Comparison of three IVC systems for mice, with focus on growth and nest building in juvenile mice

Utvärdering av tillväxt och bobyggnad hos unga möss i tre olika IVC-system

Sofie Sundgren

Husdjursagronom



Sveriges lantbruksuniversitet
Institutionen för husdjurens miljö och hälsa
Avdelningen för etologi och djurskydd

Skara 2012

Studentarbete 386

Swedish University of Agricultural Sciences
Department of Animal Environment and Health
Section of Ethology and Animal Welfare

Student report 386

ISSN 1652-280X

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Examensarbete i husdjursvetenskap på D-nivå, 30 hp, Agronom. Kurs: EX0555 Examensarbete i husdjursvetenskap – D30.

Handledare: Elin Spangenberg, Box 7068, 750 07 Uppsala
Examinator: Lena Lidfors, Box 234, Gråbrödragatan19, 532 23 SKARA

Nyckelord: IVC-system, Refine, Nest quality, Body weight, Animal welfare

Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap
Institutionen för husdjurens miljö och hälsa
Avdelningen för etologi och djurskydd
Box 234, 532 23 SKARA
E-post: hmh@slu.se, **Hemsida:** www.slu.se/husdjurmiljohalsa

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Sammanfattning

I dag är individuellt ventilerade burar (IVC-system) det vanligaste inhysningssystemet för gnagare. IVC- systemen är utrustade med HEPA-filter och utformade för att styra både in- och utmatning av luft samt temperatur och relativ luftfuktighet i burarna. Detta gör att man får säkrare försöksresultat då det skapas en optimal miljö för mössen och minimerar risken för kontaminering av oönskade partiklar inom bursystemet. Syftet med denna studie var att utvärdera hälsa och välfärd för två olika stammar av laboratoriemöss (NMRI nude och C57BL/6NCrl) i tre olika typer av IVC-system: Allentown, Arrowmight och Tecniplast. 108 musungar från varje stam (totalt 216 möss) valdes ut efter avvänjning vid en ålder av tre veckor och testats under 5 veckor. Direkta beteende-observationer gjordes i deras hemburar med fokus på aggressiva och avvikande beteende, t.ex. stereotypa beteenden så som att gnaga på galler eller hoppa mot burväggen i försök att ta sig ut. Placeringen av boet observerades samt att formen på boet poängsattes. Dessutom gjordes mätningar av vatten, födointag och viktökning. Resultaten visar skillnader i kvalitet på boet där NMRI nude stammen visade på högsta poäng för möss i Arrowmight. För C57BL/6NClr stammen så hade mössen i Allentown- och Arrowmightsystemen högst poäng medan möss i Tecniplast hade lägst. Försöket resulterade även i en skillnad i viktökning för NMRI nude stammen där möss i Arrowmight systemet hade högst ökning respektive lägst i Allentownsystemet. Dock visade resultatet för varken mat- eller vattenkonsumtion någon skillnad mellan de tre systemen. Även fast resultaten visade på skillnader i denna studie, är det fortfarande inte tillräckligt för att utesluta eller förespråka ett specifikt IVC-system. Men tillsammans med mätningar av andra relevanta parametrar, baserat på djurvälfärd, skulle det kunna ha stort inflytande på valet av IVC-system. Ju fler resultat som kan samlas in från olika parametrar, desto säkrare kan valet av IVC systemet bli. Andra relevanta parametrar för att studera djurvälfärden skulle kunna vara effekten av tekniska hjälpmedel vid hantering av djur samt burar som t.ex. bur rengöring, eller vilken typ av ljud och ljus djuren utsätts för i burarna eftersom de till exempel kan höra ultraljud. Vidare mätparametrar skulle kunna vara lämplig gruppstorlek i IVC burarna och vilken typ av miljöberikning som ska användas vilket också är viktiga aspekter att beakta eftersom mössen naturligt lever i grupper i en varierande miljö.

Summary

Today the individual ventilated cage system (IVC-system) is the most commonly used system when housing rodents. IVC systems are designed to control both supply and exhausted air at cage level, as well as temperature and relative humidity in the cages. This creates an optimal environment for the mouse and at the same time a protection against the risk of cross-infection between cages and the outside environment.

The aim of this study was to evaluate the health and welfare for two different strains of laboratory mice (NMRI nude mice and C57BL/6NCrl mice) in three different kinds of IVC-systems; Arrowmight, Allentown and Tecniplast. 108 mouse pups from each strain (in total 216 mice) were selected after weaning at an age of three weeks and tested during 5 weeks. Behavioural direct observations were made in their home cages with focus on aggressive and abnormal behavior e.g. stereotypic behavior such as gnawing on bar or jumping on cage walls trying to get out. Place and shape of nest were observed. The nest was also scored. Measurements of water and food intake and weight gain were taken. The results show differences in nest quality with a higher score for NMRI nude mice in Arrowmight system meanwhile C57BL/6NClr mice in Allentown and Arrowmight system had highest scores and Tecniplast lowest. The results also showed a higher body weight gain for NMRI nude mice in Arrowmight system and lowest in Allentown system. But neither food nor water intake showed any difference between the three systems. Although differences were found in this

study, it is still not enough to exclude or favor for a specific IVC system. But together with recordings of other relevant parameters, based on animal welfare, they could have substantial influence on the choice of IVC system. The more results that can be collected from different parameters, the more improved the choice of IVC system can be. Other relevant parameters to study could be the effect of technical aids when handling animals as well as cages at e.g. cage cleaning, or what type of sound and light the animals are exposed to in the cages since they for example can hear ultrasounds. Further, the appropriate group size in IVC cages and what type of enrichment item to use are also important aspects to consider since the mice naturally live in groups in a varying environment.

Keywords: IVC-system, Refine, Nest quality, Body weight, Animal welfare.

