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Comparative Cage Space Requirement: Cage Enrichment and Beddings of Small Laboratory Animals

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Abstract: Now a day, there is an increasing demand for high standard animal models that has led to focusing on the animal welfare. The design and choice of material for caging, enrichment and bedding can be affected by a number of factors such as social contact (i.e. degree of transparency, smell and noise), heat and noise conduction. Therefore, when choosing certain materials (e.g. wire mesh or solid flooring) other issues such as which type of ventilation or room temperature has to be carefully considered. Materials of the cage should not be harmful to the health and welfare of the animals. On the other hand, next to the amount and type of bedding material, other factors such as ventilation, relative humidity and temperature also affect the ammonia levels. Some of the small laboratory animals considered in this review are mice, rats, guinean pigs, rabbits and sheep and goats. Depending on the species, appropriate caging, enrichment, bedding and nesting material and/or sleeping structures should be provided. In addition, a solid clean and comfortable resting area should always be present for the animals in the enclosure.

Key words: Laboratory Animals · Cage Space · Enrichment · Bedding

INTRODUCTION

Cages are made of different materials that could not be harmful to the health and welfare of the animals. If careful attention is not given to design and construction of the enclosure, laboratory animals will be affected from different injuries [1]. The enclosure should be designed in such a way that each animal has sufficient space to express a wide behavioural repertoire [2, 3]. Lack of sufficient space and high stocking densities can possibly lead to endocrine stress reaction and high frequencies of aggression depending on the species [4]. Social contact like degree of transparency, smell, noise, heat and conduction can be affected by number of factors such as design and choice of material. Therefore, when choosing certain materials (e.g. wire mesh or solid flooring) other issues such as which type of ventilation or room temperature has to be carefully considered [4]. The enclosures should be able to withstand thorough cleaning and decontamination. Regular and efficient cleaning schedules for both the rooms and enclosures are necessary. The enclosure floors should be appropriate to the age and species of the animal and should enable caretakers to remove faeces [2, 3].

Hence bedding material influences the micro-environment, appropriate bedding and nesting material and/or sleeping structures should be provided according to the species difference under considerations [5]. In addition, a solid clean and comfortable resting area should always be present for the animals in the enclosure. In case of breeding animals, nesting material or structures should be provided [3]. Providing bedding material will absorb and contain urine and faeces [6]. It also provides a comfortable resting surface and enables several behaviours of rodents such as digging, chewing, nesting and hiding. Using different types of bedding in mice

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revealed differences in ammonia levels [5]. On the other hand, next to the amount and type of bedding material, other factors such as ventilation, relative humidity and temperature also affect the ammonia levels [7, 8].

European, American and Australian legislation incorporated to world helps in the use of environmental enrichment to improve the well-being of laboratory animals [2, 3]. Environmental enrichments are used to provide a more complex environment which enhances species-specific behaviour, promoting physical health and decreases abnormal behaviours as much as possible [6, 9]. Environments that are not up to standards and fail to meet the animals' needs may result in the development of abnormal behaviour, disorders, physiologic dysfunction and abnormal brain development [8]. More importantly, animals showing these types of changes will disrupt the validity, reliability and reproducibility of the research [10]. For such reasons it is important to enrich the primary animal enclosure to ensure proper scientific research standards and the welfare of the laboratory animals [10]. Barren environments should be avoided at all times except when experimental set-ups require it differently and compelling scientific evidence supports this. Furthermore, enrichment is especially important for single housed animals or aggressive males [2]. Therefore, this paper aims to review the comparative cage space, enrichment and bedding requirements of mice, rats, Guinean pigs, rabbits and sheep and goats.

MICE

Cage and Space Requirements: Providing mice with socially housing and nesting material enables them to create nests with temperatures around 30-32°C [11]. However, under special circumstances it is advisable to increase cage temperature when they are housed individually in metabolic cages or when they are recovering from general anaesthesia [12, 11]. Providing heating mats after surgery and placing half of the cages on the mat ensures that mice can move their nest away from the heat during recovery [12, 11].

