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Advances in Fish Masculinization Techniques for Sustainable Aquaculture

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Fish masculinization in aquaculture refers to a process that involves the development of secondary male sexual characteristics in female fish or the alteration of the normal sexual differentiation process. This term refers to the production of male-typical characteristics. Methods of masculinization typically involve hormonal treatment, natural variation, genetic manipulation, and temperature control. 17α-Methyltestosterone is use for hormonal treatment it is a synthetic derivative of testosterone, which is a male sex hormone (androgen). Use medicinal herbs and

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plants like papaya seed (*Carica papaya*) for natural variations. They have active ingredients like caricaine, carpasemine enzyme, and oleanolic glycoside, which has been shown androgenic effect. Genetic manipulation is done by selective breeding or genetic alteration to create fish with male sex chromosomes. For temperature control, the temperature at which eggs are incubated can influence the sex of the offspring. Aromatase inhibitors are also used as masculinization agents. They work by blocking the aromatase enzyme, which turns the hormone androgen into small amounts of estrogen in the body. The benefits of masculinization include its potential to produce monosex populations, reduce the risk of breeding issues, and promote uniform growth. However, challenges such as hormone-related side effects, environmental concerns, and long-term sustainability must be addressed. This study examines the methods and results of fish masculinization procedures in aquaculture, emphasizing their use, advantages, and difficulties for various species. It also makes recommendations for future research directions in the quest for more effective and sustainable aquaculture methods.

Keywords: Fish; masculinization; papaya seed; 17α-Methyltestosterone; aromatase inhibitor; sex-reversal.

1. INTRODUCTION

Aquaculture is considered a vital source of food, nourishment, money, and livelihood for millions globally. Fish is the most economical and excellent source of animal protein, serving as an effective solution to alleviate hunger and malnutrition in the country. India ranks as the third-largest producer of fish and aquaculture globally, accounting for around 16% of the world's inland fish production and 5% of marine fish production (Handbook on Fisheries Statistics, 2022). In total, it generated 175 lakh metric tons of fish during the period of 2021–2022, with 131 lakh metric tons originating from the inland sector and 44 lakh metric tons from the marine sector. The fisheries sector is integral to the national economy and significantly contributes to the country's foreign exchange revenues. The primary aim of aquaculture operations is to ensure sustainable production with optimal growth rates that satisfy human dietary needs. IoT solutions provide advanced methods for monitoring and controlling conditions that directly affect the success of fish masculinization (Mahamuni and Goud, 2023).

Fish masculinization refers to the process of inducing male characteristics in fish, often through hormonal treatments, to create monosex populations for aquaculture and ornamental purposes. This technique is particularly significant in species where males exhibit desirable traits such as faster growth rates or more vibrant coloration, which enhances their market value (Baron et al., 2007). The process typically involves the administration of androgens, such as aromatase inhibitors that block estrogen production, effectively reversing the natural female phenotype into a male one. Research indicates that these hormonal interventions can lead to a complete sex reversal, resulting in genetically female fish developing male reproductive organs and behaviors (Lal et al., 2023). The implications of fish masculinization extend beyond aquaculture efficiency; they raise concerns about environmental impacts and food safety due to potential hormone residues in aquatic ecosystems (Yostawonkul et al., 2023; Harlıoğlu and Farhadi, 2017).

Aromatase inhibitors are a class of pharmaceutical compounds primarily used in the treatment of hormone receptor-positive breast cancer and other conditions associated with excess estrogen production. These drugs work by inhibiting the activity of the enzyme aromatase, which plays a crucial role in the synthesis of estrogen. Aromatase inhibitors, which are used in the treat of ovarian and breast cancer among postmenopausal women (Howell et al. 2005), have a notable impact on masculinization in fish by impeding the process of estrogen-induced ovary differentiation.

Papaya (*Carica papaya*) is an important source of economical and non-toxic bioactive compounds, including antibiotics. It is widely utilized as a dietary supplement for beneficial pharmacological effects. Moreover, papaya has antibacterial, antifungal, and anti-fertility activities, serving as a medicinal plant for the treatment of various ailments in humans and animals, in addition to influencing sex ratio in fish. (Radwan et al., 2023) Alternative treatments using phytochemicals, like phytoestrogens, which act like natural fish hormones, are seen as a good replacement for 17-methyltestosterone in making tilapia populations that are all male. (Ampofo-Yeboah, 2013).

Historically and currently, the synthetic steroid 17α-methyltestosterone (17 MT) has been widely utilized as a feed ingredient in commercial farms to generate all-male tilapia fry. The 17 MT-treated feed is provided to fry at early life stages (14 days post-hatch) for a duration of 21 to 28 consecutive days. The effectiveness and expense of this treatment regimen have been recognized by aqua culturists (Karaket et al., 2021). In broad terms, estrogen treatment tends to induce feminization in genetic males, while androgen treatment promotes masculinization in genetic females. In order to create male monosex populations of Nile tilapia, farmers often use hormonal treatments, with MT being a popular option. The popularity of MT is primarily due to its simplicity, high efficiency, reliability, and cost-effectiveness (Baroiller and Cotta 2018). This method is expected to remain the predominant means of obtaining all-male offspring for a significant duration in key tilapia-producing nations. The androgen MT has undergone testing across more than 25 species within various fish families, including Salmonidae, Cichlidae, Cyprinidae, Anabantida, Poecilidae, and Cyprinodontidae. Notably, Cichlidae typically require lower androgen doses compared to other fish families (Beardmore et al., 2001).

2. BIOLOGICAL BASIS OF SEX DETERMINATION IN FISH

2.1 Natural Sex Determination Mechanisms in Fish

Fish show a diversity in sex determination mechanisms that can be broadly classified as genetic sex determination (GSD) or environmental sex determination (ESD). GSD is commonly associated with specific sex chromosomes, whereas ESD is shaped (often by temperature or social interactions) by environmental conditions. Some species are able to change sex during their lifetime, exhibiting strategies such as protandry and protogyny. This flexibility in the sexual development is a reflection of the evolutionary plasticity of the fish based on different ecological conditions (Heule et al., 2014).

