Laboratory rats and mice: omnivorous or vegetarian diet?

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Animals used in biomedical research are maintained in strictly controlled conditions. The diet is one parameter that must be managed in order to limit random variations of the phenotypic expression, ensure the reliability of the animal models. Therefore, the diet served must not only comply with palatability, stability and safety requirements, but above all it must meet the animal nutritional needs which may vary and depend upon it's physiological status. Currently there is a huge concern in providing diets to rodents which are free of any source of raw material which may contain animal product and by-product of animal origin.

What are the possible impacts of these formulas on animal physiology (reproduction, fertility), nutrigenomic orientation and the reliability of the models they represent?

1. Why is a full-vegetable diet preferable for laboratory animals?

The use of a regimen containing strictly plant raw materials for laboratory rodents is based on two major arguments:
The first argument is economical: animal protein is more expensive than vegetable protein.
- Sources of animal proteins are primarily reserved for human consumption and their added value creates high market pressures.
- Derived products are used for livestock feed and pet diets, creating global purchasing competition.
- For marine products, purchasing competition is augmented by the limited resources, in particular because of farm fish production and high added value in the cosmetics industry (Figure 1).

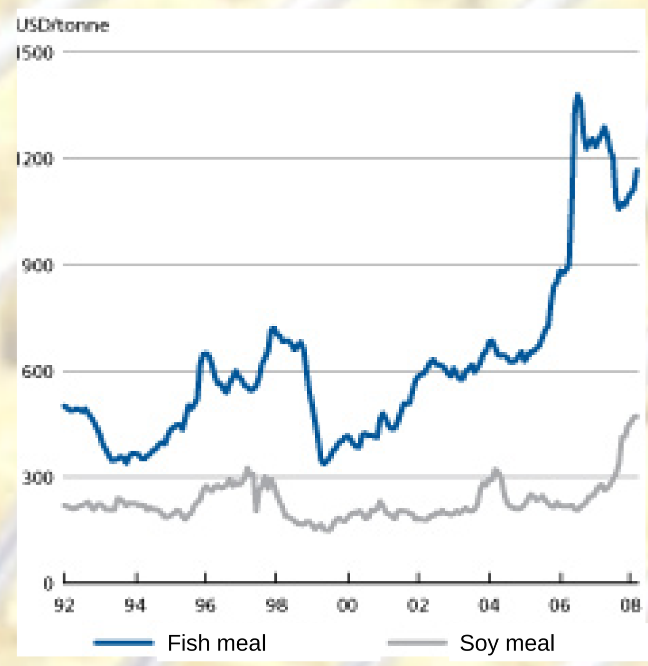


Figure 1 : Price of soy meal and fish meal (FAO, 2008).

The second argument is based on the contamination risk, resulting from the use of animal proteins.
Back in the 90's, considerable suspicion was directed toward animal protein because of the outbreak of bovine spongiform encephalopathy (BSE). This situation had serious effects on the use of animal proteins as a source of livestock feed, including for naturally omnivorous, even carnivorous animals. At the present time, in Europe, the use of animal meal is strictly regulated (EC 2001, 2008).
In the70's, Germany had an outbreak of laboratory animals diets with carcinogens (N-nitrosodimethylamin), fish meal was incriminated as the source of contaminant (Kann et al., 1977, cited by Edwards et al., 1979). It was then recommended to eliminate this raw material from any laboratory animal diet formulation (Rao & Knapka, 1987).
Nevertheless, fish meal is not the only possible source of nitrosamines contamination, since these compounds can also be detected in maize, potatoes (EPIC, 2004; Cotruvo et al., 2007) and water (Charrois et al., 2007).
There is no real logic behind the exclusion of one potential contaminant if the absence is not guaranteed from any other sources! Only a rigorous, systematic selection of raw materials, a careful selection of the ingredients before any raw material is included in a diet combined with a final control of the diet product is a suitable solution to prevent diet-borne contaminations.
This quality process is based on rigorous selection and monitoring of ingredient suppliers, as well as the stringed control of potential contaminants (heavy metals, pesticides, mycotoxins, etc.) and not on the arbitrary exclusion of families of raw materials.

2.Natural feeding behaviour and preferences of animals

The natural feeding behaviour and preference of mice (Mus musculus) and rats (Rattus norvegicus) is to depend on vegetal and animal materials for their subsistence, therefore they are true omnivorous, by opposition to herbivores such as guinea pigs (Cavia porcellus) and rabbits (Oryctolagus cuniculus) only get their protein source from plant and plant by-product. Herbivores naturally avoid animal protein sources, such as casein hydrolysates or gelatine (Figure 2, Field et al., 2009).

