INVESTIGATION OF SOME WATER USED IN LABORATORY RODENTS HUSBANDRY

J. Eberová, B. Hučko, V. Hořejšová

Czech University of Agriculture, Faculty of Agrobiology, Food and Natural Resources, Department of Microbiology, Nutrition and Dietetic, Prague, Czech Republic

Actually, standardization of feeding and drinking of laboratory animals play an important role in biomedical research. Therefore, the present study examined four types of water in male laboratory rats stock Wistar (n = 24). Namely, drinking, distilled, filtered and acidified water were tested. Also, dynamic of water consumption was followed. The experimental period took 21 days. Body weight gain and food intake were found at the beginning as well as at the end of the study. The water consumption was measured every 12 hours. There was found a significantly lower consumption of acidified water (20.3 ± 3.6 ml per day and animal), on the other hand, distilled water was the most preferable (26.2 ± 1.9 ml per day and animal) in the experimental rats (α = 0.01). The drinking evidently ran over the night activity of the experimental animals (α = 0.05). Furthermore, there was observed no significant dependence of the water intake on the body weight gain (r = 0.3019) in this case.

laboratory rat; water; biomedicine

INTRODUCTION

It was pointed out needfulness of knowledge increasing and understanding of laboratory animal feeding on the 30th symposium of Scandinavia Society of Laboratory Animal Science (ScandLAS) which took place in Stockholm, Sweden in 2000 (Lang et al., 2000). In recent times, it is very well known that standardization of housing conditions of laboratory rodents is an instrument leading its human use. "Good welfare is a prerequisite for good science," says moreover Richmond (2000). Blom (1993) reports that there is still many uncertainties as what the optimum of housing conditions for laboratory animals are. There is still a need for further evaluation of the housing conditions in order to provide a sound basic to the guidelines that are to be issued. He thinks that a preference test is one of the indirect method of estimation animal motivation and animal perception. It is important to notice that demand function is influenced by many factors. Some of them varied from the laboratory to the laboratory, some might depend on gender, genetic and physiological factors e.g. thirst, hunger, aggression or phase in oestrous cycle etc. (Andrews et al., 1995). At the present time, rats and mice are the most frequently used in biomedical research as laboratory model. They make about 60% of the total used of laboratory animals every year (Hagelin et al., 2003).

The purpose of the experiment was to determine preference for some of the tested water as well as dependence of water intake on body weight gain was studied. In addition, investigation of night drinking in rats was also important.

Table 1. Nutrition and energy contents of the diet KMK-20

Nutrition and energy contents	Unit	Amount
Dry matter	g/kg	908.3
Crude protein	g/kg	253.1
Crude fat	g/kg	37.2
Fiber	g/kg	59.1
Ash	g/kg	85.3
NaCl	g/kg	max. 3.3
N-free extract	g/kg	473.6
Organic matter	g/kg	823.0
Gross energy	MJ/kg	20.0

MATERIAL AND METHODS

The experiment was realized using 24 males of laboratory rat (*Rattus norvegicus*) stock Wistar, which were provided from conventional breeding BioTest, Konarovice. The rats were 7 weeks old (initial average body weight was 167.1 ± 10.3 g).

The rats were housed individually in plastic cages (Velaz, Praha). The non-dust hard-wood bedding was supplied by Sano, Germany. Water and the diet KMK-20 (Eypy, Havirov) were available *ad libitum*. The diet contained maximum 3.3% of sodium chloride. The nutrition and energy contents of the diet are listed in Table 1. All housing conditions respected the Czech jurisdiction i.e. the Regulation No. 311/1997 about housing and using of laboratory animals.

The experimental animals were divided into four groups at the end of the acclimatization period (8 days). Each of the groups contained 6 individuals. The experimental period lasted 21 days.

Drinking water (pH 7.3), distilled water (pH 6.5), filtered water (pH 6.8) and acidified water (pH 3.3) were offered through glass water bottles. The treated water was prepared as a potable room tempered. Water was usually stored until the another day to be free of chloride. Filtered water was produced by the instrument LIFE-ENERGY, I.B.E. Co. Ltd. Acidified water was 0.35–0.38% solution of 35–38% chlorid acid *per analysis* (Lachema, Brno). Water was measured in 21.4 °C to get its actual pH.

Water intake, food intake, body weight gain, final body weight, exterior of the rats and zoo-hygiene conditions of the animal facility were observed. The animals were weighed at the beginning and at the end of the experimental period as well as the diet was weighed at close to the dosage. Water consumption was measured every 12 hours.

Statistical programme Statgraphic *Plus* version 3.1. was applied to evaluate the data. Analysis of variance One-Way ANOVA (Turkey HSD) and simple linear regression helped to find the differences among the groups. The statistical significance level was $P \leq 0.05$ or $P \leq 0.01$.

