TECNIPLAST

ZEPHYR IVC Air Handling Unit, performance efficiency testing

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INTRODUCTION

The performance of an Air Handling Unit (AHU) serving Individually Ventilated Cage (IVC) systems was evaluated through both technical validation and in situ field trials. The field assessments focused on quantifying ventilation efficiency, defined as the system's capacity to maintain intra-cage environmental conditions that meet established welfare standards. Key parameters monitored included temperature and relative humidity stability, oxygen concentration, and the effective removal of noxious metabolic by-products such as ammonia (NH_3) and carbon dioxide (CO_2). These factors are critical for sustaining a microenvironment that supports the physiological needs and welfare of laboratory rodents, in accordance with the principles of refinement and the standardization of housing conditions.

OBJECTIVE

The objective of the trial was to monitor intra-cage environmental parameters—specifically ammonia concentration, oxygen levels, temperature, and relative humidity—under varying ambient room conditions. All measurements were analyzed in relation to the corresponding temperature and relative humidity recorded within the housing room, in order to evaluate the system's ability to maintain stable microenvironmental conditions independently of macroenvironmental fluctuations.

HOUSING

Z E P H Y R

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MATERIALS AND METHODS

- For this study, the "Zephyr" ventilation unit was connected to three racks equipped with 60, 80, and 80 GM500 IVC cages, respectively. Over the three-month testing period, the average number of occupied cages and housed mice was 203 and 620, respectively.The system was configured to operate in positive pressure mode at 75 Air Changes per Hour (ACH), with a 20% negative pressure offset.
- Three cages—one from each rack—housing 4 or 5 male transgenic mice were randomly selected for monitoring. Parameters recorded included body weight, water and feed consumption, temperature, and relative humidity. Data loggers were placed inside the cages to record these environmental parameters, while an additional logger was positioned on top of one of the racks to monitor ambient room conditions. A further logger was installed in the horizontal supply plenum near the ventilation unit.
- Ammonia, carbon dioxide (CO_2), and oxygen levels inside the three selected cages were measured on cage-change day (Day 14), across five repetitions, using a Dräger AX-7000 gas analyzer.
- Water and feed consumption were recorded at each cage change or upon bottle replacement. Mice were weighed at the beginning of the study and during each subsequent cage change.
- Throughout the three-month testing period, two Interceptor environmental microbiological monitoring systems were used. At the end of the exposure period, they were sent to the Envigo laboratory for PCR analysis, following the quarterly FELASA panel protocol.

RESULTS AND COMMENTS

Mice body weight

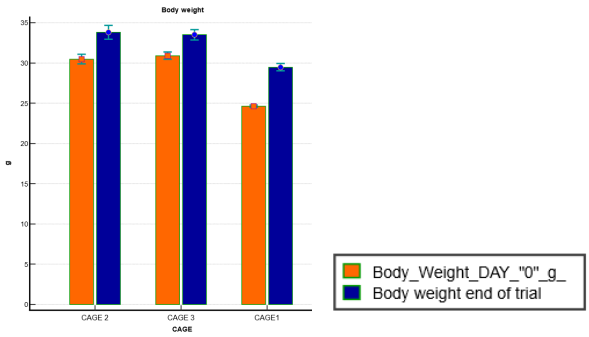


FIG. 1

BODY WEIGHT

	DAY "0" g			End of trial		
	CAGE1	CAGE 2	CAGE 3	CAGE1	CAGE 2	CAGE 3
N mice	4	5	4	4	5	4
Min	24.2	29.3	30	28.8	32.4	31.7
Max	24.9	32.5	32.1	30.8	36.9	34.7
Mean	24.6	30.5	30.9	29.5	33.8	33.5
Median	24.7	29.8	30.75	29.15	32.8	33.8
SD	0.30	1.29	0.92	0.90	1.92	1.27

TABLE 1

Fig.1 & Table 1. The mice monitored in the trial are transgenic (KO) mice for which no standard growth curves are available. However, considering that at the beginning of the test the mice were all > 10 weeks of age we still expected a slight growth before reaching the "plateau" of stabilization of body weight. Both the initial increase and the stabilization of weight occurred during the 3 months of testing.

