ENVIGO

WHITE PAPER // JULY 2018

Diet matters!

How laboratory animal diets can be a research variable and research tool

"Non-reproducible single occurrences are of no significance to science."

– Karl Popper (Popper, 1959)

Introduction

Reproducibility is a key principle in the conduct and validation of experimental science. Fundamental to the conduct of reliable, reproducible scientific research is limiting variables to those being studied. There are numerous factors that can influence research outcomes and should, therefore, be controlled. Aside from the health status of the laboratory animal, environmental factors such as diet, bedding, light cycles, noise, humidity, temperature, and personnel interactions with animals are key variables that can influence research outcomes (Reardon, 2016).

Almost 40 years ago, the National Academy of Sciences recognized that the detailed composition of laboratory animal diets used for experimental purposes is important knowledge for scientists (Institute of Laboratory Animal Resources Committee on Laboratory Animal Diets, 1978). The overall quality and consistency of diets fed to a variety of laboratory animal species was further strengthened in the late 1970s, when the U.S. Food and Drug Administration (FDA) issued regulations outlining the principles of Good Laboratory Practice (GLP).

While it has long been known that diet affects reproduction, growth, disease, and response to experimental manipulation in laboratory animals (Barnard, 2009), diet continues to be an overlooked variable in experimental investigations (Newberne, 1996; Warden, 2008). Since diet can affect the accuracy and reproducibility of scientific studies, it follows that diet can influence experimental outcomes and the conclusions drawn from the empirical data.

Careful consideration of animal diet will help support experimental designs. Broadly, the selected diet should align with nutritional requirements of the animals, have minimal batch-to-batch variation, and minimize the potential for confounding (e.g. exclusion of an ingredient that has a well-known effect on an outcome).

This article is intended to provide the reader with basic information about how diets can be both a source of experimental variation and a valuable research tool, as well as describe some key considerations when choosing a lab animal diet.

Reproducibility is a key principle in the conduct and validation of experimental science.

Types of laboratory animal diets

The first step to a more thorough understanding of diet is a review of terminology, which will be done in the next several sections and is summarized in Table 1.

Laboratory animal diets are differentiated into categories based on either the ingredient composition (i.e. natural or refined ingredients), or their end use (i.e. standard, custom, medicated). Each of these categories is described in more detail below.

Natural ingredients in laboratory animal diets refer to relatively unrefined agricultural commodities. Some examples are grains (corn, wheat, oats), grain by-products (wheat middlings, wheat bran), concentrated plant protein sources (soybean meal, corn gluten meal, alfalfa meal), and animal proteins (pork meal, fish meal). These natural ingredients are chemically complex, containing a variety of macronutrients and micronutrients as well as non-nutrients.

Figure 1. Standard diets: L to R bottom: pelleted rodent diet, extruded rodent diet. L to R top: dog, rabbit, non-human primate, cat and ferret diet.

Refined ingredients are those that have undergone further processing from their source material. As a result, they are chemically simple, as they typically provide one major nutrient and sometimes trace amounts of several other nutrients. For instance, casein is a common protein source extracted from cow's milk – it is precipitated, washed, and dried. The end result is a concentrated protein source with very little fat or carbohydrate, and the only mineral of significance with respect to formulation of diets is phosphorus. Other commonly used refined ingredients include free amino acids, sucrose, glucose, fructose, cornstarch, cellulose, and fats and oils. The non-nutrient component has generally been removed or reduced during processing.

Standard is a common term used to denote diets comprised of natural ingredients. Standard diet is also commonly referred to by other terms including grain-based, cereal-based, and chow. Standard diets have been used to feed laboratory animals for more than 75 years. For practical reasons (cost, ready availability), they are the most widely used type of laboratory animal diet. These diets come in various shapes and sizes that reflect species and method of processing (Figure 1).

They are used to support reproduction, growth, and maintenance of animal colonies. Because of the nature of the ingredients and the scale of production, there will be a greater degree of nutrient variability relative to diets using refined ingredients. Only in recent decades has the presence of non-nutrients and their variability in content, and how research can be impacted (Brown and Setchell, 2001), been appreciated.

Types of laboratory animal diets

Custom diets (Figure 2), as implied in the name, are custom formulated and generally produced in small quantities. Purified diets – also referred to as semi-purified, defined, or synthetic – are the most common type of custom diet and are made from the refined ingredients previously described. These food-grade ingredients have relatively simple chemical compositions, making it possible to manipulate individual nutrients (Reeves, 1997). The use of refined ingredients provides uniformity and reproducibility benefits, allows for tremendous versatility in diet design, and serves to reduce variation due to natural substances that may have biological activity (Kozul, 2008; Thigpen, 1999). Also quite common is the use of a complete standard diet as the primary component in a custom diet to which a variety of ingredients can be added. Custom natural ingredient diets can also be formulated using the same agricultural commodities as found in standard diets. This is less common, as the complexity of ingredient composition in natural ingredient diets limits their usefulness for a custom application. Overall, custom diets are developed and used for a defined purpose, often with input from the investigator.

