Comparative evaluation of Chinese herbal extracts and synthetic hormones for male *Oreochromis* spp. production in clay ponds

[1,4,5]Thian H. Chung, [1]Mazzaellynn Thomas, [2]Mohammad B. Munir, [1]Roslianah Asdari, [3]Md. Abdul Hannan

[1] Department of Aquatic Resource Science and Management, Faculty of Resource Science and Technology, University Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia; [2] Department of Aquaculture, Faculty of Agriculture, University Islam Sultan Sharif Ali, TB1741 Tutong, Brunei Darussalam; [3] Department of Aquatic Animal Health Management, Sher-e-Bangla Agricultural University, 1207 Dhaka, Bangladesh; [4] Honey Aquaculture, Lot 115, BLK 4, Sentah Segu Land District, 94200 Kuching, Sarawak, Malaysia; [5] VL Ocean Services, Lot 886, 43500 Semenyih, Selangor, Malaysia. Corresponding author: Md. B. Munir, mohammad.munir@unissa.edu.bn

Abstract. This study compared a Chinese herbal extract mixture to 17α-methyltestosterone (MT) for the development of male red tilapia, *Oreochromis* spp. across three generations (F0-F1, F1-F2, F2-F3). MT directly reduces natural sex differentiation, whereas herbal extracts do so via phytoandrogenic action, aromatase suppression, immunological support, and growth promotion. In the F0-F1 generation, herbal therapy produced fewer males than MT, but in the F2-F3 generation, both approaches produced 100% males. Growth and survival rates improved in succeeding generations, especially in herbally treated fish, with no negative effects on early life stages. Throughout the research, the water quality was within acceptable limits. Although early ovarian development was temporarily retarded in the herbal F0-F1 group, subsequent generations had normal reproductive timing, indicating reversible effects. Overall, the findings indicate that Chinese herbal extracts are a safe, effective, and long-lasting alternative to synthetic hormones for male *Oreochromis* spp. production.

Key Words: tilapia, Chinese herbal extracts, sex reversal, phyto-androgens, hormone-free aquaculture, growth performance, reproductive success.

Introduction. Red tilapia, *Oreochromis* spp., is the most widely farmed fish in Malaysia today, and its production has a direct impact on the country's economic development. According to Subasinghe (2017), *Oreochromis* spp*.* is the most widely produced fish in the world, and the second most significant farmed fish after carp. Furthermore, El-Zaeem et al (2011) stated that *Oreochromis* spp*.* is and will be a culturally significant species, particularly in tropical developing nations. Male *Oreochromis* spp. are often chosen by *Oreochromis* spp. breeders, just like during the cultivation stage, as they have been proven to be more advantageous due to their increased size and productivity, which saves time.

Several methods are continually employed, including the use of synthetic hormones and herbal extracts, to produce exclusively male *Oreochromis* spp. progeny. The process of producing monosex males (YY), which is frequently employed to revert sexually undifferentiated genetically improved farm *Oreochromis* spp. fry to genotypic males, involves the synthetic hormone 17-methyl testosterone (Matter et al 2024). Hormone-dependent factors are known to pose a risk to both human and environmental health (Supreeda et al 2025). Additionally, the marketing of its feed ingredient and the fishing production process may be impacted if it is used outside of the proper animal husbandry practices. Several studies have brought attention to the advantages of using medicinal herbs as a suitable and possible substitute for chemicals and medications (Green & Kelly 2009; Ghosal & Chakraborty 2014; Gabriel et al 2015; Abaho et al 2022).

Medicinal plants possess antistress, growth-promoting, appetite-stimulating, tonic, immune-stimulating, and antibacterial properties (Citarasu 2010; Chakraborty & Hancz 2011; Ghosal & Chakraborty 2014). When offered orally to animals, they have antifertility and abortifacient characteristics (Obaroh & Chionye-Nzeth 2011). Plant compounds known as phytoestrogens, which include isoflavonoids, flavonoids, lignans, and coumestans, can imitate or disrupt sex hormones. These compounds may suppress estrogen synthesis in gonadal germ cells by acting as aromatase inhibitors or estrogen receptor antagonists (Das et al 2012), making them useful for delaying development or inducing sex reversal in fish.

