Phytoestrogens as Endocrine-Disrupting Agents in Aquaculture

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1 Introduction

Many estrogen-mimicking chemicals are introduced as endocrine disruptors in the aquatic environment, which may be synthetic or natural; the synthetic ones are called xenoestrogens. The natural estrogen-mimicking compounds could be of plant or animal origin. Phytoestrogens, which resemble mammalian estrogens, particularly 17-estradiol, structurally or functionally, are sizable heterogenic group of chemical substances derived from plants. Legumes are the main source of phytoestrogens as they are involved in plant-microbe interactions and the reason to induce nodulation in the roots of legumes. Phytoestrogens are also involved in the defense mechanisms in the plant kingdom but do not involve in the endocrine system of plants. The majority of phytoestrogens are not found in seed oil, and the concentration of whole or ground seeds in a given area can vary depending on the climate, crop maturity, storage time, and region. Phytoestrogenic compounds are being introduced in the aquatic environment majorly through the feed. Some of the common plant-based ingredients used in aquaculture feed contain phytoestrogens including soybean meal and alfalfa meal.

The low molecular weight and stable structure of phytoestrogens enable them to easily pass through the lipid bilayer and interact with the enzyme and receptors of the cell and induce proestrogenic or antiestrogenic effects. Several reports suggest that phytoestrogens have beneficial effects in humans, including hepatoprotective, anti-allergic, anti-inflammatory, antitumor, antithrombotic, and antioxidant properties

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Table 1 Sources of phytoestrogens

Phytoestrogens	Source
Genistein	Soybean, broad bean, white bean, chickpeas, red bean
Daidzein	Soybean, black bean, and green split peas
Coumestrol	Alfalfa, clover, split peas, chickpeas, lima beans, soy sprouts, and pinto beans
Lignan (enterolactone and enterodiol)	Flaxseed, chickpeas, unhulled soybean, whole legumes, and cereal brans
Formononetin	Green bean, lima bean, broad bean, pink bean, mung bean, clover sprout, and alfalfa
Glycitin	Soybean, chick peas, and other legumes
Biochanin A	Clover sprout, chick peas, Chinese peas, pinto beans, and kidney bean
Resveratrol (matairesinol, secoisolariciresinol, lariciresinol, etc.)	Grape cane waste, peanut roots

(Ahmad et al. 2013; Roca et al. 2014). Phytoestrogens or their active metabolites typically have an estrogenic effect on the central nervous system and reproductive systems of both males and females in mammals and are studied extensively in mammals. This chapter concentrates mostly on the common phytoestrogens found in aquaculture feed ingredients and their effects on reproductive endocrine function.

2 Structure and Classification of Phytoestrogens

A phenolic ring and two hydroxyl groups, which are essential for binding with estrogen receptors (ER), are characteristics of phytoestrogens. The phenolic group determines whether phytoestrogens have agonist or antagonistic properties with animal estrogen (Roca et al. 2014; Torrens-Mas and Roca 2020). Based on their structural characteristics, phytoestrogens are divided into three main classes: flavonoids, lignans, and stilbenes (Cos et al. 2003; Nikolić et al. 2017). The classification of phytoestrogens is given in Fig. 2, and various sources of phytoestrogens are given in Table 1.

2.1 Flavonoids

Flavonoids have two benzene rings (A and B) in their structure, joined together by a heterocyclic (C) ring (Wang et al. 2018) as depicted in Fig. 1. Based on the position of B and C rings, oxidation and hydroxylation of the C ring, and the degree of saturation, they are typically divided into isoflavonoids, flavones, flavonols, flavan-3-ols (or catechins), flavanones, chalcones, and anthocyanins (Fig. 2).

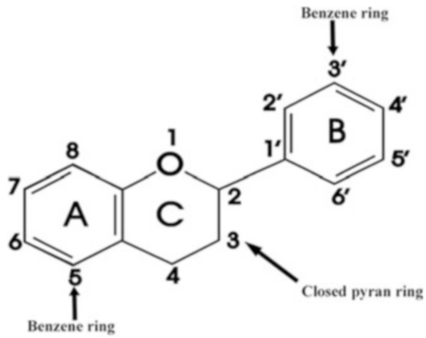

Fig. 1 General structure of flavonoids

Isoflavonoids comprise isoflavones and coumetrans. Isoflavones have a general backbone made of 3-phenylchromen-4-one. Genistein, daidzein (from soybean meal), formononetin, and biochanin A (from red clover) are included in the isoflavone category as they share the general backbone (Fig. 1). Coumestans have a 1-benzoxolo(3,2-c) chromen-6-one linked to the B ring at the C3 position instead of the C2 position.

