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Gesellschaft für Versuchstierkunde
Society for Laboratory Animal Science

Specialist information

from the Committee for Nutrition of Laboratory Animals

Feeding concepts and methods in laboratory animal husbandry and in animal experiments

- RAT -

Status October 2020

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Contents

Keywords:
Rat - Nutritional characteristics – Complete feed
Life phases - Feeding – Feeding technique - Enrichment

Preliminary remarks

In zoological taxonomy, the rat belongs to the order *Rodentia* (rodents), is related to the family Muridae (or murids) and include species such as *Rattus norvegicus* (brown rat) and *Rattus rattus* (black rat).

All rat models used in biomedical research today can be traced back to the wild form of the brown rat (*Rattus norvegicus*). This species, which originated in Eastern Asia, has gradually colonized every continent, including Central Europe in particular, since the late Middle Ages. In terms of social structure, the animals live in packs of widely varying size, sometimes in underground burrows in a variety of habits (Telle 1966).

Rats have been used for more than 150 years as laboratory animals in many areas of biomedical research, including physiology, nutrition and feeding, cardiovascular research, metabolism, and metabolic disorders. The first specific breeding experiments to elucidate pathways of inheritance were reported by Crampe in the 1880s (Robinson 1979). Defined inbred and outbred strains have been used since the 1920s and are available in large numbers today (Hedrich 2000).

Nutritional characteristics

food in differing proportions of animal and plant origin depending on the main food source and are thus classed as omnivores (Wilman et al. 2014). Food intake occurs in the form of meals that are often spread over many hours of every part of the day. In the wild, this distribution depends primarily on the undisturbed nature of the food source (Telle, 1966). These rats certainly have to put up with relatively long distances between resting place and food source. Laboratory rat strains show strain-specific rhythms in the frequency and distribution of their meals and take their food to a large extent during the dark phase (Büttner 1991, Lutz 2005). This circadian rhythmicity is largely controlled by the suprachiasmatic nucleus in the hypothalamus (Lutz 2005).

The quantity of food intake is regulated by numerous endogenous and exogenous factors. One factor to be mentioned here in particular is cholecystokinin (CCK), an intestinal peptide that temporarily reduces food intake through an interaction between CCK receptors and the brain stem (Lutz 2005). When it comes to hormonal control, leptin, and insulin act as essential lipostatic signals, as do the sex hormones, especially oestradiol (Lutz 2005, Keenan et al. 2000), which determine gender differences in growth capacity among other things. The anatomy and physiology of the stomach are essential for storing food and transporting it to the duodenum, for carbohydrate digestion, for eating behaviour and hence also for reactions to changes in the feeding regimen or composition of the food (Gärtner 1997).

The stomach is divided into a section known as the forestomach, which is devoid of glands and has an epithelial mucosa, and the actual stomach which has a glandular mucosa. An alkaline pH prevails in the forestomach with increased amylase activity (Senoo 2000). The glandular mucosa contains the gastric glands, which are often enlarged in old animals (Komarek et al. 2000). Bodyweight differences between rat strains are primarily due to genetically different growth capacities (see Keenan et al. 2000) and to effects of individual genetic variants / mutations (Shiota and Printz 2012).

An overview on the regulation of fat deposits and of the fatty cell count and size in rats is provided by Müller et al. (2010).

As with other rodents and the rabbit, coprophagy is also normal in the rat. Proteins, B and K vitamins and also biotin are returned to the body from this source.

Table 1: Data relevant to feeding in rats (Weiss et al., 2014)

Live mass	250 – 600 g, ♂ considerably more
Sexual maturity	50-72 days
Breeding maturity	90-100 days
Oestrus cycle	Every 4-6 days for 14 hours
Length of pregnancy	20 – 23 days
Litter size	6 – 12 (20) young
Live mass at birth	4 - 6 g
Weaning age	18 - 21 days
Live mass at weaning	35 – 50 g
Life expectancy	2 – 4 years
Food intake	12 – 20 g pelleted dry feed / 24 h
Water intake	15 – 35 ml/ 24 h

A general overview of the utilization of energy, protein, minerals and vitamins in the rat is provided by Keenan et al. (2000). Schug (2005) and Bielohuby et al. (2010) have conducted experimental studies on the rat-specific evaluation of feed energy at the level of metabolizable energy (ME). Experimental work has shown that the equation from pig feeding that has been used to date can be readily applied to grain-based rat feed. For purified diets, the so-called Atwater factors are more accurate (Bielohuby et al. 2010). Atwater factors are coefficients used to calculate the energy value from the energy-yielding nutrients (proteins, carbohydrates, fats, and alcohols) of the feed.