Given the extensive use of *Oreochromis* spp. and 17-methyltestosterone (MT), understanding safer alternatives is crucial. To induce masculinization, synthetic hormones derived from cholesterol and classified into subgroups can be administered via injection, implantation, or dietary supplementation (Pandi & Sheela 1995; Reis et al 2006; Guedes-Alonso et al 2014). While successful, their continued use emphasizes the significance of herbal-based methods that promote sustainable and health-conscious *Oreochromis* spp. farming.

The goal of this study was to identify existing information gaps and assess the future potential of medicinal herbal extracts as a natural alternative to synthetic hormones for inducing sex reversal in *Oreochromis* spp. aquaculture, with the ultimate goal of increasing productivity, sustainability, and product safety.

Material and Method

Research design and setup. The study's purpose was to use an herbal extract to promote 100% male *Oreochromis* spp. production in a clay pond setting. Furthermore, the herbal extract was compared to the estrogen of super male *Oreochromis* spp. The study was carried out with prior consent at the UNIMAS research partner Honey Aquaculture Farm in accordance with the standard animal ethical standards established by the University Malaysia Sarawak (UNIMAS) Animal Ethical Consent Committee (Ref # UNIMAS/AEC/R/F07/065). For the study, two clay ponds of equivalent size (L 12 m x W 24 m x D 1 m) were used, and three identical hapas (L 1 m x W 2 m x D 1 m) were set up for three replicates under two hormonal treatments (synthetic and Chinese herbal). This research was conducted in four generations, which were F0, F1, F2, and F3. The generation F0 was the *Oreochromis* spp. fish collected from the local river. F1 generations were the offspring of *Oreochromis* spp. obtained from the cross-breeding of wild broods. Only this generation was fed two hormones (synthetic and Chinese herbal extracts). F2 generations were found from the cross-breeding of F1 generations' broods. These generations were fed the commercial different sizes and protein concentrations of *Oreochromis* spp. feed, which were identically pre-starter, starter, and grower. A similar method was applied to get the F3 generation of *Oreochromis* spp. The time duration from F0 to F3 was recorded as 12 months on average.

Collection of wild brood Oreochromis* spp. *and its husbandry. A total of 20 pairs (male and female) of brood *Oreochromis* spp. were bought from the local fishermen in the Tarat River, Serian, Sarawak, Malaysia. The average weight was 150±1.9 g. All these fish were reared with commercial Uni-president® grower *Oreochromis* spp. feed (3mm size) until eggs (Figure 1) in the female *Oreochromis* spp. mouth. It was observed on a regular basis. The eggs were collected from the mouth in gentle handling using a scoop net placed in the water (Figure 1). The first eggs to appear in the first six broods of this investigation were the experimental first batch. It was for all generations. Hatching rate was determined using the following formula described by Belal et al (2012).

Hatching rate (%) = No. of eggs hatched/ Total no. of fertilized eggs x 100

Figure 1. Eggs appeared in the mouth of female brood *Oreochromis* spp. and were collected.