The latest studies have identified numerous master sex-determining (MSD) genes within the TGF-β signaling system. A few of these genes include gsdf and amhr2, which are associated with sex determination in certain teleost species (Kitano et al., 2024) and play a crucial role in initiating gonadal differentiation. The XX/XY chromosomal system in Nile tilapia (Oreochromis niloticus) (Kobayashi et al., 2012) demonstrates a distinct genetic mechanism utilized by fish to differentiate between species. In the tiger pufferfish (Takifugu rubripes), sex determination can be affected by specific genes located on these chromosomes. A single nucleotide polymorphism in amhr2 is associated with sex (Heule et al., 2014). Temperature influences sex determination in numerous fish species. The sex of the progeny is determined by the aquarium's temperature throughout critical developmental phases. Martínez et al., (2014) revealed that elevated temperatures enhance male development, whereas reduced temperatures may promote female development. Research indicates that cichlid species and other fish groups have this behavior. In addition to weather, factors influencing sex determination include water pH, dissolved oxygen levels, and modes of communication among individuals. Some species capable of sex change may be influenced by social hierarchies, determining whether an individual transitions from male to female or vice versa (Matsuda, 2003).

3. REASONS FOR MASCULINIZATION IN AQUACULTURE

3.1 Growth Advantages of Males in Specific Species

Fish masculinization is affected by numerous environmental and biological conditions, resulting in considerable consequences for growth and reproductive success in particular species. In numerous fish species, larger males possess a competitive advantage during reproduction. Females frequently favor larger males because of their capacity to offer superior resources and genetic benefits for progeny. In Atlantic cod, larger males not only produce more sperm but also display more intense wooing behaviors, enhancing their reproductive success (Uusi-Heikkilä et al., 2020). Male tilapia is favored in aquaculture due to their superior growth rate and enhanced feed conversion efficiency compared to females. This results in increased yields for aqua culturists. Hormonal therapies such as 17 alpha-methyltestosterone (MT) are frequently employed to generate all-male populations that optimize growth rates and reduce reproductive issues (Yostawonkul et al., 2023). In species where size is crucial for mating success, such as zebrafish, females may allocate greater reproductive resources to larger males. This entails generating larger egg batches when coupled with larger partners, thereby improving the survival probabilities of their progeny (Uusi-Heikkilä et al., 2020).

3.2 Prevention of Overpopulation in Farming Systems

In order to promote sustainable practices and reduce overcrowding, it is vital to prevent masculinization in aquaculture systems. Hormonal manipulation is a technique used to alter sex ratios in aquaculture. Androgens can be provided to enhance male traits, particularly in animals such as tilapia, where males generally demonstrate accelerated development rates. Conversely, estrogens are employed to feminize populations, resulting in all-female stocks that can improve production efficiency because of their accelerated growth and postponed maturation (Lal et al., 2023). This method entails administering estrogens to sexually undifferentiated fish, producing all-female populations within one generation. It is preferred for its simplicity and efficacy. This technique entails initially masculinizing genetic females with androgens, enabling them to generate sperm for conception, which then results in all-female progeny in future generations (Leet et al., 2011).

Environmental factors, including photoperiod and temperature, can profoundly affect sex determination and maturation. Regulating light exposure can postpone sexual maturation, enabling fish to attain greater size prior to harvesting. A different species may react differently to temperature changes during important stages of development, which can change the ratio of males to females (Al-Emran et al., 2024). Genetic techniques encompass triploidy, a method that produces sterility by producing fish with three sets of chromosomes. This approach inhibits reproduction while facilitating ongoing growth; hence, it mitigates overpopulation issues. Marker-assisted selection (MAS) and quantitative trait loci (QTL) mapping have made it easier to find breeding stock that can produce children with the right ratios of sexes (Budd et al., 2015).

3.3 Male Fish Often Exhibit Brighter Colors and More Vivid Patterns, Desirable for Aquariums

Male fish have more vibrant colors and intricate patterns compared to females; a feature especially evident in ornamental species. The vivid coloration in male fish is mainly attributed to specialized pigment cells known as chromatophores. These cells encompass different pigments, including melanin, carotenoids, and guanine, which contribute to the broad color spectrum exhibited in species such as guppies (Poecilia reticulata). Klann et al., (2021) reported that many chromatophore types collaborate to create intricate patterns and iridescence, augmenting the visual attractiveness of males. Vivid pigmentation fulfills essential ecological roles. In situations with fluctuating predation pressures, male fish may modify their pigmentation. For example, male guppies in predator-dense habitats typically display less vivid coloration to evade notice, whereas those in safer settings exhibit more intricate patterns to allure mates (Godin and McDonough, 2003). Coloration is crucial in sexual selection. Amundsen and Forsgren (2001) reported the female fish frequently favor males exhibiting brighter colors and more complex patterns, linking these characteristics to health and genetic viability. Female guppies preferentially select males exhibiting elevated concentrations of orange and iridescent pigments, resulting in enhanced reproductive success for vividly colored males. This desire influences the development of male attractive characteristics, facilitating speciation and diversity within aquatic habitats.

4. TECHNIQUES USED IN FISH MASCULINIZATION

4.1 Natural Phytoandrogens

Papaya (*Carica papaya*) is a plant that contains phytochemicals of significant research interest (Ampofo-Yeboah 2013). Papaya seed meal (PSM) has active ingredients like caricain, carpasemine enzyme (which stops plants from growing), and oleanolic glycoside. These ingredients have been shown to make male rats sterile (Kobayashi et al., 2008) and are used to control the over-breeding of O. niloticus (Ayotunde and Ofem 2008) by changing the fish's sex to male. Papaya is a member of the limited family Caricaceae, which comprises six genera (Ming et al., 2007). Papaya is a prevalent fruit for human consumption, available year-round in tropical regions. Commonly known as the "medicine tree" or "melon of health," papaya is abundant in nutrients (Jackwheeler, 2003). According to Rietjens et al., (2013) phytochemicals work by inhibiting the receptor sites for estrogen or by imitating the way estrogen regulates fish gonad development and sexual differentiation. The papaya seeds contain phytochemicals with estrogenic and androgenic properties, which may disrupt normal gonadal development and promote male characteristics. Additionally, these compounds can reduce reproductive performance by affecting hormone levels and gonadal histology, leading to infertility in females. (Rietjens et al., 2013).

