ORIGIN AND COURSE OF METHEMOGLOBINEMIA IN CALVES

J. Bouda, P. Jagoš, M. Skřivánek, J. Mužík and D. Supáková

Department of Diagnosis, Therapy and Control of Animal Diseases,
University of Veterinary Science, 612 42 Brno

Received May 22, 1985
Dedicated to the 60th birthday of Prof. MVDr. L. Vrzgula, CSc.

Abstract


Methemoglobinemia was studied in dependence on calf age and dose of administered KNO₃ in 34 calves. No significant increase in blood methemoglobin levels was observed in 16 calves receiving from the age of 2 - 3 days 200 mg NO₃⁻ or 500 mg NO₃⁻ per 1 litre of colostrum or milk daily for six days. The addition of NO₃⁻ doses increasing from 200 to 1 000 mg NO₃⁻ l⁻¹ of diluted milk replacer did not induce a marked appearance of methemoglobinemia within five weeks in calves from the age of 6 days. When high oral doses of KNO₃ were administered to calves on milk nutrition (0.5 g NO₃⁻. kg⁻¹ body mass) and on fodder nutrition (0.4 g NO₃⁻. kg⁻¹ body mass) a large increase of methemoglobin in blood and clinical manifestations were observed. Four to six hours after the oral application of KNO₃, methemoglobinemia reached its critical values (25 - 48 %). It was manifested by apathy, accelerated pulse and tachypnea.

Conjunctiva, mucous membrane of vagina and oral cavity were found to be cyanotic, grey-brown, blood was of chocolate brown colour.

If the content of NO₃⁻ in cattle feed is increased and methemoglobinemia is suspected, the blood sampling, from the diagnostic viewpoint, has to be carried out within 4 - 6 hours after feeding since the methemoglobin content in blood falls rather rapidly after this period. If severe cases of methemoglobinemia are treated, 1 or 2 % solution of methylene blue in 5 % glucose solution, at a dose of 10 - 20 mg. kg⁻¹ body mass proved to be quite efficient.

Calves, KNO₃, blood, vitamin A and E, thiamine, glucose, urea, AST, GMT, pH, pCO₂, base excess (BE), Na, K, Mg, Ca, inorganic P, therapy.

The increased content of NO₃⁻ in plants is associated with the overfertilization with nitrates and with improper application of herbicides. The intake of feed or water with a high content of nitrates increases the danger of methemoglobinemia development
in farm animals in the last years (Bartík and Rosival 1971; Kühnert 1981; Berschneider et al. 1979; Bouda et al. 1984).

The main toxicological manifestation following the increased intake of NO\textsubscript{3} appears in animals after the reduction of NO\textsubscript{3} to NO\textsubscript{2}. Nitrites are formed as an intermediate product during the bacterial reduction of NO\textsubscript{3} to NH\textsubscript{3} in the rumen (Bartík and Rosival 1971) or in the intestine (Knotek and Schmidt 1965). According to Berschneider et al. (1979) the extent of NO\textsubscript{3} reduction depends on the functional condition of digestive system of animals. In the case of diarrheic diseases the microflora of the intestinal tract usually changes and multiplication of bacteria reducing NO\textsubscript{3} to NO\textsubscript{2} possibly occurs (Somorà et al. 1962).

When a certain amount of NO\textsubscript{3} in feed is exceeded the rate of NO\textsubscript{3} to NH\textsubscript{3} reduction decreases and the accumulation of NO\textsubscript{2} in the intestinal tract occurs. NO\textsubscript{2} is rapidly absorbed from rumen and into blood where it transforms Fe of hemoglobin to Fe of methemoglobin thus causing the development of methemoglobinemia. Oxygen which is firmly bound in methemoglobin molecule cannot be transferred to tissues and animals thus die as a result of oxygen insufficiency (Bartík and Rosival 1971). Apart from this main toxicological effect nitrates exert a vasodilation effect which is manifested predominantly in the splanchnic region with the simultaneous decrease of blood pressure (Berschneider et al. 1979).

