Felasa – Quick reference paper on laboratory animal feeding and nutrition

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Introduction

Practical experience from teaching in laboratory animal science courses has shown that students (and their supervisors) are often not conscious (enough) about the influence of diet and dietary composition on the health of the animals and experimental results. This sometimes leads to the execution of experiments in which the diets used are such, that the results do not have any meaning and cannot be published. This is inappropriate use of laboratory animals.

This overview will hopefully add to the understanding of the importance of laboratory animal nutrition, avoiding doing experiments using inappropriate diets and thus unnecessary use of laboratory animals. Thereby this short overview is expected to contribute to the refinement of animal experiments, one of the important goals of Felasa.

I. Diet and experimental results

a. nutrient requirements
In order to provide each species with the proper nutrient levels of essential nutrients, nutrient requirements must be fulfilled (National Research Council documents describe nutrient requirements for each species). Nutrient Requirements of minipigs are currently under investigation (Ritskes-Hoitinga & Bollen 1997, 1998a). By providing each animal with their species specific essential nutrients in the proper amounts, diseases can be prevented as well as unwanted interference with experimental results. In case essential nutrient requirements are not fulfilled, unreliable conclusions may be obtained (Ritskes-Hoitinga et al. 1996, Ritskes-Hoitinga 2000).

b. nutrient requirements in different (transgenic) strains
Different species, strains, stocks and individuals can have different nutrient requirements (National Research Council 1995). Regarding the enormous development of many new transgenic strains, it must be taken into consideration that depending on the nature of a transgenic strain, nutrient requirements may vary as well.

c. standardisation
Standard commercial diets usually fulfill nutrient requirements more than sufficiently, at least when transported and stored under the proper environmental conditions. However, there can occur a large variation in composition in natural-ingredient diets (between and within brand variation) due to raw material variation, which will differentially influence experimental results (Beynen et al. 1993, Ritskes-Hoitinga et al. 1991). As between-batch variation can occur, it is advised to buy diets with a batch-analysis certificate so that one is informed about the actual composition of each batch of diet that is being used. For GLP-studies this is a necessity.

Purified diets (Beynen et al. 1993), formulated with a combination of natural ingredients, pure chemicals and ingredients of varying degrees of refinement , have a more standardised composition and give therefore more reproducible results than the use of natural-ingredient diets. However, there is a higher risk of creating shortages of unknown essential nutrients, which are present as “natural contaminants” in natural-ingredient chow diets (e.g. Chromium and Vanadium, National Research Council 1995). Moreover, certain refined ingredients can cause problems (e.g. short-type cellulose fiber can cause intestinal obstruction in rats, Speijers 1987).

For rodents a “cook-book receipe” is available for composing a purified diet, the so-called American Institute of Nutrition diet (AIN-93 diet) (Reeves et al. 1993). The AIN-93 diet fulfills the nutrient requirements for rodents as published in 1995 (National Research Council), except for the vitamin B12 level. The AIN-93 vitamin B12 level must be doubled to live up to the minimum requirements as described by the National Research Council (1995).

Ad libitum food intake is in principle determined by the energy need. This energy need changes according to the stage of life the animal is in (growth, maintenance, pregnancy, lactation). When changing the energy content of the diet (e.g. by adding fat to the test diet), one changes the dietary intake in grams. In order to make sure that only the dietary fat (and carbohydrate) intake will differ between the control and test group, one needs to apply the isocaloric exchange method (Beynen & Meijer 1993).

d. feeding level
Ad libitum feeding is considered normal practice for rodents, however it is considered bad veterinary practice for e.g. pigs, monkeys, rabbits and dogs, as they become obese (Hart et al. 1995). When feeding restrictedly, it must be secured that the restricted feeding level provides enough essential nutrients. Although ad libitum feeding of rodents is considered “normal” practice, this must be questioned intensely. Ad libitum feeding as opposed to restricted feeding has a clear negative impact on rodent health, as it shortens survival time, increases cancer incidence, shortens cancer latency period and increases the incidence of degenerative diseases in kidney and heart (Hart et al. 1995). These effects are very reproducible! Moreover, it increases the number of animals needed if sufficient animals are to survive a 2-year period in long-term toxicological studies.

