

# REVIEW ON WELFARE OF LABORATORY ANIMALS: SEVERAL THEORETICAL PRINCIPLES AND PRACTICAL ASPECTS

Han Quang Hanh<sup>2\*</sup>, Tran Thi Huong Thom<sup>1</sup>, Nguyen Chi Hieu<sup>1</sup>, Nguyen Dang Khue<sup>1</sup>, and Quach Thu Thao<sup>1</sup>

<sup>1</sup>National Institute for Control of Vaccines and Biologicals

<sup>2</sup>Vietnam National University of Agriculture

Received 02 April 2024

Accepted 30 August 2024

**Abstract:** Animal welfare is a concept that is increasingly concerned in the world and in Vietnam, including the welfare of laboratory animals. Animal welfare can be simply defined as the good treatment of animals, keeping the animals at a good physical and mental health conditions, preventing them from pain suffering. Improvement of welfare quality of laboratory animals will not only enable them to grow better and healthier, but also ensure a better result of the biological testing. To improve welfare of laboratory animals, the animal keeping centers need to pay attention to some factors related to housing and living environmental conditions, caring and handling practices, culling and culling methods. Several specific recommendations are also made to improve the welfare of rodents (guinea pig, mice and rat) when keeping them as laboratory animals.

*Keywords:* laboratory animal, humane animal treatment, guinea pig, rodent

## 1. Introduction

The use of laboratory animals in scientific research and medical testing has been common in the world in many years. First, humans use animals for many purposes, such as for food, for working, and for companion. Galen (130-201), a doctor of

Roman Emperor was the first person who performed physiological experiments on pigs, monkeys and dogs which opened a new period of using animals in research and medical test. In the 17<sup>th</sup> century, Descartes's theory indicated that living organisms were simply known as principle of mechanical

---

\* Corresponding author

E-mail address: hqhanh@vnua.edu.vn

<https://doi.org/10.56086/jcvb.v4i3.162>

machines, and animals had no thoughts as machines. However, when anesthetic medicine was invented and especially, the theory “On the Origin of Species” from Darwin was published in 1859 showed the biological similarity between human and animals, therefore animal use in research and testing had been more popular [1]. From the 20<sup>th</sup> century onwards, the use of animal in research and testing had been more common because of the development of pharmacology, toxicity, immunology, but until 1980s, the trend has been decreased because of the awareness of community as well as the policy law of some countries of the impact of animal using in research and testing. Besides that, the issue of animal welfare has been more concerned and mentioned when animal is used in research. Moreover, scientific research about laboratory animals also has been developed since 1950s to improve not only test efficiency but also animal welfare when using them.

The demand for animals used in research and teaching is huge, therefore the improvement of animal welfare is necessary. According to reports, there are about 75-100 million animals that have been used in research, teaching, in which about 70% amount used in medicine, pharmacology,

cancer research, while 30% remain amount of animals used in basic research, diagnosis and teaching. Within laboratory animals, rat takes 33%, mouse is 44%, guinea pig is only 2% and other animals such as rabbit, bird, fish [1]. When animals are used in research, testing and teaching, there are some rules, disciplines or ethical issues should be compliant to ensure animal welfare, minimize the discomfort and pain they may suffer because this is not only good for the animals, but also brings good results in testing and research.

The aim of this article is to introduce theoretical and practical principles of animal welfare as the basic to continuously improve the quality of animal welfare in research and testing facilities. The data and information of this paper is reviewed and generated from national and international scientific data.

## **2. Approaches and law on animal welfare of laboratory animals**

### ***2. 1. Definition of animal welfare***

There are many points of view about animal welfare based on different opinions of researchers about aspects of welfare or wellbeings. The animal welfare concept is also related to welfare measurement methods and human responsibility in ensuring animal welfare as well as the way they treat animals.

The definition of animal welfare of Office International des Epizooties (OIE) has a wide range of images to most countries. Since the 1960s, OIE published Terrestrial Animal Health Code, in which described the definition of animal welfare as “*Animal welfare means the physical and mental state of an animal in relation to the conditions in which it lives and dies*” . An animal has a good welfare (scientific proof) including they are “*healthy, comfortable, well nourished, safe, is not suffering from unpleasant states such as pain, fear and distress, and is able to express behaviours that are important for its physical and mental state*”. “*Good animal welfare requires disease prevention and appropriate veterinary care, shelter, management and nutrition, a stimulating and safe environment, humane handling and humane slaughter or killing. While animal welfare refers to the state of the animal, the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment*” [2], [3].

