Acta Ichthyologica et Piscatoria 55, 2025, 227–235 | DOI 10.3897/aiep.55.161420

PENSOFT

ACTA ICHTHYOLOGICA ET PISCATORIA

Feminization of a saline-tolerant tilapia hybrid, UPV SpiN (*Oreochromis spilurus* × *O. niloticus*) (Actinopterygii, Cichliformes, Cichlidae) using 17β-Estradiol: Effects of dose and administration method

Chris Jan P. DINAGA[1*], Ande Bryle N. GENCIANA[1*], Alan A. FAILAMAN[1], Maria Alexandra G. FONTANILLA[1], Regie A. ESTRADA[1], Emelyn Joy G. MAMELOCO[1], Victor Marco Emmanuel N. FERRIOLS[1,2], Rex Ferdinand M. TRAIFALGAR[1], Sanny David P. LUMAYNO[1]

1 *Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines*

2 *Philippine Genome Center Visayas, University of the Philippines Visayas, Miagao, Iloilo, Philippines*

https://zoobank.org/6713A80D-D79A-4192-9843-27AC2CCCD023

Corresponding author: Sanny David P. Lumayno (splumayno@up.edu.ph)

Academic editor: Bakhtiyor Sheraliev ♦ **Received** 7 June 2025 ♦ **Accepted** 13 August 2025 ♦ **Published** 15 September 2025

Citation: Dinaga CJP, Genciana ABN, Failaman AA, Fontanilla MAG, Estrada RA, Mameloco EJG, Ferriols VMEN, Traifalgar RFM, Lumayno SDP (2025) Feminization of a saline-tolerant tilapia hybrid, UPV SpiN (*Oreochromis spilurus* × *O. niloticus*) (Actinopterygii, Cichliformes, Cichlidae) using 17β-Estradiol: Effects of dose and administration method. Acta Ichthyologica et Piscatoria 55: 227–235. https://doi.org/10.3897/aiep.55.161420

Abstract

Application of feminization techniques using estrogen hormones can be relevant in cases where there is an insufficient supply of female stock for broodstock production. This study aimed to evaluate the feminization efficiency (FE) of 17β-estradiol (E2) at various concentrations using two different methods applied to the saline-tolerant SpiN (*Oreochromis spilurus* × *O. niloticus*) tilapia hybrid developed by the University of the Philippines Visayas (UPV). E2 concentrations of 0, 50, 100, and 200 mg · kg^{-1} were orally administered through dietary supplementation for 28 days (d) to one batch after yolk-sac absorption. Another batch, still with yolk-sacs, was immersed in E2 solutions at concentrations of 0, 50, 100, 200, and 400 µg · L^{-1} for 5 d. Soft dorsal fin tissues from phenotypically identified female tilapia were collected upon sexual maturation and processed for DNA extraction, PCR, and gel electrophoresis to determine the genotype. Selective removal of the first six dorsal spines was conducted to generate up to 63 unique codes for temporary fish identification. Treatments 2 (50 mg · kg^{-1}) and 3 (100 mg · kg^{-1}) resulted in the highest FE for dietary supplementation at 94.74% and 95.24%, respectively, while feminization through larval immersion was observed only in Treatment 3 (100 µg L^{-1}) at an efficiency of 16.67%. Neither feminization method significantly affected the survival of the fish. These results demonstrate that dietary supplementation with 50–100 mg · kg^{-1} of E2 is an effective and reliable method for feminizing UPV SpiN tilapia hybrid. PCR-based genotyping confirmed the conversion of genetic males (XY) into phenotypic females. This approach offers a practical strategy to enhance broodstock management, particularly in the development of all-female stocks for breeding. Future studies should focus on evaluating the growth and reproductive performance of sex-reversed individuals for long-term broodstock viability.

Keywords

Dietary supplementation, feminization efficiency, larval with yolk sac immersion, molecular sex markers, *Oreochromis spilurus* male × *O. niloticus* female hybrid, sex genotyping

* These authors contributed equally to this work.