It has been established that in children up to the age of three months and in very young animals (Kübler 1965) methemoglobinemia develops more easily since, in contrast to mature individuals, children do not possess sufficiently developed enzymatic reduction system (NaDH - methemoglobin reductase) capable of transforming methemoglobin to oxyhemoglobin. Severe forms of methemoglobinemia ending in some cases by death occur in infants already after the intake of water containing 50 - 80 mg NO\textsubscript{2}/l. That is the reason why the content of NO\textsubscript{3} in water used for artificially fed babies must not exceed 15 mg/l. The aim of our work has been to study the development and course of methemoglobinemia in dependence on calf age, dose and duration of interval of orally administered nitrates.

Materials and Methods

The effect of nitrates after oral administration has been verified in four experiments with 34 calves, cross-breds of Bohemian Pied and Black Pied cattle. Experiment No. 1 was conducted on two groups of calves (body mass 37 - 42.5 kg) which were from the age of 2 - 3 days given daily in the duration of 6 days 200 mg NO\textsubscript{3} per one litre of colostrum or milk, i. e. 1.4 g NO\textsubscript{3} calf and day (1st group). The other group received 500 mg NO\textsubscript{3} l\textsuperscript{-1} of colostrum (milk, i. e. 3.5 g) calf and day. Methemoglobin (MThb) content in the blood of calves was determined prior to the administration of KNO\textsubscript{3}, 5 hours after the 1st and 5 hours after the last (6th) application of KNO\textsubscript{3}.

No. 2 experiment was conducted on six calves of the average age 6 days (range 5 - 8 days) and average mass 40.2 kg at the
time of the beginning of the experiment. The feed ration contained Laktosan A milk replacer with 18% of fat which was diluted in water at the ratio 1:9.

Daily dose of diluted Laktosan was 7 litres at the beginning and 9 litres later per calf. Water and hay and concentrates were offered to calves from the 8th and 14th day of age, resp. The calves were weaned at 54 days of age.

In the period of green fodder the feed ration was represented by T concentrate mixture, good quality meadow hay and water.

The experimental group consisting of 3 calves was given nitrates (KNO₃) in the solution of Laktosan milk replacer for a period of 42 days from the age of 6 days. Further three calves were used as control. By adding lower doses of NO₃⁻ into the Laktosan we imitated the increased content of NO₃⁻ in water which is quite realistic in practice. The experimental group of calves was treated with gradually increasing doses of NO₃⁻ from 200 to 1000 mg/l of Laktosan. The total daily intake of NO₃⁻ amounted to 1.4 g at first, later it reached 9.0 g per calf. In both groups of calves observation and clinical examination were carried out daily. Blood samples from v. jugularis were taken twice a week and basic biochemical and hematological parameters (methemoglobin, AST, GMT, glucose, total protein, vitamin A and E, urea, Na, K, Mg, Ca, inorg. P, pH, BE, pCO₂, hematocrit, hemoglobin, red and white blood count) were determined. After the calves had been sacrificed, thiamine content was determined in samples of brain cortex, liver, heart and skeletal muscles.

The experiment No. 3 was carried out on 6 calves; both experimental and control group consisted of 3 calves (8 weeks old) on milk nutrition, their average body mass being 72 kg. Two calves from the experimental group received (using an eosophageal probe) 0.5 g NO₃⁻ per kg of body mass (altogether 36 g NO₃⁻) and the third calf was given 27 g NO₃⁻ (0.37 g NO₃⁻ per kg) of body mass in the form of KNO₃ diluted in 0.5 l of water. Blood samples were taken prior to KNO₃ application and then in one hour intervals after the KNO₃ administration and the content of methemoglobin and basic biochemical parameters was determined.

The experiment No. 4 was carried out on 6 calves aged 4 - 6 months, weighing 150 - 205 kg. The experimental group, consisting of 4 animals, was given (using an eosophageal probe) 400 mg NO₃⁻ per kg of body mass in the form of KNO₃ diluted in one litre of water. The control group was represented by two calves. The sampling of blood for biochemical and hematological examination was performed in the same way as in the experiment 3.