Mouse rooms usually have a 12-hour light-dark cycle with lights off during the night and light intensity during the day as high as 300 lux. Light has damaging effects on the retina, but can be particularly damaging in nocturnal species such as mice and is even more detrimental in albino strains, which lack pigment protection [13, 14]. Therefore, it is of utmost importance that light intensity be kept to a minimum. In general, for albino mice, light intensity should not exceed 60 lux at the cage level. This can be achieved by covering the highest cage shelves and by providing mice with structures, such as nesting material, that enable them to shelter from bright light [13, 14].

Ventilating cages with a rate up to 120 air changes per hour can reducing the need for frequent cage cleaning. However, health monitoring and inspection of the animals may be difficult procedures and cage cleaning might be more time-consuming, that the high intra-cage ventilation rate could induce chronic stress and heat loss due to the draft [15,16].

Mouse rooms are a constant source of sounds emitted by the mice themselves, animal technicians, caretakers and equipment. Some of these sounds, such as equipment producing ultrasound, or sudden noises, such as doors and cages opening or closing, may be a source of stress for the animals. Chronic and/or loud noises may induce impaired behaviour, cognition and immune function in mice [13, 14, 17].

Optimized and appropriate cage environment structuring is typically more beneficial than provision of a larger floor area; however, a minimum floor area is necessary to provide a structured space [18]. This enables mice to use the vertical cage dimension as well. It is difficult to scientifically specify the minimal sizes of cages for maintaining laboratory mice, as much depends on the strain, group size and age of the animals, their familiarity with each other and their reproductive condition [19]. In terms of structure, the home cage can be furnished with, for example, nest boxes, tubes, partitions and nesting material [6].

However, provision of environmental refinement should not be a process of randomly applying objects that staff consider attractive for the animals; instead, environmental refinement should be regarded as an essential component of the overall animal care program and equally important as nutrition and veterinary care [20].

Mice are highly susceptible to predation and are likely to show strong fear responses in unfamiliar situations if they cannot shelter. These responses include attempts to flee, biting when handled, or sudden immobility to avoid being detected. For this reason, cages should be provided with a shelter or hiding places. Security can be achieved via manipulable nesting material, hiding places and compatible cage mates. Even simple environmental refinement induces a robust and replicable anxiolytic-like effect in mice [20]. Moreover, providing nesting material helps mice keep their nests clean, thus always providing them with a faeces-free resting area [21, 22].

Table 1: Range of minimum enclosure dimensions and space requirements of mice

Single or group housing or breeding animals	Body weight (g)	Floor area per animal (cm^2)	Enclosure height (cm)	Minimum cage size (cm^2)
Single	Any	200	12-15	
Group	<10	38.7- 65	12-15	330
	10 - 20	60 - 77.4		
	20 - 30	60 - 100		
	30 - 40	70 - 100		
	>40	96.7 - 100		
Breeding	Pair	300 - 330	12-15	330
	Extra females + litter	For each additional female + litter 150-180 shall be added.		
Post-weaned stock at breeders (950).	<20	40	12	950
Post-weaned stock at breeders (1500)	<20	30	12	1500

Source: [1, 2, 8, 26]

The effects of space availability on the welfare of mice are not consistent. In general, aggression between male mice seems to decrease with increased crowding; however, other studies indicate that crowding increases stress-related parameters [23]. Some factors, however, can be managed by good husbandry practices, including housing mice in small, socially stable groups of three males, transferring nesting material, but not dirty bedding, during cage cleaning and avoiding exposure of male mice to (Unfamiliar) male urine [24,25].