Copyright *Dinaga et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Tilapia aquaculture is a significant industry in the Philippines, with the commodity ranking third in production volume among cultured fishery species (BFAR 2023), and Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), being the most dominantly produced species (Guerrero 2019). As a freshwater fish, Nile tilapia faces challenges due to limited water availability for freshwater aquaculture. To address this, expansion efforts focus on developing saline-tolerant hybrids and strains, as well as exploring the potential for brackish-water rearing and mariculture to maximize available resources and increase production (Jumah et al. 2016). Through crossbreeding *Oreochromis spilurus* (Günther, 1894) males with *O. niloticus* females (Fernandez 2003), the University of the Philippines Visayas (UPV) developed a saline-tolerant tilapia hybrid that has since been the subject of recent studies (Intoy and Traifalgar 2021; Huervana et al. 2022). The salinity tolerance is attributed to *O. spilurus*, while *O. niloticus* contributes traits associated with rapid growth (Fernandez 2003). Currently known as UPV SpiN, this hybrid is being cultured at the UPV multi-species hatchery for research and potential future commercial production.

Monosex culture is a common practice in tilapia farming to prevent unwanted reproduction and mitigate the negative effects of overstocking, such as disease development, increased competition, and growth regression (Abdel Fattah et al. 2020; Mwainge et al. 2021). All-male tilapia production is usually preferred due to their faster growth rates and larger size, which command a higher market value compared to females (Arriesgado et al. 2011). Sex reversal using androgen hormones to convert genetic females into phenotypic males is widely employed for this purpose (Celik et al. 2011).

However, a critical component of hatchery operations is the production and rearing of broodstock, which generate new fry for continuous culture. In this context, female tilapia is especially valuable, as fry quality and quantity are largely dependent on female breeders, particularly on indices such as maternal size, gonadosomatic index, and fecundity (Mohamed et al. 2013). Various sex ratios, such as 2–4:1 (Ly et al. 2021) and even up to 7:1 (Mashaii and Rajabipour 2022) female-to-male tilapia, can be adopted for breeding; it is important to note that more females are required relative to males. Furthermore, strategies to increase seed yield and ensure the production of high-quality eggs and fry include implementing rest periods between spawning cycles and replacing broodstock with conditioned and rested female breeders (Eguia and Eguia 2007). These considerations highlight the need for a consistent supply of female breeders, which can be achieved through feminization using estrogen hormones to meet the demand for female tilapia dedicated to breeding.

Estrogen hormones used for tilapia feminization include 17β-estradiol (E2), estradiol valerate, and 17α-ethinylestradiol (EE2), with E2 being the most commonly used due to its natural origin and ease of metabolism and excretion (Hoga et al. 2018). These hormones can be administered via three methods: dietary supplementation, immersion, or direct injection, with the first two being more practical for commercial use (Piferrer 2001). While feminization techniques have been previously applied in Nile tilapia, this study is the first to evaluate the efficacy of E2 for feminizing the UPV SpiN hybrid.

Specifically, this study aimed to evaluate the feminization efficiency of E2 at different concentrations using two administration methods (dietary supplementation and larval immersion) on the UPV SpiN tilapia hybrid. Additionally, the study sought to assess the effects of these treatments on survival rates to determine the most effective and practical approach for producing all-female broodstock dedicated to sustainable tilapia breeding programs.

Materials and methods

Swim-up fry and larvae (with yolk-sac) production. Male and female broodstock were randomly selected from the UPV SpiN population, in which sexes were maintained separately at 10–15 ppt salinity at the Multi-species Hatchery of the University of the Philippines Visayas, Miagao, Iloilo, Philippines. A total of 27 females (mean body weight [MBW] = 531.35 ± 136.33 g) and eight males (MBW = 511.44 ± 91.75 g) were used. Prior to conditioning, the selected broodstock were gradually acclimated to freshwater (0 ppt) at a rate of 5 ppt per day and maintained under these conditions for approximately one week. Females and males were then conditioned separately in freshwater for 14 days and subsequently paired in eight 1-ton tanks at female-to-male ratios of 3:1 and 4:1. For the dietary supplementation batch, tanks were checked daily for swim-up fry starting 14 days after pairing. For the larval immersion batch, larvae with visible yolk sacs were collected daily from the mouths of female broodstock beginning 10 days after pairing. Collected swim-up fry and larvae with yolk sac were acclimatized in tanks maintained at temperature 28–32°C, pH 6.5–9.0, and dissolved oxygen (DO) 5–6 ppm before being subjected to feminization.

