Dietary phytoestrogens, a source of research variation.

Ask a Nutritionist Series: Impact of phytoestrogens on research, Volume 1.

- Soybean meal, a common ingredient in laboratory animal diets, contains variable levels of phytoestrogen known as isoflavones
- Isoflavones are selective estrogen receptor modulators (SERM) because of both estrogenic agonistic and antagonistic effects
- Dietary isoflavones can affect a variety of research endpoints
  - No simple absolute threshold for physiological effects
  - Tissue responses can differ
  - Magnitude and direction of response varies
  - Responses are model dependent

Laboratory diets containing soybean meal should be avoided in research studies if the end point may be affected by dietary isoflavones.

Challenge:

Dietary isoflavones impact research.

Solution:

Envigo's minimal isoflavone Teklad Global Rodent Diets lead to reliable, repeatable research results.

Dietary soy: a natural and variable source of phytoestrogens

Phytoestrogens are plant-derived compounds which mimic both the structure and function of estrogens in mammals and can impact research results (1, 2). The main phytoestrogens (and their primary sources) include: isoflavones (mainly genistin and daidzin found in soybean protein), coumestans (mainly coumestrol found in alfalfa) and lignans (from flax and sunflower) (1). Isoflavones are glycosylated in soybean meal but will be converted within the gastrointestinal tract to the active aglycone forms, genistein and daidzein (**Figure 1**). Since analysis of isoflavones typically involves converting to the aglycone form, data are routinely reported as genistein and daidzein as they are in **Figure 2**.

Soybean meal is a common ingredient in standard laboratory animal diets, supplying primarily protein, but it also adds the isoflavones genistein and daidzein.

The isoflavone content of soybean meal can fluctuate (2 – 6 fold) due to genetics and growing conditions (3-6). Regional sourcing practices can restrict isoflavone variation to 2-fold, as demonstrated by the internal data shown in **Figure 2A** for soybean meal sourced from the upper Midwest. This same variation is then reflected in diets containing soybean meal. Dietary isoflavone level is a function of the amount of soybean meal present and the content in the source material (**Figure 2B**).

Diets containing soybean meal in typical amounts will have isoflavone levels ranging from 100 – 700 ppm (sum of genistein + daidzein, aglycone form) (7-12). Fixed formulation (adding the same amount of soybean meal in each diet lot) and regional sourcing can minimize variation in dietary isoflavones within a crop year, while year to year differences are responsible for the majority of variability.

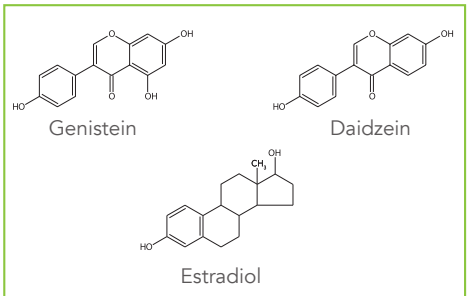

Figure 1. Structural similarity of genistein and daidzein to estradiol confers activity at the estrogen receptor.

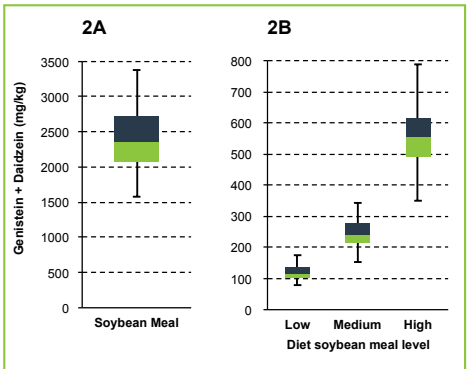

Figure 2. Box and whisker plots showing the distribution of isoflavones (genistein + daidzein) in **2A:** Soybean meal regionally sourced from the upper Midwest by Envigo from 2006 – 2016.

In **2B:** Envigo diets containing low (~5%), medium (~10%) and high (~25%) amounts of soybean meal during the same time period.

Physiological actions of isoflavones

Isoflavones may have estrogenic agonistic or antagonistic effects and are therefore termed selective estrogen receptor modulators (SERM). Structural similarities to 17 β-estradiol allow isoflavones to bind to estrogen receptors with preference for the β receptor (1, 13). Although relative potency is significantly lower than endogenous estrogens, isoflavones are able to exert multiple physiological effects due to higher circulating concentrations relative to endogenous estrogen (8, 14). Estrogen receptors are widely distributed among body tissues and various cell types, affecting a wide range of physiological systems, including the cardiovascular (15), immune (16-18), reproductive (19-21), endocrine (22, 23), and central nervous systems (24-27). While much attention has been paid to the effects of isoflavones acting through the estrogen receptor, isoflavones also have estrogen independent mechanisms such as acting as PPAR agonists (22, 28-32), activation of the cAMP/PKA pathway (33-37) and via antioxidant activity (25, 38-42).

In general, isoflavones seem to have a protective effect in animal models, modulating pathological conditions such as cancer, metabolic syndrome, and neurodegeneration.

By feeding soy-containing diets, the consequent 'protective' effect of isoflavones is likely to diminish the phenotype of animal models developed or manipulated to exhibit such pathology.

Isoflavones may also confound the effects of putative or potential therapeutic compounds.

Dietary isoflavones result in research variation

Predicting the impact of dietary isoflavones on research outcomes is difficult. Isoflavones do not illicit a classic dose response, thus there is no simple absolute threshold for the physiological effects of isoflavones and some effects have been demonstrated at low levels of phytoestrogens. For example, feeding a diet with 100 ppm genistein reduced lung metastases in athymic BALB/c mice implanted with prostate cancer cells by ~50% (43). Likewise, the proportion of TRAMP mice exhibiting prostate tumors dropped significantly with the addition of 100 ppm genistein to the diet, reducing the effectiveness of the animal model (44). Therefore, diets containing < 20 ppm have been recommended for endocrine disrupter studies because this level is thought to have only negligible endocrine effects (14).

Specific tissues respond differently to the same dose of genistein, making it difficult to define a no effect level for dietary isoflavones. In ovariectomized mice, feeding 300 ppm genistein increased uterine weight, while decreases in adipose tissue were only seen at dietary levels of 500 ppm or more and effects on body weight were only significant at 1500 ppm (45). Additionally, the magnitude and direction of isoflavone effects can vary. Similar levels of dietary genistein (~1000 ppm) suppressed (46) or enhanced (47) the action of tamoxifen in models of ectopic breast cancer in the presence of estradiol. Response to dietary isoflavones is also model dependent. In Fischer 344, but not Sprague-Dawley® rats, vaginal opening date was affected by changes in dietary isoflavone levels ranging from 7 – 431 ppm (7).

With so much uncertainty, the precautionary principle would dictate that laboratory diets that contain soybean meal should be avoided for those research studies in which the end point may be affected by dietary isoflavones.

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