Mice must be able to stand upright on hind legs and if possible be able to look outside of the enclosure, especially when they are single housed [2]. The enclosure height is the vertical distance between the floor and top of the enclosure. The space requirements should be maintained at all times, exceptions can be made for short-term housing not more than a day. Long-term procedures or experiments should take potential growth of the animals into consideration regarding proper space requirements [3, 8]. Under the circumstances that the enclosures are larger (950 to 1500 cm^2) and adequate enrichment is provided, post weaned mice can be kept under higher densities with smaller floor area per animal but only for a limited period of time [1]. The welfare of the animals cannot be compromised and aggressive behaviour, abnormal behaviour, health and physiological or behavioural stress responses should be monitored [1, 8]. Requirements were based on the European Directive Appendix III [1, 2, 8].

Floors: Solid floors or perforated floors, preferably not wire mesh or grid floors, should be used when housing mice. If wire mesh or grid floors are used than a solid (e.g. solid mat) or bedded area for the animals to rest should be provided unless the experimental specifications require it differently. Furthermore, these types of flooring can lead to serious injuries [8].

Bedding and Nesting Material: In order to provide a living environment where mice can express certain natural behaviours such as huddling, hiding, resting and breeding, bedding and nesting material should be provided to the animals regardless whether they are in stock, breeding or under procedures [27]. In addition, cleaning and sanitation are facilitated by the absorption of feces and urine. To date wood shavings or wood chips, paper or other materials have been used as bedding materials for laboratory mice. The characteristics of good bedding material will enable the mice to easily hide, build nests and should be soft. Large paper fibers fulfill these requirements and were preferred by mice out of four types of bedding [28]. Cloth bedding is proposed [29] as the alternative for paper and wood chip bedding as concerns arise about protecting the environment and natural resources and it is reusable. Furthermore, the cloth bedding is preferred by the mice over paper and wood chip bedding [29].

Enrichment: Next to bedding material, providing nesting material is an important enrichment to ensure the mice well-being. In a preference test it was shown that given the choice, mice preferred tissue nesting material on grid floor which has been avoided before over an enclosure which contained saw dust bedding material with a metal nest box which displays the importance of nesting material with regards to the animal's welfare and comfort [30]. Regarding the type of nesting material, paper derived materials such as tissues, towels and paper stripes were preferred over wood-derived material. Paper towelling was eagerly used by non-breeding female mice as nesting material which can be an inexpensive way to provide nesting material and improve the well-being of mice [31].

Besides nesting material, mice also benefit from enrichment which enables foraging, exploring and social behaviours. Therefore, providing wood sticks, cardboard,

Table 2: Evolution of cage space requirements for rats

Version of the Guide	Number/weight of rats (g)	Housing area per animal (cm^2)	Height (cm)
1963	1-3/250	185.8-650.3/animal	20.3
	4-10/250	185.8-464.5/animal	
1972 and 1974	Up to 100	110	17.8
	100-200	148	
	201-300	187	
	Over 300	258	
1978 and 1980	<100	110	17.8
	100-200	148	
	201-300	187	
	>300	258	
1985 and 1996	<100	109.68	17.8
	100-200	148.40	
	200-300	187.11	
	300-400	258.08	
	400-500	387.12	
	>500	451.64	
2011	<100	109.6	17.8
	100-200	148.35	
	201-300	187.05	
	301-400	258.0	
	401-500	387.0	
	>501	>451.5	
	Mother and litter	800	

Source: [8]

plastic tubes and opportunities to find food (e.g. hard shelled nuts, sunflower and/or sesame seeds) and social interaction will improve the welfare of the animal [9]. The use of the exercise-wheel has to be carefully monitored and perhaps provided only a limited amount of time each day as running in the wheel can sometimes develop into maladaptive behaviour or stereotypic activity. It has been shown with rats that unlimited access to a running-wheel can lead to such an intense use that substantial decrease in body weight occurs. When dealing with individually housed or aggressive mice, providing an exercise-wheel should be considered [2].