Mice body weight

	N° of Mice (cage)	Mean FEED intake g/mouse/day	Mean WATER intake g/mouse/day
CAGE1	4	3.1	5.5
CAGE2	5	3.6	5.5
CAGE3	4	3.9	6.4

TABLE 2

Table 2 provides a summary of the average food and water intake observed in the three monitored cages. These values are consistent with typical consumption patterns reported for C57Bl/6J mice. However, it should be noted that the animals used in this study were transgenic subjects derived from that strain, for which no reference data are currently available in the scientific literature.

rH%	ROOM (ENV)	CAGE 1	CAGE 2	CAGE 3	Positive Plenum
N	1933	1933	1933	1933	1933
Min	56.6	53.9	53.6	54.2	43.8
Max	81.3	72.4	83.8	78.6	71.7
Mean	**64.4**	**62.7**	**64.3**	**64.1**	**52.7**
Median	63.7	62.5	64.3	64.1	52.2
SD	2.81	3.32	3.05	3.15	2.86

TABLE 3

°C	ROOM (ENV)	CAGE 1	CAGE 2	CAGE 3	Positive Plenum
N	1933	1933	1933	1933	1933
Min	22.6	22.1	22.2	22.4	22.4
Max	25.5	27.6	27.2	27.3	25.5
Mean	**23.7**	**25.3**	**25.5**	**25,0**	**23.9**
Median	23.5	25.3	25.5	25	23.7
SD	0.56	0.66	0.66	0.66	0.42

TABLE 4

Tables 3 and 4 report the longitudinal profiles of relative humidity and temperature measured in the animal room, within the three monitored cages, and in the supply (positive pressure) plenum. The monitoring period extended over approximately two months and intermittently revealed suboptimal performance of the HVAC system in regulating these environmental parameters within the desired range. **Figures 3 and 4** provide a clear depiction of the temporal evolution of room temperature and relative humidity, underscoring the HVAC system's limited capacity to maintain appropriate humidity control—particularly during the summer months in which the monitoring took place.

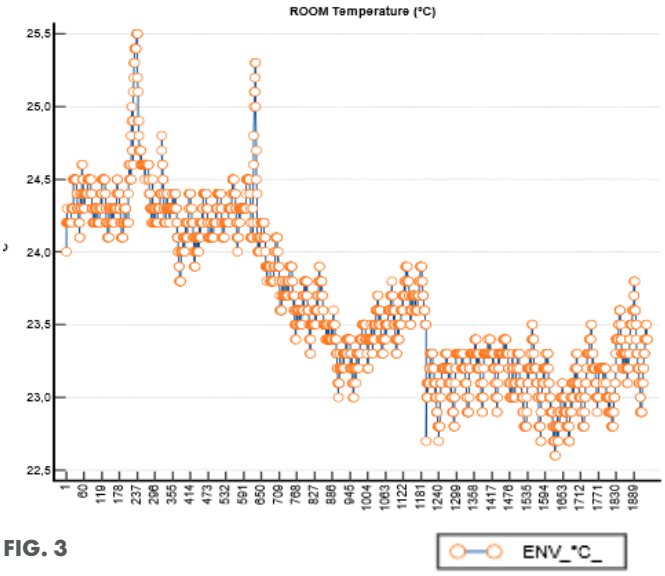


FIG. 3

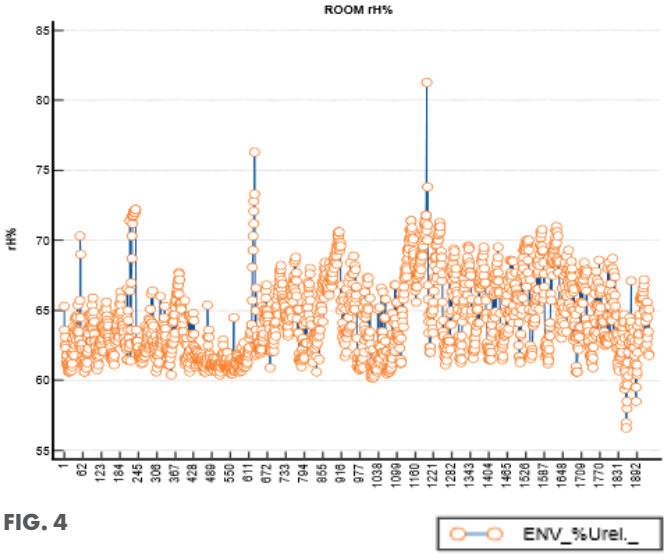


FIG. 4

rH%	CAGE 1	CAGE 2	CAGE 3	Positive Plenum
N	1933	1933	1933	1933
Min	53.9	53.6	54.2	43.8
Max	72.4	83.8	78.6	71.7
Mean	**62.7**	**64.3**	**64.1**	**52.7**
Median	62.5	64.3	64.1	52.2
SD	3.32	3.05	3.15	2.86