Collection and preparation of synthetic and Chinese herbal extract powder. The synthetic hormone was MT, and it was collected from the local aquaculture shop. The Chinese herbal extract powder is a blend of ten medicinal plant extracts. Hort identified *Sigesbeckia orientalis, Codonopsis pilosula, Citrus aurantium, Citrus sinensis, Aquilaria sinensis, Commiphora myrrha, Fen caopo, Juglans nigra, Ligusticum chuanxiong, and Cistanche deserticola*. All herbal supplies came from a qualified Chinese pharmacy. These herbs are often used in Traditional Chinese Medicine to improve male vitality, sexual function, and overall reproductive health (Bensky et al 2004; Chen & Chen 2004). Several of the identified herbs, particularly Cistanche deserticola and Codonopsis pilosula, are yang-tonifying agents with phytoandrogenic, circulatory, and immunomodulatory properties (Xing et al 2018). Although such formulations are widely used in humans, including cultural beliefs linking their use to increased male fertility and offspring sex outcomes, these effects are primarily based on traditional knowledge rather than validated clinical data (WHO 2007).

The 10 herbal root powders were mixed proportionally equal (1g each), meaning the 10 Chinese herbal mixed powder contained 10 g of each root. A Chinese locally made coating or mixing machine (Manufactured by Shandong Heng Ren Machinery Sdn Bhd) was used to mix 10 Chinese herbal extract powders into *Oreochromis* spp. commercial feeds (pre-starter, starter, and grower, Figure 2).

Figure 2. Fish feed hormone labelled as herbs and estrogen

The 10 Chinese herbals used in this study as a hormone for converting male *Orechromis* spp. are given in Figure 3.

Figure 3. 10 Chinese herbals used in this study as natural hormones.

Validation of nutrients in commercial Oreochromis* spp. *feeds. There were three types of *Oreochromis* spp. fish feeds used in this study. These were starter zero or pre-starter (40% protein), starter (35% protein), and grower (30% protein) *Oreochromis* spp. fish feed, branded by Uni-President. Each diet type (pre-starter, starter, and growth) yielded 200 g of feed. Three independent replications of each diet were collected, yielding three unique 200 g samples per feed type. Each sample was sealed in a clean plastic bag, carefully labeled, and transported to a commercial laboratory (MyCo Sdn Bhd, Malaysia) for proximate composition analysis.

Table 1

Validation of proximate composition (100g) in fish feeds used in this study as a basal

Nutrients	*Pre-starter*		*Starter*		*Grower*	
	Printed	*Validation*	*Printed*	*Validation*	*Printed*	*Validation*
Moisture (g)	11	9.99±1.09	11	10.74±0.59	11	9.82±0.88
Crude protein (g)	40	41.08±1.13	35	34.94±0.84	30	30.49±0.28
Crude lipid (g)	6	5.98±0.49	6	6.07±0.38	6	6.16±0.29
Crude fibre(g)	6	5.79±0.18	6	6.19±0.08	6	7.02±0.73
Ash (g)	16	15.81±0.91	16	16.03±0.43	16	16.19±0.09

Mono male sex identification method. This study was conducted basically in three generations to obtain 100% male *Oreochromis* spp. (Douglas 1990). F0 was the wild generation. Figure 4 shows the flow diagram.

