RESEARCH DIETS FOCUS

Mycotoxins and Endotoxin Content Vary Between Different Batches of Grain-Based Diets

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There are many variables to consider when designing an animal experiment. The animal model (strain/sub-strain), the housing and environmental conditions, and the diet are some of the common variables among different research groups. While the animal model and most environmental factors are typically well controlled, unfortunately the same cannot always be said for the diet. Diet information is typically not disclosed or when disclosed it is often underreported in experimental studies (1; 2). For the scientific community, this makes interpreting the study difficult, if not impossible, given that the diet profoundly affects phenotype.

Figure 1: A grain-based diet

Grain-Based Diets vs. Purified Diets

Rodent diets can be broken down into two basic categories: grain-based (GB) (sometimes also called standard chows, natural-ingredient diets or cereal-based diets, (Fig. 1) or purified diets) (Fig. 2). GB diets typically include agriculture-grade ingredients such as ground corn, ground wheat, wheat gluten, wheat middlings, wheat barley, ground oats, soybean meal, and alfalfa meal, as well as animal by-products such as fish meal and porcine meal, all in varying proportions (1). Most GB diets are "closed formulas" or proprietary, and therefore the actual concentrations of these ingredients are not disclosed, allowing manufacturers the freedom to alter the levels, or the sources of ingredients without disclosing these changes to researchers. Due to their unrefined nature, each ingredient provides multiple nutrients, but there are also non-nutrients that usually "ride along" with these nutrients (2). We and others have previously shown the presence of different types of non-nutrients in GB diets, including phytoestrogens, chlorophyll, bisphenol A and heavy metals such as arsenic and cadmium, all of which can profoundly impact results of different studies (3; 4; 5; 6).

In the last three to four decades, the use of purified diets (e.g. AIN-76A, AIN-93G etc.) has greatly increased in preclinical research (7). These diets are made from highly refined ingredients, each of which typically contains one main nutrient (e.g. corn starch is virtually all carbohydrate). This allows for simple modifications to macronutrient contents while keeping the diet nutritionally balanced. Purified diets are 'open' to the public and due to the refined nature of the ingredients in these diets, the presence of non-nutrients is limited (1; 2).

For the focus of this brief review, we will describe the differences in endotoxin and mycotoxin levels found in GB and purified diets, which to our knowledge has not been widely publicized.

Endotoxin and Mycotoxins

Endotoxin is a complex lipopolysaccharide (LPS) present in the outer cell membrane of gram-negative bacteria (8). In vivo, endotoxins elicit an inflammatory response in animals and can impact phenotype especially if present in large amounts in rodent diets. GB diets are known to contain much higher (and variable) levels of endotoxins compared to purified diets, and researchers (9; 10) have shown that these relatively high levels of endotoxins can significantly affect the development and function of the immune system, especially in germ-free animals (9).

Mycotoxins are secondary metabolites of fungi that can grow within or on cereal grains and are known to be present in GB diets (11). There are over 300 known mycotoxins which can impact a wide range of phenotypes including toxicity in the liver, kidney, and central nervous system (CNS). They can also elicit teratogenic, carcinogenic, immunotoxic and estrogenic effects (11; 12; 13).

Endotoxins and mycotoxins are present at relatively high levels in GB diets and are variable from one diet to another mainly because of the unrefined nature of the ingredients in these diets. However, there is limited information on whether endotoxin or mycotoxin levels vary from one batch to the next within the same GB diet and so we chose to investigate this in detail.

We measured endotoxins in at least 3 separate lots of 5 different GB diets as well as the purified diets D11112201 (15 kcal% fat), D12492 (60 kcal% fat) and D12450J (10 kcal% fat). These diets are typically used for obesity and metabolic disease research. Endotoxin levels were measured using the Limulus Amebocyte Lysate (LAL) assay. Mycotoxins were measured in these samples using a novel Randox Food Diagnostics biochip array that was customized to simultaneously detect 10 of the most prevalent mycotoxins from a single sample of diet. Mycotoxins measured included Fumonisin, Ochratoxin A, Deoxynivalenol, Zearalenone, Ergot Alkaloids, Aflatoxin G1/G2, and B1/B2, T2 Toxin and Diacetoxyscirpenol (14).

