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# The role of nutrition and care in laboratory mice used for scientific research

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## Abstract

Laboratory mice (*Mus musculus*) are the most widely used mammalian model in scientific research due to their genetic similarity to humans, rapid reproduction manageable size, and cost-effective maintenance. This review comprehensively examines the biological characteristics, behavior, housing requirements, and most importantly, the nutritional needs of laboratory mice. Adequate and species-appropriate nutrition is essential not only for animal welfare but also for the reliability and reproducibility of experimental outcomes. Nutrient requirements vary according to age, sex strain, and physiological status, such as growth, reproduction, pregnancy, and lactation. Standard diets must be formulated to provide the appropriate balance of macronutrients (protein, fat carbohydrates), micronutrients (vitamins and minerals), and water energy needs, amino acid profiles fat-soluble and water-soluble vitamin requirements, and trace mineral demands are discussed in detail supported by specific dietary formulations and intake levels. The paper also addresses the formats and administration of diets (pellet, gel, liquid), and the effects of nutritional deficiencies on physiological functions such as immunity reproduction and metabolism. Furthermore, environmental factors including cage design, ambient temperature, humidity, and light cycles are highlighted as critical elements that interact with nutrition to influence mice physiology. Proper handling and husbandry techniques are outlined to minimize stress and maintain consistent experimental conditions. Emphasis is placed on the need for standardized feeding protocols to ensure comparability across studies. In conclusion, the successful use of mice in research requires a multidisciplinary approach combining optimal nutrition, ethical care, and well-controlled environmental conditions. These factors are vital to preserving the physiological integrity of laboratory mice and generating meaningful scientific data.

**Keywords:** Animal nutrition, laboratory animals, mice, requirements

## 1. Introduction

Today, the conclusions of scientific studies have made it necessary to work with laboratory animals. Various laboratory animals are used in experiments in the fields of biology and medical sciences. The most commonly used laboratory animals are white mice, rats, guinea pigs, and rabbits. In addition, animals such as cats, dogs, hamsters, and monkeys are also used for experimental purposes. Among these, laboratory mice are the most preferred species due to their rapid reproduction, ease of care and handling, and their ability to provide a large number of experimental subjects.<sup>1</sup>

Nutrition is the most important factor affecting the growth, development, survival in laboratory mice. It also influences the expression of genetic potential in response to various stimuli. Therefore, a well structured feeding program should be implemented, taking into account the animal's biological and physiological conditions, as well as different life stages such as growth, adulthood, pregnancy, and lactation.<sup>1</sup>

In addition to feeding programs, maintaining appropriate conditions such as cage environment, hygiene, and temperature is also crucial for the care and management

of laboratory mice, as these factors can significantly influence the outcomes of research studies.<sup>1</sup>

## 2. Origin of laboratory mice

The ancestor of the laboratory mice is the wild house mice (*Mus musculus*), which typically inhabits fields and storage areas. Initially, wild mice were captured and bred by enthusiasts who were primarily interested in the variety of coat colors. These mice were often exhibited, and their rapid reproduction led to a surplus, which provided ample material for experimental studies in the early 20<sup>th</sup> century. Collaborative efforts between geneticists and mouse breeders during the early 1900s laid the foundation for the development of the inbred and outbred mice strains and stocks used in laboratories today.<sup>2</sup>

Mice belong to the family Muridae, subfamily Murinae, and genus *Mus*. The most commonly used laboratory mice originates from the North American and European house or rice field mice, a member of the subgenus *Mus*. Its scientific name is *Mus domesticus domesticus*.<sup>2,3</sup> Several subspecies of this species have also been identified. One of the most notable is *Mus batrianus*, which is found in the East Asian region.<sup>4</sup>

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Although originally spreading from Central Asia to regions such as Turkey and China in recent history, the Swiss albino mice has become the origin of today's most widely used laboratory mice strains.

