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NUTRITION OF LABORATORY ANIMALS

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Animal experimentation is a crucial step involved in biomedical research in the development of biomedical devices and drugs. The quality of the laboratory animal which is grown in controlled conditions depends totally upon the quality of its supplies in the form of day light cycle, feed, water, primary (cage) and secondary (room) enclosures, air exchange, room temperature, relative humidity, noise levels and illumination levels. Further, the quality of these supplies will decide upon the quality and authenticity of research data obtained from these animals. The broad area of laboratory animal nutrition encompasses the nutritional value of the diet, palatability, features supporting behavioral adequacies (like hard stuff for Gnawing and burrowing rodents) and the safety features of the diets such as ability to withstand sterilization and the effects of the ingredients in the diets on experiments. When compared with farm animal nutrition, laboratory animal nutrition gives a lesser weightage to cost effectiveness. It invests a lot more in quality assurance of diets. The concept of ad libitum feeding is followed generally in small laboratory animals. But studies shows that restriction of calories will increase the life span, and reduces incidences of tumors in laboratory rodents. Withdrawal of feed after a limited time offer is done to restrict calories. Cafeteria feeding is another type of feeding system used when quality of two different diets is in question. Two cups with two different types of feed are offered simultaneously to a cage and observations are made based upon preference to a particular feed type.

The guinea pig even though a rodent; needs a compulsory supplementation of dietary Vitamin-C and hence they are known as scorbutic animals.

Dietary Requirements

Nutrient requirements of various species of Laboratory animals are given in Table 1.

Feed Ingredients

Adding animal bye products to feedstuff may reduce the bioavailability of nutrients like protein and eventhough these feedstuff have a good analytical value of crude protein and energy, they may not be good for the animal's growth and reproductive performance. Pellet size is also important and usually 6mm diameter pellets are used for feeding Guinea pigs and rabbits while 10-12 mm pellets are used for rats and mice. Ascorbic acid supplementation @ 50mg Vit-C/100g diet is added to the feed of guinea pigs while compounding.

In addition, it is advisable to supplement the animals with sprouted Bengal gram, Vitamin supplements in drinking water and Lucerne grass if we are feeding animals with commercially available lab animal feed.

Vitamin Mixture Composition

a-Tocopherol acetate	12.0g
Menadione	0.15g
Thiamine	1.2g
Riboflavin	0.5g
Pyridoxine	0.6g
Niacin	1.0g
Pantothenate	1.2g
Cyanocobalamine	0.5g
Folate	0.1g

Table 1: Nutrient requirements of various species of Laboratory animals

Nutrients	Mouse	Rat	Hamster	Rabbit	Guinea pig
Crude Proteins %	18	12	15	17	18
Crude Fat %	5	5	5	2	1
Crude Fiber %	5	2-5	2-5	10-12	10-12
Digestible Energy Kcal/Kg	3000	3800	4200	2500	3000

Table 2: Components of a Laboratory animal Diet. (Courtesy- NCLAS, NIN, Hyderabad)

Sl.No.	Ingredients in Pellets	Proportion in Rats, Mice, Hamsters	Proportion in Rabbits and guinea pigs.
1.	Wheat Flour	22.5%	61.3%
2.	Roasted Bengal gram flour	60.0%	28.2%
3.	Casein	4.0%	1.0%
4.	Refined ground nut oil	4.0%	5.0%
5.	Skimmed Milk Powder	5.0%	Nil.
6.	Vit. Mixture	0.5%	0.5%
7.	Salt mixture	4.0%	4.0%

PABA	10.0g
Biotin	40.0g
Inositol	10.0g
Choline chloride	100.0g

Mineral mixture composition

Dicalcium phosphate	1250g
Calcium carbonate	555g
Sodium chloride	300g
Magnesium sulphate	229g
Ferrous sulphate	50g
Manganese sulphate	16g
Potassium iodide	1g
Zinc sulphate	2.1g
Copper sulphate	1.9g
Cobalt chloride	0.012g

Scale of compounded ration for Lab animals

Rat (g/day)	15-20
Mouse(g/day)	5
Hamster(g/day)	15
Rabbit(g/day)	50-100
Guinea pig(g/day)	25

Assessment of Diets

The proximate principle analysis forms the outline of feed quality assessment. But even if a feed meets the specifications as per proximate principle analysis, it may not be a premium feed for lab animals. The reason lies in a single term known as bioavailability. So feeding trials and data generation and assessment is also required to assess the feed quality. In India, we have a lot of commercially available lab animal feeds. These feeds are natural ingredient feeds and so generally having variations in their major and minor nutrients from batch to batch. Practically, selection of feed is done by observation of performance of the feeds in animal reproduction. Extra allowance in the form of sprouted grams, carrots and fresh grass are also used in rabbit and guinea pig rations. In conventional colonies, a low concentration (0.1%) potassium permanganate solution applied for one hour is used to destroy contaminants present in organic matter by oxidation without affecting the palatability of vegetables.