For laboratory animals, the Three-R is applied worldwide which includes Replacement –Reduction – Refinement. The Three-R concept plays an important role in guidance of animal using in testing and scientific research.

## **2.2. Animal welfare under Vietnam law**

The Law on Veterinary Medicine (2015) and Law on Animal Husbandry (2018) also have mentioned about animal welfare, including laboratory animals. Article 21 of Law on Veterinary Medicine clearly determined that “Organizations and individuals raising, cultivating and using animals have the following responsibilities::

a) Management, care, feeding, and transportation appropriate to each animal species;

b) Minimize pain and fear, treat animals ethically in animal husbandry, aquaculture, transportation, slaughter, culling, disease prevention, treatment and scientific research”.

Article 72 of Law on Animal Husbandry (2018) also mentioned about ethical treatment to laboratory animals in scientific research and other activities: “Animal using in scientific research and other activities should be treated ethically according to article 69, 70 of this Law”. Therefore, Vietnamese law also mentioned issues related to animal welfare including laboratory animals [4,5].

## **3. Some concerned issues of laboratory animal welfare**

### **3.1. Issues related to cages and living environment factors**

Diverse environment is one of the important factors in laboratory animal health care. It is also very important in quality control and testing to contribute to minimizing differences among animals. Table 1 shows five main aspects of diverse environment for laboratory animals: Society, nutrition, facility, mentality and diverse feeling as well

as environment control. Environment control includes standardize and monitorize environmental factors affected animals' activities. There are two different levels of performance:

- 1) Macro room or environment;
- 2) Micro cage or environment.

**Table 1. Aspects of diverse environment**

No.	Aspects	Factors
1	Diversity of society	Cage cleaner, caring staff
2	Diversity of nutrition	Types and sources of feed
3	Diversity of facility	Cage types, structures, devices, nesting materials
4	Diversity of mentality	Controllability
5	Diversity of sense	Hearing, sight, smell, touch

In an animal raising facility, where animals are raised under conditions differing from their natural environment, these animals are not free to move far from adverse conditions to search for food and water or to find a better housing. The rearing environment should provide physiological and behavioral needs as much as possible, otherwise the animal's health will be impaired.

There are some factors of environment: temperature, humidity, ventilation and light intensity should be maintained at an appropriate level for each species. For

example, sudden changes in humidity can have adverse impacts on rodents and rabbits. Additionally, a study on effects of facilities showed that cage cleaning frequency, light/dark cycles, noise, and daily environmental conditions affected animal health.

Light intensity inside each cage may be different based on the cage positions in the room, therefore, light intensity should be controlled for whole room (macro-environment) and for whole cage (micro-environment). The top cages should be covered to ensure uniform light intensity for

all animals in the room. Albino animals have less melanin in their retinas, light can cause retinal degeneration so animals must be provided shelter.

Noise levels should be kept in a minimum. Mice, rats and guinea pigs are particularly sensitive to sound. Therefore, caution should be exercised around devices that emit sound, such as computers and monitors. Animals may not perceive noise as well as humans, so some species are easily startled by sudden noises. Noise levels above 50 decibels can have a detrimental effect on the hearing ability of rodents and rabbits. Adverse effects included ultrasound-induced stroke in juvenile rats and reduced fertility in adult rats. Animal rooms should not conduct tests that are likely to cause fear or noise to animals.

Cages should keep animals dry, clean and warm. They need to have an adequate space to allow animals to move around normally, and they should have free to access to food and water. The cage must be made of non-toxic materials, easy to clean (disinfection if necessary), and must be sturdy enough to prevent the animal from escaping. Animals should be discouraged from fighting by providing more spaces, and when animals in one cage fight continuously, they should be kept in separate cages.

Nesting materials can be used better than mesh flooring to provide warmth and comfort and easy to clean. Nesting materials should be non-toxic, non-irritating, absorbent, easy removable, available, inexpensive, easy to use, and changed regularly to maintain a clean, odor-free environment. However, for some animals, too-clean environment can stimulate aggression when the animal does not recognize the scent of their territory. Therefore, it is necessary to add some old bedding to the new cage to prevent this situation. Nesting materials should be provided for all rodents, not just pregnant females [6].

