# PATHOLOGY OF ACUTE AND SUBCHRONIC NITRATE POISONING IN REINDEER (*RANGIFER TARANDUS* L)

Patologin vid akut och subkronisk nitratförgiftning hos ren (*Rangifer tarandus* L)

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*Abstract:* The pathology of nitrate poisoning by forest fertilizers to reindeer was studied. The post mortem picture differed with substance, dose and time of exposure.

Animals that died from acute ammonium nitrate intoxication had lesions similar to those found in acute ammonium and nitrate poisoning in sheep and cattle, without developing methaemoglobinemia. The animal that died from acute sodium nitrate poisoning probably died from acute collapse of the blood pressure without developing methaemoglobinemia and without any significant post mortem lesions. Animals dead of subchronic sodium nitrate poisoning all developed methaemoglobinemia. Animals dead within 24 hours only revealed subserous haemorrhages in the pleura and haemorrhages in musculus longissimus costarum and musculus longissimus dorsi.

Similar pleural and muscular haemorrhages were also found in animals that died 60 - 200 hours after exposure but in these animals were also found what is considered common lesions in connection with nitrate/nitrite posoning; i.e. discolorated and poorly clotted blood, cardiac hamorrhages etc.

The constant finding of these pleural and muscular haemorrhages may indicate almost pathognomonic lesions, in reindeer, in connection with nitrate poisoning of subchronic and chronic nature.

The two animals that died from voluntarily drinking ammonium-nitrate dissolved in water developed lesions indicative of a combined effect of ammonium and nitrate poisoning.

Key words: Reindeer, poisoning, ammonium nitrate, sodium nitrate, pathology.

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NORDKVIST, M., REHBINDER, C., MUKHERJEE, S. C. & ERNE, K.
Patologin vid akut och subkronisk nitratförgiftning hos ren (*Rangifer tarandus* L)

*Sammanfattning:* Patologin vid nitratförgiftning, orsakad av skogsgödselmedel, hos ren har undersökts. Obduktionsbilden varierade med gödselmedel, dosering och exponeringstid.

Djuren som dog av akut ammoniumnitratförgiftning uppvisade likartade förändringar som ses vid akut ammonium- och nitratförgiftning hos får och nötkreatur. Inget av djuren utvecklade methaemoglobinemi. Det djur som dog av akut natriumnitratförgiftning dog troligen av en akut blodtryckskollaps utan att utveckla methaemoglobinemi.

Djur vilka dog av subkronisk natriumnitratförgiftning utvecklade alla methaemoglobinemi. Djuren som dog inom 24 timmar uppvisade enbart subserösa blödningar i pleura och blödningar i musculus longissimus costarum och musculus longissimus dorsi.

Liknande blödningar i pleura och samma muskler sågs också hos djur som dog 60 - 200 timmar efter exposition. Hos dessa djur sågs emellertid också förhändringar som anses vanliga i samband med nitrat/nitritförgiftning såsom, missfärgat dåligt koagulerat blod, blödningar i hjärtat etc.

Det genomgående fyndet av dessa blödningar i pleura och dorsala rygg- bröstmuskler, hos ren, i samband med nitratförgiftning av subkronisk eller kronisk karaktär, är en indikation på synbarligen patognomoniska förändringar.

De två djur som dog efter att frivilligt ha druckit ammoniumnitrat löst i vatten utvecklade förändringar tydande på en kombinerad effekt av ammonium- och nitratförgiftning.

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NORDKVIST, M., REHBINDER, C., MUKHERJEE, S. C. & ERNE, K.
Poron (*Rangifer tarandus* L) äkillisen subkroonisen nitraattimyrkytyksen patologia.

*Yhteenveto:* Tutkittiin metsänlannoitteiden aiheuttaman nitraattimyrkytyksen patologiaa poroissa. Ruumiinavauskuva vaihteli lannoitteesta, annostuksesta ja altistamisajasta riippuen.

Äkilliseen ammoniumnitraattimyrkytykseen kuolleissa eläimissä muutokset olivat samankaltaisia kuin lampaiden ja nautojen äkillisessä ammonium ja nitraattimyrkytyksessä. Yhteenkään eläimeen ei kehittynyt methemoglobinemiaa. Eräs äkilliseen natriumnitraattimyrkytykseen kuollut eläin menehtyi luultavasti äkilliseen verenpainekollapsiin ilman methemoglobinemian kehittymistä.

