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The effect of sublethal doses $NaNO_3$ given in concentrates or by capsules on blood and vitamin A status of young red and white female cattle

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In recent years nitrate poisoning of cattle in the Netherlands comes forward as a typical soil-plant-animal problem. In the beginning the occurrence of the disease seemed to be limited to farms with turnips in the cow ration. These turnips were grown under heavy and/or late nitrogen fertilization.

In a second phase various samples of grass silage and of hay seemed to have high NO_3 contents too (more than 2.5 and 3 % NO_3 in the dry matter). It was remarkable that hay and silage with high NO_3 contents had also high crude protein contents. That means that the fodder must be young (Knol, 1970).

In practice advices of the Extension Service are based on the NO_3 content in the dry matter of the feed (te Velde, 1967). The advice for turnips is as follows:
$<$ 1.50 % NO_3 in dry matter: normal; feeding ad lib.
1.50-3.00 % NO_3 in dry matter: restricted feeding (at most 30 to 40 kg per animal per day)
$>$ 3.00 % NO_3 in dry matter: not to feed (dangerous).
There is no strong correlation between the NO_3 content of the feed and the severity of the disease, because there are various factors influencing the toxicity of NO_3. Sometimes already in the (wet) plant, but otherwise in the rumen NO_3 is reduced to NO_2 (nitrite). This reduction process continues normally in the rumen via NH_2OH (hydroxylamine) to NH_3 (ammonia). Accumulation of for instance nitrite occurs when the offered NO_3 is not completely digested by the rumen bacteria. According to Cunningham (1967) hydroxylamine aggravates the toxicity of nitrite. NH_2OH added to a ration with KNO_2 gives an increase in the methemoglobin formation.

Nitrite accumulation can be prevented by energy-rich rations with many H donators (e.g. lactic acid, glucose) for the reduction of NO_3 (Lewis, 1951).

Later on these results are repeatedly confirmed. Nitrate poisoning can be aggravated by energy-poor rations or by rations with otherwise inadequate composition (Dolge, 1967). Also the rate of feed intake is important. Practical experience says that reperated feeding of small parts of nitrate-rich feed is less dangerous than feeding of the whole portion at once. Also other substances in the plant with possibly higher toxicity than nitrate can be involved (Sprague et al., 1969). Prolamins and reduced forms of non-protein nitrogen (proteoses, peptones, peptides, amines) were mentioned. Especially in wet material such N compounds can be harmful. In high moisture silages Baumgardt (1967) determined quantities of histamine, tyramine, ethanolamine, tryptamine, serotonine and gamma-amino butyric acid as probable products of an undesirable fermentation. Also wilted (rye) grass and several clovers are sometimes suspect due to high contents of α-aminoni-

trogen (Brady, 1960) and of cyanogenetic glycosides (Garner, 1962). It is evident that in these cases NO_3 analyses in feed are at best only rough indications for the amount of all toxic substances. In blood ferroheme of hemoglobin is oxidized to ferriheme by NO_2. According to Haurowitz (1963) $> Fe^+ (H_2O)$ is in equilibrium with $> FeOH + H^+$. This product, called methemoglobin, is brown in acid solution and is incapable to carry over O_2 so that anoxia or hypoxia of blood and tissues is a real danger. However sometimes the animal can develop a compensating mechanism against hypoxia. This mechanism consists of a strong increase of the number of circulating erythrocytes and a consequent increase of the hemoglobin content (Jainudeen et al., 1964). Grant et al. (1952) considered hypoxia to be the fundamental stimulans for erythropoiesis.

According to Garner (1967) symptoms of NO_3 poisoning will occur when 30 % of hemoglobin has been converted into methemoglobin, especially when (feeding) conditions are unfavourable. In favourable conditions the same symptoms occur only when more than 50 % hemoglobin has been converted.