Medicated diets are those produced in bulk and used for veterinary purposes. These medications are added to a standard diet, and the diets are produced in facilities where equipment can be sufficiently cleaned to minimize the risk of downstream cross-contamination. Anthelmintics (a group of antiparasitic drugs) and antibiotics are the most common types of medication used in medicated diets. The purpose of these drugs in the diets is to eliminate pinworms and fur mites and to prevent infection. Importantly, drugs incorporated into a medicated diet should be stable and able to withstand degradation during production and storage. Many diets containing medications or similar compounds can be made on a small scale, and these fall into the custom category.

Figure 2. Custom diets: Purified diets (white or colored; colors are from approved food dyes, added to aid the user in differentiating diets) and custom diet with standard diet base or natural ingredients (brownish)

Types of laboratory animal diets

Table 1: Summary of diet types and various attributes of these diets

	Standard (natural ingredient) diet	Custom diet	Medicated diet (veterinary purpose)
Ingredients	+ Natural ingredients that are relatively unrefined and chemically complex + Potential for non-nutrients which can impact phenotype	+ Primarily refined and chemically simple, with limited non-nutrients + Can be made from natural ingredients or consist of standard diet plus other ingredients	Medications generally added to standard diet
Purpose	+ Reproduction, growth, maintenance, aging + Non-specific research	+ Nutrient control + Induce disease + Dose animal	+ Treat outbreak + Prophylactic + Quarantine
Variety	Relatively limited	Practically unlimited	Limited for those produced in large quantities
Scale of production	Tons	Kg	Varies
In stock	Yes	No or select few	Yes (common)
Storage	Cool, dry (<70 F, 50% RH)	4°C or colder	Cool, dry (<70 F, 50% RH)
Use by	6 or 9 months	6 months	6 months
Formula disclosure	No (for most)	Yes	Yes, for amount of drug

Careful consideration of animal diet will help support experimental design.

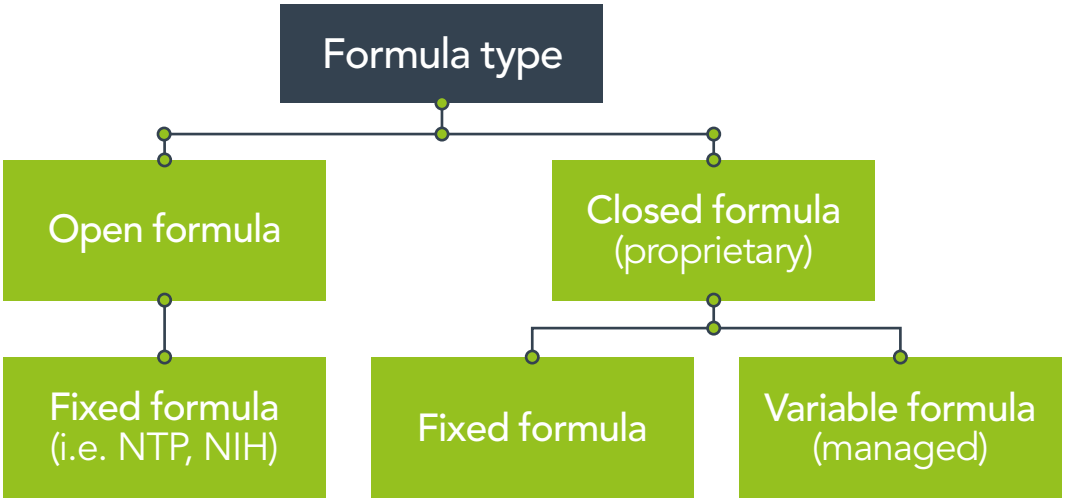
Formula types

Diet formulas for laboratory animals can be classified as open or closed (Figure 3). In open formula diets, the quantitative ingredients are published and available. Any manufacturer can produce the diet to specification, and researchers can report a full formula. In contrast, closed formulas are proprietary, meaning that while the ingredients are disclosed, the exact recipe is known only to the manufacturer. Researchers must rely on technical datasheets supplied by the manufacturer for relevant details about the diet. These closed or proprietary standard natural ingredient diets are marketed under trade names (e.g. Envigo Teklad 2018; LabDiet 5001, SDS RM3) and are widely used in facilities across the globe.

Open formulas are by definition fixed, meaning the recipe is the same each time is it produced. The most familiar examples of open, fixed formulas are standard natural ingredient diets that were created by government agencies like the National Institutes of Health (NIH) and the National Toxicology Program (NTP), such as the NIH-07, NIH-31, and NTP-2000 diets. These open formula diets are used less frequently than closed formulas, mostly due to the limited options which are not as well-suited to various research applications.