### 3. General biology

The house mouse is a rodent species that commonly co-exists with humans. It is highly adapted to a wide range of environmental conditions and is distributed across a vast area, from the poles to the tropics. Its ability to exist in large numbers over such a broad range is due to its omnivorous diet, high reproductive capacity, and short intervals between births. House mice are nocturnal animals that live in burrows dug underground.<sup>3,5</sup>

Mice have a highly developed sense of smell. In addition to detecting food and predators, they can also perceive various social olfactory signals, including pheromones. However, they have poor vision and cannot distinguish colors. Their spectral response differs from that of humans, and they are particularly insensitive to the red end of the spectrum.<sup>3,5</sup>

Due to their small body size, mice are extremely sensitive to changes in environmental conditions. Even relatively small fluctuations in ambient temperature (2-3°C) can affect their core body temperature and alter their physiology. Additionally, mice lack sweat glands in their skin.<sup>3,5</sup>

Mice can be bred as outbred, inbred, hybrid, or mutant strains, in various group sizes. Even if mice bred for several generations at different institutions originate from the same source, they may have undergone genetic changes. Similarly, clinically healthy mice obtained.<sup>3,5</sup>

### 4. Body size and lifespan

Adult mice measure approximately 12-15 cm in length from the tip of the nose to the tip of the tail, with the tail being nearly equal in length to the body. Newborn mice weigh around 1-2 grams and grow rapidly during the lactation period, steadily gaining weight. Variation in adult body weight depends on animal related variables such as genotype, sex, strain, and age, as well as external factors including diet, cage population density, and environmental temperature. By days 18-21, mice typically reach a body weight of 8-12 grams. The forelimbs have four digits, while the hind limbs have five.<sup>6,7</sup>

Both environmental and genetic factors influence the lifespan of mice. These include diet, the number of animals per cage, subclinical infections, genetic predisposition, strain, sex, and the presence or absence of harmful mutant genes. Hybrid mice typically produced by crossing two inbred strains tend to live longer than their inbred parent strains. Mice from short lived strains generally live between 5 and 16 months, while those from long lived strains can live between 24 and 36 months.<sup>3</sup>

### 5. Behavior

Laboratory mice are social animals and generally live peacefully in groups formed after weaning. However, males of certain strains (such as HRS/J, BALB/cJ, and SJL/J) may begin to fight at around 7-10 weeks of age, even if they remain in the same groups formed during the wea-

ning period. Breeding males removed from their original cages and housed together tend to engage in aggressive behavior. In contrast, adult female mice typically do not fight, even when housed together for the first time.<sup>3</sup>

Mice usually designate specific areas of the cage for sleeping, eating, urination, and defecation. They often remove food from the feeder and hoard it in a corner of the cage. Their feeding behavior is cyclical, with consumption peaking during the dark phase of the light cycle. Mice generally prefer cereal grains such as oats, rice, and wheat, as well as oily foods, meat, sugar, and dried fruits.<sup>3</sup>

Female mice that give birth construct a nest and spend most of their time near it, covering their pups with their bodies. Both female and male mice help clean and organize the newborns, removing inactive or non-viable pups from the nest.<sup>3</sup>

### 6. Use in laboratories

Laboratory mice constitute approximately 60-80% of all mammals used in laboratory research. Their widespread use is attributed to their relatively small body size, ease of handling, low maintenance cost, and anatomical similarities to humans. They are particularly utilized in bioassays and toxicity testing, as well as in the fields of microbiology, virology, radiobiology, and cancer research especially involving inbred strains.<sup>4</sup>

It is relatively easy to breed large numbers of mice, maintain their survival, and identify a wide variety of mutations. For these reasons, mice are considered the most suitable small mammalian model for genetic research. Many inbred strains of mice play a key role in various types of cancer studies. Due to their wide availability and the extensive scientific knowledge accumulated about them, they have become an indispensable component of medical research.<sup>8,9</sup>

The mouse is also a suitable animal model for toxicological studies because its lifespan is significantly shorter than that of rabbits, dogs, cats, or non-human primates.<sup>3</sup>