Rats

Cage and Space Requirements: Rats must be able to stand upright on hind legs and if possible be able to look outside of the enclosure, especially when single housed [2]. The enclosure height is the vertical distance between the floor and top of the enclosure. The space requirements should be maintained at all times, exceptions can be made for short-term housing (i.e. no more than a day). Long-term procedures or experiments should take potential growth of the animals into consideration regarding proper space requirements [3, 8]. Under the circumstances that the enclosures are larger (1500 to 2500 cm^2) and adequate enrichment is provided, post-weaned rats can be kept under higher densities with smaller floor area per animal but only for a limited period of time. The welfare of the animals cannot be compromised and aggressive behaviours, abnormal behaviours, health and physiological or behavioural stress responses should be monitored [1, 8]. The size of the basic home cage recommended differs slightly between the US and the EU and has changed through time through versions of the guide for the care and use of laboratory animals [8].

Although rats can successfully reproduce in much smaller cages than recommended [32] this may be related to the fact that they are domesticated animals who have been selected for successful reproduction under various conditions and stressors. Rats show a strong preference for a larger cage, but desires for space are subservient to the desire to have conspecifics present [33]. Juvenile rats exhibit a great deal of active play behaviour and benefit from access to more space [34]. Basic caging should also be sized so the rat has the ability to express all natural postures. Cages are often too short for rats to fully extend vertically. Cages with solid bottoms are preferred to those with wire floors [35] although wire floors with resting platforms are seemingly well tolerated. When preference testing was used to determine the strength of the preference for solid bottomed floors, results were

Table 3: Cage space recommendations for rats

	Body weight (g)	Minimum enclosure size (cm2)	Floor area per animal (cm2)	Number of animals that can be housed in minimum enclosure	Minimum enclosure height (cm)
In stock and during procedures	≤200	800	200	4	18
	≥201to 300	800	250	3	
	≥301to 400	800	350	2	
	≥401to 600	800	450	1	
	≥601	1,500	600	2	
Breeding		800 Mother and litter. For each additional adult animal permanently added to the enclosure, add 400 cm2			18
Stock at breeders in 1,500 cm2 cages	≤50	1,500	100	15	18
	≥51 to 100	1,500	125	12	
	≥101to 150	1,500	150	10	
	≥151to 200	1,500	175	8	
Stock at breeders in 2,500 cm2 cage	≥100	2,500	100	25	18
	≥101to 150	2,500	125	20	
	≥151to 200	2,500	150	16	

Source [37]

inconclusive, as animals would work just as hard to access space to explore as to access solid-bottomed caging for resting. However, when animals were not asked to work for access, a clear preference for solid-bottomed caging readily emerged [35]. Rats exhibit signs of stress in cages with wire floors, whether large or small, if no enrichment is provided [36]. One reason rats are still housed on wire-bottomed cages is to prevent Coprophagy from interfering with certain types of scientific endeavour. Coprophagy may occur both through ingestion of feces found on the cage floor as well as directly from the anus, so the utility of wire-bottomed cages in preventing all Coprophagy is questionable. Transitions from solid-bottomed to wire bottomed cages are likely to stress rats. Metabolism cages, with their wire floors, lack of cover and social isolation are likely very stressful for rats [37].

Floors: Similar requirements with regards to the flooring for mice apply to the flooring for rat enclosures. Solid floors or perforated floors, preferably not wire mesh or grid floors, should be used when housing rats. Particularly during rest, rats prefer solid floors over wired ones. If wire mesh or grid floors are used than a solid (e.g. solid mat) or bedded area for the animals to rest should be provided unless the experimental specifications require it differently. Furthermore, these types of flooring can lead to serious injuries [8]. Therefore, if wire mesh or grid floors are chosen, thorough and regular inspection of the floor and animals are necessary.