Introduction

In Europe, rodents together with rabbits represent 80 % of the total number of animals used in experiments. Mice that are the most used mammal for scientific purpose account for 59% of the total use (http://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm).
The mouse is mainly used as a model organism to better understand disease mechanisms as well as the underlying physiology and pathology (Kallnik et al., 2007).
When using mice in experiments it is essential to have healthy mice and to minimize the risk of having bacteria, virus or other unwanted infections or air born substances in the cage environment that could affect the results.
By using interventions such as barriers, isolators and systems included with filter top cages when housing mice, the health status can be kept at a high level (Höglund och Renström, 2001). To improve quality for housing animals in cage system the first individual ventilated cage system (IVC system) were introduced on the market about 40 years ago and is today the most commonly used system when housing genetically modified rodents (Khron et al., 2003). The IVC systems are designed to be a protection between cage and outside environment and developed to minimize the risk of cross-infection between cages and at the same time protect the staff from animal allergens (Renström et al., 2000; Brielmeier et al., 2006).
The IVC system is equipped with an air handling unit to control both supply and exhaust air and to register and control the temperature and relative humidity as well. Air is taken from the room, or directly from the ventilation system, and passes through a pre-filter and a HEPA filter. The clean air, supplied through pipes in the rack, is then separated out into each cage. With a constant air exchange in each cage the amount of ammonia, CO_2 and humidity is reduced as it is brought back with the exhaust air. The exhaust air is then sent through a channel to a pre-filter to trap dust and small particles such as hair from animals, thus, preventing it from leaving the room. The air exchange is controlled by the IVC system and can be manually changed depending on what kind of air changes per hour (ACH) that the manufacturer specifies or what the experiment demands. By manually shifting the air changes per hour (ACH) in the IVC system; hence affecting the air temperature in the cages which will decrease or increase, you can uphold an optimal environment in the cages. Thus, the IVC system can uphold the environmental requirements for the mouse which is a temperature between 20-40 C° and a relative humidity between 50-60 % (Van Zutphen et al., 2001).
According to Reeb-Whitaker et al. (2001) an increased airflow at 100 ACH, tended to decrease the temperature from about 24.8 °C to about 23.2 °C and thereby negatively affect

pup weight in cages with breeding pairs (one male and one female) compared to cages with breeding trios (one male and two females). As mice live in groups in the wild (Van Zutphen et al., 2001) they try to adapt to body heat loss by lying close or even on top of each other (Sjaastad et al., 2003). With close body contact the body heat loss from each individual becomes lower due to the fact that the total body surface that transports heat decreases (Sjaastad et al., 2003). As the negative pup weight gain was only seen in cages with breeding pairs and not in cages with breeding trios, Reeb-Whitaker et al. (2001) suggest that it could be due to the number of animals in the cage. Fewer mice mean less total body heat and could be the reason for affecting the pup weight.
However, when developing cage systems for mice, aspects such as natural behaviour and animal welfare have not been given that much consideration. The main goal has been on techniqual aspects, economy and hygiene (Van Zutphen et al., 2001; Baumans, 2005; Newberry, 1995). Although the mouse has adapted to a life in captivity, there are still behaviours remaining from their wild ancestors (Van Zutphen et al., 2001). By the refinement of husbandry and environmental conditions such as cage enrichment it gives the mouse a possibility to express these behaviours (Russell and Burch., 1959).Yet, there are concerns that it might affect standardization of experiment, but resent research show that increased environmental enrichment does not increase the risk to obtain unreliable results (Wolfer et al., 2004). In addition, results show that environmental enrichment gives the animal a way of coping better with their living conditions and further enhance the animal welfare (Kostomitsopoulos et al., 2007). Enhanced animal welfare with environmental enrichment such as wooden blocks, shelters or various forms of protection, as well as cardboard or paper to shred is also a benefit for science. If the behavioural and psychological needs are not satisfied due to poor refinement, it might result in diseases and abnormal behaviour.
In summary, when using mice as laboratory animals there are many ways in which the refinement of cages and cage systems can influence mice's health and welfare. To find out if there was any difference between the three cage systems in this study, measurement on the mouse pups morphological development, exploratory behaviour as well as risk assessment was made. Measurements such as water- and food consumption and weight gain were evaluated as well as behavioural direct observations and location and quality of nest.

Aim

The purpose of this study was to evaluate three different kinds of individually ventilated cage systems (IVC-system) for two different strains of laboratory mice and how it affects the animal health and welfare. When evaluating how the IVC-system affects growth, activity and behaviour, the aim of this study focused on the daily situation of the animal in the home cage with both physiological and behavioural measurements.

Hypothesis

- There is no difference in cage activity and behaviour between mice housed in the three different cage system
- There is no variation in body weight gain as well as water intake and food consumption between mice housed in the three different cage system
- There is no difference between the three cage systems when analyzing place and shape of nest.

Materials and methods

Animals and housing system

Two different strains of mice were used, NMRI nude mice and C57BL/6NCrl mice (Charles River Laboratories). In total 36 females and 18 males per strain were used as breeding animals with 12 females and 6 males in each cage system. Mouse pups from the litters were selected (in total 216) after weaning at an age of three weeks and tested during 5 weeks. The mice were housed in 3 different types of individual ventilated cages (IVC); Allentown XJ, Tecniplast sealsafe Plus and Arrowmight Maxiseal 580. The Allentown rack had a capacity of 9x7=63 cages, Tecniplast 10x7=70 cages and Arrowmight 7x8=56 cages. The frequency of air changes per hour (ACH) was: 50, 75 and 40 respectively. The cage dimensions differed between the three cage systems (Table 1). Arrowmight was equipped with full grid under the lid and a food hopper placed in the middle of the cage. The other two systems had a half grid and the food hopper was placed in the back of the cages. Air intake and outlet was placed in the top cage lid except for Allentown where air intake was placed in the back wall of the cage.

Table 1. Cage size for each of the three IVC systems. (Note: Measurements were made with no bedding material in cages, that is not the same standard as the manufacture)

		IVC system		
Cage size		**Allentown**	**Arrowmight**	**Tecniplast**
Length (cm)		35	31	32
Width (cm)		16	18	16
Height (cm)	***To lid***	13	15	13
	top of lid	17,5	23	16
Floor area (cm^2)		560	558	512
Cubic area (cm^3)		9800	12834	8192
Height to feed rack (cm)		5,8	5	7

Regrouping- start of the study

Female and male pups from the litters were separated (table 2) and housed 4 mice in each cage. Table 3 show that each of the two strains had 9 cages in each cage system with 3 cages with mice originating from Allentown, 3 cages with mice from Arrowmight and 3 cages with mice from Tecniplast (3 cages * 3 cage systems = 9 cages). In each cages system 18 of the most centered cages were used (9 cages * 2 strains). In total 54 cages were used in this study (18 cages * 3 cage systems). All the animals were randomised so there would be no animals from the same litter in the same cage during the test.

Table 2. Distribution of female and male pups between cage systems.