For the treatment of methemoglobinemia 1 and 2% solution of methylene blue in 5% glucose solution was tried. Clinical observations of all animals were carried out during all experiments. Acid-base balance of blood, i.e. pH, pCO₂, base excess (BE) and standard bicarbonate (SB) were determined in venous blood using an Astrup equilibration method on a BME 22 instrument of Radiometer. Hematocrit was determined by a routine micromethod in capillaries, hemoglobin and leukocyte number on a Coulter Counter instrument. Methemoglobin (MtHb) in blood was determined photometrically according to Homolká (1971). Na, K, Ca and Mg concentrations were determined by atomic absorption spectrophotometry on an Atomspek instrument. Inorganic P, urea, glucose,
bilirubin, total protein, activities of aspartate aminotransferase (AST), gamma glutamyltransferase (GMT) were determined photometrically on an Eppendorf instrument using Bio-test of Lachema Brno. The thiamine content was determined spectrofluorometrically by a method according to Picková (1969). Vitamin A and E was determined spectrofluorometrically on a Perkin-Elmer 204 instrument by a method of Thompson et al. (1971, 1973) applying the findings of Van Stevenick and De Goeij (Bouda 1979).

Results

The results summarized in Table 1 (experiment 1) show that the level of methemoglobin (MtHb) in the blood of all calves was found to be below 5%; in no case the addition of 500 mg NO\textsubscript{3} \cdot l\textsuperscript{-1} of colostrum or milk induced higher methemoglobinemia. It has to be pointed out, however, that in this experiment anorexia was observed in calves of the 2nd group after the addition of 500 mg NO\textsubscript{3} \cdot l\textsuperscript{-1}, the occurrence of diarrhea was more frequent and intensive than in calves of the group 1, i.e. after the addition of 200 mg \cdot l\textsuperscript{-1} of colostrum. In the last day of KNO\textsubscript{3} application diarrhea occurred in all 8 calves of the 2nd group whereas only two animals of the group 1 were affected.

Table 1

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Prior to the 1st treatment</th>
<th>After the 1st treatment</th>
<th>After the 6th treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 400 mg NO\textsubscript{3} per day</td>
<td>3.75 ± 0.38</td>
<td>3.55 ± 0.18</td>
<td>3.74 ± 0.19</td>
</tr>
<tr>
<td>n = 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>3 500 mg NO\textsubscript{3} per day</td>
<td>3.50 ± 0.19</td>
<td>3.56 ± 0.23</td>
</tr>
<tr>
<td>n = 8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± S.E.M.

In the experiment 2 neither clinical examination nor body mass gain differed significantly between the experimental and control groups. Prior to the addition of KNO\textsubscript{3} to calves in the experiment 2 the levels of methemoglobin ranged within 2.1 - 4.0 %. During the addition of KNO\textsubscript{3} the average MtHb level reached 4.8 % and 4.4 % in the experimental and control group, resp. The dynamics
of average MtHb levels after the addition of increasing KNO₃ do-
ses is presented in Fig. 1. Methemoglobinemia in one animal of
the experimental group reached the value of 8% after a daily do-
se of 4 g NO₃/calf. As far as the other followed bio-
chemical parameters of blood are con-
cerned (hemoglobin, methemoglobin, vi-
tamin A and E, AST, GMT, glucose, Na,
K, Ca, inorg. P, Mg, urea, gamma glo-
bulins, pH, BE, pCO₂, hematocrit) no significant
differences were found between the experimental and
control group. The vitamin B₃ content in all investigated
tissues of the control group was found to be higher
than that in tissues of the experimental
group of calves, the difference however being not statistically
significant (Table 2).

Table 2
Thiamine content in tissue samples of experimental
and control calves (mg . 100 g tissue)

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Heart</th>
<th>Brain</th>
<th>M. gracilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>0.19±0.08</td>
<td>0.16±0.06</td>
<td>0.24±0.06</td>
<td>0.28±0.25</td>
</tr>
<tr>
<td>Control</td>
<td>0.29±0.05</td>
<td>0.40±0.26</td>
<td>0.30±0.11</td>
<td>0.29±0.11</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M.
The differences were not significant.

