REVIEW [REVISIÓN]

EFFECTS OF PHYTOESTROGENS ON MAMMALIAN REPRODUCTIVE PHYSIOLOGY

[EFECTOS DE LOS FITOESTRÓGENOS EN LA FISIOLOGÍA REPRODUCTIVA DE LOS MAMÍFEROS]

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SUMMARY

Global consumption of phytoestrogens and their effects have increased both in animals and humans due to the augmented use of legumes in animal diets as well as the increase in vegetarian diets in some human populations. Phytoestrogens are found widely in a variety of plants and fodder, and can have adverse effects mainly on the reproductive tract in most animal species. Many phytoestrogens can act as estrogenic agonists or antagonists, and their effects can vary from an estrogenic over-response, thus increasing secretions in the reproductive tract, to infertility, also disrupting animal behavior. Therefore, many phytoestrogens are now recognized as endocrine disruptor compounds, capable of interfering with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body, which are responsible for reproduction. The effects of phytoestrogens mainly depend on the type, plant species, and amount ingested. Despite the abundant literature about the effects of phytoestrogens in humans and laboratory animal models, presently there is still little knowledge about the effects of estrogenic forage on reproductive parameters in animal production systems. Therefore, it is necessary to continue research in order to elucidate the effects of phytoestrogens, the doses at which effects are seen, the species, their disruptive or beneficial effects, as well as the mechanisms of action involved. This review focuses on the effects of phytoestrogens in the reproductive physiology of livestock and human, as well as the knowledge obtained from research in laboratory animal models.

Key words: Phytoestrogens; animal reproduction; estrogens; estrogenic agonists; estrogenic antagonists; estrogen receptors; endocrine disruptors.

RESUMEN

El consumo de dietas a base de leguminosas en los animales de granja, así como así como las dietas vegetarianas y el consumo de suplementos alimenticios con alto contenido de fitoestrógenos en los humanos se han incrementado en los últimos años. Los fitoestrógenos están ampliamente contenidos en gran variedad de leguminosas, pastos, ensilajes y hierbas, y pueden causar efectos adversos principalmente en el tracto reproductivo de la mayoría de las especies animales. Muchos fitoestrógenos pueden actuar como agonistas o antagonistas estrogénicos, por lo que sus efectos varían desde la sobre-respuesta estrogénica, aumentando las secreciones del tracto reproductivo, hasta la infertilidad, alterando además el comportamiento animal. Por lo tanto, se ha considerado a los fitoestrógenos como disruptores o desorganizadores endocrinos, capaces de interferir con la síntesis, la secreción, el transporte, la unión, la acción y eliminación de las hormonas que produce el organismo de forma natural y que son responsables de la reproducción. El efecto de los fitoestrógenos en la reproducción depende básicamente del tipo y la cantidad presente en la especie vegetal que se consuma. A pesar de la abundante literatura que muestra los efectos de los fitoestrógenos en los humanos y en los modelos animales de laboratorio, en la actualidad aún se desconoce el grado en que los parámetros reproductivos en los sistemas de producción animal se han visto afectados por el consumo de forrajes fitoestrogénicos. Por lo tanto, es necesario continuar con investigaciones que permitan dilucidar los efectos de estos compuestos con respecto a las dosis utilizadas, las especies, los efectos nocivos o benéficos, así como los mecanismos de acción

involucrados. Esta revisión se centra en los efectos de los fitoestrógenos en diversas especies de ganado y en los humanos, así como en los hallazgos obtenidos de la investigación en modelos animales de laboratorio.

INTRODUCTION

Phytoestrogens are non-steroidal compounds present in plants. These compounds are structurally similar to natural estrogens, such as 17β-estradiol, allowing them to bind with estrogen receptors and thereby to induce biologically detectable effects (Navarro, 2005). Their name comes from the roots phyto = plant + oestrous = estrous + gen = to generate. Although phytoestrogens can mimic the effects of estradiol (E_2), their effects are not necessarily identical, since phytoestrogen can produce estrogenic or antiestrogenic effects (Yildiz, 2005). These compounds compete with endogenous steroids, so the balance between estrogenic and antiestrogenic activity is determined by the phytoestrogen-estrogen ratio (Folman and Pope, 1966). This might explain why estrogenic effects of phytoestrogens predominate in livestock, whose estradiol plasma concentrations are relatively low (15 pg/ml). In comparison, antiestrogenic effects are reported mainly in humans, in which estrogen plasma levels are relatively high (50-400 pg/ml; Adlercreutz *et al*, 1991).

There are four main groups of phytoestrogens: isoflavones (genistein, daidzein, glicetin, formononetin, biochanin A and equol, an isoflavone metabolite), flavones (quercetin and campherol), coumestans (cumestrol), and lignans (enterolactone, enterodiol) (Strauss *et al*., 1998). There are also estilbenes (resveratrol) and micotoxins (zearalenol α and β), which are present in plants or in their seeds, although some fungi can also synthesize them (Moutsatsou, 2007).

When comparing the chemical structures of phytoestrogens to 17β-Estradiol, it can be seen the key structural elements that enable phytoestrogens to bind with estrogen receptors and display estradiol-like effects. These are: the phenolic ring and the distance between two hydroxyl groups. The chemical structures of 17β-Estradiol and those of some phytoestrogens are shown in Figure 1.

Phytoestrogen content varies in different foods, and may vary significantly within the same group of foods (e.g. soy beverages, tofu) depending on the processing mechanisms and the type of soybeans used. Legumes (in particular soybeans), whole grain cereals, and some seeds are high in phytoestrogens.

