A Study on Toxicological Examination and Management of Nitrate Poisoning in Cattle

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A Study on Toxicological Examination and Management of Nitrate Poisoning in Cattle

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Statement of Author

I, Md Rabiul Islam, would like to strongly assure that I have performed all the works furnished here in this report. The information has been collected from books, national and international journals, websites and other references. All references have been acknowledged duly. Therefore, I hold entire responsibility of collection, complication, preservation and publication of all data accumulation here in this report.

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Abstract

This study was conducted to diagnose the poisoning cases of two affected Holstein Friesian cows along with their clinical management at Zarif Dairy Farm, Hathazari, Chattogram. The cows were reported to consume German grass from grassland that was flooded by a nearby fertilizer company's wastewater. Based on clinical signs, toxicological data, and diphenylamine test of the supplied forage determined that the poisoning cases were "Nitrate Poisoning". Proper treatment protocol was used with 1% methylene blue (at dose rate of 20mg/kg body weight) and Ascorbic Acid (15mg/kg body weight) to decrease the methemoglobinemia level in blood. Feeding the grass was restricted, and liver tonic was also suggested to give orally for one week. After three days of treatment, the animals were recovered. Nitrate toxicity is a vital problem that may cause significant loss in livestock production. Caution must be taken while supplying forage to avoid accidental consumption of nitrate enriched grasses.

Keywords: Nitrate poisoning, methemoglobinemia, methylene blue

1. Introduction

In typical conditions, nitrate consumed by ruminant livestock such as cattle, sheep, and goats are turned to ammonia, which is then transformed into bacterial protein in the rumen by bacteria (Blood *et al*., 1991). The conversion of nitrate to nitrite is faster than the transformation of nitrite to ammonia. As a result, when more than regular amounts of nitrate are ingested, nitrite may build up in the rumen. Nitrite is then absorbed into the bloodstream, converting hemoglobin to methemoglobin, which cannot carry oxygen. As a result, an animal's death from nitrate toxicity is caused by hypoxia (Brunning-Fan & Kaneene, 1993).

Nitrate poisoning in cattle is fatal as nitrate converts into highly toxic nitrite through the reduction in the animal body. Consequently, nitrites oxidize ferrous ion (Fe2+) to ferric ion (Fe3+) and produce methemoglobin (Wood, 1980). Methemoglobin cannot carry oxygen for cellular respiration. Poisonings normally develop after ingesting nitrate-containing grasses or water or consuming nitrate-containing substances such as ammonium nitrate-containing fertilizers (Shaikat *et al.,* 2012). Consumption of nitrate at apparently 0.05% of total animals' body weight can achieve minimum lethal dose (Yeruham *et al*., 1997).

Regardless of the type of nitrogen fertilizer (including manure) utilized, plants generally take up nitrogen from the soil in the form of nitrate. When plants grow normally, very little nitrate accumulates because nitrate is readily converted to plant amino acids and protein by the stem and leaves. However, under certain circumstances, this equilibrium can be upset, causing the roots to absorb nitrate quicker than the plant can convert it to protein (Nichols, 1980).

Three factors influenced the nitrate-to-protein cycle in plants; sufficient water, sunlight energy and a temperature that favors chemical reactions. If any of these elements is lacking, the root of the plant continues to absorb nitrate at the same frequency and store it in the stalk and lower sections of the leaves. Nitrate builds up as a result of this condition. Excess nitrogen fertilization, such as in fields where farmers apply a high amount of manure, can cause nitrate accumulation in plants (Burrowns *et al*., 1987). Nitrite's absorption causes methemoglobinemia and develops anemic hypoxia. As a result, tissue is not supplied with sufficient oxygen. Moreover, nitrite is a vasodilator that may cause peripheral circulatory failure by developing tissue anoxia (Ozmeni *et al*., 2007).

