RESEARCH DIETS INC.
www.ResearchDiets.com

Laboratory animal control diets: very important, often neglected

Matthew Ricci, PhD

© 2015 Nature America, Inc. All rights reserved.
npg

Think of an animal study you've done, or read about, that used an experimental diet. Maybe the diet contained a drug or food compound, or maybe it was high in fat or low in protein or deficient in iron. Can you remember the control diet that was used in that study? If the study was designed well, then the control was identical to the experimental diet in every way, outside of the variable being studied. Unfortunately, there are many examples of research being done in which the control and experimental diets are not matched. This is like using different strains of mouse for the knockout and wild-type groups in the same study or using two completely different culture media in cell or tissue culture experiments. It's a problem because improperly matched diets can have many (sometimes dozens or more) variables different between them. Having the experimental and control diets differ only in the one variable being studied makes for simpler data interpretation, since differences in the phenotype of the animals can be attributed with confidence to the one difference between the diets. In contrast, when many dietary differences exist, how can we attribute results to only one of them?

Grain-based chows

A common example of improperly matched diets in animal studies is the use of a grain-based chow diet as a control for a purified ingredient diet. What are the differences in these two types of diets that make them inappropriate to use together? Grain-based chow diets are used in most animal facilities. They are made from a combination of relatively unrefined grains and plant materials such as corn, wheat, oat, soybean and alfalfa. Each ingredient contains multiple nutrients, with the benefit of contributing to nutritional adequacy of the diet, but also contains biologically active non-nutrients, including phytoestrogens and heavy metals such as arsenic. In addition, nutrient levels in these ingredients vary naturally. As a result, defining the content of grain-based chows is a bit like hitting a moving target.

Purified ingredient diets

In contrast, purified ingredient diets are made from highly refined ingredients that contain little outside of the nutrient itself. Examples include casein (which provides protein), corn starch and sucrose (carbohydrate), cellulose (fiber) and corn oil (fat); many other sources are also used in practice. The refined nature of the ingredients means that the content of purified ingredient diets can be controlled to meet researchers' needs, batch after batch. In fact, in the first half of the 20th century, purified ingredient diets were used to define the nutritional requirements of laboratory animals because they allowed researchers to selectively remove one nutrient at a time and then observe the effects, something not possible to do with grain-based chows. And because purified ingredient diets do not contain extraneous plant chemicals, they are considered 'cleaner' than grain-based chows.

Purified Diet Control vs. Grain-Based Chow

	Purified High Fat Diet	Purified Low Fat Control Diet	Grain-Based Chow	
	Ingredient*	Matched	NOT Matched	Reason
Fat	Lard, soybean oil	✓	X	Variable Sources
Protein	Casein	✓	X	Variable Sources
Carbohydrate	Corn starch, sucrose, maltodextrin	✓	X	Variable Sources
Fiber	Cellulose, insoluble	✓	X	Variable Sources/ 4X Higher
Micronutrients	Vitamins, minerals	✓	X	Variable Level
Phytoestrogens	NONE	✓	X	Variable Level
Heavy Metals	NONE	✓	X	Variable Level

*Ingredients typical of a purified diet, though other purified sources can be used.

FIGURE 1 | Purified diet control versus grain-based chow.

In sum, grain-based chows and purified ingredient diets differ in the sources and amounts of protein, carbohydrate, fat, fiber, vitamins and minerals (**Fig. 1**). Furthermore, grain-based chows contain phytoestrogens, heavy metals and other undefined plant compounds (**Fig. 1**). Considering this, it is easy to see why comparisons between these diets are not valid. But despite these major differences, these diets are often used side by side.

Metabolic disease

One example comes from the field of obesity and metabolic disease. In 2008, Warden and Fisler[1] noted that a substantial number of studies using purified ingredient high-fat diets did not use a matched purified ingredient low-fat control diet ('matched' means that, aside from the amount of fat and carbohydrate in the diets, all else is the same on a nutrient-to-calorie ratio; **Fig. 2**). They looked at 35 papers published in five high-impact journals in 2007 and found that 43% of these studies did not use a properly matched control diet. (It is interesting to note that a PubMed search for 'diet induced obesity mouse' in 2007 yields 203 papers while the same search for 2014 returns 1,081 papers.) Warden and Fisler correctly point out, "When comparing the effects of chow with a defined high-fat diet, the effects of the dietary fat will be confounded with the effects of other components that differ between the diets."

To be fair, nutrition training is not something many researchers have. Perhaps this explains why neither the authors nor the reviewers

Research Diets, Inc., New Brunswick, NJ. Correspondence should be addressed to M.R. (matthew.ricci@researchdiets.com).

(DIO) Formulas	60 kcal % fat		10 kcal % fat Matched diet	
Product #	D12492		D12450J	
	gm%	kcal%	gm%	kcal%
Protein	26	20	19	20
Carbohydrate	26	20	67	70
Fat	35	60	4	10
Total		100		100
kcal/gm	5.24		3.85	
Ingredient	gm	kcal	gm	kcal
Casein, 80 Mesh	200	800	200	800
L-Cystine	3	12	3	12
Corn Starch	0	0	506.2	2025
Maltodextrin 10	125	500	125	500
Sucrose	68.8	275	68.8	275
Cellulose	50	0	50	0
Soybean Oil	25	225	25	225
Lard	245	2205	20	180
Mineral Mix S10026	10	0	10	0
DiCalcium Phosphate	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0
Potassium Citrate, 1 H2O	16.5	0	16.5	0
Vitamin Mix V10001	10	40	10	40
Choline Bitartrate	2	0	2	0
FD&C Yellow Dye #5	0	0	0.04	0
FD&C Red Dye #40	0	0	0	0
FD&C Blue Dye #1	0.05	0	0.01	0
Total	773.85	4057	1055.05	4057

FIGURE 2 | Formulas for diet-induced obesity (DIO) diet (OpenSource Diet D12492) and matching control diet (D12450J).

