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Steroid hormones in fish, caution for present and future: A review

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ABSTRACT

The misuse and overuse of steroid hormones in fish is an emerging problem worldwide. The data on hormonal residue in fish was less due to a lack of effective monitoring programs on hormonal use in fish production. This review revealed the findings of previously published data on different hormonal use and their residue and impact. Steroid hormones were frequently used in fish production to promote growth and reproduction. It was suggested that hormones should be used carefully to ensure environmental, biological, and food safety. The most commonly used steroid hormones in fish production were testosterone, estrogen, progesterone, and cortisol. However, the indiscriminate use left residue in the fish flesh above the FAO/WHO permissible limits. This residue in fish caused many health hazards in consumers, like early puberty in children, advances in bone age, negative repercussions on growth, modification of sexual characteristics, and cancer development such as breast, ovarian, and prostate cancer. It also harmed fish and the aquatic environment. The most common detection methods for these hormones were GC-MS, LC-MS, and UHPLC-MS. Many countries permitted the use of hormones in fish production upon monitoring, whereas many countries prohibited it. Moreover, many countries did not have any rules and regulations on the use of hormones in fish production. Thus, this review is a wake-up call for researchers, policymakers and consumers on the impacts of hormonal residues in food commodities.

1. Introduction

The demand for healthy animal-sourced food is increasing worldwide due to population growth and the constant search for a healthy diet. Fish, as animal-sourced food, plays an essential role in our lives. It has a low-fat, high-quality protein and provides all the essential amino acids in a desirable concentration [47]. Fish is the primary source of protein in many developing countries. It provides nutrients and micronutrients essential for cognitive and physical development, especially in children [28]. There is little chance of malnutrition if people eat sufficient fish in their diet [47]. Fish is mainly obtained from natural and cultured sources. But, due to natural and man-made hazards, natural fish populations have declined in the last several decades. Therefore, more attention has been focused on culture fish production by applying new technology [106].

Aquaculture is the fastest fish-producing sector, which applies different fish production techniques like artificial breeding, sex reversal, and transgenesis [71]. Various synthetic steroidal hormones are used for sex reversal when one sex of a species is bigger than the other [31]. Seeds are constantly produced in artificial reproduction, where hormones are widely used [71].

However, using hormonal substances in fish farming may cause many adverse consequences, such as endangering public health and the environment if used indiscriminately. Therefore, consuming contaminated steroid hormone-containing fish can cause many health hazards to consumers, such as early puberty in children, advances in bone age, negative repercussions on growth, modification of sexual characteristics, and cancer development such as breast, ovarian, and prostate cancer [15]. Also, steroid hormones are small molecular pollutants that may pollute water and pose a significant threat to humans and ecosystems, even at low amounts [3].

A scientific study in China reported that steroidal hormonal residue was detected in crucian carp (*Carassius auratus*), carp (*Cyprinus carpio*), and silvery minnow (*Anabarilius alburnops*) in Dianchi Lake, which were

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the common types of fish for consumption [64]. In another study, it was found that two types of bottom layer fish of the food chain (*Boops boops* and *Sphoeroides marmoratus*) were contaminated with hormonal residue which was caught near the city of Las Palmas de Gran Canaria [40].

Thus, it became a major food safety concern related to steroid hormone use in fish production and its consequences after consumption in humans. This issue needs full attention from the scientific society for food safety purposes.

2. Steroid hormones

Hormones are chemical substances that act as messengers in the body. They are required for digestion, metabolism, growth, and reproduction. Hormones are classified as steroids and non-steroid types [29]. Steroid hormones have four cycloalkane rings and are categorized into estrogens, progestogens, androgens, glucocorticoids and mineral corticoids [40].

Different types of hormones exert their activity in various ways. Estrogens, progesterone, and testosterone improved the growth rate and reproductive capacity. Over the last 50 years, the use of estrogens in contraceptives, menopausal therapies, and growth promotion has increased significantly in medical practices. Estrogens are most prevalent in women but may also be found in men [27]. Like estrogen, natural and synthetic progestogens are also available. Progesterone is present in the estrous and menstrual cycles and plays a vital role in sustaining the pregnancy. It is mainly used as a contraceptive agent but can also be used for chemotherapy and menstrual disorders [46]. Androgens are the sex hormone produced by men, and their principal function is to regulate male sexual behavior. Androgens are classified into three types: testosterone, androsterone, and dihydrotestosterone [62].

