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The Water Delivery System Affects the Rate of Weight Gain in C57BL/6J Mice during the First Week after Weaning

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Facility planners, IACUCs, veterinary staff, and researchers make choices on water delivery systems for rodents on the basis of cost effectiveness, water quality, risk of malfunction, and potential effect on animal health and welfare. Here we compare biometrics, including weight trends, of newly arrived mice unfamiliar with automated watering; weight trends of weanlings; fecundity of mice; and risk of malfunction among 3 water delivery techniques: water bottle only, combination of automated delivery and water bottle, and automated system only. There was no statistically significant difference among the 3 experimental groups with respect to fecundity, mortality, and delivery malfunction. On the basis of body weight trends, the health and wellbeing of the mice used in these studies were not affected by the water delivery system or housing density after the first week; however, there was a significant difference in the growth rate at 21 to 28 d of age among the 3 groups of pups. The mice receiving both automated delivery and water bottles experienced higher growth rates from 21 to 28 d of age than did the other experimental groups. However, after 35 d of age, weight trends did not differ among the groups. Our results suggest that mice weaned into the same method of water delivery as their respective dams thrive equally well among the 3 tested water delivery systems.

The *Guide for the Care and Use of Laboratory Animals*[4] indicates that "animals should have access to potable, uncontaminated drinking water according to their particular requirements," and, furthermore, that "animals sometimes have to be trained to use automatic watering devices." Facility planners, IACUCs, veterinary staff, and researchers make choices on water delivery systems for rodents on the basis of cost effectiveness, water quality, risk of malfunction, and potential impact on animal health and welfare. Here we compare biometrics, including weight trends, of newly arrived mice unfamiliar with automated watering; weight trends of weanlings; fecundity of mice; and risk of malfunction between 3 water delivery techniques: water bottle only, combination of automated delivery and water bottle, and automated delivery only (Table 1).

Automated watering systems are used frequently in the research setting to provide continuous drinking water to rodents, eliminating the labor-intensive practice of changing water bottles weekly.[5] Nevertheless, automated watering for mice is not universally accepted because of concerns that leaking valves might flood cages.[3,6] Researchers and animal care providers express concern that solely using automated watering systems with mice may lead to higher mortality and morbidity of weaned mice than that associated with using a water bottle system. Researchers blame the higher mortality and morbidity to learning curve problems or young rodents having insufficient strength to trigger the automated watering system. Moreover, researchers have concerns for automated watering system because a leaky valve could easily fill a cage if not noticed in time.[1] Advantages of the automated water delivery system include: consistent water quality, constant supply of drinking water, built-in reserves for emergencies, reduced downstream costs (purchasing of replacement bottles and stoppers and storage costs), reduced washroom labor, ergonomic benefits of reduced lifting and pushing, and management information systems (alarming, reporting, accountability).[7]

Water bottles must be changed at least once weekly, which contributes to higher labor costs compared with those for cages receiving automated water supply. Given their inexperience with automated watering equipment, preweanling mice reared with dams receiving water from a water bottle alone are presumed to be at risk of dehydration when weaned onto automated water delivery systems. To safeguard against dehydration, researchers and animal care providers often offer both a water bottle and automated water delivery to new arrival and weanling mice inexperienced with automated water delivery systems. This system of double delivery of water results in additional labor associated with the water bottle approach and may even increase the risk of flooding by using 2 water supply systems at the same time. However, some argue that the cost savings of automated watering must be weighed against the time needed for mice to learn to access water and flooding problems inherent in using automated watering.[3]

The lack of peer-reviewed literature on the topic of the effects of water delivery systems on the weight trends of new arrival mice and weanling morbidity, mortality, and weight gain challenges research facilities to establish data-driven policies and guidelines. This study attempted to apply scientific methods to learn which water delivery system is the best to meet the needs of laboratory mice. The study objective was to evaluate differences in body weight, body weight trends, morbidity, mortality, and fecundity between groups of new arrival breeding pairs of mice and weanling mice reared in 3 different water delivery systems. We expected that growth rates, morbidity, mortality,

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Table 1. Definitions of groups and water delivery systems and other group characteristics

				No. of mice (male, female) at age		
group	Automated watering system?	Water bottle?	No. of breeding pairs	7 d	21 d	49 d
A	No	Yes	19	127	126 (56, 70)	126 (56, 70)
B	Yes	Yes	19	119	118 (61, 57)	118 (61, 57)
C	Yes	No	19	121	116 (50, 66)	112 (46, 66)
Total			57	367	360	356

and fecundity would be similar between the different watering systems.

Materials and Methods

This study was reviewed and approved by the University of Rochester Institutional Animal Care and Use Committee and was conducted in an AAALAC-accredited facility.