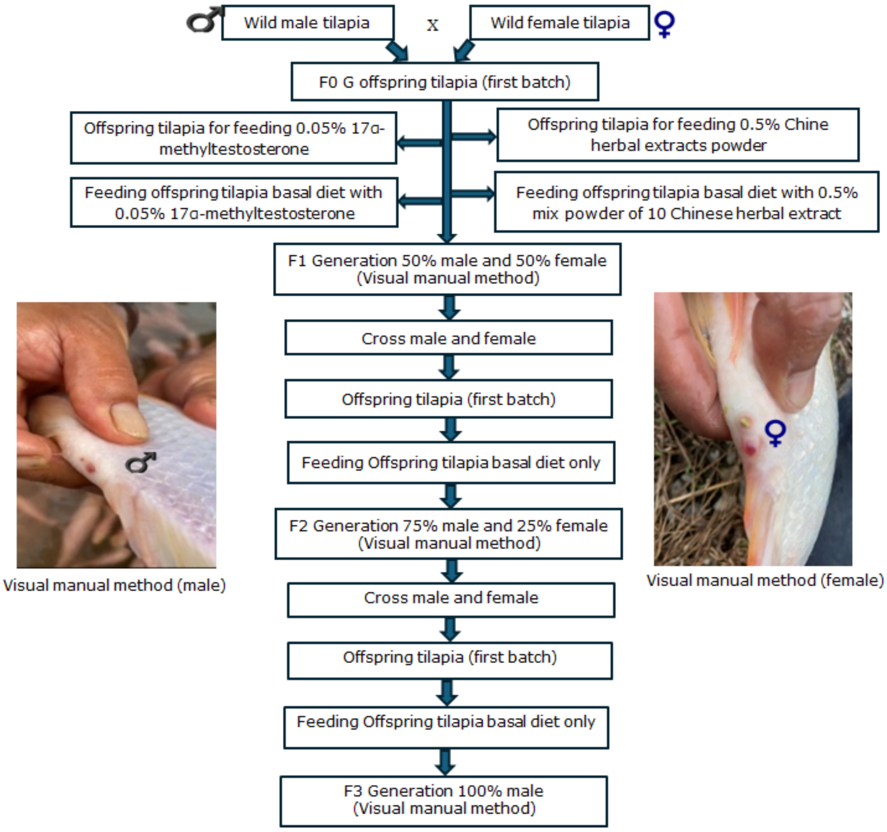

Figure 4. Schematic diagram of 100% mono male *Oreochromis* spp. production.

Physically, the male *Oreochromis* spp. has two orifices under the belly: the anus and the urogenital orifice. Meanwhile, the female has three orifices: the anus, the vaginal pore, and the urine orifice. The anus is always visible, as it is a circular hole. The urogenital orifice is a tiny point. Finally, the female's urine opening is located in a slit perpendicular to her body.

Method of Oreochromis* spp. *fry production. As a maternal mouth breeder, *Oreochromis* spp. releases their eggs when a mature male releases sperm to fertilize them. The female then returns the fertilized eggs to her mouth, where they are incubated until the young can swim on their own, a process known as swim-ups. This process is traditional, and the farmers usually find a lower number of fries directly from the mouth; therefore, this study collected the fertilized eggs directly from the female mouth and incubate to the McDonald's jars, ensuring good quality of water. Following this method, the farmers collected 7 batches of fertilized eggs. The duration of each batch is one week. From F1 to F3 generations in this study, the first batch of eggs was collected from the mouth, and those were transferred to the McDonald's incubation jars. The duration of hatching is an average of 72 hours. The fries from the McDonald's jars were stocked in the larval rearing hapa placed in the ponds labelled with 0.5% MT and 0.5% mix of 10 Chinese herbals.

Growth performance assays and survival rate. The growth performance assay was done in all generations (F0 to F1, F1 to F2, F2 to F3). It consisted of two indicators: length in cm and weight in g. The survival rate was also calculated for each treatment in each generation. It was done by counting the number of eggs, fries, and fingerlings in different events.

Water quality parameters. The water quality parameters measured in this study were water temperature, pH, nitrate, nitrite, ammonia, and turbidity. The API® Freshwater Master Test Kit was used to measure pH, nitrate, nitrite, and ammonia. Water temperature (°C) was measured using portable digital thermometers, while turbidity was measured using a HANNA turbidity portable meter (HI98703) in nephelometric turbidity units (NTU). All water quality parameters were regularly measured twice a day (morning and evening).

Data analysis. One-way analysis of variance (ANOVA) in SPSS (Version 26.0) was used to first assess the data for normality and variance homogeneity. To identify group differences, Duncan's multiple range test was employed. A P-value below 0.05 was considered significant. Every outcome was displayed as mean ± S.E.M.

Results

Water quality parameters. Water quality indicators vary throughout the generational production cycle. Hormones (MT and Chinese herbal extracts) significantly affected water quality measurements, including pH and turbidity (Table 2). *Oreochromis* spp. fish fed with Chinese herbal extracts showed higher water temperatures, nitrate, nitrite, and ammonia levels, but these differences were not statistically significant ($p < 0.05$) when compared to ponds treated with MT.