Steroid hormone detection in any sample requires standard analytical tools. In that case, radio-immunoassays (RIA) and enzyme-linked immunosorbent assays (ELISA) are commonly used to detect hormonal residues [99]. Mass spectrometry and liquid chromatography were widely used for complex matrices analysis [39].

2.1. Regulation of steroid hormone use

In many countries, regional and national laws regulate the use of hormones in animal and fish production. The usage of natural steroids like testosterone, progesterone, and 17β-estradiol has been permitted in Canada, Australia, New Zealand, Argentina, and the USA but prohibited in European Union Countries [7,11]. In Bangladesh, the use of antibiotics, growth hormones, steroids, or other harmful chemicals in animal feed for growth promotion has been banned according to the Food Act 2010 [89]. Still, there is no regulation for fish feed yet. It is essential to remember that neither any nation's legal system nor any national (European Commission) or multinational (JECFA or Codex) food safety authorities have approved the intentional use of hormones in food production [48]. Thus, the joint FAO/WHO Expert Committee set the standard of hormone for human consumption as an acceptable daily intake (ADI) of 17β-estradiol, progesterone, testosterone, zeranol, and trenbolone acetate at the rate of 0.05 μg/ kg, 30 μg/ kg, 2 μg/ kg, 0.5 μg/ kg, and 0.02 μg/ kg body weight, respectively. JECFA [53].

3. Hormonal usage in fish farming

Steroidal hormones regulate mammalian physiological processes. Almost all vertebrate and invertebrate organisms require steroid hormones for proper growth, maturity, and cellular cell division [85]. Steroid hormones are frequently used to promote fish's development and reproductive capability. Natural origin or synthetically produced estrogens and androgens are widely used in aquaculture. In aquaculture, hormonal preparations are applied to induce breeding and reversion of sex [84].

Progesterone and testosterone represent two essential sex hormones [85], and their imbalance may lead to reproductive abnormalities and infertility issues in fish [74]. Testosterone (T), androstenedione (AR), and 11-ketotestosterone (11-KT) are all produced in fish testes [88].

3.1. Growth promotion

Hormones were used in fish farming to induce sex reversal in the monosex population, which helps to increase growth rate or weight gain [93]. The use of hormone treatments for sex reversal was initiated during sex differentiation. Fish sex may be separated into genotype, determined by the genes responsible for gonad formation, and phenotype, which is the appearance of the ovary or testis. Phenotype differentiation occurs naturally, generally earlier in females than in males, during the ontogeny of the fish larvae [92]. Hormonal therapy does not often affect the fish genotype but influences the phenotypic manifestation. Some fishes have a higher sex-specific growth rate than others. The tilapia (*Oreochromis niloticus*) growth rate in males is quicker than in females [49].

Hormones regulate sex throughout early development, especially during the labile phase [23]. It is possible to achieve sexual reversal in 47 gonochoric (34 species, 9 families) by injecting one of the 31 steroids (16 androgens, 15 estrogens). The frequency of endocrine therapy for sex reversal rises in the following families in sequence: Cichlidae, Cyprinodondidae, Anabantidae, Poecilidae, Salmonidae, and Cyprinidae. The hormones and hormone modulators, namely tamoxifen (an estrogen receptor blocker), fadrozole and letrozole (nonsteroidal aromatase inhibitors), 17-methyl testosterone, 17-ethynyl testosterone, 17-ethynyl estradiol and estradiol-17β, were the most recognized for inducing masculinization or feminization [109]. It increased the fish's body weight, length, muscle composition, feed conversion efficiency, fatty acid composition, and lipid profile (Table 1). Two methods for creating monosex fish populations were indirect breeding and direct breeding. In indirect breeding, dependable breeders or progenitors were given neo-male (XX) or neo-female (XY) hormones to produce the necessary sex. Where as in direct breeding, fish hormones were given to the fish for the intended sexual orientation. Wang, Shen [109].

As a result, the goldfish in cyprinids were easy to feminize [109], but the carp need significantly greater dosages of estrogen administered for a more extended period [32]. A study has documented a unique approach for obtaining monosex female groups in 35 distinct teleosts. The guidelines covered the usage of various types of estrogen and their delivery method, dosage, therapy durations, and time frame in different

Table 1
Effects of different steroid hormones on fish growth.

