Toxicological Examination of Nitrate Poisoning in Bangladeshi Indigenous Cattle

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Toxicological Examination of Nitrate Poisoning in Bangladeshi Indigenous Cattle

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List of Acronyms Symbols Used

Abbreviation	Elaboration
Hb	Hemoglobin
MetHb	Methemoglobinemia
%	Percentage

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Abstract

In this study, seven indigenous cattle were affected by acute nitrate toxicity by consuming weeds from garlic plants. The clinical signs were ruminal tympany, dyspnea, painful breathing, frequent urination, incoordination and mucous membrane discoloration. Among seven cattle, three were in last trimester of pregnancy, two were in first and second trimester of pregnancy and others were non-pregnant. In the last trimester of pregnancy, nitrate intoxication severely affected pregnant cattle and death occurred immediately after painful breathing. Other cattle were affected but overcome the nitrate intoxication by giving primary treatment. The main purpose of this study was to diagnose the poisoning cases of the affected cattle which were all Bangladeshi indigenous breed reared by household farmers. The cows were reported to consume weeds grown near the garlic plants treated with nitrogenous fertilizer and manure. Based on the clinical signs, toxicological data and diphenylamine test of the collected weeds, there were finally determined as Nitrate Poisoning cases. Feeding the weeds strongly prohibited and to suggested fluid therapy for 3 days and liver tonic for 7 days. After 3 to 4 days of treatment, the rest of the cattle were recovered.

Key words: Nitrate poisoning, Nitrogenous fertilizer, Diphenylamine

Chapter 1: Introduction

Elevated nitrate levels in the body characterize nitrate poisoning. In case of normal digestion process of ruminants, nitrate is converted to nitrite in the rumen. Rumen microbes convert nitrite to ammonia, which is used by microbes to make amino acid and new microbial protein **(Kozloski 2009).** Nitrate poisoning in cattle usually caused by the consumption of an excessive amount of nitrate or nitrite from grazing crops, hay, silage, weeds, cultivated fodder and also from taking fertilizers, etc. Ruminal specially cattle and sheep are more susceptible to nitrate poisoning than non-ruminant species because microbes in their digestive tract favor the conversion of nitrate to nitrite. If an animal consumes large amount of nitrate, the ruminal microbes cannot convert nitrite to ammonia. That's why excessive nitrite enters the bloodstream and is absorbed by red blood cells. When nitrite is combined with oxygen carrying hemoglobin, methemoglobin is formed, and red blood cells lost their function to transport oxygen. The initial concentration of nitrate, the microbial flora, and the diet of animal, among other factors (age, sex, pregnant status) interfere in the conversion of nitrite to ammonia and result in the accumulation of nitrite **(Wang et al., 1961; Allison 1978; Kozloski 2009).**

Rural farmers mainly depend on locally available green grass they offer their cattle. There are various ways and condition by which nitrate accumulates in multiple crops and weeds. Levels of soil nitrogen, drought, light intensity and plant species were significant factors influencing nitrate accumulation under practical conditions. Nitrate is found in the highest proportion in the plant steam, leaves of different plants and in minimal quantities, only in the seed. However, rural farmers both large and household without technical guidance practice inappropriate use of technologies such as using manure without previous fermentation, or applying excessive nitrogen fertilization **(Medeiros et al., 2003; radestits et al., 2007).** These practices increase nitrate content in the plants offered to animals as bulky feed sources, which can lead to nitrate/nitrite intoxication **(Wright and Davison, 1964; radestits et al., 2007; Kozloski, 2009; Jonck et al., 2003).**

Typically, all plants may contain nitrate, and this average amount of nitrate is converted to ammonia and new-microbial protein. An excessive amount of nitrate is accumulated in forage which has been grown under conditions of excessive fertilization. Rural people also used excessive

manure and poultry litter for better production. This is one of the significant issues for accumulating high nitrate in plant and different kind of weeds **(Soomro et al., 2017**). People markedly used nitrogen as a fertilizer on agricultural land to improve both quality and yield of crops around the world **(Malhi et al., 2004).** As a result, plants accumulate excessive amounts of nitrate resulting in high livestock mortality rates **(Soetan et al., 2010)**. The Outbreak of nitrate poisoning occur in livestock worldwide by taking different fodder which may contain excessive nitrate. Outbreak also occur by taking different kind of weeds which may contain excessive amount of nitrate. Many species of livestock are susceptible to nitrate and nitrite poisoning, but cattle are considered most vulnerable because of the rapid conversion of nitrate to the more toxic nitrite form by rumen microorganisms **(O'Hera and Fraser 1975; Tokarna et al., 2002, Ozmen et al., 2005)**. Sudden death, abortion, infertility, decrease milk yield, reduce performance, interference with the conversion of carotene to vitamin A, and decrease growth rate have been attributed to nitrate toxicity **(Mcllwain and Schipper, 1963).**

