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ORIGIN AND COURSE OF METHEMOGLOBINEMIA IN CALVES

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Abstract

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Methemoglobinemia was studied in dependence on calf age and dose of administered KNO_3 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 NO_3^- or 500 mg NO_3^- per 1 litre of colostrum or milk daily for six days. The addition of NO_3^- doses increasing from 200 to 1 000 mg NO_3^- . 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 KNO_3 were administered to calves on milk nutrition (0.5 g NO_3^- . kg^{-1} body mass) and on fodder nutrition (0.4 g NO_3^- . . 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 KNO_3 methemoglobinemia reached its critical values (25 - 48 %). It was manifested by apathy, accelerated pulse and tachypnea.

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

If the content of NO_3^- 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, KNO_3, blood, vitamin A and E, thiamine, glucose, urea, AST, GMT, pH, pCO_2, base excess (BE), Na, K, Mg, Ca, inorganic P, therapy.

The increased content of NO_3^- 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_3^- appears in animals after the reduction of NO_3^- to NO_2^-. Nitrites are formed as an intermediate product during the bacterial reduction of NO_3^- to NH_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_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_3^- to NO_2^- possibly occurs (Somora et al. 1962).

When a certain amount of NO_3^- in feed is exceeded the rate of NO_3^- to NH_3 reduction decreases and the accumulation of NO_2^- in the intestinal tract occurs. NO_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 insufficience (Bartík and Rosival 1971). Apart from this main toxicological effect nitrites 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_3^-/l. That is the reason why the content of NO_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_3^- per one litre of colostrum or milk, i. e. 1.4 g NO_3^- calf and day (1st group). The other group received 500 mg $NO_3^- . l^{-1}$ 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_3, 5 hours after the 1st and 5 hours after the last (6th) application of KNO_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 im 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 (KNO_3) 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 NO_3^- into the Laktosan we imitated the increased content of NO_3^- in water which is quite realistic in practice. The experimental group of calves was treated with gradually increasing doses of NO_3^- from 200 to 1 000 mg/l of Laktosan. The total daily intake of NO_3^- 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, pCO_2, 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 NO_3^- per kg of body mass (altogether 36 g NO_3^-) and the third calf was given 27 g NO_3^- (0.37 g NO_3^- per kg of body mass in the form of KNO_3 diluted in 0.5 l of water. Blood samples were taken prior to KNO_3 application and then in one hour intervals after the KNO_3 administration and the content of methemoglobin and of 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 NO_3^- per kg of body mass in the form of KNO_3, 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, pCO_2, 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 H o m o l k a (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 aspartete 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_3^- . l^{-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_3^- . l^{-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 . l^{-1} of colostrum. In the last day of KNO_2 aplication diarrhea occurred in all 8 calves of the 2nd group whereas only two animals of the group 1 were affected.

Table 1

Mean methemoglobin levels (per cent of total hemoglobin) in the blood of calves after 6 d treatment with KNO_3

Group 1	Prior to the 1st treatment	After the 1st treatment	After the 6th treatment
1 400 mg NO_3^- per day n = 8	3.75 ± 0.38	3.55 ± 0.18	3.74 ± 0.19
Group 2 3 500 mg NO_3^- per day n = 8	3.50 ± 0.19	3.56 ± 0.23	3.99 ± 0.48

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_3 to calves in the experiment 2 the levels of methemoglobin ranged within 2.1 - 4.0 %. During the addition of KNO_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 KNO_3 doses is presented in Fig. 1. Methemoglobinemia in one animal of the experimental group reached the value of 8 % after a daily dose of 4 g NO_3^-/calf. As far as the other followed biochemical parameters of blood are concerned (hemoglobin, methemoglobin, vitamin A and E, AST, GMT, glucose, Na, K, Ca, inorg. P, Mg, urea, gamma globulins, pH, BE, pCO_2, hematocrit) no significant differences were found between the experimental and control group. The vitamin B_1 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).