Fish species	Name of steroid hormone	Effect	Reference
Zebrafish	17β-estradiol (E2)	Impair juvenile development, delay breeding age and adult fecundity	Chaturantabut et al. [25]
Salmonid fish	Cortisol	Suppresses somatic growth	Breves et al. [21]
Alpine white fish	17α ethinylestradiol (EE2) and estrogen	Increase in body weight	Brazzola et al. [20]
Juvenile Coho Salmon, Nile Tilapia (*Oreochromis niloticus*)	Testosterone, 17α-methyltestosterone and progesterone	Increases in both body weight and length, muscle composition, sex conversion	McBride and Fagerlund; Ajiboye et al. [6,69]
Tongue Sole (*Cynoglossus semilaevis*)	17β-estradiol and testosterone	Accelerates the fish weight gain, feed conversion efficiency, fish body composition and fatty acid composition of fish body lipids	Zhang et al. [115]

fish species [8]. In aquaculture, the development of YY male tilapia was considerably favoured for population increase and preventing excessive breeding/overcrowding [79]. Tilapia fry was often feminized to produce YY males by giving hatchlings with 17-βestradiol [26]. When XY neo-females were bred with XY men, 25 % YY males were obtained, which progeny testing may recognize. These YY males were subsequently feminized with 17-β estradiol therapy to produce males (YY) [93]. Thus, farmers could also manually select sexes, hybridize chromosomal alteration, and hormone sex inversion to produce entirely male tilapia [70].

For growth promotion, hormones were delivered via nutritional supplementation or systemically (direct injection and silicone-based implants) for sex conversion treatment [87]. Immersion and feeding were still the most effective therapies since both techniques address a significant number of fish. The method involving systemic transmission was overpriced on the market and needed technical expertise to be implemented for the fish [102,22]. To achieve all-female or all-male population, 17-estradiol, estradiol valerate, 17-methyl testosterone, or 17-methyl dihydrotestosterone were used by immersion and diet approach. However, androgen was also used to masculinize young females and produce no males [44].

3.2. Reproduction

Hormones were introduced in the aquaculture industry to make artificial reproduction, or embryo generation, essential in managing gonad development and ovulation. Sex steroid hormones (testosterone, 17-estradiol), maturation-promoting hormone (17, 20-dihydroxy-4-pregnen-3-one, or DHP), and cortisol significantly affect reproduction. These had different levels and feedback processes since they revealed the fish's reproductive and physiological state [96]. In artificial reproduction, the manipulation of final maturation and ovulation in the gonad occurs. Exogenous hormones can accelerate or slow fish maturation and cause spawning to occur a few months earlier or later than usual (Table 2). Environmental factors can also affect fish reproduction naturally [80].

Estrogens are steroid hormones that control reproductive cycles and various other activities in vertebrates [82]. Besides the regulatory actions in many organ systems, estrogen regulates oogenesis, vitellogenesis, gonadotropin control, testicular function, and other reproductive features [59,66]. Hormonal techniques for fish breeding were based on intramuscular or intraperitoneal injection in brood stocks [97]. The oldest and still the most used hormone was the crude extract of mature fish's pituitary gland (PE) (carp and salmon). Another broadly used hormone was the gonadotropin-releasing hormone (GnRH) of both mammalians (mGnRH) and salmon (sGnRH) [34].

Table 2
Effects of different steroid hormones on fish reproduction.

Fish species	Name of steroid	Effect	Reference
Rainbow trout and lake trout	Estrogen, 17β-Estradiol	Development and reproduction	(Tapper, 2020)
Sturgeon	Testosterone, Progesterone and Estrogen	Gonadal maturation	Barannikova et al. [9]
Zebrafish	Estradiol, androgen	Regulation on reproduction, ovulation, vitellogenesis and yolk formation, spermatogenesis	Tokarz et al. [101]
Teleost	Cortisol	Ovulation, oocyte maturation, gonadal corticosteroid genesis	Milla et al. [72]
Marine fish	Estrogens and Androgens	Increase fish production based on sexual dimorphism	Tripathy et al. [103]
Marine fish	Progesterone deoxy cortisol	Gonadal maturation and production of other endogenous steroids	Tripathy et al. [103]

4. Detection techniques

Different analytical techniques were used to detect hormonal residue in fish samples [40]. The most commonly used detection and sample preparation techniques are given below.