The first and most striking symptoms appear often some hours after feeding. Dyspnea and in some cases nervous or locomotion disorders are noticed. Not all animals die but besides of a typical chocolate-coloured blood post mortem examination shows congestion of the sub-mucosa of the (fore-) stomach and petechial hemorrhages on the serous surfaces. Also abortion is observed (Merck, 1961). Besides hemoglobin also vitamin A and/or carotene can possibly be oxidized. According to Hoar et al. (1968) a significant decrease of the vitamin A content of bloodplasma and a tendency to a lower vitamin A content of the liver was caused in lambs by 1.8 % NO_3 in the ration during a period of extra carotene and vitamin A supply after a preceding vitamin A depletion. This repletion is a possible explanation why not in all circumstances an influence from NO_3 on vitamin A can be found (Jones, 1966).

It is very difficult to confirm the diagnosis of NO_3 poisoning in practice by methemoglobin analysis, because 50 % of the methemoglobin is reconverted to hemoglobin within 24 hours (Henry, 1966).

Further investigations can say something about the value of nitrite analyses in blood plasma in this respect.

Administration of methylene blue intravenously which through its leukoform aids in the reduction of ferri- to ferroheme is used as therapy.

The experiment described below was conducted to determine the effects of different levels of sublethal doses $NaNO_3$ on young cattle fed on winter ration. It is a preliminary investigation.

Experimental

Ten female Meuse-Rhine-Yssel (MRY) animals with an average age of 1 year and a bodyweight of 250 kg in the middle of the experiment were used. The division into 5 groups of 2 animals was random. The division of 5 treatment steps of $NaNO_3$ over the groups was random too. The steps were 0, 75, 150, 225 and 300 mg NO_3 per kg bodyweight. The NO_3 supplementation was adjusted to the real bodyweight every 14 days. The experiment had 2 parts: from 18-11-1969 until 16-3-1970 a 50 % $NaNO_3$ solution in water, mixed with concentrates (pappy mixture) was administered once a day, from 16-3-1970 until 8-4-1970 cristalline $NaNO_3$ in gelatin capsules was given orally with a 'pill shooter' once a day just before the concentrate feeding.

The concentrates were given at 9 a.m.; at 4 p.m. the animals got dry sugar-beet pulp;

Table 1. Average dry matter content and nutritive value of ration components.

	Dry matter (%)	Starch equivalent per kg product (small units)	Digestible crude protein per kg product (g)
Hay	83.0	340	65
Concentrate	84.0	674	175
Pulp	90.0	600	40

after 16-2-1970, however, for a higher rate of feed intake the beet pulp was mixed with the concentrates and given also in the morning. The hay was given in the morning after the concentrate feeding and at 5 p.m. The animals were always fed individually. The nutritive value of the feed is given in Table 1 and the percentage of NO_3 in the dry matter of the concentrate mixture (expressed in the same manner as in the introduction for turnips, hay or silage) are given in Table 2. The quantity of NO_3 given by capsules is also mentioned. The NO_3 content of the hay was 0.07 %. The average hay intake is given in Fig. 2. All animals had a average intake of 1.6 kg concentrates and 0.8 kg pulp during the whole period. The concentrates were composed of 25 parts maize gluten, 20 parts citrus pulp, 14 parts soybean meal, 12 parts cottonseed meal, 12 parts rapeseed meal, 7 parts manioc meal, 6 parts cane sugar molasses, 2.5 parts minerals for cattle, 1 part soybean oil and 0.5 part salt with vitamin AD_3. Blood and liver biopsy samples were taken every month and hemoglobin (Hb), methemoglobin (metHb), Packed Cell Volume (PCV), carotene and vitamin A were determined. Also respiration rate, bodyweight and water intake were registered. Blood samples were taken 3 to 4 hours after nitrate feeding when high metHb values were expected (Jainudeen et al., 1964). The modified cyanid method of Evelyn and Malloy (Henry, 1966) was used for the metHb analysis. These analyses were carried out about 5 hours after blood sampling. The animals were housed in the experimental farm Cranendonck at Maarheeze.