TABLE 5

°C	CAGE 1	CAGE 2	CAGE 3	Positive Plenum
N	1933	1933	1933	1933
Min	22.1	22.2	22.4	22.4
Max	27.6	27.2	27.3	25.5
Mean	**25.3**	**25.5**	**25,0**	**23.9**
Median	25.3	25.5	25	23.7
SD	0.66	0.66	0.66	0.42

TABLE 6

Tables 5 and 6 describe the trends in relative humidity and temperature measured inside the three monitored cages and within the system's positive pressure plenum. The mean values for both parameters remained within the acceptable ranges defined by international guidelines; however, occasional peaks, particularly in relative humidity (%) were recorded, reflecting the same issues previously observed at the room level. As is well known, IVC systems do not condition air with respect to temperature and relative humidity; control of these parameters depends entirely on the quality of the room-supplied air as conditioned by the HVAC system.

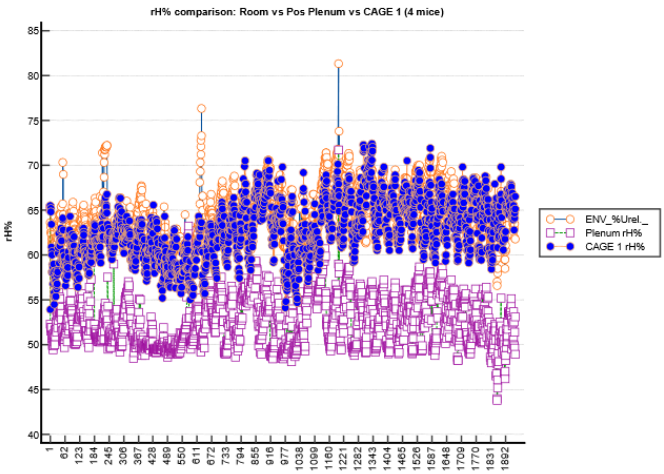


FIG. 5

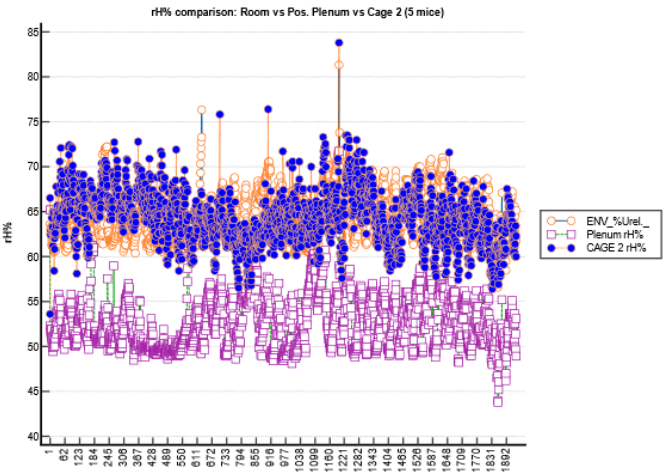


FIG. 6

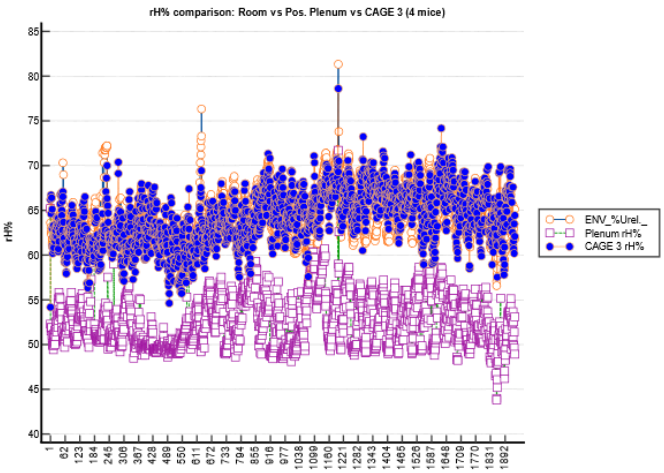


FIG. 7

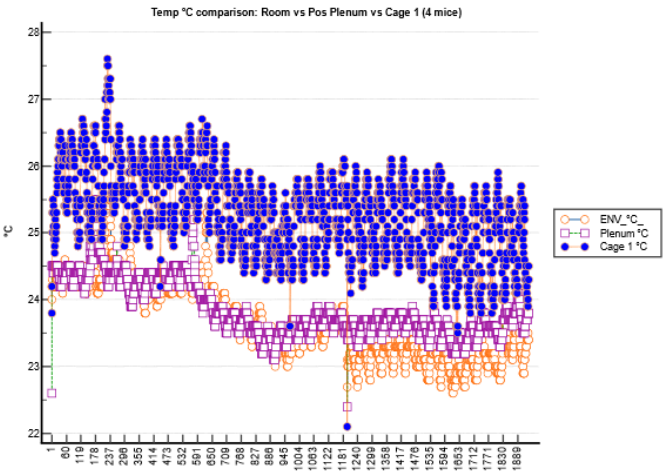


FIG. 8

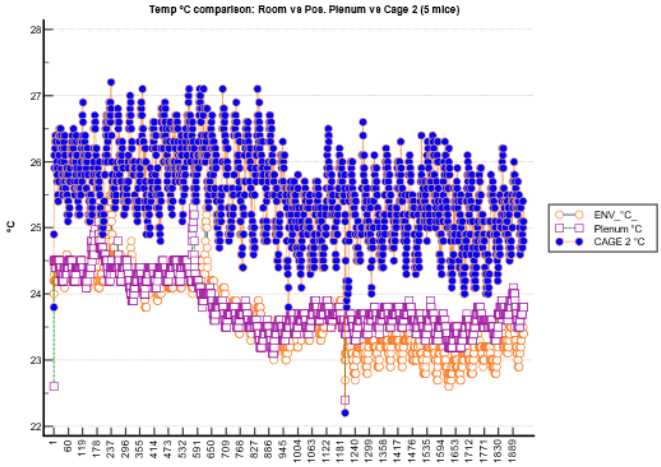


FIG. 9

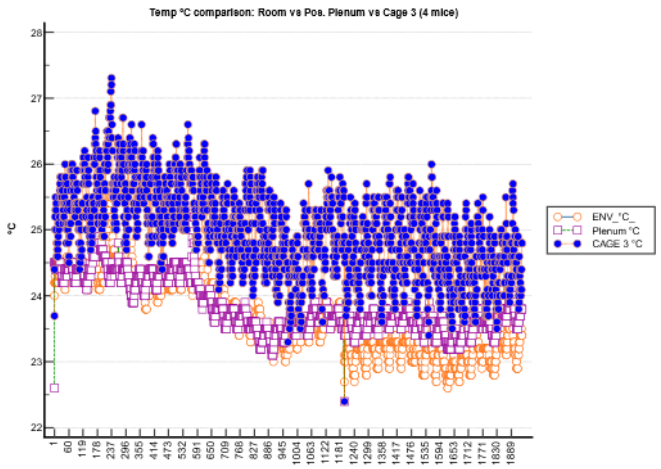


FIG. 10

Figures 5 through 10 illustrate the relationship between the Room, the Positive Pressure Plenum, and the three animal-housed cages with respect to temperature and relative humidity. This pattern highlights the passive role of the ventilation unit in modifying the thermal and hygrometric profile of the supplied air, reinforcing the notion that environmental conditions inside IVC cages are primarily determined by the quality of room air and by internal metabolic heat and moisture production. These findings underline the importance of precise room-level HVAC regulation when aiming to ensure microenvironmental stability and compliance with animal welfare standard. A slight increase in both parameters is observed within the cages themselves, attributable to the metabolic activity of the mice.

Ammonia, CO_2 and O_2

In-cage gas measurements were conducted exclusively on cage-change day (Day 14). Ammonia concentration typically peaks between the twelfth and fourteenth day of housing, particularly under conditions of high animal density per unit surface area, as was the case in this study (4 to 5 mice per cage). Across the five sampling sessions, the average relative humidity in the housing room was 69.3% (SD ±2.25), while intra-cage values ranged from 67.0% to 68.4% across the three monitored cages.