RESULTS AND DISCUSSION

The effect of the treated water on some zootechnic parameters was studied in detail, whereas there had demonstrated no significant impact of the level of water intake on the food intake, the body weight gain and the final body weight in the experimental rats (results and standard deviations are provided in Table 2). Even though, there were observed some slight intergroup variations, respectively the rats which were watered by filtered water shown lower level in all of the followed parameters. However, the standard deviations were high, that means the response to filtered water was largely unbalanced in the group.

Analysis of variance helped to detect differences in the total water intake between the groups. The water intake is summarized in Table 3. Evidently different consumption was noticed between acidified water and distilled water ($\alpha = 0.01$). Apparently, acidified water was not so much-favoured because of its organoleptic properties in contrast to the others, mainly thanks to slightly an acidic taste. Moreover, the apparent disadvantage of long term drinking of acidified water is a negative effect on dental health (Heine, 1998). Nevertheless, macroenvironment and microenvironment, such as temperature, humidity, ventilation, foot composition, foot schedule etc. may impact the water demand function (NRC, 1996). Additionally, during night period was recorded significantly lower the demand function for acidifed water which was different from the demand

Table 2. The food intake, the body weight gain and the final body weight when compared to the type of used water

Parameter	Drinking water	Distilled water	Filtered water	Acidified water
	Mean (S.D.)			
Food intake (g)	28.3 (7.3)	36.1 (1.8)	30.4 (5.6)	36.2 (1.6)
Body weight gain (g)	103.2 (20.1)	108.4 (15.0)	91.6 (19.5)	103.5 (10.2)
Final body weight (g)	281.6 (30.8)	287.0 (13.4)	258.2 (33.6)	282.0 (7.4)

Table 3. The water consumption in the experimental rats

Parameter	Drinking water	Distilled water	Filtered water	Acidified water
Water consumption (ml)	24.8	26.2	23.9	20.3
Standard deviation	2.3	1.9	2.6	3.6
Minimum	20.7	23.7	21.0	16.0
Maximum	25.9	27.6	27.4	25.9
Analysis of variance	–	B–D	–	B–D
Water consumption – daytime (ml)	8.1	8.6	6.8	6.2
Standard deviation	1.3	0.8	1.1	0.6
Minimum	7.0	7.4	4.9	5.3
Maximum	9.6	9.5	8.2	7.0
Analysis of variance	a–d	b–c, B–D	b–c	a–d, B–D
Water consumption – darktime (ml)	16.4	17.4	15.3	13.2
Standard deviation	0.9	1.2	1.3	1.9
Minimum	15.1	15.6	14.1	10.8
Maximum	17.6	18.4	17.3	16.0
Analysis of variance	A–D	B–D	–	A–D, B–D