Table 2. NO_3 from $NaNO_3$ in dry matter of concentrates (from 16/3 mixed with beet pulp) during every sampling time. Average % of total ration and intake in kg per day of concentrates with $NaNO_3$ are given (on dry matter basis) between brackets.

Group	mg NO_3 per kg bodyweight	15/12 % NO_3 in conc. (0.8 kg, 23.9%)	19/1 % NO_3 in conc. (1.1 kg, 23.5 %)	16/2 % NO_3 in conc. (1.7 kg, 38.4 %)	16/3 % NO_3 in conc. (2.6* kg, 53.6 %)	8/4 g NO_3 in casules before conc. (2.6* kg, 56.8 %)
0	0	0	0	0	0	0
1	75	2.14	1.69	1.16	0.81	21
2	150	4.37	3.50	2.45	1.74	46
3	225	6.54	5.17	3.56	2.51	65
4	300	7.81	6.38	4.35	3.08	81

* 1.7 kg dry matter of concentrates + 0.9 kg dry matter of betet pulp.

Results

The results of the observations at different times are given in Fig. 1, 2 and 3. Within the groups and also between Groups 0, 1 and 2 the animals had comparable reactions. For ease of survey the individual observations are thus averaged for the lowest three NO_3 groups.

Fig. 1 shows that after 15/12 the methemoglobin levels suggest an adaptation of the animals to the NO_3 feeding. The effect of NO_3 given by capsules was comparatively high.

The hemoglobin and hematocrite values of Groups 3 and 4 were slightly increased over the whole period.

Fig. 2 shows no influence of NO_3 on respiration rate. The high rate at 18/11 was possible caused by bringing the animals indoors. The respiration rate was estimated 4 hours after nitrate feeding and twice a week; the results were averaged per period.

The hay intake of the animals varied during the experiment. Also the rate of intake of concentrates varied. The high NO_3 groups wanted 1-2 hours for a complete intake. To stimulate the rate of intake of concentrates with $NaNO_3$ the quantity of hay was restricted in the beginning. At the end of the experiment a decrease of hay intake was

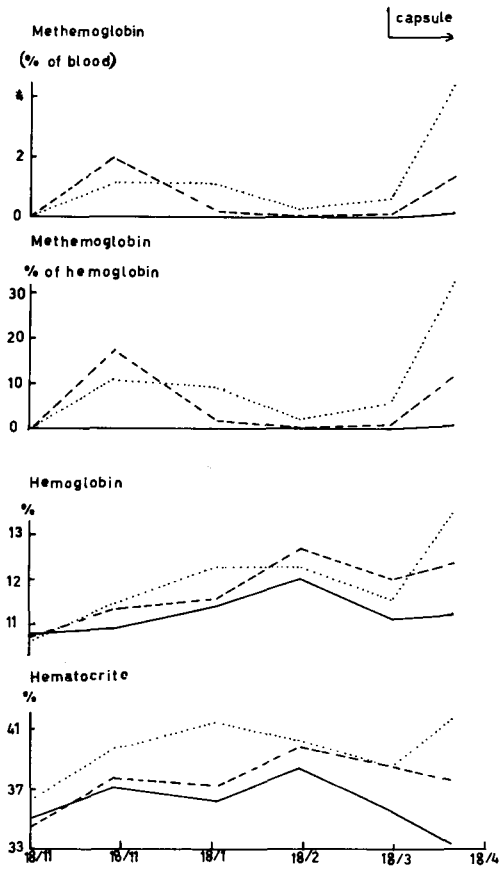

Fig. 1. Course of results of the combined groups 0, 1, 2 and of Groups 3 and 4. .... Group 4; - - - Group 3; ——— Group 0, 1, 2.