Nitrate toxicity in cattle is severe, especially during the pregnancy period. Excessive dietary intake of nitrate through feed, forage and different weeds has been associated with last trimester bovine abortion and other reproductive difficulties. But no sign of abortion has been observed in the first trimester **(Ozmeni et al., 2007).** Excessive intake of nitrate from forage, fodder, or weeds may cause rapid decline in transplacental oxygen to transfer fetal blood may cause intrauterine death and abortion. Therefore, nitrogen retention in both the fetal and uteroplacental tissue, becomes very important, especially in the last trimester **(Johnson et al., 1983; Sanli et al., 1983).**

Chapter 2: Materials and Method

2.1 Study area: The study was carried out for periods of 3 months. The data was collected from household farmer in Saidpur Upazilla under the Nilphamary district of Bangladesh. Data was collected through face-to-face interview methods with cattle owners and carefully observed the affected cattle.

2.2 Number of cases: In this study, the total number of affected cattle was seven. Among seven cattle, the number of pregnant was five and rest of the cattle non-pregnant. Among five pregnant cattle, three were in last trimester of pregnancy, one in the first and another in the second trimester of pregnancy.

2.3 Mortality: Mortality means the number of dead cattle among the total population. In this study, the number of affected cattle was 7, and the number of cattle that died was 3. Therefore, mortality rate was 42.85 %.

2.4 Morbidity: Morbidity means number of affected cattle among the total population. In this study, all cattle were affected with an associated history of consumed weeds of garlic plants. So, the morbidity rate was 100 %.

2.5 Clinical history: Three cattle died with oozing, partially clotted blood around the rectal opening. All affected cattle were the same feeding history. In addition, all cattle consumed same weeds the household owner collected from his garlic plant. Between 2 to 6 hours, all cattle showed clinical sign respectively.

2.6 Clinical sign: All affected cattle showed clinical sign after ingestion of nitrate containing weeds. Death may occur which were in last trimester of pregnancy. Clinical sign associated with:

- Rapid and painful breathing
- Prominent sound heard during breathing (severe dyspnea)
- Tympany
- Inco-ordination
- Dark color blood found in rectal region
- Frequent urination

- Discoloration of mucous membrane

2.7 Toxicological Investigation: For toxicological examination, fresh weeds sample were collected that were offered to the cattle. To find out the actual cause, I perform the diphenylamine test. I firstly gathered all the necessary chemicals and materials for this test. After collecting the chemical, prepared the reagent to complete the test.

Reagent Preparation:

Firstly, taken 500 mg Diphenylamine in a measuring cylinder

Add 20 ml distilled water

Gently shaking for proper mixing

Add concentrated sulfuric acid up to 100 ml

Reagent ready for working or further use

Perform the Test: After chopping the weed into small pieces, grinding had been done using mortar and pastle. Finally add few drops of prepared reagent to the weed sample. Within 1 minute developed deep blue discoloration of the sample indicate severely positive result of nitrate poisoning.