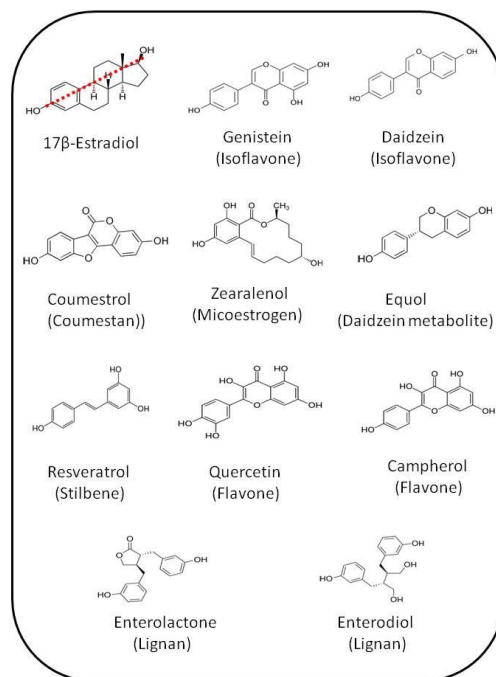

Figure 1. 17β-Estradiol and some examples of phytoestrogens (the name of the group to which they belong is in brackets).

Isoflavones are the most prevalent phytoestrogen in legumes, especially soybeans (103,649.3 μg/100 g) and soy-based foods (Thompson *et al*., 2006), including tofu (27,118.5 μg/100 g), soy infant formula (3,200 – 4,600 *μ*g/100 ml; Chen and Rogan, 2004; Setchell *et al*., 1998), and soy milk (2,944.2 μg/100 g); detectable levels are also present in fruits, vegetables, whole grains (Reinli and Block, 1996), and even some alcoholic beverages (Lapcik *et al*., 1998). Dietary supplements containing high levels of isoflavonoid phytoestrogens are now widely available (Setchell *et al*., 2001; Thompson *et al*., 2007). Lignans are the primary source of phytoestrogens found in flaxseed (379,012.3 *μ*g/100 ml), nuts (186.6 *μ*g/100 ml) (Thompson *et al*., 1991, 2006; Kuhnle, *et al*., 2008), and in lower concentrations in cereals, legumes, fruits and vegetables (Price *et al*., 1985). Coumestans occur predominantly with germination; for example beans sprouts and also in fodder crops (Price *et al*, 1985). The total phytoestrogen, lignans, isoflavones, and coumestans content in several foods and drinks are shown in Table 1. Stilbenes are present in red grapes and other fruits (Gehm *et al*., 1997), and their contents are shown in Table 2.

Table 1. Total phytoestrogen, lignans, isoflavones, and coumestans content in vegetables, legumes, soy products, fruits, nuts and drinks. n.d. = non detectable. Modified from Thompson *et al*. (2006).

Food items 100 g	Lignans content (µg/100 g)	Isoflavones (µg/100 g)	Coumestans (µg/100 g)	Total phytoestrogen (µg/100 g)
Vegetables				
Soy bean sprouts	2.2	787.5	n.d.	789.6
Garlic	583.2	20.3	0.1	603.6
Alfalfa sprout	44.8	394.1	2.5	441.4
Winter squash	113.3	0.3	0.0	113.7
Green beans	66.8	39.0	0.0	105.8
Collards	97.8	1.9	1.5	101.3
Broccoli	93.9	0.2	0.0	94.1
Cabbage	79.1	0.9	0.0	80.0
Legumes				
Lentils	26.6	9.5	0.3	36.5
Baked beans	15.3	1.3	0.0	16.6
Kidney beans	6.5	1.6	0.0	8.1
Chick peas	2.9	1.7	0.9	4.7
Fruits				
Dried dates	323.6	5.1	0.8	329.5
Dried apricot	400.5	39.8	4.2	444.5
Dried prunes	177.5	4.2	1.8	183.5
Peaches	61.8	2.6	0.1	64.5
Strawberry	48.9	2.4	0.3	51.6
Raspberry	37.7	9.3	0.5	47.6
Watermelon	2.9	0.1	0.0	3.0
Nuts and oil seeds				
Flaxseed	379012.3	321.4	46.8	379380.4
Pistachios	198.9	176.9	6.7	382.5
Sunflower seed	210.3	5.7	0.1	216.0
Chestnuts	186.6	21.2	2.4	210.2
Walnuts	85.7	53.3	0.6	139.5
Almonds	111.7	18.0	1.5	131.1
Cashews	99.4	22.1	0.4	121.9
Hazel nuts	77.1	30.2	0.3	107.5
Peanuts	27.1	7.3	0.1	34.5
Beverages				
Wine, red	37.3	16.5	0.1	53.9
Tea, green	12.0	0.7	0.3	13.0
Wine, white	8.0	4.7	0.1	12.7
Tea, black	8.1	0.6	0.2	8.9
Coffee, decaf	4.8	0.7	0.0	5.5
Beer	1.1	1.6	n.d.	2.7
Milk, cow	0.9	0.3	0.0	1.2
Soy products				
Soybeans	269.2	103649.3	1.5	103920.0
Tofu	30.9	27118.5	0.7	27150.1
Soy yogurt	46.6	10227.8	0.5	10275.0
Soy milk	12.3	2944.2	0.6	2957.2
Cereals and breads				
Bread, flax	7239.3	300.8	0.6	7540.6
Bread, multigran	4785.6	12.6	0.5	4798.7
Bread, rye	142.9	3.4	0.0	146.3

Table 2. The stilbene resveratrol in food and beverages. Modified from Hurst *et al*. (2008).