2.1.1 Isoflavones

Isoflavones can be found in large quantities in soybean and its byproducts. Majorly three types of isoflavones have been found from soybean meal which exists in four chemical forms, namely:

1. Aglycones: genistein (4′,5,7-trihydroxyisoflavone), daidzein (4′,7-dihydroxyisoflavone), and glycitein (4′,7-dihydroxy-6-methoxy isoflavone).
2. Glucosides: genistein, daidzein, and glycitein.
3. Acetylglucosides: 6′-O-acetylgenistin, 6′-O-acetyldaidzin, and 6′-O-acetylglycitin.
4. Malonylglucosides: 6-O-malonylgenistin, 6-O-malonyldaidzin, and 6″-O-malonylglycitin.

According to Chakraborty et al. (2014), most dietary forms of soybean meal have a mixture of three bioavailable forms of aglycones, namely, genistein, daidzein, and glycitein. Glycoside forms are biologically inactive, which can become bioactive absorbable forms at the intestinal lumen, aglycones, when hydrolyzed by bacterial glucosidases (Setchell 1998). Genistein can be further metabolized to *p*-ethyl phenol, while daidzein can be processed to equol and O-demethyangolensin in animals (Soukup et al. 2016); however, the efficiency of metabolism varies in different organisms. Isoflavone content is known to vary based on the crop and products (Carrera et al. 2011; Medic et al. 2014). The total isoflavone concentrations in soybean meal were estimated to be 15.70–30.75 mg/g dry matter (Flachowsky et al. 2011).