Mice. C57BL/6J mice (n = 120; 60 male and 60 female; age, 42 to 49 d; The Jackson Laboratory, Bar Harbor, ME), an inbred strain that is commonly used as a background in genetically modified mice, were chosen for the study to generate strain-specific results and recommendations. The mice, which were accustomed to receiving water via water bottles only, were randomly assigned into 3 groups each consisting of 20 breeding pairs. The breeding pairs were weighed and randomly assigned on arrival to 1 of 3 water delivery systems. All of the mice were weighed 1 wk after arrival. Female mice were monitored weekly for evidence of pregnancy (body weight and appearance). The length of time from arrival to birth of the first litter was compared between the 3 experimental groups of mice. The number of mouse pups per litter; weaning weights; and first, second, third, and fourth week postweaning weights were compared among the 3 experimental groups.

Housing. Mice were housed in 7.75×12×6.5 in., polycarbonate, solid-bottom cages (Allentown Caging Equipment, Allentown, NJ) with filtered microisolation tops on HEPA-filtered, direct-exhaust, ventilated racks (product no. MDJU140MVPCD1, Allentown Caging Equipment). Mice were fed a commercial laboratory diet (PMI Lab Diet 5010 Rodent Chow, PMI International, Brentwood, CO), that was autoclaved with the caging and were exposed to a 12:12-h light:dark photocycle. Cages contained with less than 1 in. of corncob bedding (Bed-o'cobs, The Andersons, Maumee, OH) and 1 Nestlet (Ancare Company, Bellmore, NY). Cages were changed on a top in the animal housing room every 2 wk, with the exception of cages housing dams with litters younger than 21 d old, which were not disturbed for the first 3 d after birth and then were changed at 2-wk intervals.

Water was supplied by the City of Rochester after chlorination to 0.5 ppm and treatment by reverse osmosis. Water in the automated delivery system was treated by reverse osmosis. Water bottle water was treated by reverse osmosis and autoclaved. Mice assigned to groups A and B received water in polysulfone low-profile bottles (Allentown Caging and Equipment Company) equipped with a 2.5-in., stainless steel sipper tube in a size 8.5 neoprene cork. Mice in groups B and C were placed on racks fitted with automated watering systems and connected to the manifold (Edstrom Industries, Waterford, WI). Automated water lines were flushed daily automatically and then every 6 mo after 45 min of contact with 20 ppm sodium hypochlorite.

Study design. All cages of mice in the animal room were visually checked daily by animal care staff for morbidity, mortality, and water delivery malfunction. Water delivery malfunction was identified by discovering excessively wet bedding. For the first 21 d of age, all mice were weighed weekly to evaluate growth trends and to determine pregnancy. Male mice were euthanized when pregnancy was confirmed by weight gain and appearance (prominent nipples and a large abdomen) of the female mice. Without opening or removing them fully from the rack, cages were checked daily for pups. Pups were counted without removal from the nest during the first 14 d of life to avoid disturbing the dam and possibly leading to maternal neglect or cannibalization.[8]

Weanling mice from each of the 3 study groups were housed in single-sex, same-litter groups after weaning at 21 d of age, with a housing density of 1 to 5 animals per cage; this housing scheme is the preferred method for studies involving inheritance of body weight.[2] All mice at weaning received approximately 4 pieces of water-moistened rodent chow in the front left cage floor per IACUC-approved policy to ease access to chow and water. Mice were maintained in these housing groups for the duration of the experiment (49 d of age).

Sentinel mice were tested serologically quarterly for mouse hepatitis virus, mouse parvovirus, and mouse minute virus and were screened by perianal tape test and zinc sulfate fecal floatation for endoparasites and fur pluck for ectoparasites. Sentinels were tested annually after a terminal blood collection for mouse hepatitis virus, mouse parvovirus, mouse minute virus, cilia-associated respiratory bacillus, *Mycoplasma pulmonis*, mouse rotavirus, ectromelia virus, Theiler disease virus, lymphocytic choriomeningitis virus, mouse adenovirus 1 and 2, pneumonia virus of mice, polyoma virus, reovirus, and Sendai virus. In addition, parasite screening involving a perianal tape test, fecal floatation, and cecal exam for endoparasites and fur pluck tests for ectoparasites was performed.

Statistical analysis. Data analysis was completed by using SAS 9.2 (SAS Institute, Cary, NC). For cross-sectional (single time point) comparison among the groups, Kruskal–Wallis statistics were used. For analysis of longitudinal data, generalized estimating equations, which do not assume normality of the error and thus are more robust, were used. ANOVA was used to compare the body weight growth rates among all the groups. A P value of less than 0.05 was regarded as statistically significant.