Fig-1: Grass Sample

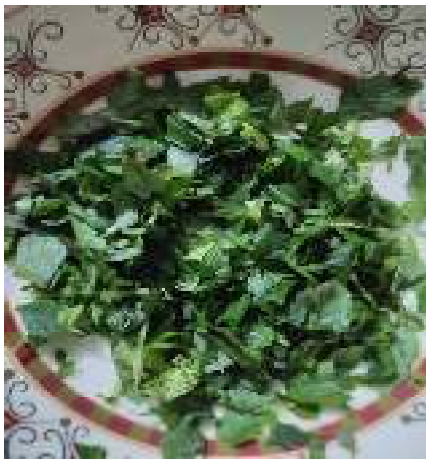

Fig-2: Chopping of Grass

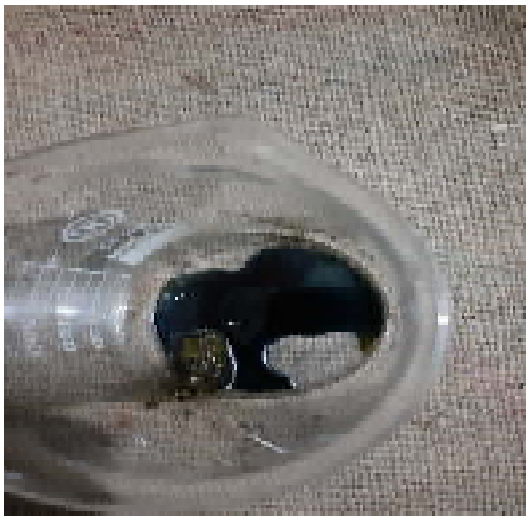

Fig-3: Diphenylamine Test

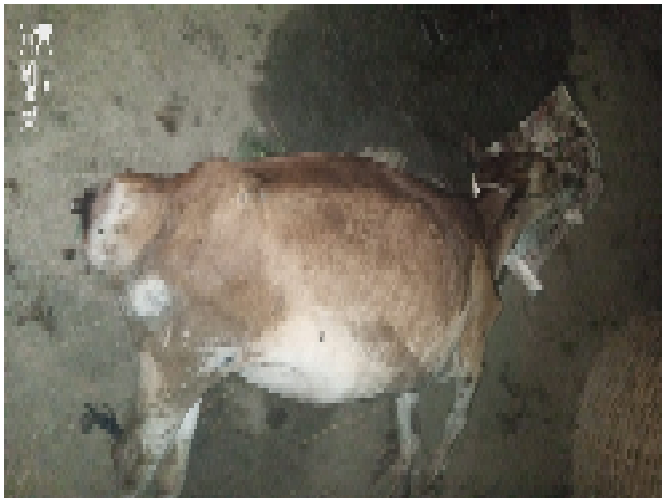

Fig-4: Death Pregnant Cattle

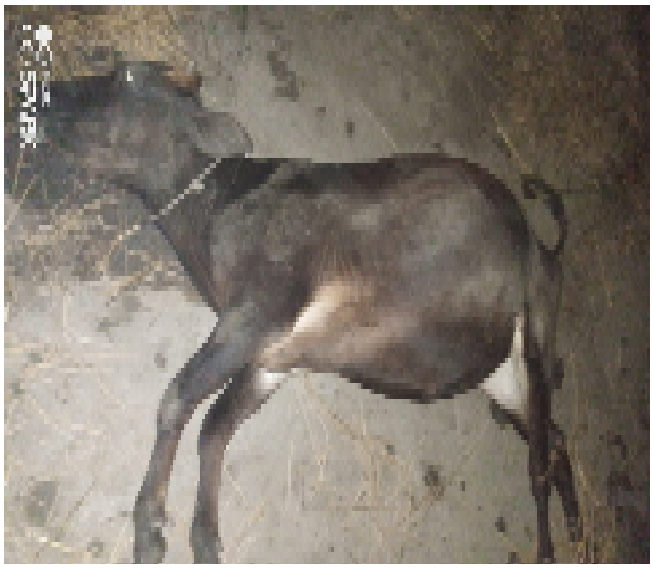

Fig-5: Death Pregnant Cattle

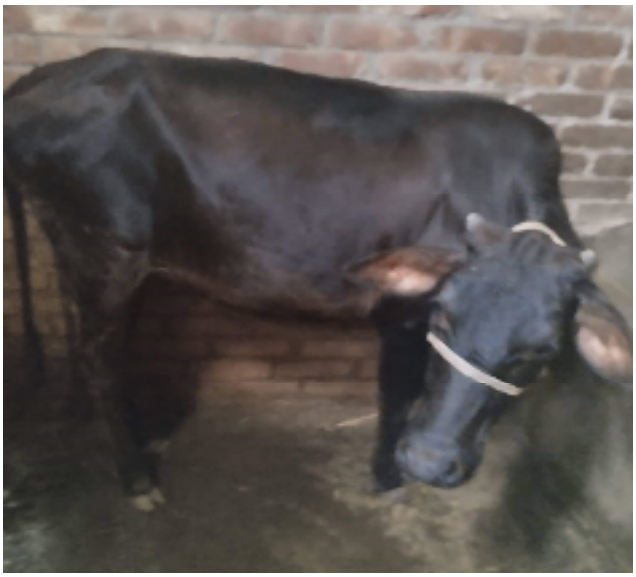

Fig-6: Live Animal After Recover

Chapter 3: Result and Discussion

Clinical examination (sign, symptoms), toxic effect, and Diphenylamine test of the weeds (Showing in figure) concluded to diagnoses these causes as "Severely Nitrate Poisoning".

Table 1: The test method and the results of nitrate poisoning

Type of sample	Animals	Nitrate Poisoning Test
Collected weeds	All animal	Severe Positive

Diphenylamine oxidized twice and help to interpret the test result. Firstly, diphenylamine reacts with nitrate/nitrite to form colorless diphenyl benzidine. After that, diphenyl benzidine is further oxidized and give a violet or bluish color, which indicates nitrate positive.