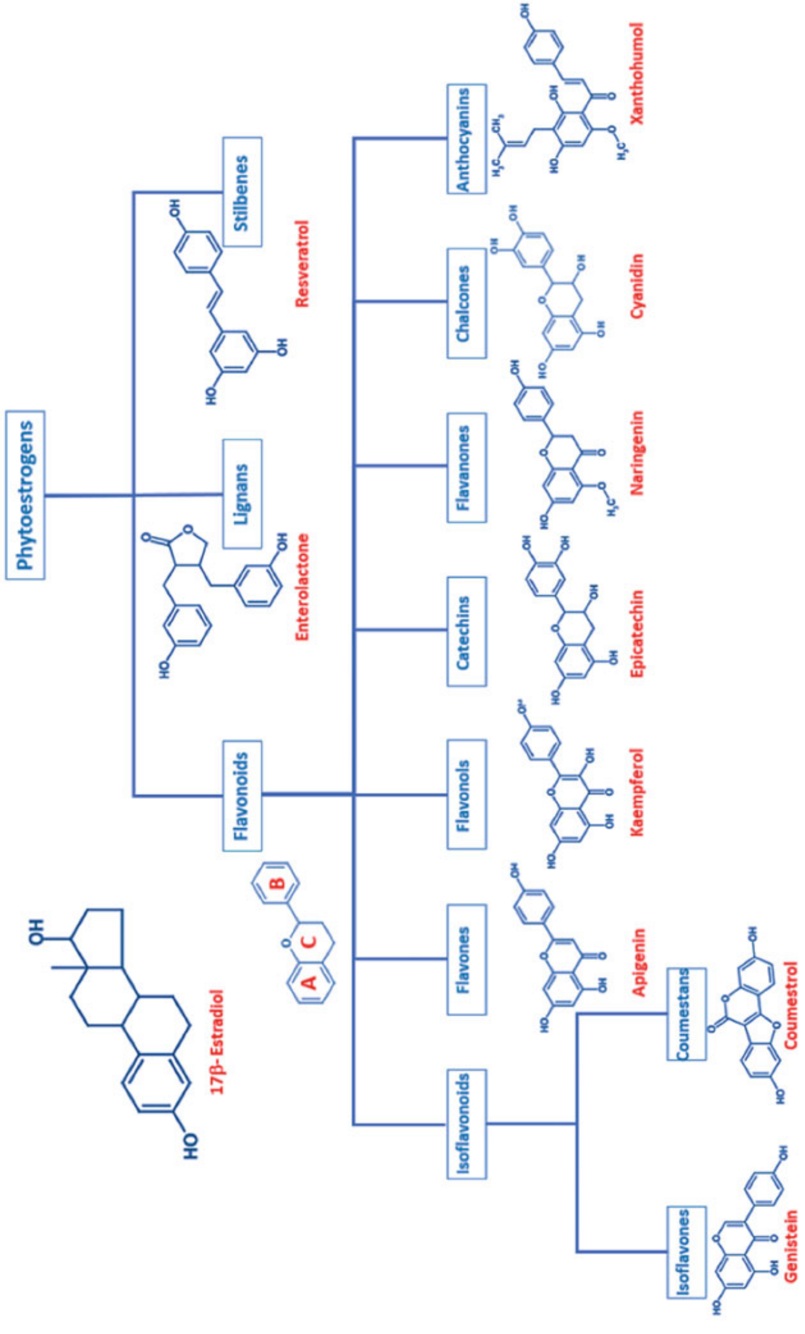

Fig. 2 Classification and structure of phytoestrogens (Torrens-Mas and Roca 2020)

Genistein

Among the total isoflavone content in soybean meal, genistein is recorded as the predominant one. According to reports, genistein content in soybean meal varies from 2.6 to 22.4 mg/100 g of soybean meal (Chen and Wei 2008) based on the crop, season, and type of processing. Since soybean meal is the widely utilized leguminous plant source of protein in feed, it is relevant to describe the genistein content reported in fish feed worldwide. The carp commercial diets were reported to have 6.76–23.71 mg, the trout diets were estimated to be 4.16–5.02 mg, and the diet of medaka was reported to have 0.93–5.85 mg of genistein/100 g feed (Xiao et al. 2018). Feeds manufactured in various countries, namely, Japan, the UK, and Korea, were determined to contain genistein 3.97–11.89 mg/100 g (Inudo et al. 2004; Xiao et al. 2018).

Daidzein

7,4′-dihydroxy isoflavone (daidzein) is found widely in plants belonging to legumes. Recent studies on humans have demonstrated the efficacy of daidzein in treating menopause, osteoporosis, and high blood cholesterol and reducing the risk of some hormone-related cancers and heart disease. Despite the known health advantages, it is also known for its potential to affect fertility and cause developmental toxicity to the reproductive tract in female rats. The effect of daidzein on fish reproductive endocrine system is still at the preliminary face.

Glycitein

In soy food products, glycitein, an O-methylated isoflavone, makes up 5–10% of all isoflavones. Glycitein comes under phytoestrogens similar to other soy isoflavones in that it has a weak estrogenic activity compared to other isoflavones.

Biochanin A and Formononetin

Biochanin A (5,7-dihydroxy-4-methoxy-isoflavone) is derived from edible and herbal plants such as peanuts, alfalfa sprouts, soy, and red clover. Formononetin, otherwise known as biochanin B, is a 7-hydroxyisoflavone substituted by a methoxy group at position 4′. The plant metabolite acts as an estrogenic or antiestrogenic compound in animals. It is a member of 7-hydroxyisoflavones and a member of 4′-methoxyisoflavones. It is functionally related to daidzein.

2.1.2 Coumestrol

Coumestrol was first discovered as an estrogenic compound in 1957 by E. M. Backoff in ladino clover and alfalfa. The compound is known to have high estrogenic activity and high binding affinity with estrogen receptors (binds with 94% affinity to ERα and 185% affinity to ERβ, when related to the binding affinity of estradiol to both). The compound, however, is having much less activity than estradiol while 30–100 times more activity than isoflavones. The compound is normally found in a limited range of food, and alfalfa sprouts are one among them.

2.2 Lignans

A large class of low molecular weight polyphenols called lignans is present in many types of plants, especially grain, nuts, coffee, tea, cocoa, flaxseed, and fruits. Lignans have two phenylpropane groups linked by a -β′-β′ bond formed between the central atoms of their side chains (eighth or position). Lignans function as anti-nutrients in the defense of seeds and plants against herbivores and are precursors to phytoestrogens. Lignans and lignin have different molecular weights; lignin is a high polymer that is indigestible, whereas lignans are small and soluble in water. High levels of lignans can be found in sesame and flax seeds. Cereals (rye, wheat, oat, and barley), soybeans, tofu, cruciferous vegetables (like broccoli and cabbage), and some fruits, particularly apricots and strawberries, are other foods that contain lignans. Lignan and its estrogenic effects are well-studied in humans and rats (Tou et al. 1998), especially in delaying puberty, lengthening diestrus, affecting the reproductive development of offspring, and so on. However, research on the effect of lignan in fish is null, so far (according to the Web of Science).