4.1. Extraction method

Solid-phase extraction (SPE) was the most commonly used method due to its simplicity, rapidity, and ability to extract large samples with high recovery. The majority of the study used SPE to purify hormones from animal samples. SPE used relatively low amounts of organic solvents, and most contaminants were eliminated throughout the clean-up process. Despite that, SPE coupled with solvent extraction, solvent vortex extraction, matrix solid-phase dispersion, and solvent ultrasonic extraction were widespread [31,77]. Recent developments in sample preparation included the use of more rapid, simple, and ecologically friendly methods in addition to more conventional extraction techniques [64]. As a result, a solvent-less or solvent-reduced version of conventional solid-phase extraction had been introduced that was called solid-phase microextraction (SPME), which requires just a few microliters of extractant. Though SPME was effective and solvent-free, it has some drawbacks, such as delicacy, cost, and commercialized fibre [100]. To meet these challenges, the researcher used adaptable fibres that can be reused more than once. Furthermore, SPE incorporated molecularly imprinted polymers (MIPs) to improve adsorption capacity and accuracy [55,4]. Mass polymerization produced encoded MIPs, which had applications in offline molecularly imprinted solid-phase extraction (MISPE) [54].

4.2. Detection method

Most hormonal residue analyses used immunoassays like RIA or ELISA [47]. ELISA was a straightforward and cost-effective method for screening many biochemically active compounds with high sensitivity. ELISA did not need hazardous reagents that were harmful to humans and ecosystems. Researchers used a commercial kit of ELISA to quantify the residual concentration of testosterone in tilapia samples at ng/g level. In that case, 69.9 % recovery values were found [52]. Therefore, there were no severe issues with sample disposal, but this technique had some limitations. Commercial ELISA kits are expensive and sometimes give false positive and negative results. Furthermore, it was impossible to quantify multiple steroid residues in complicated matrices [114].

In addition, Radioimmunoassay was a sensitive analytical process. This technique's selectivity and specificity were very high. But, this technique requires highly skilled technicians to handle hazardous, radioactive particles [83]. It had restrictions in its applicability since not all hormones could be detected with it, and internal hormones could interfere with it. However, after purification, lipids might confound with the test result and create an incorrect estimate of steroid levels [60].

The most extensively used analytical technique for determining steroid hormones was gas chromatography-mass spectrometry (GC/MS) [98]. However, this procedure was complicated and time-consuming because it involved an extensive purification process, such as extraction, clean-up, and derivatization [35]. This approach effectively analyzed market samples due to their high linearity, precision, and accuracy [112]. Using this technique, a research study can establish the influence of steroids on aquatic organisms, even at ng/L concentrations. This method identified and quantified the 17-estradiol residues in the muscle of various fishe products. It was found that GC-MS detected different steroid hormones in the plants, eggs, fish, milk, other fermented alcoholic beverages and meat of poultry, bovine and porcine, and their products. The residual levels ranged from 0.03 to 1.41 μg/kg

[48].

Analytical liquid chromatography techniques (LC) with tandem mass spectrometry (LC-MS/MS) were often used to quantify hormonal residue in fish products. Electrochemical, ultraviolet diode array (DAD), fluorescence, UV-Vis, and tandem mass spectrometry (MS/MS) were the most used detectors attached to liquid chromatography (LC) for hormone analysis [90]. It has emerged as a powerful technology for analyzing hormonal residues at low levels in complicated matrices even without the requirement for derivatization [42,5,86]. Due to its high specificity and detection range in various matrices, this technique has gained popularity and was commonly used in food analysis [19,114]. Furthermore, different ionization techniques, including electrospray ionization (ESI) [36] or atmospheric pressure chemical ionization (APCI), were also performed to analyze steroid hormones [50,107].

