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## SHORT COMMUNICATION

# Successful Management of Nitrite Poisoning in Crossbred Dairy Calves

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## ABSTRACT

Nitrate/ nitrite poisoning in dairy cattle occurs when there is an abrupt change in diet from the introduction of a bulk feed source with high nitrate levels. Over fertilization of fodder with untreated manure and occurrence of drought season followed by a rainy season could result in higher level of accumulation of nitrate in plants. A sudden onset of illness was reported in eight crossbred dairy calves (between 8-11 months of age) at University Livestock Farm and one among the affected calf died after showing symptoms of discomfort, excessive salivation, tympany, dyspnoea, sternal recumbency progressing to lateral recumbency, pedalling movements and, stiffness of the limbs and neck. On clinical examination, the affected animals exhibited sialorrhoea, tympany, increased pulse and respiratory rates, laboured breathing, brownish discoloration of mucous membrane and loss of co-ordination. Microscopical examination of rumen fluid revealed normal protozoan motility. Blood colour was dark chocolate brown in all the affected animals and coagulation time was delayed. Animals had a history of *ad libitum* feeding with chopped lush green fodder grasses namely hybrid Napier and fodder maize that were harvested from cattle slurry irrigated fodder plots. Based on the history and clinical signs noticed, the case was presumptively diagnosed as nitrate/nitrite poisoning, later confirmed with diphenylamine test. All the animals were treated with intravenous administration of 1 per cent methylene blue @ 1-2 mg/kg BW. Subsequently the dairy calves showed an uneventful recovery from the condition.

**Key words:** Methylene blue, Nitrate, Nitrite, Sodium thiosulphate

Nitrate/ Nitrite poisoning is the one of the most widespread toxic condition in ruminants. Fodder crops namely maize, sorghum, brassicas, Italian rye grass, tama and green oats are considered as the principal accumulators of nitrate that could reach toxic levels for bovines. Accumulation of nitrate is more in rapid growing plants with reduced photosynthesis, over fertilization of the soil with excessive use of animal manure and poultry litter are the principle cause of nitrate accumulation in forages (Follet and Hatfield, 2001) and the accumulation is more in young and rapidly growing plants as compared to the mature forages (Haliburton and Edward, 1978).

The occurrence of a rainy season followed by a draught period, less adaptation of the ruminants to a nitrite rich diet and *ad libitum* feeding of bovines with nitrite rich plants have increased the risk of nitrate toxicity in ruminants (Gontigo *et al.,* 2017). Ruminants like cattle, goat and sheep are more prone to nitrate toxicity as the rumen bacteria reduce nitrate to a more toxic nitrite and when this conversion ratio is faster than the conversion of nitrite to ammonia, the excess nitrite that accumulates in the rumen is progressively absorbed into the blood stream. Nitrite in the blood reduces the oxygen carrying haemoglobin to a brownish black substance called methemoglobin that cannot carry oxygen and imparts the above colour to the blood (Osweiler 1996). Nitrite also causes vasodilatation that leads to a reduction in blood pressure and circulatory shock and foetal death in pregnant animals due to

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hypoxia. Affected animals show clinical signs when 20 per cent of haemoglobin is converted to methemoglobin and death results when it reaches to 60-80 per cent (Jones 1988). Clinical signs include chocolate brown discoloration of the mucous membranes, severe abdominal pain, ataxia, incordination, muscle tremor, weakness, severe dyspnoea, cyanosis, collapse and recumbency. In this paper we report a case of spontaneous occurrence of nitrate or nitrite intoxication in cross bred dairy heifers and its' successful management.

A sudden onset of illness was reported in eight crossbred dairy calves (8-11 months of age) in University Livestock Farm, Mannuthy and one among the affected calves died after showing symptoms like sudden onset of discomfort, excessive salivation, tympany, dyspnoea, sternal to lateral recumbency, pedalling movements and stiffness of the limbs and neck. It was reported that all calves were healthy and they were fed *ad libitum* chopped lush green fodder (hybrid Napier and fodder maize) harvested from cattle slurry irrigated fodder plots.

Detailed clinical examination was carried out on all the affected animals and observations were recorded. The affected animals exhibited sialorrhoea, ataxia, tachycardia, tachypnoea, dyspnoea, cyanotic mucous membrane and loss of balance. Microscopical examination of rumen fluid revealed normal protozoan motility. Blood colour was found to be dark chocolate brown in all affected animals.

Based on the feeding history and clinical signs noticed, the case was presumptively diagnosed as nitrite poisoning and treatment was carried out with 1 % methylene blue. The diphenylamine test was performed in grass samples by adding one drop of the reagent solution (0.5 g diphenylamine, 20 ml distilled water, and 80 ml concentrated sulfuric acid) to three drops of the plant extract on glass slides. The extract was obtained by manual pressure (Tokarnia *et al.,* 2012). The results of the diphenylamine test associated with clinical signs and epidemiological data helped to conclude the diagnosis of nitrate/nitrite intoxication. All the affected animals were treated with intravenous administration of 1% methylene blue @ 1-2 mg/kg BW. Subsequently the dairy calves showed an uneventful recovery from the condition.