Table 2

Water quality parameters in *Oreochromis* spp. 1st generation with two hormones

Water quality parameters	*17α-methyltestosterone treated pond water*	*Chinese herbal extracts treated pond water*
Temperature (°C}	27.14±2.11	28.03±1.29
pH	6.53±0.08^{b}	5.45±0.05^{a}
Nitrate (mg L^{-1})	0.65 ±0.03	0.67±0.07
Nitrite (mg L^{-1})	0.30±0.01	0.33±0.03
Ammonia (mg L^{-1})	0.011±0.09	0.014±0.06
Turbidity (NTU)	3.39±3.91^{a}	8.26±1.44^{b}

Superscript showing a significant difference at the level of $p < 0.05$, n=3.

The water quality measurements for the *Oreochromis* spp. fish F1-F2 and F2-F3 generations' rearing ponds showed no significant ($p < 0.05$) variation, except for turbidity during the F1-F2 generation (Table 3).

Table 3

Water quality parameters in *Oreochromis* spp. 2nd and 3rd fish generations without hormones

Water quality parameters	*Fish generation*	*17α-methyltestosterone treated pond water*	*Chinese herbal extracts treated pond water*
Temperature (°C}	F1-F2	27.54±1.21	28.47±0.94
	F2-F3	28.11±0.53	28.24±0.79
pH	F1-F2	6.99±0.49	6.45±0.35
	F2-F3	7.31±0.13	7.41±0.05
Nitrate (mg L^{-1})	F1-F2	0.35±0.17	0.40±0.09
	F2-F3	0.28±0.08	0.31±0.11
Nitrite (mg L^{-1})	F1-F2	0.15±0.08	0.17±0.01
	F2-F3	0.14±0.04	0.15±0.03
Ammonia (mg L^{-1})	F1-F2	0.005±0.03	0.007±0.02
	F2-F3	0.004±0.002	0.005±0.01
Turbidity	F1-F2	1.34±0.21^{a}	1.99±0.14^{b}
	F2-F3	1.18±0.01	1.20±0.04

Superscripts indicate significant differences at $p < 0.05$, n=3; F0 = parental generation, F1 = first generation from F0; F2 = second generation from F1, and F3 = the final generation obtained from F2 male and female crossing.

Fertilized eggs, their hatching and survival rate in generations: The present study collected fertilized eggs from the mouth of the *Oreochromis* spp. female brood. This applied for F0 to F1, F1 to F2, and F2 to F3 generations.

Table 4

Survival status from fertilized eggs to grower in 3 fish generations during the study

Particulars	*17α-methyltestosterone*			*Chinese herbal extracts*		
	Generations			*Generations*		
	F0-F1	*F1-F2*	*F2-F3*	*F0-F1*	*F1-F2*	*F2-F3*
Fertilized eggs	296.7±5.1[a]	315.3±5.5[c]	316.3±7.0[c]	303.3±12.3[ab]	304.0±7.5[b]	313.3±9.1[c]
Hatching fries	258.0±7.2[b]	276.7±6.5[c]	292.3±5.9[d]	244.7±6.0[a]	269.0±5.0[c]	290.0±6.2[d]
Hatching percentage	87.0±3.8[b]	87.7±1.9[c]	92.4±0.9[d]	80.7±2.2[a]	88.5±0.6[c]	92.6±0.7[d]
Fingerlings	223.0±6.6[b]	242.0±7.2[c]	271.7±13.1[d]	193.3±9.5[a]	242.3±5.9[c]	274.0±7.9[d]
Fingerlings survival(%)	86.4±1.6[b]	87.5±1.8[b]	92.9±2.9[c]	79.0±1.9[a]	90.1±1.4[c]	94.5±0.7[d]
Grower	198.3±3.8[b]	220.0±9.5[c]	253.3±16.0[d]	162.3±13.3[a]	224.3±3.1[c]	260.3±8.1[d]
Grower survival rate (%)	89.0±1.1[b]	91.0±5.1[b]	93.2±1.6[b]	83.9±2.9[a]	92.6±1.7[b]	95.0±0.3[c]

Superscripts indicate significant differences at $p < 0.05$, n=3; F0 = parental generation, F1 = first generation from F0; F2 = second generation from F1, and F3 = the final generation obtained from F2 male and female crossing.