4.2 Hormonal Ttreatments

The use of 17α-methyltestosterone (17α-MT) to masculinize tilapia fish has attracted a lot of interest since it successfully creates all-male populations. These populations are preferable for aquaculture because they grow quicker and have better feed conversion ratios. Research has shown that when 17α-MT is given orally or by immersion, it can achieve a high rate of masculinization, with reports suggesting that fry handled properly can produce as much as 98% male offspring. According to Susano et al., (2020), the usual doses can vary from 30 mg/kg in feed to 500 µg/L in immersion, and larger concentrations usually yield the best outcomes. Although the hormone is known to enhance male development, there are concerns about possible histopathological changes in important organs like the gills, liver, and kidneys. As a result, it is necessary to closely monitor residual hormone levels to make sure they stay within safe limits (Suseno et al., 2020). Although there are advantages to utilizing 17α-MT, its environmental impact and long-term consequences on fish health are important factors to consider. If not handled appropriately, the synthetic nature of 17α-MT could have harmful genetic and ecological effects due to its excessive or incorrect use. The rates of masculinization are amazing; however, there may be intersex people and sexual maturity growth retardation as a result of long-term exposure to high hormone levels, according to research (Farias et al., 2023; Asad et al., 2020). Hence, although 17α-MT is still an effective method for increasing tilapia output in aquaculture, it needs to be used carefully to strike a balance between financial gains, environmental preservation, and fish health.

4.3 Aromatase Inhibitors

Aromatase inhibitors (AIs) are a class of pharmaceutical agents crucial for the treatment of hormone receptor-positive breast cancer, one of the most prevalent forms of the illness. These inhibitors are designed to precisely target the aromatase enzyme, which regulates the conversion of androgens into estrogens in postmenopausal women. As estrogen stimulates the proliferation of hormone receptor-positive breast cancer cells, the suppression of estrogen production is a crucial aspect of treatment. Aromatase inhibitors have emerged as viable tools in this endeavor (Arora and Potter 2004). Three primary aromatase inhibitors utilized in clinical practice are anastrozole, letrozole, and exemestane. These medications function by blocking aromatase; hence, they decrease estrogen levels in the body. Gonnelli and Petrioli (2008).

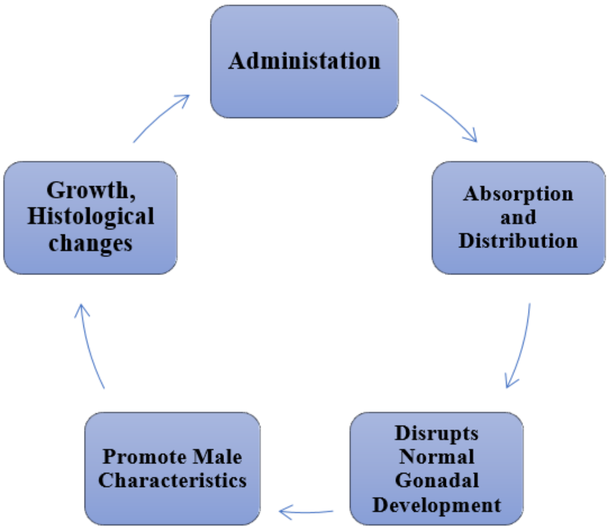

Fig. 1. Mechanism action of papaya seed on fish masculinization

Table 1. Plant-based androgen agent used for fish masculinization in aquaculture

Fish species	Administration methods	Dose	Duration	Findings	References
Nile tilapia (*Oreochromis niloticus)*	Oral administration	2g/kg	20 Weeks	Fish fed with dietary PSE increased the sex ratio in favour of male fish by achieving a 77% male phenotype	Radwan et al. (2023)
Oreochromis mossambicus	Oral (in feed)	20g and 30g PSM/kg	120 days	77.8% masculinization observed after the fed of papaya seed	*Omeje* et al. (2018)
Oreochromis niloticus	Oral (in feed)	6 g PSP /kg diet	45 days	different levels of PSP showed that all growth performance, survival and feed utilization parameters were significantly increased	Farrag et al. (2013)
Nile Tilapia	Oral (in feed)	4.27 g/kg	28 days	82% masculinization was observed in Nile tilapia when fed with 4.27 g/kg of PSM	Ugonna et al. (2018)
Oreochromis Niloticus (gift)	Oral (in feed)	6g PSM per kg diet	60 days	the use of seeds of Carica papaya as a productiveness control agent for the male of *O. niloticus*	Christopher et al. (2021)
Oreochromis andersonii	Oral (in feed)	15g and 35 g of PSE / kg diet	120 days	Sexually undifferentiated fry with 15 g/kg resulted in 82% masculinization, while levels above this could inhibit growth performance	Iipinge et al., (2019)
Oreochromis niloticus	Oral (in feed)	150g/kg	30 days	Papaya seeds, control the reproduction of Nile tilapia and overcome the problem of early maturation, instead of expensive chemical hormones	Yadav et al. (2021)
Oreochromis mossambicus	Oral (in feed)	15 g/kg	60 days	65% males were observed after they were fed 15 g of pawpaw seed powder per kg of basal diet in *O. mossambicus*	Ampofo-Yeboah, (2013)

4.4 Environmental Manipulations

In temperature-dependent sex determination (TSD), the early developmental phase during which temperature can influence gonadal sex is referred to as the thermosensitive period (TSP). As the temperature changes within a certain range, some fish species, like tilapia (Oreochromis niloticus) (Baroiller et al., 1995), channel catfish (*Ictalurus punctatus*) (Patino et al., 1996), common carp (*Cyprinus carpio*) (Barney, 2008), and *clarias gariepinus* (Santi et al., 2016), change sex. Initial research indicated that temperatures exceeding 36 °C were lethal for common carp, but temperatures below 35 °C did not influence gonadal sex differentiation (Nakamura et al., 2015). However, in Nile tilapia and African catfish, elevated temperatures (specifically 36 °C) biased the sex ratio in favor of the male phenotype (Santi et al., 2016). The methods to monitor and maintain optimal conditions for fish masculinization. For example, it can support the argument that real-time monitoring of environmental parameters ensures the efficacy of hormonal or genetic treatments (Goud et al., 2020).

4.5 Genetic and Genomic Approaches

In aquaculture, genetic and genomic methods for masculinizing fish, especially tilapia and rainbow trout, have become crucial. Many different genetic pathways underlie sex determination in fish, with intricate interplay between key genes and other variables. As an example, while the sdY gene in rainbow trout is known to play a significant role in defining sex, there has been evidence of spontaneous masculinization in XX females, which could indicate the presence of other genetic factors. Genome-wide association studies (GWAS) have found quantitative trait loci (QTL) that are connected to this spontaneous maleness. These QTL include genes like fgfa8 and cyp17a1 that may be involved in this process. Piferrer et al., (2012) and Fraslin et al., (2020) reported that sex determination in fish is multifactorial and that these genetic insights could be used to efficiently adjust sex ratios in aquaculture.