© 2015 Nature America, Inc. All rights reserved.

npg

of these papers knew that comparing purified ingredient diets to grain-based chow is problematic. Another telling statistic from the Warden and Fisler article is that one-third of the time (34%), there was insufficient description of the diets in the methods section of these papers. Even though authors are supposed to include enough information for readers to judge and perhaps even replicate their experiment, details of the diets fed to animals are often left out. To this point, Warden and Fisler suggest, "Just as it is essential that mouse strains be specified, constituents of experimental diets must be specified."

Unfortunately, using mismatched diets in the metabolic disease field is still fairly common. This practice might lead researchers to conclude that phenotypic differences between animals fed a high-fat purified ingredient diet and those fed a low-fat grain-based chow group are due to the difference in dietary fat level, when they could be the result of the many other differences between these diet types.

Gut microbiota

Another example highlighting the necessity of a proper control diet can be found in the growing number of animal studies looking at gut microbiota. An exciting and rapidly growing field, the intestinal microbiome (the total complement of bacteria, fungi and viruses living in the small and large intestine) is important in many different areas of study including metabolism, immunology and inflammation among many others[2–4]. Since survival of gut microbes depends, in large part, on the source and amount of indigestible dietary fiber consumed by the animal, it is crucial to understand the differences in dietary fiber between purified ingredient diets and grain-based chows for gut microbe work.

Purified ingredient diets have historically contained about 5% cellulose as the only fiber source (though this is easily changed at the diet formulation step). Cellulose is an insoluble source of fiber that is not readily fermented by gut bacteria, meaning that it has little to no ability to promote bacterial growth. In contrast, grain-based chows contain 4 to 5 times more total fiber (20–25%), about 15–20% insoluble and 3–5% soluble fiber. Soluble fiber is fermentable by gut bacteria and has effects on gut morphology, inflammation and microbe populations[5–7]. Owing to the differences in fiber alone, one would expect gut microbiome data from animals fed grain-based chows and animals fed purified ingredient diets to be different, regardless of whatever other variables were intentionally changed (e.g., high versus low fat content). Unfortunately, there are examples in the published literature, some in top-tier journals, in which a high-fat diet is compared to a grain-based chow and the resultant differences in gut bacteria populations are attributed only to the differences in dietary fat. Use of a matched control diet will allow researchers to more confidently assign cause to their measured effects at the end of their experiments.

Company profile

At Research Diets, Inc., we have been formulating and making purified ingredient OpenSource diets for over 30 years. Our team of scientists has decades of collective experience in formulating experimental and control diets, so please contact us at Info@ResearchDiets.com and we would be glad to consult with you.

Research Diets, Inc. has formulated over 20,000 original diets and regularly incorporates compounds. In addition, we manufacture the BioDAQ Food and Liquid Intake Monitor for rats, mice and non-human primates. Visit our website at www.ResearchDiets.com.

1. Warden, C.H. & Fisler, J.S. Comparisons of diets used in animal models of high-fat feeding. *Cell Metab.* **7**, 277 (2008).
2. Bäckhed, F., Manchester, J.K., Semenkovich, C.F. & Gordon, J.I. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc. Natl. Acad. Sci. USA* **104**, 979–984 (2007).
3. Cerf-Bensussan, N. & Gaboriau-Routhiau, V. The immune system and the gut microbiota: friends or foes? *Nat. Rev. Immunol.* **10**, 735–744 (2010).
4. Kuo, S.M., Chan, W.C. & Hu, Z. Wild-type and IL10-null mice have differential colonic epithelial gene expression responses to dietary supplementation with synbiotic *Bifidobacterium animalis* subspecies *lactis* and inulin. *J. Nutr.* **144**, 245–251 (2014).
5. Kuo, S.M., Merhige, P.M. & Hagey, L.R. The effect of dietary prebiotics and probiotics on body weight, large intestine indices, and fecal bile acid profile in wild type and IL10-/- mice. *PLoS ONE* **8**, e60270 (2013).
6. Le Blay, G., Michel, C., Blottière, H.M. & Cherbut, C. Prolonged intake of fructo-oligosaccharides induces a short-term elevation of lactic acid-producing bacteria and a persistent increase in cecal butyrate in rats. *J. Nutr.* **129**, 2231–2235 (1999).
7. Pan, X.D., Chen F.Q., Wu. T.X., Tang, H.G. & Zhao, Z.Y. Prebiotic oligosaccharides change the concentrations of short-chain fatty acids and the microbial population of mouse bowel. *J. Zhejiang Univ. Sci. B.* **10**, 258–263 (2009).

This article was submitted to *Lab Animal* by a commercial organization and has not been peer-reviewed. *Lab Animal* takes no responsibility for the accuracy or relevancy of the information provided therein.