### 7. Housing: shelter, cages, drinkers, and feeders

Mice housing facilities should be designed to optimize both animal welfare and operational efficiency. The spacing between cages should be wide enough to allow personnel to move comfortably. Work tables and waste disposal channels should be available within the facility. Ideally, the housing area should have two doors: one leading to the laboratory and the other designated as an emergency exit. As a general rule, the corridor between cage rows should be at least 1.5 times the width of the table used for cage cleaning and changing.<sup>10,11</sup>

Cages may vary in material, structure, and size. A cage designed to house a breeding pair and their offspring should measure approximately 14 cm in width, 30 cm in length, and 12-15 cm in height.<sup>3,11</sup>

The design, size, and construction of mice cages can differ depending on the purpose. Most cages feature either a solid (hardbottom) base or a wire mesh base. Solid bottom cages, often referred to as "shoebox cages" due to their shape, are commonly made from pol-

ycarbonate, polypropylene, or polystyrene. Bedding or nesting material is placed directly on the solid floor. In contrast, wire bottom cages typically hang above a tray filled with absorbent material, which helps to collect and contain feces and urine. These cages are especially useful when continuous collection of excreta is required. However, due to mice's natural nesting behavior, solid bottom cages are generally preferred, as nesting success is limited in wire bottom cages even when nesting material is provided.<sup>3,12</sup>

Each cage should be equipped with a basket or bucket shaped feed container positioned approximately 5 cm above the cage floor. The feeder may be integrated into the cage lid or attached to the side of the cage. Mice can access feed through openings 3-5 mm wide.<sup>3</sup>

If the feeder is made of wire in a basket form, mice may climb onto it and urinate or defecate into the feed, leading to contamination. Therefore, the feeder should hold enough food to last until the next scheduled cage change.<sup>3</sup>

Water is supplied either through individual bottles placed in each cage or via an automated watering system. Bottles may be made of glass or plastic, with larger capacities preferred. Both materials are easy to clean, but plastic bottles are often favored due to their lighter weight and resistance to breakage.<sup>3</sup>

## 8. Handling techniques

One of the most commonly used methods for handling mice is to grasp them by the tail, either with the hand or using forceps. However, it is important to hold them by the middle of the tail rather than the tip, to avoid injury and ensure proper control.<sup>13</sup>

In another method, the animal is placed on a hard surface. Mice are then grasped by the skin over the shoulders using the thumb and index finger of one hand, while the tail is secured against the palm with the tip of the index finger. This restraint technique allows for intramuscular or intraperitoneal injections to be administered with the other hand.<sup>13</sup>

## 9. Physiological and anatomical structures of mice

### 9.1. Thermoregulation and water balance

Due to their large surface area to volume ratio, mice lose body heat rapidly. They are highly sensitive to environmental temperature and are unable to tolerate drops in room temperature, often expending significant energy to maintain their body temperature.<sup>14-16</sup>

Mice are also extremely sensitive to water loss. Because they do not sweat or pant to reduce heat, water retention plays a vital role in thermal regulation. If the body loses water through evaporation to prevent overheating, excessive dehydration can lead to shock. In the wild, mice exhibit thermoregulatory behaviors such as burrowing and hiding underground to conserve body heat. However, these behaviors are difficult to replicate in laboratory settings. Therefore, it is crucial to provide optimal environmental conditions.<sup>14-16</sup>

The recommended environmental parameters in laboratory conditions include a temperature range of 19-

25°C, relative humidity of 40-70%, and a 12 hour light/dark cycle. Light intensity should be 350-400 lux at bench level, while 60 lux is sufficient within cages particularly for albino mice to prevent retinal damage. Mice lack sweat glands and have a limited ability to salivate, further emphasizing the need for precise environmental control.<sup>14-16</sup>

### 9.2. Digestive system

The tubular portion of the digestive system of mice consists of the esophagus, stomach, small intestine, cecum, and colon. The proximal part of the stomach is keratinized, while the distal part is glandular. Gastric secretion is continuously active, regardless of food presence.