Furthermore, environmental variables have a substantial effect on fish sex determination and masculinization. Zebrafish and Nile tilapia are among the many species that have shown evidence of sex reversal caused by temperature changes during pivotal stages of development. The signaling pathways involved in sex differentiation have been better understood thanks to the integration of modern genomic techniques like next-generation sequencing (NGS). Innovative breeding tactics that increase male output without using hormone therapies alone are now within reach, thanks to these technologies that enable researchers to thoroughly examine gene expression profiles. To optimize fish output while ensuring sustainability, it will be crucial to utilize these genetic and genomic techniques as aquaculture keeps changing (Martínez et al., 2014; Sukumaran, 2023).

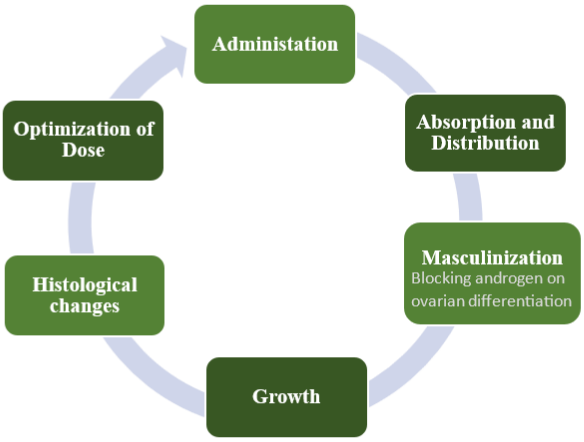

Fig. 2. Mechanism action of 17alpha methyl testosterone on fish masculinization

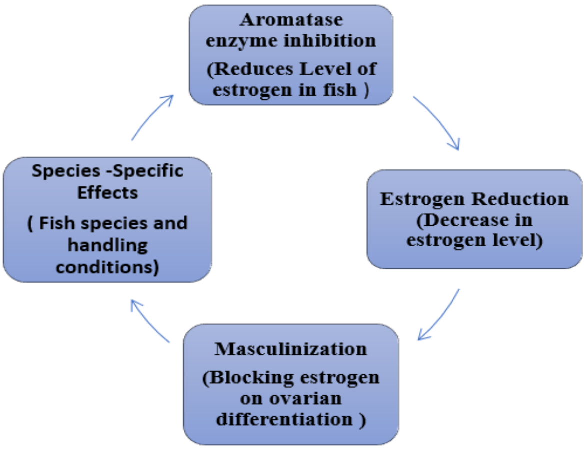

Fig. 3. Mechanism action of aromatase inhibitor on fish masculinization

Table 2. The 17α-methyltestosterone hormonal utilization in aquaculture

Fish Species	MT Administration Method	Dose (mg/kg or mg/L)	Duration of Treatment	Effectiveness (%)	Notes	References
Nile Tilapia	Oral (in feed)	30–60 mg/kg feed	21–28 days	95–100	Most common species for MT use; highly effective for sex reversal.	El-Greisy and El-Gamal, (2012)
Mozambique Tilapia	Oral (in feed)	30–50 mg/kg feed	21–28 days	90–98	Requires warm temperatures (25–30°C) for optimal results.	Bhujel, (2013)
Rainbow Trout	Immersion	10–15 mg/L	2–4 hours daily for 7 days	80–95	Effective but less commonly used due to environmental concerns.	Weber and Leeds, (2022)
Asian Sea Bass (Barramundi)	Oral (in feed)	40–60 mg/kg feed	21 days	90–95	Timing of administration is crucial during early developmental stages.	Meachasompop et al., (2023)
Channel Catfish	Oral (in feed)	50 mg/kg feed	28 days	85–95	Male catfish exhibit better growth and feed efficiency.	Hossain, M. A. (1998).
Common Carp	Oral (in feed)	30–50 mg/kg feed	28 days	85–90	Less commonly used; requires higher doses for consistent results.	Mallik et al. (2023)
Grouper (Epinephelus spp.)	Immersion	10–20 mg/L	2 hours daily for 10 days	85–95	Effective for marine species when applied during larval stages.	Jung et al., (2024)
Zebrafish	Immersion	25 mg/L	2 hours daily for 10 days	90–95	Often used in research; requires precise handling to avoid toxicity.	Modarresi Chahardehi et al., (2020)
Guppy	Oral (in feed)	20–30 mg/kg feed	14–21 days	85–90	Effective for ornamental fish production; lower doses required.	Lewbart, (2008)
Snakehead (Channa spp.)	Oral (in feed)	50–60 mg/kg feed	21–28 days	85–90	Results in increased male-biased populations, enhancing aquaculture.	Gao et al., (2024)

Table 3. The fish masculinization using aromatase inhibitor agents

Fish Species	Aromatase Inhibitor	Dosage	Treatment Duration	Method of Administration	Results/Notes	References
Nile Tilapia (*Oreochromis niloticus*)	Letrozole	50-200 µg/L	30 days	Water immersion	Increased male ratio; higher dosages linked to adverse effects like reduced survival or growth rates.	Sarker et al., (2022)
Zebrafish (*Danio rerio*)	Exemestane	100 µg/L	21 days	Water immersion	Significant male-biased sex ratio observed without noticeable toxicity at optimal doses.	Lal et al. (2024)
Common Carp (*Cyprinus carpio*)	Anastrozole	0.5 mg/kg feed	60 days	Oral administration	Enhanced male population; growth rates were unaffected when combined with balanced diets	Tzchori et al., (2004)
Guppy (*Poecilia reticulata*)	Fadrozole	25-50 µg/L	14 days	Water immersion	Improved male ratios; requires precise timing to align with critical sex differentiation window.	Hallgren et al., (2006)
Dwarf gourami, (*Trichogaster lalius)*	Anastrozole-17α-methyltestosterone	140 AI + 60 MT ppm		Oral administration	89.70% masculinization observed after the fed of anastrozole-17α-methyltestosterone in diet and inhibit the synthesis of estradiol and promote the synthesis of testosterone	Katare et al., (2018)
Dwarf gourami, (*Trichogaster lalius*)	Anastrozole	200 ppm	50 days	Oral administration	90.32% masculinization observed after the fed of anastrozole	Katare et al., (2021)
Dwarf gourami, Trichogaster lalius	Anastrozole	1000 µg/L.	50 days	Immersion methods	100% masculinization observed after the immersion treatment of anastrozole	Katare et al., (2021)
Large mouth bass (*Micropterus salmodies*)	Letrozole	20 mg/kg feed	60 days	Oral administration	86.67% observed after the fed of letrozole and that effects on growth, male ratio, and gonadal development	Zhang et al., (2024)

