

Phytoestrogens – Uninvited, Troublesome Guests in Scientific Research

2018 - Brief Scientific Literature Review

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There are two main categories of research animal diets: grain-based (GB) diets (commonly referred to as “chows”) and purified diets. GB diets are made with agricultural and animal byproducts. Because of their less-refined nature, each ingredient provides multiple nutrients which are inseparable from one another. Additionally, most GB diets are “closed-formula,” meaning that the researcher only knows what ingredients are in the diets but does not know how much of each ingredient is included. Thus, ingredient composition can vary between different batches of the same GB diet. Purified diets, in contrast, are made with refined ingredients and a fixed amount of nutrients. Each purified ingredient contains one main nutrient, and the concentration of each ingredient added is available to the researcher. With purified ingredient diets, therefore, the scientist has complete control over the ingredients, including the addition or subtraction of any nutrient in the diet.



**Is Your Diet
Out of Control?**

Grain-based chows contain variable levels of phytoestrogens, heavy metals, pesticides and BPA. Gain control of your experiments with OpenSource purified diets from Research Diets, Inc. Contact our Resource Center at info@researchdiets.com.

Phytoestrogens (PE) are plant-derived, estrogen-like compounds that interact with estrogen receptors and elicit estrogenic or anti-estrogenic effects (1). They can compete with endogenous or exogenous estrogens, endocrine disruptors, or other molecules that bind to estrogen receptors. Since estrogen receptors are present in many tissues and are associated with a variety of physiological pathways, exposure to PE can alter gene expression, phenotypical outcomes, and the sensitivity of animals to certain treatments (2–4). Two types of PE are abundant in the ingredients of commonly used GB diets: coumestrol in alfalfa and isoflavones (eg. genistein) in soybean meal (5). Jensen et al. reported that isoflavone concentrations in different GB diets can vary from 100 ppm to 700 ppm (Fig 1)(6). Moreover, PE levels in the same GB diet formula can vary 2- to 5- fold between different lots (7). Not surprisingly, PE were found in the blood and urine of rodents after consuming a GB diet containing soybean and alfalfa (8). When pregnant and lactating dams were fed the same diet, PE were also found in the blood of both newborn and nursing pups (8).

The variability of PE in GB diets has likely caused inconsistent results, leading to researcher frustration and/or misinterpretation of data. The following sections briefly summarize how these variable PE concentrations in GB diets can impact many research areas.



Reproductive Development

PE have been shown to affect reproductive development in both male and female rodents. Variability in the PE content between different batches of the same formula has caused different timing of vaginal opening and disparate induction of estrogen-responsive genes (3,9). PE also influence the male reproductive system (10) and early exposure to isoflavones is capable of influencing organ development and behavior in both sexes (11,12).

Cancers Rodents consuming isoflavones have shown fewer tumors and/or a delay in tumor development in different cancer diseases (13). However, some researchers have observed the opposite effect in ovariectomized mice, a mouse model of postmenopause. Diets supplemented with genistein or soy protein isolate have been shown to promote progression of estrogen receptor positive human breast cancer cells and chemically-induced mammary tumors in ovariectomized rodents (14,15).

PE also interfere with anti-estrogens/selective estrogen receptor modulators (SERM) in breast cancer studies. Diets with isoflavones abrogated the effects of tamoxifen on the growth of breast tumors in some studies, while synergistically enhance the effect of tamoxifen in other studies (16,17). Furthermore, PE reduce the efficacy of aromatase inhibitors, which inhibit estrogen production and are commonly used to treat hormone receptor-positive postmenopausal breast cancer (18). Given the inconsistent effects of PE on SERM, researchers using a tamoxifen-inducible cre transgenic mouse model need to avoid hidden and variable PE in their diets.