Chronic nitrate poisoning is a form of nitrate toxicity where no pathognomonic signs are observed. It may reduce growth rate, lower milk production, inappetence, and make animals susceptible to infections. Nitrate consumption at a level of 0.5-1.0% of the feed (on a dry matter basis) normally develops chronic nitrate toxicity in cattle (Ishigami & Inonue, 1976).

Excess dietary intake of nitrate via feed and forage has been associated with last trimester bovine abortion and other reproductive difficulties. But no signs of abortion have been observed in the first trimester (Ozmeni *et al*., 2007). In a study, it has been found that nitrate or nitrite causes a rapid decline in transplacental oxygen to fetal blood. Intrauterine death and abortion occur due to a rapid decrease of oxygen transfer to fetal blood. Due to an expeditious rate of nitrogen retention in both the fetal and uteroplacental tissue, it becomes very important, especially in the last trimester (Johnson *et al*., 1983. Sanli *et al*., 1983).

This article reports about nitrate poisoning cases observed in two cattle where one was a pregnant cow and another one was a dry cow. This study aims to describe toxicological findings of cattle suffering from nitrate poisoning, nitrate detection in forage, and management to withdraw the toxicity.

2. Materials and Methods

2.1 Case Definitions:

A 2 years old pregnant Holstein Friesian cross cow and a 3.5 years old Holstein Friesian cross milch cow at Zarif Dairy Farm, Hathazari, Chattogram, Bangladesh were investigated based on owner complain as suspected poisoning (Figure 1). These animals were observed on the DVM internship program 2021 under Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh and asked for clinical investigation and treatment.

The manager of the farm stated that the animals had been fed German grass from grassland and after 6 hours of feeding animals were showing some clinical signs (Inappetence, diarrhea, distended abdomen). The owner also administered anti-diarrheal drugs (DD Nil) and cholera saline but the animals didn't respond to treatment. After 24 hours the animals were observed, clinical history and clinical signs had been recorded. It had been found that the grassland from where the animals were fed was flooded by a nearby fertilizer industry's wastewater.

Figure 1: Affected pregnant cow (Left) and milch cow (Right)

Figure 2: Fecal content of affected cow

Toxicological Investigations:

For toxicological examination, fresh grass had been collected from four different places of that grassland and brought to the Department of physiology, biochemistry, and pharmacology, CVASU, Chattogram, Bangladesh. Nitrate poisoning test and cyanide poisoning tests had been performed to detect the nitrate and cyanide, respectively.

Nitrate Poisoning test:
After chopping the grasses into small pieces, grinding had been done using mortar and pestle. Then few drops of prepared reagent mixture (Diphenylamine 500mg, Concentrated Sulphuric Acid 100ml, and Distilled Water 20ml) was added with the sample. After 2-3 minutes, blue or violet discoloration of the sample indicate the positive result (Figure 3).

Cyanide Poisoning Test:
Collected sample (5gm) had been grinded and transferred to a test tube. After that, 2-3 drops of chloroform had been added to test tube. After that, a piece of moistened sodium-picrate paper was inserted in it without contacting the sample. After few minutes or an overnight incubation (37-39^{o}C), gradual orange or brick red discoloration of the sodium picrate paper indicate the positive result.

Figure 3: Diphenylamine Test

3. Result and Discussion

Clinical examinations, some surrounding toxicological data, and the Diphenylamine test of the grasses (shown in Figure) concluded to diagnose these cases as ‘‘Nitrate Poisoning”.

Table 1: The test methods and the results of cyanide and nitrate poisoning

Type of sample	Animals	Nitrate Poisoning Test	Cyanide Poisoning Test
German Grass	**Cow 1**	+	-
	Cow 2	+	-

Diphenylamine reacts with nitrate/nitrite to form diphenyl benzidine which is colorless. This diphenyl benzidine is further oxidized to form 2nd stage of diphenyl benzidine which is violet or bluish. On the contrary, the result of the cyanide poisoning test was negative for that sample.