Shortly after birth, more than 100 types of bacteria colonize the gastrointestinal tract of the offspring, establishing the gut microbiota. This results in the rapid formation of a complex ecosystem that contributes to essential functions such as the production of amino acids, maintenance of homeostasis, and the development of resistance to certain intestinal pathogens.<sup>12</sup>

### 9.3. Respiratory system

The respiratory system of mice is divided into three main sections. The upper respiratory tract includes the nostrils, nasal cavity, and nasopharynx. The middle portion consists of the larynx, trachea, and bronchi, while the lower portion comprises the lungs. The left lung consists of a single lobe, whereas the right lung is bilaterally divided into four lobes: superior, middle, inferior, and postcaval.<sup>12,17</sup>

At rest, a mouse consumes approximately 3.5 mL of O<sub>2</sub> per gram of body weight per hour about 22 times the oxygen consumption rate of an elephant. To meet this high metabolic demand, mice have evolved adaptations such as elevated alveolar pressure, rapid respiratory rate, short airway length, and a high erythrocyte concentration.<sup>12,17</sup>

### 9.4. Excretory system

The urinary system consists of the kidneys, ureters, urinary bladder, and urethra. The kidneys are located dorsally, against the abdominal wall. Typically, the right kidney lies anterior to the left kidney. Inbred male mice have heavier kidneys than females. The glomeruli are relatively small. Mice urine is excreted in one or two drops and is highly concentrated. Excess nitrogen is eliminated through the urine. Taurine and creatinine are consistently present in mice urine, while tryptophan is not always detectable.<sup>12</sup>

### 9.5. Skeletal system

The skeletal system is divided into two main parts. The axial skeleton includes the skull, vertebrae, ribs, and sternum. The appendicular skeleton comprises the pectoral and pelvic girdles as well as the limbs. The standard vertebral formula for mice is C7 T13 L6 S4 C28, although variation can occur in the thoracic and lumbar regions across different strains. The mouse brain exhibits a typical mammalian structure.<sup>12</sup>

Mice have one incisor and three molars in each quad-

rant of the mouth. The third molars are the smallest teeth in both jaws.<sup>12</sup>

### 9.6. Reproductive system

Mice reach puberty at around two months of age, when their body weight ranges between 20-35 g. Their reproductive competence typically lasts for 1-1.5 years, during which they can produce 6-10 litters. Adult males usually weigh between 20-40 g, and females between 25-40 g. Reproduction begins at around 50 days of age in males and 50-60 days in females. In females, the ovaries are located posterior to the kidneys and enclosed in a transparent ovarian capsule. Mice have three pairs of thoracic and two pairs of abdominal inguinal mammary glands.<sup>18,19</sup> The onset of sexual maturity depends on factors such as species, season, growth rate, litter size, and nutritional status.<sup>20</sup>

Sex determination in mice is based on the length of the genital papilla and the anogenital distance.<sup>21</sup> Neonatal males can be distinguished from females by their longer anogenital distance. Pale testes can often be seen along the abdominal wall. Genital papillae are also more prominent in males than in females. Additionally, the presence of nipples in females from day 9 postnatally is a distinguishing characteristic.<sup>20</sup>

Laboratory mice are polyestrous year round. Their estrous cycle lasts 4-5 days, with estrus typically lasting 9-20 hours. Estrus, mating, and ovulation generally occur during the night. Mating is confirmed by the presence of a vaginal plug. In the final stages of pregnancy, the female prepares a nest for her offspring. The gestation period lasts 19-21 days, with litters averaging 1-12 pups. Lactation continues for about 21 days (Table 1).<sup>21</sup>