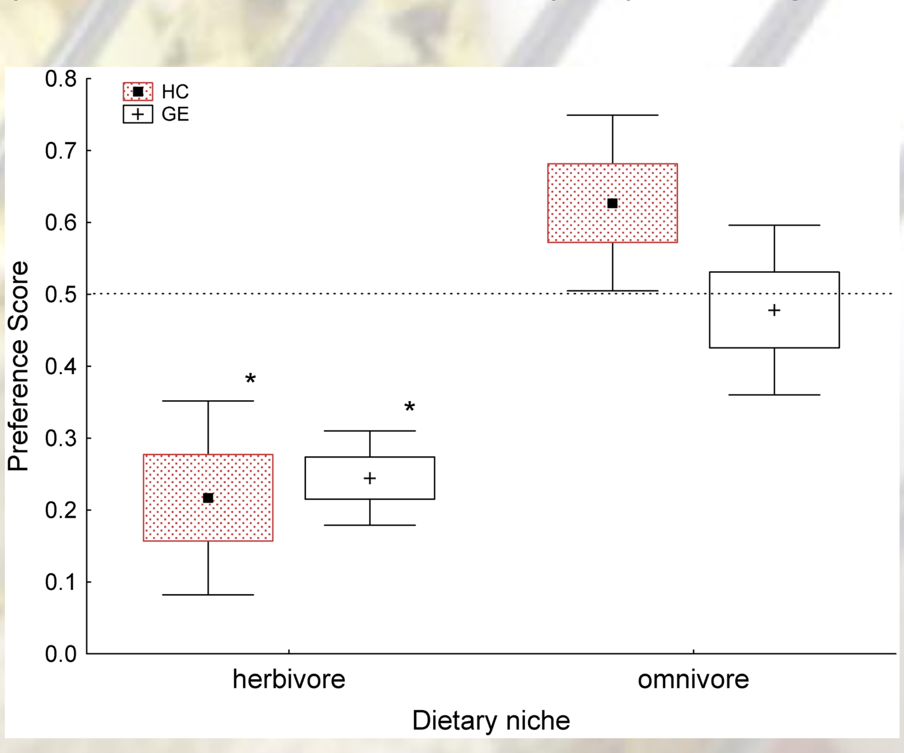


Figure 2 : Dietary preferences of herbivores (*Aplodontia rufa, Cavia porcellus, Microtus townsendii, Oryctolagus cuniculus, Thomomys mazama*) and omnivores (*Peromyscus maniculatus, Mus musculus, Rattus norvegicus, Canis latrans*) for casein hydrolysate (CH) or gelatine (GE) (Field et al., 2009).

3. Nutritional needs and responses of laboratory rodents

In order to formulate diets, the initial control of raw material nutrients is essential to meet individual animal needs; in order to select the best ingredient, analytic and industrial tools help to precisely quantify the nutritional and composition value of each raw material required for the precise dosage of each diet. The quality of raw material impact the animal phenotypic determinism (more than 50 in rats: NRC, 1995).

The replacement of animal proteins by plant sources is very frequent in livestock feed, as an example, pig feed formulation for (omnivores) is prepared in a manner where it is uniquely manufactured from plant sources with no adverse effects on animal production (Aumaître and Lambert, 1969; Bouard et al., 1979). One indispensable parameter to any substitution in the recipe is the amino acids among the formulation, along with anti-nutritional factors contained in plant raw materials, such as gossypol in cotton meal ,glucosinolate levels in canola, lignin contents in fibre-rich raw materials, etc.