Food (100 g)	Total resveratrol (µg/100 g)
Peanut butter	15.5 - 50.4
Peanuts (raw)	71.42 - 178.0
Cocoa Powder	140.0 - 230.0
Red grapes	150.0 - 781.3
Peanuts (boiled)	177.7 - 711.1
Beverages	**Total resveratrol (µg/ml)**
White wine	0.05 - 1.8
Rose wine	0.43 - 3.52
Red grape juice	1.14 - 8.69
Red wine	1.92 - 12.59

There are reports indicating that most of the legumes commonly used for feeding livestock contain from 5 to 25 % phytoestrogens; their concentrations vary depending on environmental factors, such as: temperature, humidity, light, age of the plant, amount of fertilizer, and pathogens (Adams, 1995). Alfalfa (*Medicago sativa*) (Bora and Sharma, 2011), white clover (*Trifolium repens*), subterranean clover (*Trifolium subterraneum*) (Sakakibara *et al*., 2004) and red clover (*Trifolium pretense*) (Sabudak and Guler, 2009), as well as soybean (*Glycine sp.*) (Setchell *et al*., 2002) contain high quantities of phytoestrogens. Alfalfa produces coumestrol (Adler and Trainin, 1960), especially when suffering from foliar disease by fungal pathogens (Loper *et al*., 1967), and the concentration found is 1,500 - 22,500 µg/100 g. Subterranean clover contains isoflavones including genistein, formononetin, and biochanin A (Kallela *et al*., 1984). The main estrogenic compound in red clover is formononetin. The isoflavones content found in this legume varies from 778,600 - 1,074,400 µg/100 g (Sivesind *et al*., 2004). Soy and its products contain isoflavones as well (total concentration: 103,649.3 µg/100 g), mainly genistein, daidzein and glycetin (Kallela *et al*., 1984; Lundh *et al*., 1988; Thompson *et al*., 2006); they also contain significant amounts of lignanes (30.9 - 269.2 µg/100 g) (Thompson *et al*., 2006).

ABSORPTION, BIOAVAILABILITY, AND METABOLISM

The metabolism of isoflavones has been well characterized in sheep and other mammalian species, including humans (Lindsay and Kelly, 1970; Lundh, 1995). Isoflavones are naturally found as biologically inactive glycoside conjugates, containing glucose or carbohydrate moieties, and they become active compounds when the sugar residue is removed in the gut by bacteria. Once consumed, they are rapidly metabolized and absorbed, entering systemic circulation predominantly as conjugates with limited bioavailability. The unconjugated form (aglycone) is the bioactive form (Patisaul and Jefferson, 2010).

In sheep, formononetin and biochanin A are biotransformed by ruminal bacteria to the demethylated intermediates daidzein and genistein, and then to the estrogenic isoflavone equol (Setchell *et al*., 2002). Despite the considerable degradation of isoflavones in the gut, plasma equol concentrations in plasma are significant (20 mmol/L in sheep, Setchell *et al*., 2002) which account for the pathophysiological effects on reproduction.

Following absorption, isoflavones are reconjugated in the liver, mainly to glucuronic acid (Lundh, 1995). In bovines, rumen microorganisms convert daidzein and genistein into two active metabolites: equol and para-ethyl-phenol, respectively, although no pathophysiological effects due to equol have been observed in cattle (Lundh, 1995).

In humans, plasma concentrations of daidzein, genistein and equol in adults consuming soy food range from 10 nM - 10 µM (Setchell and Cassidy, 1999; Ren *et* al., 2001; Moutsatsou, 2007). Young girls, 8 - 14 years old, have very high urinary isoflavone excretion (142.1 nmol/mg creatinine) compared to adult women (44 nmol/mg creatinine), which suggests less intestinal degradation and/or greater absorption of isoflavones in young people (Maskarinec *et al*., 2005). Lignans are metabolized to enterolactone and enterodiol in the gut, resulting in variable plasma concentrations (15 - 41.4 nmol/L) (Adlercreutz, 2002; Murkies *et al*, 1998).

Circulating concentration of isoflavones in 12 - 24 months infants fed soy-based formulas (daidzein 295 ng/ml and genistein 684 ng/ml) can be 20,000 - 50,000 times higher than plasma estradiol concentrations (6.5 – 14.3 pg/ml) in early life and may exert biological effects. In addition, infants fed soy-based formula maintain high steady state plasma concentrations of isoflavones, which may possibly be explained by reduced intestinal biotransformation, as evidenced by low or undetectable concentrations of equol and other metabolites (Setchell *et al*., 1997; 1998).

Many of the metabolites of phytoestrogens and the flavonoids formed both in the gut and liver may be also biologically active and may mediate estrogen-signaling (Mueller *et al*., 2004). Thus, the bioavailability of dietary phytoestrogens determines their activity in vivo.

Despite the route of administration used in animal experiments, it has been observed that phytoestrogens

administered orally or subcutaneously have similar effects (Jefferson *et al*., 2009b). It takes about 80% of the subcutaneous dose to cause the same level of uterine weight gain as the oral dosage, suggesting that these compounds are readily absorbed and reach the target organs. Therefore, the peak and total circulating levels may contribute to the overall biological effect. Furthermore, similar levels are attained both in rodents and humans after consumption or administration of phytoestrogens (µM range).