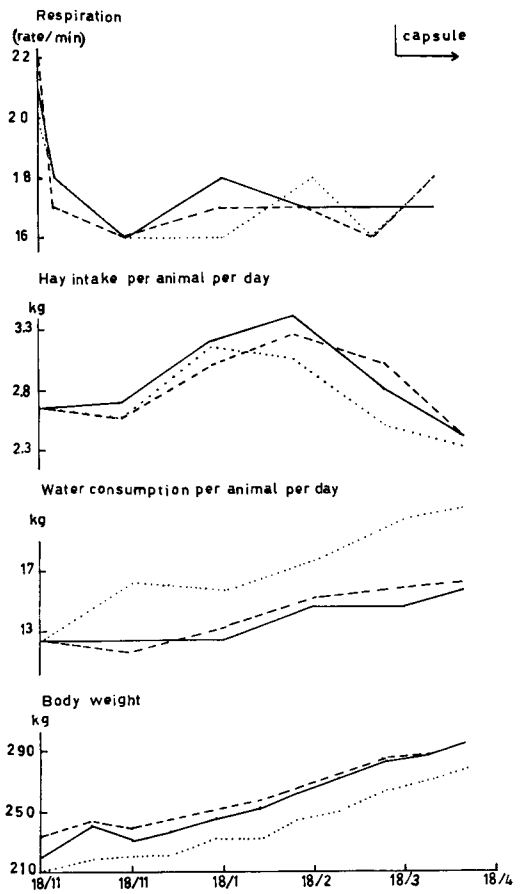

Fig. 2. Course of results of the combined groups 0, 1, 2, and of Groups 3 and 4. .... Group 4; - - - Group 3; ——— Group 0, 1, 2.

attended with the highest level of concentrates intake (Table 2). Moreover the hay uptake of the animals in Groups 3 and 4 was a little depressed during the experiment. This had however no measurable effect on weight gain but by establishing this we neglect somewhat the 30 % higher water consumption of the animals in Group 4 at the end of the experiment and the possible influence of this on bodyweight. The average daily weight gain of all animals was 500 g. The vitamin A and carotene levels in Fig. 3 are not markedly influenced by NO_3 feeding. Analysis of variance of the results however gives a significant depression of the vitamin A content of blood plasma in the highest NO_3 group (see Table 3).

Discussion

In Table 2 NO_3 is expressed as mg NO_3 per kg bodyweight and as % NO_3 in the dry matter of concentrates in which it was mixed (except on 8/4). However a possible influence of roughage (rumen content) and of feeding level on the symptoms especially on

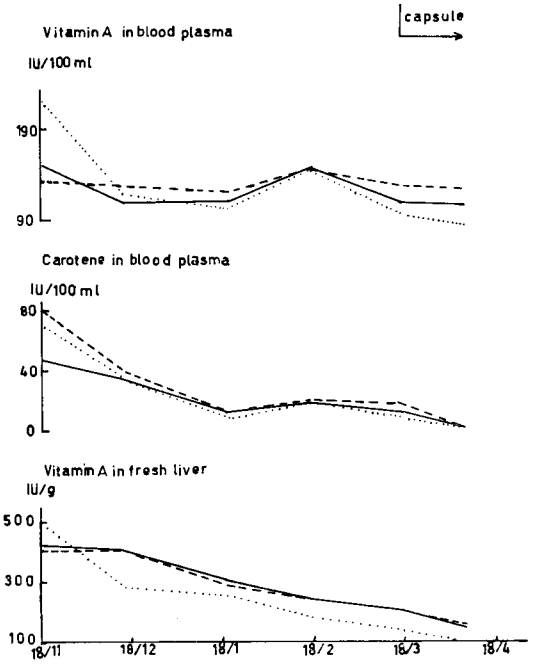

Fig. 3. Course of results of the combined groups 0, 1, 2 and of Groups 3 and 4. .... Group 4; --- Group 3; —— Group 0, 1, 2.