The commonly used purified diets AIN-76A, AIN-93G, and AIN-93M are open formulas that were developed by a committee of scientist members of the American Institute of Nutrition (now called American Society for Nutrition). Most manufacturers of purified diets fully disclose the ingredient composition and inclusion rates to the end user.

Figure 3: Schematic of formulation terminology



Formulation practices and impact on nutrient variation

Closed formulas can be either **fixed** or **variable** (Figure 3). For both types, ingredients should be listed on the manufacturer's technical data sheet. Beyond this, it is relevant to make some comparisons between the approaches.

In fixed-formula diets, the ingredients and their relative proportions are not altered. This factor is coupled with stringent ingredient sourcing and monitoring practices in order to manage variability. The rationale for using fixed formulation is that natural ingredients contain a variety of nutrient and non-nutritive components, both of which can influence research results. The overall goal is to satisfactorily minimize nutrient variation and manage non-nutrient variation to the extent possible, all while maintaining the integrity of the formula.

Manufacturers that use variable formulation do so because they believe macronutrient variability in raw materials could lead to significant variation in the finished diets. Therefore, inclusion rate changes may be necessary. These changes are made based on the indirect assay of ingredients using near infrared spectroscopy (NIRS) to achieve stability in a set of nutrients (protein, fat, crude fiber, and perhaps a few macro-minerals) that can be predicted relatively accurately by NIRS. These changes are made in real time, and not all nutrients can be monitored. End users will not be aware whether or how a variable formula diet has changed over time. It is plausible that this practice will introduce more variability in nutrients that are not monitored, and in the amount and type of non-nutrients. This may have considerable impact on study results (Thigpen, 2004; Thigpen, 2007).

The nutrient content of plants does vary with harvest location and across growing seasons, and although geographic and seasonal variations may impact ingredient nutrient levels, the effects on levels in the finished feed are often overstated.

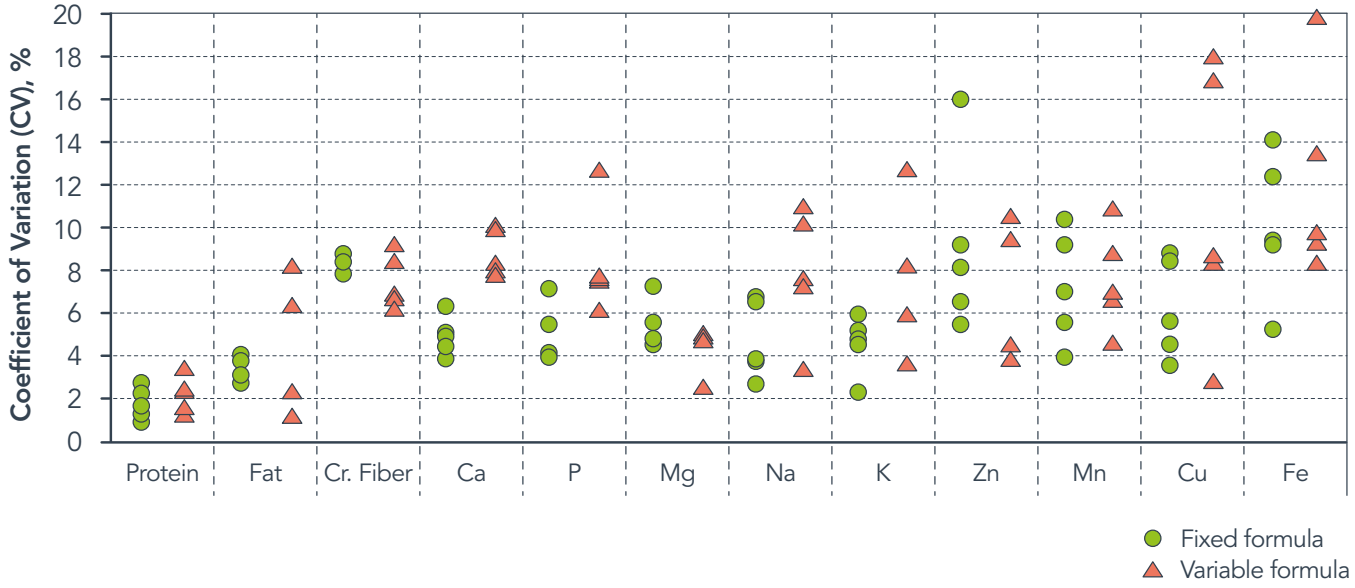
The results from a study of the quality and variation in the average protein content of soybean meal sources from six highly varied countries revealed a maximum difference of just of 2.8% (Thakur, 2007). In most feeds, this would represent a finished product variation of 0.2-0.7% protein – a small variation that would likely be balanced by protein variations in other ingredients.