### **3.2. Issues related to caring, technical operation**

Laboratory animal care includes all aspects of animal care aimed to ensure health of the animal herds. This is important because it benefits not only the animals, but also it helps improve the reproducibility of the test results. Good care means to make use of care conditions, reasonable and thoughtful resources for the good development of animals. It includes essential facilities for animal caring regularly by providing, monitoring, and maintaining facilities, resources, and essential caring practices. Besides, it is necessary to regularly monitor

environment, sources and devices that exposed to animals.

A good caring program means to ensure a good nesting system and good animal caring practices that helps animals can be developed, mature, reproduced, and good health. There is criteria of housing and management had designed not only for sufficient physiological needs but also social needs and behaviors of laboratory animals. With well-trained staff, animal health care can be ensured even under basic facility and devices.

Not only physiological needs is provided, animals should be allowed to express their distinct behaviors as much as possible, including playing and social contacts with others in a group. Social reaction with other members in the same species is one of the important aspects of laboratory animals. If they do not have a chance to express distinct behaviors of their species, it will be harmful to their health and development, for example, an animal repeated abnormal behavior is called stereotyped action. A diversified environment is important for animal health and development than other factors based on demand of species' behaviors.

Capture, movement, operation of animals are important and concerned issues.

Capture is the first step in the most experiments. Capture methods used based on types of cages and animal behaviors. All the rodents can bite as a natural response to fear. Therefore, it is necessary to ensure the animal and human health, especially for new imported animals as they may contain pathogens, bacteria and virus.

Animal capture is use for a purpose of testing, sample collecting, medical administration, or other technical testing. In mostly applied researches, animal is captured only in short period of time. When using animal capture devices, make sure that they are suitable of size, design and performance to minimize uncomfot and injury to animals. They should be designed specially to complete research purposes or avoid injury for not only animal but also technicians.

Application the following principle when using animal capture devices:

- Do not consider them as normal cages
- Do not used as simple as optimized solution for animal capture or management
- Minimize capture time
- Animals should be trained to get used to staffs and devices
- Animals should be observed at suitable period of time

- Veterinary health care is needed, if there is an injury or disease, it is necessary to stop capturing or combine with animal capture devices.

Veterinary staffs should minimize reaction of animals with humans' approach and avoid sudden movement. Most of rodents has tails to support themselves. Tails should be captured at roots and whole body of animals should be kept quickly [6].

### **3.3. Issues related killing and culling**

Killing and culling are concerned in all over the world. Euthanasia (In Greek:  $\epsilon\upsilon\theta\alpha\nu\alpha\sigma\acute{\iota}\alpha$  =  $\epsilon\upsilon$  (good, pleasant) +  $\theta\acute{\alpha}\nu\alpha\tau\omicron\varsigma$  (death)) refers to the practice of a life-end with the aim of reducing the animal's pain and physical suffers. The word "euthanasia" means a death with no pain. This is clearly not possible if the animal struggles or writhes in terror during surgery or culling. Euthanasia really depends on rapid unconsciousness and maintains this state until death occurs. The British House of Lords Select Committee on Medical Ethics defined euthanasia as a deliberate intervention made with obvious intention of a life-end, to relieve intractable suffering. Euthanasia is applied to destroy, kill animals with less pain and stress.

In a laboratory animal raising facility, there must be a place to keep animals while waiting for culling that is in good hygiene condition. animals should be provided with enough water, not be beaten or tortured, limiting fear, stress, and pain for animals or not be allowed to witness the culling of their species. When culling is applied, the staffs still play an important role in euthanasia to ensure a humane culling. Therefore, suitable staffs should be selected under monitoring of experienced experts [7].

## **4. Improvement of welfare of some laboratory animals species**

### **4.1. Animal welfare for guinea pigs**

#### **4.1.1. Feed and caring**

Guinea pigs should be fed at a fixed time everyday and ensured their adequate nutritional needs. They do not nest but prefer burrows in good quality, cool, soft hay. It helps to protect newborn animals if the temperature is low. In laboratory, guinea pigs should be provided hiding places to avoid sudden light and noise. In general, guinea pigs are easily agitated and frightened when disturbed by sudden noises. They tend to run around when they are frightened. Therefore, background music can be turned on in animal rooms to hide noises, and hiding places are provided for animals to take refuge. Guinea pigs cannot synthesize vitamin C themselves

leading to lack of vitamin C, so it is important to supplement vitamin C for them. Every week, fiber from hay or vegetables such as carrots and cucumbers should be supplied to them, not only to ensure fiber content but also to satisfy their chewing habits [9].

