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THE FACTS ABOUT ISOFLAVONES

A LOOK AT THE BIG PICTURE

from Purina Mill's LabDiet® Team

Isoflavones, the most common form of phytoestrogens, are detectable in most all diets fed to laboratory animals. Isoflavones are not bad for animals; however more and more researchers need to verify the levels contained in the diets for specific study areas. Before making your decision about isoflavones, there is more that you should know.

FACTS ON ISOFLAVONES IN DIETS

- Isoflavones (IF) are a common class of phytoestrogens. They are plant-derived compounds capable of estrogenic or antiestrogenic effects on the animals consuming them. Isoflavones are structural mimics of endogenous 17ß-estradiol (Seielstad, et al., 1995).
- IFs are nothing new. As early as the 1920s, IFs were found to affect body metabolism and in the 1940s, IFs found in red clover were found to affect sheep fertility.
- Since the early 1990s, with an increase in endocrine disruptor (ED) awareness, IFs have been identified as contributing factors that may affect the outcome of such studies.
- In the lab community, some advocate feeding low IF diets to all of their animals. However, others feel the low IF diets should only be used where appropriate.

The following information should assist you in making the correct choice for your lab animals.

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Isoflavones are found in numerous ingredients (wheat, corn, oats, alfalfa, corn gluten meal and soybeans) commonly used in diets for laboratory animals.

INGREDIENT	TOTAL IF*
Wheat	1
Corn	12
Oats	12
Alfalfa Meal	36
Gluten Meal	36
Soybean Meal	1400

*Genistein+Daidzein+Glycitein = Total IF, ppm
(Nestle Purina Analytical Labs, St. Louis, MO)

For over 50 years, soybean products have been used as the main protein source in natural-ingredient laboratory diets. During that period, countless laboratory animals (of numerous species) have been bred, raised and maintained on that type of diet without obvious detrimental results.

There are numerous forms of IFs, however those of primary concern are genistein, daidzein and coumestrol. Their potencies relative to estradiol (mammalian estrogen) are shown below:

RELATIVE ESTROGENIC POTENCIES

MOLECULE	POTENCY
Estradiol	100.000
Coumestrol	0.202
Genistein	0.084
Daidzein	0.013

As shown, the estrogenic activity of the IFs is several-fold lower than estradiol. However, they can interact with mammalian estrogen receptors (ER) to cause positive and negative responses in the tissue/organs targeted by the ERs.

ISOFLAVONES ARE A COMPLEX ISSUE

In vitro (cell culture) estrogenic potencies of IFs can vary in different types of studies and do not predict *in vivo* (whole animal) potency (Whitten and Patisaul, 2001). Recombinant cell cultures can contain many more estrogen receptors than those found in an intact animal system. This increased number of receptors could lead to more efficient utilization of the estrogenic activity from IFs, and result in the miscalculation of lower levels of IFs required to produce detectable estrogenic activity in an *in vivo* system (Yang and Bittner, 2002).

Depending on their concentration and relative potency, IFs can antagonize estrogenic activity in one instance and amplify it in another. Also, IFs can be biphasic in their effects; meaning they can be antiestrogenic in a high-estrogen environment and estrogenic in a low estrogen environment. IFs can have different effects in mammals, depending upon the stage of life. Developing mammals are very sensitive to hormones, including those with estrogenic activity.

The growth of numerous types of cancer cells *in vitro* has been suppressed by including the IF, genistein, in the growth medium (Yanagihara et al., 1993; Jing et al., 1993, Messina et al., 1994). However, according to Allred et al. (2004), the anti-proliferative effects of genistein have not been shown *in vivo.* Those same investigators reported that it is unlikely that dietary IF consumption will result in plasma genistein concentrations required for anti-proliferative effects reported *in vitro.*

Several investigators have published articles regarding the effects of diets containing various levels of IFs. A review article entitled, "Isoflavone levels in common rodent diet can interfere with the value of animal models and with experimental results" (Jensen, M.N. and M. Ritskes-Hoitinga, Laboratory Animal (2007) 41:1-18) cited the following: "…attention must be paid to the phytoestrogen content of animal diets, and the use of diets with high levels of phytoestrogens should be avoided for studies of hormone-sensitive endpoints" (Thigpen et al., 1999; Degen et al., 2002; Owens et al., 2003). Simply, choosing the right diet is essential.