MECHANISM OF ACTION

Phytoestrogens exert their effects via multiple mechanisms: they interact with both ERα and ERβ, thereby inducing weak estrogenic or antiestrogenic actions (Kuiper *et al*., 1998; Mitchell *et al*., 2001). The key structural elements that enable phytoestrogens to bind with high affinity to estrogen receptors and display estradiol-like effects are: the phenolic ring that is indispensable for binding to the estrogen receptor; the ring of isoflavones mimicking a ring of estrogens at the receptor binding site; low molecular weight, similar to estrogens (MW=272); the distance between two hydroxyl groups in the isoflavone nucleus is similar to that in estradiol; and optimal hydroxylation pattern (Yildiz, 2005).

Phytoestrogens have lower affinity for estrogen receptors (ER) than estradiol (E_2), and most of them exhibit a higher affinity for ERβ than for ERα (Whitten and Naftolin, 1998; Turner *et al*., 2007) by approximately 30 fold. Other phytoestrogens, such as resveratrol, bind to ERβ and ERα with comparable affinity, but with a 7,000-fold lower activity than E_2 (Bowers *et al*., 2000). In comparison, E_2 recruits the co-regulators of both types of receptors in a non-selective way (An, *et al*., 2001). The ligand-receptor complex generated is capable of inducing transcriptional activity (Kuiper *et al*., 1998) (figure 2). However, the concentration required for isoflavones (genistein, daidzein, glicetin and equol) to induce transcriptional activity is 10^4 higher than E_2, and their activity is lower than that of the steroid. This lower transcriptional activity of phytoestrogens is offset by their higher bioavailability, since the free circulating fraction is more than 50%, compared with the 4.5% for E_2. Furthermore, phytoestrogen circulating levels are one order of magnitude higher than those of E_2 (ng/ml versus pg/ml). This greater accessibility to ERs explains why in the presence of endogenous estrogens, isoflavones behave as estrogenic antagonists, while in the absence of estrogens they behave as weak agonists (Navarro, 2005). In addition to the interaction with ERs, phytoestrogens may also modulate the concentration of endogenous estrogens by binding or inactivating some enzymes, such as P450 aromatase, 5α-reductase, 17β-hydroxyesteroid dehydrogenase (17β-OHDH), topoisomerases, and tyrosine kinases.

ESTRADIOL AND PHYTOESTROGENS MECHANISM OF ACTION
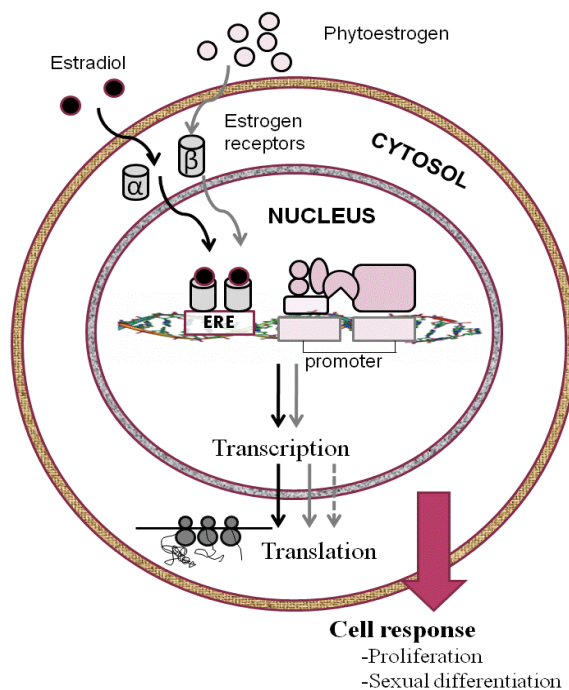

Figure 2. Phytoestrogens have weaker estrogenic activity than estradiol. They exhibit higher affinity for ERβ than for ERα but with a lower activity than E_2. ERE=estrogen response elements. Black lines: estradiol mechanism of action. Grey solid and discontinuous lines: phytoestrogens mechanisms of action.

Phytoestrogens may also affect the bioavailability of sex hormones by binding or stimulating the synthesis of sex hormone binding globuline (SHBG) (Johnston, 2003). Some phytoestrogens exert an inhibitory effect on steroidogenic enzymes (Strauss *et al*., 1998). For example, isoflavonoids and lignans inhibit 5α-reductase activity, thereby reducing the conversion of testosterone to the active form DHT (Evans *et al*., 1995).

Global consumption of phytoestrogens has increased both in animals and human beings due to the augmented use of legumes in animal diets (Rachuonyo *et al*., 2005) and the increase of vegetarian diets in some human populations (Patisaul *et al*., 2010). Even though the general opinion and that of clinicians toward phytoestrogens is mainly positive, many phytoestrogens are now recognized as endocrine disruptor compounds (EDCs) (Patisaul *et al*., 2010). EDCs are natural or synthetic compounds that may alter hormonal function by numerous mechanisms, including: 1) direct stimulation or inhibition of the

endocrine system; 2) mimicking or blocking the body response to endogenous steroid hormones; or 3) altering the biosynthesis, secretion, transportation, binding, action, degradation or elimination of endogenous hormones that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior (Dickerson and Gore, 2007; Phillips and Tanphaichitr, 2008).

EFFECTS OF PHYTOESTROGENS ON REPRODUCTIVE PHYSIOLOGY.