#### 4.1.2. Ensuring suitable environment conditions

According to the recommendations of the European Community (EC, 1986) [9], the

optimal temperature for guinea pigs is 18-22 °C and the relative humidity is 50-60%. Therefore, make sure to minimize loud noises because it will scare the mice and they may get injured. Lighting intensity should also be kept stable or little change. Standards for environmental factors for guinea pigs according to GMP, Korea (Korea Food & Drug Administration, 2009) and Netherland (Rivm, 2000) as below in table 2 [6, 8]:

**Table 2. Specification of environment condition for laboratory animal**

Factors	Specification (GMP, Korea)	Specification (Rivm, Netherland)
Temperature	18 – 26°C	20 – 24°C
Humidity	40 – 60% (no less than 30%, no more than 70%)	50 – 70 %
Air changing	15 – 20 times/hour	10 – 15 times/hour
Air velocity	0,13 – 0,18 m/s	
Ammonia concentration	< 20 ppm	
Light	150 – 300 lux, distance 40 – 80 cm from the floor	
Noise	< 60dB	

#### 4.1.3. Ensuring suitable cages for guinea pig

Guinea pigs can be kept on the floor or in cages because the most important thing is to ensure enough space for them. Minimum area for each animal is 2500cm<sup>2</sup> (according to EC, 1986, minimum 600cm<sup>2</sup>/animal), minimum cage height is 30cm. It is able to keep 1 male 1 female together, 1 male and a few females or keep in groups of few males and few females [9].

Guinea pigs should be kept in cages with solid, well-padded floors. Wire mesh cages maybe not suitable for guinea pigs because they are difficult to add bedding to and easily lead to foot problems. Soft, absorbent materials such as shavings and hay should be added to suit their natural behavior of snuggling and exploring their environment.

#### 4.2. Animal welfare of mice, rats



Rodents were used quite commonly as laboratory animals, has started in the late 19th century and has become more common in the 20th century. Rodents used as experimental animals often gradually adapt to laboratory conditions, so they have different environmental requirements and behavioral expressions than wild animals. Therefore, when raising and using rodents as experimental animals, it is necessary to pay attention to improving their welfare by improving environmental conditions, caring and feeding.

#### 4.2.1. Maintain stable microclimate conditions of rearing room

The raising mice rooms need to provide the best living conditions for them, including five main factors: temperature, humidity, ventilation, lighting and noise. The recommended microclimate conditions for mouse breeding rooms are temperature 20-24°C, humidity 55± 10%, (according to European Community (EC), 1986) or from 18-26°C and 30-70% [10], ventilation 15-20 air changes/hour (EC) or 10-15 air changes/hour [10], minimum noise level (EC) or below 85 dBA [10], low lighting (EC) or 325-400 Lux [10]. The microclimate standards of rearing room also depend on other factors such as cage types and animal density as well as age of the mice, but the

general requirement is to minimize fluctuation in these microclimate conditions.

#### 4.2.2. Cages improvement

Mice need to be kept in cages with enough space for them to move around and express natural behaviors. The cage should be large enough for them to stand or lie down comfortably without hunching over their body or tail, and large enough to provide playing materials for the mice. According to recommendations of European Community [11], mouse less than 200g needs a minimum area as 200 cm<sup>2</sup>/animal; mouse less than 300g needs a minimum area as 250 cm<sup>2</sup>/animal and mouse less than 400g needs a minimum area as 350 cm<sup>2</sup>/animal. Minimum cage height for all three types of mice is 18cm.

The material used to make the cage should be harmless to animals, inedible, easy to clean and disinfect, unaffected by waste and flexible to take animals out [11,12]. Polycarbonate or polypropylene cage materials are recommended over stainless steel because they are lighter, quieter and provide a better insulation. The bottom of the cage should be designed as a solid block instead of mesh. [10], animals readily exhibit nesting or burrowing behaviors when bedding material is provided. If cage bottom is made of wire mesh, an additional area with

a pad should be added to satisfy their habits and reduce foot injuries.