Bedding and Nesting Material: Typical laboratory rat beddings include: wood shavings, wood chips, corncob processed to various diameters, cellulose and wood pulp. Rats prefer wood-based bedding with a larger particle size [38]. Corncob bedding has been shown to affect rats' physiology with changes in estrous cyclicity associated with corn's natural estrogenic compounds, as well as disruption of slow-wave sleep [39]. Being reared on corncob bedding has also been shown to reduce measures of anxiety in male rats [40]. Cellulose-based bedding is well tolerated by rats but does not provide the absorption of some other types of bedding [41].

As mentioned before in the 'bedding and nesting material' section on mice, providing proper bedding and nesting material is a crucial aspect to the welfare of the animal. Not only does it contribute to the expression of several natural behaviours of the rat, it also facilitates sanitation and cleaning of the enclosure [8] and influences the ammonia levels [5, 42]. Like mice, nest building is common for rats and observed in wild and pet rats when nesting material is provided [43]. However, there is a difference between mice and rats regarding the use of nesting material. When nesting material is provided to rats for the first time in their adult life, their initial response is to chew and eat it. Rats need to learn how to build nests from their mother and when they do, providing nesting material is a suitable type of environmental enrichment. Furthermore, the type of nesting material can help in avoiding chewing and eating of it, for example Enviro-dri (Paper-fibers) is eaten less

often than Kleenex tissues [43]. Next to nesting material, providing a nesting box should be considered a suitable and important enrichment for rats. With regards to bedding material, rats prefer the type of bedding on which they were raised except for corn-cob bedding [44].

Enrichment: Providing enrichment for rats can have beneficial effects on aggression in a group. Enriching standard cages of laboratory rats physically can help them to increase the control over their environments and to promote their species-specific behaviour [51]. Group housed rats in the enriched condition (Rope, ladder, crawl ball, shelter etc.) showed lower levels of agonistic behaviours compared to rats living in non-enriched cages [45]. Some means of enriching rat cages have become standard, such as providing rats with gnawing items made of nylon, wood, or plastic [46]. Although their incisors wear mainly on the surfaces, rats are motivated to gnaw and will chew objects placed in their cage for that purpose. They will also gnaw at shelters, food crocks, or other objects placed in their cages. Although rats rarely injure themselves on sharp edges they create, objects that have sharp edges from gnawing should be removed. Tubes, boxes and/or pipes will make the environment more complex and enable foraging and hiding opportunities. In addition, PVC pipes should be given to individually housed or aggressive rats [2]. In other words, enrichment entails objects or interactions that should be readily provided in a standard home cage. Standard enrichment more likely to benefit a greater number of rats overall [33, 45]. Providing pumpkin seeds can function as foraging enrichment as well [2].Wood sticks can also be used as enrichment for chewing and gnawing [4].

Other factors may need to be considered before implementing enrichments such as foraging or running wheels. Providing rats with a foraging enrichment (Food hidden under gravel in a metal dish) decreased aggression and allowed rats to perform species-specific feeding behaviours, but also increased rates of obesity [47]. Rat will spontaneously use running wheels if provided and the frequency of use and effects on the rat and research will differ by sex, strain and age [48]. Standard housing results in sedentary rats with poorer performance on tests of agility and strength than rats housed in large pens, running wheels may be one way to manage rats' metabolic abnormalities [48].

Cage cleaning affects rats by placing them in a new environment from which all pheromonal markers have been removed [49] provides a review of how lab procedures may disrupt pheromonal communication in rodents and schedules of cage cleaning are reliant on types of caging used, with frequencies varying from once every 2 weeks to three times per week for various types of solid-bottomed cages. An additional disruption is that this usually takes place during the day, when a nocturnal animal is resting [45].

Rat behaviour is disrupted for about an hour after cage change. This disruption may be related to novelty and handling rather than disruption of pheromones, as nonbreeding rats show no preference for scent-marked cages and changing the cage of a rat close to parturition or with new-born pups may result in cannibalism [41].

