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Laboratuvar Kemirgenleri, Sindirim Fizyolojisi ve Beslenmeleri ile İlgili Önemli Konulara Genel Bir Bakış

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ÖZET

Biyomedikal araştırmalardaki mevcut eğilimler, çeşitli hastalıkların altında yatan mekanizmaları anlamak açısından uygun deneyler planlanmasında önemli katkıları bulunan deney hayvanlarının kullanımına küresel olarak güvenmektedir. Çoğunlukla kemirgen olan bu hayvanlar; köklü gen haritaları, kolay uygulanabilirliği ve ıslahta çok sayıda yavru üretme yeteneği gibi bazı özelliklere sahip olması yönünden biyomedikal araştırmalarda geniş ölçüde tercih edilmektedir. Bu hayvanlar karakteristik sindirim sistemi yapıları nedeniyle farklı diyetlere karşı yüksek uyumluluğa sahip olup insanlara zarar verebilecek enfeksiyöz olmayan ya da genetik hastalıklara karşı yüksek duyarlılık göstermektedir. Kemirgenler üretim ve deneysel amaçlara bağlı olarak her bir tür ve suşa özgü yaşama payı ve verim payı gereksinimlerini karşılayabilen diyetlerle beslenmektedir. Bitki ve/veya hayvan kaynaklı çeşitli yem ham maddelerinden oluşan pelet haline getirilmiş veya ekstrüde edilmiş formdaki ticari diyetler, bu hayvanların beslenmesinde oldukça yaygın şekilde kullanılmakta olup yapılan araştırmadaki gereksinimlere göre hazırlanmaktadır. Bu derleme çeşitli amaçlarla kullanılan laboratuvar hayvanlarının orjini, tarihi, yemleme davranışları ve suşları ile ilgili detaylı bilgi vermektedir. Ayrıca bu hayvanların besin madde gereksinimleri; diyetlerinin özellikleri, tipi, içeriği, fiziksel formu ve çevresel faktörlerin diyetlerine etkisi tartışılmaktadır.

An overview about Laboratory Rodents, Digestive Physiology and Important Issues regarding Their Nutrition

Review

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ABSTRACT

The current trends in biomedical research globally rely upon experimental animals for their pivotal contribution in proper designs of experiments to understand the underlying mechanisms of various diseases. These animals which are mostly rodents; are broadly preferred in biomedical research for certain characters like well established gene maps, easy adaptability and ability to produce large number of offsprings at breeding. Besides, these animals are well known for high compliance to different diets beacuse of their characteristic digestive system structure, and high susceptibility to targeted non-infectious or genetic diseases which may cause harm to humans. Rodents are fed with different diets that meet their maintenance and productivity requirements specific to each specie and strain, highly depending upon the production or experimental purposes. Commercial diets in pellet or extruded form consisting of various feed raw materials of plant and / or animal origin, are the most widely used diets for feeding and special diets are also prepared according to the requirements of research. This review deals in comprehending the details about orgin, history, feeding behaviours and strains of variously used laboratory animals. In addition, nutrient requirements of this animals; characteristics, type, content, physical form

of diet and effects of environmental factors on their diet have been discussed.

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1. Introduction

Laboratory animals have been opted on the basis of results of various experiments and are being used to counter and eliminate the potential adverse health effects of various chemicals and health issues encountered in other living things (humans) or to shed light on the solution of these problems. Besides, in order to assess the safety and efficacy of various products like immunotherapeautics, drugs, food additives, industrial chemicals, both herbisides and pestisides, the regulatory and monitoring testing requires the use of laboratory animals with the aim to safeguard human and animal health [1].

There has been a long history of using laboratory animal models of human diseases for drug testing and validation and still is the common practice among biomedical researchers and scientists [2]. The increasing use of these animals nowadays has played a crucial role in the accuracy and reliability of the results of the studies in terms of reproducibility, which is one of the basic reliability criteria of the studies [3]. Experimentalts researchesand trials on laboratory animals are mostly conducted in the fields of medicine, biology and veterinary medicine. According to the trials performed on rodents in the fields of biomedical researches such as ethics, physiology, microbiology, immunology, oncology, pharmacology, behavioral sciences, neurology and surgical sciences, the results obtained are more reliable in order to switch the trials to human models. It is stated that these animals are the most suitable options for biological and behavioral studies. Besides, physiological or established pathological conditions could be searched easily and can be applied as models to other animal and human health fields [4].

This review emphasies on the need, significance and choice of proper laboratory animal suiting the requirements of good experimental designs and their essential components, along with systematic reviews about history, housing and feeding of laboratory animals being used in biomedical research.

