Vitamin and Mineral Modifications Using Purified Ingredient Diets

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Animal models have been, and continue to be crucial in understanding the etiology of metabolic diseases in humans. Nutritional science research during the 20th century has shown that diet is a powerful environmental tool capable of changing the phenotype of an animal, and thus improve our understanding of different diet-related metabolic diseases. Examples include obesity, diabetes, dyslipidemia, non-alcoholic steatohepatitis, atherosclerosis, and hypertension. To study these disorders in animals, researchers typically use diets that contain excess levels of fat and sucrose, among other modifications. Animal diets can also be modified to create vitamin and mineral deficiencies which allows researchers to define the requirements of these nutrients.

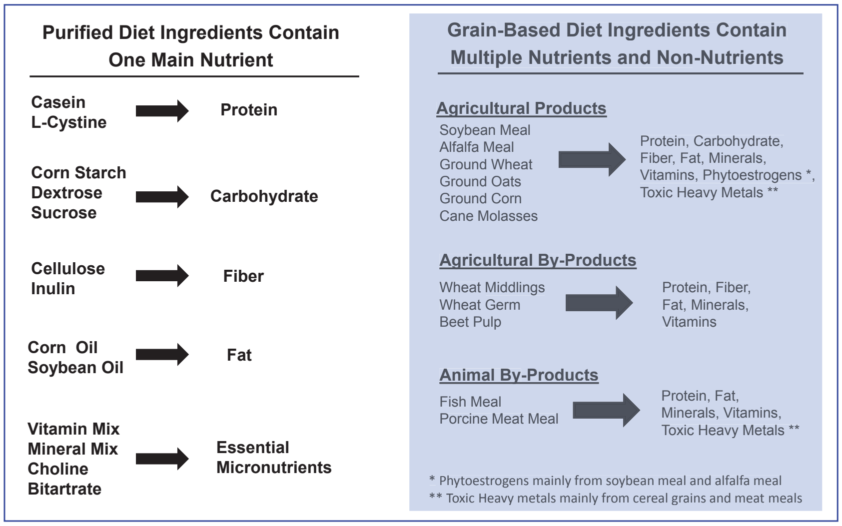

Figure 1: Common ingredients in purified diets and grain-based diets and their nutrient/non-nutrient contributions. Adapted from Pellizzon, MA and Ricci, MR, 2014, Biomarkers in Toxicology, Chapter 36, Pages 629-643.

Rodent diets are typically broken down into two main categories:

1. Grain-based (GB) or cereal-based rodent diets *(commonly referred to as "chows")*
2. Purified diets.

GB diets are made with unrefined plant/grain-based or animal ingredients that contain multiple nutrients and non-nutrients. GB diets typically include agricultural grade ingredients such as ground corn, ground wheat, wheat gluten, wheat barley, ground oats, soybean meal, alfalfa meal, and animal by-products such as fish meal and porcine animal meal in varying proportions (Figure 1). Most GB diets are "closed formulas" or proprietary, and therefore the actual concentrations of these ingredients are not disclosed to the end-user. This allows companies that make these diets the freedom to alter levels or sources of ingredients without disclosing these changes to researchers. On the other hand, purified diets are "open and fixed formulas" made with refined ingredients that contain only one main nutrient (Figure 1 and Table 1). Given their open nature, these formulas are reportable and their nutrient contents are more easily defined than those of diets made using less refined grains and animal by-products.

Product #	**D11112201**	
	OpenStandard Diet	
	gm%	*kcal%*
Protein	19	*20*
Carbohydrate	63	*65*
Fat	7	*15*
Total		*100*
kcal/gm	3.80	
Ingredient	**gm**	***kcal***
Casein	200	*800*
L-Cystine	3	*12*
Corn Starch	381	*1524*
Maltodextrin 10	110	*440*
Dextrose	150	*600*
Cellulose, BW200	75	*0*
Inulin	25	*25*
Soybean Oil	70	*630*
RDI Mineral Mix	45	*0*
Vitamin Mix V10001	10	*40*
Choline Bitartrate	2	*0*
Yellow Dye #5, FD&C	0.025	*0*
Red Dye #40, FD&C	0	*0*
Blue Dye #1, FD&C	0.025	*0*
Total	**1071.05**	***4071***