Kaikkiin subkrooniseen natriumnitraattimyrkytykseen kuolleisiin eläimiin kehittyi methemoglobinemia. Kahdenkymmenenneljän tunnin sisällä kuolleissa eläimissä ilmeni ainoastaan rintakalvonalaisia verenvuotoja sekä verenvuotoja Musculus longissimus costarumissa ja M. longissimus dorsissa.

Samankaltaisia verenvuotoja rintakalvossa ja samoissa lihaksissa nähtiin myös niissä eläimissä, jotka kuolivat 60 - 200 tuntia altistamisen jälkeen. Näissä eläimissä havaittiin kuitenkin myös nitraatti/nitriittimyrkytyksen yhteydessä tavallisina pidettäviä muutoksia kuten väriltään muuttunut ja huonosti hyytynyt veri, verenvuotoja sydämessä jne.

Porojen subkroonisessa tai kroonisessa nitraattimyrkytyksessä säännöllisenä löydöksenä tavatut verenvuodot rintakalvossa ja ylemmässä selkärintalihaksistossa viittaavat siihen, että muutokset ilmeisesti ovat tälle myrkytykselle ominaisia.

Niihin kahteen eläimeen, jotka kuolivat juotuaan vapaaehtoisesti veteen liuotettua ammoniumnitraattia, kehittyi ammonium - ja nitraattimyrkytyksen yhteisvaikutukseen viittaavia muutoksia.

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## INTRODUCTION

The toxicity of nitrate forest fertilizers to reindeer has been investigated by Nordkvist & Erne 1983. The present investigation deals with the pathological lesions found in animals autopsied during the above mentioned study.

## MATERIALS AND METHODS

*Experimental animals*

All animals used (12) were males, 14 - 18 months old. In the experiments optimally as well as poorly fed animals were used. The optimally fed animals were given 1 - 1.5 kg of commercial reindeer fodder (SLR:s renfor), per animal per day, and in addition hay *ad lib.* The poorly fed animals were given 0.5 kg lichen and 0.1 - 0.2 kg of hay per animal per day.

*Experimental procedure:*

The experiments were divided into 3 parts;

1. Acute toxicity
2. Subchronic toxicity
3. Palatability test

*Chemicals*

For acute toxicity ammonium nitrate ($NH_4NO_3$ pro analysi (Merck)) and sodium nitrate ($NaNO_3$ pro analysi (Merck)) were used.

For the palatibility test a commercial fertilizer «skog-AN» (Norsk Hydro AS) was used ($NH_4NO_3$). It was dissolved in water in a 2.5% = (14.44 mg $NO_3$/l) and 1% + (5.78 mg $NO_3$/l) solution.

The chemicals were in the acute and subchronic toxicity tests administered, as an aqueous solution, by means of a drencher (JCJ Super Drencher). In the experiment of subchronic toxicity the dose was divided into 2 parts administered morning and evening, respectively. In the palatability test a 2.5 % and a 1 % water solution was offered animals as the only water supply. These animals had had water withdrawn for 20 hours before the experiment and were poorly fed.

*Pathological investigations*

During the experiments 12 animals were autopsied; in the investigation of acute toxicity 4 animals, in the experiment of subchronic toxicity 6 animals and in the palatability test 2 animals.

Treatments, doses etc are seen in Table 1.

Tissues for histopathological investigation were fixed in 10 % formaldehyde embedded in paraffin, cut in 5 µm thick sections and stained with haematoxylineosin and van Gieson stains.

*Methaemoglobin measurements*

Blood samples were collected from the jugular vein using 10 ml vacutainer tubes (Becton-Dickinson). Methaemoglobin was measured spectrophotometrically according to Evelyn & Malloy (1938).

## RESULTS

### *Acute toxicity*

The three animals that recieved $NH_4NO_3$ showed a somewhat different picture from the animal that received $NANO_3$. The animals which received $NH_4NO_3$ had a pH in the rumen of 8 - 8.3, although the content appeared normal. The blood was dark and only to a very small extent coagulated. Petechial, partly confluent haemorrhages were present subendo- and subepicardially along with minor intramuscular haemorrhages and minor areas of muscular degeneration (Fig. 1) characterized by loss of striation and granulation. The lungs were emphysematous and one animal revealed minor petechial haemorrhages in the submucosa of trachea. The livers showed a mild to moderate acute stasis and mild centrolobular, vacuolar degeneration of hepatocytes. The kidneys were hyperaemic at the cortico-medullary junction and with mild degenerative vacuolating changes in some proximal tubules.