Overall, irrespective of formulation approach, rigorous quality control by feed manufacturers can significantly reduce the magnitude of batch-to-batch nutrient variation in a finished product.

Formulation practices and impact on nutrient variation

It is not necessary to alter ingredient inclusion rates to manage nutrient variability, as shown in Figure 4. The plot is a comparison of the coefficient of variation (CV) over a period of 5 years, comparing a variable formula and a fixed formula. The plot shows both the magnitude and the distribution of variation for these nutrients (<5% for protein and 5-15% for most minerals) and demonstrates that fixed and variable formulation strategies can result in similar nutrient stability in the finished product.

Figure 4. Comparison of macro- and micro-nutrient variation in a fixed vs. variable formula diet over a period of 5 years (2009-2013). Source: Envigo



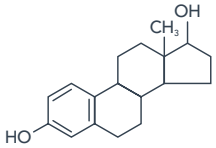
Ingredient selection and monitoring to minimize the presence of specific non-nutrients

In addition to the effects a diet may have on research studies as a result of its nutrient profile, the presence of non-nutrients can also affect endpoints (Kozul, 2008). Plant and animal origin materials in natural ingredient diets contain many compounds that are not nutrients. Some fall into the category of contaminants which are introduced from the environment, while others are inherent in the ingredients. Some have been identified, can be reliably measured, and have recognized physiological effects. Others are yet to be identified.

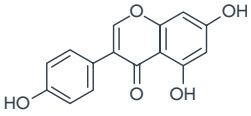
Examples of contaminants include heavy metals (arsenic, lead, mercury, cadmium), certain pesticides (list varies by region of the world), and mycotoxins. The disruptive potential of some contaminants is recognized (Oller, 1989; Rao 1987; Greenman, 1980), and limits are placed on their levels in certified diets used for GLP purposes. The common practice of screening corn and wheat ingredients for mycotoxins prior to unloading at the feed mill ensures that finished diets will not exceed a limit. Exclusion of fish meal will minimize nitrosamines, a potential carcinogen (Edwards, 1979), and result in lower arsenic levels in a standard diet.

Many rodent diets, formulated years ago but still commonly used, contain small amounts of alfalfa meal. More recently, it has been appreciated that the naturally occurring component chlorophyll in alfalfa meal interferes with fluorescent imaging in live animals (Troy, 2004). Autofluorescence in the abdominal region due to chlorophyll obscures the image. Use of either a standard diet without alfalfa meal or a purified diet greatly reduces background autofluorescence, making it possible to quantify the signal of interest (Inoue, 2008).

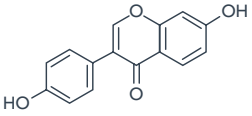
Among those non-nutrients that are inherent, phytoestrogens receive the most attention. Phytoestrogens are plant-derived compounds that mimic both the structure and the function of estrogen in mammals. The primary types of phytoestrogen present in laboratory animal diets are the isoflavones genistein and daidzein, found in soybean meal, and coumestrol, found in alfalfa meal (Thigpen, 1999). Their structural similarity to estrogens allows interaction with the estrogen receptors and the elicitation of estrogenic or antiestrogenic effects. For this reason, they are referred to as selective estrogen receptor modulators (SERMs) (Brown 2001, Thigpen 2004).



estradiol



genistein



daidzein

Ingredient selection and monitoring to minimize the presence of specific non-nutrients

Phytoestrogens have both hormonal and non-hormonal properties capable of modulating cell function and therefore influencing a very broad range of physiological systems (Barnes, 2010). The effects of dietary isoflavones at levels commonly found in traditional rodent diets have been noted in animal models used to study reproductive and endocrine disruptors (e.g. Boettger-Tong, 1998; Makela, 1995), cancer (e.g. Ju, 2001; Allred, 2004; Lakshman, 2008), bone metabolism (e.g. Picherit, 2000; Droke, 2007), behavior (e.g. Lephart, 2004; Lund, 2001), cardiovascular system (e.g. Nagarajan 2008; Peluso, 2000), endocrine (e.g. Cederroth, 2008; Mezei, 2003), immunology (e.g. Cooke, 2006; Vasiadi, 2014) among others. See Table 2 for an abbreviated summary.

Unfortunately, these effects are not predictable; isoflavone content varies considerably, both between different formulations and also from batch to batch of the same product (Jensen, 2007). In addition, there is no recognized dose-response relationship or threshold for the various research effects. A number of confounding factors (e.g. timing and duration of exposure, level and type of isoflavone, animal model, endpoints monitored) also contribute to the difficulty in predicting the magnitude or direction of effects for any phytoestrogen exposure, which may lead to apparently conflicting results.

Therefore, the best approach to minimize the impact of dietary phytoestrogens on biomedical research is to minimize their presence in laboratory animal diets used in research studies where there is a reasonable likelihood that they could impact an outcome.