Feminization through dietary supplementation. Four experimental diets containing increasing levels of E2 were prepared by dissolving the appropriate amount of hormone in 200 mL of ethanol per kilogram of feed. The hormone solution was sprayed onto and thoroughly mixed with commercial tilapia fry feed (≥42% crude protein, ≥8% crude fat, =5% crude fiber, =16% crude ash, =12% moisture, =0.25 mm particle size). The treated feed was then sieved and placed in a fume hood to allow ethanol evaporation for at least 72 h before use. The inclusion levels of E2 were as follows: Treatment 1 (control, 0 mg · kg^{-1}), Treatment 2 (50 mg · kg^{-1}), Treatment 3 (100 mg · kg^{-1}), and Treatment 4 (200 mg · kg^{-1}). A total of 480 Day-0 tilapia swim-up fry were randomly distributed into 12

culture tanks (180 L each), with three replicate tanks per treatment and 40 fry per tank. The tanks operated under a flow-through system with a flow rate of 100 mL · min^{-1}. The feeding trial lasted for 28 d. Feeding rates were adjusted weekly based on weight data. Fry were fed ad libitum six times daily from days 0–7. From days 8–14, feeding was 20% of mean body weight (MBW), six times daily. From days 15–28, feeding was reduced to 15% MBW, administered four times daily to match the fry's growth and feeding efficiency. Daily siphoning and partial water changes were conducted, while water quality parameters (temperature, DO, pH, ammonia, and salinity) were monitored three times per week. During the experimental period, the mean water temperature was 27.84 ± 0.09°C, pH 8.65 ± 0.03, DO 5.62 ± 0.04 ppm, ammonia 0.05 ± 0.01 mg · L^{-1}, and salinity 0.00 ± 0.00 ppt. All values are presented as mean ± standard deviation (SD). After the trial, fish from each culture tank were transferred to 1-ton tanks per replicate for grow-out rearing. Salinity was gradually increased at a rate of 2 ppt per day until reaching 20 ppt, and subsequently maintained within the range of 10–20 ppt. A commercial diet (≥28% crude protein, ≥6% crude fat, ≥5% crude fiber, =12% crude ash, =12% moisture, ≥1.50 mm particle size) at ≥3% MBW without hormones was used until the fish reached 100–150 g, at which point, manual phenotypic sex sorting and genotyping were conducted.

Feminization through larval (with yolk-sac) immersion. Five treatments of increasing E2 dosage were set up by mixing the corresponding amount of hormone in plastic containers holding 10 L of freshwater. Concentrations of E2 were: Treatment 1 (control, 0 µg · L^{-1}), Treatment 2 (50 µg · L^{-1}), Treatment 3 (100 µg · L^{-1}), Treatment 4 (200 µg · L^{-1}), and Treatment 5 (400 µg · L^{-1}). To accommodate all treatments with three replicates each, 15 containers were prepared, with 30 larvae with yolk-sac stocked per container, totaling 450 tilapia larvae. The tilapia larvae were immersed for 5 d until the yolk sac was entirely absorbed and were then fed with commercial tilapia fry feed (≥42% crude protein, ≥8% crude fat, =5% crude fiber, =16% crude ash, =12% moisture, =0.25 mm particle size) for about 28 days at ≥15% MBW without hormones. Water quality parameters were not measured; however, daily siphoning and partial water changes were carried out to maintain rearing conditions. To promote faster growth, the tilapia were then transferred to 1-ton tanks each replicate and were fed with a commercial diet (≥28% crude protein, ≥6% crude fat, ≥5% crude fiber, =12% crude ash, =12% moisture, ≥1.50 mm particle size) at ≥3% MBW until the fish reached 100–150 g for manual phenotypic sex sorting and genotyping.

Survival and feminization parameters. The survival rate (SR [%]), phenotypic female ratio (P_{fe} [%]), and feminization efficiency (FE [%])—also referred to as sex-reversal efficiency or male-to-female conversion rate—were determined using data obtained during sampling. The following formulas were used to calculate these respective indices:

$$SR = 100(N_{FF} \times N_{IF}^{-1})$$

$$P_{fe} = 100(N_{fe} \times N_{IF}^{-1})$$

$$FE = 100(N_{XYfe} \times N_{TXY}^{-1})$$

where, N_{IF} is initial number of fish, N_{FF} is the final number of fish, N_{fe} is the total number of phenotypic females, N_F is the total number of fish, N_{XYfe} is the number of phenotypic females with XY genotype, and N_{TXY} is the total number of all fish with XY genotype (including phenotypic males).