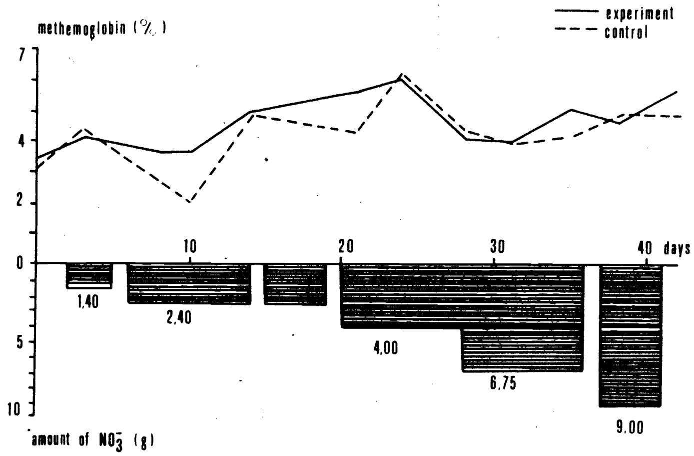

Fig. 1. Average methemoglobin levels in blood after the longterm administration of NO_3^- in the form of KNO_3. Amount of NO_3^- in g/day and calf.

Table 2

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

Group	Liver	Heart	Brain	M. gracilis
Experimental n =	0.19±0.08	0.16±0.06	0.24±0.06	0.28±0.25
Control n =	0.29±0.05	0.40±0.26	0.30±0.11	0.29±0.11

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 NO_3^- (0.36 g NO_3^- . kg^{-1} of body mass) were intraruminally applied to a calf. Doses 36 g NO_3^- (0.5 g NO_3^- . 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 % methemoblobinemia showed malaise, apathy but cyanotic discolouration of mucosa was not marked. Calf, in which MtHb reached 35 %, was strikingly apathetic already 3 hours after the application of KNO_3, later its gait was reeling, stability of posture unsteady, it leant its head against manger, fore limbs were placed too far back. After 5 and 6 hours after the KNO_3 administration the calves fell and lay in lateral position, pulse and breething 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 KNO_3 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 KNO_3. In one calf clinical manifestations of intoxication after the aplication of KNO_3 have not appeared which suggests a different individual sensitivity of animals (Fig. 3). Apathy, markedly frequent pulse and

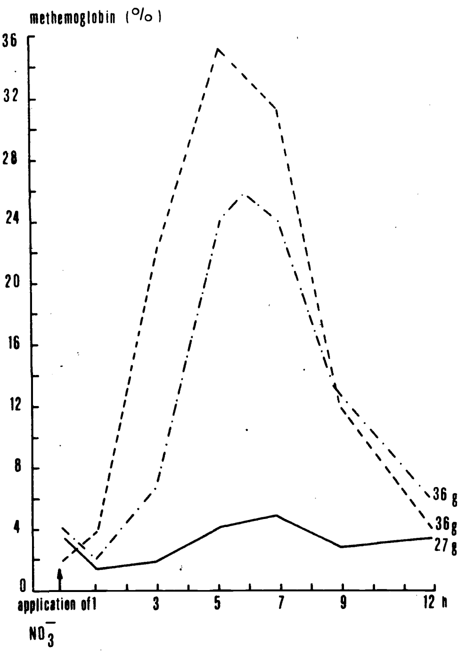

Fig. 2. Dynamics of methemoglobin levels in blood of calves on milk nutrition following the application of 500 mg NO_3^- (in the form of KNO_3) /kg of body mass.

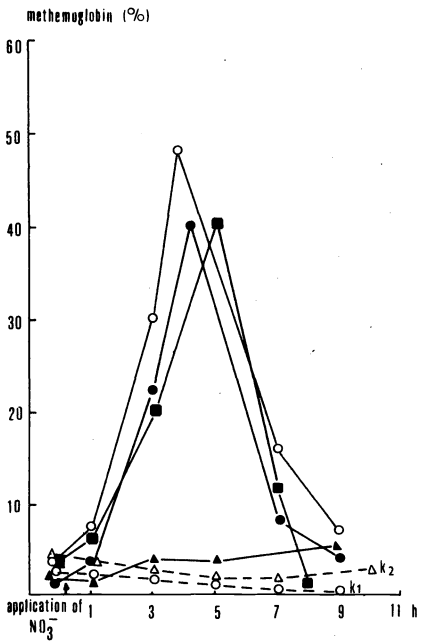

Fig. 3. Dynamics of methemoglobin levels in blood of control calves (denoted by dashed line), of experimental calves on fodder nutrition following the applications of 400 mg NO_3^- (in the form of KNO_3) /kg of body mass. Experimental calves (■▲●○).

breathing were observed in other calves of the experimental group usually 3 - 4 hours after the application of 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 discolouration 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 coffein 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 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 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 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 NO_3^- in water.