This requirement could be met by choosing a standard natural ingredient diet that excludes soybean meal and alfalfa meal, or by choosing a purified diet.

Table 2: Summary of biological effects of soy and/or soy isoflavones reported in the literature for a number of research areas.

Research area	Effects described in the literature
Oncology	Modulate tumor growth, latency, multiplicity, metastasis; diminish action of drugs such as tamoxifen and letrozole
Reproductive	Increase uterine weight, accelerate vaginal opening, affect response to exogenous estrogens/xenobiotics
Endocrine	Differences in body weight/adiposity, glucose/insulin homeostasis, bone density, blood pressure
Neuroscience	Performance differences on tests measuring anxiety behaviors and response to pain stimuli
Immunology	Modulate cytokine production and immune organ development

Use of custom diets

Custom diets, by their very nature, are research tools, as they can be designed and manufactured to accurately control nutrient levels, induce disease, and dose an animal. However, they need to be chosen correctly, and there is significant value to collaborating with a nutritionist when selecting a custom diet. The following sections highlight some common uses of custom diets.

Diet can be used to manipulate nutrient intakes. This has been a tool used in research for a very long time. For example, various sugar, starch and fiber-adjusted diets have been fed to differentiate the effects of carbohydrate type. Certain fibers and resistant starches are commonly fed to investigate changes in the intestinal microbiota. Diets with low or high protein levels are used to study malnutrition, gestational protein restriction, or renal physiology. Amino acid defined diets are used to study inborn metabolic errors. Using a variety of fat sources, the fatty acid profile can be manipulated to contain different proportions of saturated, monounsaturated, or polyunsaturated fatty acids. Custom diets can be formulated to be deficient in a given vitamin or mineral. Then, specific amounts of a given nutrient can be added back. Custom diets can be designed to mimic human dietary intakes, such as "western" or "Mediterranean." These can be relatively subjective, so it is important to convey your specific objectives to the nutritionist who develops the formulation.

Custom diets can be designed to induce disease. This is often in conjunction with a genetic modification, surgical procedure, or other intervention. Some examples include western and atherogenic diets where the presence of saturated fat, cholesterol, and cholate are common features. Diets containing 40-60% of kcal from fat (most often from lard or milkfat) are commonly used to induce obesity. Key dietary factors in the variety of diets used to promote fatty liver disease include sucrose, saturated fat, cholesterol, methionine, and choline. Cuprizone, a copper chelator, added to a standard diet base causes demyelination in a model of multiple sclerosis.

Diet can reliably and conveniently be used to dose an animal. Use of diet overcomes welfare concerns and technical limitations, although a drawback is that incorporation into diet does not offer as precise a delivery as oral gavage. A regular approach is the addition of doxycycline or tamoxifen to a standard diet, to control genetics in certain transgenic models. All sorts of ingredients can be and have been added to custom diets, including those supplied by customers. This requires that safety has been vetted and stability through the manufacturing process considered. In determining the inclusion rate of a drug, extract, or other type of material, considerations include purity, expected food intake, and body weight. If the dose is being extrapolated from humans or other species, it is suggested that the rodent's metabolic rate be considered in order to properly calculate the inclusion level.

Selecting an appropriate diet

The goal of diet selection is to minimize the potential for diet to be a confounding factor when interpreting data. Furthermore, the introduction of new technologies, such as genomics and proteomics, which have broad biological implications, will likely increase the importance of controlling environmental variables such as diet in research studies.

Below are some key considerations for selecting an appropriate diet. Notably, it is often worthwhile to consult a nutrition expert familiar with laboratory diet options to ensure you are making the best choice possible based on your experimental requirements.

Choose a diet that generally aligns with nutritional requirements. This is where your diet vendor can be helpful, as can general resources (Tobin, 2007; National Research Council, 1995; Ritskes-Hoitinga, 2004; Keenan, 2000). A variety of standard diets are commercially available, designed for various life stages and/or suitable for specific research applications.

Within the broader range of standard diets, some further divisions can be made (Table 3). The diets used by commercial breeders contain about 18-19% protein and 5-6% fat by weight.

This is suitable for breeding of most stocks and strains. Higher-fat standard diets (8-11% fat) have increased energy content and can be beneficial in specific circumstances when animals are not robust or are not thriving on typical diets, but these diets can also lead to excess weight gain and increased fat deposition that can ultimately reduce breeding performance. Many diets are described as multi-purpose; they contain a range of protein levels and moderate fat content. Diets designed primarily for maintenance and aging have lower protein and fat levels.

The goal of diet selection is to minimize the potential for diet to be a confounding factor when interpreting data.

Selecting an appropriate diet

Table 3: Range of macronutrient levels for commercially available standard natural ingredient diets.