2.3 Stilbenes

The stilbenes (Fig. 2) have a 1,2-diphenylethylene nucleus and a polyphenolic structure. The stilbene resveratrol, which can be found in a variety of foods like nuts, grapes, berries, and wine, has received the most research. The most well-known stilbene, resveratrol, is used to prevent disease, particularly obesity. The compounds are well known for their antiaging activity in humans, and they act as an antioxidant and inhibit cyclooxygenases in zebrafish (Kowalska et al. 2011). Other stilbenes and their metabolites are also thought to be potential anti-obesity candidates. The main stilbene in grape plants is called piceid (5,4′-dihydroxystilbene-3-O-β-glucoside), and its concentration in red grape juice is almost six times higher than that of resveratrol monomers. Red wine also contains astringin (5,4′,3-trihydroxystilbene-3-O-β-glucoside) and isorhapontin (5,4′-dihydroxy-3′-methoxystilbene-3-β-Dglucoside), though they are rarely found in higher concentrations than trans-piceid or resveratrol monomeric forms. The studies on endocrine disruptive effects of stilbenes in fishes are limited to a few, which are conducted on medaka and zebrafish (Cavalcante et al. 2017; Rohmah et al. 2022).

3 Phytoestrogen on Reproduction Mechanism of Endocrine Disruption

A concern of successful reproduction and development of seeds for enhancing aquaculture production has been discussed in the recent past. The endocrine-disrupting compounds have the potential ability to perturb the sensitive hormone pathways that take part in reproduction. Phytoestrogen acts on the endocrine axis by (1) disrupting the enzymes involved in various hormone synthesis, action, and metabolism, (2) mimicking the endogenous estrogen due to the structural similarity of phytoestrogen with estradiol and competing for the receptor binding sites, and (3) inhibiting cell signaling activities at ER-deficient cells and consecutively inducing apoptosis (Sassi-Messai et al. 2009). These actions consequently result in hormone imbalance, reduced gonad size, decreased fertility and fecundity, lowered egg production, impaired sexual differentiation, and apoptosis of fish embryos (Tables 2, 3 and 4). However, it is known that the impact of these chemicals on fish reproductive function varies depending on several factors, including species, sex, age, type, and dose of phytoestrogens. The detailed account on mechanism of phytoestrogen on the reproductive endocrine system is given in the following subsections.

3.1 Effect on Gonadotropins and Thyroid Homeostasis

Gonadotropins are secreted from the adenohypophysis by the action of gonadotropin-releasing hormone (GnRH) from the hypothalamus to secret reproductive steroids from gonads. It is believed that phytoestrogens either by directly contributing or by disrupting the estrogen-degrading enzymes increase the circulating level of total estrogen, which in turn reduces the FSH secretion from the pituitary gland. Pelissero et al. (2001) noticed a slight decrease in βFSH levels in plasma at the end of spermatogenesis in male and ovulating female fishes fed with a diet containing 500-ppm genistein. Similarly, feeding Turkey berry leaf extract (which contains solasodine, a phytoestrogen) to *C. carpio* disrupted the balance of the gonadotropin hormone (Rahmadiah et al. 2019).

The enzyme 5′-iodothyronine deiodinase is responsible for the bioconversion of thyroxine (T4) in animals, resulting in most circulating triiodothyronine (T3). T3 is comparatively high in biological activity and induces vitellogenesis in female fishes

Table 2 Effect of phytoestrogens on female fish

Compound and dose	Effect	Species	Reference
100% mixed plant protein containing soybean meal	Reduced proportion of vitellogenic and mature oocytes	*Oreochromis niloticus*	Fontaınhas-Fernandes et al. (2000)
500 ppm of genistein	Increase in plasma VTG, decreased testosterone levels, βFSH, and βLH level	*Oncorhynchus mykiss*	Pelissero et al. (2001)
Genistein at 750 and 30,000 ng/fish	Increased testosterone and estradiol production by the ovaries	*Oryzias latipes*	Zhang et al. (2002)
1000 times higher phytoestrogen than E2 concentration	Binds with estrogen receptors and impairs its function	*Acipenser baeri*	Latonnelle et al. (2002)
1000 μg/L of genistein	Atretic oocytes, enlarged ovarian lumen, somatic stromal tissue proliferation, delayed oocyte maturation, and the presence of primordial germ cells in the ovary	*O. latipes*	Kiparissis et al. (2003)
Genistein at 415,800 ng/g	Increase in plasma Vtg	*Ictalurus punctatus*	Kelly and Green (2006)
Soybean meal at 160 and 340 g/kg of feed	Reduction in the average number of spawned eggs	*Carassius auratus*	Bagheri et al. (2013)
Genistein at 75.83 mg/g with daidzein at 67.82 mg/g	Increase in plasma E2	*C. auratus*	Bagheri et al. (2014)
Genistein 1.6 g per kg of diet	No significant changes in the DHP level	*Huso huso*	Jourdehi et al. (2014)
10 mg/L of genistein and daidzein	Decreased content of ovarian ERβ levels	*Cyprinus carpio*	Sarasquete et al. (2017)
17.5 and 35% of soybean meal	Reduced proportion of vitellogenic and mature oocytes	*C. carpio*	Banani (2019)
Genistein at 1,3, 6, 9 mg/100 g of feed	Increased serum estradiol and testosterone, reduced vitellogenesis and expression level of ovarian aromatase and hepatic estrogen receptors, and reduced vitellogenic oocytes in the ovary	*C. carpio*	Nuzaiba et al. (2020)
Genistein at 10 mg/L in water	Decreased levels of ERβ in the ovary	*Danio rerio*	Sarasquete et al. (2020)
Cacao bean meal at 10 g/kg diet	Increased oocyte granulation and follicle numbers	*C. auratus*	Al-Khalaifah et al. (2020)