	Allentown(nr of cages)		**Tecniplast (nr of cages)**		**Arrowmigh (nr of cages)**	
Gender\Strain	*C57BL/6NCrl*	*NMRI Nude*	*C57BL/6NCrl*	*NMRI Nude*	*C57BL/6NCrl*	*NMRI Nude*
Male	*7 cages*	*2 cages*	*3 cages*	*4 cages*	*4 cages*	*5 cages*
Female	*2 cages*	*7 cages*	*6 cages*	*5 cages*	*5 cages*	*4 cages*

Table 3. Distribution of pups between cage systems

Strain	*Breeding environment*	*Change of environment (**K** is control groups)*		
		Allentown	**Arrowmigt**	**Techniplast**
C57BL/6NCrl	**Allentown**	*12 pups(**K**)(3 cages)*	*12 pups(3 cages)*	*12 pups(3 cages)*
C57BL/6NCrl	**Techniplast**	*12 pups (3 cages)*	*12 pups(**K**)(3 cages)*	*12 pups(3 cages)*
C57BL/6NCrl	**Arrowmight**	*12 pups(3 cages)*	*12 pups(3 cages)*	*12 pups(**K**)(3 cages)*
NMRI Nude	**Allentown**	*12 pups(**K**)(3 cages)*	*12 pups(3 cages)*	*12 pups (3 cages)*
NMRI Nude	**Techniplast**	*12 pups(3 cages)*	*12 pups(**K**)(3 cages)*	*12 pups(3 cages)*
NMRI Nude	**Arrowmight**	*12 pups(3 cages)*	*12 pups(3 cages)*	*12 pups(**K**)(3 cages)*

Animal room and caging system

In the 4 *8 m mouse room the temperature was 22 ± 1° C and the air humidity at 40-50%. Cage air pressure was positive with respect to the room. The cages were equipped with aspen shavings as bedding material (140-150 g in each cage) and sizzle nest as nesting material (16 ± 0.2 g in each cage). New nesting material was always placed in the back of the cage at cage change. All the animals had access to water and food *ad libitum*. The mice were fed a commercial diet (CRM, Pelleted Rat and Mouse Breeder and Grower Diet; Special Diets Services, UK) and given water in bottles**.** After regrouping the pups the food intake was recorded weekly and tap water intake twice a week for five weeks. The animals were weighed once every day for 5 days after regrouping and after that they were weighed once weekly. The mice were given clean cages once weekly. The light:dark cycle was 12:12h with lights on between 24.00-12.00 (with no twilight periods). The animal room was also equipped with a changing station (CS5 changing station, Tecniplast, Italy) supplied with a HEPA filter to make the environment as sterile as possible when changing cages.

Nest location and behavioural observations

During five weeks, nest location was observed for 20 days (four days a week) with a total number of 132 observations for each cage system with 36 observations from day one until day four, and 96 observations during the rest of the 20 observation days. To record the behaviour of mice in their home cages observations were made 3 times a day (6.00, 12.00 and 18.00) during the first four days and thereafter two times a day (8.00 and 16.00) for four days a week. Every observation period lasted for 20-30 minutes (5-10 minutes for each caging system) with one observer recording the cages in ranked order Tecniplast, Arrowmight and Allentown. The observer started with the cage from top left in the caging system and ended bottom right. In each cage the observer registered location of the nest. The observer also registered behaviour with focus on aggressive interactions and deviate behaviour to see how mice are able coop with the environment (Table 4). Observations of nest location are presented in the results as well as behavioural observations. To make the observations easier the cage was divided into 3 zones; back, middle and front. During dark period the room was lit by red-light lamps (6 x 25 W) to make the observation as easy as possible without disturbing the animals.

Table 4. Ethogram of the behaviour of the young mice in their home cage

Behaviour	**Description of behaviour**
Deviate behaviour	Stereotypic behaviour such as gnawing on bar or jumping on the walls to get out.
Aggressive interactions	Kicking, biting or chasing other mice.

Nest scoring

Nest scoring was measured during a 5 week period. In each cage 16 ± 0.2 gram of nesting material was located in the rear end of the cage and replaced once a week. During first week the nests were scored at day 1 after cage change as well as day 2,3,4 and 7. Then the nests were scored two times per week, 3 days after cage change and again on day 7, just before cage change, according to the presence of walls and height of the nest and the similarity of a cup or a bowl in shape. The scores went from 0 to 5 where a higher score indicates a better built nest and a lower score means less complete nest (Table 5). Recordings were performed by looking through the cage wall or taking the lid off.

Table 5. Score system for nest building (modified from Hess et al., 2008)

Score	Description of nest
0	Undisturbed: Nesting material has not been moved, touched or affected.
1	Disturbed: Nesting material has been moved, touched or affected (for example chewed or spread around). No clear sign of built nest, however nest material can be gathered with a hole in the bedding material.
2	Flat nest: Nesting material with a cavity in the middle. Absence of or incomplete walls.
3	Cup shaped: Nesting material shaping a cup or bowl with a cavity in the middle as well as small walls surrounding it. The wall is not high enough to reach the widest point of a globe.
4	Incomplete nest: Nesting material with a cavity as well as walls so high that they reach the widest point on a globe and that they almost meet again.
5	Complete nest: Nesting material with complete walls shaping a globe/dome with an exit hole at the top or on the side of the nest.

Statistical analysis

Results from the 9 cages for each strain in each cage system have been put together regardless of earlier cage system and sex to be able to get groups of sufficient sizes for the statistics. Data were tested using General Linear model (GLM) comparing the three different housing systems in food and water intake, weight gain and nest scoring. Differences were regarded as significant at a level of $P<0,05$ and results are presented as means ± standard deviation (SD). Nest location was summarized as percentage of the total number of observations during the experimental period. Results from behavioural observations were summarized as total number of observations per week.

Two cages (ArTec1 and ArAr2) with C57BL/6NCrl mice only had three animals from start because there were too few mice in the litters after weaning. The same problem for NMRI nude mice resulted in three nude mice and one NMRI nude with fur in each cage.

Two males (ArTec5 and AllAll5) and one female (TecTec5), all NMRI nude, were excluded after 5 days, 5 weeks respectively 2 weeks. Thus, their individual results are not included in the results.

Results

Body weight

Differences were found in body weight for NMRI nude mice during week 3 ($P<0.05$), week 4 ($P<0.05$) and week 5($P<0.01$). They had a significantly higher body weight gain in the Arrowmight system compared to the Allentown system (figure 2). No differences were found in body weight for C57BL/6NCrl mice between the three cage systems (Figure 3).