	% weight	% kcal
Protein	14 - 24%	17 - 30%
Carbohydrate	40 - 50%	50 - 75%
Fat	4 - 11%	10 - 25%

Category	Protein, % weight	Fat, % weight
Breeding	18 - 19%	5 - 6%
Higher energy	17 - 21%	8 - 11%
Multiple purpose	16 - 24%	4 - 6%
Maintenance	14 - 16%	3 - 4%

Selecting an appropriate diet

Macro- and micronutrient levels in these standard diets are in relative excess of estimated requirements, as they have to be suitable for the entire recommended use period, taking into consideration variable effects of processing and storage. The typical use period is 6 months in North America and 9-12 months in other parts of the world. This is largely based on longstanding practices and experience rather than an abundance of specific information about nutrient degradation or diet quality at these time points.

Study vendor's technical datasheet.
However, be aware of limitations, particularly for standard diets. It is important to have reasonable expectations about the accuracy and precision of the nutrient information. The estimates are derived from a variety of sources and should be used only as guidelines. If the interpretation of an experiment depends on accurate knowledge of a nutrient level, it should be measured; this is advisable whether a natural ingredient or purified diet is used.

Evaluate the list of ingredients.
Careful consideration should be given to the use of readily available and economical standard natural ingredient diets that have been formulated to contain minimal levels of isoflavones by omitting soybean meal, particularly when there is supporting literature for such use (Table 2). These newer formulations, which reflect a better understanding of nutrient requirements and nutrient utilization, were developed with an appreciation of the impact of non-nutritive components on study outcomes.

Consider the formulation approach when choosing a standard diet.
Does the formula stay the same over time or can it change? Fixed formulas do not change, but variable ones can. Fixed formulas have the combination of stable nutrient levels and stable ingredient composition.

Selecting an appropriate diet

Evaluate the diet manufacturer.
It is common practice at the institutional level to purchase standard, natural ingredient diets from a primary vendor. The selection of vendor is typically made by a group consisting of veterinarians, institute directors, representative researchers, and procurement personnel. Those with this responsibility should ensure that due diligence is conducted to ascertain quality systems encompassing ingredients, manufacturing, biosecurity, and distribution network, as well as technical support. Site audits and requests for nutrient and contaminant data from manufacturers will provide valuable information to aid decision-making.

Choose a custom diet when research objectives demand it. For instance, a purified diet is necessary when precise control of nutrient levels is needed. Search the literature for precedent, review manufacturer websites, and contact the company nutritionists for advice and formulation assistance.

Invest time upfront during the experimental design phase to carefully select diet(s). Appropriate planning before beginning your experiments can pay-off in the long-term. The following example demonstrates that diet type and specifically the degree to which diets are matched can significantly impact study conclusions.

Hubbard et al. (2013) conducted a study to determine whether liver iron stores could be increased through dietary iron supplementation, using pregnant mice to mimic human oral iron supplementation through pregnancy. The mice were fed a control purified diet with 45 ppm iron, a level that was not expected to maximize liver Fe stores. In earlier work, this purified diet was compared to a standard natural ingredient diet containing approximately 220 ppm iron, and liver iron stores were not increased with higher dietary iron.

To control for differences in ingredients and iron source, both of which likely affected iron bioavailability, the authors included a purified diet, matched to the first except containing 220 ppm. Using this diet design, they found that liver iron stores could be increased through dietary iron supplementation when using otherwise equivalent diets.

Appropriate planning before beginning your experiments can pay-off in the long-term.

Conclusions

In summary, diet can be a research variable due to its potential for nutrient and non-nutrient variability, which are influenced by quality practices, formulation approaches, and types of ingredients present. Diet can also be a variable if the chosen diet is not aligned with life stage or study objectives.

On the other hand, diet is a research tool when it is carefully selected. For instance, ingredients of concern are avoided and the diet is chosen to support study objectives. There will always be some unknowns, but the opportunity to perform quality work is maximized.

Diet manufacturers must provide accurate information and produce high-quality products suitable for use in research. In turn, individual end users – particularly those in a position to make decisions about diet on behalf of an organization – bear responsibility for making rational choices regarding the selection of a diet vendor as well as the specific diet or diets.

The benefit to investing time and effort in diet choice is better quality research and improved translatability from animal models to humans.