Table 3. Average data per group at the beginning (18/11) and the end of NO_3 supply by concentrates (16/3), and by capsules (8/4).

mg NO_3 per kg bodyweight	Group	18/11	16/3	8/4	18/11	16/3	8/4	18/11	16/3	8/4
		Methemoglobin (% of blood)			Methemoglobin (% of Hb)			Hemoglobin (%)		
0	0	0.0	0.0	0.0	0	0	0	10.9	10.8	11.0
75	1	0.0	0.0	0.0	0	0	0	10.4	11.5	11.2
150	2	0.0	0.0	0.3	0	0	2	11.3	11.0	11.2
225	3	0.0	0.1	1.4[2]	0	1	12[2]	10.8	12.0	12.4[2]
300	4	0.0	0.7[1]	4.3[1, 2]	0	6[1]	32[1, 2]	10.7	11.6	13.4[1, 2]
		Hematocrite (%)			Respiration (rate/min)			Body weight (kg)		
0	0	35.5	34.4	33.2	20	22	17	215	271	286
75	1	34.2	37.4	33.6	22	16	16	219	280	294
150	2	35.6	35.1	33.0	20	17	16	227	299	306
225	3	34.5	38.5	37.6[2]	22	20	17	234	287	295
300	4	36.4	38.5	41.7[1]	20	22	18	209	265	279
		Vit.A (IU/g fresh liver)			Vit.A (IU/100 ml blood plasma)			Carotene (IU/100 ml blood plasma)		
0	0	482	278	200	127	101	97	96	12	2
75	1	434	180	126	157	116	117	82	12	2
150	2	342	145	120	167	113	115	84	12	2
225	3	400	211	158	136	126	124	80	18	2
300	4	495	136	101	224	96[1]	85[1]	70	12	2

[1] Significant ($\leqslant 5$ %) difference between the averages of group 4 and of groups 0, 1, 2, 3 (corrected for initial content at 18/11 in analysis of variance).

[2] Significant ($\leqslant 5$ %) difference between the averages of groups 3, 4 and of 0, 1, 2 (corrected for initial content at 18/11 in analysis of variance).

Table 4. NO_3 expressed for Group 3 and 4 as g per animal per day and as % of dry matter of total ration during every sampling time. The average dry matter intake per animal per day of both groups is given between brackets.

Group	mg NO_3 per kg bodyweight	15/12 g NO_3 per animal per day	19/1 g NO_3 per animal per day	16/2 g NO_3 per animal per day	16/3 g NO_3 per animal per day	8/4 g NO_3 per animal per day
3	225	55	56	60	65	65
4	300	66	69	73	80	81
		% NO_3 of total ration (3.4 kg)	% NO_3 of total ration (4.6 kg)	% NO_3 of total ration (4.2 kg)	% NO_3 of total ration (4.8 kg)	% NO_3 of total ration (4.5 kg)
3	225	1.61	1.24	1.38	1.31	1.42
4	300	1.94	1.50	1.76	1.73	1.80

the methemoglobin formation should also be borne in mind. When NO_3 is expressed as g per animal per day and as % of total ration like in Table 4, then there appear other difficulties. Now one neglects too much the way of administration, like the possible influences of H-donators in concentrates (our experiment) and how many times a day or how fast the NO_3 is administered. See the remarkable effect of capsules on 8/4!

For the explanation of the methemoglobin formation (Fig. 1) the expression of NO_3 in g per animal per day or in mg NO_3 per kg bodyweight is not very useful. From Table 4 one can derive that between 1.50 and nearly 2 % NO_3 in the dry matter of the total ration only variable methemoglobin levels were caused, dependent of feeding level, animal variation in susceptibility, way of administration (capsules), etc. Some figures are difficult to interprete. From Table 2 one can derive that more than 6 % NO_3 in concentrates, which formed about 25 % of the total ration (on dry matter basis), had produced methemoglobin levels above 1 % of blood (about 10 % of Hb). The lower methemoglobin levels, we have measured in our experiment, were accompanied with lower NO_3 contents (< 6 %) in concentrates. When this interpretation is true it emphasizes that 6 % NO_3 in 1 kg concentrates has not the same effect as 3 % NO_3 in 2 kg concentrates.