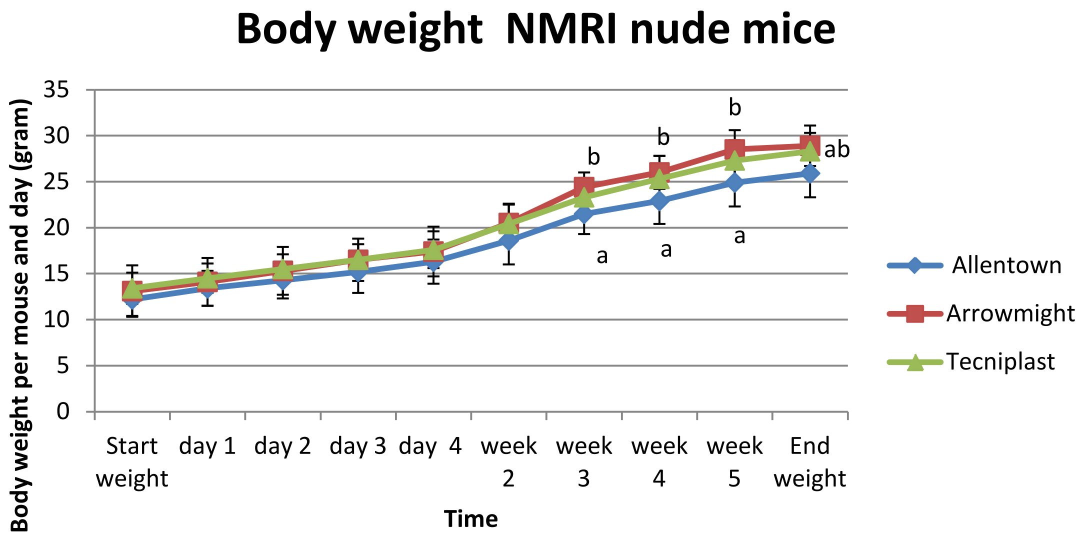

Figure 2. Mean body weight (± SD) for NMRI nude mice during 5 weeks. Significant differences between cage systems are designated by different letters.

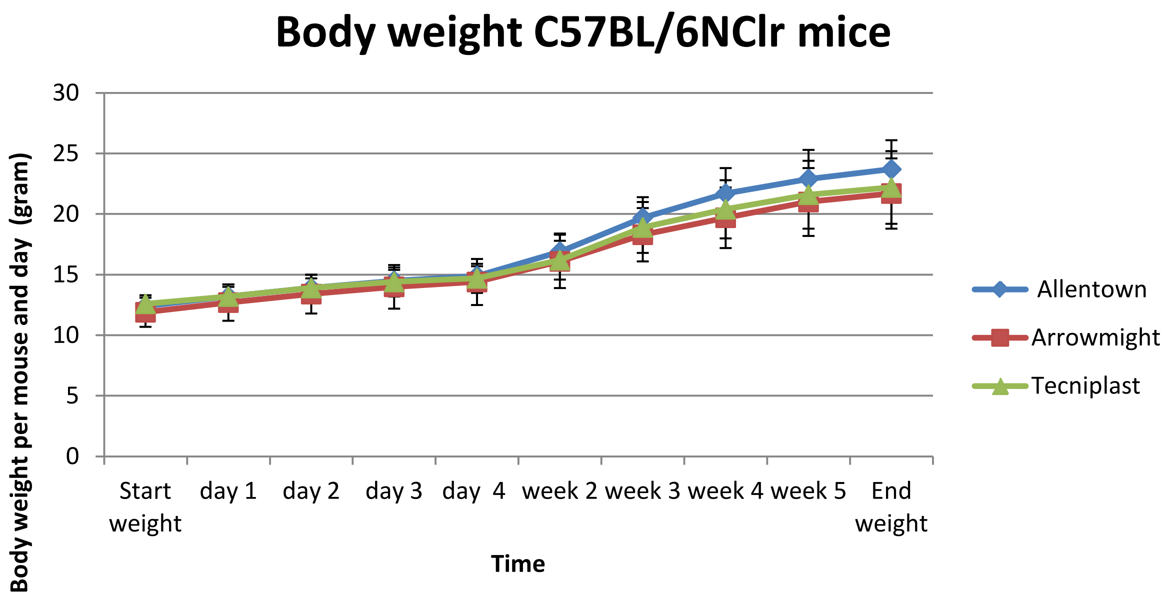

Figure 3. Mean body weight (± SD) for C57BL/6NCrl mice during 5 weeks.

Food and water intake

No significant differences were found in food (table 7) or water intake (table 8) within strain between the three different IVC systems during the whole period of the experiment. Note that there is a difference in feed and water consumption between the two strains as NMRI nude mice are drinking 19 % more as well as eating 34 % more than C57BL/6NClr mice estimated as mean value for all 5 weeks.

Table 7. Mean values (±SD) of feed consumption in gram per mouse and day

	week 1	**week 2**	**week 3**	**week 4**	**week 5**
NMRI nude	**Mean ±stdev (g)**	**Mean ±stdev (g)**	**Mean ±stdev (g)**	**Mean ±stdev (g)**	**Mean ±stdev (g)**
Allentown	3.7±0.6	4.4±0.7	4.5±0.7	4.7±0.7	4.9±0.6
Arrowmight	4.0±0.4	4.7±0.5	4.9±0.6	5.0±0.6	4.9±0.7
Tecniplast	3.9±0.4	4.7±0.5	4.9±0.6	5.3±0.6	5.1±0.4
C57BL/6NCrl					
Allentown	2.7±0.2	3.1±0.3	3.3±0.4	3.3±0.4	3.4±0.5
Arrowmight	2.7±0.3	3.0±0.3	3.2±0.3	3.2±0.4	3.1±1.5
Tecniplast	2.6±0.6	3.0±0.4	3.2±0.4	3.3±0.5	3.0±0.6

Table 8. Mean values (±SD) of water intake in gram per mouse and day

	week 1	**week 2**	**week 3**	**week 4**	**week 5**
NMRI nude	**Mean ±stdev (g)**	**Mean ±stdev (g)**	**Mean ±stdev (g)**	**Mean ±stdev (g)**	**Mean ±stdev (g)**
Allentown	4.5±0.7	5.1±0.9	4.8±1.1	5.3±0.8	5.7±0.8
Arrowmight	4.8±0.4	5.4±0.8	5.3±0.8	5.5±0.8	5.2±2.2
Tecniplast	4.9±0.6	5.5±0.8	5.8±0.9	6.2±0.8	6.3±0.6
C57BL/6NCrl					
Allentown	3.7±0.4	4.2±0.7	4.2±0.8	4.6±0.9	4.8±0.9
Arrowmight	4.2±1.1	4.1±0.5	4.3±0.6	4.5±0.7	4.7±0.8
Tecniplast	3.5±0.4	4.1±0.7	4.4±0.9	4.7±0.9	4.7±0.9

Behavioural observations

During 5 weeks, behavioural observations was recorded for 20 days (4 days a week) with cages observations 3 times a day during week 1, and 2 times a day during week 2 to week 5. With 9 cages/strain in each system the total number of observation week one was 108 (9 cages *3 times a day*4 days) and 72 observations each week rest of the 4 weeks respectively (9 cages* 2 times a day * 4 days).
Results show that the number of observations of both aggressive interactions and deviate behavior are higher for mice in Tecniplast and Arrowmight comparing to Allentown for both C57BL/6NClr and NMRI nude mice (Figure 4 and 5). Highest number of observations of aggressive interactions was observed in Tecniplast for NMRI nude mice (Figure 4) and highest number of observations of deviate behaviour such as gnawing on bar or jumping on the walls to get out was seen in Arrowmight for NMRI nude mice (Figure 4).

