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Bacteriological quality and intake of acidified drinking water in Wistar rats is pH-dependent

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Introduction

Drinking water in laboratory animal facilities is often acidified with hydrochloric acid (HCl) to a pH of about 2.5, in order to reduce bacterial growth (*Tober-Meyer and Bieniek*, 1981a). Pseudomonas Aeruginosa in particular easily multiplies in water at room temperature and can become an undesirable source of bacterial infection (*Koopman et al*, 1978). Acidification of drinking water with HCl to pH 2 resulted in a significantly reduced urine volume in Wistar rats, when compared to intake of untreated tap water or acidified water at pH 3 (*Clausing and Gottschalk*, 1989). Acidification of drinking water with HCl to pH 2.2 caused teeth enamel and dentin to dissolve (*Karle et al.* 1980). The question arises of as to what would be an acceptable pH when acidification is chosen as a means of reducing bacterial growth in drinking water in the laboratory animal facility. Acidification of drinking water must neither lead to a disturbed wellbeing, nor undesirable interference with experimental results. The following two experiments examined whether the acidification of drinking water with HCl influences water intake in rats. A reduced water intake can be considered an indicator of disturbed welfare and is known to influence experimental results (*Clausing and Gottschalk*, 1989). The water was acidified with HCl to three different pH levels (2.5, 3.0 and 3.5). Total bacterial counts were monitored.

Materials and Methods

Experiment 1.

Thirty male SPF Wistar rats (HsdCpb:WU, Zeist, the Netherlands) were housed individually in stainless steel cages in 3 groups of 10 animals. The animals were divided into groups in such a way that the mean body weights in all groups were similar (around 470 g). The animals were housed conventionally (before personnel could enter the animal unit, they had to change shoes and put on a white coat). The temperature in the animal room was 22.0-24.0° C, relative humidity 45-65%, and the lights were on from 7 am until 7 pm. Diet (RMH-B flour, Hope Farms, Woerden, The Netherlands) and water were provided ad libitum throughout the experiment. Drinking water was provided in clean bottles. Two times a week the animals received new clean bottles with fresh drinking water. If necessary, bottles were filled up in the in-between periods of changing bottles. During the first 9 days all rats received tap water (acclimatization period). After day 9 the schedule of provision of drinking water was as follows:

group "TW": tap water;
group "HCl2.5": tap water acidified with HCl to pH 2.5;
group "DW": demineralised water.

After day 30 all animals received tap water. Water consumption was measured twice weekly (or more often when necessary) by weighing the bottles before and after consumption. After water had been in the bottles being used by the rats for a period of 3 days, water samples were taken from 5 water bottles per group. These samples were incubated at 30° C for 3 days and examined for total aerobic plate count (TAPC). Initial reference bacterial counts were obtained from bottles filled with demineralised water and directly examined for TAPC.

Experiment 2.
Four groups of 8 male Wistar rats from the same source, were housed under the same conditions as in Experiment 1. During the first 14 days, all rats received tap water (acclimatization). After day 14, the schedule of drinking water provision was as follows:

group "TW": tap water;
group "HCl2.5": tap water acidified with HCl to pH 2.5;
group "HCl3.0": tap water acidified with HCl to pH 3.0;
group "HCl3.5": tap water acidified with HCl to pH 3.5.

After day 45, all animals received tap water. Water consumption was determined twice weekly, as in Experiment 1. After the drinking bottles had been used for a period of 4 days, samples from 5 bottles per group were taken for TAPC measurements (see above) and for Enterobacteriaceae counts. Fresh water samples and samples from bottles that had been used by the rats for 4 days were measured for pH.

Statistical Analysis
The results were compared by one-way analysis of variance (ANOVA, Dunnett). The level of significance was pre-set at 0.05.

Results

Experiment 1.
The results of the water intake (g/day) are given in table 1. Water intake of tap water was similar to the consumption of demineralised water. Consumption of drinking water with pH 2.5 was significantly reduced on days 13-20 and 27-30, when compared to animals drinking non-acidified water. The mean water intake in the group "HCl2.5" was also lower on days 20-27, but this was not significant. After reverting from acidified to non-acidified drinking water after day 30, there were no longer significant differences in water intake between the groups. TAPC analyses revealed that bottles filled with tap and demineralized water contained high numbers of microorganisms after 3 days consumption by the rats (>$5x10^5$ per ml). Addition of HCl to the drinking water reduced the number of microorganisms (TAPC ranged from $2.4x10^2$ to $2.7x10^3$ per ml). Direct investigation of reference bottles filled with demineralised water showed numbers of microorganisms below 1000 per ml (range: $4.7x10^2$ to $7.9x10^2$ per ml).