Sex identification through PCR-based detection of sex-specific genetic markers. Prior to genotypic sex identification, samples were first classified based on external phenotypic sex characteristics. Male tilapia displays two openings located anterior to the anal fin: the anus and a single urogenital pore. In contrast, the female possesses three distinct openings: anus, genital pore, and urinary pore (Setthawong et al. 2024). Fin tissue samples were extracted from the soft dorsal rays of phenotypic female UPV SpiN tilapia hybrids. A sedation tank and a recovery tank were prepared prior to fin extraction. Tilapia sampled were sedated with a solution of 3 mL of phenoxyethanol per 10 L of water. Soft dorsal fins weighing 25–30 mg were collected from UPV SpiN phenotypic females after they were anesthetized. Fin extraction was performed by selectively excising the first six dorsal spines to assign a unique identifier to each tilapia sample (e.g., _23456, 1_3_56, 12_ _ _6), where missing numbers indicate the excised fin positions. This exision pattern can generate up to 63 unique identification codes (Supplementary Fig. 1). Fig. 1 shows representative examples of the coding scheme employed. Following excision, the fish were immediately transferred to a recovery tank. Using a modified Wizard® Genomic DNA Purification Kit (Promega) protocol, tissue samples were digested in a lysis buffer with Proteinase K and incubated overnight at 65°C to digest. Samples were treated with RNase A, followed by protein precipitation and DNA isolation using isopropanol. DNA pellets were washed with 70% ethanol, air-dried, and rehydrated. DNA purity and concentration were assessed using a NanoDrop spectrophotometer, with acceptable 260/280 ratios between 1.8 and 2.0. Final DNA extracts were stored at –20°C for further analysis.

A 10 µL PCR reaction mix was prepared using 1 µL of DNA template (300 ng · µL^{-1}), 5 µL GoTaq® Colorless Master Mix, 0.5 µL each of forward and reverse primers, and 3 µL nuclease-free water. PCR cycling conditions included initial denaturation at 94°C (3 min), 34 cycles of denaturation (94°C, 30 s), annealing (53.8°C, 30 s), extension (72°C, 1.5 min), and final extension at 72°C (10 min). PCR amplicons were separated using 1% agarose gel electrophoresis, stained with GelRed, and visualized under a

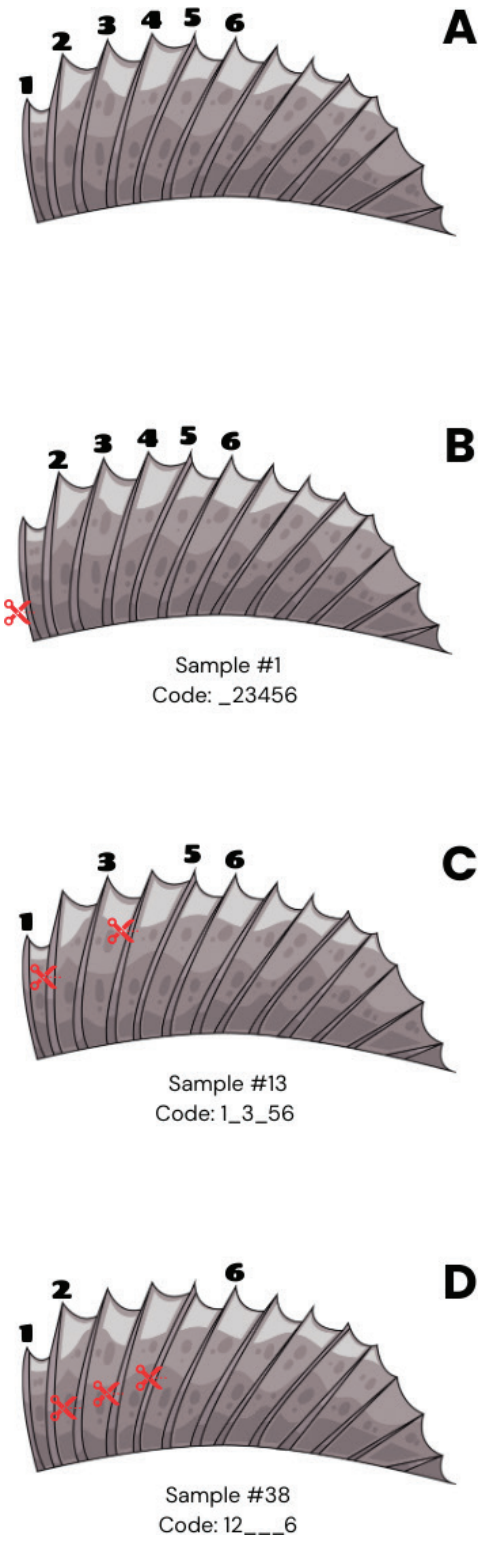

Figure 1. Representative examples of the fin excision coding scheme used to uniquely identify UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*) samples. (**A**) The first six dorsal spines were assigned numbers 1–6, respectively; excision of selected spines generates a unique identifier for each sample. (**B**) Sample #1; Code: _23456 (spine 1 excised). (**C**) Sample #13; Code: 1_3_56 (spines 2 and 4 excised). (**D**) Sample #38; Code: 12_ _ _6 (spines 3–5 excised). Selective removal of the first six dorsal spines produces up to 63 unique codes, with missing numbers indicating excised spines.