Livestock

Females

The use of pasture legumes has widely increased in animal farming throughout the world (Adams, 1995; Rachuonyo *et al*., 2005; Sabudak and Guler, 2009), and reproductive abnormalities due to phytoestrogens have been described in cows and sheep.

The endocrine disrupting potential of phytoestrogens was first reported in Australia, when abnormally high rates of infertility, abortion, and reproductive abnormalities in newborn lambs were observed in ewes grazing on clover rich pastures. This syndrome was called "clover disease" (Bennetts *et al*., 1946). Aside from these disturbances, very low lambing rates, prolapse of the uterus and dystocia, severe metritis, pyometron and *hydrops uteri* have been observed. Furthermore, reduced ovulation and conception rates, as well as permanent infertility were described (Lightfoot and Wroth, 1974). It was eventually determined that the steroid-like phytoestrogens, most notably formononetin and coumestrol, were responsible for the observed effects (Braden *et al*., 1967; Adams, 1995). Decades later, a particular case of infertility and liver disease in captive cheetahs that had been placed on a soy-based diet was ultimately attributed to the same class of compounds (Setchell *et al*., 1987).

Red clover silage containing isoflavones, and alfalfa containing coumestans have been reported to cause infertility in cattle. Many cows suffered from cystic ovaries, with behavioral abnormalities including irregular estrous, nymphomania and anestrous (Kallela *et al*., 1984). In other studies, cows fed alfalfa containing high amounts of coumestrol showed an estrogenic syndrome characterized by repeated estrous, abortions, endometriosis, cystic ovaries, false estrous, swollen vulvae and uterine enlargement (Adler and Trainin, 1960). Also, an estrogenic syndrome was described in dairy cows after ingesting significant concentrations of coumestrol, with normal levels of E_2 in plasma. The syndrome was characterized by repeated breeding, abortions, metritis and ovarian cysts, as well as increased cervical mucus

fluidity and swollen uteri. The study was carried out for one year in 608 cows, in which 1264 inseminations resulted only in 376 gestations. Of these, 102 had a swollen uterus and 36 ended in abortion; only 238 had normal pregnancies (Romero *et al*., 1997).

In ewes, aside from the "clover disease" syndrome, described by Bennets in 1946, very low lambing rates were seen in addition to prolapsed uteri and dystocia, severe metritis, pyometron and *hydrops uteri*. Furthermore, reduced ovulation and conception rates were described, as well as permanent infertility (Lightfoot and Wroth, 1974). In addition to ovarian and endometrial cysts observed in females, pytoestrogens can induce other morphological effects in livestock. In ovariectomized ewes fed with red clover silage, changes in teat length and color of the vulva, similar to those in ewes with estradiol implants were observed (Cantero *et al*., 1996).

Long term consumption of a soy diet significantly increases mean insemination and infertility rates (Woclawek-Potocka *et al*., 2005), and inhibits LH-stimulated progesterone (P4) secretion. In consequence, cows fail to ovulate and get pregnant (Piotrowska *et al*., 2006). Isoflavone-dependent infertility is occurring more often without observable symptoms and can only be detected by measuring phytoestrogens in the diet or by observation of their effects on the animal's reproductive health and efficiency (Adams, 1995). Furthermore, long term feeding of cows with soy based diets has hazardous effects during early pregnancy (Piotrowska *et al*., 2006), such as abortions during the first three months (Jonker, 2004). It has been proposed that isoflavone active metabolites (equol and para-ethyl-phenol) could be involved in these effects, since they are in higher concentrations during early pregnancy compared with late pregnancy and estrous cycle (Woclawek-Potocka *et al*., 2008).

Hyperestrogenism and reproductive problems have also been found in grain fed pigs, where the grains have been linked to contamination by saprophytic fungi, such as *Fusarium*. These grains are considered the major source of pasture contamination in Australia and New Zealand. Zearalenone is a fungal compound with estrogenic potency identified as a major cause of these problems. Zearalenone and its metabolites α-zearalenol and β-zearalenol, which are formed *in vivo* in the rumen, elicit significant estrogenic activity in animals. Zearalenone concentrations are commonly 50 - 500 μg/100 g during autumn in New Zealand (Reed *et al*., 2004). This mycoestrogen causes hyper-estrogenism and low fertility, with a decreased ovulation rate and cycle length, increased duration of estrous, without affecting the pregnancy rate and embryonic loss in ewes (Smith *et al*., 1990). Higher dosages and longer exposure cause significant

reduction in estrous, ovulation and lambing (Reed *et al*., 2004).

In heifers, zearalenone causes infertility by reducing the conception rate (Reed *et al*., 2004), increasing embryonic resorption and early abortion (Kallela and Ettala, 1984).

Studies *in vitro* have shown that daidzein and quercetin act directly on the swine ovary by decreasing the synthesis of progesterone and 17β-estradiol (Santini *et al*., 2009) and increasing the expression of ERβ (Nynca *et al*., 2009). All this evidence shows the deleterious effects of phytoestrogens on reproductive processes in female species of economic importance.

Males

Interest in the effects of phytoestrogens on male fertility has increased in recent years as it has been demonstrated that estrogens play an important role in the male reproductive system (Rochira *et al*., 2005). Although there are only few reports, it has been shown that phytoestrogens also cause reproductive disruption in males. In bovines, ingestion of fodder with high quantities of coumestrol causes glandular metaplasia in both prostate and bulbourethral glands (Lenis *et al*., 2010), gynecomastia and even galactorrhea (Romero *et al*., 1997). In cryopreserved bull spermatozoa, genistein can affect a protein tyrosine phosphorylation-independent signal transduction pathway that is involved in sperm capacitation, the acrosome reaction and sperm- pellucid zone binding, thereby decreasing 40-50% the acrosome reaction (Menzel *et al*., 2007). All of these events, which are considered to be similar to the effects of DDT in wild bird populations, have raised questions regarding the potential risk phytoestrogens might pose not only to livestock but also humans.