DIETARY ISOFLAVONES: YES OR NO?

An increasing amount of research has been done in hopes of determining whether IFs are always negative in rodent diets.

Dr. Retha Newbold, a long-time investigator in developmental reproductive biology at the NIEHS Laboratory of Molecular Toxicology, has spent years investigating the effects of phytoestrogens and endocrine-disrupting chemicals. The author stated, "There are some experimental animals that simply thrive and reproduce better on diets with phytoestrogens…..it should be up to the investigators themselves to determine if their particular experiment calls for a phytoestrogen-free diet or not…." (EHP, 114:11:A641; Nov. 2006).

Negative effects of low (or no) IFs on phenotypic expression and reproductive system development have been reported in a recent article written by noted biomedical investigators (Ruhlen et al., 2008). They used a term called "Fetal Estrogenization Syndrome (FES)."

- FES indicates fetal exposure to elevated estradiol *in utero* and subsequent elevated serum estradiol in the offspring, resulting in the following observations:
  - Female offspring – Early puberty and increased uterine responsiveness to estrogen.
  - Male offspring – Reduced size in testis, epididymis and seminal vesicle. Enlarged prostate. Impaired glucose regulation.
  - Both sexes – Lighter birth weight, became obese as adults with elevated serum leptin levels.

Ruhlen et al. (EHP, 116:322-328; March, 2008) compared high-phytoestrogen diets to a low-phytoestrogen (soy-free) diet and their effect on reproductive performance and subsequent development of the offspring. Some highlights of their findings are shown below:

- Permanent adverse effects on the reproductive system in male and female offspring of dams fed the low-phytoestrogen diet, resulting in FES as a result of elevated fetal estradiol.
- A soy-based diet can be beneficial by reducing fetal serum estradiol, protecting against FES.
- Low-phytoestrogen diet resulted in prediabetic traits of excess fat and impaired glucose tolerance relative to the phytoestrogen-rich diets.
- Low-phytoestrogen diet produced fetuses with lighter birth weight relative to offspring from dams fed soy-rich diets.
- By adulthood, the low-phytoestrogen offspring were obese. Other researchers (Cederroth et al., 2007) had similar results. CD-1 mice receiving feed without soy IFs were fatter and showed impaired glucose tolerance.
- CONCLUSIONS: Removing all IFs from feed leads to alterations that could disrupt many types of biomedical research.

HOW DO ISOFLAVONES INFLUENCE THE REPRODUCTIVE, SKELETAL, CENTRAL NERVOUS AND CARDIOVASCULAR SYSTEMS OF RODENTS?

Reproductive System

- Low dietary levels of IFs can depress reproductive performance in Sprague-Dawley rats (Casanova et al., 1998).
- Female CF1 mice fed "regular" diet (>300 ppm IF) were immediately sexually receptive when housed directly with males, and their conceptions occurred earlier than females fed in IF-deficient diet (Khan et al., Physiol. Behavior, 2008).
- Male Sprague-Dawley rats fed IF-free vs. 200 ppm IF diets had no difference in prostate weight or testosterone levels (Weber et al., Proc Soc Exp Biol Med. 1999).
- Perinatal and developmental exposure to dietary isoflavones does not affect later responsiveness of the uterus to exogenous estrogen administration (Wade et al., Food and Chem Tox, 2003).