The animal receiving $NaNO_3$ had a watery rumen content with a pH of 6.5. The lungs were markedly emphysematous and the liver showed a mild acute stasis along with some mild centrolobular parenchymatous degeneration. The blood was of normal colour and well coagulated. Methaemoglobinemia was not present at death (Table 1).

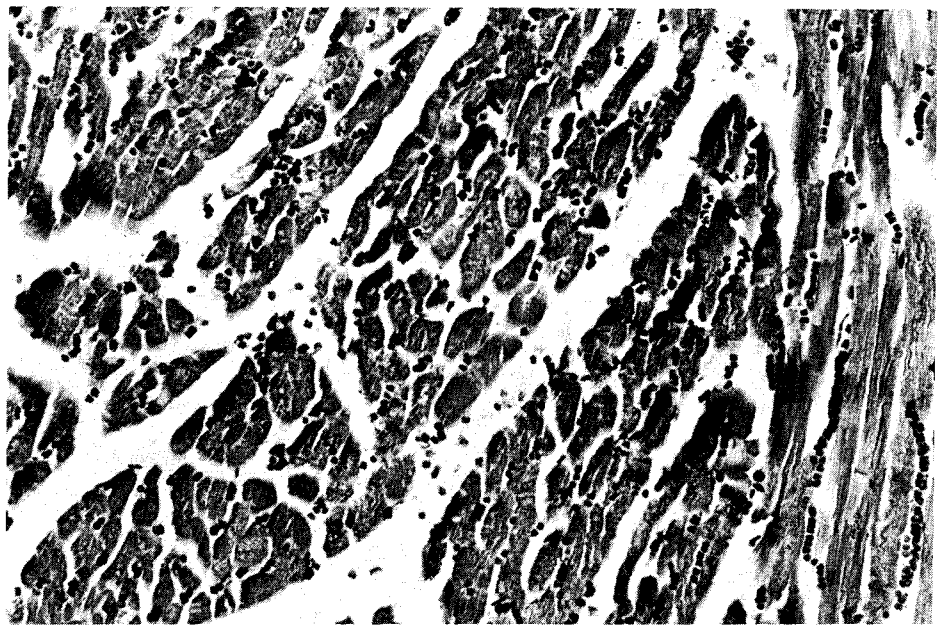

Fig. 1. Myocard: Degeneration of myofibrils characterized by loss of striation, swelling and granular appearence. Note also congestion of capillaries and haemorrhages. HE x 280.

*Myocard: Degeneration karakteriserad av förlust av striering, svullnad och kornighet hos muskelceller. Notera också blodstockning i kapillärer och blödningar. HE x 280.*

### *Subchronic toxicity test*

As seen in Table 1 three of the six animals died after 24 hours while remaining animals, three, died after 60 - 200 hours. Accordingly the three animals of each time interval developed somewhat different pathological picture.

In the three animals that died after 24 hours the rumen content was dry with a pH of 6.0 - 6.2. The blood was of normal colour and coagulated. The carcasses were somewhat dehydrated and of normal colour. Subserous haemorrhages pin point - 1 x 1 cm were found dorsally in thorax located at the site of the costo-vertebral articulations. In addition haemorrhages were found in longissimus costarum and the cranial part of longissimus dorsi.

The three animals that died after 60 - 200 hours developed more prominent lesions. The content of the rumen was normal with a pH of 7.0 - 7.7. The blood was poorly coagulated and dark. The myocards revealed minor intramural subepi- and