**Table 1.** Biological features of mice.

Adult body weight	M: 20-40, F: 18-40
Feed Consumption	15g/100g BW
Water Consumption	15ml/100g BW
Lifespan (years)	1.5-3
Rectal temperature (°C)	38-39
Respiration rate (per minute)	60-220
Puberty (days)	28-40
Gestation period (days)	19-21
Birth weight (g)	1-1.5
Estrous cycle (days)	4-5
Litter size	6-12

Abbreviation: M, Male; F, Female;

### 10. Nutrient requirements

All animals require nutrients such as protein, carbohydrates, fat, and minerals for their development and growth, skeletal development, tissue repair, blood formation, and muscle growth. Feeds serve as energy sources for the chemical reactions essential to all physiological processes and for generating body heat. Water is equally as important a nutrient as feed. Nutrient requirements increase significantly in young animals during periods of rapid growth and in females during pregnancy.<sup>22</sup>

Appropriate diets should be formulated to meet the nutritional needs of animals based on their physiological status, metabolic activity, age, duration of handling, and genetic background. For example, the dietary requirements of an adult mice housed in a small cage differ from those of the same mice housed in a larger cage. Environmental factors such as temperature and humidity also influence nutrient requirements. Therefore, when formulating a diet for a mouse, not only these criteria but also potential nutrient deficiencies or excesses should be considered. Table 2 provides the levels of selected nutrients in the diet.<sup>12</sup>

**Table 2.** Nutrient requirements of mice (%).

Nutrient	Concentration in the Diet (%)
Crude Protein	20-25
Fat	5-12
Carbohydrate	45-60
Crude Fiber	2.5

Mice are typically fed ad libitum. For this purpose, a completely pelleted diet is used, stored in elevated silos to avoid fecal contamination. Mice consume 3-5 grams of pelleted feed per day to meet their nutritional needs. Common feed ingredients include barley, wheat, oats, sunflower seeds, and bread. Additionally, oil, sugar, meat, fruits, and vegetables may be provided. Vegetable oil serves as the main fat source, while protein sources include fish meal, milk powder, and soybeans. Furthermore, the feed should be supplemented with essential vitamins, minerals, and salt. The amounts of vitamins, calcium, and phosphorus should be increased in the feed given to lactating females.<sup>23,24</sup>

Cube shaped pellets are provided ad libitum to mice housed in cages. Pellets with a diameter of 12-18 mm and a longer length help minimize feed waste. Average daily feed consumption is listed in Table 3.<sup>25</sup>

**Table 3.** Average daily feed consumption.

Week	Percentage of Body Weight (%)
Week 3	16.5%
Week 4	15%
Week 5	13%
Week 6	11%
Week 8	9%
Week 14	6%
Week 52	3.5-4%

### 10.1. Energy requirement

The term energy refers to the components in feed that serve as fuel for the animal's physiological activities such as thermoregulation, respiration, and physical movement. Mice primarily derive their energy from carbohydrates, followed by fats and, to a lesser extent, proteins.<sup>22</sup>

After weaning, mice consume approximately 3.5 grams of feed per day for 14 days. Among the diets tested, the one providing 14.5 Kcal of metabolizable energy (ME) per day resulted in satisfactory growth. It has been reported that 5 grams of feed (equivalent to 18 Kcal ME)



per day is required to achieve rapid growth during the post weaning period (21-42 days), while 3.8 grams of feed (14 Kcal ME) per day is adequate for normal growth.<sup>22</sup> The energy requirements during reproductive stages are calculated as follows: 1.2 times the maintenance level at the beginning of pregnancy, 2.4 times at the end of pregnancy, and 3 times the maintenance level during lactation. The total ME requirement during pregnancy and lactation is approximately 4.2 Mcal.

