and peristaltic activity of intestines. The thirst and straining may be due to alkalosis. It is reported that alkalosis is produced by soluble oxalates after detoxification into carbonates and bicarbonates in the rumen. Alkalosis also causes the inflammation of the alimentary tract which may lead to inappetance in the animals.

Nitrate Poisoning

Existence of nitrate and its distribution

Nitrates are ubiquitous in feed, food and water and are essentially nontoxic. Nitrate (NO_3) becomes toxic when reduced to nitrite (NO_2) and can be a serious health hazard to ruminants grazing pastures or offered harvested feed and fodder which contain nitrate in excess of one percent on DMB. Similarly, drinking of water that contains 1500 ppm of nitrate can cause acute toxicity. Water from deep wells also contains higher levels of nitrate.

As plants mature, nitrate content rises until the prebloom stage, peaks and then begins to decline. The nitrate concentration has also been shown to increase during the first ten days of regrowth. The level of nitrate is highest in roots and stems, lower in leaves and almost negligible in flowers and seeds. Ensiled fodder usually contains less nitrate than fresh crop.

A number of plants are known to accumulate nitrates at potentially toxic levels under certain circumstances. For example, forage crops such as maize, kikuyu grass, johnson grass, oat hay, and alfalfa hay. Nitrate is the principal inorganic nitrogen source for plants. Generally, only small quantities of nitrates accumulate because they are rapidly reduced (by nitrate reductase) and combined with carbohydrates to form amino acids.

Factors that favour accumulation of nitrates in plants

The most common factors favouring accumulation of nitrates in plants include high nitrate content of soils, low intensity of light, drought, herbicide treatment with phenoxyacetic herbicides such as 2, 4-D., etc.

1. Application of excessive nitrogen fertilizers may increase nitrate levels in some forages to toxic levels.
2. Nitrate tends to accumulate in plants on cloudy days and in cool weather and at night. Cloudy weather may also inhibit reduction of nitrate to nitrite. So nitrate gets accumulated. Enough soil moisture may be present at night, even during drought, to allow uptake of nitrate and this gets accumulated because the activity of nitrate reductase is low during night.

- Ruminants appear to adapt to continuing ingestion of sublethal levels of nitrate through the compensatory erythropoietic response of increasing haemoglobin levels, haematocrits and blood volumes.
- Nitrate toxicity is influenced greatly by the diet of livestock. Adequate levels of readily fermentable carbohydrates increase nitrite disappearance in the rumen and protect the animal. Fasted animals are more susceptible to nitrite intoxication than are fully fed animals. It was reported that fasted animals eat more rapidly and may become intoxicated on forages grazed safely by fed animals.
- Harvested forage fed to livestock may be more toxic due to a more rapid rate of nitrate ingestion.
- Physical form of the feed can also influence toxicity. Nitrates are readily reduced in silages and moist hays.
- Animals can adapt to relatively high nitrate levels in the diets if the level of nitrate incorporation is increased gradually. This adaptation is due to an increase in the rate of nitrite reduction in the rumen.

Rumen microbes for nitrite reduction

Fodders ingested by ruminants often contain high levels of nitrate and hence, prevention of nitrite accumulation is particularly important. However, nitrate reduction rate is much higher than nitrite reduction rate, which causes nitrite accumulation. High levels of nitrite might cause an acute intoxication to host animals. High levels also inhibit the growth of many kinds of ruminal microbes, especially cellulolytic bacteria, methanogenic bacteria and protozoa. T. Hino and co-workers from Japan conducted several studies on these aspects and the relevant details are presented here. Addition of fumarate, lactate or formate to cultures of mixed ruminal microbes stimulated nitrate reduction and nitrite reduction also, to a greater extent. This stimulation is possible due to increased numbers of bacteria that reduce fumarate, nitrate and nitrite, such as *Selenomonas ruminantium*, *Veillonella parvula* and *Wolinella succinogenes*. The numbers of nitrate-reducing bacteria in the goat rumen were estimated by competitive polymerase chain reaction. The cell number of *S. ruminantium* was the largest of the three nitrate-reducing bacteria (10^7 cells/ml ruminal fluid) followed by *V. parvula* and *W. succinogenes*, which were less than 10^4 cells/ml. Therefore, *S. ruminantium* appears to be the most predominant in the rumen, but both the subspecies, i.e., *ssp. lactilytica* and *ssp. ruminantium* are not able to reduce nitrate and nitrite equally. *S. ruminantium ssp. lactilytica* only is able to ferment lactate and glycerol while the other is unable to ferment them.

problems including suppressed appetite, rate of weight gain, milk production (Undersander et al., 1999) and in severe cases, acute toxicity and possibly death. With timely and accurate assessment of nitrate concentration in forage and water sources, potential risks of livestock exposure to excessive nitrate intake may be properly managed or avoided.

