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The analysis of volatile N-nitrosamines in the rumen fluid of cows

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Summary

After supplying nitrate to the rumen fluid of cows a temporary nitrite accumulation occurs.

The formation of volatile N-nitrosamines during that period was analysed. No high levels could be detected.

Introduction

After the discovery of the carcinogenic properties of N-nitroso compounds (Magee and Barnes, 1956) many investigations have been performed on the presence and formation of these compounds in the environment (Preussmann et al., 1979).

Much attention is paid to the formation in-vivo of N-nitrosamines after consumption of nitrate or nitrite, especially in man. Recently, the formation of N-nitrosamines in the blood of humans was shown after the ingestion of nitrate-rich food (Fine et al., 1977).

In our institute research is carried out on nitrate poisoning of cattle after feeding on nitrate-rich feed. Because of the anaerobic and reducing conditions in the rumen, nitrate is reduced to nitrite. After high nitrate ingestions a temporary nitrite accumulation occurs in the rumen (Kemp et al., 1977, 1978). Under these conditions formation of N-nitrosamines seems likely. Experiments of Juszkiewicz & Kowalski (1976) with goats showed that only very low concentrations were formed. They found a pH dependency with the highest nitrosamine concentrations at pH ~ 5, but low in the normal pH range of the rumen fluid (pH 6-7). A minor transfer of these nitrosamines was found into the blood and milk (Juszkiewicz & Kowalski, 1974).

In the first experiments (van Broekhoven & Stephany, 1978) we found a relation between nitrosamine formation and nitrite concentration in the rumen fluid of a cow after intake of nitrate. Concentrations up to 0.5 μg/kg of dimenthylnitrosamine, diethylnitrosamine and nitrosopyrrolidine were found. Because of

the high nitrite concentrations combined with relatively low nitrosamine concentrations, artefact formation was likely.

Other experiments to study the possibility of artefact formation showed that the method of analysis used in the first experiments was not reliable. We tried to stop arteficial nitrosamine formation after sampling. Of all the methods tested extraction directly after sampling with an extraction cartridge was the most convenient (van Broekhoven & Davies, 1980).

This paper gives the results of the analysis of rumen fluid of two cows after intake of nitrate and sampling with extraction cartridges.

Materials and methods

Two non-lactating Friesian cows were used. Both were fitted with a rumen fistula. The animals were fed on hay low in nitrate. During one week before the experiment the cows were habituated to a higher nitrate content by adding daily about 80 mg/kg body weight of nitrate as potassium nitrate to the rumen (Kemp et al., 1977). Then 120 mg/kg body weight of nitrate as potassium nitrate was given into the rumen of the cows.

After this nitrate treatment a rumen fluid sample was taken every quarter of an hour. One part was used for determination of nitrite, from the other part two samples were taken for determinations of volatile N-nitrosamines.

For a crude estimation nitrite indicator strips (Merckoquant 10 007, Merck) were used. The samples for more precise nitrite determination were treated with a lead acetate solution and further stored and analysed as described by Vertregt (1977). The samples for the analysis of volatile N-nitrosamines were worked up immediately after they were taken. As extraction cartridges Preptubes (Thermo Electron Corp.) were used. An extra sample was taken occasionally to estimate recovery or to study artefact formation. Directly after sampling a mixture of volatile nitrosamines was added (dimethylnitrosamine, diethylnitrosamine, di-n-propylnitrosamine and nitrosopiperidine) to a final concentration of 1 μg/kg to estimate recovery or 1 mg/kg morpholine was added to study artefact formation (Rounbehler et al., 1980).

At the beginning of the habituation period also some samples were taken. To determine the blank values the whole procedure was run with water instead of rumen fluid.

The chemicals used were all analytical grade. Dichloromethane (Baker) was distilled before use. The analyses of the volatile N-nitrosamines were performed on a specific nitrosamine detector, the Thermal Energy Analyzer model 502 LC (Thermo Electron Corp.) coupled to a gas chromatograph (Packard Becker 427). The whole procedure was described before (van Broekhoven & Davies, 1980).

Results and discussion

The changes in the nitrite content of the rumen fluids after supplying nitrate to

Table 1. Changes in the contents of nitrite and dimethylnitrosamine (NDMA) in the rumen fluid after nitrate supply to the rumen on the first day of habituation and after one week.

