Laboratory Animal Nutrition and Experimental Results

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Introduction

The composition of the diet and feeding practice can influence the health status, performance and metabolism of experimental animals. Changes in diet and their effects are the objective of nutritional studies. The results of other kinds of animal experiments can be unintentionally affected by the composition of the diet. Such effects relate to undesirable and unknown variations in the dietary constituents. These variations can adversely affect the accuracy and precision of experimental results. Consequently, animals are sacrificed unnecessarily and time and resources are wasted.

Variation in the concentration of dietary components, nutrients and contaminants, might cause clinical signs. Symptoms of deficiency or toxicity are readily observed. Although harmful to the animals, in these instances no biased results will enter the literature because the experiment will be stopped. However, relatively small variations in diet, which occur more frequently, are not always obvious. Nevertheless, the metabolism on the cellular level of the animals could be affected, which in turn may influence the outcome of the experiment. Small variations in diet might cause that undesirably and unknownly biased results end up in scientific journals.

Between- and withing-brand variation

Two types of diet variation should be distinguished: differences between diets from different manufacturers and differences between batches of one brand.

Table 1 illustrates the magnitude of between-brand variation of commercial diets based on natural ingredients. Essentially all diets were of the closed formula type, that is, they are manufactured and marketed by a company that claims the quantitative ingredient composition of the diet is privileged information. It is clear from Table 1 that commercial rat diets can differ considerably with respect to their composition.

Different batches of one brand of diet based on natural ingredients can also differ markedly in their composition (Table 2). The variation in protein concentration can be higher than that indicated in Table 2. *Gärtner et al* (1974) computed that the variation, corrected for analytical error, in the contents of crude protein of batches of one brand was as high as 12% (expressed as relative standard deviation). Different batches of an open formula diet, which by difinition is manufactured in accordance with a formulation that is readily available, also show considerable variation in composition (*Knapka* 1985). Fluctuations of dietary components may be minimal when using purified diets based on refined, standardized ingredients (*Wise* 1982).

Variations in the diet of one brand as shown in Table 2 can be caused by errors during preparation of the diets, by changing the source and quality of the ingredients and by differences in processing and storage conditions. Different manufacturers use different formulas and thus make foods with different nutrient composition as shown in Table 1. Investigators should not choose a brand of commercial diet on the basis of catalogue values, since such values do not agree with chemical analyses (*Wise & Gilburt* 1981).

Impact of variations in diet composition

Thus commercial laboratory animal diets can vary markedly between brands. If these differences in diet composition affect experimental results, the use of different brands may introduce a systematic bias to the results and decrease their accuracy. The use of a certain diet can

Table 1. Variation of the analysed composition of commercial rat diets from different manufacturers

Component	No. of manufacturers	Mean ± SD	Range	Ref.
Protein (g/kg)	4	206 ± 40	155 - 268	(Clapp 1980)
Riboflavin (mg/kg)	4		9 - 56	(Wolff et al 1981)
Calcium (g/kg)	4	8.9 ± 4.1	4.1 - 13.0	(Clapp 1980)
Zinc (mg/kg)	4	149 ± 67	56 - 233	(Wise & Gilburt 1981)
Selenium (mg/kg)	3	0.08 ± 0.04	0.03 - 0.11	(Newberne 1975)
Lignin (g/kg)	5	18.5 ± 3.4	14.5 - 22.4	(Wise & Gilburt 1980)
DDT (mg/kg)	3	1.2 ± 1.0	0 - 2.5	(Newberne 1975)

Table 2. Variation of the analysed composition of different batches of one brand of commercial rat diet

Component	No. of batches	Mean ± SD	Range	Ref.
Protein (g/kg)	65	198 ± 0.7	182 - 213	(Topham & Eva 1981)
Calcium (g/kg)	65	10.4 ± 0.1	8.0 - 14.0	(Topham & Eva 1981)
Zinc (mg/kg)	65	50 ± 10	24 - 77	(Topham & Eva 1981)
Selenium (mg/kg)	148	0.34 ± 0.15	0.04 - 0.66	(Greenman et al 1980)
DDT (mg/kg)	148	0.03 ± 0.05	0 - 0.3	(Greenman et al 1980)
Cadmium (mg/kg)	65	0.43 ± 0.16	0 - 0.9	(Topham & Eva 1981)
Nitrosodimethylamine (ug/kg)	6		0.2 - 21.3	(Silverman & Adams 1983)

either enhance or mask the response of animals to a given stimulus. This in turn can lead to false interpretation of the results. From a scientific point of view this is undesired. Diet-induced bias of experimental results also implies that the results of a given experiment are no longer comparable with those of other studies. This will increase the need to repeat experiments. Clearly, this does not contribute to reduction of animal use.

Diet-induced bias of results essentially refers to the comparison of experiments in different laboratories. However, if in the course of current studies the brand of diet is altered, this bias can also refer to experimental results obtained within one laboratory.

Between-batch variation within one experiment can decrease the precision of results. In other words, the standard deviation increases, which lowers the statistical power. As a result, more animals will be needed in order to obtain valid information.

Diet variation and experimental results

Feeding of different commercial diets to rats will give rise to different results. Growth rates differ (*Clapp* 1980, *Wolff et al* 1981) as well as the incidence of disorders such as nephrocalcinosis (*Clapp* 1980) and the development of tumors (*Pickering & Pickering* 1984). The brand of diet has also been shown to affect the outcome of potency tests of bacterial vaccins (*Knight & Lucken* 1980) and the toxicity to paraquat in mice (*Evers et al* 1982). *Koopman et al* (1987) have shown that the brand of commercial diet significantly affects the amount of segmented filamentous bacteria in small intestine and *Enterobacteriaceae* in feces of mice.