(Nelson and Habibi 2016). Genistein disrupted thyroid homeostasis by disrupting the enzyme 5′-iodothyronine deiodinase (Schiller et al. 2013). Genistein also competes with thyroxine for binding to thyroxine-binding globulin (Schiller et al. 2013).

Table 3 Effect of phytoestrogens on male fish

Compounds and dose	Effects	Species	Reference
500 ppm of genistein	Increased vitellogenin synthesis, decrease in testosterone levels, plasma βFSH and βLH and 17a,20b(OH)2	*O. mykiss*	Pelissero et al. (2001)
1000 ppm of genistein	Decreased sperm motility and spermatocrit	*O. mykiss*	Pelissero et al. (2001)
Genistein at 750 and 30,000 ng/fish	E2 level was increased Testosterone level from the testis was decreased	*O. latipes*	Zhang et al. (2002)
1000 μg/L of genistein	Low densities of spermatozoa; 72% of male medaka showed feminized secondary sex characteristics	*O. latipes*	Kiparissis et al. (2003)
Genistein at 58.5 ± 0.6 μg/g with daidzein at 37.3 ± 0.2 μg/g	VTG production was induced, but there was no negative impact on the success of reproduction	*O. latipes*	Inudo et al. (2004)
1–10 mg/L genistein	Induced VTG gene expression and circulating vitellogenin	*O. latipes*	Scholz et al. (2004)
Genistein at 50–100 μg/mL	Increased circulating vitellogenin and a decrease in 11-KT level	*C. auratus*	Ishibashi et al. (2004)
Genistein at 415,800 ng/g	Increased in plasma Vtg	*I. punctatus*	Kelly and Green (2006)
Soybean meal at 46.4 g/100 g feed	Reduction in plasma Vg	*O. mossambicus*	Davis et al. (2009)
Soybean meal at 500 mg/kg	Lowered gonadosomatic index (GSI)	*C. carpio*	Turker and Bozcaarmutlu (2009)
Genistein, 75.833 μg/g with daidzein, 67.821 μg/g	Decrease in plasma testosterone level, while 17-estradiol levels were increased	*C. auratus*	Bagheri et al. (2013)
Soybean meal at 160 and 340 g/kg of feed	Reduction in sperm quality	*C. auratus*	Bagheri et al. (2013)
Genistein at 3 mg/L	Disrupt thyroid homeostasis	*C. auratus*	Nelson and Habibi (2016)
Daidzein at 10 mg/L in water	Increase in expression of the bromodomain testis-specific gene (BRDT) in gonad	*D. rerio*	Sarasquete et al. (2020)
10% inclusion of cacao bean meal	Increased testosterone levels	*C. auratus*	Al-Khalaifah et al. (2020)
1-mg genistein in 100-g feed	Increased serum estradiol and vitellogenin and expression of vtgb2 in the liver	*C. carpio*	Nuzaiba et al. (2022)