Humans

The fact that soy consumption has been widely promoted as being healthful (Adlercreutz and Mazur, 1997) is a reason for studying the potential endocrine disrupting properties of isoflavonoids, mainly genistein and daidzein (Patisaul *et al*., 2009). Emerging evidence indicates that exposure to these compounds may in fact be risky to infants (Cao *et al*., 2009) and the unborn (Rozman *et al*., 2006). Fetal exposure could have potential consequences, because genistein and other phytoestrogens can cross the placenta (Todaka *et al*., 2005). Daily intake of isoflavones in soy infant formula is estimated at 1 - 8mg/kg bw/day in infants fed soy formula (Rozman *et al*., 2006), which is four to seven times higher than the amount consumed by adults on a traditional soy-based Asian diet (Barnes, 1995). Longer menstrual bleeding and menstrual discomfort have been reported in young

women fed soy-based infant formula compared to those who were fed non-soy based formula as babies (Strom *et al*., 2001). Nonetheless, little is known about how exposure to soy phytoestrogens, either in the prenatal life or in infancy, impacts female reproductive health or behavior in humans (Patisaul *et al*., 2009).

Despite the health benefits attributed to phytoestrogens, clinical and experimental studies have generated ambiguous and often conflicting results (Patisaul *et al*., 2010). It is considered that phytoestrogens provide beneficial effects on perimenopausal symptoms, including hot flushes and night sweats. However, demonstrable evidence is weak and most clinical trials show minimal or no amelioration (Jacobs *et al*., 2009; Shen *et al*., 2009).

Therapeutic actions of phytoestrogens in hormone dependent cancers and carcinogenesis (Peeters *et al*., 2003), atherosclerosis, reduced risk of cardiovascular disease (Clarkson, 2002), and osteoporosis (Cassidy *et al*., 2006) have also been stated. However, there is no strong evidence confirming these beneficial effects (Liu *et al*., 2009), and in some cases only marginal benefits have been observed (Cooke, 2006). Thus, health benefits frequently attributed to phytoestrogens may be exaggerated (Jacobs *et al*., 2009).

Concerning breast cancer, it has been shown that endogenous and exogenous estrogens can stimulate the growth of breast cancer cells. Consequently, these cells are called estrogen-dependent or estrogen-receptor positive, and they have a relatively large quantity of estrogen receptors. Phytoestrogens, mainly flavones and isoflavones, are considered to have a protective effect on the initiation or progression of breast cancer by inhibiting the local production of estrogens from circulating precursors in breast tissue. Isoflavones are capable of inhibiting aromatase, an enzyme that converts androgens to estrogens, thus causing a decrease in circulating estrogen concentrations (Rice and Whitehead, 2006). It is important to mention that over 60% of breast carcinomas express this enzyme (Miller, 1991).

Isoflavones have also been shown to exert inhibitory effects on 17β-hydroxysteroid dehydrogenase (17β-HSD) type 1, the enzyme that converts estrone to estradiol, in breast cancer cells (Brooks *et al*., 2005). Phytoestrogens such as daidzein, biochanin A, and genistein in low doses stimulate growth of some types of cancer cells, but at higher doses, growth is inhibited (Dampier *et al*., 2001; De Lemos, 2001). However, results from both *in vitro* and *in vivo* studies, both in animal models and in humans, have been inconsistent (Enderliln *et al*., 2009).

The overall evidence shows no consistent effects of dietary phytoestrogens on indicators of cell

proliferation in normal human breast tissue, although phytoestrogens may increase proliferation in existing breast cancer. Furthermore, there is still no conclusive evidence that a high dietary intake of phytoestrogens is related with the reduced incidence of breast cancer (Rice *et al.*, 2006). Other reviews of studies in humans suggest a modest inverse relationship between risk and high soy intake (Trock *et al.*, 2006; Wu *et al.*, 2008) but this trend is generally not supported by data from the animal literature (Warri *et al.*, 2008).

To date, no clear consensus has been reached on whether or not phytoestrogens are helpful or harmful, or when they might be contraindicated for some groups. Thus, the degree to which phytoestrogen consumption confers meaningful health benefits for human remains to be solved (Mense *et al.*, 2008).

Concerning men, several studies suggest that global semen quality has declined over the past 50–60 years (Bostofte *et al.*, 1983; Carlsen *et al.*, 1992; Swan *et al.*, 2000). One of the possible causes of this problem could be the increased exposure of humans to environmental chemicals called "endocrine disruptors" (McKinney *et al.*, 1998). A significant decline in sperm count, from 113 million/ml in 1940 to 66 million/ml in 1990, among men without a history of infertility, associated to a decrease in mean seminal volume (3.4 ml to 2.75 ml) was reported, thereby decreasing further the total sperm count. It was proposed that such remarkable changes in semen quality might be due to environmental factors, such as estrogens or compounds with estrogen-like activity (Carlsen *et al.*, 1992). However, no conclusive evidence exists in this regard since there is only one study measuring the effects of phytoestrogens on semen parameters in men. Healthy males received short-term phytoestrogen supplementation containing 40 mg of phytoestrogens isoflavones genistein, daidzein, and glycitein daily for 2 months. Serum E_2, testosterone, FSH, or LH were not influenced by phytoestrogen supplementation; nor did seminal volume, sperm concentration, sperm count, and sperm motility (Mitchell *et al.*, 2001). To date, evidence linking dietary consumption of phytoestrogens and reduced semen quality is insufficient and requires further study (Phillips and Tanphaichitr, 2008).