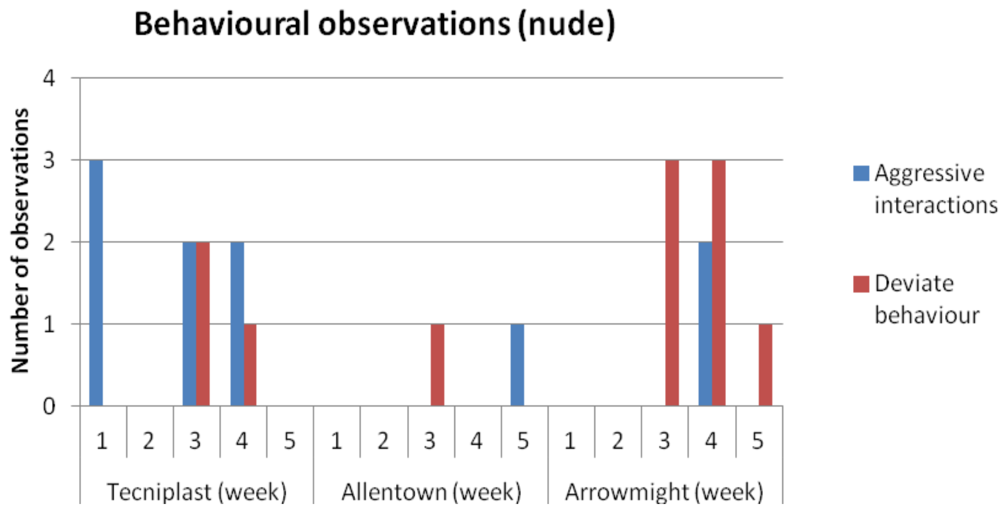

Figure 4. Total number of behavioural observations of NMRI nude mice during 5 weeks.

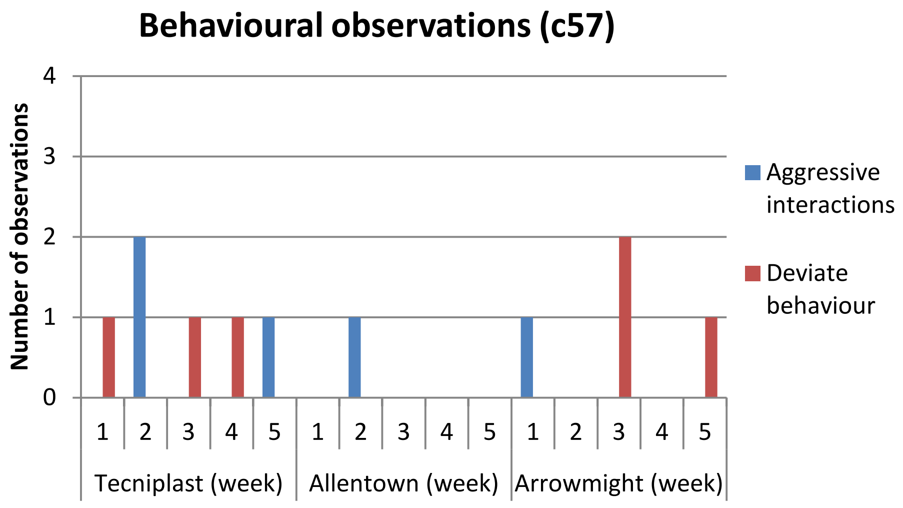

Figure 5. Total number of behavioural observations of C57BL/6NCrl mice during 5 weeks.

Nest scoring

In this study, the results imply that type of cage system affected the nest quality. Significant differences ($p < 0.05$) were observed during day 2 ($p < 0.05$) and day 3 ($p < 0.001$) and day 7($p < 0.05$) (week 1) with a higher score for Arrowmight and Tecniplast compared to Allentown, and day 7 (week 4) with a higher score for Allentown and Arrowmight compared to Tecniplast for C57BL/6NCrl mice (figure 6). Significant differences were also found day 7 ($p < 0.05$) during week 1 and day 3($p < 0.01$) during week 3 with highest score for Arrowmight system for NMRI nude mice (figure 7). With 9 cages from each system, the numbers of observations for each point in the diagram is 9 observations.

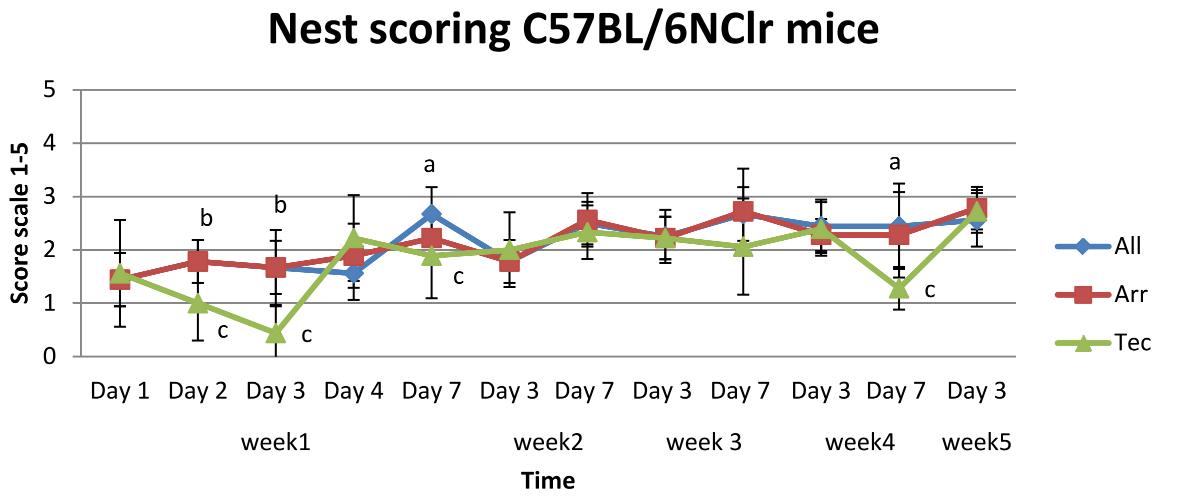

Figure 6. Average nest scoring (±SD) for C57BL/6NClr mice in all three IVC systems (n = 18).

