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Choice of laboratory animal diet influences intestinal health

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The importance of diet choices

When designing experiments with animal models, the importance and variety of dietary choices is typically overlooked, especially if diet is not the main focus of the study. This can be seen in the methods sections of many publications in lab animal science, where it is common to read vague terms such as 'standard chow' or 'regular diet' which do not provide readers with useful information. There are many reasons to consider the diet carefully for each and every experiment, especially given that dietary factors are known to affect nearly every phenotype, including health to disease characteristics.

'Standard chow' (**Fig. 1**) commonly describes a variety of grain- or cereal-based diets, or 'chows' for short, which are made with ingredients such as soybean meal, ground corn, fish meal and animal byproducts. With very few exceptions, these are 'closed' formulas, meaning that the formula for the chow—the amounts of each ingredient used—is proprietary and not shared with the research community. Furthermore, some manufacturers will change the amount of an ingredient in the chow from one batch to another, depending on the nutrient levels in those particular batches of ingredient. It is important to note that there are typically very high levels of both soluble and insoluble fiber in chow, often making up about 20% of the chow and coming from multiple sources. In addition, chows frequently contain non-nutritive but biologically active components such as phytoestrogens, and toxic heavy metals such as arsenic, at biologically relevant levels[1,2]. Because there can be changes in the composition of ingredients from one batch to the next, it is not possible to maintain consistent nutrient and non-nutrient profiles between batches or between studies using chows[1–4].

In contrast to chows, purified diets (**Fig. 2**) are formulated with refined ingredients, and the formulas are 'open' to researchers so that they can know the relative amount of each ingredient in the formula. The refined ingredients of purified diets allow complete control over their nutrient compositions. The use of ingredients such as casein (mainly protein), corn starch (carbohydrate), soybean oil (fat) and cellulose (fiber) means that the researcher can report the formula and nutrient composition of the study's diet, replicate the composition from batch to batch, and revise the diet with ease, when necessary. As such, purified diet formulations can be individually tailored to meet the needs of any researcher. For example, manufacturers can selectively remove or alter individual dietary components in purified diets, such as the fiber source; this is not possible in chows, given that fiber in chow diets comes from multiple grain ingredients.

FIGURE 1 | Example standard chow diet. Standard chows are often made from proprietary formulas that can change from one batch to the next.

Purified diets have been used since the 1920s, and they were key to the discovery of the essential nutritional roles of vitamins and minerals[5]. In 1976, a committee of nutrition researchers assembled to develop an open, fixed formula for a diet with purified ingredients and low in fat, which could be fed to mice and rats at all life stages. This diet formula is called the AIN-76A diet, and it was revised in the early 1990s to produce the AIN-93 diet series[6]. These AIN formulas were capable of meeting the nutritional and experimental needs of studies in many areas of biological research, and they can be—and have been—adapted and modified to meet any research goal.

For example, purified high-fat diets for studies of diet-induced obesity (DIO) in rodents were formulated by replacing calories from the carbohydrate source with those from the fat source. Purified high-fat diets provide a nutritionally balanced way to study DIO, and when compared to a properly matched low-fat diet, they allow the researcher to understand the effects of higher levels of fat on an animal's phenotype. However, although they are extremely useful, no purified diet formula is perfect. Despite imperfections in dietary formulas, and sometimes even because of such imperfections, the research community has made some serendipitous discoveries about lab animal health using purified diets.

Profound effects of diet choice on gut health

It is well known that diet profoundly affects the health of the gut. In fact, the process by which high-fat purified diets initiate DIO and

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metabolic disease in rodents likely begins in the gut. Transplantation of microbiota from mice with DIO into the gut of germ-free mice has been shown to increase the susceptibility of those germ-free mice to obesity[7]. Additionally, feeding mice a high-fat diet increases metabolic endotoxemia, a state of inflammation that is thought to precede the development of obesity and insulin resistance[8].

However, carefully controlled studies also suggest that metabolic disease induced by high-fat diets might not be solely caused by the level of fat in the diet: fiber plays a key role as well. As mentioned above, chow composition is about 20% total fiber that is a mix of soluble and insoluble fiber; in contrast, most purified diets historically are composed of about 5% total fiber that is comprised of insoluble fiber in the form of cellulose. This is important because gut bacteria can ferment soluble fiber, whereas insoluble fiber is largely not fermentable. Bacterial fermentation of soluble fiber releases short chain fatty acids (SCFAs), which supply a major amount of energy to colonocytes and are thought to provide other benefits, including prevention of diet-induced obesity, decreased adipose tissue storage and improved insulin action[9]. An increase in SCFAs can change the pH of the gut, which can in turn decrease the populations of pathogenic, pH-sensitive bacteria. Therefore, one should expect that the differences in dietary fiber between chows and purified diets, and the resultant changes in gut bacteria, should produce different phenotypes in the animal. This is also one of many reasons why a chow diet should never be used as a control for a purified diet.

Recent research has underscored the importance of fiber, in particular, in shaping gut health and the development of obesity. In one study, mice that were fed purified diets with cellulose developed more adiposity and had lower cecum and colon weights than those that were fed chow diets, regardless of whether the purified diet was low or high in fat[10]. These effects occurred rapidly over several days. Gut microbiota were shown to be important contributors to this effect, since an antibiotic treatment administered in the drinking water reduced adiposity and partly restored cecum mass and colon length in mice fed the low-fat purified diet. The addition of cellulose to the chow diet did not cause significant changes in the cecum and colon, demonstrating that cellulose did not cause the adiposity or atrophy of cecum and colon tissues that was observed in mice that were fed the purified diets. Rather, these results suggested that chow contained something protective that was lacking in the purified diet.

Because there are known differences between purified diets and chows, particularly with respect to the types and relative amounts of fiber that they contain, researchers subsequently added to the low- and high-fat purified diets either non-fermentable fiber in the form of cellulose or fermentable fiber in the form of inulin . They found that the addition of inulin to low- or high-fat purified diets increased cecum and colon weights and significantly reduced body weights of mice. Adding inulin to chow also increased colon length and cecum weights in mice with conventional microbiota whereas this effect was absent in germ-free mice. This suggests again that microbiota are required for inulin to positively affect cecum and colon morphology. SCFAs were dramatically higher in mice that were fed chow compared to those that were fed purified diets, and the addition of inulin to a purified diet increased the proportion of one specific SCFA, acetate, significantly. In their discussion, the study's authors suggest that fermentation of soluble fiber by gut bacteria maintained gut health and that this effect might be mediated in part by an increase in SCFA levels.

FIGURE 2 | Example purified diet. Purified diets are made from open formulas with refined ingredients that are maintained consistently from one batch to the next.

These data show that gut morphology and, most likely, gut health as well differ greatly between mice that are fed a low-fat chow and those that are fed an equally low-fat purified diet whose only source of fiber is cellulose. Furthermore, the simple addition of inulin to the formulas of current purified diets can prevent many of these gut changes while simultaneously providing a stable and defined control diet. It is very important that researchers know and understand that the fiber content of their control diet or any other diet affects bacterial growth and health in the cecum and colon of laboratory animals. As a result, researchers should choose their control diets very carefully and take care not to compare chow diets and purified diets against one another. In order to maintain the highest degree of control over their studies, researchers should consider using diets with purified ingredients that can be formulated to meet their specific needs.

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