Figure 2: Purified diets made from refined ingredients

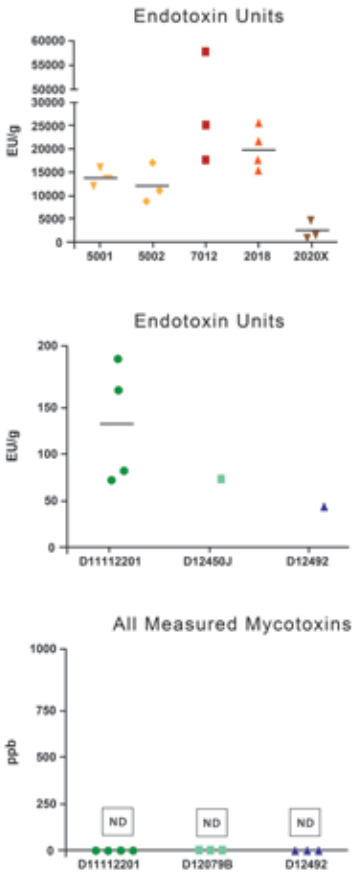

Figure 3: Mycotoxins and endotoxins in purified and grain-based diets

We observed that GB diets contain about 200-300 times the levels of endotoxins compared to the purified diets (Fig. 3) (14). Different GB diets contained different levels of endotoxins, which agrees with previously published information (9) and there were significant differences (about 20-60% variation) among different batches of the same GB diets (Fig. 3). Given that endotoxin levels vary batch-to-batch; this adds an additional variable that could impact study design.

We found that most grain-based diets contained 4-5 different mycotoxins (Fig. 4) (14). Although levels of these mycotoxins are low, combinations of mycotoxins could elicit a larger phenotypical response, which has been shown in vitro (11; 13). All the purified diets tested negative for the mycotoxins analyzed (14), clearly showing that the refined nature of the ingredients minimizes contamination of the diet by toxins coming from unrefined grains (Fig.3).

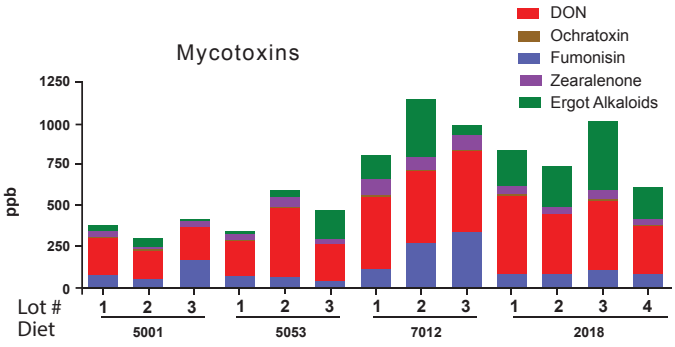

Figure 4: Diverse combinations of mycotoxins in each grain-based diet lot

In conclusion, these results strongly suggest that since GB diets contain endotoxins and mycotoxins, at variable levels, and thus, researchers should choose the diet wisely as the presence of these compounds can have a profound effect on the rodent phenotype. Furthermore, it is impossible to know how the combination of these contaminants with other known contaminants in GB diets (e.g. phytoestrogens, heavy metals, pesticides, pollutants, BPA) can influence the rodent phenotype. What is certain is that purified diets provide a clean, consistent nutrient profile and minimize the presence of any contaminants that can affect the phenotype of valuable animal models. A well-controlled diet free from biologically active contaminants may reduce the variation of studied parameters, and thereby reduce the number of animals needed in experimental research (2; 15).

For further information regarding any of these data, please contact us at info@researchdiets.com.

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