References

1. **Allred CD, Allred KF, Ju YH, Clausen LM, Doerge DR, Schantz SL, Korol DL, Wallig MA, Helferich WG. (2004)** Dietary genistein results in larger MNU-induced, estrogen-dependent mammary tumors following ovariectomy of Sprague-Dawley rats. Carcinogenesis. Feb;25(2):211-8.
2. **Barnard, DE, Lewis, SM, Teter, BB, Thigpen, JE. (2009).** Open- and closed-formula laboratory animal diets and their importance to research. Journal of the American Association for Laboratory Animal Science 48(6), 709–713.
3. **Barnes, S. (2010).** The biochemistry, chemistry and physiology of the isoflavones in soybeans and their food products. Lymphat Res Biol, 8(1), 89-98.
4. **Boettger-Tong H, Murthy L, Chiappetta C, Kirkland JL, Goodwin B, Adlercreutz H, Stancel GM, Mäkelä S. (1998).** A case of a laboratory animal feed with high estrogenic activity and its impact on in vivo responses to exogenously administered estrogens. Environ Health Perspect. Jul;106(7):369-73.
5. **Brown NM, Setchell KD (2001).** Animal models impacted by phytoestrogens in commercial chow: implications for pathways influenced by hormones. Lab Invest 81:735-747.
6. **Cederroth CR, Vinciguerra M, Gjinovci A, Kuhne F, Klein M, Cederroth M, Caille D, Suter M, Neumann D, James RW, Doerge DR, Wallimann T, Meda P, Foti M, Rohner-Jeanrenaud F, Vassalli JD, Nef S. (2008).** Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism. Diabetes 57:1176-1185.
7. **Cooke PS, Selvaraj V, Yellayi S. (2006).** Genistein, estrogen receptors, and the acquired immune response. Journal of Nutrition 136:704-708.
8. **Droke EA, Hager, KA, Lerner MR, Lightfoot SA, Stoecker BJ, Brackett DJ, Smith BJ. (2007).** Soy isoflavones avert chronic inflammation-induced bone loss and vascular disease. Journal of Inflamm 4:17.
9. **Edwards GS, Fox JG, Policastro P, Goff U, Wolf MH, Fine DH. (1979).** Volatile nitrosamine contamination of laboratory animal diets. Cancer Res 39:1857-1858.
10. **Greenman DL, Oller WL, Littlefield NA, Nelson CJ (1980).** Commercial laboratory animal diets: toxicant and nutrient variability. J Toxicol Environ Health 6:235-246.
11. **Hubbard, A. C., Bandyopadhyay, S., Wojczyk, B. S., Spitalnik, S. L., Hod, E. A., & Prestia, K. A. (2013).** Effect of dietary iron on fetal growth in pregnant mice. Comparative medicine, 63(2), 127.
12. **Inoue Y, Izawa K, Kiryu S, Tojo A, Ohtomo K. (2008).** Diet and abdominal autofluorescence detected by in vivo fluorescence imaging of living mice. Mol Imaging 7:21-27.
13. **Institute of Laboratory Animal Resources Committee on Laboratory Animal Diets (1978).** Control of diets in laboratory animal experimentation. Washington (DC): National Academies of Sciences.
14. **Jensen MN, Ritskes-Hoitinga M (2007).** How isoflavone levels in common rodent diets can interfere with the value of animal models and with experimental results. Laboratory Animals, 41(1), 1–18.

References

15. **Ju, Y. H., Allred, C. D., Allred, K. F., Karko, K. L., Doerge, D. R., & Helferich, W. G. (2001).** Physiological concentrations of dietary genistein dose-dependently stimulate growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in athymic nude mice. Journal of Nutrition, 131(11), 2957-2962.

16. **Keenan et al.** Chapter 5 - Nutrition, In Handbook of Experimental Animals, edited by Georg J Krinke,, Academic Press, London, 2000, Pages 57-75, ISSN 1874480X, The Laboratory Rat, ISBN 9780124264007.

17. **Kozul CD, Nomikos AP, Hampton TH, Warnke LA, Gosse JA, Davey JC, Thorpe JE, Jackson BP, Ihnat MA, Hamilton JW (2008).** Laboratory diet profoundly alters gene expression and confounds genomic analysis in mouse liver and lung. Chem Biol Interact 173:129-140.

18. **Lakshman M, Xu L, Ananthanarayanan V, Cooper J, Takimoto CH, Helenowski I, Pelling JC, Bergan RC. (2008).** Dietary genistein inhibits metastasis of human prostate cancer in mice. Cancer Res 68:2024-2032.

19. **Lephart ED, Setchell KD, Handa RJ, Lund TD. (2004).** Behavioral effects of endocrine-disrupting substances: phytoestrogens. ILAR J. 45(4):443-54.

20. **Lund TD, Lephart ED. (2001).** Dietary soy phytoestrogens produce anxiolytic effects in the elevated plus-maze. Brain Res 913:180-184.

21. **Makela, S. I., Pylkkanen, L. H., Santti, R. S., & Adlercreutz, H. (1995).** Dietary soybean may be antiestrogenic in male mice. The Journal of nutrition, 125(3), 437.

22. **Mezei O, Banz WJ, Steger RW, Peluso MR, Winters TA, Shay N. (2003).** Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW 264.7 cells. J Nutr 133:1238-1243.