Table 1. Experiment 1. Water Consumption (average g/day ± sem)

Day no.	Tap water	HCl, pH 2.5	demi water
2 - 6	27.8 (2.3)	27.7 (1.7)	25.4 (1.0)
6 - 9	28.1 (2.2)	27.8 (1.6)	27.4 (1.4)
9 - 13	28.5 (2.4)	24.0 (1.2)	28.2 (1.7)
13 - 16	30.6 (2.5)	25.9 (0.6)*	28.6 (2.4)
16 - 20	32.9 (3.0)	25.7 (0.7)*	32.5 (2.7)
20 - 23	32.7 (2.9)	27.4 (0.7)	31.1 (2.8)
23 - 27	30.5 (2.2)	27.7 (0.9)	32.6 (2.3)
27 - 30	33.1 (2.5)	23.8 (0.5)*	30.5 (1.7)
30 - 34	32.5 (2.8)	32.7 (2.1)	31.2 (2.2)

ANOVA, Dunnett, * $p < 0.05$

Experiment 2

The results of the water intake are given in Table 2. The water consumption of rats receiving "HCl2.5" was significantly lower from days 21-42, when compared to animals receiving non-acidified drinking water. After reverting from "HCl2.5" to non-acidified drinking water, the water intake was still significantly reduced from days 45-49. Thereafter, it normalized to the level of the control animals. The water consumption in the groups "HCl3.0" and HCl3.5" was not significantly different from those animals drinking non-acidified tap water.

Table 2. Experiment 2. Water Consumption (average g/day ± sem)

Day no.	Tap water	HCl, pH 2.5	HCl, pH 3.0	HCl, pH 3.5
3 - 7	23.4 (1.0)	20.5 (0.4)	21.8 (1.3)	22.8 (1.0)
7 - 10	24.3 (1.4)	22.3 (1.1)	21.8 (0.7)	22.6 (0.9)
10 - 14	23.3 (1.3)	21.1 (0.8)	22.4 (2.1)	22.9 (1.3)
14 - 17	22.5 (0.8)	21.6 (1.0)	24.9 (1.7)	23.6 (1.1)
17 - 21	23.6 (1.5)	21.3 (0.9)	24.2 (1.4)	23.4 (1.0)
21 - 24	23.3 (1.0)	18.7 (1.1)*	23.1 (1.3)	22.5 (1.3)
24 - 28	23.2 (1.2)	18.6 (0.8)*	23.7 (1.3)	24.0 (0.9)
28 - 31	24.3 (1.9)	17.1 (0.5)*	23.1 (1.3)	23.8 (1.1)
31 - 35	23.2 (1.0)	19.3 (0.8)*	22.7 (1.1)	22.8 (0.9)
35 - 38	23.0 (1.1)	18.7 (0.7)*	23.3 (1.1)	23.0 (1.5)
38 - 42	24.4 (1.1)	19.1 (0.7)*	23.7 (1.2)	24.5 (1.2)
45 - 49	23.8 (1.2)	18.5 (0.5)*	23.4 (1.1)	23.8 (1.0)
49 - 52	24.2 (0.9)	23.3 (0.7)	24.0 (1.6)	25.7 (0.9)
52 - 56	24.0 (1.4)	21.3 (0.6)	23.4 (1.4)	23.6 (1.4)

ANOVA, Dunnett, * $p < 0.05$

TAPC's were higher than $1x10^5$ per ml in non-acidified drinking water, after bottles had been in the animal room for 4 days. All 5 bottles from the "HCl2.5" group had TAPC's below 20 per ml. TAPC's from "HCl3.0" varied from 10 to 120 per ml and from "HCl3.5" from 40 to $1.3x10^5$ per ml. Enterobacteraceae counts in all bottles from all groups were < 1 per ml.