Euthanasia. According to IACUC policy, mice were euthanized by using CO_2 at a regulated flow equating to 20% of the cage volume per minute (Euthanex, Allentown, PA) followed by cervical dislocation after euthanasia.

Results

Three female mice, one assigned to each study group, failed to conceive or may have repeatedly cannibalized pups and were removed from the study, leaving 19 litters for analysis per study group.

Weight trends for new arrival mice. The rate of weight gain in the new arrival mice over their first 7 d after arrival did not depend on the watering system (all P values greater than 0.05). In addition, the rate of weight change of both male and female mice differed significantly ($P < 0.001$) due to sex.

Morbidity/mortality. All mice appeared healthy throughout the study. Eight preweaning mice, representing all groups, died between 7 and 21 d of age. All preweanlings that died were from litters of 7 or 8 pups.

Four weanling mice in group C (automated watering system only) died between 21 and 28 d of age weeks of age. Three of the 4 mice that died between weaning and 1 wk after weaning were in group C and were more than 1 SD below the mean weight of all mice in the project at weaning date. There was no statistically significant difference between the 3 experimental groups with respect to pup mortality ($P > 0.05$). In addition, the relation between death and group, sire's weight, and dam weight was not significant in the prediction of deaths in pups. However, the P value for the dam's weight was 0.0649 in the prediction of death in pups.

Fecundity. There is no significant difference in the average days to birth among the groups (group A, 35.6 ± 11.3 d; group B, 35.3 ± 8.9 d; group C, 34.0 ± 8.4 d; $P = 0.8604$ [ANOVA], 0.8133 [Kruskal–Wallis]).

Weight change of the pups. There were differences among groups regarding weight change in pups. There was a strong group×week interaction ($P = 0.0007$). Difference in the weight change of the pups occurred mainly between 21 to 28 d of age. Sex also had a significant ($P < 0.0001$) effect. Weanlings in group B (double delivery system) experienced greater ($P = 0.0007$) growth rates on average than did those in groups A (water bottle only) and C (automated system only). Furthermore, pups in group A experienced greater ($P = 0.0007$) growth rates than did those in group C.

Regression analysis of weight change between 21 to 28 d of age indicated that pups in group A had an average weight gain that was 8.1% greater ($P = 0.0007$) than that of those group C, and mice in group B had an average weight gain of 12.4% greater ($P = 0.0007$) than that of those in group C. Regression analysis of weight change between 21 to 35 d of age indicates that weanlings in group A had an average weight gain that was 4.4% greater than that of those in group C, and those in group B had an average weight gain of 4.0% greater than that of group C, but both of these differences were not statistically significant ($P = 0.1674$). Regression analysis of weight change between 21 to 49 d of age indicates that mice in group A had an average weight gain of 3.5% greater than that of group C, and mice in group B had an average weight gain of 1.6% less than that in group C. However, both of these differences were not statistically significant ($P = 0.3573$).

There were no significant differences in rates of weight gain between the groups from weaning to 21 to 35 d of age ($P = 0.1674$), although male mice, as expected, became heavier ($P < 0.0001$) than did female mice, and litter size had a significant ($P < 0.0720$) effect on rate of gain. Furthermore, rates of weight gain did not differ between the groups from weaning to 21 to 49 d of age ($P = 0.3573$), although male mice, as expected, became heavier than female mice, and the number of mice in the weaned cages had a significant ($P = 0.0017$) effect on weight gain.

Final weights. The weight of mice (mean ± 1 SD) at termination of the experiment was 20.18 ± 2.36 g for group A, 20.30 ± 2.68 g for group B, and 20.22 ± 2.57 g for group C (Figure 1).

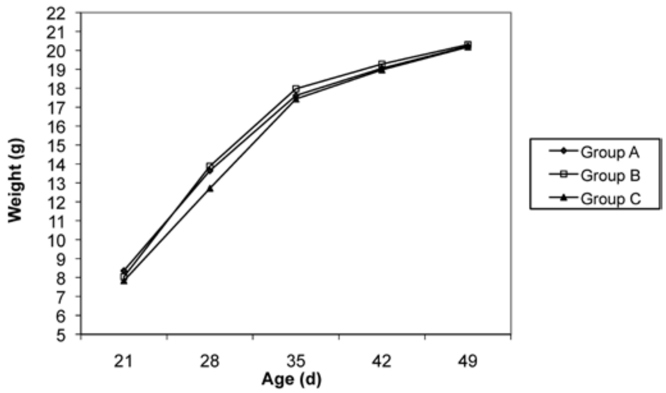

Figure 1. Average weight of mice over study duration.