Life phases

Pregnancy

For the pregnant female, feed requirements are determined by the requirements for the animal's subsistence; in young breeding animals, they are determined by the need for the young animals' own growth and, especially in the last trimester, by the fetal growth and maternal tissue growth.

According to data from the National Research Council (NRC) (1995), the additional energy requirement (ME = metabolizable energy) for pregnancy up to Day 16 is estimated at about 30% in addition to the energy required for subsistence; from Day 17 to parturition it is estimated at 2.5 times the subsistence requirement.

Lactation

During lactation, the nutrition requirements are higher than in any other phase of life. The feed must cover the subsistence needs of the lactating female, possibly its further growth and, in particular, the need for milk production, which in view of the high energy content of rat milk is very important and also determined by the number of young to be suckled. In the case of permanent mating, a new pregnancy may have to be considered. During lactation, the total nutrition requirement may be two to three times the subsistence needs of the animal. The energy requirement (ME) increases up to four times the subsistence needs of the female, depending on the number of young.

Growth

From the time of birth onwards, rats show extreme growth, which continues beyond weaning at the age of 3 weeks until about 12 weeks of age and the slowly levels off depending on the strain. In most strains, a significant sexual dimorphism is observed with regard to the increase in body mass, the male animals becoming much heavier than the females. The body mass growth in the adult males is primarily due to fat accumulation.

Lipid synthesis in the liver and fatty tissue is low up to weaning age, then rises in both tissues up to the age of 50 days, remaining high in the liver of the adult animal, while fatty and other tissue continue to play only a minor role as sites of lipid synthesis (Gandemer et al. 1982). They then serve predominantly as storage sites. According to NRC data, the energy requirement (ME) for growth is about twice the requirement for the animal’s subsistence (NRC 1995).

Adult Phase

The adult phase is defined as the period between (principal) growth and the natural end of life. If no experimental procedures take place during this period, there are no special nutrition requirements for the animal apart from its subsistence needs. For this reason, the risk of abnormal fatty degenerating is particularly high in such cases and, depending on the strain, can result in a shortened lifespan, premature occurrence of age-related tumours and other metabolic disorders (diabetes, etc.) (Roe et al. 1995, Keenan 1996, Keenan et al. 1996, Ryle et al. 1995, Klinger et al. 1996). Therefore, the monitoring and regulation of feeding in this part of an animal’s life is especially important.

Table 2: Raw nutrient content[1)] of commercially available complete feed for rats

Raw nutrients in % (OS)	Maintenance	Breeding
Raw protein	14.0 – 19.3	18.8-26.5
Raw fat	3.5 – 4.5	3.8 – 6.0
Raw fibre	4.3 – 6.1	3.0 - 4.7
Raw ash	6.0– 6.9	5.7 – 7.1
N_2-free extract (NfE)	52.9 – 61.2	47.5 – 54.6
Starch	34.0 - 36.8	29.0 - 36.0
Sugar	2.0 – 4.9	2.0 – 5.4

1) according to manufacturer’s data (OS original substance)

An energy value of about 500 kJ ME/kg LM0.75 is assumed for the day-to-day subsistence needs of the rat (NRC, 1995). The formula used by the NRC for calculating energy comes from Kleiber (1967). The daily food intake in the adult phase varies between 12 and 20 g dry feed.

Certain strains, such as nude rats, may have an increased energy requirement in view of their genetic modification and the resulting increase in their thermal needs.

Water supply

Drinking water must be provided *ad libitum*. An adequate supply of water is especially required when feeding with dry feed. Acidified drinking water (phosphoric or hydrochloric acid for the reduction of secondary contamination) can lead in some rat strains to negative effects on the tooth enamel (Karle et al. 1980) or in correspondingly predisposed rat models (e.g. PKD) to increased blood pressure (Gretz et al. 1996, McCallum et al. 2015). Information on the preparation of drinking water can be found in the GV-SOLAS booklet "*Drinking water supply for laboratory animals*".

Provision of feed and feeding techniques

It is customary to use complete feed products for rats as a result of standardization efforts in laboratory animal housing. Commercial feed is predominantly used ad libitum in pelleted or extruded form. Standard pellets for rat feed have a diameter of 10 or 15mm. When using extruded feed, care must be taken to make sure the animals do not become fat, because the animals like this form of feed and will consume increasing amounts of it.