The pH of fresh "TW" was 7.91. The pH of freshly made "HCl2.5", "HCl3.0" and "HCl3.5" were 2.5, 3.0 and 3.5 respectively. After bottles had been used by the rats for 4 days, the average pH±SD of "TW" was 7.32±0.64 (5 bottles), of "HCl2.5" was 2.60±0.02 (8 bottles), of "HCl3.0" was 3.15±0.06 (8 bottles) and of "HCl3.5" was 3.83±0.13 (8 bottles).

Discussion

TAPC's of fresh demineralised water were too high when compared to the guidelines for human consumption (EC-guideline 80/778/EEC). No minimum bacteriological quality requirements are available for drinking water of laboratory animals (*Kroon et al.*, 1994). The number of microorganisms after 3 days of consumption, was not influenced by the type of non-acidified drinking water (tap water and demineralised water gave the same high TAPC figures in Experiment 1). Acidification of drinking water by HCl to pH 2.5 and 3.0 clearly reduced the number of microorganisms when compared to non-acidified water. Acidification to pH 3.5 did not always prevent high TAPC's. If the number of microorganisms in the drinking water is to be reduced by acidification with HCl, then a pH of 3.0 and lower is advised.

The measured pH of fresh tap water was alkaline (7.9). After 4 days the average pH was 7.3 (the lowest measured value was pH 6.3, while the

highest value was pH 7.8). A variable pH in drinking water bottles might interfere with experimental results. The pH of acidified drinking water remained almost constant during the 4-day period in the animal room (Experiment 2). When taking the pH and TAPC's into consideration, acidified drinking water can provide a more constant environmental variable than nonadicified water.

In both experiments water intake was reduced significantly by acidifying to pH 2.5. Acidification to pH 3.0 and 3.5 did not influence water consumption when compared to tap water. In Experiment 2, the water intake remained at a lower level for 4 days after animals had received nonacidified water again (days 45-49). The reason for this is not clear. It might indicate that animals had adapted to a lower water intake, and/or that metabolic changes had occurred, leading to a lower water consumption. A reduced water intake may indicate that the well-being of animals is affected. Reduced water intake in itself can interfere with experimental results (*Clausing and Gottschalk*, 1989). A reduced consumption of acidified water may be strain-related. Karle et al. (1980) measured a reduced water intake in Wistar rats, but not in Sprague-Dawley and Cara rats. The reduction in water consumption in Wistar rats, was caused by a reduced frequency of water consumption (*Karle et al.*, 1980). Tober-Meyer et al. (1981b) did not find any change in 12 measured parameters in male Han:Wistar rats, when acidified drinking water (pH 2.3-2.5) was compared to untreated drinking water over a 7 months period. Unfortunately, water intake was not measured (*Tober-Meyer et al.* 1981b). Acidification of drinking water to pH 2 interfered with experimental results in male Ico/Shoe:Wistar rats, as it resulted in a reduced urine volume (*Clausing and Gottschalk*, 1989). Unexpectedly, water consumption had not changed (*Clausing and Gottschalk*, 1989). The method of measuring water consumption and the way in which urine was collected might explain this discrepancy.

Conclusion.

Acidification of drinking water with HCl to pH 3.0 gave a virtually constant water pH during a period of 4 days, stable low bacterial counts, as well as a "normal" water intake in male HsdCpb:Wistar rats over a 25-day period. Acidification to pH 2.5 led to a reduced water consumption. Drinking water with pH 3.5 led to higher water microbial counts after 4 days. On the basis of our results, a pH 3.0 should be the pH of choice, when acidification of drinking water with HCl is considered to be necessary.

Summary

The effects of acidification of drinking water on bacteriological quality and water intake in adult, male Wistar rats, was studied in 2 consecutive experiments. HCl was used to acidify water to pH 2.5, 3.0 and 3.5. Control groups received untreated tap or demineralized water. Acidification of water with HCl to pH 2.5 effectively prevented growth of aerobic bacteria in the drinking water bottles after a number of days, but also caused a reproducible decline in water intake when compared to untreated water. A reduced water intake may indicate disturbed wellbeing and may interfere with experimental results. Acidification to pH 3.0 also kept bacteriological counts low and did not reduce water intake when compared to rats drinking non-acidified water. Acidification to pH 3.5 led to high bacteriological counts after a few days. On the basis of these 2 experiments, acidification of drinking water with HCl to pH 3.0 is advised.

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