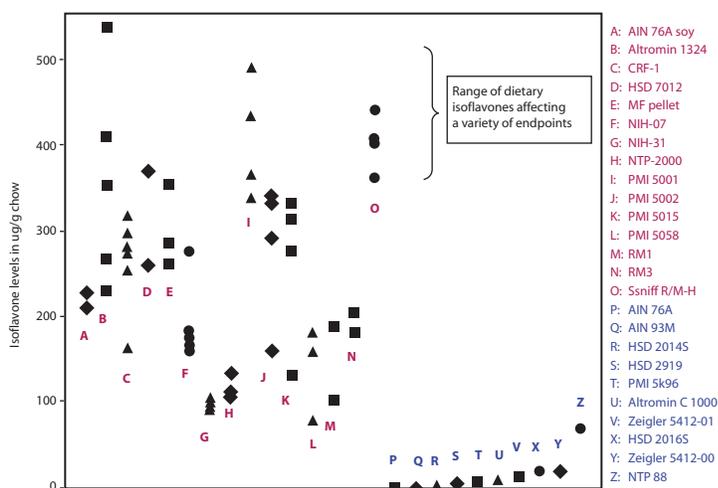


FIGURE 1. Isoflavone content in a number of commonly used laboratory rodent diets. Each data point represents a batch of the diet formulations listed on the right. Diets P-Y are formulated without soy and have negligible amounts of isoflavones. Graphic representation - for details see reference (6).

Metabolic Syndrome Estrogen directly modulates glucose and lipid metabolism via estrogen receptors, and regulates whole body energy homeostasis (19). Studies in male rodents observed beneficial effects including weight loss, reduced triglyceride levels, and improved insulin sensitivity after feeding soy (1). Reduced body weight and fat mass were also observed in female and ovariectomized rodents fed a GB diet rich in isoflavones (20). Additionally,

genistein attenuated the development of hypertension in spontaneously hypertensive rats (21). However, female rats exposed to genistein from postnatal day 1 to day 22 developed adiposity and smaller muscle fiber perimeter, while no such effects were observed in male rats (22).

Endocrine Disruptors Endocrine disruptors are synthetic chemicals that can affect numerous endocrine functions including reproductive, neuronal and immune system functions and are associated with certain cancers (23). While more studies are needed to fully understand the effects of endocrine disruptors, variations in PE levels between different lots of the same GB diets or those from different manufacturers have impeded this progress (24,25). When diethylstilbestrol, a synthetic form of estrogen, was added to a GB diet that inherently contained relatively low levels of PE, early vaginal opening in female mice was observed in a dose-dependent manner (4). However, the effect disappeared when diethylstilbestrol was added to a GB diet with a higher amount of PE.



Gut Microbiota While interactions between gut microbiota and PE are known to be reciprocal, like most areas of research involving gut microbiota, these too are not yet fully understood. The gut microbiota can metabolize isoflavones to S-equol, and equol has been shown to be more estrogenic and to possess stronger antioxidant activity compared to its parent structure (26). Since rodents have the ability to convert isoflavones to equols,(27) the biological effects of PE in GB diets may greatly affect the phenotypes of the animals although these effects may be difficult to predict given this bacterial-driven conversion to S-equol. On the other hand, changes in fecal bacteria communities have been observed in human after consuming soy products (28). It is unclear how PE affect the gut microbiota of lab animals.

Other areas A systematic review reveals that PE may prevent bone loss by increasing bone mineral density in ovariectomized rodents (29). Additionally, PE can cross the blood-brain barrier and exert neurobehavioral effects on such parameters as food and water intake, learning and memory, social interaction, anxiety-related behaviors, locomotor activity and pain sensitivity (30–32). It has also been shown that MRL/lpr mice, a mouse model of systemic lupus erythematosus, developed more severe glomerulonephritis when fed a commercial GB diet with higher amount of isoflavones compared to a purified diet with no detectable PE (33).

Does removing the phytoestrogen source from grain-based diet fix the problem?

In recognition of the large body of evidence supporting the effects of PE, some GB diet manufacturers now sell GB diets without soybean or alfalfa meal. While the PE in these diets are minimized, they are not eliminated. Importantly, these ingredients are not refined, and may still possess other contaminants or non-nutrients that can alter biology, such as mycotoxins, heavy metals and pesticides (34, 35).

A cautionary tale

Unfortunately, many researchers still use GB diets as ‘controls’ for purified diets (36) (37). Given the vast differences between purified diets and GB diets, rather than a ‘control’ diet, a GB diet should be viewed as another treatment diet. In addition, scientists studying the efficacy of drugs on a variety of diseases should take into account the potential effects of PE and consider the diet choice carefully.

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