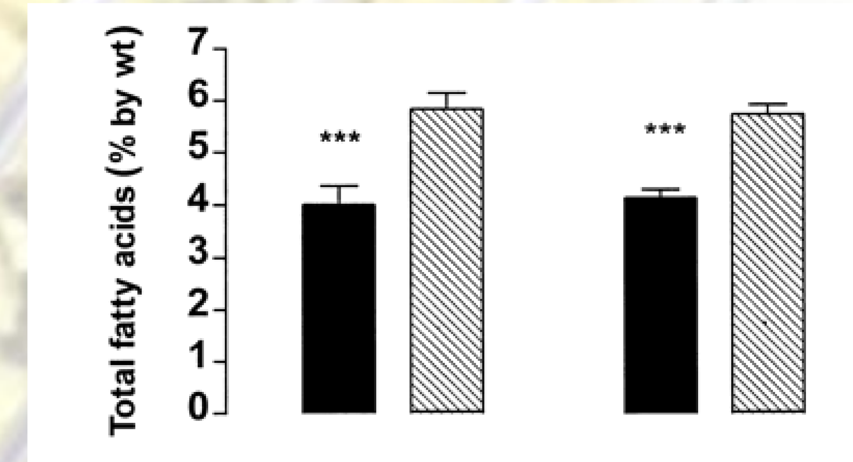
Furthermore, replacing animal raw materials by plant sources has a significant impact on the availability, digestibility and bio absorption of elements to the organism, in another way elements provided in an inorganic form may lower bioavailability than it's organic counterparts.
In addition, the nutritional value of certain components may be either poorly understood or difficult to dose in regard of what is perhaps under estimated today and will reconsidered tomorrow. These substances have been catalogued by the NRC as "potentially beneficial dietary components" (NRC, 1995). Thus, in the section devoted to rats, chromium was downgraded from "indispensable nutrient" to "potentially beneficial component" between the second edition of this reference (NRC, 1972) and the fourth (NRC, 1995); by opposition, the interest for dietary fibres appears only in the 4th edition. It is thus entirely possible that in the coming years certain substances such as taurine, hydroxyproline, bioactive peptides or certain long chain fatty acids will be considered as indispensable for defined physiological stages.

In absence of exhaustive knowledge of laboratory animals nutritional needs, only a formula blending diversified ingredients source that are consistent with the animals natural behaviour and digestive physiology, will guarantee that the model is stable and representative.

4. Risks of using strictly full- vegetal diets - example of DHA

The use of diets for laboratory rodents formulated solely from plant raw materials creates the risk of shortfalls between nutritional needs and supplies. As an example, docosahexaenoic acid (DHA, omega-3), is present primarily in ingredients of animal origin, in particular fish and sea products (Innis, 2007); this fatty acid can be synthesised by animals ingesting alpha-linolenic acid (ALA) found in plant sources such as flax.
This route of supply, however, is insufficient for embryonic growth since the capacity to transform ALA to DHA is lower than required during gestation to ensure normal foetal development, especially the brain and cardiac tissues. In humans, the effects of a mother's vegetarian diet on the baby have shown plasma fatty acid contents in the umbilical cord (Figure 3, Sanders, 1999).

Figure 3 : Mean proportions of docosahexaenoic acids in plasma and arterial phospholipids in the umbilical cord of babies born at term from vegetarian (black bars) or omnivorous mothers (hatched bars). ***P < 0.001 (Sanders, 1999)



In animals, the reduced supply of brain DHA in the foetus and neonate has been linked to deficits in dendritic development of neurons, in neurotransmission and to elevations of indicators of anxiety, aggression and depression.(Bertrand et al., 2006; McNamara & Carlson, 2008).

The supply of DHA is also indispensable for reproduction in order to guarantee rodent fertility. Thus, sexual maturity (first ovulation) may be artificially delayed by an essential fatty acids dietary deficiency (Smith et al., 1989). The between omega-6 and omega-3 fatty acids ratio in the diets improves the ovulation rate of rats (Trujillo & Broughton, 1995). Fish oil is used to feed poultry in order to improve sperm quality (Hudson & Wilson, 2003). When using raw materials rich in omega-3, a special attention must be paid to storage conditions in order to prevent fatty acids oxidation which in return may have potential negative effects on fertility and health (Zidkova et al., 2004).

The use of a variety of ingredients in diets, consistent with the natural feeding habbits and digestive physiology of laboratory animals, contributes to the diversity of nutrient supplies and thus to minimising the potential risk of a deficiency or shortfall of a given nutrient essential for experimental protocols. As humans, the principal laboratory rodents (Mus musculus and Rattus norvegicus) are natural omnivores. This behaviour makes them particularly well suited models for human biomedical research. Published data stress the importance of diet for the expression of genotype (Kozul et al., 2008). They show that the use of feeds for these rodents that contain no animal raw materials may lead to drift and even major physiological dysfunction (neuronal, cardiac, etc.) starting at the earliest stages of development. The results could be negative for producers because of reduced reproduction performance and for users of the animals in experimental conditions. Changes in the perinatal diet can in fact influence the expression of genotype and affect adult phenotype.

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