Table 1: D11112201 – Open Standard Diet

RDI Mineral Mix			
Ingredient	**gm**	**Amt in 45 gm Mineral Mix**	**Nutrient**
Calcium Phosphate, Dibasic	260	6.0 g	Calcium
29.5% Ca, 22.8% P		3.0 g	Phosphorus
Calcium Carbonate	110		
40% Ca			
Magnesium Oxide	8.38	0.5 g	Magnesium
60.3% Mg			
Magnesium Sulfate, 7H2O	51.52	0.33 g	Sulfur
9.87% Mg, 13% S			
Potassium Citrate, 1H_2O	330	6 g	Potassium
36.2% K			
Sodium Chloride	51.8	1 g	Sodium
39.3% Na, 60.7% Cl		1.6 g	Chlorine
Chromium Potassium Sulfate, 12 H_2O	0.385	2 mg	Chromium
10.4% Cr			
Copper Carbonate	0.21	6 mg	Copper
57.5% Cu			
Potassium Iodate	0.007	0.2 mg	Iodine
59.3% I			
Ferric Citrate	4.2	37 mg	Iron
17.4% Fe			
Manganese Carbonate Hydrate	2.45	59 mg	Manganese
47.8% Mn			
Sodium Selenite	0.007	0.16 mg	Selenium
45.7% Se			
Zinc Carbonate	1.12	29 mg	Zinc
52.1% Zn			
Sodium Fluoride	0.04	0.9 mg	Fluorine
45.2% Fl			
Ammonium Molybdate, 4 H2O	0.06	1.6 mg	Molybdenum
54.3% Mo			
Sucrose	179.821		
TOTAL	**1000**		

Table 2: RDI Mineral Mix

An example of a purified diet is the Open Standard Diet (OSD) (Table 1) that is based on the historical AIN based diets (AIN-93G and AIN-76A) with some improvements. Unlike the AIN diets, this diet contains minimal sucrose similar to a GB diet, and contains more fiber and a source of soluble fiber (inulin). As seen from Table 1, all the ingredients of this diet are reported (open formulation). Given the refined nature of the ingredients, it is easier to study micronutrient modifications (hyper supplementation and/or deficiencies) in rodents using purified diets. Nutrient deficiency studies are relatively straightforward using purified diets since these diets are generally simple to revise. In contrast, since each plant ingredient in GB diets can contain more than one nutrient (Figure 1), removing a single nutrient from these diets is not possible. For example, one could not remove the iron from any or all of the plant or animal ingredients - it is like trying to remove the sugar from a baked apple pie.

The detailed breakdown of vitamin and mineral mixes used in purified diets are also available for researchers to report in the methods section of publications (Tables 2 and 3). Each vitamin and mineral is added individually to this diet. Thus, virtually limitless modifications can be made to purified diets. Vitamin and mineral mixes can be modified easily to remove one or more micronutrients. For example, a vitamin B12 deficient vitamin mix (contains no cyanocobalamin) is presented in Table 3. Diets made using this vitamin mix would contain only trace levels of B12 (coming from other purified ingredients such as casein). Similarly, it is very easy to remove ferric citrate from the RDI Mineral Mix (Table 2) to formulate a diet with no added iron – such diets are typically used to study iron deficiency anemia.

Ingredient:	V10001		V15911	
	gm	Amount in 10 gm	gm	Amount in 10 gm
Vitamin A Acetate (500,000 IU/gm)	0.8	4000 IU	0.8	4000 IU
Vitamin D3 (100,000 IU/gm)	1	1,000 IU	1	1,000 IU
Vitamin E Acetate (500 IU/gm)	10	50 IU	10	50 IU
Menadione Sodium Bisulfite, 62.5%	0.08	0.5 mg	0.08	0.5 mg
Biotin, 1.00%	2	0.2 mg	2	0.2 mg
Cyanocobalamin, 0.10%	1	10 µg	**0**	**0 µg**
Folic Acid	0.2	2 mg	0.2	2 mg
Nicotinic Acid	3	30 mg	3	30 mg
Calcium Pantothenate	1.6	16 mg	1.6	16 mg
Pyridoxine-HCl	0.7	7 mg	0.7	7 mg
Riboflavin	0.6	6 mg	0.6	6 mg
Thiamin HCl	0.6	6 mg	0.6	6 mg
Sucrose	978.42		979.42	
Total	**1000**		**1000**	

Table 3: Vitamin Mixes. V10001 - Complete Vitamin Mix (used in AIN-76A Diet). V15911 – Vitamin Mix with No Added B12

These modifications are not possible using GB diets, and thus, purified diets provide a reliable method to study such phenotypes. In fact, ~60-70 years ago, purified diets were used to define the nutrient requirements of lab animals because of their ability to specifically control the dietary levels of micronutrients. Furthermore, given the defined nature of the ingredients added to these diets, we can precisely supplement specific nutrient doses of one or more nutrients using purified diets.

Matched Control Diets

In spite of the many differences between GB diets and purified diets, it is surprising how often researchers do not use a matched control diet. Purified diets and GB diets should never be compared against each other since there are far too many differences (Figure 1) between these diet types to make meaningful comparisons. If, for instance, a researcher finds that certain genes in a microarray are differentially expressed on a high-fat purified diet compared to a low-fat GB diet, it is tempting to conclude that gene expression was altered due to the difference in fat levels between the diets. However, just about everything else in the diets is different too, including the sources and amounts of vitamins, minerals, protein, fat, carbohydrate and fiber. Please contact us to discuss your (control) diet needs.

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