Keenan et al. (1999) state that ad libitum overfeeding of rodents is at present one of the most poorly controlled variables affecting the current rodent bioassay. Moderate dietary restriction (70-75% of adult ad libitum food intake) is advised as a method that will improve uniformity, increase exposure time and increase statistical sensitivity of chronic bioassays to detect true treatment effects (Keenan et al. 1999). However, moderate dietary restriction will only improve uniformity in individually housed animals, where there is control of individual food intake. A restricted amount of food in group-housed animals is expected to increase variation due to differences in individual food intakes, based on the hierarchy in the group. It will be the challenge to find restricted feeding schedules in group-housed animals, in order to fulfill the animals social needs as well.

e. contaminants
There are several documents stating maximum allowed concentrations of contaminants (GV-Solas 1980, Barqa 1992). One of the guidelines that give maximum limits, to which all toxicologists all over the world are referring to, are issued by the Environmental Protection Agency (1979). As different guidelines state different levels, what to choose as the “correct” maximum tolerated levels? Firstly, one has to decide which guidelines are most appropriate in the experimental setting one is working in. One might even have to develop specific institutional guidelines. Secondly, for each experiment one can do a literature search to figure out whether contaminants, and if yes, which will interfere with the specific purpose of that study. That way concrete maximum levels of specific contaminants can be established. Purified diets have lower contaminant levels than natural-ingredient diets.

II. Diet and well-being

a. welfare and enrichment
From preference testing it is known that rats prefer to work for food instead of obtaining it just like that. For each species there are certain species specific essential needs connected to searching and finding food (e.g. rooting of pigs). If these essential needs are not fulfilled, abnormal behaviour like stereotypies can occur (pigs can develop sham chewing).
Enrichment of the environment is possible by letting the animals work and or search for food. Knowledge of the natural feeding time and behaviour are important factors to consider. The time of day at which a restricted amount of food is giving can be an important tool in providing a better welfare (e.g. in rabbits, Krohn et al. 1999). Giving food rewards are important tools to learn and train animals. Which food rewards are chosen and in what amounts need careful consideration: is there interference with the experimental results and or health of the animal?
Certain dietary schedules require individual housing. As individual housing opposes the well-being of social living species, alternative ways of feeding need to be considered. E.g. the animals can be individually fed for a certain period each day and then socially housed for the remaining part of the 24-hour period.

b. transport and acclimatisation
Knowledge of the species is important when transporting animals. Before transport, getting specialist advice for each particular species is needed: e.g. (mini)pigs will vomit when being fed just before transport. Rats and mice will acclimatise faster after transport, when food and water has been provided during the transport (van Ruiven 1996).

III. Diet and animal models

a. Choice of model and experimental conditions

Knowledge and choice of species and experimental (including dietary) conditions will have a major impact on results. The effect of linoleic acid on mammary tumour development in animal models depended on the model system used and type of parameters measured (Ritskes-Hoitinga et al. 1996). Feeding fish oil to rabbits to examine the possible positive influence of fish oil on atherosclerosis, resulted in liver pathology and more atherosclerosis on higher doses of fish oil. This was thought to be the result from the inability of the herbivorous rabbit liver to cope with the long-chained unsaturated fatty acids from fish oil (Ritskes-Hoitinga et al. 1998b).
Feeding by gavage is expected to cause stress, influences metabolism and will therefore lead to other results than voluntary intake (Vachon et al. 1988). Vachon et al. proved that voluntary intake of a certain meal gave results similar to the human, whereas giving the same meal by gavage, did not (Vachon et al. 1988)!

b. Diet and pharmacological studies
The effect/pharmacokinetics of pharmacological substances (e.g. oral antibiotics) are largely dependent on the time of administration in relation to the time of feeding. How long animals need to be fasted before the "bare" effect of pharmacological substances tested can be judged, is an important animal welfare issue (Claassen 1994). A rat will have an empty stomach already after 6 hours (Vermeulen et al. 1997). Fasting for longer periods led to increased locomotory and grooming behaviour (Vermeulen et al. 1997).