2. Physiological Characteristics of Digestive System in Rodents

All activities of the digestive canal and related glands that involve disintegration and breakdown of feed particles, the absorption of nutrients and by products and the removal of residual products starting from the mouth and ending with the anus, is called digestion. This event involves the certain physicochemical events that are usually initiated and triggered by smaller fraction of the food received into the body. The main task of the digestive tract is to provide water, electrolyte and nutrients to the body continuously. Chewing of nutrients in rodents occurs very quickly. The engraving and gyration functions are well developed by the forward and reverse movements of the lower jaw [5, 6]. The digestive system in rodents consists of glands associated with the digestive tract [7]. For digestion and absorption of nutrients, nutrients must pass through digestive tract at an appropriate rate [8]. Physical, chemical and microbiological factors play an important role in the breakdown of nutrients taken into the digestive tract into the foundation stones that can be utilized by the organism [6, 9].

In this way, after the carbohydrates and polysaccharides are broken down into monosaccharides, proteins to amino acids, fats to fatty acids and glycerol, after passing through the mucous membrane of the gastrointestinal tract, these are absorbed into blood and lymph. In monogastric animals such as rats and mice, the microbial activity in the large intestine is low. These animals mainly process food compounds through digestive enzymes and acids [6].

In rats or mices, the functionality of the digestive system and the secretion of digestive enzymes is limited to the hydrolysis of the components from the maternal milk. Serous glands of the tongue produce a lingual lipase that is important in the digestion of milk triglycerides [10]. Salivary glands both in neonatal rats and mice, are not functionally developed compared to serous glands. Saliva secretion is a reflex process involving various receptors, afferent nerves, saliva centers, secretory and vasomotor efferent nerves, blood vessels and gland cells. When the feed is placed in the stomach or intestine of the laboratory animal without running mental reflexes, the saliva secretion increases. Especially when irritation-causing foods are swallowed, or in case of nausea and disgust as a result of any gastrointestinal disorder, salivation secretion increases by these reflexes. Swallowed salivation can attenuate the effects of harmful substances by diluting and neutralizing them to overcome the rising disorders because of these harmful agents [5, 6].

The composition of the diet changes the function of the digestive system. Intestinal hydrolases enzymes like maltase, sucrase, isomaltase and trehalase which are involved in the digestion of carbohydrates cannot be detected in the intestines of rats during the first two weeks postnatal, but their activity keeps on increasing. Amylase, chymotrypsin, trypsinogen, and lipase activitiesare found to be little changed before weaning butare observed to be increased during weaning [11]. Gastric secretion of acids and pepsinogen reaches to same level as that of adults in third and fourth postnatal weeks and coincides with the shift and transition to solid food [10].

Although, the enzymatic changes in the gastrointestinal tract linked with weaning seem to be directly related to the shift from milk to solid food, there is also evidence that the major cause of this change is of no dietary origin. However, glucocorticoids, thyroxine, glucagon, gastrin, cholecystokinin, prostaglandins and insulin also affect the postnatal development of the gastrointestinal tract. The digestive capacity is almost constant with normal feeding in older rats [11].

3. Nutrient Requirements of Rodents

While formulating a diet and nutrition for rodents, it is noteworthy that the maintenance requirements necessary to carry out vital processes necessary for survival of rodents must be considered. Besides, the productivity requirement which include productive processes such as growth, reproduction and lactation (pregnancy, milk and reproductive fertility) must be met. Therefore, these animals need to be fed with diets containing sufficient and balanced amounts of energy and nutrients they need for maintenance and productivity [11, 12]. Feeding of animals with an inadequate diet in terms of content or quantity adversely affects growth, reproductive performance, as well as resistance to diseases due to their immune response. In fact, some anatomical and physiological changes caused by a deficiency or a large amount of feeding directly affect the results of biomedical research in laboratory animals well before changes occur in animal performance [11]. In general, the nutritional requirements of rodents are influenced by various factors, same as in other animals. These factors include the rodent’s type, sex, breed, strain, age, live weight, gender, physiological status [6, 11, 12]. It is stated that dietry components and feeding behaviours can affect the physiological and metabolic patterns in laboratory animals and it can also affect the results of end experiments [13, 14]. In fact, individual differences in animals during the study may affect the outcome of the study and should be taken into consideration, especially in the metabolism studies, in which the nutrient contents cannot meet their needs. Genetic diversity prevailing among rat strains, stocks, sexes and individuals may affect nutrient requirements [12]. For instance, it has been stated that genetic differences in growth potential among different strains and sexes notebaly influence the daily requirements for amino acids and other nutrients in laboratory animals. In some studies [15, 16] which include the genetic samples, the emergence of non-overlapping results in feeding with the same nutrients suggests that the nutrition physiology of this animals should be well known. Therefore, it should be noted that there are differences between individuals up to 15% or 20% for many nutritional parameters in individuals with similar age, sex and genetic structure [11].