Central Nervous System

- Sprague-Dawley rats fed IF-free vs. 200 ppm IF diets had no difference in food/water intake, locomotor activity or brain aromatase activity (Weber et al., Proc Soc Exp Biol Med. 1999).
- Long-term exposure to diets containing a minimum of 200 ppm IF appeared to be more docile than rats fed the IF-free diets (Lephart et al., ILAR Journal, 2004).
- Consideration needs to be made to ensure the appropriate dietary IF level is selected for the protocol dealing with the central nervous system.

Cardiovascular System

- High IFs were beneficial to obese Zucker rats in platelet sensitivity, lipid metabolism and liver function (Banz et al., FASEB, 1999).

Skeletal System

- OVX rats fed 440 ppm IF compared to those fed 40 ppm IF had a slight reversal in OVX-induced femoral bone loss, but not in lumbar vertebra (Arjmandi et al., Amer J. Clin Nutr, 1998).
- OVX rats fed 300 ppm dietary IF, or more, had higher femoral bone mass density than those fed an IF-free diet (Picherit et al., Br. J. Nutr, 2001).
- OVX rats fed a soy-based diet containing 450 ppm IF had less bone loss compared to those receiving a casein-based diet containing a very low level of IF.
- In general, bone loss studies should be conducted with diets containing less than 300 ppm IFs.

HOW DO ISOFLAVONES INFLUENCE CANCER RESEARCH?

- When diets with increasing levels of genistein are fed to athymic mice, the surface area of breast cancer tumors was significantly increased (Ju et al., J of Nutr, 2001).
- When exposed to pre- and post-natal dietary IF, the growth of colon cancer tumor in male Sprague Dawley rats was suppressed (Raju et al., J of Nutr, 2009).
- Isoflavones delay mammary tumorigenesis in mice that are predisposed to develop mammary tumors (MMTV-neu mice) (Jin and McDonald, J of Nutr, 2002).
- Genistein inhibits the growth of bladder tumors in severe combined immunodeficient mice (Singh et al., Canc Res, 2006).
- When fed to athymic mice, dietary genistein inhibits the metastasis of human prostate cancer (Lakshman et al, Canc Res, 2008).

CHOOSING THE CORRECT DIET FOR YOUR STUDIES

Contact the researchers at Purina LabDiet® to help you choose the right Verified Diet for your research. As noted in the literature cited earlier, rushing to use a "low" IF diet may in fact have detrimental effects on your animals and studies. Instead, consider using the same scientific approach for selecting your diet as you would for selecting your research model and experimental design.

Clearly, some research dictates the usage of diets with controlled levels of IFs, while other research does not. When choosing the proper diet for your studies, consider this:

- Understand your response variables. Are they sensitive to IFs?
- Define what "low" means to YOU, because diets can range from 0 to 600 ppm IFs.
- Know the IF content of every batch of feed that your animals consume. Knowing that your diet is "low" in IFs is not sufficient. "Low" is a relative term and tells you nothing about the IF content of the diet. Some studies will require a "low" IF content of less than 10 ppm, while other studies may require a "low" IF content of less than 150 ppm.
- What is right for your research? Use a scientific approach to choose a diet that is right for you and gives you precise and repeatable data.

Don't always believe what you hear, get the facts! Contact the nutrition experts at info@labdiet.com.

LABDIET® ADVANCED PROTOCOL® VERIFIED DIETS

5K96*	Advanced Protocol® Verified Casein Diet 10 IF
5V5R**	PicoLab® Select Rodent Diet 6F/50 IF
5V5M**	PicoLab® Select Mouse Diet 9F/50 IF
5V75	Verified Rodent Diet Pelleted 75 IF
5V75	Picolab® Verified Rodent Pelleted 75 IF
5V12	Verified Rodent Diet Extruded 75 IF
5V12	PicoLab® Verified Extruded 75 IF

Verified = Verified Isoflavone Levels
** TestDiet® custom product*
*** Available as conventional pellets or extruded particle*

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