23. **Nagarajan, S., Burris, R.L., Stewart, B.W., Wilkerson, J.E., and Badger, T.M. (2008).** Dietary soy protein isolate ameliorates atherosclerotic lesions in apolipoprotein E-deficient mice potentially by inhibiting monocyte chemoattractant protein-1 expression. J Nutr 138, 332-337.

24. **National Research Council.** Nutrient Requirements of Laboratory Animals, Fourth Revised Edition, 1995. Washington, DC: The National Academies Press, 1995. doi:10.17226/4758.

25. **Newberne P and Sotnikov A (1996).** Diet: The neglected variable in chemical safety evaluations. Toxicological Pathology 24:746-756.

26. **Oller WL, Kendall DC, Greenman DL (1989).** Variability of selected nutrients and contaminants monitored in rodent diets: a 6-year study. J Toxicol Environ Health 27:47-56.

27. **Peluso MR, Winters TA, Shanahan MF, Banz WJ. (2000).** A cooperative interaction between soy protein and its isoflavone-enriched fraction lowers hepatic lipids in male obese Zucker rats and reduces blood platelet sensitivity in male Sprague-Dawley rats. J Nutr 130:2333-2342.

28. **Picherit C, Coxam V, Bennetau-Pelissero C, Kati-Coulibaly S, Davicco MJ, Lebecque P, Barlet JP. (2000).** Daidzein is more efficient than genistein in preventing ovariectomy-induced bone loss in rats. J Nutr 130:1675-1681.

References

29. **Popper, KR (1959).** The logic of scientific discovery. Hutchinson, London, United Kingdom.

30. **Rao GN, Knapka JJ. (1987).** Contaminant and nutrient concentrations of natural ingredient rat and mouse diet used in chemical toxicology studies. Fundam Appl Toxicol 9:329-338.

31. **Reardon, S. (2016).** A mouse's house may ruin studies. Environmental factors lie behind many irreproducible rodent experiments. Nature News (12 February).

32. **Reeves PG (1997).** Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 127(suppl 5):838S–841S.

33. **Ritskes-Hoitinga, R.** Chapter 28 - Nutrition of Laboratory Mice, In The Laboratory Mouse, edited by Hans J Hedrich and Gillian Bullock, Academic Press, London, 2004, Pages 463-479, ISBN 9780123364258

34. **Thakur M, Hurburgh CR (2007).** Quality of US Soybean Meal Compared to the Quality of Soybean Meal from Other Origins. Journal of the American Oil Chemists' Society 84:835-843.

35. **Thigpen JE, Setchell KD, Ahlmark KB, Locklear J, Spahr T, Caviness GF, Goelz MF, Haseman JK, Newbold RR, Forsythe DB (1999).** Phytoestrogen content of purified, open- and closed-formula laboratory animal diets. Lab Anim Sci 49:530-536.

36. **Thigpen JE, Setchell KD, Saunders HE, Haseman JK, Grant MG, Forsythe DB (2004).** Selecting the appropriate rodent diet for endocrine disruptor research and testing studies. ILAR J 45:401-416.

37. **Thigpen JE, Setchell KD, Padilla-Banks E, Haseman JK, Saunders HE, Caviness GF, Kissling GE, Grant MG, Forsythe DB (2007).** Variations in Phytoestrogen Content between Different Mill Dates of the Same Diet Produces Significant Differences in the Time of Vaginal Opening in CD-1 Mice and F344 Rats but Not in CD Sprague-Dawley Rats. Environ Health Perspect 115:1717-1726.

38. **Tobin et al.** Chapter 10 - Nutrition, In The Mouse in Biomedical Research (Second Edition), edited by James G. Fox, Muriel T. Davisson, Fred W. Quimby, Stephen W. Barthold, Christian E. Newcomer and Abigail L. Smith, Academic Press, Burlington, 2007, Pages 321-383, ISBN 9780123694546.

39. **Troy T, Jekic-McMullen D, Sambucetti L, Rice B. (2004).** Quantitative comparison of the sensitivity of detection of fluorescent and bioluminescent reporters in animal models. Mol Imaging 3:9-23.

40. **Vasiadi, M., Newman, J., & Theoharides, T. C. (2014).** Isoflavones inhibit poly (I: C)-induced serum, brain, and skin inflammatory mediators-relevance to chronic fatigue syndrome. Journal of neuroinflammation, 11, 168-168.

41. **Warden C and Fisler J (2008).** Comparisons of diets used in animal models of high fat feeding. Cell Metab 7:277.

ENVIGO

Learn more about the difference of a Teklad diet through an Envigo nutritionist consultation at envigo.com/askanutritionist

This publication is brought to you by Envigo.

Envigo provides essential research services, models and products for pharmaceutical, crop protection, and chemical companies as well as universities, governments, and other research organizations.

envigo.com

Follow us