4. The Importance of Diet in Rodent Nutrition

Diet is one of the most important environmental factors affecting the health, well-being, reproduction, growth and response to experimental manipulation in rodents [3, 17]. Rodents need a diet containing 50 essential nutrients. The adequacy of the diet is indicated by growth, maintenenance and reproductive performance. However, the nutritional maintenance requirements for rodents in long term studies are practically unkown [18]. The rats in their growing stage have greater protein and amino acid requirements than older rats in state of maintaninence [19].

A nutritionally balanced diet should contain all the relevant entities of those nutrients that meet the daily needs of the animals with high bioavailability of nutrients, should be delicious and free from biological and chemical pollutants. Besides good transport and storage conditions must be provided for the prepared diet being fed to animals. Unfortunately, except protein, the dietary requirements for maintenance of adult rats have not been carefully studied [18]. The minimal dietary concentration of protein needed for growth, reproduction and maintenance have been approximated to be 15%, 15% and 5%, respectively [12]. Nephropathy is often associated with overfeeding of protein, a common diet-related disease observed in Sprague-Dawley, Wistar and F-344 rats. However, it has been found that limiting protein intake without restriction of calories had only minor influence on renal disease and longevity [20, 21]. Therefore, the diet used should be prepared for production or for experimental purposes. Such mechanisms affect the reproducibility, which is one of the reliability criteria of the research results [17].

Although, there is no definite carbohydrate requirements for rats but laboratory animals need carbohydrates for reproduction and lactation. Rats don't possess the enzymes to digest fibers so if they are getting any calori from fibers, the reason is because of bacterial fermentation in caecum. The effects of fiber depend on its source and properties (solubility viscosity, fermentability). Fiber enhances rats fecal bulk and the change in size and weight of cecum and colon is also associated with fiber [20]. Rat well utilize starch and glucose but if they are fed with sucrose and fructose it can lead to metabolic abnormalities in both male and female rats and neprocalcinosis in female rats. Lipids are also necessary as these are major sources of essential fatty acids like omega-3 and omega-6 and are involved in absoroption of fat soluble vitamins except their use as extensive energy source [12]. Diet lipid contents are expressed as 5% by NRC for the growth and maintenance requirements of rats. Besides, mega-nutrients, minerals like calcium and phospohrus and their dietary ratio of is of major significance.

The relative increase in nonphytate phosphorus levels than calcium in the diet, results in nephrocalcinosis with mineral deposits at the cortico-medullary region [22]. This process causes renal hypertrophy and degenerative renal tubular changes, especially in female rats. Generally, nephrocalcinesis is more common when rats are fed purified diets The NRC [12] recommend a calcium to phosphorus molar ratio of 1.3 to prevent nephrocalcinosis. Nutrient bioavailability must also be validated in providing the proper amount of nutrients for rats because the nurients found in any natural diet are not 100% bioavailable.

Laboratory rodents are usually fed *ad libitum* [23]. In this type of feeding, animals will be able to regulate/adjust their feed consumption to meet their energy requirements. When the energy requirement is met, the animal usually stops eating. If high-energy diets (an excess of fat or carbohydrate and an increase in energy density) are used, nutrient quantities in the diet must be increased in order to compensate for reduced feed intake, otherwise may lead to nutritional deficiencies. In addition, feed consumption may affect the energy density of a diet and the capacity of the gastrointestinal tract, as well as reduced consumption in cases of insufficiency [24, 25]. Hyperglycemia occurs when non-diabetic, weak and active rodents living in wild and difficult conditions are fed on an energy-rich diet [26].

Feeding rodents *ad libitum* leads to long-term health problems, such as increased obesity, shorter survival, more degenerative kidney and heart diseases and increased cancer at an earlier age. Therefore, the restricted feeding program, which means limiting the amount of food accessible to animals, is implemented in rodents instead of *ad libitum* feeding. It is stated that restricted feeding in rodents is more effective on health [27] and to increase resistance to experimental stress conditions [13]. Furthermore, gavage / stomach tube is often utilized to avoid the possible adverse effects of a bad taste on food intake in order to determine the effect of compounds such as drugs or nutrients [23]. Orogastric gavage is a common technique used in rodent toxicology and pharmacology studies [28]. However, this application has some disadvantages, such as it eliminates the physical effects of chewing and the effect of salivary enzymes in the digestive process besides causing stress in animals [23, 28].