Nitrate Toxicity may be Classified into Acute and Chronic Type

Acute toxicity

In acute type the affected animal may die within hours of ingestion of a lethal dose. Animals die of hypoxia from lack of oxygen due to methaemoglobinemia. Ruminants develop a brownish discolouration of nonpigmented skin and vaginal membranes, and discolouration of the vaginal membranes in females is a reliable indicator before other clinical signs are visible. Staggered gait, accelerated pulse, frequent urination and laboured breathing, followed by collapse are the other symptoms. Lethal intoxications usually result in coma and death within two to three hours after the first appearance of symptoms. The blood is dark and red to coffee brown and clots poorly.

Chronic toxicity

Considerable controversy exists with regards to the symptoms of chronic nitrate ingestion. Symptoms are depressed appetite, reduction in weight gains, decreased milk production, abortion, vitamin A deficiency and hypothyroidism. Apart from nitrate to nitrite and eventual toxicity, the nitrate has a direct caustic action on stomach and intestinal mucosa and causes gastroenteritis. Fatal nitrite poisoning in pigs showed no external signs; postmortem shows discoloured tarry blood.

Diagnosis of Nitrate Poisoning

Analysis of nitrate content in feed and water is important to confirm the source of poisoning. A simple test 'diphenylamine blue test' is available for detecting nitrate in feed samples. Stock solution for test is prepared by dissolving 500 mg diphenylamine in 20 ml water and then adding sulphuric acid to make 100 ml volume. A working solution is made by mixing one part stock solution to one part 80% sulphuric acid. Both the solutions should be stored in brown glass bottles. Suspected feed materials are tested by placing a drop of working solution inside the cut stem at a node or joint. Development of deep blue colour within 10 seconds indicates 2% or more nitrate.

Studies of Yoshii *et al.* (2003) revealed that the cell number of *S. ruminantium* (*ssp. ruminantium*) that reduces nitrate and nitrite in the rumen was usually 8-10% of the total number of *S. ruminantium* while, the lactate-using *ssp. lactilytica* was less than 1% of the total number. The percentage was not affected by the roughage/concentrate ratio or nitrate content of the diet in 2 weeks. However, feeding a high nitrate diet for 12 weeks increased the percentage. Nitrate reduction by *S. ruminantium* was enhanced by the coexistence of amylolytic bacteria. (*Streptococcus bovis* and *Prevotella ruminicola*) in a medium containing starch and nitrite accumulation increased; nitrite might not be reduced because of exhaustion of starch. Coexistence of cellulolytic bacteria (*Ruminococcus albus* and *Fibrobacter succinogenes*) facilitated the growth of *S. ruminantium* in a medium containing cellulose and nitrite reduction increased. These results suggest that **fibre digestion is important to decrease nitrite accumulation in the rumen**, because fibre digestion may provide sugars and electron donors to nitrite-reducing bacteria after readily fermentable carbohydrate are exhausted. Hence, it appears that measures followed to enhance fibre digestion may suppress nitrite accumulation in the rumen and save the ruminant animal from nitrate poisoning.

Symptoms of Nitrate Toxicity

Nitrate contained in forage may pose performance and health risks to ruminants. Nitrate risk to ruminants can be affected by various animal factors such as rumen microbes, age and condition of the animal, diet offered and the environmental stresses and water quality, as well as several plant factors including nutrient management, species, growth stage, environmental stresses, and nonstructural carbohydrate level. Forages containing less than 1000 mg NO_3^- N/ kg (dry weight basis) usually pose no risk for cattle (Undersander et al., 1999). Low levels of ingested nitrate are reduced by rumen bacteria to nitrite and then ammonia (Cowley and Collings, 1977), and any excess ammonia absorbed by the blood stream is excreted in the urine as urea. However, when high levels of nitrate are ingested, the capacity of the normal nitrate conversion process becomes overloaded and a portion of the nitrate is absorbed by the blood stream as nitrate and nitrite. Some of the nitrate that is absorbed recycles back to the rumen through saliva thereby adding again to the nitrate pool in the rumen. In contrast, absorbed nitrite inhibits the oxygen transporting capacity of red blood cells by oxidizing the ferrous iron of haemoglobin to ferric iron (methaemoglobin) leading to chronic animal performance