### 10.2. Protein and amino acid requirements

Proteins are essential nutrients involved in nearly all metabolic processes and are composed of amino acids. Both the quality and quantity of dietary protein are critical. Therefore, protein content is always specified in diet formulations. A high protein content can sometimes compensate for deficiencies in other dietary components.<sup>26</sup> When rapidly growing hybrid mice were fed a diet containing 12.5% protein (in the form of casein and amino acids), optimal growth was achieved. This level is considered the growth requirement. The maintenance protein requirement was calculated as 200 mg of nitrogen. For diets with high digestibility and protein quality, the required protein level is approximately 130 g/kg of dry matter. Most commercial diets contain between 17-30% crude protein. Diets composed of natural feed ingredients with 20-25% crude protein are generally sufficient for growth and reproduction. Additionally, variations in dietary fat levels can influence protein concentration requirements. It is important to emphasize that protein quality is more critical than protein quantity. In conclusion, a protein level of 18% is considered sufficient for normal reproduction in mice, and higher levels are not typically necessary.<sup>27,28</sup> There is limited information on the specific amino acid requirements of mice. Hybrid mice require approximately 1.3 grams of amino acids per day for maximum growth. Both D and L isomers of methionine and D-L phenylalanine contribute to growth. However, only the L-isomers of valine, leucine, isoleucine, and threonine are effective. The D-isomers of tryptophan, histidine, and lysine are ineffective for promoting growth (Table 4).<sup>27</sup>

**Table 4.** Amino acid requirements of mice.

Amino Acid	In Diet (%)
Arginine	0.3
Histidine	0.2
Tyrosine	-
Isoleucine	0.4
Leucine	0.7
Lysine	0.4
Methionine	0.5
Phenylalanine	0.4
Tryptophan	0.1
Valine	0.5

### 10.3. Fat requirement

If the diet is below the metabolizable energy (ME) requirement for normal growth and reproduction, carbohydrates in the diet are reduced and replaced with fat. Normally, mice diets contain approximately 5% fat.

Mice diets should contain linoleic acid and arachidonic acid. However, the exact required amounts have not been clearly determined. Similarly, mice also require linolenic acid, but the precise amount is unknown.<sup>27</sup>

### 10.4. Mineral requirements

Mice primarily need minerals such as calcium, phosphorus, manganese, iron, zinc, and potassium (Table 5). They also require other minerals that are not listed here, although the exact quantities have not been definitively stated. However, it is assumed that these needs are met through their diet.<sup>12</sup>

**Table 5.** Mineral composition of mice diets.

Mineral	In Diet
Calcium, %	1.23
Magnesium, %	0.18
Phosphorus, %	0.99
Potassium, %	0.85
Sodium, %	0.36
Iron, mg/kg	255.50
Iodine, mg/kg	1.9
Zinc, mg/kg	50.3
Copper, mg/kg	16.1

#### 10.4.1. Calcium and phosphorus

Calcium and phosphorus are usually considered together due to their shared physiological roles. They are mainly involved in the structure of bones and teeth. The calcium requirement for mice is reported to be 0.5% in purified diets and 2.1% in natural diets. Phosphorus levels in various diets range from 0.3% to 1.2%. The Ca/P ratio in the diet is important for normal mineral metabolism and varies depending on age. In general, a ratio of 2:1 or 1:1 is recommended.<sup>22</sup>

Calcium deficiency reduces body weight gain, bone development, and serum calcium levels. However, mice have a more effective adaptation mechanism, making calcium deficiency less impactful. They compensate in two ways: 1) By increasing the concentration of calcium binding protein in the duodenal mucosa. 2) By slowing down skeletal development to conserve calcium. As a result, poor growth is more commonly observed in mice than osteoporosis.<sup>12,27</sup>

#### 10.4.2. Iron

Mice also require iron for growth and reproduction. A dietary iron level of 25-100 mg/kg is sufficient for normal growth and hematopoiesis but may be inadequate for storage. Iron deficiency can lead to characteristic symptoms of anemia, reduced birth weight, and decreased litter size.<sup>12,27</sup>

#### 10.4.3. Magnesium

Magnesium is another essential dietary element for mice, though its optimal intake level has not been clearly defined. A purified diet containing 0.07% magnesium supports normal growth and development. Although 0.04% magnesium is sufficient for basic survival, a minimum of 0.07% is required to meet the increased demand.