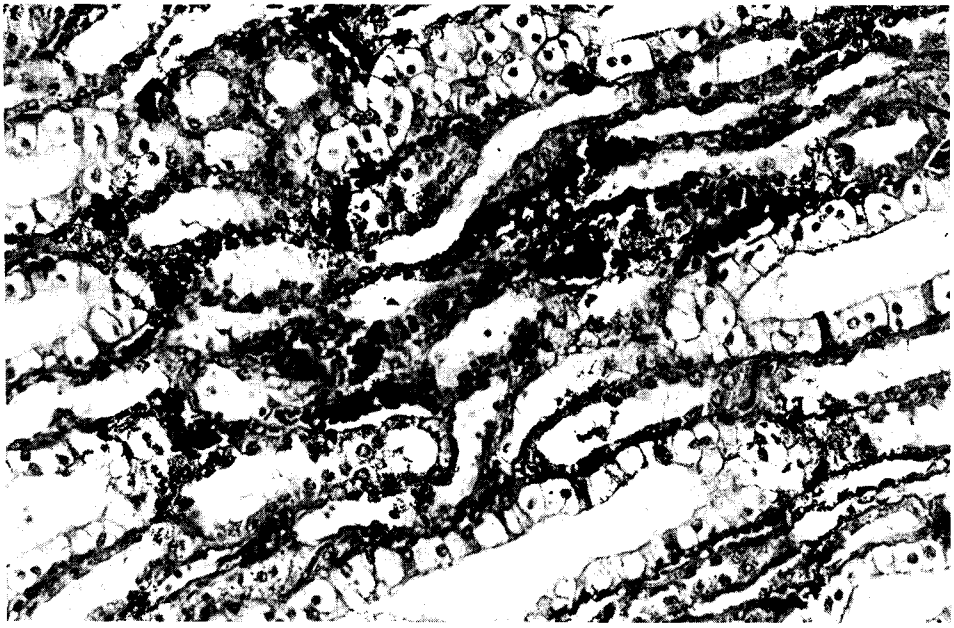

Fig. 2. Kidney: Degeneration of tubular epithelium and interstitial haemorrhages. HE x 450.

*Njure: Degeneration av tubuli epitelet och interstitiella blödningar. HE x 450.*

subendocardial haemorrhages but also minor to marked degenerative lesions of the muscle-cells characterized by loss of striation and a swollen granular appearence (Fig. 1). The kidneys showed degeneration of the epithelium of the proximal tubules and marked engorgement of glomerular capillaries. (Fig. 2) Also these animals had similar subserous haemorrhages dorsally in the thorax ventrally of the costovertebral articulations and haemorrhages in longissimus costarum and the cranial part of longissimus dorsi. The carcasses were dehydrated and of normal colour. The level of methaemoglobinemia at death varied considerably (Table 1).

### *Palatability test*

The two animals that died in this experiment showed almost the same lesions. The rumen content was watery, with an acid smell and had a pH of 7.6. The blood was poorly coagulated and dark. The hearts revealed petechial to large 1 x 1 cm partly confluent subepicardial haemorrhages and areas of degeneration of the muscle cells characterized by loss of striation, swelling and granulation. The livers showed acute moderate stasis. The kidney were markedly hyperaemic at the cortico - medullary junction and with pronounced degeneration and necrosis of the epithelium of the proximal tubules.

In one animal (no. 105) was found subserous haemorrhages dorsally in the thorax located beneath the costo-vertebral junctions and also bleedings in musculus pectoralis. Minor petechial haemorrhages in the aboral part of the tracheal mucosa were also noted. The levels of methaemoglobinemia at the death were relatively low (Table 1).

## DISCUSSION

It is apparent, from the present experiments, that the post mortem picture in reindeer differs with substance, dose and time of exposure.

### *Acute toxicity*

The three animals dead, in the acute toxicity experiment with $NH_4NO_3$, did not develop methaemoglobinemia and showed pathological lesions in most respects similar to those found in acute ammonium intoxication (Singer & McCarty 1971) and strong similarities with ammonium - nitrate poisoning in cattle (Horner 1982).

It thus seems reasonable to conclude that the main toxic substance acting on these animals was ammonium. The high alcaline levels of the rumen content supports this statement. Furthermore, the reduction of nitrate to ammonium (Holtenius

1957, Deeb & Sloan 1975) by the rumen flora may initially have increased the amount of ammonium.

The animal that died after being dosed with $NaNO_3$ did neither develop methaemoglobinemia nor did it develop any prominent post mortem lesions but mild lesions indicative of circulatory failure. This animal probably died from an acute collaps of the blood pressure due to the vasodilatory effect by nitrite (Holtenius 1957).

### *Subchronic toxicity*

The three animals that died 24 hours after dosing developed methaemoglobinemia but few pathological lesions. The only notable lesions were the subserous haemorrhages in the thorax, located beneath the costo-vertebral articulations, and haemorrhages in musculus longissimus costarum and musculus longissimus dorsi. These haemorrhages could be a result of forced breathing as the death of the animals most probably was due to acute oxygen shortage (anoxia) caused by the methaemoglobin formation (Asbury & Rhode 1964).