5. Types of Diets for Rodents

The type of diet used in rodents varies depending on the production or experimental purposes [11, 12, 23]. In these animals, generally four types of diets are used. These include:

Natural ingredient diets which contain whole grains (eg, corn, ground wheat), mill by-products (eg, wheat bran, wheat middlings, corn gluten meal), high protein meals (eg, soybean meal, fishmeal), mined or processed mineral sources (eg, ground limestone, bonemeal and other ingredients etc (eg, dried molasses, alfalfa meal). Commercial diets are the most widely used natural diets in the feeding of rodents and some special diets are prepared according to the requirements in the researches. These type of diets are relatively inexpensive, palatable and variable nutrient, but are at risk of contamination with pesticide residues, heavy metals or other substances [12].

Fixed formula diets are prepared from clear formulas which consist of fixed amounts and igredients which usuallay do not change, formulas are clearly prepared. These diets may contain many sources of protein, fat and carbohydrates, and also bioavailability of nutrients is high. Any information regarding the changes in these diets is not disclosed to the public becuase of proprietary concerns [3].

Purified diets are pure in nutrient contents, having a less risk of chemical contamination, which are less variable and easier to control by formulations. In these formulations, pure and invariant ingredients are used. In these diets, the casein and soy protein isolates afre used as protein source, sugar and starch as carbohydrate sources, vegetable / animal fats as a source of fat, a chemically extracted cellulose as a source of fiber and also chemically pure inorganic salts and vitamins are used [12]. The composition of these diets, which consist of expensive, more purified macronutrients and pure chemicals and are less delicious than diets with natural ingredients and have an unpelletable form. Feeding the diet to rodents in powder form can cause incisor teeth to be too long which adversely affect the health of rodents [29]. These diets, are often used in studies with specific nutritional deficiencies and excesses. Therefore, considering the fact that the standard feed cannot be used in every study, it is important for these animals to use purified diet in which different feeds are used for each research [3, 29].

Chemically defined diets are formulated using chemically pure and synthetic nutrients such as amino acids, fatty acid esters, glucose, vitamins and mineral salts. The intake of these diets is difficult by many laboratory animals and these are relatively very expensive diets [12]. The nutritional contents of laboratory animal diets in our country (mostly for rats and mice) are generally as follows: crude nutrients (crude ash, crude fiber, crude fat, crude protein, nitrogenfree extractives) and moisture, also metabolized energy, carbonhydrates, vitamins, minerals, trace minerals, aminoacids and fatty acids. However, it is noteworthy that these diets cannot meet the requirements of every physiological period in which the animal exists and the information about the feed raw materials used in its production and presentation of the content information to the market is not clearly stated [24].

6. Feedstuffs Used in Rodent's Diets

Mice and rats are omnivorous. These animals mostly consume vegetable materials such as various cereal grains (such as barley, wheat, corn, oats, sunflower seeds, cannabis seeds). These rodents also feed on germinated cereal grains and salad, lettuce, beet in summer, carrots in winter, as well as animal origin materials such as meat-fish meal, milk, even bread soaked in water or milk. Milk containing fish oil can also be given [12].

Herbivorous guinea pigs (Guinea pigs) are fed with salads, lettuce, puffer, clover and alfalfa as green feed, as well as carrot, turnip, hay, barley, oat and bran. Since the vitamin C level in concentrated feeds should be 10 times the normal requirement, guinea pigs can be given germinated grain feeds as a source of vitamin C, as well as fresh green feeds such as clover, clover grass, cabbage and spinach. Hamsters eat various plants, their seeds, fruit and meat. They prefer cereals such as wheat, corn and sunflower, pumpkin seeds, fresh greens, vegetables, carrots, cabbage, snapdragon and lettuce. Insects, scrambled eggs and meat can also be given. Gerbils prefer cereals, sunflower seeds and vegetables such as carrots and cucumbers. They feed on plants, seeds and insects in the wild [30].

In addition, the incidence of coprophagia, which refers to oral ingestion of the secum contents in rodents, is observed. The secal feces are softer and lighter in color than normal feces, and animals can distinguish these feces from normal feces. In fact, cecal feces constitutes 50% of the daily diet of these animals [31]. Caprophagia allows the animals to benefit from vitamin K and B_{12}, digest the microbial proteins and restore the microbes to the digestive tract [5, 6]. Therefore, the prevention of coprophagia for laboratory animals reared in germ-free environments affects the content and quantity of prepared diets, and a richer diet should be formulated with extra vitamins keeping in mind the requirement of an animal [32].