Laboratory Animal Models

Females

Data from animal research have provided much information about the effects and mechanisms by which phytoestrogens modify the reproductive physiology of mammals. There is abundant information on the effects of these compounds in females. Most of the studies have evaluated the effects of genistein and daidzein, which are the isoflavones

consumed by humans in soy beans and soy products. However, there are many other phytoestrogens present in foods awaiting evaluation.

The research on phytoestrogens demonstrates that their effects on the female genital tract depend on the age at which exposure occurred and its duration. Neonatal exposure results in severe alterations in the reproductive physiology of females (Burton and Wells, 2002). Perinatal exposure to genistein in female mice (10 mg/Kg) or rats (0, 5, 100, 500 ppm) accelerate vaginal opening and altered estrous cycles advances pubertal onset, increases the length of the estrous cycle (Delclos *et al.*, 2009), accelerates the onset of persistent estrus, causes abnormal estrous cycles, decreases fertility, delays parturition (Jefferson *et al.*, 2009a) and decreases the number of live pups in adulthood (Jefferson *et al.*, 2005). Neonatal genistein can also interfere with ovarian differentiation resulting in ovarian malformations indicative of impaired fecundity such as multi-oocyte follicles, and attenuated oocyte cell death (Jefferson *et al.*, 2006; 2009b). Ovarian defects, including the absence of corpora lutea, the presence of large antral-like follicles with degenerating or no oocytes and numerous ovarian cysts have also been observed following neonatal genistein exposure in rats (Kouki *et al.*, 2003). Similar extended estrous cycles in adult females have been observed in experimental animal models with perinatal exposure to other phytoestrogens such as resveratrol, zearalenone, and Bisphenol A (Nikaido *et al.*, 2004). These abnormalities could result from organizational disruptions anywhere within the Hypothalamus-Pituitary-Gonadal (HPG) axis, including the ovary.

Early sexual differentiation of the developing brain is also vulnerable to neonatal endocrine disruption by phytoestrogens, which modify the organization of sexually differentiated neural pathways within the hypothalamus. Neonatal exposure to genistein impairs the ability to stimulate GnRH neuronal activity following hormone priming in ovariectomized females (Bateman and Patisaul, 2008), thus masculinizing the female HPG axis. GnRH neurons receive hormonal and environmental signals from estrogen-responsive kisspeptin neurons from the anterior ventral periventricular (AVPV) and arcuate (ARC) nuclei in the hypothalamus (Polston and Simerly, 2006). These neurons coordinate pubertal onset and steroid feedback on GnRH neurons in many species, including humans (Kauffman *et al.*, 2007). AVPV kisspeptin neurons are more numerous in females than in males and are thought to be essential for steroid positive feedback and the initiation of the preovulatory GnRH surge (Clarkson *et al.*, 2008). Neonatal exposure to genistein decreases the density of neuronal fibers immunolabeled for kisspeptin in the AVPV of female rats (Bateman and Patisaul, 2008; Patisaul *et al.*, 2009) indicating that disrupted organization of the kisspeptin

signaling pathways may be a mechanism by which reproductive abnormalities are induced including disrupted timing of pubertal onset, irregular estrous cycles and premature anovulation. In the same way, exposure to coumestrol during lactation causes reduction in ovarian weight and increased apoptosis in ovarian granulose cell in young adult females (Moon *et al*., 2009).

All of these effects are supposed to be mediated by ERs which are present in the reproductive female tract. In female rodents, ERα is present in both the epithelium and stroma of the uterus and vagina; ERβ expresses at a lower level than ERα. ERα is present in the female tract from day 15 of gestation, and continues increasing throughout gestational development and reaches a maximum at day 21. In contrast, the expression of ERβ is much lower than ERα. ERβ expresses in the ovary, pituitary, nervous system, and mammary gland (Okada *et al*., 2005).

In rodents, it has been shown that soy isoflavones increase uterine weight, and the thickness of endometrial and vaginal epithelium (Gallo *et al*., 1999; Santos *et al*., 2010). In adult ovariectomized female rats, genistein (50 mg/Kg) administered during three months increases uterine weight, endometrial proliferation, vaginal epithelium growth, and has slight effects on mammary glands, in a similar way to E_2. These findings corroborate the estrogenic effects of this isoflavone (Rimoldi *et al*., 2007).