Statistically, the 3 groups did not differ ($P > 0.05$) in mean body weight at 49 d of age.

Water leaks. During the study there were 2 water leaks: one from a bottle and another from an automated valve. In both cases, the animals were transferred to new cages before adverse effects occurred (for example, hypothermia and drowning). The leak event occurrence was too low to become statistically significant. There was no statistically significant difference ($P > 0.05$) between the 3 experimental groups with regard to water delivery malfunction.

Discussion

On the basis of body weight trends, the health and wellbeing of the mice used in these studies were not affected by the water delivery system after 28 d of age. By 49 d of age, there was no significant difference in mean body weight between the 3 experimental groups. These results can assure future investigators that mice experiencing only automated water delivery tend to be as healthy as those receiving water bottles only or a combination of water bottles and automated water delivery. However, investigators measuring weight trends in mice from weaning to 28 d should examine their measurements closely, because differences in measured growth rates may reflect the water delivery system used.

The rate of weight gain in the new arrival mice over the first week after arrival did not depend on the watering system. Although the results of this study are strain-specific, investigators can feel comfortable about directly switching newly arrived C57BL/6J adult mice (42 to 49 d of age) from the water delivery system used at the vendor facility to any of the 3 water delivery systems mentioned herein.

We did not observe aggressive or injurious behavior among the mice in our study. Preweaning deaths represented all study groups. Past studies involving 70-d-old C57 BL/6JIco × CBA/JIco first-time dams experienced 4% (81 of 2030 pups) preweaning mortality.[9] In our current study, preweanlings died due to unknown reasons and represented the following mortality rates: group A, less than 1% (1 of 127); group B, less than 1% (1 of 119); and group C, 4% (5 of 121). All of these rates are within the expected preweaning mortality. These preweanlings were found dead, desiccated, not cannibalized, and were poor necropsy subjects. A single cage (no. 7) in group C accounts for 4 of the 7 preweanling deaths and may reflect problems with the individual dam or that litter, such as lactation cessation or congenital defects. Physical examination of this dam revealed no abnormalities. The preweaning mortality rate was not statistically significant ($P = 0.07$) and was within the normal limits found in the above mentioned study. [9]

Four postweaning mice from group C died within the first week after weaning, and 3 of these 4 mice were more than 1 SD lower than the mean weaning weight of all groups (8.06 ± 1.61 g). Two of the weanlings that died were littermates, whereas the other 2 represented 2 other litters and were singly housed at the time of death because they were only male mice in their respective litters at the time of weaning. A review of our data revealed that weaning weights of 14 group A, 11 group B, and 17 group C mice had weights that were more that 1 SD lower than the mean weight at weaning for all groups. This finding suggests that weight is not the only factor in determining postweaning mortality. Therefore, future investigators may want to consider the contributions that weaning body weight (even though the difference was not significant in our study) and housing density have on mortality. Perhaps mice weighing less than 8 g or pups housed singly at weaning should receive water bottles to reduce possible mortality; however, further studies must evaluate this hypothesis.

Average days to birth did not differ among the groups. This conclusion was expected because this parameter is multifactorial. In particular the fecundity of dams older than 42 to 49 d of age was not affected by the watering system. Therefore, colony managers need not be concerned that the water delivery system used might lower fertility or fecundity.

The growth rate of mice between weaning and 28 d of age revealed a strong interaction between group and week ($P = 0.007$), with mice in group B (double delivery system) having higher growth rates from 21 to 28 d of age. Perhaps having more available watering sources allowed for more drinking opportunities or less competition between cagemates for water resources. However, after 35 d of age and at study termination (day 49) average weights did not differ significantly between groups. Nevertheless, investigators measuring weight trends in mice from weaning to 28 d of age should examine their measurements closely, because any apparent differences might reflect the water delivery system used during the study.

The 2 malfunctions in watering systems were not statistically significant; the incidence of malfunction did not differ among water delivery systems. A future study looking at greater numbers of cages and spontaneous water leaks might better assess the risk of leaking among the 3 systems.

In conclusion, our results suggest that mice weaned into the same system of water delivery as their respective dams thrive equally well among the 3 tested water delivery systems over 49 d of age. After 28 d of age, mice receiving only automated water tended to be as healthy as those receiving water bottles only or a combination of water bottles and automated water delivery. However, growth rates from 21 to 28 d of age were statistically reduced when pups were offered the automated water delivery system only. In addition, investigators may want to consider possible contributions of weaning body weight and housing density on mortality. With the exception of specialized studies that require water additives to individual cages, we find that automated water delivery without supplementation with bottles is an acceptable option for average-weight C57BL/6J pups.

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