Guinea Pigs

Cage and Space Requirements: Originally from South America, guinea pigs are a diurnal crepuscular species, being active in early morning and evening with intermittent periods of rest, activity and nibbling of food during the day and night. Grass is the natural diet of guinea pigs. In the Andes, their natural habitat, they live in herds or small groups of 5 to 10 animals and exhibit a definitive social hierarchy with a dominant male and female. They are very alert for predators and frequently seek shelter in the burrows of other animals, as well as in crevices and tunnels formed by vegetation. Guinea pigs typically live an average of 4-5 years, but may live as long as 8 years [4].

Being prey animals, guinea pigs easily panic when an unfamiliar or unseen person comes into their room. Cages or pens with open sides of metal wire are recommended so that the animals have good visual contact with their environment. They will not panic when familiar caretakers enter their room, if they are able to see them. Theses enclosure types also provide the caretakers with easy observation of the animals and better ventilation. Being heavy rodents, weighing close to 1 kg, solid-bottom caging is highly preferred to help prevent pressure sores and pododermatitis that can develop if housed on wire bottom cages [4].

Adult guinea pigs measure up to approximately 30 cm in length and require at least 3 cm additional horizontal space to allow for free expression of the stretching posture. They are poor jumpers and diggers but greatly enjoy burrowing in hay. The hay provided should be soft to avoid eye injuries [4].

The guinea pig should be able to stand upright on hind legs. Preferably, the minimum height of 23 cm should be used as it enables juvenile guinea pigs in expressing playful behaviour (e.g. frisky hops) and adult guinea pigs

Table 4: Range of minimum enclosure dimensions and space requirements of guinea pigs

Single, group housing or breeding animals	Body weight (g)	Floor area per animal (cm^2)	Enclosure height (cm)	Minimum cage size (cm^2)
Single	<250	300 - 700	18 - 20	
	250 - 550	650 - 900	22 - 23	
	>550	650 - 1000	22 - 23	
Group	<200	200 - 500	17.8 - 23	1800
	200 - 350	387 - 500		1800
	350 - 550	450 - 800		1800
	550 - 700	600 - 800		2500
	>700	600 - 900		2500
Breeding	Female + litter	1200	23	2500
	Breeding pair + litter	2500	23	
	Extra female	For each additional female in harem add 1000	23	

Source: [1, 28, 26]

can fully stand on hind legs at this height In order for the animals to optimize the floor space provided, appropriate shelters should be placed within the enclosure. The space requirements should be maintained at all times, exceptions can be made for short-term housing [4].

When housing guinea pigs in large pens it is important to provide open sides or wire mesh sides, next to closed sides like opaque materials or metal, to enable the guinea pigs to see the people approaching but also provide hiding opportunities [50]. Sudden noises or unexpected movements could cause these animals to panic and stampede and possible injuries could arise [26, 50].

Even though it is thought that the use of floor pens are taking up to much space and are difficult to thoroughly clean, they are inexpensive, easy to construct and flexible. If cages are preferred than it is not recommended to use only transparent or translucent materials or wire mesh cages. Partial transparent or wire mesh sides of a cage can be used as this will allow the animals to see people approaching [50].Requirements are based on the different directive of international legislation [8, 26, 50].

Floors: Similar requirements with regards to flooring for mice and rats apply to the flooring for guinea pig enclosures. Solid floors or perforated floors, preferably not wire mesh or grid floors should be used when housing guinea pigs. Especially when housing breeding animals or for long-term purposes, wire floors are not suitable and solid floors are necessary [4]. If wire mesh or grid floors are used then at least a solid (e.g. solid mat) or bedded area for the animals to rest should be provided unless the experimental setup require it differently [4]. Furthermore, these types of flooring (i.e. grid and wire floors) can lead to serious injuries [8]. Therefore, if wire mesh or grid floors are chosen, thorough and regular inspection of the floor and animals are necessary. Also, advise on requirements of using wire mesh floors exist and are based on the weight of the animal. Guinea pigs fewer than 350 g require a mesh of approximately 10 mm (3/8 in) made of 10-12 gauge wire; larger animals need a 16 mm (5/8 in) mesh of 9-10 gauges [26].

