Case Study

ACUTE NITRATE / NITRITE INTOXICATION IN BEEF CATTLE

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ABSTRACT

Acute death of 44% of a mixed-Simental adult beef cattle herd occurred in January 2024, in the Western Galilee region, near Fassuta village, in the north of Israel. The grazing area where cattle was held, was characterized by majority of young *Silybum marianum* plants, also known as "milk thistle". This nitrophilic plant tend to thrive in heavily grazed range lands. The acute nature of this event characterized by high mortality, methemoglobinemia and high nitrates concentration in samples, indicated plant-origin intoxication as a result of massive consumption of young thistle plants. Indeed, laboratory analysis revealed 6.3% nitrate content (on DM basis) in the plants found in that pasture, and high concentrations of nitrate were found in three samples of intra-ocular fluid from three different cows: the first sample tested contained 100 ppm, and two additional samples contained 80 ppm nitrate. Additionally, an intra-ocular sample from a fetus found in a dead cow contained 25 ppm. When cattle ingest nitrate-containing plants, nitrite ions are produced by ruminal micro-organisms. Nitrite ions then combine with hemoglobin to produce met-hemoglobin, blocking the transport of oxygen. The outcome is a form of oxygen deprivation, which led to this acute intoxication.

Key words: nitrate-nitrite intoxication, cattle, milk thistle, pasture.

INTRODUCTION

Nitrate intoxication of grazing ruminants, especially cattle, is a well-known phenomenon (1-3). Specific intoxication in cattle caused by grazing *Silybum marianum* plants, also known as milk thistle, was described as early as 1955 (4). This nitrophilic plant encroach rangeland areas where animals gather and defecate, in particular around watering and feeding points (5). Density of thistles is affected by cattle grazing management, such as continuous versus rotational grazing (6), but generally in midEastern rangelands, increased cattle grazing density is associated with a higher frequency of thistles (such as the milk thistle and the syrian thistle) (7). Livestock generally avoid entering dense areas of mature thistle but they will graze young, immature plants. Cattle will not graze thistle beyond the late bud stage and grazing milk thistle is deemed dangerous for cattle because of high and possibly lethal concentrations of nitrates (8, 9).

How often such intoxications occur? In a retrospective study of all suspected bovine intoxications submitted to the California Animal Health and Food Safety Laboratory in the years 2000-2011, Nitrate/nitrite poisoning was the most commonly diagnosed plant-associated intoxication (46% of plant associated intoxications) (10).

CASE DESCRIPTION

A herd of a mixed Simental cattle breed, that was held in a pasture near Fassuta village in the Western Galilee, suffered

Figure 1. Group of animals found dead in the field.

Figure 2. Heamorrage from the eyes in an infected cow

Figure 3. Another affected dead cow, lying in a pasture dominated by thistle plants.

Figure 4. The dead bull.

from high mortality rate of 22 out of 50 cows (44%), during one night in January 2024.

The event occurred from the afternoon hours throughout the night, while in the previous day no clinical signs were observed. At morning, the animal carcasses were scattered all around the field (Fig. 1), while they were bloated and a dark blood was excreted from the eyes and nozzles (Fig. 2 & 3). The blood from the nozzles was foamy. During a venous blood sampling it was noticed that the blood didn't coagulates, and its color was indeed red-dark, which indicates met-hemoglobinemia. No signs of diarrhea were found.

This herd has been grazing in the same pasture for the last three months, during this time the cattle received additional poultry manure and potatoes. These cows have been vaccinated against FMD, botulism, rabies, clostridium and babesiosis. Pesticides were not used in the period close to the case. The affected cattle were mature females and the herd's bull (Fig. 4). Young individuals were not affected (Fig. 5 & 6). No clinical signs were observed in the other animals in the herd. Also, no abortions or stillborn were observed during this period. However, a day after the incident, the farmer reported one stillborn (Fig. 7).

The size of the pasture area (which is demonstrated

Figure 5. Living calf among poisoned dead cows.

Figure 6. Living calf near its dam.

Figure 7. Aborted calf a day after the poisoning event.

in Fig. 8) is about 30-50 acre and it was previously used by three different herds. Topographically, the area is rocky and sparsely covered with trees and bushes that are located only around it. In the pasture, young herbs were present and a high quantity of young milk thistle have grown after several days of rain (fig. 9 & 10). Although the herd was accompanied by shepherd dogs that seemed to have eaten or licked the affected cows (fig. 11), there were no clinical symptoms in the dogs. Furthermore, there was no evidence that other animal species such as wild life, have been affected, as generally happens in other acute intoxications such as carbamate or organophosphorus poisoning, due to primary or secondary poisoning. In addition, the breeder reported that already in 2017, the herd suffered from nitrate poisoning.