It follows from the experiment 1 that even after relatively high doses of 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 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 Piskač 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 resistence 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, nutrient 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 (Somora 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 addapted 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 of 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 anamnesis, 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 of body mass, with a simultaneous i. v. or i. m. application of coffein has proved to be very successful.

It follows from our work that calves are not as sensitive as children towards the intoxication by NO_3^-. Lower sensitivity towards NO_3^- and NO_2^- was also observed by D v o ř á k (1984) in piglets and growing pigs. It follows that calves and piglets tolerate higher doses of NO_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_3^- and NO_2^-.

Vznik a průběh methemoglobinémie u telat

Methemoglobinémie byla studována u 34 telat v závislosti na jejich věku a dávce podaného KNO_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 NO_3^-, resp. 500 mg NO_3^-, nedošlo k významnému zvýšení hladin methemoglobinu v krvi. Přidávání vzestupných dávek NO_3^- od 200 do 1 000 mg NO_3^- . l^{-1} ředěné mléčné náhražky nezpůsobilo u telat od stáří 6 dnů po dobu 5 týdnů výraznější vzestup methemoglobinémie. Po velkých jednorázových perorálních dávkách KNO_3 u telat na mléčné výživě (0,5 g NO_3^- . kg^{-1} ž. h.) a na rostlinné výživě (0,4 g NO_3^- . kg ž. hm.) došlo k vysokému vzestupu methemoglobinu v krvi a ke klinickým projevům. Za 4 - 6 hodin po perorální aplikaci KNO_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 čokoládově hnědou barvu.

Při zvýšeném obsahu NO_3^- v krmivu pro skot a při podezření na methemoglobinémii je třeba z diagnostického hlediska provádět odběr krve za 4 - 6 hodin po nakrmení zvířat, neboť po této době dochází k rychlému poklesu methemoglobinu v krvi. Při léčbě těžkého stupně methemoglobinémie se velmi dobře osvědčil 1% nebo 2% roztok metylenové modři v roztoku 5% glukózy, v dávce 10 - 20 mg . . kg^{-1} ž. h.

Изучение возникновения и протекания метгемоглобулинемии телят

Метгемоглобулинемия изучалась у 34 телят в зависимости от их возраста и дозы подаваемой KNO_3. У 16 телят, которым в возпасте 2-3 суток добавляли ежедневно в течение 6 суток 1 литр молозива или молока 200 мг NO_3^- или 500 мг NO_3^-, существенного повышения уровня метгемоглобулина в кровы не произошло. Добавлением увеличивающихся доз NO_3^- от 200 до 1000 мг NO_3^- на один литр молочного заменителя у телят в возрасте 6 суток в течение 5 недель не было вызвано более значимого увеличения метгемоглобулинемии. После одноразовой дачи большой дозы KNO_3 перорально телятам на молочном питании (0,5 г $NO_3^-.кг^{-1}$ живого веса)и на растительном корму (0,4 г $NO_3^-.кг^{-1}$ живого веса) произошло существенное повышение метгемоглобулина в крови и появилиць клинические признаки. Через 4-6 часов после пероральной дачи KNO_3 метгемоглобулинемия достигала критических величин (25-48%). Метгемоглобулинемия проявлялась апатией, учащенным пульсом и дыханием, соединительные оболочки слизистой влагалища и полости рта были цианотичные, серо-копичневые, кровь - шоколадно-коричневого цвета.

При повышенном содержании NO_3^- в кормах для скота и при подозрении в метгемоглобулинемии необходимо с диагностической точки зрения взять кровь через 4-6 часов после кормления животных, так как по истечении указанного срока происходит быстрое понижение метгемоглобулина в крови. В ходе лечения тяжелой метгемоглобулинемии хорошо зарекомендовал себя 1% или 2% раствор метиленового синего в пастворе 5% глюкозы дозой 10-20 мг.кг.$^{-1}$ живого веса.

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