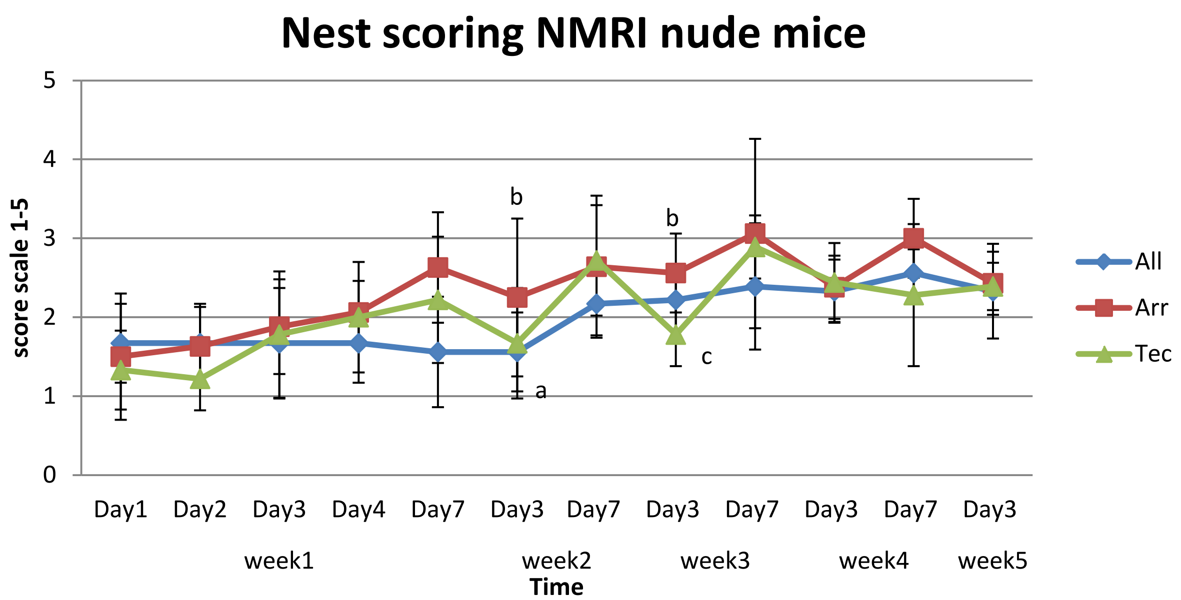

Figure 7. Average nest scoring (±SD) for NMRI nude mice in all three IVC systems (n=18).

Nest location

The results for NMRI nude mice show that the lowest numbers of observations were found in the front and in the middle of the cages for all three systems with 0 up to 18 % observations (Figure 8) and 0 up to 28 % observations respectively (Figure 9). Cages named: ArTec, TecTec, AllTec = mice origin from Tecniplast. ArAr,TecAr, AllAr = mice origin from Arrowmight and ArAll, TecAll, AllAll = mice orgin from Allentown

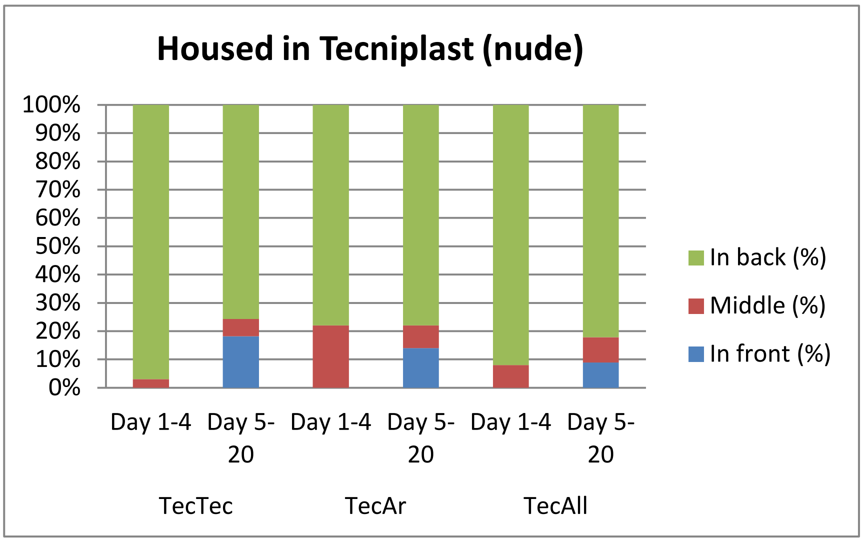

Figure 8. Nest location for NMRI nude mice with 36 observations day 1-4 and 96 observations Day 5-20 during a 5 week period.

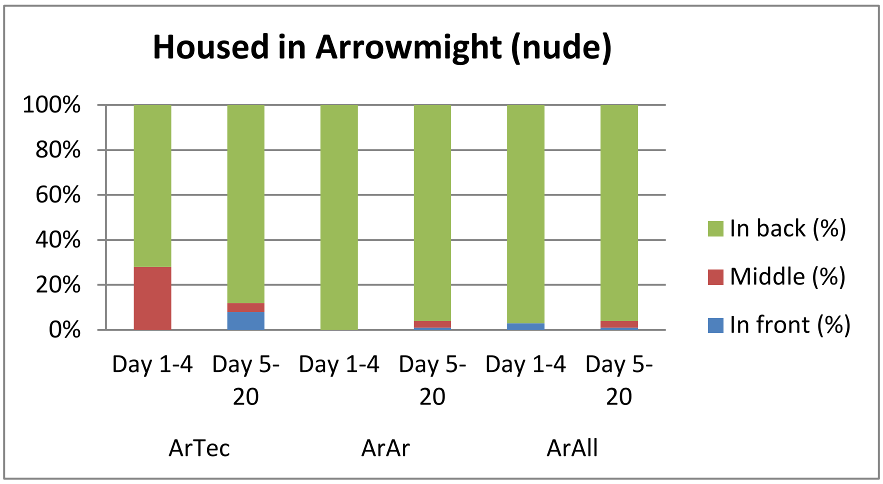

Figure 9. Nest location for NMRI nude mice with 36 observations day 1-4 and 96 observations Day 5-20 during a 5 week period.