7. Physical Form of Diets

Diets for laboratory rodents are prepared in different physical forms. The most common form used in the diet of these animals is pelleting [12]. Pelleting is the combination of powder feeds through a molding process with a mechanical process under humidity, temperature and pressure.

Many factors affect the pellet quality. In general, these factors can be grouped as factors related to eating (physical and chemical properties of feed, formulation) and applied technology (water vapor application, tempering, oil addition, matrix properties and cooling). The particle size of the powder feed to be pelleted from the physical properties of the feed has the most important effect on pellet quality. Quality pellets are often produced by pelletizing mixed feeds containing smaller particles. It helps in increasing the particle surface being exposed to water vapor used during pelleting procedures [18, 33]. Moisture, fat, starch, cellulose and protein content in mixed feed are also very important in terms of pellet quality. The increased protein content of the feed positively affects the pellet quality, while the cellulose content adversely affects the pellet quality. Raw materials such as wheat, barley and canola can be easily pelleted due to the substances that bind the feed particles in its structure, while corn is relativley difficult to pellet. The pelleted feed has advantages such as; easy handling and storage, in preventing dehomogenization, decreased feed loss, increased feed density, increased digestion of starch and protein, increased palatability of feed, decreased pathogenic microorganisms and reduced transport costs [33].

Another diet form used in rodents is an extruded diet, which is similar to the pelleted diets [12]. Extrusion in the production of this diets is a process in which the feed is squeezed and pushed through the tapered holes under pressure. This process generally allows the grains to be ground and then wetted to soften and to force the material to pass through a steel tube under pressure by means of a screw. The starch contained in the exuded material is highly (80%) gelatinised. The extrusion process is effective in inactivating antinutritional factors and increasing the utilization of fat energy [32, 33]. However, extruded diets are not widely used in laboratory rodents due to increased waste during feeding and high production costs [12].

8. Effects of Environmental Factors on Diet

The environments in which the rodents are reared and the social and physical enrichment of these environments should be established in such a way that rodents could easily demonstrate their specific behavior and meet their physiological requirements [34, 35]. Environmental conditions that depend on seasonal changes such as temperature, lighting and ventilation affect the feeding and drinking systems used in rodents. From such conditions, energy requirements in animals exposed to temperatures below the lower threshold of the thermo-neutral zone are increasing, because energy is required to consume energy to maintain a constant body temperature. High temperature, disturbing stimuli, social conflict or other environmental factors that reduce feed consumption require increased amounts of nutrient in the diet due to reduced feed consumption [12]. If diets contain highly unsaturated fats such as fish oil, it is suggested to give fresh feed daily to animals or antioxidants should be used to prevent oxidation of fats [11].

Cage systems should be developed according to the characteristics rodent species and the animals should be fed with suitable feeds for these cage types [36]. Accordingly, it is stated that animals can be fed with standard diets in conventional cages, while they can be fed with sterile feeds in hepa filtered and fully protected systems such as individually ventilated cages. In the individually ventilated cages system, it is preferable to use standard diets that are sterilized by autoclaves instead of sterilized special diets for financial reasons [37].

In the autoclaving method, which is among the most practical sterilization procedures, the temperature duration should be taken into consideration in order to prevent the loss of nutrients in the diet. Otherwise, because of the nutrient losses that may occur in diets during autoclaving sterilization method, a decrease in body weight in certain weeks in mice consuming this diet was observed [38]. In addition, diets should be kept under suitable cold and dry storage conditions where wild rodents could not enter [11]. Besides, palatability of feed is an important factor that ensures regulate the intake of the minimum level of nutrients. If an unknown substance is added to the diet, the palatability may change. Therefore, in order to evaluate the effect of the substance to be used, a few animals must be acclimated during a certain period of time before the experiment is executed in rodents [29].

9. Conclusion

The nutrition of rodents is the most preferred subject and has many postive effects while using them as laboratory animals in biomedical researches. It is of great importance that diets of laboratory animals must be prepared while keeping in mind the socially and physically challenged environments. The formulation should also make sure the high bioavailability of nutrients, deliciousness and it should lack of biological and chemical pollutants. The diet should be sufficient in terms of content and quantity of well-known chemical and natural ingredient in order to meet the maintenance and productivity requirements of laboratory animal depending on production or experimental purposes for each species and species-specific strains.

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