ds of lactating mice.<sup>12,27</sup>

#### 10.4.4. Zinc

Zinc is an essential element for mice. When found in a natural feed based diets at levels of 50-58 mg/kg, it is sufficient to support optimal growth and reproduction. Zinc deficiency can result in hair loss on the shoulders and neck, cachexia, and decreased catalase activity in the liver and kidneys.<sup>12,27</sup>

#### 10.4.5. Sodium and chloride

Sodium is typically added to diets as sodium chloride (NaCl) at rates between 0.5-1.0%. It has been shown that levels between 0.36-0.49% are adequate for normal growth and reproduction. Deficiency in sodium can lead to reduced appetite, slower growth, and decreased storage of energy and nutrients in the body.<sup>12,27</sup>

#### 10.4.6. Selenium

Selenium and vitamin E are known to have overlapping biological activities across all animal species. Mice can tolerate up to 40 mg/kg of selenium in the diet. The immune response in mice is reported to be optimal when dietary selenium levels are around 1.25 mg/kg. Both lower and higher levels have been associated with a reduced immune response.<sup>12,27</sup>

#### 10.4.7. Potassium

The potassium requirement for growth in mice has been determined to be approximately 0.2%. A natural feed based diet containing 0.82% potassium supports healthy growth and reproduction. Symptoms of potassium deficiency include dull eyes, coarse fur, dry and flaky tails, and general weakness.<sup>12,27</sup>

### 10.5. Vitamin requirements

Vitamins are generally not synthesized by animals and must be obtained from the diet. The required amount varies depending on the mouse's ability to absorb nutrients, the presence of antagonistic compounds, and the balance with other dietary components.<sup>29</sup>

Estimated vitamin requirements for mice vary. The recommended levels are presented in Table 6.<sup>29</sup>

**Table 6.** Vitamin requirements of mice.

Vitamin	In the Diet
A, IU/kg	15.000
D, IU/kg	5.000
E, IU/kg	37
B 12, mg/kg	0.03
Riboflavin, mg/kg	8
Niacin, mg/kg	82
Thiamine, mg/kg	17
Pyridoxine, mg/kg	10

#### 10.5.1. Vitamin A

The daily vitamin A requirement for all species is estimated at 25-39 mg per kg of body weight. This corresponds to approximately 1-2 IU/day or 250-500 IU/kg of diet.

Most standard mice diets contain higher levels, typically 4000-5000 IU/kg. Vitamin A deficiency may lead to symptoms such as tremors, diarrhea, coarse fur, abscesses, growth retardation, abortion, and infertility in males.<sup>12,27</sup>

#### 10.5.2. Vitamin D

No signs of vitamin D deficiency have been observed in albino mice fed natural diets containing 150 IU/kg of vitamin D. Most natural diets contain around 5000 IU/kg, which has been found to be adequate for reproduction.<sup>12,27</sup>

#### 10.5.3. Vitamin E

In females fed diets deficient in vitamin E, fetal resorption was observed, though testicular degeneration was not found in males. It has been reported that a minimum of 350 µg of  $\alpha$ -tocopherol per day is required during the first pregnancy, equivalent to approximately 70 mg/kg in the diet. Vitamin E deficiency can cause convulsions, heart failure, fetal resorption, muscular dystrophy, and hyaline degeneration. Since vitamin K is synthesized by intestinal microbes, it generally does not need to be added to the diet.<sup>12,27</sup>

#### 10.5.4. Water soluble vitamins

##### 10.5.4.1. Thiamine

For normal growth, a diet must contain 3 mg/kg of thiamine. For reproduction and lactation, this requirement increases to approximately 20 mg/kg. In cases of thiamine deficiency, severe convulsions, circular movements, cerebral hemorrhaging, decreased feed intake, stunted growth, and early death can occur especially when the animal is held by the tail for a few seconds.<sup>12,27</sup>

