RESEARCH DIETS FOCUS

The Importance of a Proper Control Diet

by Matthew Ricci, Ph.D., VP, Science Director - Research Diets, Inc.

The goal of most laboratory animal studies is to study the effect of an intervention or treatment on phenotypic outcomes. Often this means that the experimental group of animals is fed a special diet. For example, this could be a high-fat diet, a diet lacking a nutrient or a diet with an added compound.

As scientists, we are all taught early in our careers how to minimize variability between experimental and control groups. We do this because reducing variability means that we will have greater power in our statistics to show phenotypic differences and ultimately be able to use fewer animals. In lab animal studies, we aim to reduce variability between groups by housing all of the animals in the same room, using the same number of animals per cage and using the same water, bedding, enrichment and diet. So, when *experimental* animals are fed a special diet, the *control* animals should be fed a diet matched in every way to the special diet, except of course for the dietary variable that the researcher is studying.

Matched Control Diets

It is therefore surprising how often researchers do not use a matched control diet. One unfortunately common example is the use of a low-fat grain-based (GB) chow as the 'control' for a high-fat purified ingredient diet. Purified ingredient diets and GB chows should never be compared against each other since there are far too many differences between these diet types to make comparisons meaningful. If, for instance, a researcher finds that certain genes in a microarray are differentially expressed on a high-fat purified ingredient diet compared to a low-fat GB chow, it is tempting to conclude that gene expression was altered due to the differences in fat levels between the diets. However, just about everything else in the diets was different too, including the source and amount of vitamins, minerals, protein, fat, carbohydrate and fiber.

Types of Fiber

It is worth briefly discussing differences in fiber content between these diet types, given the current widespread research on the effects of gut microbiota on various disease states. 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 which is not readily fermented by gut bacteria, meaning that it has little to no ability to promote microbial growth. In contrast, GB chows contain about 20-25% total fiber (~20% insoluble and ~5% soluble). Soluble fiber is fermentable by gut bacteria and has significant effects on gut morphology, inflammation, and microbe populations (1-3). So, it is *especially* important in gut microbiota research to avoid purified diet vs. GB chow comparisons. Gut microbiome data from GB chows and purified diets would be expected to differ simply due to the differences in fermentable fiber, not to mention any other differences between the diets (e.g. high-fat vs. low-fat).

Aside from differences in nutrients (fat and fiber, for example), GB chows contain many plant-derived chemical entities that are absent from purified ingredient diets. Examples include phytoestrogens and toxic heavy metals (e.g. arsenic), both of which can have real and measurable effects on the animal's phenotype. For example, dietary phytoestrogens can affect sexual maturation, bone metabolism, behavior and cancer (4) while arsenic in the diet can affect tissue gene expression (5). As a result, researchers are starting to avoid the use of chows so as to limit their animals' exposure to varying levels of unnecessary non-nutrients.

What's in the Literature

The good news is that the use of poor control diets is being recognized and discussed in the literature. In a 2008 correspondence in Cell Press, Warden and Fisler note how few papers included a matched low-fat control diet in comparison to a purified ingredient high-fat diet (6). They found that almost half (43%) of the 35 papers using mice and high fat diets published in five high-impact journals in 2007 used a GB chow and not a purified, matched, low-fat control diet. The authors state:

"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."

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.

The fact that 'mismatched' diet studies are published in high-impact journals suggests that neither the authors nor the reviewers were aware of the problems inherent in comparing data from groups of animals fed completely different diets. Perhaps equally alarming is that in the same group of papers, Warden and Fisler found that 34% of the time, there were insufficient data about the diets in the methods section. In other words, fully one-third of the time, describing the diets in detail was not considered important by either the authors or the reviewers. To this Warden and Fisler suggest that:

"Just as it is essential that mouse strains be specified, constituents of experimental diets must be specified."

Recently, Benoit and colleagues (7) studied this issue directly by feeding mice a high-fat purified ingredient diet and comparing them to groups fed either a matched, low-fat purified ingredient diet or a low-fat GB chow. Not surprisingly, they found that some parameters such as insulin sensitivity and body weight were affected by the choice of control diet. The authors state that,

"…conclusions on the lipid-related effects of HFDs [high fat diets] must be formulated with great care because some end points are profoundly affected by the ingredient composition of the diet rather than by fat content."

They go on to say in the final sentence in their paper:

"Therefore, further studies using "pairs" of control diet/HFD ***matched with similar ingredients*** *should now be used to identify the respective effects of fibers, carbohydrates, and fatty acids on metabolic disorders in HFD-induced obesity." (emphasis added)*

So why do some researchers use GB chows as controls for purified ingredient diets? One reason simply is that they are not aware of how mismatched dietary groups can affect the conclusions they make from their data. It's safe to say that no researcher would intentionally set up an experiment such that conclusions from their data would be suspect. Another reason sometimes given is cost. There is no doubt that purified ingredient diets cost more than GB chows; this is mainly due to the inherent costs of the raw materials. So to use a properly matched purified ingredient diet does add costs to the study. But how much money is actually saved if the use of a GB chow brings into question the conclusions made from the data?

No diet is perfect, including purified diets. But if a purified ingredient diet isn't producing the desired phenotype, it can be easily modified – this is one of the clear advantages purified ingredient diets have over GB chows. The bottom line is that by not using a properly matched, purposely designed control diet, it is simply not possible to know how to interpret the data at the end of the experiment. This not only holds true for high- and low-fat diet comparisons, but any time a purified ingredient diet is compared to a GB chow. Unfortunately, such comparisons can lead to erroneous conclusions and, ironically, the need to spend more time and money repeating the study.

(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

References

1. 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(3):e60270, 2013.
2. 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.
3. 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(4):258-63, 2009.
4. Jensen M.N., Ritskes-Hotinga, M. How isoflavone levels in common rodent dies can interfere with the value of animal models and with experimental results. *Lab Animals.* 41:1-18, 2007.
5. Kozul C.D., Nomikos A.P., Hampton T.H., Warnke L.A., Gosse J.A., Davey J.C., Thorpe J.E., Jackson B.P., Ihnat M.A., Hamilton JW. Laboratory diet profoundly alters gene expression and confounds genomic analysis in mouse liver and lung. *Chem Biol Interact.* 173(2):129-40, 2008.
6. Warden C.H., Fisler J.S. Comparisons of diets used in animal models of high-fat feeding. *Cell Metabolism,* 7(4): 277, 2008.
7. Benoit B., Plaisancié P., Awada M., Géloën A., Estienne M., Capel F., Malpuech-Brugère C., Debard C., Pesenti S., Morio B., Vidal H., Rieusset J., Michalski M.C. High-fat diet action on adiposity, inflammation, and insulin sensitivity depends on the control low-fat diet. *Nutrition Research,* 33 (11):952-60, 2013.

RESEARCH DIETS INC.™
www.ResearchDiets.com
Where NutriPhenomics Begins

Research Diets, Inc.
20 Jules Lane
New Brunswick, NJ 08901 USA
Tel: 732.247.2390
Fax: 732.247.2340
info@researchdiets.com