The F0-F1 generation from wild broodstock generated considerably fewer eggs ($p < 0.05$) compared to the F1-F2 and F2-F3 generations. This lower performance was consistent across hatching success and survival rates for fry, fingerlings, and growers (Table 4). The F2-F3 generation of broodstock treated with Chinese herbal extract had the highest grower survival rate ($p < 0.05$) compared to the F0-F1 generation. Hormonal supplementation was used exclusively throughout the F0-F1 generation.

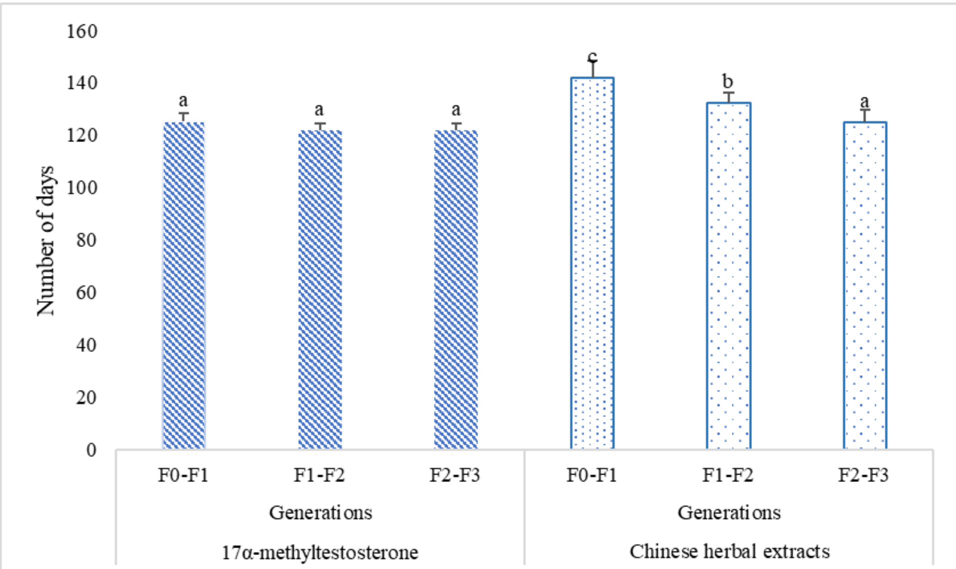

Figure 5. Time span to collect fertilized eggs from the mouth of *Oreochromis* spp. broods in three generations.

Figure 5 shows the time required to obtain fertilized eggs from brood female *Oreochromis* spp., across three generations. In the F0-F1 generation, egg collection was substantially faster ($p < 0.05$) in broods administered MT than in those fed Chinese herbal extracts. There were no significant changes ($p > 0.05$) between the three generations (F0-F1, F1-F2, F2-F3) of the MT treatment or the F2-F3 generation of the Chinese herbal extract treatment. The F0-F1 generation had the longest egg collection time ($p < 0.05$), followed by the F2-F3 generation treated with Chinese herbal extracts.

Sex ratio in different generations. *Oreochromis* spp. treated with MT showed no variation in sex ratio (male:female) across three generations compared to those fed 10 Chinese medicinal preparations (Table 5). The number of males and females changed with each generation. The F0-F1 generation given herbal extracts had the lowest number of males (81), significantly less ($p < 0.05$) than the 100 males in the MT group. There were no significant changes between treatments in the F1-F2 and F2-F3 generations when progeny were fed commercial fish feed.