UV Gel Documentation System. This study used the Marker-5 primer set (Jiang et al. 2022), which targets the major sex-determining gene *amhy* located on linkage group 23 (LG23), for genotyping Nile tilapia strains. Bands were measured based on the expected amplicon sizes generated by the specific primers used. The Marker-5 primer pair (forward: 5′–ATGGCTCCGAGACCTTGACTG; reverse: 5′–CAGAAATGTAGACGCCCAGGTAT) specifically amplifies fragments of approximately 1422 bp from the X chromosome and 982 bp from the Y chromosome.

Statistical analysis. Data were reported as mean ± SD of the mean. Following the Shapiro–Wilk test and Levene's test to check for the data's normality and homogeneity of variance, one-way analysis of variance (ANOVA) was used to determine, separately, if a significant difference ($P < 0.05$) exists between treatments of each batch. For significantly different data, Tukey HSD multiple comparison post-hoc test was then conducted to determine which treatments are different.

ARRIVE 2.0 Reporting Compliance. The study was conducted and reported in accordance with the ARRIVE 2.0 (Animal Research: Reporting of in vivo experiments) guidelines, which promote transparency, reproducibility, and completeness in reporting animal research methodologies (Percie du Sert et al. 2020).

AI assistance. Portions of the manuscript, including language and grammar refinement, were revised with the assistance of ChatGPT (OpenAI 2025 version). All intellectual content and analysis remain the responsibility of the authors.

Results

Survival rate and phenotypic female ratio. UPV SpiN fry subjected to dietary supplementation with E2 exhibited high survival rates (99.17%–100%), with no significant differences observed among treatments ($P > 0.05$), indicating that the E2 concentrations did not adversely affect survival.

In terms of the phenotypic female ratio (P_{fe}), dietary supplementation of E2 resulted in a significantly higher proportion of females compared to the untreated control ($P < 0.05$), as shown in Table 1. The control group produced only 49.44% females, while all hormone-treated groups showed a marked increase. Treatment 2 yielded the highest P_{fe} at 97.94%, followed by Treatment 3 at 97.28% and Treatment 4 at 93.97%. However, no significant difference ($P > 0.05$) was found among the three E2-treated groups despite the differences in E2 concentrations.

Similarly, UPV SpiN larvae (with yolk sacs) treated with E2 via immersion exhibited high survival rates during both periods. Survival ranged from 85.56% to 100% during the 5-day immersion period with E2, and slightly decreased to 83.33% to 98.89% during the subsequent culture period without E2, likely due to accumulated mortality. No significant differences in survival were observed among treatments in either period ($P > 0.05$).

Table 1. Survival rate (SR) [%], and phenotypic female ratio (P_{fe}) [%] of UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*) after treatment with estradiol-17β (E2) through dietary supplementation.

Treatment	Survival rate [%]		Phenotypic female ratio [%]
	Day 0–28 (with E2)	Day 29–75 (no E2)	
1 (0 mg · kg^{-1})	100 ± 0.00^{a}	99.17 ± 0.58^{a}	49.44 ± 16.51^{a}
2 (50 mg · kg^{-1})	99.17 ± 1.44^{a}	96.64 ± 1.53^{a}	97.94 ± 1.79^{b}
3 (100 mg · kg^{-1})	99.17 ± 1.44^{a}	98.33 ± 1.15^{a}	97.28 ± 2.58^{b}
4 (200 mg · kg^{-1})	100 ± 0.00^{a}	100 ± 0.00^{a}	93.97 ± 6.44^{b}

Values are presented as mean ± standard deviation (SD). Within the same column, means with different superscript letters are significantly different ($P < 0.05$).

However, in terms of the P_{fe}, the treated groups (Treatments 2–5) did not differ significantly from the control (Treatment 1), even at the highest observed value of 72.84% in Treatment 2 (Table 2).

Table 2. Survival rate (SR) [%], and phenotypic female ratio (P_{fe}) [%] of UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*) after treatment with estradiol-17β (E2) through larval (with yolk-sac) immersion.