Table 2: Animal status and toxic effect of nitrate

Types of samples	Animals	Effect of Nitrate Poisoning in Affected Cattle
Weed sample	Last trimester pregnant cattle (3)	Severely affected and death occurred
	First and second trimester pregnant cattle respectively (2)	Affected and showed clinical symptoms but survived without abortion
	Non-pregnant cattle (2)	Expressed clinical symptoms but recovered

The pregnant cattle, which were in last trimester of pregnancy, they were severely affected and died immediately after breathing difficulty. Other pregnant cattle in the 1st and 2nd trimesters of pregnancy were affected badly but safely and gave birth to healthy offspring after gestation period.

Further, no case of abortion and congenital abnormalities had been noticed in the affected cattle. Non pregnant cattle only showed clinical signs, but no severe health effect occurred.

Nitrate and nitrite are closely related to the effect of nitrate toxicity/poisoning. Ingestion of excessive amount nitrates may cause inflammation of the rumen and intestine. Through this route, toxic products are absorbed before and after absorption. Nitrates are converted to more toxic form nitrite by bacteria of digestive tract. Nitrite is an intermediary product of nitrate toxicity. On the other hand, nitrite is converted to ammonia in the same way as nitrate to nitrite conversion. If nitrite-ammonia transformation is not satisfactory, it may occur, blood nitrite ions increase and can cause methemoglobinemia by co-oxidation of iron in hemoglobin and anemic hypoxia result **(Smith, 1995; Blood, 1991).**

The severity of nitrate toxicity depends on the intake rate of nitrate rich fodder, crops or weeds. The seriousness of nitrate toxicity differs from animal to animal due to body condition or individual physical status. In case of pregnant animal, severe nitrate toxicity may cause abortion and death of the animal. Because of the body's immunity status, the pregnant cattle were more susceptible to nitrate toxicity. After taking nitrate rich weeds, affected cattle showed clinical sign between 2 to 6 hours. These include dyspnea, painful breathing, Inco-ordination, frequent urination, tympany and discoloration of mucous membrane and death of pregnant cattle in just few hours which is a similar finding to **Stober (2006).** Within 2-3 hours after taking nitrate rich substance, much increase MHb concentrations may occur in the blood. In this study, affected cattle showed discoloration of mucous membranes, similar to **(Kemp et al., 1976),** who reported that when MHb concentration may increase in the blood, it has been associated with discoloration of mucous membrane of affected cattle or animals. The cattle died without convulsion, which is not a typical symptoms of nitrate toxicity, a similar finding was also reported by **Sperman (1989)**.

Diagnosis of nitrate/nitrite toxicity was based on clinical signs (painful breathing, dyspnea, frequent urination, incoordination, discoloration of mucous membrane), laboratory findings (diphenylamine test), helped to diagnoses that nitrate/nitrite toxicosis was the cause the problem. The clinical sign of nitrate toxicity in this study was similar to what was reported previously **(Van Dijk et al., 1983; Slenning, Galey and Anderson 1991; Ozmen 2003).** The Sudden death of three cattle indicated severe nitrate intoxication and was also attributed to acute nitrite poisoning,

presumably caused by the sudden dilation of blood vessel **(Jones 1993)**. Among seven cattle, five cattle survived to overcome nitrate toxicity. The variation of toxicity depends on the rate at which the microflora of rumen reduces nitrate to nitrite or ammonia **(Kemp, Guerink and Malestein 1977).**

Chapter 4: Limitation

I did not measure the hematological and biochemical parameters of the affected cattle. Besides this, a post-mortem examination was not performed on the dead cattle. The course of the treatment was not also observer on time.

Chapter 5: Conclusion

The clinical sign and laboratory findings suggested that three pregnant cattle were affected, and sudden mortality occurred due to severe nitrate toxicity. Nitrate/nitrite accumulates in the plants and weeds due to excessive use of nitrogenous fertilizer and manure. Nitrate toxicity is a serious problem that may cause significant loss of livestock production. Therefore, caution must be taken while using nitrogenous fertilizer, and weeds for livestock should not be used without knowing anything.

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Biography

I am Mahafuzul Alam, from Nilphamary. I passed Secondary School Certificate in 2014 (G.P.A- 5.00) and Higher Secondary Certificate examination in 2016 (G.P.A-5.00). I am a student of 22 Batch and now I am an intern student under the faculty of Veterinary Medicine in Chattogram Veterinary and Animal Sciences University. In future I would like to a field Veterinary Practitioner.