Many of the reproductive disruption caused by phytoestrogens are related to the hypothalamo-pituitary-ovary (HPO) axis. Phytoestrogens (flavones, isoflavones and coumestanes) mimic E_2 negative feedback to inhibit the activity of the hypothalamic GnRH pulse generator and thereby serum LH levels in rats (Christoffel *et al*., 2006).These effects are mediated through ERβ (Bowe *et al*., 2003). Phytoestrogens can also inhibit gonadotropin (LH and FSH) release acting through ERα (Jacob *et al*., 2001; Trisomboon *et al*., 2007). In the ovary, phytoestrogen genistein inhibits oocyte nest breakdown and attenuates cell death during mouse oocyte development (Jefferson *et al*., 2006). At the same time, genistein is capable of decreasing P_4 and E_2 concentrations in plasma (Tiemann *et al*., 2007)

Males

In male animal models, phytoestrogens are capable of causing disruptive effects on reproductive parameters. Experimental evidence indicates that long term administration of daidzein to male rats causes a decrease in plasma levels of testosterone as well as erectile dysfunction in a similar way to E_2; and these effects are possibly mediated through ER (Srilatha and Adaikan, 2004). Similar reduced testosterone levels

have been obtained in adult male rats exposed to a high-dose of coumestrol (Tarragó-Castellanos *et al*., 2006). This decrease in serum testosterone is due to decreased steroidogenesis in testicular Leydig cells caused by isoflavones (Akingbemi *et al*, 2007). Long term dietary administration of genistein in rats, suppresses the steroidogenic response of Leydig cells to hCG and cAMP by down regulating the expression of the cytochrome P45017α-hydroxylase/C17-20 lyase enzyme (P450scc), which initiates steroidogenesis in these cells (Svechnikov *et al*., 2010) reducing serum levels of testosterone and androstenedione (Weber *et al*., 2001).

A high phytoestrogen diet in male rats can also block spermatogenesis, induce germ cell apoptosis (Assinder *et al*., 2007), and decrease the expression of ERα and AR in the cauda epididymis as well as increase lipoperoxidation in epididymal sperm. These effects are possibly mediated by disruption of the steroid regulation of the epididymis, resulting in decreased quality of sperm, and thereby reducing fecundity (Glover and Assinder, 2006). On the other hand, chronic dietary exposure to genistein in combination with vinclozoline (a fungicide considered an endocrine disruptor) reduces sperm count and motility, litter size and increased post-implantation loss (Eustache *et al*., 2009). *In vitro* studies show that mouse and human spermatozoa exposed to genistein and daidzein in different doses, alone or in combination, accelerate capacitation (Adeoya-Osiguwa *et al*., 2003) and acrosome loss, which may possibly impair fertility (Fraser *et al*., 2006).

ERα and ERβ are also distributed in the rodent male reproductive tract and glands. ERα is present in ductuli efferent, caput epididymis, coagulating glands, urethral glands, and prostate. ERβs are located in the epithelial cells of the prostate, the epithelial cells of the corpus and cauda epididymis, ductus deferens, and in the stroma cells of the urethral glands. These findings indicate that species and tissue differences should be taken into careful consideration in assessing the physiological and pharmacological effects of sex steroids (particularly estrogens and phytoestrogens) on the reproductive tissues of male rodents (Yamashita, 2004).

Similarly to females, exposure to phytoestrogens in early age disrupts reproductive function in adulthood. Neonatal exposure to genistein in male mice reduces the expression of ERα and androgen receptor (AR) in testes in adulthood, without affecting sperm count and motility (Shibayama *et al*., 2001). High dose of daidzein (100 mg/Kg) administered to juvenile rats show impaired penile erection in adulthood (Pan *et al*., 2008). Perinatal exposure to phytoestrogen diets can also decrease steroidogenesis and androgen secretion

by testicular Leydig cells in the adult rat (Akingbemi *et al.*, 2007).

The mechanisms by which genistein and the other phytoestrogens may influence reproductive physiology may involve: a) genomic effects on ERα, ERβ and other nuclear receptors (P_4, androgen), b) inhibition of enzymes involved in steroidogenesis, such as 3β- and 17β-hydroxysteroid dehydrogenase, aromatase; c) stimulation of sex hormone-binding globulin; d) inhibition of protein tyrosine kinase, important for signal transduction; e) inhibition of DNA topoisomerases I and II, enzymes essential for DNA replication; and f) antioxidant activity (Okura *et al.*, 1988; Dusza *et al.*, 2006) and may also work through epigenetic mechanisms involving both hyper and hypo-methylation (Tang *et al.*, 2008; Zhao and Mu, 2011).

CONCLUSION

Despite the growing literature regarding the effects of phytoestrogens in humans, livestock and animal models, it remains unclear if these compounds are deleterious or beneficial for animal species. In human beings, data are limited to characterizing the biological effects of soy products and of the isoflavones genistein and daidzein, while only a few data exist on lignans. However, there are many more phytoestrogens, such as coumestanes, stilbenes, and flavones, identified in nature; thus the spectrum of phytoestrogens is expanding and their biological role awaits evaluation.

The experimental methodology followed is of high importance in defining the estrogenic effect of phytochemicals. *In vitro* studies provided the first data on estrogenic action, whereas animal models are useful to evaluate the true estrogenic potential of phytoestrogens. However, phytoestrogen biological activity depends on multiple factors, such as the chemical form of the phytoestrogen, the route of administration, the metabolism, the intrinsic estrogenic status, the age, and the time and the level of exposure. Therefore, the consequences of phytoestrogen intake in humans may not necessarily be beneficial and in some cases may actually increase disease risk. Well-designed, prospective studies are necessary to assess the effects of phytoestrogens in reproductive physiology. Further investigations into the pharmacokinetics and age at consumption of isoflavones are needed in order to more accurately evaluate the effects and risks of phytoestrogen consumption in both humans and livestock.

Finally, the experimental evidence obtained from research in animal models should be considered to evaluate the disrupting effects phytoestrogens in the reproductive physiology of livestock. It should be considered the possibility that some of the reproductive problems observed in those animals could be caused by the consumption of phytoestrogens and micoestrogens in fodder.

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