a, b, c, d – $P \leq 0.05$
A, B, C, D – $P \leq 0.01$

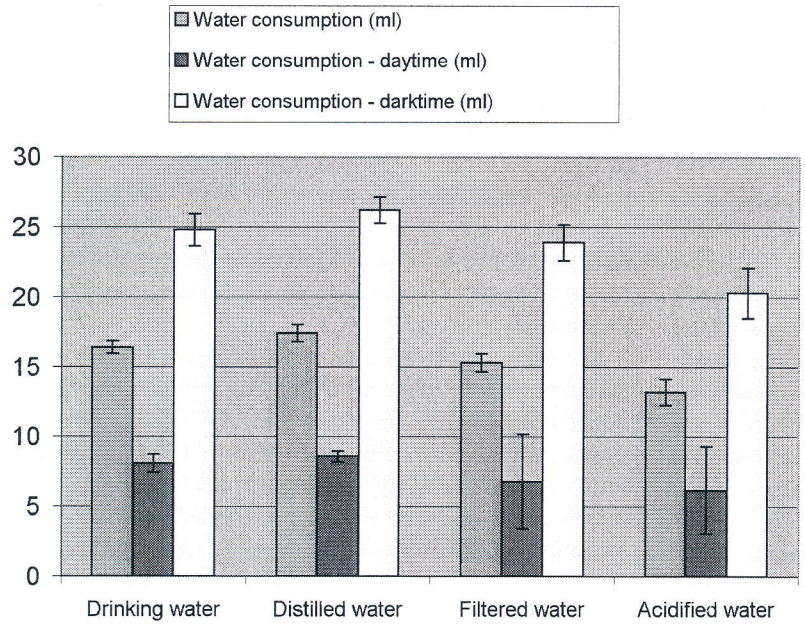

Fig. 1. Overview of the dynamic of water consumption in the experimental rats

function for drinking water. Confidence interval was equal $\alpha = 0.01$. Alike, Sørensen et al. (2001a) who tested sugar, distilled and acidified water, found different preferention in two inbred strain of laboratory rats i.e. Long Evans and Wistar Kyoto ($\alpha = 0.05$). The intake of acidified water was as little as 13.2 ± 1.9 ml per day and animal, but the consumption of drinking water was 16.4 ± 0.9 ml per day and animal and distilled water 17.4 ± 1.2 ml per day per animal. However, it is important to mention the benefit of acidification which refer Lane-Petter and Pearson (1971). Respectively, low pH (pH between 2 and 3) kills a number of sensitive bacteria, such as *Pseudomonas aeruginosa*, a very common contaminant of water supplies and it inhibits the growth of great many more e.g. *Escherichia coli* without killing them. It was also confirmed notice of Sýkora et al. (1983). These authors defined water consumption at 10–20 ml of water per day and rat (body weight 100–250 g, fed by pelleted diet-water contents 10%). Similarly, the publication Laboratory Animal Medicine written by Fox et al. (2002); Waynforth and Flecknell (1992) said that the water intake is 8–11 ml per 100 g of body weight per day. Perhaps, preference for distilled water could also cause because of its quite small contents of mineral substance. Also, Sørensen et al. (2001b) found higher demand function for distilled water than for acidified water. On the other hand, long term intake of distilled water could influence erythrocytes because generally, the effect of hypotonic medium on the cell structure and incidently the cells blow out (Alberts et al., 1998) is known.

It is supposed that applied acidified water had not been preferred in the experimental rats. On the other hand, drinking water seems to be a good source of liquid for laboratory rodents in spite of its lower popularity when compared to the distilled water consumption, because this type keeps much more favourable quality for their healthy effects.

The dynamics of water consumption was calculated as a mean of water intake all of the tested water – separately for the total consumption, the daytime consumption as well as for the darktime consumption (Fig. 1). A significantly different consumption was found between day period and night period in the experimental rats ($\alpha = 0.05$). The water consumption corresponded to 7.4 ml at day period ($s_{\bar{x}} = 1.4$; min. 4.9 ml; max. 9.6 ml). The demand function for water was equaled to 23.7 ml per rat at night period ($s_{\bar{x}} = 3.3$; min. 16.0 ml; max. 27.6 ml). Investigation agreeable to many of literature sources, e.g. Hau and Van Hoosier (2003); Olds and Olds (1979) who said that rodents are typical nocturnal animals, therefore they prefer night drinking instead of daytime.

The dependence of the total water intake on the body weight gain was tested by simple linear regression. In this case, the correlation coefficient was relatively low, the value equals $r = 0.3019$ ($\alpha = 0.1$). No important correlation between followed parameters was obtained.

The rats in general appeared to be affected neither by the experimental regime, nor by other environmental factors, as well as their exterior has not manifested any signs of discomfort. It is supposed that the welfare of the experimental rats has not been impaired by the treated water.

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EBEROVÁ, J. – HUČKO, B. – HOŘEJŠOVÁ, V. (Česká zemědělská univerzita, Fakulta agrobiologie, potravinových a přírodních zdrojů, katedra mikrobiologie, výživy a dietetiky, Praha, Česká republika):

Prověření různých druhů napájecí vody používaných v chovu laboratorních hlodavců.

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V současné době hraje výživa a napájení laboratorních zvířat v biomedicíně významnou roli. V této studii byla sledována preference pro některý ze čtyř testovaných druhů napájecí vody u laboratorního potkana. Otázka dynamiky napájení byla v tomto experimentu rovněž zkoumána. Konkrétně byla studována obliba pitné vody z vodovodního řádu, vody destilované, filtrované a acidifikované na samcích laboratorního potkana outbredního kmene Wistar (n = 24). Experimentální období bylo stanoveno na 21 dní. Příjem vody byl sledován každých 12 hodin a granulovaná dieta KMK-20 byla vážena vždy těsně před podáním. Tělesná hmotnost potkanů a příjem krmiva byly zjišťovány na začátku a na konci pokusu.

Statisticky významně bylo zjištěno (α = 0,01), že acidifikovná voda byla u experimentálních potkanů nejméně preferovaná (20,3 ± 3,6 ml za den na zvíře) v porovnání s konzumací destilované vody (26,2 ± 1,9 ml za den na zvíře). Dále bylo jednoznačně prokázáno, že potkani upřednostňovali napájení během noční aktivity (α = 0,05). Závislost množství přijaté vody na přírůstku tělesné hmotnosti nebyla v tomto případě stanovena jako statisticky průkazná (r = 0,3019).

napájecí voda; laboratorní potkan; biomedicína

Contact Address:

Ing. Jitka Eberová, Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, katedra mikrobiologie, výživy a dietetiky, Kamýcká 957, 165 21 Praha 6-Suchdol, Česká republika, tel.: +420 224 382 678, fax: +420 220 921 640, e-mail: eberova@af.czu.cz