

## Phytoestrogens: Fact, fiction, and the future

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Phytoestrogens are nonsteroidal compounds produced by many plants and contained in many natural dietary products, such as soybeans, wheat, barley, corn, alfalfa, and oats. Structurally, they are similar to endogenous estrogens and share a similar mechanism of action through their affinity for and binding to estrogenic receptors. Although not steroids, phytoestrogens mimic or antagonize some of the actions of endogenous estrogens, but their potency is much lower than that of steroidal estrogens. Phytoestrogens have been ascribed certain putative health benefits against osteoporosis, heart disease, and some cancers.

Soybean meal is also a common component of laboratory animal food and has been used for many years in thousands of experiments. These diets have been the mainstay in the vast majority of scientific experiments that form the baseline data for many scientific knowns. Recently, with the increased use of inbred and transgenic animals in studies of cancer, immune responses, and endocrine disruptors, phytoestrogens in animal diets have been the subject of increased interest.

Various mammalian species, particularly rodents, have served as hosts in the evaluation of phytoestrogen effects on target tissues, such as the uterus, breast, prostate, and blood vessels. A myriad of effects have been reported, but not all have been consistent. The specific effect and relative potency of phytoestrogens are known to depend on numerous factors, including the type of assay (in vivo vs in vitro), type of species, age and endocrine status of the animal, route of administration, and dosage, which is evident in the inconsistencies in published literature.

### EFFECTS ON TUMOR CELLS

High soy intake has been suggested to account for lower breast cancer incidence in Asian, compared to European, women. However, a recent meta-analysis of results from case-control and cohort studies indicates that high soy intake does not reduce cancer risk, at least in post-menopausal women (Trock et al, 2000). Animal studies also mimic these inconsistencies. The soy isoflavone, genistein, at a concentration of 20  $\mu\text{mol/L}$ , inhibited proliferation of estrogen-independent human breast cancer cells in culture by approximately 50% (Santell et al, 2000). However, dietary genistein (750 ppm) fed to athymic mice either before or after injection of tumor cells had no effect on tumor growth (Santell et al, 2000). The authors of this study concluded that it would be unlikely to achieve plasma concentrations required to inhibit cancer cell growth from a dietary dosage of genistein. Similarly, in the chemically induced mammary tumor model in the rat, dietary soy protein containing up to 1070 ppm genistein failed to inhibit tumor growth (Cohen et al, 2000). These results, in conjunction with those of other studies, led the authors to conclude that the aggregate data, while suggestive in some cases of inhibitory effects, do not provide compelling evidence in support of soy isoflavones, administered in the physiological range, as potent chemopreventive agents in mammary carcinogenesis.

### EFFECTS ON THE REPRODUCTIVE TRACT

Semipurified diets containing 375 ppm to 750 ppm genistein fed to ovariectomized rats induced uterotrophic effects, as measured by increased uterine weight (Santell et al, 1997). Mammary gland regression following ovariectomy was inhibited by genistein 750 ppm but not 375 ppm. In contrast, intact immune rats, in response to coumestrol in acute (subcutaneous) or chronic (multiple injections) doses, showed a decline in estradiol response (Markaverich et al, 1995), leading the authors to conclude that the potential estrogenicity of phytoestrogens requires a careful reassessment in intact and ovariectomized animals before the impact of phytoestrogens on reproductive function can be understood. While in the young neoDES mouse, phytoestrogens have been reported to reduce prostate tumors, in the mature animals, phytoestrogens have no effect on the development of epithelial metaplasia or the expression of c-fos protooncogene (Mäkelä et al, 1995). Male and female rhesus monkeys fed phytoestrogen-intact soy protein diets had significantly lower LDL and VLDL concentrations, and significantly higher HDL concentrations, than animals on the phytoestrogen-free diet (Anthony et al, 1996). However, in the same study, phytoestrogens had no effect on the reproductive systems of either the males or females, as evaluated by reproductive hormone concentration and organ weight at necropsy (Anthony et al, 1996).

### THE JURY IS STILL OUT

To summarize the aggregate data on specific effects of phytoestrogens, either estrogenic or antiestrogenic, the evidence is inconclusive. The results vary based on the type of assay, the species, maturity and endocrine status of the animal, route of administration, and dosage. In particular, extreme caution is warranted with results from studies that have utilized pharmacological doses of phytoestrogens intended to induce a response. Such results should not be generalized to the potential effects of a normally occurring dietary intake in the laboratory. This fallacy is well illustrated in a recent publication by Brown and Setchell (Brown and Setchell, 2001).

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### CAUTION! A CASE STUDY

Brown and Setchell have studied the bioavailability of isoflavones in rats and mice fed commercial rodent diets. The authors report a daily food intake of 30 g for an adult rat (which may be higher than generally reported) and 3 g for an adult mouse, and suggest that these correspond to 24.3 mg and 2.4 mg, respectively, of isoflavones, "...amounts comparable to those producing a wide range of physiological effects." These physiological effects, however, were noted with specific isoflavones, such as genistein, that have been administered by a variety of methods, including force-feeding, injection, or dietary supplementation. In no way do these parameters represent the normal daily intake and feeding of laboratory animals. For example, rats with chemically induced breast tumors were force-fed genistein or daidzein 50 mg/kg/day for 4 weeks. To consume that amount of daidzein or genistein in a standard diet, a 400 g rat would need to consume 63 to 76 g of Purina 5001 daily, nearly 3 times the normal amount. In another example, the results of a study on the effects of isoflavones on LDL in mice following a dose of 3.6 mg/g diet represents an exposure level to soy isoflavones more than 4 times that reported in the article for Purina 5001 diet. These examples underscore the importance of careful and relevant comparisons in science.

While these authors call for a wider use of phytoestrogen-free diets, they fail to acknowledge that low levels of phytoestrogens in the diet also may be deleterious. It is conceivable that diets lacking phytoestrogens may retard normal growth and maturation of the animal. To date, quantification has not established optimum serum and urinary levels of phytoestrogens in rodents. In addition, a phytoestrogen-free diet has the negative potential of shifting baseline data and diminishing the value of historical comparisons. Therefore, valid scientific data regarding optimum phytoestrogen levels must be collected and evaluated before blanket dietary recommendations can be made.

### THE FUTURE:

Continued dedication and excellence

For the past 60 years, Purina Mills LabDiet® scientists have partnered with the research community to advance the understanding of laboratory animal nutritional needs. The tradition of vigilance and quality assurance at Purina Mills has led to the genesis of a surveillance database on the levels of nutrients and non-nutrients, including phytoestrogens, in raw ingredients and complete diets. The information has been used to maintain Constant Nutrition™ in all products, as well as to support recommendations to the scientific community, whose research might be sensitive to exogenous sources of estrogens.

For years, scientists at the Purina Mills Gray Summit Research Center have been conducting studies in rats and mice on the effects of phytoestrogens formulated into normal daily diets. The outcome measurements from these studies will evaluate growth, organ weight, and reproduction in males and females, intact and ovariectomized. These studies will also determine the proper dietary levels that should be maintained. Based on the information generated from these studies, specific nutritional recommendations will be made.

With this issue, and many others that address animal nutrition, Purina Mills feels strongly that decisions should be based on sound scientific evidence.

*Your work is worth it!*

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