Neither clinical manifestations nor the increase of MtHb in
blood (Fig. 2) were observed in the experiment No. 3 when 27 g
NO₃ (0.36 g NO₃ . kg⁻¹ of body mass) were intraruminally applied
to a calf. Doses 36 g NO₃ (0.5 g NO₃ . kg⁻¹ body mass) produced
in two calves after three hours steep rise of MtHb in blood, the
maximum values (26 and 35 %) being reached 5 hours following the administration (Fig. 2). Calf with 26 % methemoglobinemia showed malaise, apathy but cyanotic discoloration of mucosa was not marked. Calf, in which MtHb reached 35 %, was strikingly apathetic already 3 hours after the application of KNO₃, later its gait was reeling, stability of posture unstable, it leant its head against manger, fore limbs were placed too far back. After 5 and 6 hours after the KNO₃ administration the calves fell and lay in lateral position, pulse and breathing were markedly frequent; conjunctiva, vaginal mucosa and mucous membrane of the mouth were cyanotic and grey-brown, blood was of chocolate brown colour. Seven hours after the KNO₃ administration the MtHb levels started to decrease gradually and general condition of calves improved. Next day the MtHb levels as well as clinical condition were again within the norm.

In the experiment No. 4, in which calves on fodder nutrition were used, methemoglobinemia reached critical values (25 - 48 %) within 3 - 6 hours after the administration of KNO₃. In one calf clinical manifestations of intoxication after the application of KNO₃ have not appeared which suggests a different individual sensitivity of animals (Fig. 3). Apathy, markedly frequent pulse and

![Fig. 2. Dynamics of methemoglobin levels in blood of calves on milk nutrition following the application of 500 mg NO₃ (in the form of KNO₃) /kg of body mass.](image)

![Fig. 3. Dynamics of methemoglobin levels in blood of control calves (denoted by dashed line), of experimental calves on fodder nutrition following the applications of 400 mg NO₃ (in the form of KNO₃) /kg of body mass. Experimental calves: (● ▲ ○ )](image)
breathing were observed in other calves of the experimental group usually 3 - 4 hours after the application of \( \text{KNO}_3 \). After 4 - 5 hours static and kinetic ataxy was noted, twitch of thoracic, and limb muscles appeared and later the animal collapsed and therapy had to be applied. Cyanotic to grey-brown discoloration of mucous membrane was dependent on the degree of methemoglobinemia.

When treating methemoglobinemia, the intravenous application of 1 or 2 % solution of methylene blue in the solution of 5 % glucose, administered in the dose 10 - 20 mg . kg\(^{-1}\) body mass in combination with routinely applied i. m. dose of caffeine proved to be very satisfactory. Marked improvement in health condition of calves was observed already 5 minutes from the start of administration, within 5 - 10 minutes following the application of methylene blue the animals were usually able to stand. Calves accepted food usually within 4 - 6 hours after the treatment. Within several minutes after the application saliva, ocular secretion and urine were coloured blue. One hour following the application of methylene blue blood was of bright red colour. Blue-green colouration of urine, caused by methylene blue, outlasted till the 3rd day after its application.

**Discussion**

The problems of nitrates and nitrites in relation to methemoglobinemia have been studied particularly in mature ruminants (Wiesner et al. 1979; Lotthammer et al. 1982; Berschneider et al. 1979; Kühnert 1981). It has been known from human medicine that even a small amount of \( \text{NO}_3^- \) (over 15 mg/l) in water causes methemoglobinemia in infants since their reduction enzymatic systems are not sufficiently developed. Fetal hemoglobin in a newborn child represents 60 - 80 % of total hemoglobin. The ability to reduce methemoglobin with the aid of NADH-methemoglobin reductase is low in the case of fetal methemoglobin (Kübler 1965). Literature data have also been presented stating that feeding rations with a high content of \( \text{NO}_3^- \) causes abortions in breeding dams or calves with lowered viability are born (Weissbach and Hein 1976). In our experiments, particularly in No. 1 experiment and partially also in No. 2, we have tried to evoke conditions which we can encounter in practice.

By additions of various doses of \( \text{NO}_3^- \) into colostrum, milk and Laktosan, amounting up to 500 mg/l and 1 000 mg/l in the experiment 1 and 2, resp., we have imitated the increased content of \( \text{NO}_3^- \) in water.