For C57BL/6NCrl mice the number of observation for place of nest varied during the whole period with nest location in the back ranging from 81 to 100% in Tecniplast (Figure 10), 39 to 83% in Arrowmight (Figure 11), and 24 to 92 % in Allentown (Figure 12). Thus, the results for mice in Allentown diverge from Arrowmight and Tecniplast with as low as

24 % of the observations in the back. Cages named: ArTec, AllTec, TecTec = mice origin from Tecniplast. ArAr, AllAr, TecAr = mice origin from Arrowmight and ArAll, AllAll, TecAll = mice origin from Allentown .

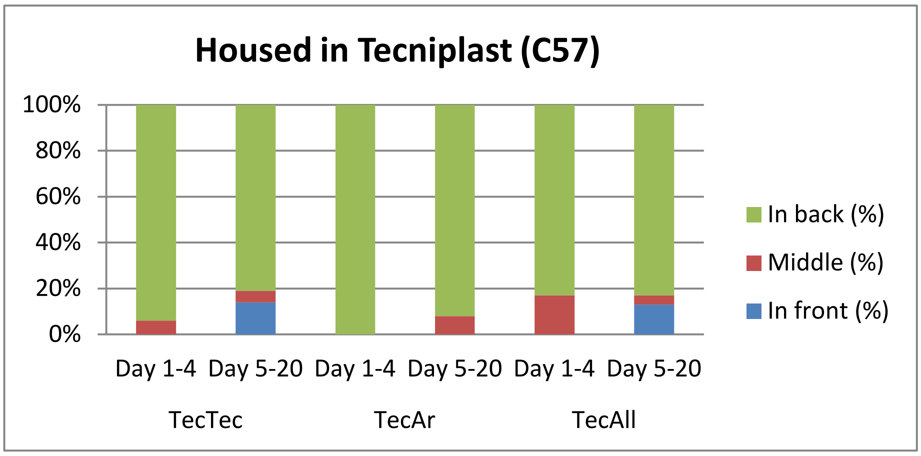

Figure 10. Nest location for C57BL/6NClr mice with 36 observations day 1-4 and 96 observations Day 5-20 during a 5 week period.

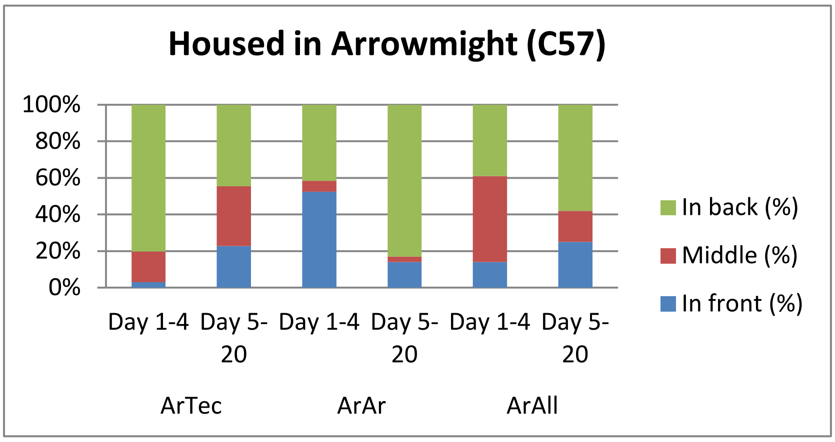

Figure 11. Nest location for C57BL/6NClr mice with 36 observations day 1-4 and 96 observations Day 5-20 during a 5 week period.

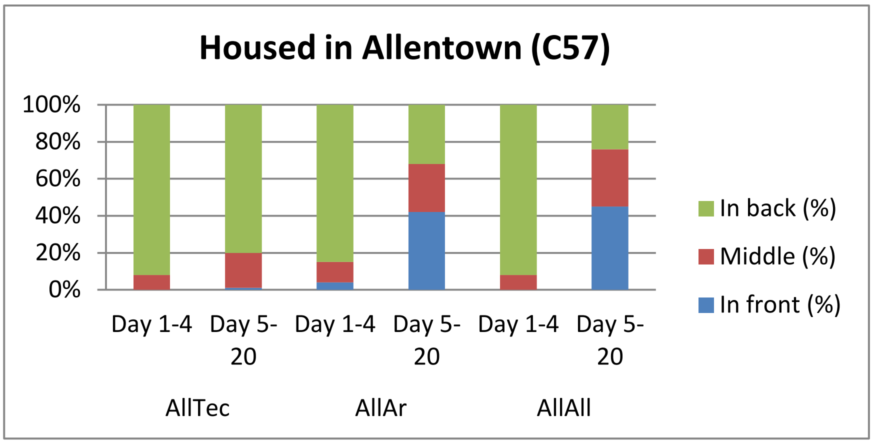

Figure 12. Nest location for C57BL/6NClr mice with 36 observations day 1-4 and 96 observations Day 5-20 during a 5 week period.

Discussion

There are many ways to evaluate the animal welfare and housing conditions for mice in IVC systems with studies for example on location of nest box, concentration of ammonia and carbon dioxide or the effect of the cage change frequency (Kostomoitsopoulos et al., 2007; Silverman et al., 2008; Reeb et al., 2001). Some studies focus on psychological aspects such as behavioural observations and effects of IVC housing on results in behavioural tests (Kallnik et al., 2007; Ennaceur et al., 2006; Mineur & Crusio., 2009) whereas other studies focus on physiological measurement such as food and water consumption or body weight (Memarzadeh et al., 2004; Höglund & Renström., 2001)
With focus on growth and nest building in the present study the results show no significant differences between the three cage systems in terms of feed consumption and water intake. The results are consistent with those of Memarzadeh et al. (2004) who also was unable to detect significant differences in feed consumption comparing cages with different mechanically ventilation designs.
The fact that food and water consumption diverge between NMRI nude mice and C57BL/6NCrl mice is due to the genetic differences were NMRI nude mice have no fur and therefore need more food and water to uphold the body functions, to grow and maintain the thermoregulation at the same level as C57BL/6NCrl mice (Sjaastad et al., 2003) when both strains are living in an environment with the same temperature and air humidity.
In addition, this study suggests that type of cage system could have an effect on body weight gain for NMRI nude mice where mice in the Allentown system had lower body weight gain. One possible reason could be the different cage size (In this study measured with no bedding material in the cages). Tecniplast have a floor area of 512 cm^2 and a cubic area of 8192 cm^2, comparing to Allentown with 560 cm^2 and 9800 cm^2 and Arrowmight with 558 cm^2 and 12834 cm^2 respectively. Significant differences in weight gain are similar to the results obtained by Höglund & Renström (2001) in their study of evaluating two different IVC systems in cage environment and animal health aspects. In addition, they suggest that larger individual space increase dominant behaviour and could have an effect on weight gain or weight loss. In this study the floor area is bigger in Allentown compared to Tecniplast but when it comes to the cubic area it is much larger in Arrowmight. Since mice are able to climb and thereby not only confined to the floor area, thus, the dominant behaviour should occur more common in Arrowmight because of the bigger total area to