The feed is normally provided via in-built feed racks (stainless steel) in the wire lid of the housing cage. It may also be administered in bowls (stainless steel, ceramic) in the cage, particularly in the case of soaked feed for young animals that have not yet been weaned.

The rate at which feed is lost as a result of being scattered or thrown around in play depends primarily on the structure and hardness of the pellets. A loss rate of approximately 10% must be expected. The amount scattered may also be much higher in individual cases. For this reason, the quantity of feed must be checked regularly even when provided *ad libitum*.

In the case of practical feeding by the use of breeding and maintenance diets, the differing nutrition needs of the individual life phases are only very roughly taken into account: with permanently mated animals, breeding feed is normally given throughout the breeding period, but is actually only recommended for the mother animal from the second trimester onwards and during lactation. The weaned animals are given maintenance diets from the age of four weeks, depending on the facility. This feed has a lower raw fat and raw protein content (Table 2) and provides thus less metabolizable energy, which is the crucial factor for gaining body fat.

Feeding in experiments

Depending on the objective of an experiment, the dosage form, the composition and the feeding technique here may differ completely or in part from the usual approach in breeding and housing. For example, it is possible that the intake of nutrients and active ingredients have to correspond exactly to requirements in individual experiments, which may lead to a further differentiation in feeding in individual cases. In experiments individual nutrient groups, such as fat or protein, are often needed in non-physiological concentrations in order to induce or stimulate certain phenotypes. In most cases, these so-called experimental diets are commercially available according to specific requirements. In the manufacture of these feed products, however, production-related mixing thresholds occur, e.g. in fat content. Further information on this can be found in the GV-SOLAS booklet "*Characterization and production procedures of laboratory animal nutrition*".

Depending on the subject of the question being addressed in the experiment, it may be possible to deviate from the otherwise customary *ad libitum* approach to feeding and switch to rationing or mealtime feeding, e.g. in order to record the exact intake and utilization of the feed. In this case, the feed is provided in dosed quantities and/or at defined intervals, taking into consideration the needs of the individual animal. In the case of restrictive feeding, less feed is offered than the quantity consumed when the feed is made available *ad libitum*. The restriction may be quantitative by reducing the quantity of the feed or qualitative by reducing the nutrient content.

If identical quantities of feed intake are necessary in experimental and control groups, so-called pair-feeding is used. Here, the feed intake is measured in the individual animal in the experimental group and the same quantity provided to the corresponding control animal the next day.

As with all species, when rewards are given, their effect on hygiene and standardization must be taken into account (see "Statement from the Committee for Nutrition concerning the use of non-standardized feed in laboratory animals").

In the experimental administration of non-pelleted feed (meal, pap, liquid feed), the animals' need for wearing down their incisors means they must be provided with material they can gnaw on, preferably non-treated wood.

For procedures under anaesthesia, there is a clear recommendation not to have a period of fasting (GV-SOLAS Committee for Anaesthesia and Analgesia 2012).

In the event of deviations from this recommendation, the need for an interruption of feeding must be carefully reviewed in each individual case to minimize any resulting energy deficit and hypoglycaemia.

Nutritional disorders

Spontaneous nutritional disorders are often attributable to an inadequate supply of energy as a result of *ad libitum* feeding. Depending on the strain, this can lead to an increase in early-onset spontaneous processes, such as obesity, diabetes, tumours, degenerative diseases, or a shortened lifespan (Keenan et al. 2000).

To counteract such effects, experimental data are required on the precise nutritional needs in the various phases of life of the animals and their use in experiments. Only insufficient data of this kind have been obtained to date, if any at all.

In general, reducing the energy intake (rationed feeding or increased raw fibre content) can delay the onset of pathological processes and significantly reduce their degree of severity

(Keenan et al. 2000). A decrease in the energy intake compared with ad libitum feeding is therefore recommended in principle for the maintenance phase of the rats. The possible influence on the expression of model-related target characteristics in the individual strains must, however, be taken into account.

An oversupply of protein is regarded as a cause of nephropathy in a variety of strains (Sprague-Dawley, Wistar, F344; Keenan et al. 2000). But the interaction with the energy content of the feed must always be borne in mind here. Deficient abrasion of tooth enamel and malocclusion can have a massive effect of animal welfare (e.g. occurrence of overgrown incisors). The resulting pain leads to a refusal to eat and consequently a loss of bodyweight, which can result in the death of the animal if it is not kept under sufficient observation. Regular and careful observation of the animals is therefore absolutely essential.