The lack of what is considered almost pathognomonic post mortem lesions i.e. discolorated and unclotted blood, cardiac haemorrhages and congestion of liver, kidneys and intestine (Sinclair & Jones 1967, Deeb & Sloan 1975) is striking. Apparently the animals died too rapidly to develop more prominent lesions.

The animals that died after 60 - 200 hours however showed most of the above mentioned common lesions but for haemorrhages in the mucosa of the trachea reported to be a common finding in cattle (Johannsen & Kühnert 1969). Neither did they show irritation of abomasum and intestines which has been reported to be a common finding in connection with nitrate poisoning (Bradley et al. 1974, Hibbs 1979) but by Johannsen & Kühnert (1969) reported to be an inconsistent finding. The kidney lesions, characterized by degeneration of the epithelium of the proximal tubules and engorgement of capillary tufts have been observed in connection with ammonium poisoning (Osebold 1947, Singer & McCarty 1971) but also in connection with ammonium nitrate poisoning (Horner 1982) and experimental nitrate poisoning (Deeb & Sloan 1975). Kidney lesions thus have to be regarded as a consistent finding in connection with subchronic or chronic poisoning with fertilizers containing ammonia and/or nitrates. Also in these three animals haemorrhages in the pleura and musculus longissimus costarum and musculus longissimus dorsi were a prominent finding. The constant finding of these haemorrhages indicates an almost pathognomonic lesion in reindeer in connection with nitrate poisoning of subchronic or chronic nature.

### *Palatability*

The two animals were exposed to a common forest fertilizer dissolved in water. Both animals developed methaemoglobinemia but the lesions found at autopsy are indicative of a combined effect of ammonium and nitrate poisoning, being rather similar to the findings reported in suspected ammonium nitrate poisoning in cattle (Horner 1982).

The lack of haemorrhages in the muscles of longissimus costarum and longissimus dorsi and the fact that only one of the animals revealed subserous haemorrhages dorsally in the thorax would thus indicate that anoxia was not the major cause of death in these animals but an important contributary factor.

Table 1. Animals dead during the experiments.
*Tabell 1. Djur som dött i samband med experimenten.*

| Animal No. *Djur Nr.* | Experiment — « — | Hours of survival *Timmar överlevnad* | Substance *Substans* | Dose g $NO_3$/kg body-weight *Dos i g $NO_3$/kg kroppsvikt* | Maximum Met-haemoglobin % — « — | Methaemoglobin at death % *Methaemoglobin vid döden %* | Food regimen *Utfodring* |
|---|---|---|---|---|---|---|---|
| 109 | Acute *Akut* | 2 | $NH_4NO_3$ | 1.2 | 0 | 0 | poor *mager* |
| 121 | Acute *Akut* | ½ | $NH_4NO_3$ | 2.1 | 0 | 0 | poor *mager* |
| 206 | Acute *Akut* | ½ | $NH_4NO_3$ | 1.1 | 0 | 0 | optimal |
| 218 | Acute *Akut* | 11 | $NaNO_3$ | 2.0 | 0 | 0 | optimal |
| 106 | Subchronic *Subkronisk* | 180 | $NaNO_3$ | 2 x 0.4 | 70.6 | 0.5 | poor *mager* |
| 111 | Subchronic *Subkronisk* | 24 | $NaNO_3$ | 2 x 0.5 | 57.6 | 36.7 | poor *mager* |
| 112 | Subchronic *Subkronisk* | 24 | $NaNO_3$ | 2 x 0.25 | 48.6 | 48.6 | poor *mager* |
| 114 | Subchronic *Subkronisk* | 24 | $NaNO_3$ | 2 x 0.5 | 67.2 | 67.2 | poor *mager* |
| 207 | Subchronic *Subkronisk* | 60 | $NaNO_3$ | 2 x 0.4 | 26.4 | 26.4 | optimal *optimal* |
| 212 | Subchronic *Subkronisk* | 200 | $NaNO_3$ | 2 x 0.4 | 80.8 | 70.8 | optimal *optimalr* |
| 105 | Palatability *Smaklighet* | 180 | $NH_4NO_3$ | 1 % sol | 74.6 | 26.3 | poor *mager* |
| 122 | Palatability *Smaklighet* | 42 | $NH_4NO_3$ | 2.5 % sol | 31.3 | 31.3 | poor *mager* |

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