use for the mice. As the study of Höglund & Renström (2001), imply it would mean that lower body weight should occur in the Arrowmight system and not in the Allentown system which appeared in this study.
Another difference when looking at the layout of the three cage systems is the place of supply and exhaust air which are both placed in the lid of Arrowmight and Tecniplast whereas in Allentown the supply air vent is placed in the lower back end of the cage and exhaust air in the top lid. Studies shows that human perceive discomfort when air speed at 0.2 m/s occurs and thereby experience it as draught (Lipman et al., 1999). Implying the same for mice it could have a relevant influence on the body weight depending on place and air flow for the ventilation system. As for all cages with ventilation systems, an increasing airflow may affect the internal temperature and cause draughts (Batchelor et al., 1998), which could affect the body weight gain for mice in cages were air intake is placed at the floor level.
The fact that the results in this study showed a difference in body weight gain for NMRI nude mice and not for C57BL/6NCrl mice could be the reason of nude mice being more sensitive to a change in the internal temperature in the cages. As mentioned earlier, the supply air vent is placed in the lower back in Allentown. This IVC system also had lowest results in body weight gain. Perhaps the mice experience it as draughty by the food hopper when trying to eat. By upholding the body functions and maintain the thermoregulation at the same level as mice with fur, it will then affect the body weight gain. In addition, the shape of the cages in the Arrowmight system is different from the cages in the other two systems. The design of cages in Arrowmight has a valve configuration which does not result in a linear air speed for the supply and exhausted air through the cages as the other cage configurations. Could then cages in Arrowmight perhaps be experienced as more draughty for the mice due to the different configuration when creating a dissimilar airstream? Perhaps the results from the behavioural observations where deviating behaviour was more common in Arrowmight for NMRI nude mice is an effect of the shape of the cage.

Results in this study show that there is a contradiction between feed consumption and body weight gain as the feed consumption is similar between the three cage systems but the body weight gain show a significant difference for NMRI nude mice. Once again, the results from the behavioural observations show that NMRI nude mice in both Arrowmight and Tecniplast had a higher number of observations of aggressive interactions as well as deviate behavior. Thereby these results cannot explain the fact that mice in Allentown for NMRI nude mice had lowest results in body weight which suggest that other behavioural observations such grooming, eating, drinking or time spent on building nest could be of interest to see if there is any difference between the three IVC-systems.

As earlier mentioned this study also focused on nest quality and nest location to see if there were any differences between the three cage systems. As the nesting material was placed in the back of the cages at cage change the results show that the mice spent less time trying to move the nest to another area in the cage. For NMRI nude mice the nest location showed a more equal result reaching from 72 up to 100% of the observations in the back for all three systems. Nest location for C57BL/6NCrl mice seemed to be most common in the back of the cages for Tecniplast with 81 up to 100 %. In Arrowmight the observations in front were as high as 53 %. The fact that nest location were more common in front in Arrowmigt could be explained by looking at the layout of the cages in the IVC systems as the food-hopper was located in the back in Allentown and Tecniplast and in the middle for Arrowmight which could indicate that the location of the food-hopper affects the choice of place for the nest. This theory is similar to the results observed for Nevison et al. (1999) in the effect of nest location for mice showing that most mice pulled the nest material under the food-hopper. The results for C57BL/6NCrl mice in Allentown diverge from Arrowmight and Tecniplast with as low as 24 % of the observations in the back of the cages. Place of air intake at floor level perhaps not only have an effect on body weight gain as mentioned earlier in this discussion. Perhaps it also have an effect on the place of nest as an increasing airflow may cause draught (Batchelor et al., 1998), thus, by preventing it the mice move the nest.

When it comes to nest building the results presents a difference, at four occasions, in nest quality between the three IVC systems were the nest quality for mice in the Tecniplast system were poorer for the C57BL/6NCrl strain. The variation in nest quality found in cages for NMRI nude mice appeared at two occasions and differed between the cage systems and is thereby not specified to one system. Overall, cages with NMRI nude mice had higher nest scoring rate. There are studies showing a relationship between environmental enrichment and increasing aggression (Nevision et al., 1999). Since C57BL/6NCrl mice are one of those genetic lines with higher levels of aggression (Parmigiani et al., 1999), thus, they are more easily influenced to increase their aggressive behaviour when having access to nesting material as environmental enrichment. However, the results from behavioural observations in this study show the opposite with less number of observations of aggressive interactions for C57BL/6NCrl mice compared to NMRI nude mice. When comparison was made between the three cage systems in this study, the sexes were mixed in the results, but were not further evaluated if it could have an impact on the results.

A non significant result were found between sexes in a strain in the choice of different nesting material and therefore both sexes can be analyzed together without affecting the results according to Van de Weerd et al. (1997).

Although differences were found in this study with a lower body weight gain in Allentown for NMRI nude mice as well as differences between cage systems in nest quality the results from the present study are not enough to recommend or discard any of the tested systems. But together with recordings of other relevant parameters, based on animal welfare, they could have substantial influence on the choice of IVC system. The more results that can be collected from different parameters, the more improved the choice of IVC system can be. Other relevant parameters to study could be the effect of technical aids when handling animals as well as cages at e.g. cage cleaning, or what type of sound and light the animals are exposed to in the cages since they for example can hear ultrasounds. Further, the appropriate group size in IVC cages and what type of enrichment item to use are also important aspects to consider since the mice naturally live in groups in a varying environment.

Conclusion

The results show that nest quality and body weight gain was affected but is not enough to exclude or favor for one of the three IVC systems in this particular study. As a suggestion, further studies with perhaps more measurements on individual level as well as internal- and external cage environment aspects can improve the results in this study and further improve the development of IVC systems and animal welfare.

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Faculty of Veterinary Medicine and Animal Science
Department of Animal Environment and Health
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SE-532 23 Skara, Sweden
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