DIAGNOSIS

Blood, intra-ocular fluid, internal organs and ruminal content were sampled and sent to the toxicology laboratory at Kimron Veterinary Institute.

NITRATE DETERMINATION IN OCULAR FLUID

Sample Collection and Stability

Ocular fluid specimens, collected postmortem, were chosen as the appropriate sample for nitrate determination. These specimens exhibit stability with respect to nitrate concentration for up to 60 hours after death. When refrigerated, stability is maintained for at least 1 week, while storage at -20°C extends stability for up to one month.

Figure 8. Location of the herd.

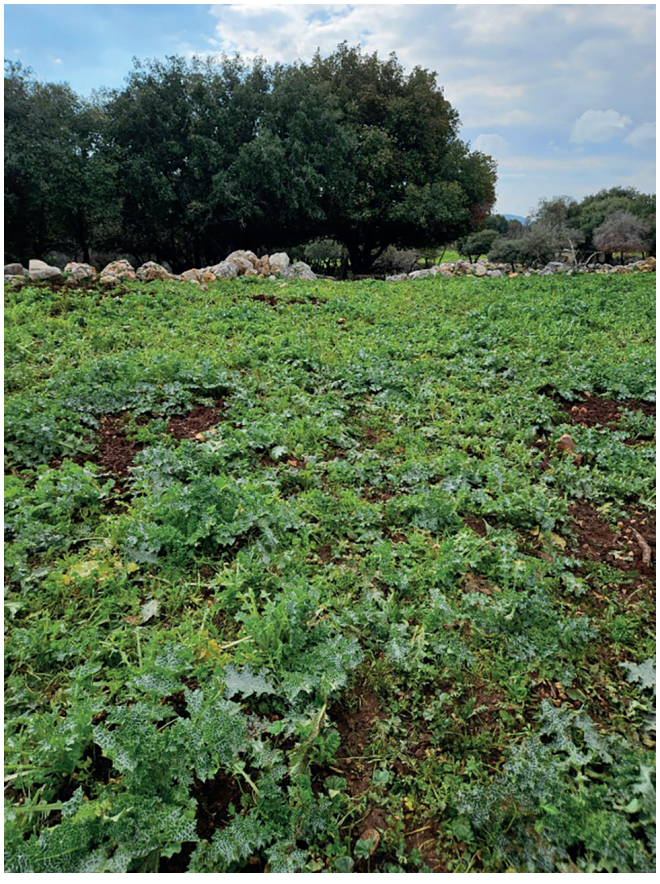

Figure 9. Domination of thistle plants in the pasture.

Figure 10. Flowering thistle.

Testing Method

The MQuant® Nitrate test strips (Merck KGaA, Darmstadt, Germany) were employed for the semi-quantitative determination of nitrate concentrations in ocular fluid. These strips facilitate a quick and easy monitoring process. Nitrate ions in the ocular fluid are reduced to nitrite ions by a reducing agent present on the strip. The resulting nitrite ions react with an aromatic amine to form a diazonium salt, which in turn reacts with N-(1-naphthyl)-ethylene-diamine to form a red-violet azo dye. The concentration of nitrate is estimated by

Figure 11. Herd's dog that fed from a carcass

visually comparing the reaction zone of the test strip with a provided color scale. Additionally, the presence of nitrite ions is indicated by a color change in the "alert zone" of the strip.

Nitrate Determination in Plant Material (*Silybum marianum*)

Identification and quantification of nitrate was measured based on the method described by Buck (11) and Buxton (12).

Sampling and Sample Preparation

Transport of *Silybum marianum* plant samples to the laboratory and preparation of samples for analysis were conducted following the toxicology lab standard operating procedure (SOP). Initially, 50 grams of plant material were cut and dried at 60°C for 24 hours. Subsequently, 1 gram of chopped dried plant material was placed in a 50 ml test tube for further processing.

Extraction Procedure

Extraction of nitrate from the plant material was achieved by adding 20 ml of diluted acid (1% HCL) to the test tube containing the plant material. A 1 ml aliquot was then taken from the filtered extract for subsequent analysis.

Colorimetric Reaction

To facilitate the colorimetric determination of nitrate, 9 ml of 20% acetic acid was added to the aliquot. Furthermore, 0.4 grams of a color reagent powder were dissolved in the solution to convert any nitrate to nitrite, resulting in the development of a red color indicative of the total nitrate content in the extract. Following vortexing and centrifugation, the intensity of the red color was measured.

Measurement and Calculation

The measurement of nitrate concentration was performed using a colorimetric method employing visible spectrophotometry. Absorbance readings were taken at a wavelength of 520 nm. The concentration of nitrate in the extract was calculated based on the absorbance values obtained. Calibration was performed using reagent blanks and a reference standard solution of 25 ppm KNO3.