Among rodents, the thumb rule is that if the feed meets the requirements of a lactating mouse, we may assess the diet to be good for rats and mice in any physiological status. Most of the commercial feed manufacturers use animal bye products like fish meal and waste from slaughter houses to raise the crude protein content in the diet. Such sources have poor bioavailability and can also act as a potent source of Salmonellosis in the colony. So, it is better to conduct a study with the available diet in animals on their performance. Evaluate animal feeds by feeding trials is the most appropriate and practical means. Usually studies are done in breeding mice especially the mice intensively bred by making use of post partum estrous (Here, mice are bred and males are not separated; after giving birth to young ones and while lactating the pups, they exhibit

an estrous in which the male will mate and the animal will again get pregnant. So at the day of weaning of the first set of young ones, next set of young ones are delivered by the mother and again lactation starts- so no rest). Mice are used because of its highest metabolic rate in comparison with any other laboratory animal. Feed these animals with the diet in question, and take data (Rate of conception, Birth weight, litter size, mother's weight, weaning weight, pre weanling mortality etc.) of 2-3 litters. This data may be evaluated to assess any feed.

A wide array of purified diets and natural ingredient laboratory animal diets of assured quality is available in European and US markets. They offer medicated diets also for example Fenbendazole diet is available for treatment of pin worms in rodents. The drawbacks in depending on any feed company situated outside our country is that, practically it is difficult to ensure an uninterrupted supply and the high cost of feeds and its import.

Storage of Food

The natural ingredient diets can be used up to about 6 months after manufacture while Vit-C in diets has only 3 months of shelf life. Exposure to temperatures above 21°C can deteriorate the feed quality. Food shall be stored off the floor in racks/ trolleys/slabs for better cleaning and viewing. Torn/ unsealed bags of pelletted feeds shall ideally be rejected or if not viable shall be checked for fungal growth, insects or any sort of spoilage and ideally be irradiated or autoclaved before usage. Insects are notorious for their capabilities as intermediate hosts for rat tapeworm Rodentolepis diminuta in any rodent colony. Storage of a newly received consignment of feed bags shall be kept under the older set of feed bags since the older consignment obviously reaches the expiry date first (this requires proper supervision, motivation and training to animal care staff) and hence the older bags from top portion of the pile will always be used up first.

Quality Assurance

Quality assurance of laboratory animal feed has to be done in order to analyze the proximate principles as well as chemical and microbial contaminants. Ideally, batchwise microbiological screening of feed (Especially for Salmonella and E. coli) immediately after opening the feed packets has to be done. In a barrier maintained facility with gnotobiotic/specific pathogen free laboratory animals, any supply will pass through a sterilization/ decontamination procedure and so does the animal feed and steam sterilization is the most commonly equipped tool for sterilization of animal feeds. It is well known that, heat will coagulate proteins and hence essential amino acids will get affected. It also will destroy vitamins especially Vitamin-A, Vitamin-C and Thiamine. So the nutritive value will be reduced. So if autoclaving is done, extra allowances for vitamin supplementation and a decontaminated source for protein like sprouted Bengal gram has to be given. Also sticking together of feed pellets and charring also is seen along when we perform autoclaving. Palatability also is reduced by autoclaving. An alternative to autoclaving is ?-irradiation. Eventhough effective, this method is very costly. "RODENT-IR" is an irradiated commercial feed available in India for rodents. Companies supply irradiated rabbit and guinea pig feed upon request if the requirement is considerable and economic for them.

Heavy metal and pesticide contamination should be periodically checked. Aflatoxin testing also is very important in lab animal feed. Analysis of the presence of natural toxicants is an emerging area of importance. This includes estimation of phytoestrogens like genistein and diadzein. It is known that the phytoestrogens concentrations can affect certain experimental results. Feed with low phytoestrogen concentrations are already available in European and American markets. They are available in India on request. Soybean and alfalfa are examples of ingredients with high phytoestrogen concentrations.

Water

Water has to be supplied ad libitum to lab animals. Animals deprived of water will not consume feed and will show considerable a loss of condition within days. The supplied drinking water shall be free from pesticide and heavy metal contamination. Microbiological analysis for potent pathogens also shall be done ideally. Pseudomonas aeruginosa can

cause a condition known as "circling/rolling" in rats. This middle ear infectious agent is transmitted through water. It is advised to use reverse osmosis water or UV sterilized water in conventional colonies. Detergents and antiseptics may be used for water bottle washing and shall be done routinely to prevent bacterial growth and transmission. Acidification of water is done with HCl to make the pH =2.5 to 3.0. Autoclaving can also be used to ensure a sterile water supply. Care also has to be taken to replace the water bottles to its respective cages after refilling if topping up is done as a routine practice.

Conclusion

Laboratory animal nutrition has a major role not only in the growth and reproduction of animals but also in the reliability of experimental results. In conventional laboratory animal colonies fed with indigenously available commercial pelletted diets, deficiency diseases like Hunch back and hypoproteinemia in rats and mice, fatty liver in pregnant rabbits, pregnancy ketosis in guinea pigs, and Vit-C deficiency are commonly seen. Supplementation with natural sources of major nutrients and synthetic vitamin-mineral concentrate supplements is also required to keep the growth, reproduction and well being of laboratory animals fed with indigenously available diets. In laboratory animal nutrition, quality assurance and documentation of the laboratory animal feed and water also is equally important as providing the animal with a healthy diet.

References

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