Bedding and Nesting Material: With the exception of short-term experimental protocols, guinea pigs should be kept on solid-bottom caging with bedding. "When grid or perforated floors are used, a solid resting area must be provided" [1] that is large enough to allow all animals to lie on it simultaneously. To maintain a hygienic cage environment, bedding should be dust-free, seasoned soft wood and changed at least twice per week. Minimum environmental and feeding enrichment should include the bedding, a hide box and fresh, high-quality hay given daily [1].

When housing guinea pigs, wood shavings or chips are frequently used and satisfactory for bedding [4]. It is not recommended to use sawdust alone or any other small particle type of bedding due to the possibility of it adhering to the vulva, scrotum and prepuce which can cause irritation and obstruction resulting in reduction of fertility. A nest box or any other type of shelter or refuge is necessary to house guinea pigs, especially when females have to give birth [50]. Unlike rats and mice, guinea pigs do not burrow but may use burrows from other animals in the wild [4, 9].

Tubes, pipes like PVC or shelters have to be present in the cage or pen to allow the animals to climb onto or hide under them as they are easily frightened [9]. When using piping as refuge, these must be short enough

to prevent more than two guinea pigs inside the piping. Allowing more than two animals inside the pipe could cause the animals in the middle to be smothered and suffocated [4].

Enrichment: The most important enrichment for the guinea pig is the social group and social interaction, see 'social housing'. It is recommended to provide hay to satisfy the need for roughage and concealment [1, 9]. Providing enough hay or placing hay in hay racks positioned just above the floor, or giving Lucerne hay biscuits enables tunnelling and could function as temporary shelter or nest box if needed [50]. Furthermore, the presence of hay may help in preventing behaviour problems such as hair damage caused by barbering and will assist in maintaining tooth care. Hay must be renewed frequently though, dirty or mouldy hay is not beneficial for the welfare if the animals. With regards to chewing and gnawing, wood sticks, small softwood blocks and/or pop sticks can be used as enrichment [1, 4].

Rabbits: Despite the overall decline in the number of rabbits used for research over the last several decades, the number of publications citing rabbit models is steadily increasing. While rabbits are commonly used for polyclonal antibody production, they are also widely used in other research disciplines, including infectious disease, cancer and cardiovascular disease. Domestic breeds of rabbits (Including the New Zealand White (NZW), the most frequently used in research) were selectively bred from the European rabbit (*Oryctolagus cuniculus*). With the advent of the eighth edition of the Guide for the Care and Use of Laboratory Animals (Guide), many institutions are revisiting how they house and care for rabbits [8].

Cage and Space: As rabbits will stand on their hind legs to survey their surroundings, cages must be tall enough to easily accommodate this behaviour. This behaviour can be encouraged by hanging treats or toys from the top of the cage. While providing boxes and elevated platforms to climb on increases cage complexity and addresses the need to view the environment, it does not necessarily allow the rabbit to stand fully upright position [8].

Bedding: Bedding should be safe to eat, example, dust free, straw (Hay). Rabbits need regular access to suitable toileting places. If providing litter, trays use newspaper, hay/straw, shredded paper and/or paper based nonclimbing and non-expanding cat litter are very important [8].

Enrichment: As a prey species, wild rabbits create and then live in burrows; similarly, domestic rabbits will readily utilize hiding places. These can be complex structures built into cages or simple cardboard boxes or paper bags. Being able to hide or perch also provides a mechanism for coping when scared or stressed. Perching and hiding places are particularly important for socially housed rabbits, as they provide a means to create microenvironments and escape dominant animals. One important consideration for socially housed rabbits is the provision of multiple hiding spaces so as to avoid creating a resource that can be guarded [8]

Chewing and gnawing behaviours represent up to 20% of wild rabbit activity. For domesticated rabbits, chew toys that are not easily swallowed or cannot entrap the rabbit's head or appendages can encourage this natural behaviour. These can include untreated cardboard tubes or wood blocks; hard plastic dumbbells, Kong toys, balls, or rings; or stainless steel rattles, oversized chains, or rings. Some plastic baby toys, like oversized keys, can be repurposed for rabbit enrichment, wooden blocks or sticks, in particular, significantly increase locomotion and intake behaviour [8].