It follows from the experiment 1 that even after relatively high doses of \( \text{NO}_3^- \) in very young calves the increase of methemoglobin level over the reference value (Jagoš and Bouda 1981) has not occurred. If higher doses of \( \text{NO}_3^- \) were applied anorexia in calves in the experiment 1 has however appeared, the incidence of diarrhea being more frequent than in the control animals. According to Kühnert (1981) this diarrhea could appear as a result of local irritation of mucosa of digestive system and osmotic effect (Bartík and Pískáč 1974). On the other hand, in the experiment No. 2 mild subclinical methemoglobinemia appeared in one calf out of three older ones following the dose of
500 mg l\(^{-1}\). As far as other studied clinico-biochemical parameters are concerned, no significant difference have been found between the experimental and control group. High resistance of older calves against NO\(_3\) is dealt with in the experimental work of Berend et al. (1979). These authors administered to calves on milk nutrition aged from 10 weeks doses of NO\(_3\) ranging from 15 to 370 mg kg\(^{-1}\) body mass in the duration of 8 weeks. They found no marked effect upon the weight gain, nutritient conversion, methemoglobin and hemoglobin content and blood erythrocyte count, not even in the group of calves with the highest NO\(_3\) intake.

The toxic effect of NO\(_3\) is dependent in a high degree both on the composition of feed ration and on the health condition of an animal and individual difference in metabolization of NO\(_3\). Also in our previous studies we found higher degree of methemoglobinemia in diarrhea of calves when comparing with healthy ones. According to literature data the reduction of NO\(_3\) to NO\(_2\) occurs also due to bacterial intestinal flora (Somor'a et al. 1962). Also Escherichia coli, Pseudomonas fluorescens, Bacillus subtilis and Staphylococcus albus, which can be present in milk replacers, are responsible for the reduction of NO\(_3\) to NO\(_2\) (Selenka 1970). On the other hand, the bacteria of lactic fermentation suppress the reduction of NO\(_3\). It follows from the presented data that the sensitivity of calves towards intoxication by NO\(_3\) could be substantially higher than in our experiments if quality of nutrition is not good and diarrhea is present.

We have also found that calves when gradually adapted to NO\(_3\) tolerate substantially higher doses of NO\(_3\), from 1.4 to 9.9 g of NO\(_3\) (i. e. from 200 mg to 1 000 mg NO\(_3\) l\(^{-1}\) of Laktosan) without apparent clinical signs and increase in methemoglobin content in blood over the reference value. On the other hand, a high increase of methemoglobin in blood and clinical manifestation of methemoglobinemia were observed after single doses of NO\(_3\) in experiment 3 and 4. Methemoglobinemia reached critical values (25 - 48 %) following peroral application of KNO\(_3\) within 3 - 6 hours. Neither clinical manifestations nor more significant increase of methemoglobin appeared in one calf on fodder nutrition receiving high dose 0.4 g kg\(^{-1}\) body mass, which demonstrates the individual sensitivity of animals towards the intoxication by NO\(_3\). Dynamics of methemoglobin levels in blood of calves on milk and fodder nutrition has been studied both from the diagnostic and therapeutic point of view.

It follows from our experiments that the highest methemoglobinemia was reached within 4 - 6 hours following the application of KNO\(_3\). From this reason the blood sampling has to be carried out 4 - 6 hours after feeding if methemoglobinemia is suspected since prior to or after this time interval the methemoglobin values are significantly lower.

From the finding of methemoglobinemia in its subclinical form the determination of methemoglobin in blood is decisive. From the clinical point of view anamnnesis, cyanosis to brownish discolouration of mucosa (conjunctiva, vaginal mucosa) and chocolate brown colour of blood are of great significance (Bouda et al. 1984). As an therapeutic agent an intravenous application of 1 or 2 % solution of methylene blue in the solution of 5 % glucose, at a dose of 10 - 20 mg kg\(^{-1}\) body mass, with a simultaneous i. v. or i. m. application of coffeein has proved to be very successful.
It follows from our work that calves are not as sensitive as children towards the intoxication by NO\textsuperscript{3}. Lower sensitivity towards NO\textsuperscript{3} and NO\textsuperscript{2} was also observed by Dvořák (1984) in piglets and growing pigs. It follows that calves and piglets tolerate higher doses of NO\textsuperscript{3} than children. Since in ethiopathogenesis of many diseases of young farm animals more factors are usually involved it is henceforth necessary to devote due attention to the problems of NO\textsuperscript{3} and NO\textsuperscript{2}.