The table below reports the mean concentrations of carbon dioxide (CO_2), ammonia (NH_3), and oxygen (O_2) recorded during the experimental period.

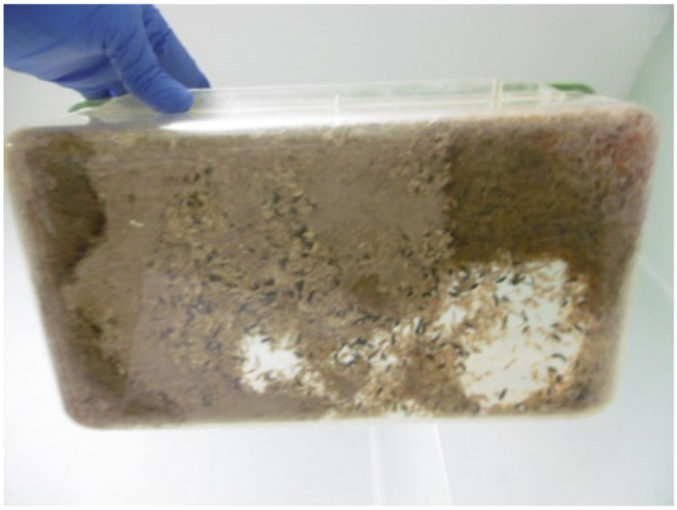
Cage	N° of Mice	O_2 (%)	CO_2 (%)	NH_3 (ppm)
CAGE 1	4	20.9	0.07	40.2
CAGE 2	5	20.9	0.07	53.6
CAGE 3	4	20.9	0.08	57.2

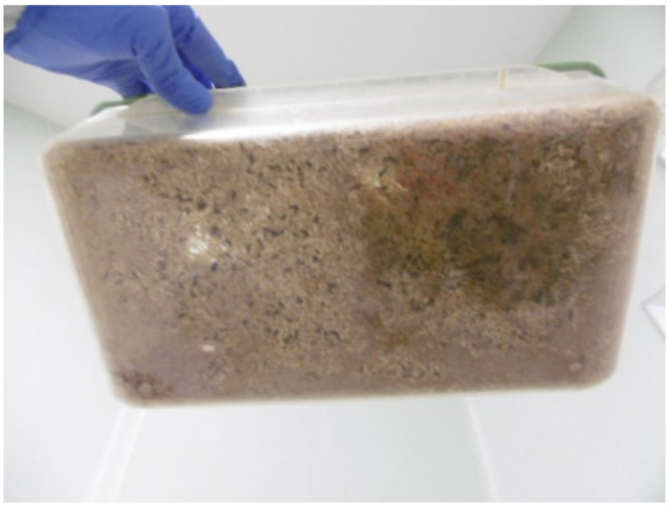
TABLE 7

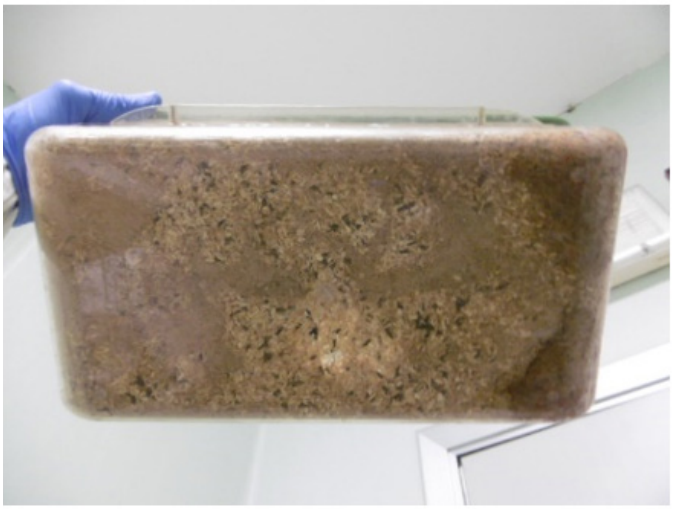
- The oxygen concentration within the cages remained stable at 20.9% throughout the entire testing period. This level, consistent with ambient atmospheric air, was unaffected by the presence of other gases such as ammonia (NH_3) and carbon dioxide (CO_2), and no oxygen depletion was observed. CO_2 concentrations consistently remained well below the established safety threshold of 0.5% under ventilated cage conditions and did not pose any concern during the study. These findings align with previously published data indicating that properly functioning IVC systems typically maintain CO_2 levels below those considered harmful to laboratory rodents [2–4].