When our experimental situation is compared with practice it is amazing that no more serious symptoms were observed. Therefore one may wonder if true NO_3 poisoning is very frequent in practice. At least the influence of some amines and/or reduced NPN compounds should also be taken in consideration (Sprague et al., 1969). Our animals had a good physical condition, the concentrate (+ pulp) ration contained probably many H-donators and the rate of feed intake of the high NO_3 groups was lowered, so that the intake of NO_3 was spread over a long period. On the other hand no alarming symptoms did occur with NO_3 given by capsules at once. However in this experiment capsules were administered shortly before the concentrate feeding. Jainudeen et al. (1964) gave 180 g NO_3 per animal per day to heifers of 270 kg without observing general respiratory distress. The quantity was administered however twice a day, sprinkled over the hay and 1 animal died. Some animals appeared to be healthy although 70 % of their Hb was converted into metHb. These animals had also very high PCV values. Cunning-

ham (1967) finally had a positive result about the influence of NH_2OH on the poisonousness of KNO_2. In this stage we can conclude that it is probable that apart from NO_3 other poisons are involved in practice, but it has not been proved at the moment.

In our experiments the increase of PCV did completely compensate for the metHb formation. This is clearly proved by the unaltered respiration rate.

The rate of feed intake was decreased even in the period with capsule administration. This can be caused by lesions in the (fore-) stomach (Merck, 1961). Reduced palatability due to NO_3 is probably not the only factor.

The influence of NO_3 on vitamin A content of blood plasma was only significant at the highest NO_3 level. The tests of significance were selected after scrutiny of the data and the results must therefore be interpreted with caution. The condition of repletion as in the experiments of Hoar et al. (1968) was not present in this experiment. The vitamin A status namely decreased in all animals during the experiment (Fig. 3, liver).

The result of the determination of methemoglobin depends very strongly on the time between analysis and sampling. With an average methemoglobin content in blood of the animals of Group 4 of 4.33 % the recovery after 20 hours was 44 %; with 1.43 % methemoglobin in Group 3 33 %; and with 0.27 % methemoglobin in Group 2 only 22 %. Although the losses appear to decrease with the higher NO_3 levels, the low recoveries are unsatisfactory. The samples were stored at room temperature.

Our experiment was based on the assumption that the decrease of the methemoglobin content in 5 hours was always systematically and not accidentally. That means that analysis of variance is possible although the real figures in the cow have to be considered with caution again.

Summary and conclusions

In a preliminary investigation 5 groups of 2 young Meuse-Rhine-Yssel heifers got during 4 months 0, 75, 150, 225 and 300 mg NO_3 per kg bodyweight per day, respectively. The nitrate was mixed as a water solution of $NaNO_3$ with the concentrate. The highest group got at most 7.8 % NO_3 in the dry matter of the concentrate or 1.94 % NO_3 in the dry matter of the total ration.

The clinical symptoms were limited to a depression of the rate of feed intake and an increase of water consumption in the higher NO_3 groups. The animals of these groups converted 10-16 % Hb in metHb with also a compensatory increase of PCV and Hb values of the blood. However NO_3 values below 6 % in dry matter of concentrates (conc. = 25 % of total ration) did not produce metHb above 10 % of Hb. During 3 weeks after this experiment the same amounts of $NaNO_3$ were given with gelatin capsules orally daily. The same depression of appetite was noticed, but metHb, Hb and PCV values increased more (maximum of 32 % metHb). Apart from concentrate there is a possible influence on metHb formation from hay, feeding level and rate of intake of NO_3.

Respiration rate, body weight and vitamin A content of the liver were not influenced. Vitamin A content of plasma decreased significantly (but only in the highest NO_3 group). Toxicity of $NaNO_3$ was discussed in comparison with NO_3 poisoning in practice. Apart from NO_3 other substances as various amines or reduced NPN compounds may play a role under farming conditions.

The reconversion of metHb in Hb depends on the metHb level in the blood and is not linear.

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