#### 4.2.3. Supplement bedding and nesting

Bedding should be added to absorb moisture and waste (urine, feces). Straw, corn husks, and paper can be used as bedding, but it should be changed regularly. Adding dry and absorbent materials such as paper towels and wood shavings to make a nest for mice will have more positive effect on mouse welfare than other types of materials because mice have the habit of making nests [14].

#### 4.2.4. Material addition as enrichment of living environment for animals

Mouse is a spiece that enjoys discovering surrounding environment, therefore, it is needed to add some materials to enrich their habitats such as adding toys, pipes or places for them to crawl. When the environment is enhanced, cognitive ability and flexility of animal is better [15,16]. However, it is necessary to choose safe, suitable materials and slowly add so the animal easily gets used to it.

### 5. Conclusion and suggestion

Many countries in the world has changed of awareness and enforced law and regulations on animal welfare into practices. Animal welfare for laboratory animal is very important for laboratory animal raising centres, not only help animals have a better

living condition but also ensure a result of experiments and testing. Some solutions to improve animal welfare include cages and living condition improvement, enhanced nourishment, suitable caring for each animal species, minimize technical operations and interventions that cause pain and fear, and humane killing and culling if necessary. The animal raising centre should apply Vietnamese standards and international specifications to take appropriate measures to improve animal welfare of laboratory animals.

### References

- [1] Baumans, V.(2005).Science-based assessment of animal welfare: laboratory animals. *Revue scientifique et technique-office international des epizooties*, 24(2), 503.
- [2] OIE (World Organization for Animal Health], 2011. *Terrestrial Animal Code*. [http://web.oie.int/eng/normes/mcode/en\\_chapitre\\_1.7.8.htm](http://web.oie.int/eng/normes/mcode/en_chapitre_1.7.8.htm), access on **15 March 2024**
- [3] OIE (World Organization for Animal Health, 2022). *Terrestrial animal health code 2022*. Retrieved from <https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access> on **16 June 2023**

- [4] National Assembly of Vietnam (2015) Law on Veterinary: Number 79/2015/QH13
- [5] National Assembly of Vietnam (2018) Law on Animal Husbandry: Number 32/2018/QH14.
- [6] National Institute of public Health and the environment (2000). Laboratory Animal Science and Husbandry in Vaccine Quality Control.
- [7] College of Veterinary Medicine (1989). The Biology and Medicine of Rabbits and Rodents. The United states of America.
- [8] Korea Food & Drug Administration (2009). Good Manufacturing Practices and Quality Control for Vaccines.
- [9] Council of Europe (1986). European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123). Council of Europe, Strasbourg 1986
- [10] National Research Council (1996). Guide for the Care and Use of Laboratory Animals, 7th edition. National Academy Press, Washington, 1996; 22
- [11] Council of Europe (2022). Revision of Appendix A of the Convention. Draft Species specific provisions for rodents and rabbits. Working Party for the Preparation of the Multilateral Consultation of parties to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. Strasbourg, 14 October 2002; <http://www.coe.int>
- [12] Wolfensohn S, Lloyd M. Handbook of Laboratory Animal Management and Welfare. Oxford University Press, Oxford, UK 1994
- [13] Allmann-Iselin I. Husbandry. In The Laboratory Rat. The Handbook of Experimental Animals. Krinke GJ ed, Academic Press 2000
- [14] Brain PF, Büttner D, Costa P, Gregory JA, Heine WOP, Koolhaas, J, Militzer K, Ödberg FO, Scharmann W, Stauffacher M. Rodents. In The Accommodation of Laboratory Animals in Accordance with Animal Welfare Requirements. Proceedings of an International Workshop held at the Bundesgesundheitsamt, Berlin 17-19 May 1993. O'Donoghue PN, ed, Bundesministerium für Ernährung, Landwirtschaft und Forsten, Bonn,

Germany 1993;  
1-14

[15] Patterson-Kane EG, Hunt M, Harper D.  
Behavioral indexes of poor welfare in  
laboratory rats. J Appl Anim Welfare Sci  
1999; 2:97-110

[16] Young D, Lawlor PA, Leone P,  
Dragunow M, Furing MJ. Environmental  
enrichment inhibits  
spontaneous apoptosis, prevents seizures  
and is neuroprotective. Nat Med 1999;  
5:448-453