Treatment	Survival rate [%]		Phenotypic female ratio [%]
	Day 1–5 (with E2)	Day 6–60 (no E2)	
1 (0 µg · L^{-1})	100 ± 0.00	98.89 ± 1.92	65.91 ± 13.82
2 (50 µg · L^{-1})	98.89 ± 1.92	98.89 ± 1.92	72.84 ± 10.90
3 (100 µg · L^{-1})	97.78 ± 1.92	97.78 ± 1.92	64.00 ± 12.17
4 (200 µg · L^{-1})	85.56 ± 13.47	83.33 ± 17.32	69.62 ± 11.36
5 (400 µg · L^{-1})	95.56 ± 5.09	93.33 ± 5.77	71.02 ± 1.63

Values are presented as mean ± standard deviation (SD). No significant differences were observed across all treatments ($P > 0.05$).

Sex identification through PCR-based detection of sex-specific genetic markers and feminization efficiency [%]. Sex identification was performed using PCR amplification targeting sex-specific genetic markers, which successfully produced two distinct bands: as shown in Figs 2, 3. DNA bands were separated on 1% w/v agarose gel, with a 1 kb DNA ladder as the molecular weight reference. Samples displaying both bands were identified as XY females, whereas those exhibiting only the ~1422 bp band were classified as XX females. XY individuals exhibiting phenotypic female characteristics were classified as sex-reversed and included in the calculation of FE.

As shown in Table 3, all three E2-treated groups exhibited high FE, ranging from 88.71% to 95.24%. The presence of multiple double bands in Treatment 3 (Fig. 2) provides additional support for the feminization of genetically male (XY) individuals, confirming phenotypic sex-reversal induced by the treatments.

In contrast to the other hormone administration method, feminization via the larval (yolk-sac) immersion approach showed limited success, with only a few XY individuals exhibiting phenotypic sex reversal (Fig. 3). Among the immersion treatments, only Treatment 3 resulted in successful feminization (Table 4).

Discussion

The presently reported study demonstrated that dietary supplementation of E2 is an effective method for inducing feminization in the saline-tolerant UPV SpiN tilapia hybrid. As shown in Table 1, significant differences ($P < 0.05$) in phenotypic female ratio were observed between the control and all E2-treated groups,

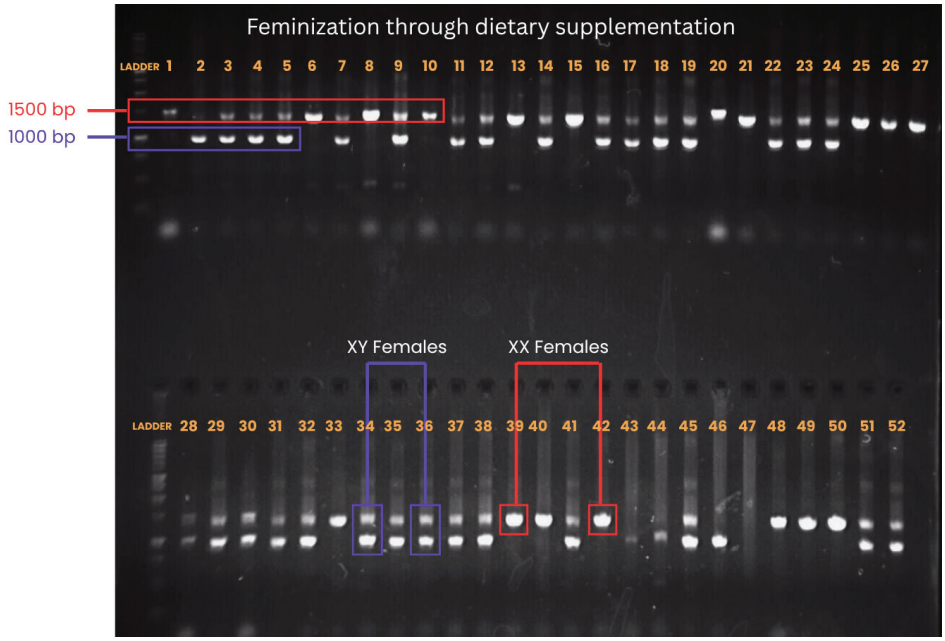

Figure 2. Representative PCR products from the feminization experiment of UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*) via dietary supplementation (Treatment 3), visualized under UV using a Gel Doc system on a 1% agarose gel. A 1 kb DNA ladder was used as a molecular size reference.