LABORATORY RESULTS

Thistle plants were indeed found in the ruminal content. Laboratory analysis revealed 6.3% nitrate content (on DM basis) in the plants found in that pasture. High concentrations of nitrate were found in three samples of intra-ocular fluid from three different cows. The first sample tested contained 100 ppm. In the following day, two additional samples were tested, each containing 80 ppm nitrate. An intra-ocular sample from a fetus found in a dead cow contained 25 ppm. Laboratory findings indeed indicated an acute nitrate intoxication.

DISCUSSION

Nitrate poisoning can arise in animals, especially in ruminants. In cattle, which is the most affected species, nitrate toxicosis can occur by several ways, all of them are by ingestion. Ingestion of excess of nitrates can occur from plants (in the field or with forage given), water containing nitrates (for example whey) or nitrate-containing fertilizers. Due to the rumen flora activity, nitrate ions are converted to nitrite ions, which are ten times more toxic than nitrate ions. The same process can occur in the cecum of horses but in a less extent.

Acute nitrite toxicosis, as we had in this case, is manifested by met-hemoblobin formation (which is brown), due to the oxidation of iron atoms in the hemoglobin, from Ferrous iron (Fe+2) to Ferric iron (Fe+3), rendering the hemoglobin unable to carry oxygen. This process can lead to a severe generalized lack of oxygen in organs called methemoglobinemia. In this status, as we have seen in this outbreak, the animal exhibits brown mucous and blood.

Nitrate poisoning in cattle can act very quickly and symptoms may not be seen before the animals are found dead. However, symptoms that can be seen in some cases are weakness, unstable gait, shallow and rapid mouth breathing, rapid and weak pulse, frequent urination, blue or brown discoloration of mucous membranes, tremors, coma and death. Subacute or chronic nitrate poisoning in cattle can result in infertility and abortions.

There are several factors that can affect the quantity of nitrates in the plants.

Some of the most important factors are:

1. The species of the plant. It is known that milk thistle tends to accumulate a high quantity of nitrates.
2. Stalks are usually higher in nitrates concentrations than leaves and grains.
3. Young or immature plants are with higher nitrate contents than mature plants (as we had in this case).
4. Environmental conditions that reduce plants growth such as cloudy or cold weather, can stimulate nitrate accumulation.
5. The presence of nitrate in the plant is higher in night that in the day time.
6. Manure and fertilizers containing nitrogen can lead to excessive storage of nitrates in the plant.
7. **Pathogenesis**
8. The "milk thistle", or in his Latin name *"Silybum marianum"*, belongs taxonomically to the Asteraceae family. It has various common names including milk thistle, blessed milkthistle, Marian thistle, Mary thistle, Saint Mary's thistle, Mediterranean milk thistle, variegated thistle and Scotch thistle. This species is an annual or biennial plant, originally a native of Southern Europe through to Asia, it is now found throughout the world. Typical thistle has red to purple flowers in spring and shiny pale green leaves with white veins (fig. 4). Milk thistle has been used in traditional medicine for centuries (13). Nitrate concentrations in plant dry matter greater than 1.5 NO3 are widely regarded as potentially hazardous to grazing ruminants (14, 15). These guideline values were based on an oral LD50 estimate for cattle of about 0.3 g/kg body weight for nitrate drenched as an aqueous solution (15).
9. Following nitrate-containing plants consumption, nitrate ions are reduced to nitrite ions by the ruminal flora, and are rapidly absorbed and bind to the ruminant's hemoglobin converting it to met-hemoglobin, which inhibits oxygen transport. This process can lead to abortions, fatigue, dyspnea, cyanotic mucous membranes, weakness, and if severe, death due to anoxia. Ruminants are more susceptible because rumen flora can rapidly reduce nitrates to nitrites.

SUMMARY

In this case, 22 of 50 beef cattle succombed due to an acute nitrates poisoning. The herd entered an area with young milk thistle an ingested this plant which was proven by laboratory tests to contain high quantity of nitrates. PM examination revealed a large amount of this plant in the rumen content. The death was quick since it occurred in all the affected animals during one night. Laboratory tests found also nitrates in the occular fluids and blood of several animals . Other laboratory tests to pesticides that can lead to an acute death manifestation like in this case, revealed negative results.

In the field, there was no evidence of other affected animal species such as wildlife or dogs that ate from the carcasses. Furthermore, yound cattle were not affected, due to the lack of rumen and ruminal microorganisms.

This case study demontrates the importance of pasture management and proper knowledge relatting to potential hazardous plants. The natural grazing environment cannot be ignore, rather adressing the cows and the pasture as one holistic eco-system.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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