Fish Species	**Aromatase Inhibitor**	**Dosage**	**Treatment Duration**	**Method of Administration**	**Results/Notes**	**References**
Rainbow trout (*Oncorhynchus mykiss)*	Chitosan nanoparticles to letrozole	1.5 mg/kg	60 days	Oral administration	76.66% male population observed because inhibiting the production of estradiol, increasing the amount of testosterone	Alijani et al., (2022)
Siamese Fighting Fish (*Betta splendens*)	letrazole	500 and 1000µg/L	50 days	Water immersion	Increased proportion of males; effective during the early gonadal differentiation phase.	Katare et al., (2015)
Mozambique Tilapia (*Oreochromis mossambicus*)	Fadrozole	50 µg/L	25 days	Water immersion	Achieved male-biased populations; treatment timing critical for effective results.	Gale et al., (1999)

5. ADMINITRATION METHODS OF MASCULINIZATION AGENTS

5.1 Oral Administration

A growing interest in the effectiveness of natural extracts as alternatives to synthetic hormones can be seen in the oral administration methods for fish masculinization in Nile tilapia (Oreochromis niloticus). A study examining Basella alba extract revealed that both oral administration and immersion methods can successfully promote masculinization. Even though immersion techniques had higher sex reversal rates than oral feeding, the latter still had notable results, with a masculinization rate of about 64.96% for fry fed hormone-treated food. This indicates that although immersion may be more efficacious, oral delivery is still a feasible alternative, particularly in situations when immersion is unfeasible or less preferable (Cungihan et al., 2024). Subsequent study underscores the feasibility of integrating androgens into feed as a strategy for sex reversal. Male hormones, like 17α-methyltestosterone (17α-MT), can be added to feeds and given orally. This is known to be an effective way to get high masculinization rates, sometimes exceeding 99% when combined with proper feeding practices. Nonetheless, issues such as water pollution from leftover feed and the requirement for accurate hormone administration must be resolved to enhance effectiveness and ecological sustainability (Fuentes-Silva et al., 2013; Hoga et al., 2018). These findings emphasize the necessity of investigating various administration methods to improve male tilapia production while reducing dependence on synthetic hormones.

5.2 Immersion Techniques

Immersion methods to stimulate fish masculinization use synthetic androgens, specifically 17α-methyltestosterone (MT), to induce sex reversal in numerous fish species. Studies have shown that synthetic androgens, including 17α-methyltestosterone (MT) and mesterolone, are effective when applied by immersion procedures. A study on flowerhorn fish found that exposing them to 250 µg/L of mesterolone led to a considerably greater male ratio (84.94%) compared to the control group (Silarudee and Kongchum, 2008). Similarly, research on common carp found that MT immersion procedures might produce up to 95% male individuals when appropriate hormone concentrations and immersion times were used (Asad et al., 2020). In addition to synthetic hormones, natural compounds have been studied for masculinization. A study on rainbow fish (*Melanotaenia boesemani*) used bee resin extract, indicating that varying dosages could effectively skew sex ratios toward males, with the highest results reported at 1 ppm (Albasa et al., 2019). Furthermore, studies on Nile tilapia demonstrated that short-term exposure to androgens such as MDHT could result in nearly full masculinization of fry (Gale et al., 1999). These improvements emphasize the adaptability of immersion approaches, not only in terms of synthetic hormone use but also in the use of natural alternatives, extending the range of options accessible for fish sex reversal in aquaculture practices.

6. ADVANTAGES OF FISH MASCULINIZATION

The benefits of fish masculinization in aquaculture include increased growth rates and enhanced production efficiency. All-male populations, generated by YY male technology, exhibit accelerated growth and attain greater sizes than females, resulting in enhanced profitability for aqua culturists. This method is also useful in averting undesirable reproduction, which might result in overpopulation and resource depletion in farming systems.

1. Improved Feed Conversion Efficiency (FCR): Males have faster growth rates and higher FCR than females, which leads to more efficient feed consumption. The results of this research suggest that male tilapia have an 11.7% higher FCR than females.
2. Cost Reduction: Enhanced FCR leads to lower feed costs and overall production expenses, benefiting both economic and environmental aspects of fish farming (Kause et al., 2022).
3. Higher Yield: By producing all-male populations, aquaculture operations can maximize growth rates and reduce the time to market, increasing overall yield (Yostawonkul et al., 2023).
4. Energy Allocation: By producing all-male populations, energy that would typically be diverted to reproductive processes in females can instead be used for growth, enhancing overall biomass production.
5. Growth Rates: Males often exhibit faster growth rates compared to females,

resulting in a more efficient feed conversion and shorter time to market.

6. Minimized Early Maturation: Masculinization helps prevent early maturation, which can lead to reduced growth and increased energy expenditure on gonadal development (Budd et al., 2015).

7. CONCLUSIONS

The practice of fish masculinization in aquaculture has emerged as a critical strategy for enhancing production efficiency and ensuring sustainability in the industry. By producing all-male populations, farmers benefit from faster growth rates, uniformity, and improved feed conversion ratios, thereby increasing economic returns. Hormonal treatment, particularly using androgens like 17α-methyltestosterone, has proven effective and widely adopted, though concerns over environmental and consumer safety persist. To address these challenges, ongoing research is exploring alternative techniques, such as genetic manipulation, environmental regulation, and natural plant-based hormones. Additionally, stricter regulations and monitoring systems are necessary to minimize ecological and health risks associated with hormonal residues. comprehensive approach integrating scientific innovation, regulatory oversight, and stakeholder collaboration is essential to ensure the long-term viability of this aquaculture practice.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Albasa, A. R. H., Hakim, R. R., & Setyono, B. (2019). Masculinization of Rainbow Fish (Melanotaenia boesemani) through immersion the larvae with extract of Bee Honey Resin as a natural substance with different doses. IJOTA *(Indonesian Journal of Tropical Aquatic)*: 2(1), 33-39.

Al-Emran, M., Zahangir, M. M., Badruzzaman, M., & Shahjahan, M. (2024). Influences of photoperiod on growth and reproduction of farmed fishes-prospects in aquaculture. Aquaculture Reports, 35, 101978.

Ampofo-Yeboah, A., 2013. *Effect of phytogenic feed additives on gonadal development in sMozambique tilapia* (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Amundsen, T., & Forsgren, E. (2001). Male mate choice selects for female coloration in a fish. Proceedings of the National Academy of Sciences, 98(23), 13155-13160.

Arora, A. and Potter, J.F., 2004. Aromatase inhibitors: current indications and future prospects for treatment of postmenopausal breast cancer. *Journal of the American Geriatrics Society*, 52(4), pp.611-616.