Transport

The animals must be supplied with food and liquid at the time when they are placed in containers for shipment. Opening of containers later is only permitted with the agreement of the shipper or recipient. The quantity of food provided must be sufficient for twice the schedule transport time to ensure that supplies remain available in the event of the transport being returned or delayed. The components of the food provided must not perish during the transport. In general, materials should be used that do not negatively influence the hygiene status of the rodents.

The provision of supplies must take into account the timespan between the first animal being placed in the transport container by the sender and the last animal being removed by the recipient. It is therefore recommended that the animals are already provided with food during a net transport duration of 3 to 4 hours. A positive factor for the animals is that the supply of food during transport also keeps them occupied at the same time. The food supplied to the animals during transport should be the same as that used in the housing facility, either pelleted or extruded. The food may be provided loose in the transport container; for reasons of space alone, it is advisable not to include a feeding device.

A supply of liquid is advisable for all net transport times of more than 3 to 4 hours. For short transport times (< 8 hours) soaked feed is sufficient as a source of moisture. For reasons of hygiene and to avoid any change of diet, no apples, carrots or other fruit and vegetables, whether cooked or not, should be added.

The addition of jellied water in the form of a pad has become accepted as a source of liquid. The water has been stabilized with agar or colloid, rendering into a form that can be transported. This is commercially available with quality certificates and thus safeguards the hygiene of the animals. The instructions of the manufacturer/supplier must be observed here; the outer packaging must often be slightly cut or removed before the start of transport, so that the animals recognize and accept the source of liquid.

For safety reasons, containers with liquid water must not be used (risk of leakage with negative consequences for the animals).

Enrichment

A structured housing environment indisputably influences neurobiological, behavioural, and stress-related physiological characteristics of the rat and, with a certain degree of caution, also allows conclusions to be drawn as to the wellbeing of the animals. It can be assumed that feeding behaviour and utilization of food are also influenced by enrichment measures. The actual effects of individual changes, however, have not yet been sufficiently investigated; in particular, the influence on the variability of parameters in experiments has yet to be validated. In general, therefore, all measures for structuring the housing environment should be scientifically monitored (see also GV-SOLAS Committee for Animal-Appropriate Housing).

This applies in particular also to GLP-regulated studies, for which an enrichment must always first be clarified, the measures duly validated (Savidis-Dacho et al. 2007) and the GLP rules described accordingly in order to avoid uncontrollable effects e.g. on food intake.

References

Bielohuby, M., Bodendorf K, Brandstetter H, Bidlingmaier M, Kienzle E. 2010. Predicting metabolisable energy in commercial rat diets: physiological fuel values may be misleading. Br J Nutr 103(10):1525-1533.

Büttner D. 1991. Chronobiologie in der Versuchstierkunde - Methodische Probleme. *In:* Gärtner K (ed.), Qualitätskriterien in der Versuchstierforschung, Verlag Chemie Weinheim. (GERMAN)

Gandemer G, Pascal G, Durand G. 1982. In vivo changes in the rates of total lipid and fatty acid synthesis in liver and white adipose tissue of male rats during postweaning growth. Int J Biochem 14:797-804.

Gärtner K. 1997. Zur Funktion des Vormagens bei Muriden. 34th Meeting of the GV-SOLAS, Sep 8. - 11., Jena. (GERMAN)

Gretz N, Kränzlin B, Pey R, Schieren G, Bach J, Obermüller N, Ceccherini I, Klöting I, Rohmeiss P, Bachmann S, Hafner M. 1996. Rat models of autosomal dominant polycystic kidney disease. Nephrol Dial Transplant 11(6): 46-51.

GV-SOLAS Committee for Anaesthesia and Analgesia. 2012: Withdrawal of food in the anaesthesia of laboratory animals. www.gv-solas.de

GV-SOLAS Committee for Nutrition of Laboratory Animals. 2012. Use of non-standardized feed in laboratory animals. www.gv-solas.de

GV-SOLAS Committee for Nutrition of Laboratory Animals. 2016. Drinking water supply for laboratory animals. www.gv-solas.de

GV-SOLAS Committee for Humane Housing of Animals. 2004. Species-appropriate housing of laboratory rats. http://www.gv-solas.de/fileadmin/user_upload/pdf_publikation/Tierhaltung/Rat_housing-12_2019.pdf www.gv-solas.de

Hedrich HJ. 2000. The History and Development of the Rat as a Laboratory Model. *In:* Krinke G (*ed.*), The Laboratory Rat, Academic Press, London.