Table 4 Effect of phytoestrogens on early life stages

Compound and dose	Effect	Species	Reference
4 mg/g of genistein	Decreased circulating vitellogenin	Striped bass, *Morone saxatilis* juvenile	Pollack et al. (2003)
2 and 8 mg/g of genistein	Increase in circulating vitellogenin	Striped bass, *Morone saxatilis* juvenile	Pollack et al. (2003)
2 mg/kg of phytoestrogens	Increased proportion of females by 55%	*Anguilla anguilla* juvenile	Tzchori et al. (2004)
50% dilution of effluent water from pulp mill	Elevated concentrations of vitellogenin, male-biased sex ratios, and occurrence of inter-sex gonad	*Danio rerio* juvenile	Örn et al. (2006)
Genistein (1×10^{-4} M, 0.5×10^{-4} M, 0.25×10^{-4} M) for 60 h	Teratogenic effects in the embryo including dead cells in the brain, spinal kyphosis, yolk sac edema, and pericardial edema	*D. rerio* at 24 h postfertilization	Kim et al. (2009)
10 μM of genistein	Apoptosis in the embryo	*D. rerio* from 5 days post-hatch	Sassi-Messai et al. (2009)
Genistein at 2, 4, and 8 mg/g	Increased proportions of phenotypically male individuals	*Ictalurus punctatus* at 60 and 150 days post-hatch	Green and Kelly (2009)
17% of soybean meal with 0.05 g/100 g of genistein	Increased proportion of males	*Clarias gariepinus* fry	Ahmed et al. (2015)
3 and 10 mg/L genistein	Reduce thyroxine peroxidase and transthyretin (transfer protein)	*Senegalese sole* early life stages	Sarasquete et al. (2017)
Genistein at 500 ng/L	Increased 17β-estradiol and aromatase activity	*Rutilus kutum* Post-fertilized embryo	Mohammadrezaei and Nematollahi (2018)
Genistein and daidzein (from 1.25 mg/L to 20 mg/L)	Upregulation of estrogen receptor, *cyp1a* transcript levels, and death receptors (*fas*)	*D. rerio* at 2–3 h post-fertilization	Sarasquete et al. (2020)
Genistein @ 4.41 mg/L or daidzein 65.15 mg/L	The lethal concentration of genistein and daidzein	*D. rerio* at 2–3 h post-fertilization	Sarasquete et al. (2020)
Turkey berry (*Solanum torvum*) leaf extract at 300 mg/L	Increased the percentage of female common carp larvae	*Cyprinus carpio* post-hatching larvae	Rahmadiah et al. (2019)

3.2 Effect on Steroidogenesis

Sex steroid synthesis occurs in the gonads, the interrenal gland, and the brain on the action of gonadotropins. The enzymes including aromatases, dehydrogenases, and side chain cleavage enzymes involved in the biosynthesis of various steroids (including 17β-estradiol, progesterone, dehydroepiandrosterone, testosterone and 11-Keto testosterone) from the common precursor, cholesterol. Most of the steroids impart in gonad growth, maturation, ovulation, permeation, spawning, and fertilization. Phytoestrogens, especially isoflavones, are reported to block the transfer of cholesterol (Stevenson et al. 2011) and disrupt certain enzymes of the steroid synthesis pathway, including aromatases (cyp19a and cyp19b), 3-hydroxysteroid dehydrogenase (3βHSD), and 20β-hydroxysteroid dehydrogenase (20βHSD) enzymes and enzymes involved in estrogen-inactivating pathways. This disruption action of phytoestrogen induces an imbalance in steroid hormone levels in fish.

The gonad and bran aromatase enzymes are involved in converting androgens to estrogens, hence testosterone to estradiol during steroidogenesis. Disruption of aromatase enzymes causes an increase in total testosterone and a reduction in total estrogen levels, consequently disturbing the steroidogenesis pathway. An increase in testosterone was observed in *H. huso* (Jourdehi et al. 2014). According to Weber et al. (2002), genistein induced the level of endogenous testosterone as an indication of aromatase inhibition in rainbow trout. Serum level of testosterone was significantly increased in males *C. auratus* fed on the 10% inclusion of cacao bean meal (Al-Khalaifah et al. 2020). The enzyme 3βHSD catalyzes the biosynthesis of the steroids progesterone from pregnenolone, 17-hydroxyprogesterone from 17-hydroxypregnenolone, and androstenedione from dehydroepiandrosterone (DHEA) in the steroid-producing glands. Zhang et al. (2019) reviewed that flavones and isoflavone are interfere with testicular isoforms of 3βHSD and possibly with androgen synthesis. Synthesis of active progesterone in fish, 17α,20β-dihydroxy-4-pregnen-3-one (DHP), is catalyzed by the enzyme 20βHSD from 17-α-hydroxyprogesterone. DHP plays an important role in the final maturation of prophase I-arrested oocytes (Senthilkumaran 2011), testicular recrudescence (Sreenivasulu et al. 2012), and spermiation (Miura et al. 1992). A dose-dependent reduction in the expression of 20βHSD is observed in male *C. carpio* when genistein (1–9-mg/100-g feed) included in the diet (Nuzaiba et al. 2022). Supplementation of dietary genistein at 500 ppm reduced the DHP level in both males and females of *O. mykiss* (Pelissero et al. 2001).