HPLC (high-performance liquid chromatography) was also a valuable tool for detecting hormones. Due to high selectivity, specificity, and sensitivity, it was a potential technology for hormonal evaluation. It has a column, a detector and a monitor system. Compared to HPLC, the column length is shorter than that of UHPLC (ultra). UHPLC was mainly used to identify trace chemicals in various matrices due to its excellent sensitivity, reduced solvent sample, and time requirements [45]. In addition, UHPLC-MS was a fast method without solvent extraction procedures [68]. A broad spectrum of steroidal hormones could be detected and quantified using this approach, which had a minimum limit of detection (LODs) and quantification limits (LOQs) (Table 3). Guedes-Alonso et al. [40] detected fifteen steroid hormones in fish tissues by microwave-assisted extraction coupled with ultra-high performance liquid chromatography-mass spectrometry (Fig. 1). Nineteen endocrines disrupting was found in certain fishes processed by QuEChERS (quick, easy, cheap, effective, rugged, and safe) [51].

5. The impact of hormonal usage

The increasing demands of culture fish production require appropriate culture techniques to improve production to an economically viable level. A few studies regarding growth promotion by steroid hormones revealed the potential advantages of this strategy [58]. However, using hormones indiscriminately modifies the sexual behaviour of aquatic creatures, primarily fish and harms the environment, ecology, and public health [86]. Over the past several years, the use of hormones in aquaculture has been of great importance. After they were used, a significant part was excreted into the aquatic environment. This indicates that if all the endocrine disruptors were not appropriately managed, they might move into water, prey, and the food chainTop of FormBottom of Form

. It was observed by a study that exposure to both natural and manufactured chemicals could pose a hazard to people and wildlife [5]. Theoretically, if hormonal treatment in fish is appropriately done by maintaining a withdrawal period, the fish muscle should not contain hormonal residue at a hazardous level [86].

5.1. Impact on aquatic environment

Fish obtain steroids and their equivalents from their diet and the water they live in. Steroid hormones can be found in fishmeal and feed. The amount of these steroids varies with the type of materials used. Secondly, chemically synthesized hormones are used in different products and enter the aquatic system via sewerage system outputs [65]. Steroids can persist in the low stomach P^H of fish [94]. The presence of steroidal hormones has been reported in common fish species, i.e., crucian carp (*Carassius auratus*), carp (*Cyprinus carpio*), and silvery minnow (*Anabarilius alburnops*), in Dianchi Lake of China [64]. Steroid hormone was bound with the blood's steroid-binding proteins (SBP) to form a complex that resists steroidal degradation and elimination [75]. Certain hepatic enzymes converted weak estrogenic substances to strong ones. In this way, marketed diets enhanced the retention and bioaccumulation of steroids in fish bodies [86].

A research study revealed the E2 concentration in sewerage systems ranged between 0 and 150 ng/L [12,111]. Likewise, other researchers claimed that a range of 1–10 ng/L estrogen (E2) might induce the

Table 3
Methods for determining hormonal residues in fisheries products using liquid chromatography and various detectors.

Compounds /chemicals	Sample	Stationary Phase	Mobile Phase	Detection technique	Limit of detection (LOD)	Limit of quantification (LOQ)	References
Epitestosterone, 17α-methyltestosterone, Testosterone 17-propionate, Medroxyprogesterone, Progesterone, Estrone−3sulfate, 17α-ethynylestradiol, 17β-estradiol, and Estriol	Fish muscle	Supelco Discovery C18 column	ACN, H2O 0.3 mL/min	MS/MS	0.06–0.22 ng/g	0.12–0.54 ng/g	Xu et al. [113]
Corticosterone, Estrone, 17β-oestradiol, Estriol, Medroxyprogesterone, Progesterone, 17α hydroxyprogesterone, Testosterone, and 19-nortestosterone,	Fish muscle	Zorbax Eclipse C18 column	ACN, H2O 0.8 mL/min	MS/MS	0.03–0.15 ng/g	0.11–0.47 ng/g	Wang et al. [110]
Progesterone, Estrone, 17β-estradiol, Estriol, Estrone−3-sulfate	Fish muscle	Acquity UPLC BEH C18 column	MeOH, H2O (pH 9) 0.4 mL/min	MS/MS	0.002–3.09 ng/g	0.005–9.26 ng/g	Jakimska et al. [51]
Progesterone, Testosterone, Estrone, 17β-estradiol, Estriol, Prednisone, and Norethisterone	Fish muscle, skin, viscera	Acquity UPLC BEH C18 column	MeOH, H2O	MS/MS	0.14–49.0 ng/g	3.95 μg/g	Guedes-Alonso et al. [40]