Sheep and Goat: Sheep (*Ovis aries*) have been domesticated for thousands of years. They are used in biomedical research in the United States in studies ranging from investigations of asthma and respiratory disease to development of novel cardiac interventions [20]. There are significant numbers of sheep currently used in laboratory and likely to increase in the future [52]. This review will discuss some characteristics of sheep that are important to consider in the research laboratory and will provide recommendations for refining research protocols involving sheep.

Cage and Space: Regulations regarding minimum cage sizes for sheep and goat have been promulgated, but animals must also be provided with adequate space for normal ambulation. Pen sizes should be large enough, or cleaned frequently enough, so that all members of the group may simultaneously lie in clean, dry areas of the pen [1, 9].

Bedding: Hay and straw are highly recommended bedding options for sheep and goat, not only on farms, but also in the research setting. Wood chips, corn cobs and paper products have been used in indoor facilities and these alternative bedding options may have advantages in terms of moisture absorption and cleaning needs.

Table 5: Overview and summary of enrichments

Species	Enrichment
Mice	Nesting material, shelters/refuges, running wheel, cardboard, (PVC) pipes, wood sticks, hard shelled nuts, sunflower seeds, sesame seeds
Rats	Nesting material, tubes, boxes, (PVC) pipes, crawl balls, rope ladders, shelters/refuges, pumpkin seeds, wood sticks
Guinea pigs	Shelters/refuges, (PVC) pipes, hay, wood sticks, softwood blocks, pop sticks
Rabbits	Chains, rattles and kongs elicit temporary interest in some rabbits. Card board boxes are for sure are very entertaining for rabbits. Providing plenty of free choice hay can also decrease barbering behaviour in socially housed rabbits
Sheep and goats	Grooming materials, mirror and playing ball etc.

However, straw and hay provide the animal with more opportunity for foraging, so should be used whenever possible [8].

Enrichment: Grooming/petting, mirrors (make sure it is not a stress. male species may get very agitated with a mirror. Also position in an area that it cannot be broken), walks/exercise, large handled exercise balls, hang a plastic container in the stall hanging from the ceiling with carrots or apples inside and arrange the placement of the pasture incorporating a rock wall [28].

CONCLUSION

Cages of small laboratory animals can be made up of different materials that cannot be harmful to the health and welfare of the animals. If someone doesn't give careful attention during design and construction of the enclosure, laboratory animals will be affected by different injuries. Small laboratory animals require resources to carry out natural behaviours, such as exploring, foraging, running, escaping hiding and hygiene maintenance. Indicators that small laboratory animal housing conditions are improving include the availability of commercially produced resources for nesting and shelter. Bedding material influences the micro environment, appropriate bedding and nesting material and/or sleeping structures. In addition to the amount and type of bedding material, other factors such as ventilation, relative humidity and temperature also affect the ammonia levels. Environmental enrichments are used to provide a more complex environment which enhances species-specific behaviour, promoting physical health and decreases abnormal behaviours as much as possible

Recommendations:

- The size and type of cage, bedding, enrichments and also the type of feed must be prepared based on the behaviour and species of laboratory animals.
- The cage should not be against the health and welfare of laboratory animals.
- Cage/bedding/nest should have appropriate space, ventilation, relative humidity and temperature.
- Enrichments should enhance species-specific behaviour, promoting physical health and decreases abnormal behaviours as much as possible.
- Generally, laboratory animals should be handled in accordance with the international legislations of laboratory animals' welfare.

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