##### 10.5.4.2. Riboflavin

The riboflavin requirement for normal growth in mice is 4 mg/kg in the diet. A level of 7 mg/kg is considered optimal, providing sufficient support for both lactation and reproduction.<sup>12,27</sup>

Mice fed a diet containing only 0.4-0.6 mg/kg of riboflavin become weak, grow slowly, and typically die within 9 weeks. Deficiency symptoms include atrophic or hyperkeratotic epidermis, spinal cord myelin degeneration, and corneal vascularization with ulceration.<sup>12,27</sup>

##### 10.5.4.3. Pyridoxine

A dietary level of 1 mg/kg of pyridoxine is sufficient to support growth in mice. However, pyridoxal and pyridoxamine are found to be less effective. Pyridoxine deficiency can result in slow growth, hypersensitivity, hind-limb paralysis, and hair loss.<sup>12,27</sup>

##### 10.5.4.4. Vitamin B12

For normal growth, reproduction, and lactation, a dietary level of more than 5 mg/kg of vitamin B12 is necessary. Since vitamin B12 is synthesized by intestinal bacteria, exact requirements are difficult to determine. The minimum effective level has been reported as 5 mg/kg. However, higher amounts may be needed if the animal is treated with antibiotics, raised in a germ free

environment, or if coprophagy is prevented. In cases of deficiency, young mice exhibit stunted growth, kidney atrophy, stillbirth, or neonatal mortality.<sup>27</sup>

### 11. Water requirements

Clean and continuous access to water is essential. The presence of vegetables or moist foods in the diet reduces the need for water intake. Water restriction decreases feed consumption. Environmental temperature also affects water consumption. For instance, mice on a dry diet kept without water for just one day at 24-26 °C may die. A 25 gram mice typically requires 6-7 ml of water per day. Sick mice consume even less and dehydrate quickly.<sup>12,29</sup>

Water consumption is influenced by factors such as ambient temperature, altitude, feed composition, and quantity. Water is a potential source of pathogenic microorganisms; thus, it should be microbe free, particularly free from *E. coli*. Chemical contamination risks should be considered, and contamination levels should be monitored. Water should also be free of toxic minerals.<sup>12,29</sup>

### 12. Types and forms of mice diets

Mice diets are formulated in various forms pellets, flour, gel, or liquid. Pellet feeds are the most convenient and minimize waste. Flour based diets are typically used when test components are added but are generally less suitable for routine feeding. Gel diets are created by mixing 3% agar with flour in equal parts and are often used in toxicological studies. Liquid diets are developed to meet specific needs, such as filter sterilization.<sup>3,27</sup>

Standard mice diets are designed to meet the needs of maintenance, reproduction, and growth. Maintenance diets are low in fat (4-5%) and contain 12-14% protein, whereas reproductive diets require 17-19% protein.<sup>3</sup> These diets should also be appropriately balanced for amino acids, vitamins, and minerals. Nutrient levels are often based on studies conducted in rats, as their nutritional requirements are similar.<sup>30</sup>

Modern mice diets generally come in pellet form with diameters ranging from 8-12 mm. These include one or more cereal grain sources, protein sources such as fish meal, meat meal, or dried milk, and a premixed blend of vitamins and minerals. Some diets clearly specify ingredient proportions, while others only list nutrient names without quantities.<sup>30</sup>

### 13. Conclusion

Successful research depends on maintaining mice in optimal health and providing high quality care and nutrition. The physical condition and performance of laboratory mice are directly influenced by their environment and feeding. Therefore, proper husbandry and diet are critical in all experimental studies to ensure valid and reproducible results.

### Ethical approval

This study does not require approval from the Ethics Committee.

### Authors contribution

TMB: Research, Planning, Article scanning. TŞ: Writing original draft and Review. The author approved the final version submitted.

### Conflict of interest

There are no conflicts of interest associated with this research publication, according to authors.

### Data availability

The data that support the finding of this study are available from the corresponding author upon reasonable request.

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