Table 5

Male and female ratios in different generations

Particulars	*17α-methyltestosterone*			*Chinese herbal extracts*		
	Generations			*Generations*		
	F0-F1	*F1-F2*	*F2-F3*	*F0-F1*	*F1-F2*	*F2-F3*
Grower brood *Oreochromis* spp.	198.3±3.8[b]	220.0±9.5[c]	253.3±16.0[d]	162.3±13.3[a]	224.3±3.1[c]	260.3±8.1[d]
Male *Oreochromis* spp.	100±0.6[b]	165.3±4.3[c]	253.3±9.3[d]	81.3±3.8[a]	168.3±1.5[c]	260.3±4.7[d]
Female *Oreochromis* spp.	98.3±2.2[d]	54.7±1.2[a]	0.0	81.0±3.8[c]	56.0±0.6[b]	0.0
Male (%)	50	75	100	50	75	100
Female (%)	50	25	0	50	25	0

Superscripts indicate significant differences at $p < 0.05$, n=3; F0 = parental generation, F1 = first generation from F0; F2 = second generation from F1, and F3 = the final generation obtained from F2 male and female crossing.

Discussion. This study examined the efficiency of a Chinese herbal extract combination as a natural alternative to MT in producing 100% male *Oreochromis* spp. across three generations (F0-F1, F1-F2, F2-F3). The findings have important implications for growth performance, sustainable *Oreochromis* spp. farming, and hormone-free sex reversal. MT, a synthetic androgen, inhibits normal sex differentiation in *Oreochromis* spp., resulting in phenotypic males from both genetic males (XY) and females (XX) (Robson et al 2023). In contrast, Chinese herbal extracts increase male production by phyto-androgenic action, aromatase inhibition, immunostimulation, and growth promotion (Abaho 2022).

In the F0-F1 generation, the sex ratios were equivalent between treatments, demonstrating that the herbal extract mixture can produce a high proportion of males similar to MT. Initially, the herbal group had fewer males than the MT group, but this difference disappeared in the F1-F2 and F2-F3 generations. Both treatments produced a completely male population (100% males) in the F2-F3 generation, with no females reported in any group. This finding implies that once a male-biased broodstock is formed, future generations retain male dominance even when given commercial diets. The persistence of male bias across generations may be due to genetic or epigenetic impacts linked with sex-reversed brooders, a phenomenon previously described in *Oreochromis* spp. culture systems (Mair et al 1991; Phelps & Popma 2000). Bioactive chemicals in herbs, such as *Cistanche deserticola, Commiphora myrrha,* and *Codonopsis pilosula*, have been related to reproductive control in mammals, supporting their possible androgenic or estrogen-blocking actions (Xing et al 2018).

The survival rate improved throughout generations in both treatments, most likely due to domestication effects and enhanced broodstock quality (Samuels et al 2024; Diakos et al 2025). The F2-F3 generation derived from herbal-treated broodstock had the highest survival rates during egg, fry, fingerling, and grow-out stages, demonstrating that herbal extracts had no negative impacts on early life stages. In contrast, MT has been shown to have a detrimental effect on long-term broodstock fitness and reproductive capacity, making herbal therapies a safer, more sustainable option for multigenerational breeding projects.

During the F0-F1 phase, water quality measurement revealed small changes in pH and turbidity, with herb-treated ponds having slightly lower pH and higher turbidity levels. However, nitrate, nitrite, and total ammonium nitrogen levels remained within safe limits for *Oreochromis* spp. development, and the variations did not persist in the following generations. This is consistent with recent research showing that herbal-based additives are suitable for sustainable *Oreochromis* spp. production without impacting water quality (Fujaya et al 2022).