Even though the chopped lush green grass was fed to animals in all the age group the intoxication occurred only in the heifers group, whose diet was almost exclusively composed of fodder that corresponded to approximately 60-80% of the total dry matter intake. Dairy cows, on the other hand received larger amounts of concentrate, and the calves received milk and ate a small amount of concentrates and grass. Hybrid Napier and maize fodders are good accumulator nitrates (Medeiros *et al.,* 2003). The condition was further aggravated the fact that the fodder were harvested from plots that were fertilized with large amount of untreated manure and pig slurry. Additionally, the site experienced a long period of drought

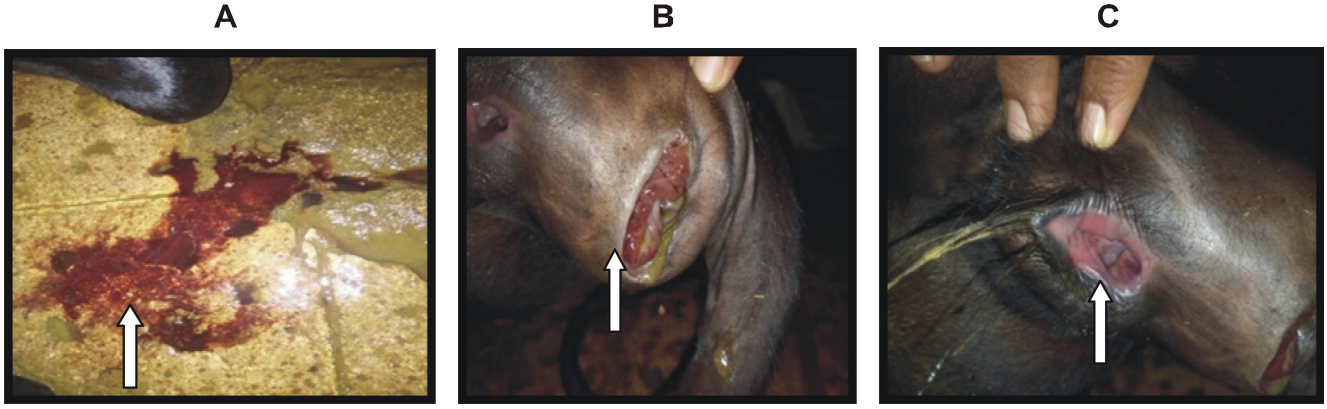


**Fig. 1 Brownish discoloration of the blood (A), mucus membrane of vulva (B) and rectum (C)**

without rain followed by high rainfall during the incident. All these factors might have contributed to the accumulation of nitrate in the fodders offered to the animals and bulk consumption of these fodder caused the intoxication.

In ruminants, nitrate/nitrite toxicity occurs when high nitrate levels in the feed overwhelm the animal's digestive system to the extent that the rate of conversion of nitrate to nitrite is faster than the conversion of nitrite to ammonia (Al Qudah, 2009). Microbes in the rumen reduce nitrate contents in the ingested plants in to nitrite, which is five to six times more toxic than nitrates (Rogers and Hope-Cawdery, 1980). Following its absorption into blood through rumen capillaries, nitrite oxidizes the haemoglobin to methaemoglobin which is unable to transport oxygen and also results in vasodilatation (Leng, 2008). These two predominant effects of nitrite lead to tissue hypoxia and circulatory collapse (Haymond *et al.,* 2005). The amount of nitrate ingested by the animal and methemoglobin formed influences the onset of clinical signs, disease progression and death (Jonk *et al.* 2013). The amount of methemoglobin formed in each animal depends on its nitrate recycling rates in the rumen and on the drop in nitrite levels, reflecting different levels of nitrate tolerance presented by the animals (Ozmen *et al.,* 2005).

The poisoned heifers exhibited a cyanotic or brownish discoloration of mucosa of conjunctiva, vaginal vestibule and rectum; dyspnoea, sialorrhoea, tympany, and progression to sternal recumbence and death. Similar findings were also reported by Medeiros *et al.* (2003) and Jonck *et al.* (2013). Among the intoxicated animals one heifer died after showing symptoms like stiffness in limbs, neck and jaw, tympany and pedalling movements. Necropsy was performed soon after the animal's death. A strong nitrous odour was noticed while opening the carcass. The blood was dark chocolate brown in colour with difficult coagulation, all the compartments of stomach were distended due to gas accumulation and a brownish discoloration was observed in lungs, kidneys and liver. These necropsy findings were in accordance with the report of Tokarnia *et al.* (2012) who noticed brownish discolouration of visceral organs in intoxicated animals. Chocolate-coloured and poorly clotted blood and intensely red coloured skeletal and cardiac musculature as observed in the present case were compatible with those described by Jonck *et al.* (2013).

The drug of choice for nitrate/nitrite poisoning is intravenous administration of 1% methylene blue at the dosage of 1 to 2 mg/kg body weight. Methylene blue acts by reducing $Fe^{3+}$ to $Fe^{2+}$, and receiving an $H^{+}$ from NADPH (becoming leukotolene blue) and donating it to methemoglobin, which is converted to haemoglobin. Since the time taken to metabolize the methylene blue is short, the poisoned animal shows recovery within minutes after its administration. The rapid response to the treatment is another form of diagnosis of the intoxication (Radostits *et al.,* 2007; Jonck *et al.,* 2013).

The clinical signs, necropsy findings, epidemiological data and the positive diphenylamine test confirmed the nitrate/nitrite intoxication. The sudden change in diet, with introduction of a bulky food source containing high nitrate levels, the over fertilization of grass with untreated manure, and the occurrence of a drought period followed by a rainy season were some of the causes of the intoxication. Intra venous administration of one percent methylene blue @ 1-2 mg/kg body weight resulted in uneventful recovery of the animals.

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