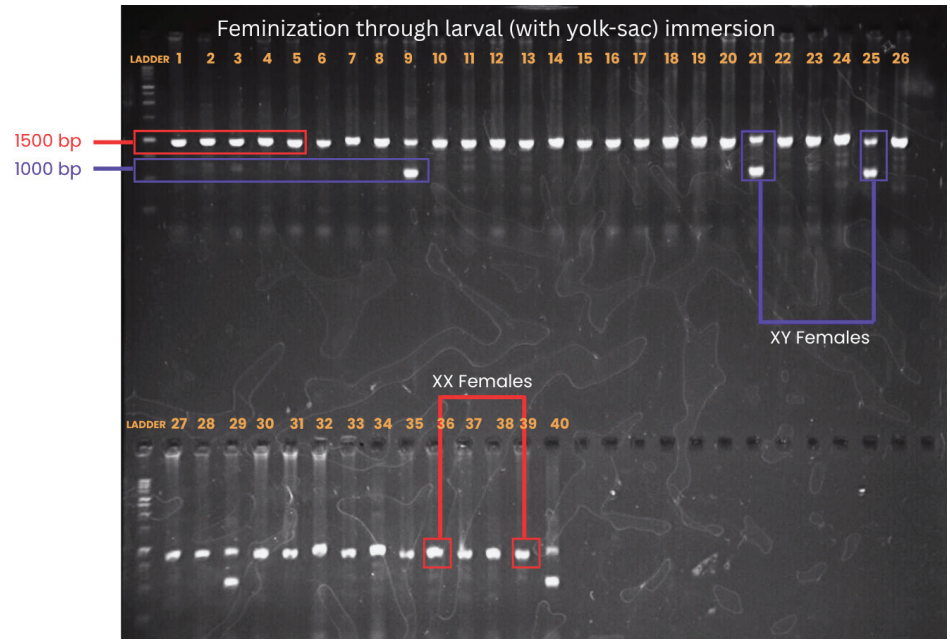

Figure 3. Representative PCR products from the larval (yolk-sac) immersion feminization experiment (Treatment 3) of hybrid tilapia (*Oreochromis spilurus* × *O. niloticus*), visualized under UV light on a 1% agarose gel, with a 1 kb DNA ladder used as a molecular size reference.

Table 3. Phenotypic sex ratios and feminization efficiency (FE) of UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*) from the batch treated with varying 17β-estradiol (E2) through dietary supplementation for 28 days. Shown are the number of surviving individuals at the time of PCR-based genotypic sex determination, phenotypic sex counts, total phenotypic females confirmed by PCR, and number of XY individuals exhibiting a female phenotype. Each treatment was replicated three times with an initial sample size of 40 per replicate.

Treatment	Replicates	Initial sample size	Survival count (at the time of PCR)	Phenotypic male number	Phenotypic female number	Female survival count (at the time of PCR)	Phenotypic female with XY genotype	Feminization efficiency [%]
	1	40	22	12	10			
1 (0 mg · kg^{-1})	2	40	37	12	25	47	—	—
	3	40	34	22	12			
	1	40	34	1	33			
2 (50 mg · kg^{-1})	2	40	29	0	29	88	36	94.74
	3	40	31	1	30			
	1	40	33	1	32			
3 (100 mg · kg^{-1})	2	40	35	0	35	100	60	95.24
	3	40	39	2	37			
	1	40	39	5	34			
4 (200 mg · kg^{-1})	2	40	38	2	36	101	55	88.71
	3	40	40	0	40			

regardless of concentration. Even at the lowest dose of 50 mg · kg^{-1}, the treated group showed a markedly higher proportion of phenotypic females (97.94 ± 1.79%) compared to the control (49.44 ± 16.51%). The highest female ratio was achieved at 50 mg · kg^{-1} (97.94%), with a marginally lower ratio observed at 100 mg · kg^{-1} (97.28%). Importantly, feminization efficiency was calculated as the proportion of genetically male (XY) individuals that appeared as phenotypic females. Only individuals genotyped as XY were included in the FE calculation to ensure that observed feminization resulted from successful sex reversal rather than natural sex ratio variability. The use of molecular sex markers thus provided a robust assessment of the true impact of hormonal treatment.

Among the dietary treatments, FE was highest at 100 mg · kg^{-1} (95.24%), indicating that a large proportion of genetic males were successfully feminized. The treatment with 50 mg · kg^{-1} also showed a high FE of 94.74%, while 200 mg kg^{-1} did not result in further improvement. These results suggest that 100 mg · kg^{-1} is an optimal dose, achieving high feminization while avoiding unnecessary excess hormone use. This plateau in response aligns with findings in other fish species, such as *Oreochromis mossambicus* (Peters, 1852) (see Hekimoğlu et al. 2019) and *O. niloticus* (see Alcántar-Vázquez et al.