Vznik a průběh methemoglobinémie u telat

Methemoglobinémie byla studována u 34 telat v závislosti na jejich věku a dávce podaného KNO\textsubscript{3}. U 16 telat, kterým bylo od stáří 2-3 dnů po dobu 6 dnů přidáváno denně na 1 litr mleziva nebo mléka 200 mg KNO\textsubscript{3}, resp. 500 mg KNO\textsubscript{3}, nedošlo k významnému zvýšení hladin methemoglobinu v krvi. Přidávání vzestupných dávek KNO\textsubscript{3} od 200 do 1 000 mg KNO\textsubscript{3} 1-1 oděděné mléčné náhražky nezpůsobilo u telat od stáří 6 dnů po dobu 5 týdnů výraznější vzestup methemoglobinémie. Po velkých jednorázových perorálních dávkách KNO\textsubscript{3} u telat na mléčné výživě (0,5 g KNO\textsubscript{3}, kg-1 ž. h.) a na rostlinné výživě (0,4 g KNO\textsubscript{3}, kg ž. h.) dosáhlo k vysokému vzestupu methemoglobinu v krvi a ke klinickým projevům. Za 4-6 hodin po perorální aplikaci KNO\textsubscript{3} dosahovala methemoglobinémie kritických hodnot (25-48 %). Methemoglobinémie se projevila apatií, zrychlením pulsu a dechu, spojivky sliznice poševní a dutiny ústní byly cyanotické, šedohnědé, krev měla čokoládovou barvu.

Při zvýšeném obsahu NO\textsuperscript{2} v krmivu pro skot a při podezření na methemoglobinémii je třeba z diagnostického hlediska provádět odběr krve za 4-6 hodin po nakrmení zvířat, nebot po této době dochází k rychlému poklesu methemoglobinu v krvi. Při léčbě těžkého stupně methemoglobinémie se velmi dobře osvědčil 1% nebo 2% roztok metylenové modř v roztoku 5% glukózy, v dávce 10 - 20 mg . kg-1 ž. h.

Изучение возникновения и протекания метгемоглобинемии телят

Метгемоглобинемия изучалась у 34 телят в зависимости от их возраста и дозы подаваемой KNO\textsubscript{3}. У 16 телят, которым в возрасте 2-3 суток добавляли ежедневно в течение 6 суток 1 литр молозива или молока 200 мг KNO\textsubscript{3} или 500 мг KNO\textsubscript{3}, существенного повышения уровня метгемоглобина в крови не произошло. Добавлением увеличивающихся доз KNO\textsubscript{3} от 200 до 1000 мг KNO\textsubscript{3} на один литр молочного заменителя у телят в возрасте 6 суток в течение 5 недель не было вызвано более значимого увеличения метгемоглобинемии. После одноразовой дачи большой дозы KNO\textsubscript{3} перорально телятам на молочном питании (0,5 г KNO\textsubscript{3}, кг-1 живого веса) и на растительном корму (0,4 г KNO\textsubscript{3}, кг-1 живого веса) произошло существенное повышение метгемоглобина в крови и появились клинические признаки. Через 4-6 часов после пероральной дачи KNO\textsubscript{3}, метгемоглобинемия достигала критических величин (25-48%). Метгемоглобинемия проявлялась апатией, учащенным пульсом и дыханием, соединительные оболочки слизистой влагалища и полости рта были цианотичные, серо-копычневые, кровь — шоколадно-коричневого цвета.
При повышенном содержании NO\textsuperscript{2-} в кормах для скота и при подозрении в метемоглобинемии необходимо с диагностической точки зрения взять кровь через 4-6 часов после кормления животных, так как по истечении указанного срока происходит быстрое понижение метемоглобина в крови. В ходе лечения тяжелой метемоглобинемии хорошо зарекомендовала себя 1% или 2% раствор метиленового синего в пастыре 5% глюкозы дозой 10-20 мг.кг. \textsuperscript{-1} живого веса.

References