IV. The impact of a regular feeding schedule on circadian rhythms(T) of physiological and behavioral functions

[(T): Some chronobiological terms are explained at the end of the text]

When individuals of several strains / species of rodents and rabbits are fed a long time ad libitum they tend to become fat, especially so with increasing age and limited space for physical workout (e. g. NZW rabbits kept in cages during longtime maintainance). In order to prevent excessive fattening, the quantity of food, thus, often is restricted: usually a limited amount of food is replenished every day during the working hours. Restricted animals start to eat immediately when food is presented and, in consequence, many biochemical and physiological functions of the gastrointestinal tract and even of the whole organism are phase-shifted in nocturnally active rodents and rabbits. Since the impact of shifted or even inverted circadian rhythms on experiments usually is underestimated this paragraph compiles some basic informations on that.
Supplied with food ad libitum, nocturnally active animal species like mouse, rat, hamster and rabbit are consuming almost all of their food during the hours of darkness. Correspondingly many follow-up parameters are on a significantly higher level during the hours of darkness. The differences between the regular minimum and maximum as a rule are so great (can be up to several hundreds of percentages!) that it would be an artefact to ignore them. Few examples would be: mucosal enzymes in the small intestinal tract (Saito et al. 1975), carbohydrate absorption (Hara and Saito 1989), bile flow and composition (Ho and Drummond 1975), serum gastrin and cholecystokinin (Pasley et al. 1987) or serum insulin (Rubin et al. 1988).
When the time of food access is restricted, those functions which are coupled more or less directly to food ingestion are shifted to the time of food access, whether it is during some hours of light or of dark time. This means, that periodic food access can override the light:dark regimen which usually is the main ´zeitgeber´ **(T)** for circadian rhythms of animals and men (Philippens et al. 1977, Rubin et al. 1988, Saito et al 1976 a, b, Saito et al. 1980, Stevenson et al. 1975, Stevenson and Fierstein 1976). However, even many of those functions which are not obviously coupled to food intake, e. g. the 24 h rhythm of locomotor activity (Boulos and Terman 1980,

Boulos et al. 1989, Honma et al. 1983, Jilge et al. 1987, Jilge 1992, Jilge and Staehle 1994, Jilge and Hudson 2001), core body temperature (Jilge et al. 2000), corticosterone (Krieger 1974, Morimoto et al. 1979, Takahashi 1979), heart rate and blood pressure (van den Buuse 1999) are phase-shifted by a shifted feeding regimen.
There are functions, however, which are exclusively synchronized by the light-dark zeitgeber: the enzymes N-acetyltransferase and hydroxyindol-o-methyltransferase and the endproduct catalyzed by them in the pineal organ, melatonin (Reiter 1993, Tamarkin et al. 1985), the disc shedding rhythm of photoreceptors (LaVail 1976) and the mitotic index of the cornea (Burns et al. 1976).

There are two ways how restricted food access affects circadian rhythms:
1. masking and 2. entrainment[(T)] (Aschoff 1986, Aschoff et al. 1982, Aschoff and von Goetz 1986, Mrosovsky 1996, 1999, Pittendrigh and Daan 1976).

1. Masking

Masking means that a periodic environmental factor acts directly upon the overt rhythm without affecting the circadian oscillator[(T)] driving it. As a result the rhythm is synchronized immediately, without transients. When the circadian rhythm of locomotor activity, free-running in constant conditions, is exposed to scheduled food access, the activity rhythm immediately stops to free-run[(T)] and re-assembles around the phase of food access. When - e. g. several weeks later - food is offered ad libitum again, the circadian rhythm continues to free-run at the phase which it had without an interspersed food regimen. That means: periodically restricted food access has an effect on the activity rhythm without affecting the circadian oscillator (Abe et al. 1989; Aschoff and von Goetz 1986).