Time after supplying nitrate (h)	Cow 1		Cow 2		
	NO_2^- (mmol/l)	NDMA* (μg/kg)	NO_2^- (mmol/l)	NDMA* (μg/kg)	NDMA** (μg/kg)
First day					
1.00	ND	0.2	ND	0.4	–
2.00	ND	0.2	ND	0.4	–
After habituation					
0	–	0.6	–	0.4	–
0.25	0.7	0.5	0.4	ND	ND
0.50	1.4	ND	0.6	ND	0.3
0.75	1.9	0.2	1.0	ND	0.7
1.00	3.0	0.3	1.7	ND	1.0
1.25	3.9	ND	2.1	ND	0.9
1.50	3.8	ND	2.6	ND	0.8
1.75	3.5	ND	2.9	0.3	0.8
2.00	2.5	0.2	2.7	0.3	0.3
2.25	1.3	0.3	1.7	0.3	0.3
2.75	0.1	0.1	0.1	0.3	–

Blank (water): 0.2 μg NDMA /kg (4 samples).
Direct extraction of the water with dichloromethane did not show a measurable amount of volatile N-nitrosamines.
* Means of duplicates. SD from combined duplicates: 0.1 μg/kg
** Samples treated with lead acetate and stored at 4 °C (see text).
ND = not detected: below limit of detection (0.1 μg/kg).
– = not determined.

the two cows are given in Table 1. A maximum concentration of 3.9 and 2.9 mmol nitrite/litre in each cow, was found after 1.5-1.75 hours. This is in good agreement with the values found by Kemp et al. (1977, 1978).

In the samples used for the analysis of volatile N-nitrosamines only dimethylnitrosamine (NDMA) was found.

The results of the blanks and the samples taken at the beginning of the habituation period and the results of the experimental day are given in Table 1. The values found were very low (less than 0.6 μg/kg). The results were corrected for recovery of NMDA. Percentage recoveries of the various N-nitrosamines added to the rumen fluid and limit of detection of NDMA are given in Table 2.

The samples with the added morpholine did not show an increased nitrosomorpholine peak, compared with a blank. So, most probably no artefacts were formed during the analyses.

The present results show that only very limited amounts of volatile N-nitrosamines were formed during the formation of considerable amounts of nitrite in the rumen after nitrate supply. There is also no difference between these results and those obtained at the beginning of the habituation, when according to

Table 2. Percentage recoveries of N-nitrosamines added to the rumen fluid.

Spike (μg/kg)	n	Mean recovery/SD (both in %)			
		NDMA	NDEA	NDPA	NPIP
1	4	91/17	92/10	70/17	70/14

NDMA: dimethylnitrosamine; NDEA: diethylnitrosamine; NDPA: di-n-propylnitrosamine; NPIP: nitrosopiperidine.
n = number of samples.
Limit of detection for NDMA: 0.1 μg/kg.
Volume of extract injected was about 10 μl.

Kemp et al. (1977) the reducing capability is not yet maximal. The concentrations are more or less the same as the blank values and also around the limit of detection.

The present findings even indicate a tendency for lower nitrosamine content during the nitrate reduction. A breakdown of volatile N-nitrosamines could occur in a rumen fluid with a high reducing activity. The reason for the higher levels of NDMA at the beginning of the experiments is not clear. In the hay samples no volatile N-nitrosamines could be detected. The saliva could be the origine. The formation of N-nitrosamines may take place further on in the digestive tract and pass through the blood into the saliva.

Earlier experiments with a multifistulated cow showed, that the nitrite concentration decreased rapidly after the rumen. Measurements after rennet stomach showed a high nitrate concentration, but no measurable amount of nitrite. Nitrosamines could be formed in the rennet stomach, where the pH is about 3.

Some of the samples of cow 2 used for the nitrite determinations were also analysed for nitrosamine content. These samples were treated with lead acetate and stored at 4 °C until analysis. After filtration the samples were worked up in the same way as the fresh rumen fluid samples by the Preptube method. The results in Table 1 clearly show that this analysis will lead to artefacts.

The lack of nitrosable amines in the rumen fluid could be due to the feed that was used. So, experiments with other feeding regimes should also be performed.

In future studies attention will also be paid to the formation in vivo of N-nitrosamines in the blood.

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