Asad, F., Qamer, S., Ashraf, A., Ali, T., Shaheen, Z., Akhter, S., ... & Mustafa, G. (2020). Masculinization in common carp (Cyprinus carpio) by androgen immersion: The interaction effect of hormone concentration and immersion time. Brazilian Journal of Biology, 81, 285-290.

Ayotunde, E.O. and Ofem, B.O., 2008. Acute and chronic toxicity of pawpaw (Carica papaya) seed powder to adult Nile tilapia (Oreochromis niloticus Linne 1757). *African Journal of Biotechnology*, *7*(13).

Barney, M.L., 2010. *Molecular investigations on sex determination and differentiation pathways in the common carp, Cyprinus carpio* (Doctoral dissertation, University of Tasmania).

Baroiller, J.F. and D'Cotta, H., 2018. Sex control in tilapias. *Sex control in aquaculture*, pp.189-234.

Baroiller, J.F., Chourrout, D., Fostier, A. and Jalabert, B., 1995. Temperature and sex chromosomes govern sex ratios of the mouthbrooding cichlid fish Oreochromis niloticus. *Journal of Experimental Zoology*, *273*(3), pp.216-223.

Baron, D., Montfort, J., Houlgatte, R., Fostier, A. and Guiguen, Y., 2007. Androgen-induced masculinization in rainbow trout results in a marked dysregulation of early gonadal gene expression profiles. *BMC genomics*, *8*, pp.1-18.

Beardmore, J.A., Mair, G.C. and Lewis, R.I., 2001. Monosex male production in finfish as exemplified by tilapia: applications, problems, and prospects. *Reproductive* *biotechnology in finfish aquaculture*, pp.283-301.

Bhujel, R. C. (2013). On-farm feed management practices for Nile tilapia (Oreochromis niloticus) in Thailand. *On-farm feeding and feed management in aquaculture*, *583*, 159-189.

Budd, A. M., Banh, Q. Q., Domingos, J. A., & Jerry, D. R. (2015). Sex control in fish: approaches, challenges and opportunities for aquaculture. Journal of Marine Science and Engineering, 3(2), 329-355.

Conover, D.O. and Heins, S.W., 1987. Adaptive variation in environmental and genetic sex determination in a fish. *Nature*, *326*(6112), pp.496-498.

Conover, D.O. and Kynard, B.E., 1981. Environmental sex determination: interaction of temperature and genotype in a fish. *Science*, *213*(4507), pp.577-579.

Cungihan, L. L., Chinaman, V., Cariño, V., & Torio, H. (2024). Efficacy of Basella alba L. Extract on the Masculinization of Nile Tilapia (Oreochromis niloticus Linnaeus, 1758) Using Immersion and Oral Administration Techniques. Eurasian Journal of Agricultural Research, 8(1), 56-65.

Donaldson, L.R. and Olson, P.R., 1957. Development of rainbow trout brood stock by selective breeding. *Transactions of the American Fisheries Society*, *85*(1), pp.93-101.

Ekanem, S.B. and Okoronkwo, T.E., 2003. Pawpaw seed as fertility control agent on male Nile tilapia.

El-Greisy, Z. A., & El-Gamal, A. E. (2012). Monosex production of tilapia, Oreochromis niloticus using different doses of 17α-methyltestosterone with respect to the degree of sex stability after one year of treatment. *The Egyptian Journal of Aquatic Research*, *38*(1), 59-66.

Farias, R. D. S., Oliveira, K. R. D. S., Souza, M. E. D., Ferreira, D. A., Silva, A. A. D. N., Silva Júnior, V. A. D., ... & Coimbra, M. R. M. (2023). Effect of dosage of orally administered 17α-methyltestosterone on sex reversion of the yellowtail tetra Astyanax lacustris (Lütken, 1875). Animal Reproduction, 20, e20220080.

Feist, G., Yeoh, C.G., Fitzpatrick, M.S. and Schreck, C.B., 1995. The production of functional sex-reversed male rainbow trout with 17α-methyltestosterone and 11 β-hydroxy androstenedione. *Aquaculture*, *131* (1-2), pp.145-152.

Fitzpatrick, M.S., Contreras-Sanchez, W.M., Milston, R.H. and Schreck, C.B., 1999. Fate of the masculinization agent methyltestosterone in the pond environment. In *V Central American Symposium on Aquaculture* (pp. 249-250). San Pedro Sula.

Fraslin, C., Phocas, F., Bestin, A., Charles, M., Bernard, M., Krieg, F., ... & Quillet, E. (2020). Genetic determinism of spontaneous masculinisation in XX female rainbow trout: new insights using medium throughput genotyping and whole-genome sequencing. Scientific Reports, 10(1), 17693.

Fuentes-Silva, C., Soto-Zarazúa, G. M., Torres-Pacheco, I., & Flores-Rangel, A. (2013). Male tilapia production techniques: a mini-review. African journal of Biotechnology, 12(36).

Gale, W. L., Fitzpatrick, M. S., Lucero, M., Contreras-Sánchez, W. M., & Schreck, C. B. (1999). Masculinization of Nile tilapia (Oreochromis niloticus) by immersion in androgens. *Aquaculture*, *178*(3-4), 349-357.

Gao, R., Yan, H., Zhou, H., Hu, M., Ding, Y., Shen, X., Wang, J., Wang, C., Wang, L., Jiang, C. and Liu, Y., 2024. Administration of mifepristone can induce masculinization and alter the expression of sex-related genes in Takifugu rubripes. *Aquaculture Reports*, *36*, p.102172.

Godin, J. G. J., & McDonough, H. E. (2003). Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. Behavioral Ecology, 14(2), 194-200.

Gonnelli, S. and Petrioli, R., 2008. Aromatase inhibitors, efficacy and metabolic risk in the treatment of postmenopausal women with early breast cancer. *Clinical interventions in aging*, *3*(4), pp.647-657.

Goud, C. S., Das, S., Kumar, R., Mahamuni, C. V., & Khedkar, S. (2020). Wireless sensor network (wsn) model for shrimp culture monitoring using open source iot. In 2020 Second International Conference on Inventive Research in Computing Applications (ICIRCA) (pp. 764-767). IEEE.

Hallgren, S. L., Linderoth, M., & Olsén, K. H. (2006). Inhibition of cytochrome p450 brain aromatase reduces two male specific sexual behaviours in the male Endler guppy (Poecilia reticulata). *General and Comparative Endocrinology*, *147*(3), 323-328.