Steroid catabolism occurs in two phases mainly in the liver with some contributions from the gills, intestine, and kidney. In phase I, catabolism occurs through monoxygenation, hydroxylation, and esterification, while phase II catabolic pathway majorly occurs through glucuronidation and sulfonation (James 2011). Enzymes involved in estrogen-inactivating pathways (phase II steroid estrogen-metabolizing enzymes) are glucuronosyltransferase (UGT) and sulfotransferase (SULT). The UGT catabolism of 17β-estradiol is reported in *C. carpio* and in red mullet (Solé et al. 2003; Daidoji et al. 2006; Martin-Skilton et al. 2006)), and the expression is higher in male and juvenile fishes. Similarly, 17β-estradiol catabolism by SULT has been found in zebrafish, Siberian sturgeon, channel catfish, red mullet, and four-spotted megrim (Perdu-Durand and Cravedi 1989; Martin-Skilton et al. 2006; Wang and James 2007; Yasuda et al. 2008). Expression of these enzymes is susceptible to xenobiotic exposure (James 2011), and genistein exposure to the salmonid fish disrupted estrogen-catabolizing enzymes (Ng et al. 2006). Degradation of estrogen-inactivating enzymes by phytoestrogens may significantly increase the total estrogen level in male and juvenile fish (Nezafatian et al. 2017; Mohammadrezaei and Nematollahi 2018).

3.3 Effect on Vitellogenesis in Fish

Estrogen secreted by the gonads is transported through the blood to the liver to synthesize vitellogenin in female fishes. ERβ1 and ERβ2 are two cytoplasmic estrogen receptors reported to exist in teleosts. Hepatic vitellogenesis is based on the type of vitellogenin, and they are more sensitive to the induction by E2. Vitellogenin is normally synthesized from the liver of sexually mature females and is released to the bloodstream and stored in the developing oocytes through receptor-mediated endocytosis (Wahli et al. 1981). Still, in the male, the vitellogenin-producing gene remains silent until they are exposed to any type of xenoestrogens. Hence, the measurement of plasma vitellogenin levels, mainly in males and immature females, is used as a biomarker of exposure to estrogenic compounds in aquatic environments (Cheek et al. 2001; Matozzo et al. 2008). Vitellogenin also aids in hemagglutinating and bacteriostatic functions in male fish if present (Reading et al. 2011).

Phytoestrogens may either have agonistic effects with endogenous estrogen (estrogenic or proestrogenic effect) or act antagonistically to E2 (antiestrogenic effect) (Pelissero et al. 2001; Green and Kelly 2009). The effect is reported to depend on the ratio of phytoestrogens to endogenous estrogens, aromatase activity, species, and reproductive status, length of exposure, and method of administration in fish (Tsai et al. 2000; Trant et al. 2001). Differential effects in both male and female fish conducted in recent studies indicated that the action of phytoestrogens, particularly genistein, depended on the endogenous estradiol level. The agonistic effect of phytoestrogen with animal estrogen is observed when the endogenous level of estradiol is low. Phytoestrogens disrupt the estrogen-metabolizing enzymes, and as a result, the total circulating estradiol level is increased, significant enough to induce the following cascade, including vitellogenesis in a low estradiol environment. The estrogenic potency of these compounds in fish has been documented during the last decades. Various types and levels of phytoestrogens are known to stimulate the vitellogenin concentration in fishes, viz., injection of genistein at 50–100 μg/mL increased the circulating vitellogenin in male *C. auratus* (Ishibashi et al. 2004; Nezafatian et al. 2017; Nezafatian and Zadmajid 2018), 1–10 mg/L of genistein-exposed juvenile and adult males and primary cultures of male liver cells of

O. latipes (Scholz et al. 2004), yearlings of *A. baeri*-fed soybean meal included diet (Pelissero et al. 1991), 1 mg genistein in 100-g feed in adult male *C. carpio* (Nuzaiba et al. 2022), and 2 and 8 mg/g of genistein in juvenile-striped bass, *Morone saxatilis* (Pollack et al. 2003).