Below, I give a few examples of biological effects caused by changes in the concentrations

of dietary components within their range of fluctuation in practical situations. The protein content of the diet (within the range 30-300 g/kg) affects demethylation and hydroxylation of certain xenobiotics by rat liver homogenates (*McLean & McLean* 1966). Dietary selenium (within the range 0-0.1 mg/kg) affects growth performance and erythrocyte and liver glutathione peroxidase activity in rats (*Hafeman et al* 1974). Cadmium intake (within the range 0-1 mg/kg of diet) affects systolic blood pressure (*Perry & Erlanger* 1974) and renal vasculature in rats (*Fowler et al.* 1975). Residues of DDT in the diet (within the range 0-1 mg/kg) influence the activity of certain hepatic microsomal enzymes of the rat (*Kinoshita et al.* 1966). Thus unknown fluctuations of diet in such studies can lead to incorrect interpretation of the experimental results.

Diet standardization?

It is clear that a standard diet for rats (and other laboratory animals) does not exist (*Wise* 1981). Should efforts be made to design guidelines so as to produce an international, standardized diet? This is not opportune because specific research programs may require specific diets. *Knapka* (1985) concludes that the standardization of laboratory animal diets must involve the concept of standard reference diets rather than a single standard diet. In this way there is flexibility with regard to changing the concentrations of one or more components while keeping constant the remainder of the diet. In order to bring to effect this approach it needs to be supported by a high degree of international harmony and collaboration.

Practical approach to diet variation

What should we do about variation in the composition of laboratory animal diets? First, it should be realized that, depending on the parameters under study, in many experiments the results may not be affected by small changes in the concentrations of dietary components. However, one should be aware of the possible adverse influences of variations in the composition of animal diets. In order to deal with this problem, two steps might be appropriate (*Beynen* 1985). Investigators should search the literature and identify components of the diet which may affect the parameters to be measured in the experiment. The diet to be used should be analysed to measure the concentrations of those components identified. The concentrations of these components should then be kept constant throughout the experiment. Furthermore, diets of experimental animals should be described as extensively as possible in scientific papers. Repetition of experiments can only be meaningful if these guidelines are followed. Furthermore, it could then be possible to track down the effect of diet, if any, when experiments turn out not to be reproducible.

Abstract

There is considerable variation in the composition of commercial diets for laboratory rodents. This variation refers to differences between diets from different manufacturers and differences between batches of one brand. Examples are given of biological effects caused by diet variation. Thus results obtained in experiments with laboratory animals can be influenced differently, and therefore they may not be reproducible of comparable. Moreover, the use of a certain diet can either enhance or mask the response of animals to a given stimulus, which leads to false interpretation of the results. Investigators should be aware of the potential influence of diet variation on experimental results. It is suggested that investigators analyse diets beforehand for those components which may affect the parameters under study. If necessary the diet can be rejected for use. The concentrations of the critical components should be kept constant throughout the course of the experiment.

Dansk sammendrag

Der er betydlig variation i sammensætningen af kommercielle foderblandinger til forsøgsdyr. Denne variation kan både skyldes forskelle imellem forskellige fabrikater og forskelle på grund af batch variation. Variationer kan påvirke resultater fra dyreforsøg og sammenligneligheden af dyreforsøg samt deres reproducerbarhed. Endvidere kan brugen af bestemte foderblandinger enten tilsløre eller forstærke dyrenes respons på en given stimulus, hvilket kan føre til forkert tolkning af resultater. Det foreslås at analysere foderblandinger for komponenter, som kan have indflydelse på de undersøgte parametre. Koncentrationen af kritiske komponenter bør holdes konstant under forsøget.

Finsk abstract

Yhteenveto / K. Pelkonen

Laboratoriojyrsijøille tehdyissæ kaupallisissa rehuissa on huomattavia keskinæisiæ eroja. Kirjoittajat antavat esimerkkejæ biologisista vaikutuksista ja toteavat ettæ erot saattavat johtua erilaisita rehuista. Lopuksi tekijæt ehdottavat, ettæ tutkijoiden tulisi analysoida kæyttæmænsæ rehut niiden komponenttien osalta, joilla voi olla vaikutusta ko. Tutkimuksen tulokseen.

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Scand-Las møde i København

(Scandinavian Federation of Laboratory Animal Science) inviterer til en foredragseftermiddag om forsøgsdyrfoder *onsdag d. 28. oktober 1987, kl. 14.15 til ca. kl. 17.00.* DAM auditoriet, Panum Instituttet, Blegdamsvej 3 B (hovedindgangen), 2200 Kbenhavn N.

Program:

Kl. 14.15 Nutritionist, phil. cand. *Pia Lindeskog,* Institut for Medicinsk Næringslære, Huddinge Sjukhus, Sverige:
»Diet, constant or variable?
Do you really have to think of the nutrition of laboratory animals?«

Kl. 15.15 Pause.

Kl. 15.45 *John Eva,* B. Sc., S.R.S.C., C. Chem., og
Michael Rickett, L.R.S.C.,
Special Diets Services, England:
»Newly developed diets for laboratory animals.«

Foredragene holdes på engelsk.

Firmaet SCANBUR ApS byder på en forfriskning i pausen og er sponsor for foredragsholdernes ophold.

Tilmelding med oplysning om antal deltagere senest 18. oktober 1987 til:
Scanbur ApS, 03 82 02 31 (Marianne Kallesen) eller
Panum Instituttet, 01 35 79 00, lok. 2824 (Hans-J. Skovgaard Jensen).