Handbook on Fisheries Statistics, 2022

Harlıoğlu, M. M., & Farhadi, A. (2017). Feminization strategies in crustacean aquaculture. Aquaculture International, 25, 1453-1468.

Heule, C., Salzburger, W., & Böhne, A. (2014). Genetics of sexual development: an evolutionary playground for fish. Genetics, 196(3), 579-591.

Hoga, C. A., Almeida, F. L., & Reyes, F. G. (2018). A review on the use of hormones in fish farming: Analytical methods to determine their residues. CyTA-Journal of Food, 16(1), 679-691.

Hörstgen-Schwark, G., 1993. Initiation of tetraploid breeding line development in rainbow trout, Oncorhynchus mykiss (Walbaum). *Aquaculture Research*, *24*(5), pp.641-652.

Hossain, M.A., 1998. *Optimization of feeding and growth performance of African catfish (Clarias gariepinus Burchell, 1822) fingerlings*. University of Stirling (United Kingdom).

Howell, A., Cuzick, J., Baum, M., Buzdar, A., Dowsett, M., Forbes, J.F., Hoctin-Boes, G., Houghton, J., Locker, G.Y. and Tobias, J.S., 2005. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet*, *365* (9453), pp.60-62.

Jackwheeler, M.N., 2003. *Health mate Papaya* [online]

Jung, H. K., Lee, C. H., & Lee, Y. D. (2024). Characteristics of Morphological Changes in Fins according to Larval Growth of Red Spotted Grouper, Epinephelus akaara. *Development & Reproduction*, *28*(3), 95.

Karaket, T., Reungkhajorn, A. and Ponza, P., 2023. The optimum dose and period of 17α-methyltestosterone immersion on masculinization of red tilapia (*Oreochromis* spp.). *Aquaculture and Fisheries*, *8*(2), pp.174-179.

Katare, M. B., Basavaraja, N., Joshi, H. D., & Archana, C. (2015). Effect of letrozole on masculinization of Siamese fighting fish (*Betta splendens*). *Journal of Applied and Natural Science*, *7*(1), 425-433.

Katare, M. B., Lakra, W. S., Chadha, N. K., Kumar, M., Basavaraja, N., Gupta, S., ... & Swain, S. (2018). Synergistic effect of anastrozole-17α-methyltestosterone on masculinization of dwarf gourami. *Journal of Experimental Zoology India*, *21*(2).

Katare, M. B., Pai, M., & Mogalekar, H. S. (2021). Effect of anastrozole on masculinization in ornamental fish, dwarf gourami, Trichogaster lalius (Hamilton, 1822). *Indian Journal of Experimental Biology (IJEB)*, *59*(09), 581-589.

Kato, K., 2023. Breeding studies on red sea bream Pagrus major: mass selection to genome editing. *Fisheries Science*, *89*(2), pp.103-119.

Kause, A., Nousiainen, A., & Koskinen, H. (2022). Improvement in feed efficiency and reduction in nutrient loading from rainbow trout farms: the role of selective breeding. Journal of Animal Science, 100 (8), skac214.

Kincaid, H.L., Bridges, W.R. and Von Limbach, B., 1977. Three generations of selection for growth rate in fall-spawning rainbow trout. *Transactions of the American Fisheries Society*, *106*(6), pp.621-628.

Kitano, J., Ansai, S., Takehana, Y., & Yamamoto, Y. (2024). Diversity and Convergence of Sex-Determination Mechanisms in Teleost Fish. Annual Review of Animal Biosciences, 12(1), 233-259.

Klann, M., Mercader, M., Carlu, L., Hayashi, K., Reimer, J. D., & Laudet, V. (2021). Variation on a theme: pigmentation variants and mutants of anemonefish. EvoDevo, 12(1), 8.

Kobayashi, H., Wang, C. and Pomper, K.W., 2008. Phenolic content and antioxidant capacity of pawpaw fruit (*Asimina triloba* L.) at different ripening stages. *HortScience*, *43*(1), pp.268-270.

Kobayashi, Y., Nagahama, Y., & Nakamura, M. J. S. D. (2012). Diversity and plasticity of sex determination and differentiation in fishes. Sexual Development, 7(1-3), 115-125.

Lal, J., Biswas, P., Singh, S.K., Debbarma, R., Deb, S., Yadav, N.K. and Patel, A.B., 2023. Effects of dietary aromatase inhibitors on masculinization of rosy barb (*Pethia conchoniu* s): Evidence from growth, coloration and gonado-physiological changes. *Plos one*, *18*(11), p.e0287934.

Lal, J., Vaishnav, A., Singh, S. K., Biswas, P., Mehta, N. K., Meena, D. K., & Waikhom, G. (2024). The potential of aromatase inhibitors in fish masculinization: a comprehensive review of applications, mechanisms and future perspectives. *International Aquatic Research*, *16*(2), 113-142.

Leet, J. K., Gall, H. E., & Sepúlveda, M. S. (2011). A review of studies on androgen and estrogen exposure in fish early life stages: effects on gene and hormonal control of sexual differentiation. Journal of Applied Toxicology, 31(5), 379-398.

Lewbart, G., 2008. *Rapid Review of Exotic Animal Medicine and Husbandry: Pet Mammals, Birds, Reptiles, Amphibians and Fish*. CRC Press.

Mahamuni, C. V., & Goud, C. S. (2023). Unveiling the Internet of Things (IoT) Applications in Aquaculture: A Survey and Prototype Design with ThingSpeak Analytics. Journal of Ubiquitous Computing and Communication Technologies, 5(2), 152-174

Mallik, S.K., Shahi, N., Pathak, R., Kala, K., Patil, P.K., Singh, B., Ravindran, R., Krishna, N. and Pandey, P.K., 2023. Pharmacokinetics and biosafety evaluation of a veterinary drug florfenicol in rainbow trout, Oncorhynchus mykiss (Walbaum 1792) as a model cultivable fish species in temperate water. *Frontiers in Pharmacology*, *14*, p.1033170.

Mankiewicz, J.L., Godwin, J., Holler, B.L., Turner, P.M., Murashige, R., Shamey, R., Daniels, H.V. and Borski, R.J., 2013. Masculinizing effect of background color and cortisol in a flatfish with environmental sex-determination. *Integrative and comparative biology*, *53*(4), pp.755-765.

Martínez, P., Viñas, A.M., Sánchez, L., Díaz, N., Ribas, L. and Piferrer, F., 2014. Genetic architecture of sex determination in fish: applications to sex ratio control in aquaculture. *Frontiers in genetics*, *5*, p.340.