When the endogenous estradiol level is high, as, in the case of female fish, antagonistic effects of phytoestrogens are observed, the increase in the estradiol level due to the disruption of estrogen-metabolizing enzymes becomes insignificant. Phytoestrogens in the circulation compete with endogenous estradiol for binding with its membrane and nuclear receptors (ERs), which in turn reduce the estradiol action (antagonistic activity of phytoestrogen). Low level of circulating vitellogenin on administering dietary phytoestrogens was observed in previtellogenic to vitellogenic phase in female *O. mykiss* (Bennetau-Pelissero et al. 2001; Pastore et al. 2018), female *C. carpio* (Turker and Bozcaarmutlu 2009; Nuzaiba et al. 2020), and female *O. latipes* (Zhang et al. 2002). Reduced vitellogenin protein and mRNA concentration in the liver were later described based on the activity and expression of estrogen receptors. Downregulation of *erβ* expression is observed on feeding phytoestrogens (Fritz et al. 2002; Sarasquete et al. 2017).

3.4 Effect on Gonadal Development and Sex Reversal

The effect of environmental estrogenic compounds, including phytoestrogens, is known for impairing gonadal maturation and inducing female characteristics in their male counterparts. The ovary of a 100% plant protein-based diet-fed female *O. niloticus* was observed to contain comparatively a lower number of vitellogenic oocytes (Fontaınhas-Fernandes et al. 2000). A similar result was obtained when soybean meal was fed to *C. carpio* at 17.5 and 35% by Banani (2019). At the same time, dietary cacao bean meal supplementation increased granulation and follicle numbers (Al-Khalaifah et al. 2020). Histological examination of the long-term effect of dietary soybean meal fed in *C. auratus* showed an impact on oocyte maturation progress and spermatogenesis process in female and male fish, respectively (Bagheri et al. 2013), at the inclusion of 35 and 65%. The mean number of eggs spawned by females and sperm quality in males was also reduced on feeding soybean meal at 35 and 65% (Bagheri et al. 2013). In his long-duration study, Bagheri et al. (2014) observed that isoflavone contents affect the GSI of males. Isoflavone extract from soybean-fed *C. carpio* lowered male gonadosomatic index (GSI) but female GSI at low-level extract up to 500 mg/kg supplementation (Turker and Bozcaarmutlu 2009). The fish gonadosomatic index (GSI) did not significantly differ among the three experimental groups; when *O. mykiss was* fed with 500- and 1500-ppm genistein (Pelissero et al. 2001), they also reported delay in spawning in long-term genistein-exposed fishes. Similarly, reproductive success and egg viability of soybean meal included diet in *C. auratus* (Bagheri et al. 2013) and flame angelfish, *Centropyge loriculus* (Callan et al. 2014.

Genistein feeding increased the proportion of females in juvenile eel, *Anguilla anguilla*; Southern flounder, *Paralichthys lethostigma*; and *C. gariepinus* (Tzchori et al. 2004; Ahmed et al. 2015). Kiparissis et al. (2003) observed oocyte development in the testes of adult male *O. latipes*. A greater number of male and intersex populations were observed by Örn et al. (2006) when *D. rerio* was fed with pulp mill effluent rich in xenoestrogens. Sayed et al. (2012) observed an abundance of 77% female population when *C. gariepinus* was fed with 4-nonylphenol.

4 Beneficial Effects of Phytoestrogens

Apart from the endocrine-disrupting activity, phytoestrogens are also reported to induce some beneficial effects in fish. Its effect on the endocrine system can be employed for modulating the endocrine system in super intensive aquaculture system. Phytoestrogens like genistein have induced aromatase inhibition and consecutively produced a monosex population of tilapia. The estrogenic effects of these compounds can be explored to produce a single-sex population in species that shows a higher growth rate in females than male counterparts, as observed in salmon and trout. The antioxidant property of these phytochemicals is focused on in recent research. The dietary genistein (100–500 mg/kg) was found to be instrumental in improving growth, antioxidant capacity, and lipid metabolism in common carp (Yang et al. 2022).

5 Conclusion

Phytoestrogens have been known to exert a major effect on the fish endocrine system and reproductive outcomes. Though the impact would not be visible in aquaculture grow-out systems, loss of awareness on the effect of individual compounds and transgenerational effects are yet to be evaluated. Studies indicated that the accumulation of phytoestrogens in the muscle of fish is insignificant to induce any effect in fish consumers. Further oil extraction does not remove the phytoestrogen contents from the feed ingredients, as these compounds are not readily soluble in lipids. Further, studies on their beneficial roles and use as sex reversal agents also need to be explored with dose and duration specificity for the oral route of administration.

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