Karle EJ, Gehring F, Deerberg F. 1980. Trinkwasseransäuerung und ihre schmelzschädigende Wirkung auf Rattenzähne. Z Versuchstierk 22:80-88. (GERMAN)

Keenan KP. 1996. The uncontrolled variable in risk assessment: *ad libitum* overfed rodents - fat, facts and fiction. Tox Pathol 24 (3):376-382.

Keenan KP, Laroque P, Soper KA, Morrissey RE, Dixit R. 1996. The effect of overfeeding and moderate dietary restriction on Sprague-Dawley rat survival, pathology, carcinogenicity, and the toxicity of pharmaceutical agents. Exp Toxic Pathol 48:139-144.

Keenan KP, Ballam GC, Haught DG, Laroque P. 2000. Nutrition *In:* Krinke G (ed.), The Laboratory Rat, Academic Press, London.

Kleiber M (ed.). 1967. Der Energiehaushalt bei Mensch und Haustier. Verlag Paul Parey, Hamburg, Berlin. (GERMAN)

Klinger MM, MacCarter GD, Boozer CN. 1996. Body weight and composition in the Sprague Dawley rat: comparison of three outbred sources. Lab Anim Sci 46(1):67-70.

Komárek V, Gembardt C, Krinke A, Mahrous TA, Schaetti P. 2000. Synopsis of the organ anatomy. *In:* Krinke G (ed.), The Laboratory Rat, Academic Press, London.

Lutz T. 2005. Regulation of food intake of laboratory animals. 43rd Meeting of the GV-SOLAS, Berlin.

McCallum L, Lip S, Padmanabhan S. 2015. The hidden hand of chloride in hypertension. Pflugers Arch 467(3):595-603.

Müller G, Wied S, Dearey E-A, Wetekam E-M, Biemer-Daub G. 2010. Lipid storage in large and small rat adipocytes by vesicle-associated glycosylphosphatidylinositol-anchored proteins. *In:* Meyerhof W, Beisiegel U, Joost H-G (eds), Sensory and metabolic control of energy balance. Springer, Berlin, Heidelberg.

National Research Council. 1995. Nutrient requirements of laboratory animals. 4th rev. ed., National Academy Press, Washington.

Robinson R. 1979. Taxonomy and Genetics. *In:* Baker HJ, Lindsey JR, Weisbroth SH (eds.), The Laboratory Rat, Vol. I, Academic Press, New York, London.

Roe FJC, Lee PN, Conybeare G, Kelly D, Matter B, Prentice D, Tobin G. 1995. The biosure study: influence of composition of diet and food consumption on longevity, degenerative diseases and neoplasia in Wistar rats studied for up to 30 months post weaning. Food and Chemically Tox. 33 (Suppl. 1), Pergamon Press, Headington Hill Hall, Oxford OX3 0BW, UK.

Ryle PR, Harling RJ, Brennan C, Begg SE, Gopinath C. 1995. Survival in rat carcinogenicity studies: a comparison of Wistar rats obtained from two sources. Int. Congress of Tox.-VII, Seattle, USA.

Savidis-Dacho H, Schmidt H, Ilk R, Holasek M, Oberfeichtner M, Livey I, Barrett N. 2007. Evaluation of cage enrichment for the rat in the pharmaceutical industry. 6th World Congress on Alternatives and Animal Use in the Life Science, 21. – 25.08 2007, Tokyo, Japan.

Schug K. 2005. Untersuchungen zur Energiebewertung in Standardfuttermitteln für Ratten. Inaug. Diss., Faculty of Veterinary Medicine, Ludwig Maximilian University Munich. (GERMAN)

Senoo H. 2000. Digestion, Metabolism. *In:* Krinke G (ed.), The Laboratory Rat, Academic Press, London.

Shiota M, Printz RL. 2012. Diabetes in Zucker diabetic fatty rats. *In:* Joost H-G, Al-Hasani H, Schürmann A (eds), Animal Models in Diabetes Research, Humana Press, Springer, New York, Heidelberg.

Telle H-J. 1966. Beitrag zur Kenntnis der Verhaltensweise von Ratten, vergleichend dargestellt bei *Rattus norvegicus* und *Rattus rattus*. Z Angewandte Zool 53:129-196. (GERMAN)

Weiss J, Becker K, Bernsmann E, Chourbaji S, Dietrich H. 2014. Versuchstierkunde: Tierpflege in Forschung und Klinik, 4. Auflage, Enke Verlag, Stuttgart. (GERMAN)

Wilman H, Belmaker J, Simpson J, de la Rosa C, Rivadeneira MM, Jetz W. 2014. Elton traits 1.0: Species-level foraging attributes of the world's birds and mammals. Ecology 95:2027.

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