Matsuda, M. (2003). Sex determination in fish: Lessons from the sex-determining gene of the teleost medaka, Oryzias latipes. Development, growth & differentiation, 45 (5-6), 397-403.

Meachasompop, P., Bunnoy, A., Keaswejjareansuk, W., Dechbumroong, P., Namdee, K. and Srisapoome, P., 2023. Development of immersion and oral bivalent nanovaccines for streptococcosis and columnaris disease prevention in fry and fingerling Asian seabass (*Lates calcarifer*) nursery farms. *Vaccines*, *12*(1), p.17.

Mei, J. and Gui, J.F., 2015. Genetic basis and biotechnological manipulation of sexual dimorphism and sex determination in fish. *Science China Life Sciences*, *58*, pp.124-136.

Ming, R., Wang, J., Moore, P.H. and Paterson, A.H., 2007. Sex chromosomes in flowering plants. *American Journal of Botany*, *94*(2), pp.141-150.

Moav, R. and Wohlfarth, G., 1976. Two-way selection for growth rate in the common carp (*Cyprinus carpio* L.). *Genetics*, *82*(1), pp.83-101.

Modarresi Chahardehi, A., Arsad, H., & Lim, V. (2020). Zebrafish as a successful animal model for screening toxicity of medicinal plants. *Plants*, *9*(10), 1345.

Nakamura, K., Harada, A., Kanno, T., Inagaki, R., Niwano, Y., Milleding, P. and Örtengren, U., 2015. The influence of low-temperature degradation and cyclic loading on the fracture resistance of monolithic zirconia molar crowns. *Journal of the mechanical behavior of biomedical materials*, *47*, pp.49-56.

Omeje, V.O., Lambrechts, H. and Brink, D., 2018. Effect of pawpaw (Carica papaya) seed meal on sex determination, growth, and survival, of Oreochromis mossambicus fry. *Israeli Journal of Aquaculture-Bamidgeh*, *70*.

Pandian, T.J. and Sheela, S.G., 1995. Hormonal induction of sex reversal in fish. *Aquaculture*, *138*(1-4), pp.1-22.

Piferrer, F., Ribas, L., & Díaz, N. (2012). Genomic approaches to study genetic and environmental influences on fish sex determination and differentiation. Marine biotechnology, 14, 591-604.

Radwan, M., Darweesh, K.F., Ghanem, S.F., Abdelhadi, Y., Kareem, Z.H., Christianus, A., Karim, M., Waheed, R.M. and El-Sharkawy, M.A., 2023. Regulatory roles of Pawpaw (*Carica papaya*) seed extract on growth performance, sexual maturity, and health status with resistance against bacteria and parasites in Nile tilapia (Oreochromis niloticus). *Aquaculture International*, *31*(5), pp.2475-2493.

Radwan, M., El-Sharkawy, M.A., Alabssawy, A.N., Ghanem, S.F., Mohammadein, A., Al Malki, J.S., Al-Thomali, A.W., Manaa, E.A., Soliman, R.A., Yassir, S. and Mekky, A.E., 2023. The synergy between serious parasitic pathogens and bacterial infestation in the cultured Nile tilapia (*Oreochromis niloticus*): a severe threat to fish immunity, causing mass mortality and significant economic losses. *Aquaculture International*, *31*(5), pp.2421-2449.

Rietjens, I.M., Sotoca, A.M., Vervoort, J. and Louisse, J., 2013. Mechanisms underlying the dualistic mode of action of major soy isoflavones in relation to cell proliferation and cancer risks. *Molecular nutrition & food research*, *57*(1), pp.100-113.

Santi, S., Gennotte, V., Toguyeni, A., Mélard, C., Antoine, N. and Rougeot, C., 2016. Thermosensitivity of the sex differentiation process in the African catfish, Clarias gariepinus: Determination of the thermosensitive period. *Aquaculture*, *455*, pp.73-80.

Sarker, B., Das, B., Chakraborty, S., Hossain, M. A., Alam, M. M., Mian, S., & Iqbal, M. M. (2022). Optimization of 17α-methyltestosterone dose to produce quality mono-sex Nile tilapia Oreochromis niloticus. *Heliyon*, *8*(12).

Silarudee, S., & Kongchum, P. (2008). Masculinization of flowerhorn by immersion in androgens. Science, Engineering and Health Studies, 26-32.

Sukumaran, S. (2023). Genetic and genomic tools in fisheries and aquaculture. International Workshop-cum-Training on Fisheries Management and Aquaculture. ICAR-Central Marine Fisheries Research Institute. 128-153.

Suseno, D. N., Luqman, E. M., Lamid, M., Mukti, A. T., & Suprayudi, M. A. (2020). Residual impact of 17α-methyltestosterone and histopathological changes in sex-reversed Nile tilapia (*Oreochromis niloticus*). Asian Pacific Journal of Reproduction, 9(1), 37-43.

Tzchori, I., Zak, T., & Sachs, O. (2004). Masculinization of genetic females of the common carp (*Cyprinus carpio* L.) by dietary administration of an aromatase inhibitor.

Uusi-Heikkilä, S. (2020). Implications of size-selective fisheries on sexual selection. Evolutionary Applications, 13(6), 1487-1500.

Valenzuela, N. and Lance, V.A., 2019. *Temperature-dependent sex determination in vertebrates*. Smithsonian Institution Scholarly Press.

Weber, G.M. and Leeds, T.D., 2022. Effects of duration and timing of immersion in 17α-methyltestosterone on sex reversal of female rainbow trout. *Aquaculture Reports*, *23*, p.101014.

Yostawonkul, J., Kitiyodom, S., Supchukun, K., Thumrongsiri, N., Saengkrit, N., Pinpimai, K., Hajitou, A., Thompson, K.D., Rattanapinyopituk, K., Maita, M. and Kamble, M.T., 2023. Masculinization of red tilapia (*Oreochromis* spp.) using 17α-methyltestosterone-loaded alkyl polyglucosides integrated into nanostructured lipid carriers. *Animals*, *13*(8), p.1364.

Younes, M. and Honma, N., 2011. Estrogen receptor β. *Archives of pathology & laboratory medicine*, *135*(1), pp.63-66.

Zhang, D., Li, S., Tian, T., Du, J., Lei, C., Zhu, T., ... & Song, H. (2024). Effects of 17α-methyltestosterone and letrozole on growth and gonadal development in largemouth bass (*Micropterus salmodies*). *Frontiers in Physiology*, *15*, 1444918.

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