide access to plenty of fresh water and good quality roughage.

4) Urea containing feeds should not contain urea levels which exceed 1/3 of the total protein content of the product. In other words, if a feed contains 20 percent protein, no more than 6.6 percent should come from non-protein nitrogen.

5) Make sure that feeds containing urea are well mixed and evenly distributed in the bunk.

SUMMARY OF BEST PRACTICE :-

· If cattle have not been previously supplemented, start with pure salt; slowly and then gradually introduce urea supplement – increasing it slowly and gradually to about 0.1g/kg body weight/day. (35-40 g/day for a 400 kg cow).

· Ensure that cattle get regular (daily) access to supplement once supplementation has started.

· If cattle unavoidably miss out on urea supplementation for a couple of days, then restart them at a lower intake level.

· Prevent over-consumption of supplement mix or blocks (e.g. by using salt to regulate intake).

· Feed supplement mixes or blocks under a roof to prevent urea getting wet and dissolving.

· Suspect urea poisoning if cattle are found dead close to the supplement.

2. Materials and Method

2.1.Chemicals:-

-Ethanol (96%).

- Concentrated Hydrochloric acid.

-potassium Ferro cyanide.

-Zinc acetate.

-Di methyl amino benzaldhyde.

-Pure urea.

-Distilled water.

-Activated charcoal.

-Acetic acid(1.040g/cm^3).

2.2. Equipments:-

-sensitive balance. (shimdzu Ay120).

-Spectrophotometer. (Jen way6300).

-Magnetic stirrer. (scott science UK).

-Beakers.

-Volumetric flask 100 ml.

-Volumetric flask 500 ml.

-Filter paper.

-Funnel.

-water bath.

2.3. Methods:-

A. Prepare of Reagent:-

1. DMAB reagent:-

1.76 gm of Di methyl amino benzaldhyde was weighted in a beaker and dissolved in mixture 100ml of ethanol (96%) and 10ml concentrated Hydrochloric acid.

2. Carrez solution (1):-

21.79 gm of zinc acetate was weighted in a beaker and dissolved in 3 gm of acetic acid with little Distilled water, the solution was transformed to volumetric flask 100ml and the volume was completed to the mark with Distilled water.

3. Carrez solution (2):-

10.76 gm of potassium Ferro cyanide was weighted in a beaker and dissolved in Distilled water the solution was transformed to volumetric flask 100 ml and the volume was completed to the mark with Distilled water.

B.stock solution:-

1 gm of pure Urea was weighted in a beaker and dissolved,by water the solution transformed to volumetric flask 100ml and the volume was completed to the mark with Distilled water.

C. Prepare of standard solutions:-

1- Standard solutions (0.02%):-

2 ml from stock solution were taken to volumetric flask 100 ml and the volume was completed to the mark with Distilled water.

2- Standard solutions (0.04%):-

4 ml from stock solution were taken to volumetric flask 100ml and the volume was completed to the mark with Distilled water.

3- Standard solutions (0.06%):-

6 ml from stock solution were taken to volumetric flask 100 ml and the volume was completed to the mark with Distilled water.

4- Standard solutions (0.08%):-

8 ml from stock solution were taken to volumetric flask 100 ml and the volume was completed to the mark with Distilled water.

5- Standard solutions (0.1%):-

10 ml from stock solution were taken to volumetric flask 100 ml and the volume was completed to the mark with Distilled water.

D-Prepare of blank solution:-

1g of Activated charcoal was weighted and added to volumetric flask 500ml, 5ml from carrez solution(1) were added to the contain of volumetric flask , then 5ml from carrez solution(2) were added to the contain of volumetric flask and the volume was completed to the mark with Distilled water.

The volumetric flask was put at Magnetic stirrer for 30 minutes the contain of volumetric flask was filtrated, 5ml from filtrated solution were taken in tube, 5ml from DMAB were added to tube, the tube put at 20c□ in a water bath for 15 minutes little amount of solution was taken and was measured the absorbance in wave length 420nm

E- Prepare of samples:-

2g of sample was weighted in a beaker and taken to volumetric flask 500ml ; 1g of Activated charcoal was weighted and added to that flask 5ml from carrez solution(1) was added to the contain of volumetric flask ,5ml from carrez solution(2) was added to the contain of volumetric flask and the volume was completed to the mark with Distilled water.

The volumetric flask was put at Magnetic stirrer for 30 minutes the contain of volumetric flask was filtrated, 5ml from filtrated solution was taken in tube, 5ml from DMAB was added to tube, the tube put at 20c□ in a water bath for 15 minutes little mount of solution was taken and was measured the absorbance in wave length 420nm.abefor calibrated the Spectrophotometer with blank solution. The experiment repeated to the 4 samples.

F-Measuring the absorbance of standard solutions:-

1. Standard solution (0.02%):-

5ml of standard solution (0.02%) were taken in tube, 5ml of DMAB solution had been added to it and the tube put at 20c^{0} in a water bath for 15 minutes little mount of solution was taken and was measured the absorbance in wave length 420nm.