***ACN(Acetonitrile), MeOH (Methanol)

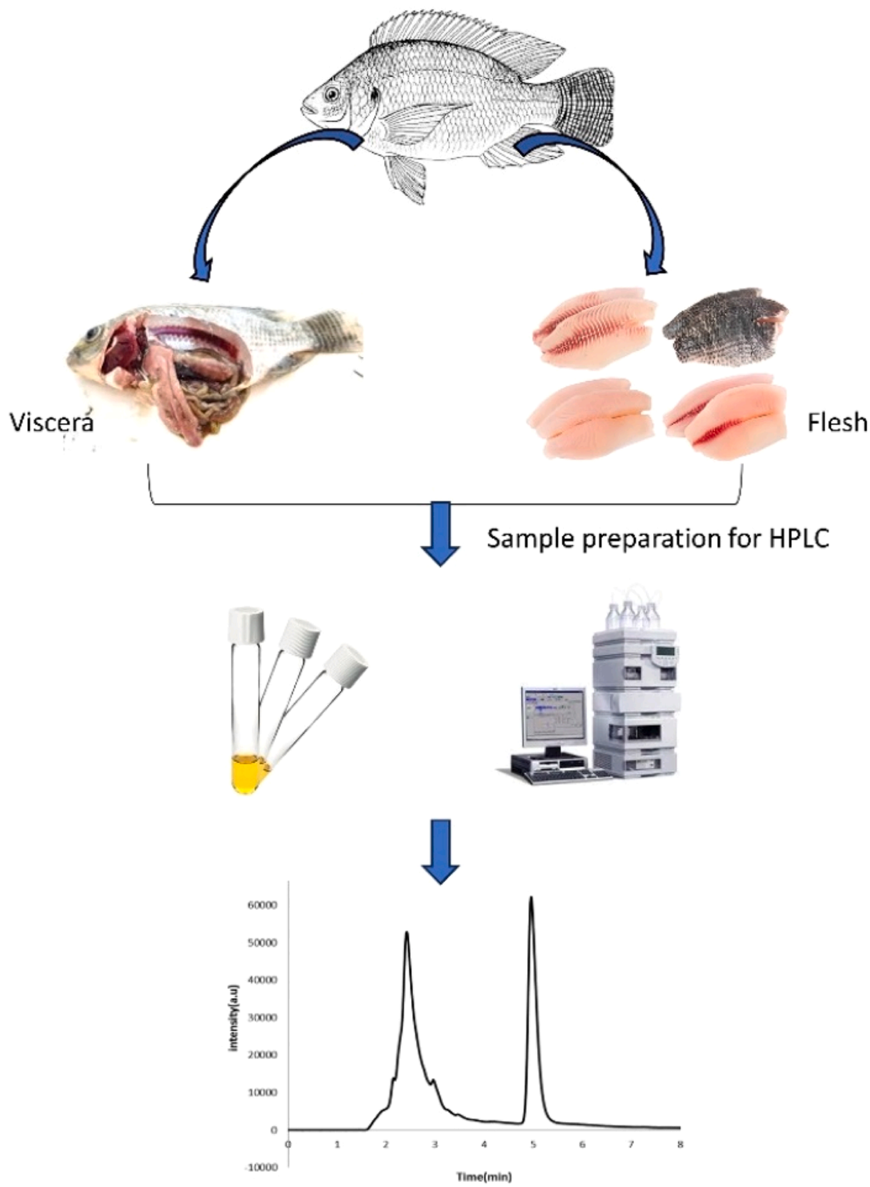

Fig. 1. Illustration of the detection of steroid hormonal residue by UHPLC-MS. It is an Ultra-high-performance liquid chromatography-electrospray ionization mass spectrometry (UHPLC-MS) technique for quantitatively evaluating hormonal residues in fish.

feminization of male fish [12]. However, due to estrogen-based therapy, several synthesized and native estrogens had been released into the environment, most notably into seas and coastlines and cause harm to the aquatic species [81].

It was found by a study that hormones used for the treatment of fish were converted into water-soluble metabolites by the liver. A few hours or days after sex reversal therapy, 90 percent of the hormone was digested, and discharged via biliary and urinary route into the water [43,67]. If the water in which steroidal hormone-treated fish was not appropriately managed, the sex reversal process can contaminate the ecosystem. The adverse effects are such as vitellogenesis (plasma vitellogenin induction), feminization, and hermaphrodism (male and female gonads in a single individual) in fish [10].