Nitrate toxicity can be treated with reducing chemicals such as methylene blue and ascorbic acid. Methylene blue is given intravenously in doses ranging from 4 to 22 mg/kg B. weight, depending on the severity of the poisoning (Smith, 1990). 2 doses of 1% Methylene blue were administered intravenously at a dose of 20mg/kg in this current investigation. Besides, ascorbic acid (15mg/Kg B. weight) and liver tonic was also administered in intravenous and oral route, respectively in both cases. Feeding the forage from that grassland was withdrawn and after three days, all clinical signs was subsided (Dijk et al., 1983).

The pregnant cow gave birth to a healthy offspring after 1.5 months of the incident. Further, no case of abortion and congenital abnormalities had been noticed on that farm.

Because of poisoning effects, nitrate and nitrite have close relations. Besides, excessive nitrate intake may cause inflammation of the rumen and intestines. They are the routes of providing more toxic components, before and after absorption. Nitrate is converted to nitrite, an intermediary product, by bacteria of the ruminant digestive tract. On the contrary, nitrite is converted to ammonium in the same way. If nitrite-ammonium transformation is not adequate, blood nitrite ions increase and may cause methemoglobinemia by co-oxidation of iron in hemoglobin and anemic hypoxia results (Klooster *et al*., 1990).

Nitrate has direct irritating action on the mucosa of the gastrointestinal tract and can induce gastrointestinal inflammation. Diarrhea occurred 24 hours after ingesting nitrates, which was related to gastro-intestinal irritation in both cases (Brown *et al*., 1990).

Nitrate and nitrite are potential toxicants that are becoming more common in the environment. The widespread use of nitrates as agricultural fertilizers, along with the increasing amounts of nitrogenous waste produced by industries, and feedlots, are vital causes for the increase of nitrogenous compound distribution in the environment, particularly in surface and groundwater (Issi *et al.*, 2008). Human and animal exposure to nitrate in environment is possible, but excessive nitrate concentration in feed and forage poses a greater risk of poisoning (Rankins *et al.*, 2004).
Depending on numerous circumstances, ruminant animals can tolerate a wide range of nitrate levels. The major aspects that make nitrate less toxic:

- If the increase is gradual, the animal will get adapted to ingest increasing amounts of high-nitrate feed.
- Animals in good health are less prone to be affected than animals in bad health.
- Because carbohydrates aid in the conversion of nitrate to microbial protein, an adequate supply of accessible carbohydrates (grain) allows the animal to eat more nitrate.

As nitrate toxicity may create life-threatening conditions, concern should be taken in providing forage. Grassland around fertilizer industries would be a potential source of nitrate poisoning in cattle through ingestion of grasses from there. It is recommended to perform nitrate poisoning test of grasses before feeding the cow.

4. Limitation

The hematological and biochemical parameters of the affected cows were not measured. Besides, clinical variables, which were attributed to the course of the treatment were not also observed accordingly timely manner.

5. Conclusion

In conclusion, the clinical examination, laboratory data, and treatment response suggested that these animals were suffering from nitrate poisoning, which could be treated with methylene blue alone. Nitrate poisoning is a serious problem that may cause significant loss in livestock production. Caution must be taken while using nitrogen fertilizers to avoid environmental contamination and animal exposure to lethal quantities of nitrates.

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Biography

This is Md Rabiul Islam, the youngest child of Md Nurul Amin and Bulbul Akther, doing his graduation on Doctor of Veterinary Medicine (DVM) at Chattogram Veterinary and Animal Sciences University under the Faculty of Veterinary Medicine. He passed the Secondary School Certificate Examination (SSC) in 2012 from Sermon High School, Chattogram, and got GPA 5.00 out of 5.00 and then Higher Secondary Certificate Examination (HSC) in 2014 from Omarghani M.E.S. College, Chattogram and got GPA 5.00 out of 5.00. Currently, he is doing his year-long internship. He has a great enthusiasm for research and researches the molecular basis of emerging pathogens.