- Ammonia concentrations measured on Day 14 (cage-change day) were within expected ranges and did not raise concerns regarding the animals' physiological or behavioral well-being. These results are consistent with prior studies showing that short-term exposure to ammonia concentrations below 25 ppm in latrine-free areas—and even occasional exposure to levels between 50 and 100 ppm—does not produce adverse effects on respiratory health, organ function, or behavior in rodents housed under standard IVC conditions [1,5,6,8].

- From a behavioral standpoint, the mice consistently partitioned their cages into distinct zones, designating specific areas for latrine use and maintaining separate, cleaner zones for other activities [5] (Fig. 11, 12, 13).







An abnormal increase in ammonia (NH_3) concentrations within Individually Ventilated Cages (IVCs) is often observed under conditions of elevated relative humidity (rH > 65%). This phenomenon is multifactorial, involving biochemical, microbiological, and physical mechanisms:

1. Enhanced Urease Activity and Microbial Growth Elevated humidity promotes the proliferation of urease-producing bacteria within bedding materials. These microorganisms hydrolyze urea present in rodent urine into ammonia via the urease enzyme. Increased moisture enhances both bacterial metabolism and enzymatic activity, thereby accelerating ammonia production [1].
2. Loss of Bedding Absorptive Capacity - Under high humidity conditions, bedding materials can become saturated, significantly reducing their ability to absorb urine and volatile nitrogenous compounds. This saturation leads to increased volatilization and accumulation of free ammonia within the cage microenvironment [2].
3. Reduced Gas Diffusion and Impaired Air Exchange Efficiency - High relative humidity alters the physicochemical properties of air, potentially reducing the diffusion rate of gaseous ammonia and compromising convective gas exchange. In ventilated systems, this may result in the formation of microzones with impaired ventilation and gas stagnation [3].

Maintaining relative humidity below 60–65% is critical to limiting in-cage ammonia accumulation. Environmental monitoring systems should therefore include humidity control and dynamic ventilation feedback mechanisms to safeguard animal welfare and ensure data reproducibility. In most research facilities, humidity regulation is managed by the central HVAC system; however, as previously noted, our facility experienced significant failures in maintaining consistent humidity levels.

Interceptor and the EAD Microbiological monitoring

Over the course of the three-month evaluation period, two Interceptor filters designed for environmental microbiological monitoring were positioned within the dedicated housings integrated into the Zephyr ventilation unit. The microbiological health status of the three racks connected to the Zephyr system was well-characterized prior to testing. The primary aim of this study was to validate the established efficacy of the Interceptor system in supporting the detection of pathogenic and opportunistic microorganisms via polymerase chain reaction (PCR) analysis.

The six microorganisms, previously documented within the microbiological unit comprising the three racks connected to the Zephyr ventilation system, were successfully identified on both Interceptor filters following a three-month exposure period.

The identified microorganisms included: Helicobacter ganmani, Helicobacter mastomyrinus, Mouse Norovirus (MNV), Rodentibacter pneumotropicus, Trichomonas spp., and Tritrichomonas muris.

The complete concordance of microbial profiles between the two Interceptor devices supports the analytical reliability of the dedicated Exhaust Air Dust (EAD)-based detection system. This approach enables the routine implementation of a secondary, or "backup," Interceptor to confirm laboratory findings obtained from the primary sampling unit, thereby enhancing the robustness of environmental health monitoring protocols in IVC systems.

CONCLUSIONS

The field performance assessment of the Zephyr Air Handling Unit (AHU) demonstrated no adverse effects on the welfare of mice housed in Green Line GM500 cages. The system exhibited stable and uniform air distribution within the cages, with intra-cage environmental parameters showing consistent repeatability—even in the presence of significant room relative humidity fluctuations caused by HVAC system limitations.

Physiological indicators, including feed and water consumption and body weight, along with behavioral observations—such as the absence of aggressive interactions (e.g., fighting), regular grooming behavior, and the consistent spatial segregation of cage areas into latrine and clean zones—collectively support the conclusion that the ventilated system maintained an in-cage microenvironment conducive to the sustained welfare of laboratory mice.

References

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