5.2. Impact on human health

Humans commonly ingest fish because of their great dietary value. The indiscriminate use of steroid hormones in fish, followed by residue in fish and fish products above the ADI level, might cause human health hazards. Different experiments further proved that they detected hormone residue in the edible parts of fish and aquatic products above the ADI level [78]. By consuming these contaminated fish, steroid hormone residue enters the human body and exerts its effect by binding with different body receptors [78]. The consumers might be in danger if they intake hormone residue-contaminated fish. It may cause cancer and myocardial illness in humans whenever taken in quantities beyond the recommended levels [108]. Prolonged intake of hormones in humans could lead to unwanted health hazards such as hepatotoxicity and fetotoxicity (Fig. 2). Due to ecosystem contamination, bioaccumulation of steroid hormones in fishes could have dangerous impacts on humans and animals [62]. Elevated estrogen concentrations have been associated with greater incidences of mammary cancer in women [61] and prostrate carcinoma in men [18]. Estrogen in food and water could promote puberty, menopause, and premature ejaculation [38]. In humans, steroidal estrogens, alone or in conjunction with progesterone, decreased intraocular pressure (IOP) following menstruation, which might elevate the chance of future glaucoma [105].

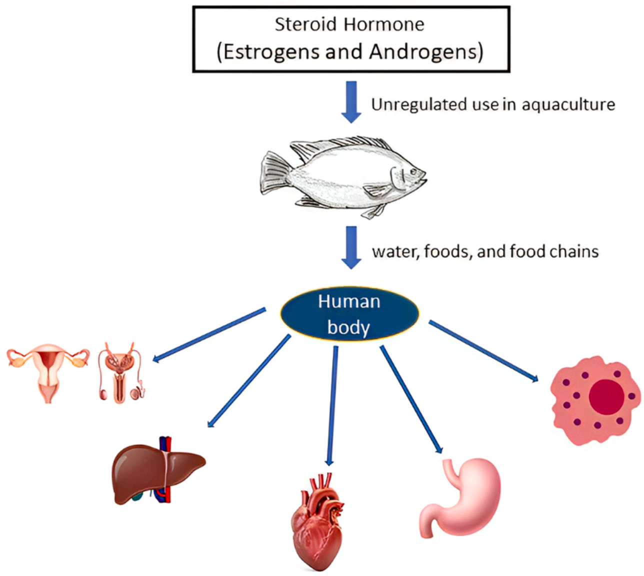

Fig. 2. A plausible mechanism of translocation and accumulation of steroid hormones into the human body and making an intended impact on various systems (Digestive, Reproductive and Hematopoietic systems).

5.2.1. *Effect of steroids on the immune system*

Steroidal hormone reduces T-cell function, B-cell proliferation and immune regulation [73]. Though estrogens tend to have multiple effects on antibody production in general, they also mainly suppress specific thymocyte subgroups (Benagiano *et al.,* 2019). Even though thymocytes regulate immune function, it was no surprise that estrogens suppress immunological responses. Sex Steroid hormones might serve as tumor enhancers [56].

5.2.2. *Effect of steroids on the digestive system*

The steroidal hormone impacted energy demand, gastrointestinal function, metabolic activity, development, and body mass [104]. According to epidemiological and clinical investigations, steroidal hormones were vital in regulating food consumption and energy homeostasis in humans and animals. Obesity was more common in post-menopausal women since their estrogen levels have dropped than in typical female cyclists [57]. A survey report found that about 40.4 % of people experienced digestive system abnormalities in steroid-treated cases. Among them, 57.6 % felt digestive burn feelings, while 18.6 % had stomach discomfort [1].

5.2.3. *Effect of steroids on the reproductive system*

Various sex steroids had effects on the reproductive tract of human beings. Exogenous steroids from different sources could affect the blood hormone level. Ultimately, it can cause intersexuality, modified gender ratios, femininity of men [76], and masculinity of women, as well as decreased fertility (Table 4) [17,95]. Elevated testosterone levels suppressed spermatogenesis in men and ovarian activity and menstruation in women [44]. The formation of non-cancerous prostate hyperplasia and prostate carcinoma was facilitated by androgens [2]. It also diminished women's psychological empathy, which results in low social intelligence, lower longevity rate of embryos and ineffective interactions [63].