2. Entrainment

Entrainment means that an external variable like periodic food access has zeitgeber properties (for the definition of zeitgeber see: Aschoff 1958, 1960; Pittendrigh 1960). Scheduled feeding, thus, acts on the oscillator system itself which controls the timing of overt rhythms. The time needed for entrainment – following the instatement of a zeitgeber schedule - depends on the phase relation between the free-running rhythm and the zeitgeber schedule, i. e. the ´re-arrangement´ of the rhythm around the phase of food access occurs via transitory periods. Their number correlates with the phase relation between zeitgeber and circadian rhythm. As a general rule, the greater the phase difference between function and zeitgeber, the longer the time needed for entrainment. In general the time necessary for entrainment can last up to 50 – 60 days (Pittendrigh and Daan 1976; Jilge et al. 1987, Jilge and Staehle 1993, Jilge 2000). When returning to ad libitum food access again, a free-running rhythm starts out from the phase of the preceding food regimen. In that case, the period length of the free-running circadian rhythm is affected for a couple of cycles by the period length of the preceding zeitgeber. While the honey bee was the first animal in which entrainment of an oscillator with scheduled feeding had been proven (Beling 1929) a ´feeding-entrainable oscillator´ (FEO) was shown to exist in some strains of mice, the hamster, rat, rabbit, pigeon, house sparrow and some marsupial species, the parameter recorded most frequently being the activity rhythm (Stephan 1986, Jilge et al. 1987, Jilge and Stähle 1993, Jilge and Hudson 2001, Coleman et al. 1989, Kennedy et al. 1991, Hau and Gwinner 1992, Jilge 1992, Mistlberger 1993, Philipps et al. 1993, Rashotte and Stephan 1996, Marchant and Mistlberger 1997, Challet et al. 1998, Stephan and Davidson 1998, Mistlberger and Marchant 1999, Lax et al. 1999). The FEO is a circadian oscillator in addition to and separate from the ´light entrainable oscillator´ (LEO): even when the LEO had been destroyed, hamsters were entrained by periodic food access (reviewed by Mistlberger 1994). While the LEO in mammalian species is known to be located in the suprachiasmatic nuclei of the hypothalamus lying above the chiasma opticum and bilaterally symmetric to the third ventricle, we have no information so far about the location of FEO nor of its afferent and efferent pathways.

In those animals being entrained by periodic food access, in first instance some functions are rearranged immediately after the implementation of scheduled feeding, while simultaneously, but requiring a much longer time, entrainment and restitution of homeostasis of other functions is taking place "unnoticed" (so-called "masking"). The masking of physiological functions appears to be necessary for maintaining vital functions during the time-consuming process of achieving homeostasis for functions implying complete circadian reorganization.

Thus, when food access is restricted to only some hours during the day, one should keep in mind that many digestive and metabolic functions are brought out of phase, especially when nocturnal animals are fed during some hours of the light period. The process of re-entrainment around the phase of food access can require 50 – 60 days and physiological functions like locomotor activity, digestive functions and urine excretion will be affected during this time (Jilge and Stähle 1993)

There are however functions, e. g. the mitotic index of the corneal epithelium and the rhythm of pineal melatonin production which neither are entrained nor masked by periodic feeding but rather remain entrained with the light:dark zeitgeber. Different functions may become permanently internally desynchronized by restricted feeding schedules: the DNA synthesis of the thymocyte for example is coupled to restricted food access whereas the mitotic index in the cornea is not altered by restricted feeding (Pauly et al. 1976). So far we do not know enough about the consequences of the permant temporal displacement of functions e. g. on reproductive, immunologic, intermediary-metabolic or behavioral parameters. It may be that follow-up studies come to the conclusion that (certain) restricted feeding schedules threaten homeostasis.

- circadian rhythm (CR): periodic biological function with a frequency of 1 cycle per 24 ± 4 h. CR´s are generated endogenously in the suprachiasmatic nuclei (SCN) of the hypothalamus.
- circadian oscillator(system): Neurons generating a CR with a period of about but significantly different from exactly 24 h. The SCN are considered to be the ´masterclock´ of mammals which is entrained by an external zeitgeber. Since the light:dark cycle, entering the SCN via the retinohypothalamic tract (RHT) is the main zeitgeber for mammals, the SCN are referred to as light-entrainable-oscillator (LEO). As delineated above, in some species an additional oscillator has been described so far, which is entrainable by periodic food access. Hence, the name feeding entrainable oscillator (FEO) has been suggested.
- entrainment: synchronization of a CR by an external (or internal) periodic variable within a limit of 24 ± 4 h.
- free-running rhythm: circadian rhythm (e. g. of locomotor activity) in the absence of any external zeitgeber.
- zeitgeber: external, periodic variable entraining a circadian oscillator.

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