2. Standard solution (0.04%):-

5ml of standard solution (0.04%) were taken in tube, 5ml of DMAB solution had been added to it and the tube put at 20c^{0} in a water bath for 15 minutes little mount of solution was taken and was measured the absorbance in wave length 420nm.

3. Standard solution (0.06%):-

5ml of standard solution (0.06%) were taken in tube, 5ml of DMAB solution had been added to it and the tube put at 20c^{0} in a water bath for 15 minutes little mount of solution was taken and was measured the absorbance in wave length 420nm.

4. Standard solution (0.08%):-

5ml of standard solution (0.08%) were taken in tube, 5ml of DMAB solution had been added to it and the tube put at 20c^{0} in a water bath for 15 minutes little mount of solution was taken and was measured the absorbance in wave length 420nm.

5. Standard solution (0.1%):-

5ml of standard solution (0.1%) was taken in tube, 5ml of DMAB solution had been added to it and the tube put at 20c^{o} in a water bath for 15 minutes little mount of solution was taken and was measured the absorbance in wave length 420nm.

3. Results and Discussion

3.1 Results of using spectrophotometer Technique:-

Standard calibration curve:-

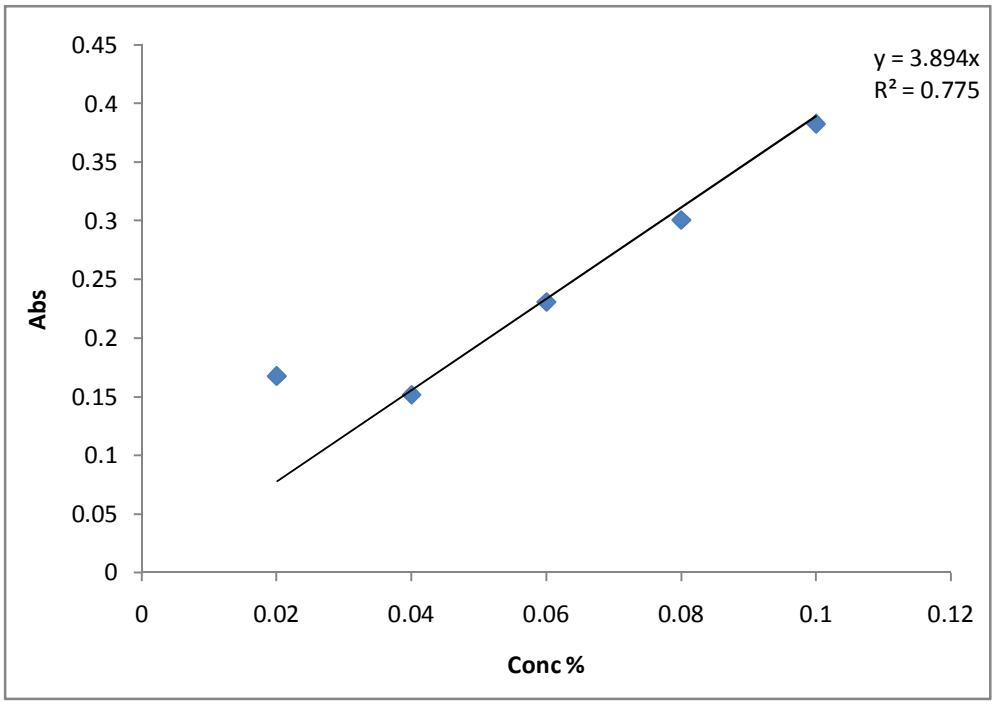
The calibration curve for Urea was given by using standard Urea solution (0.02%, 0 .04%, 0 .06%, 0.08%, and 0.1%) and showed the spectrum of standard solution.

Table (3.1): show the absorbance of urea in dietary:

Conc.%	Abs
0.02	0.168
0.04	0.152
0.06	0.231
0.08	0.301
0.1	0.383
Sample1	0.084
Sample2	0.086
Sample3	0.019
Sample4	0.012

3.2Calibration curve:-

The calibration curve for urea by using standard urea solution (0.02%, 0 .04%, 0 .06%, 0.08%, and 0.1%).



From calibration curve:-

The conc. Of sample1=0.0215 (w/v) %

The conc. Of sample2=0.0220 (w/v) %

The conc. Of sample3=0.00487 (w/v) %

The conc. Of sample4=0.00308 (w/v) %

The real conc. Of urea in sample by ***(w/w) % = (w/v) %×5×100 /2***

5 ≡ dilution factor.

2 ≡ weight of sample.

The conc. Of sample1= 5.375 (w/w) %

The conc. Of sample2= 5.50 (w/w) %

The conc. Of sample3= 1.2175 (w/w) %

The conc. Of sample4= 0.77 (w/w) %

3.3 Discussion

The concentrations of urea in the samples 1&2 are too high lead to poisoning in cattle if they are used for cattle either directly.

As for samples 3&4 contain a good proportion of urea where it can be used directly for cattle.

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