5.2.4. *Effect of steroids on hematopoietic system*

Steroid hormones cause cell death of hematopoietic cells such as monocytes, macrophages, and T lymphocytes [14]. It has been reported that estrogens interact with various cells (monocytes, bone marrow stromal cells, and osteoblasts) to help in the cytokine release of these cells and reduce their function [16]. When testosterone was consumed in large doses, various adverse effects occurred in men like edema, hypercalcemia, decreased glucose tolerance, bone growth, and skeletal weight [37]

Likewise, serum cholesterol and steroid level alterations have also been recorded following estrogen exposure. Some documented side effects included alterations in neurodevelopment,anemia, lymphopenia, and neutropenia with impairments in lymphocyte function [13,30].

6. Conclusion and future remarks

People generally rely on fish protein to meet their daily protein needs as the demand for protein rises. Farmers employ steroid hormones in fish production to promote artificial reproduction and sexual conversion to meet the rising demand and make a decent profit. Finally, this uncontrolled hormone enters the environment and human food chain, causing irreversible damage to the aquatic environment and human body, including the reproductive, immunological, and cardiovascular systems, if they exceed the permissible limits.

However, the authorities should take proper steps to reduce the effect of such unregulated use of steroid hormones. National legislators should consider public health and environmental safety and assess whether or not to allow the use of steroid hormones for sexual conversion. Moreover, farmers shouldundergo training about proper management and withdrawal period of hormones. The highest level of caution should be exercised to prevent the ecological imbalance posed by hormonal residues and ensure food safety. To fully understand these hormones, more study is required. As a result, it is recommended that the number of steroid hormones in freshwater and river running water,

Table 4
Effects of steroid hormone on the human body.

Fish Species	Specimen	Hormone	Detected Level	Effects	References
1. White Amur Bream (*Parabramis pekinensis*)	Muscle	Estrogen estrone Diethylstilbestrol (DES)	0.33 ng/g 10.9 ng/g	Prostate, breast and testicular cancer, hepatotoxicity, fetotoxicity, glaucoma, suppress T cell and B-cell function	Zhou et al. [116,62,73]
2. Common Carp (*Cyprinus carpio*)	Muscle	Estrogen estrone Diethylstilbestrol (DES)	1.04 ng/g 14.6 ng/g	Metabolic activity and obesity Scorching or abdominal discomfort	Butera [24] Alparslan, Kapucu [1, 116]
3. Mandarin Fish (*Siniperca chuatsi*)	Muscle Liver	Diethylstilbestrol (DES) Estrogen estrone	10.1 ng/g 23.7 ng/g	Osteoporosis and Exhaustion	Zhou et al., [1,116]
4. Common Roach (*Rutilus rutilus*)	Bile, Plasma	Estrone, testosterone 11-ketotestosterone	1–29 ng/L	The femininity of men, the masculinity of women, suppression of spermatogenesis, ovarian activity and menstruation	([33,91]; Moreno-Pérez *et al.,* 2012)
5. Guinean Puffer (*Sphoeroides marmoratus*)	Muscle Viscera	Progesterone Progesterone	0.56 µg/g 3.26 µg/g	Carcinogenic effect	Guedes-Alonso et al., [41,47]
	Skin	Progesterone	3.95 µg/g		

particularly on the surface of waters, need to be assessed routinely using appropriate scientific methodsto control and regulate hormone usage in fish culture.

CRediT authorship contribution statement

Kazi Rafiq: Writing – review & editing. **Salman Shahriar Nibir:** Writing – review & editing. **Purba Islam:** Writing – review & editing, Visualization, Supervision, Methodology, Formal analysis, Conceptualization. **Md. Anwarul Islam:** Conceptualization, Visualization. **Popy Khatun:** Writing – review & editing, Methodology, Formal analysis. **Md. Imran Hossain:** Writing – original draft, Visualization, Methodology, Formal analysis. **Rony Ibne Masud:** Writing – review & editing. **Mahir Anjum:** Writing – review & editing. **Shadia Tasnim:** Writing – review & editing. **Md. Zahorul Islam:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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