Herbal supplementation temporarily slowed ovarian development in the F0-F1 generation, causing broodstock to generate fewer viable eggs than the MT-treated and control groups. The herbal mixture's phytoestrogen-like substances most likely reduced ovarian activity by modifying endogenous steroidogenesis and delaying vitellogenesis (Patisaul & Jefferson 2010; Sirotkin & Harrath 2014). This reproductive delay was reduced in later generations, with F2-F3 broodstock laying eggs on a timeline similar to MT-treated fish, suggesting that early suppression of reproduction was reversible and did not impact long-term gametogenesis. Similar trends were documented in investigations when phytoestrogens' endocrine-modulating effects were transient (Skinner 2014; Farooq et al 2025).

Sex ratios did not alter significantly between MT and herbal treatments after three generations. During the initial F0-F1 exposure, the herbal group generated fewer males (81) than the MT group (100), indicating that herbal bioactive substances have milder androgenic effects than synthetic hormones (Citarasu 2010; Chakraborty & Hancz 2011). Following the cessation of food exposure, both F1-F2 and F2-F3 generations had 75% males and 100% males, respectively. This suggests that masculinization was a transitory phenotypic impact caused by food intake rather than a genetically fixed feature. The reversibility of sex ratios is consistent with previous findings that endocrine manipulation, whether by phytochemicals or synthetic androgens, usually results in transitory phenotypic changes rather than stable genetic sex modifications (Citarasu 2010; Chakraborty et al 2014). Overall, herbal supplements displayed equivalent masculinization efficacy to MT across multiple generations while promoting growth, survival, and reproductive success with no long-term physiological or environmental side effects. Herbal bioactive substances offer a safer, sustainable alternative to synthetic hormones by integrating growth promotion, immunostimulation, and sex reversal in *Oreochromis* spp. cultivation (Citarasu 2010).

Conclusions. A mixture of 10 Chinese herbal extracts demonstrated potential as an alternative to MT in producing 100% male *Oreochromis* spp. in the F3 generation. The initial reproductive turnover was decreased, but subsequent generations had equivalent or improved sex ratios, growth, and survival. These results show a promising herbal-based method for aquaculture, but further investigations on safety, efficacy, and protocol modification are needed before broad adoption.

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Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:
Thian Hai Chung, Department of Aquatic Resource Science and Management, Faculty of Resource Science and Technology, University Malaysia Sarawak, Datuk Mohd Musa Road, 94300 Kota Samarahan, Sarawak, Malaysia; Honey Aquaculture, Lot 115, BLK 4, Sentah Segu Land District, BT 13, Kuching Street, Serian, 94200 Kuching, Sarawak, Malaysia; VL Ocean Services, Lot 886, Sungai Lalang Street, Batu 27, 43500 Semenyih, Selangor, Malaysia, e-mail: alexthianhoney@gmail.com
Mazzaellynn Thomas, Department of Aquatic Resource Science and Management, Faculty of Resource Science and Technology, University Malaysia Sarawak, Datuk Mohd Musa Road, 94300 Kota Samarahan, Sarawak, Malaysia, e-mail: mazzaellynn@gmail.com
Mohammad Bodrul Munir, Department of Aquaculture, Faculty of Agriculture, University Islam Sultan Sharif Ali, Tutong Road Km 33, TB1741 Tutong, Sinaut, Brunei Darussalam, e-mail: mohammad.munir@unissa.edu.bn
Roslianah Asdari, Department of Aquatic Resource Science and Management, Faculty of Resource Science and Technology, University Malaysia Sarawak, Datuk Mohd Musa Road, 94300 Kota Samarahan, Sarawak, Malaysia, e-mail: aroslianah@unimas.my
Md. Abdul Hannan, Department of Aquatic Animal Health Management, Sher-e-Bangla Agricultural University, Sher-Bangla Nagar, 1207 Dhaka, Bangladesh, e-mail: sohagbsmrau@gmail.com

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