Table 4. Phenotypic sex ratios and feminization efficiency (FE) of UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*) from the batch treated with estradiol-17β (E2) through larval (with yolk-sac) immersion for five days. Shown here are the number of surviving individuals at the time of PCR-based genotypic sex determination, phenotypic sex counts, total phenotypic females confirmed via PCR, and number of XY individuals exhibiting a female phenotype. Each treatment was replicated three times with an initial sample size of 30 per replicate.

Treatment	Replicates	Initial sample size	Survival count (at the time of PCR)	Phenotypic male number	Phenotypic female number	Female survival count (at the time of PCR)	Phenotypic female with XY genotype	Feminization efficiency [%]
	1	30	22	6	16			
1 (0 μg · L^{-1})	2	30	18	9	9	46	—	—
	3	30	28	7	21			
	1	30	28	11	17			
2 (50 μg · L^{-1})	2	30	25	6	19	54	0	0
	3	30	22	4	18			
	1	30	24	12	12			
3 (100 μg · L^{-1})	2	30	20	6	14	44	5	16.67
	3	30	25	7	18			
	1	30	17	6	11			
4 (200 μg · L^{-1})	2	30	23	4	19	46	0	0
	3	30	26	10	16			
	1	30	26	8	18			
5 (400 μg · L^{-1})	2	30	21	6	15	54	0	0
	3	30	29	8	21			

2015). Similar patterns were also observed in other aquaculture species like *Silurus glanis* Linnaeus, 1758 (see Król et al. 2014) and *Penaeus* (*Fenneropenaeus*) *merguiensis* De Man, 1888 (see Ikhwanuddin et al. 2019), where hormone concentrations exceeding a certain threshold did not improve feminization outcomes and, in some cases, resulted in adverse effects such as intersex development, morphological anomalies, and reduced survival (Hunter et al. 1986; Melard 1995; George and Pandian 1996; Piferrer 2001). On the other hand, E2 administration through yolk-sac larval immersion proved to be ineffective for feminization of the UPV SpiN tilapia hybrid. As presented in Table 2, although survival rates remained high across all treatments during and after the 5-d immersion period (85.56%–100%), no significant differences ($P > 0.05$) were observed in the phenotypic female ratio or feminization efficiency among treatments. FE remained low or absent across all immersion treatments, with only the 100 mg · L^{-1} group showing a low efficiency of 16.67%. This discrepancy can be attributed to several factors, including the short exposure duration, early developmental stage, and physiological limitations in hormone uptake at the yolk-sac stage.

This finding contrasts with previous studies, such as Kim et al. (2001), where *Silurus asotus* Linnaeus, 1758 exhibited a dose-dependent response to E2 immersion, although their exposure duration was longer (15 d). Similarly, Gennotte et al. (2014) reported that EE2 immersion in Nile tilapia embryos induced feminization, but the efficiencies varied widely depending on the age at treatment and exposure duration. The absence of significant feminization in the present study may therefore be attributed to the short exposure time, physiological limitations of hormone uptake at early larval stages, or species-specific differences in hormonal sensitivity (Piferrer 2001; Gennotte et al. 2014).

While phenotypic sex assessment is practical, it can sometimes be confounded by ambiguous external characteristics or developmental variability, leading to potential misclassification (Baroiller et al. 1999; Piferrer 2001; Devlin and Nagahama 2002). The use of molecular sex markers with PCR-based confirmation provides stronger evidence of E2's effectiveness in converting genetic males (XY genotype) into phenotypic females. Confirming the presence of XY genotypes among phenotypic females not only validates the accuracy of the feminization process but also broadens its practical applications. To facilitate accurate matching of samples during genotyping, a simple yet effective identification system was employed. By clipping dorsal fin spines in distinct patterns, the need for electronic PIT tags or dye marks was eliminated. The excised spines heal naturally within a couple of weeks, but since DNA extraction and analysis are completed within a few days after sampling, the temporary mark is sufficient to maintain identity until genotyping is finished. This innovative, low-cost approach ensures that even in large batch breeding experiments, each specimen's test result can be confidently matched to the correct individual. In particular, the development of all-female broodstock offers a strategic advantage in increasing fry production, which is critical in addressing the gradual decline in tilapia supply observed in recent years (Guerrero 2019; BFAR 2023). Furthermore, identifying and selecting sex-reversed XY females is a critical step in producing YY supermales, which can be used to generate all-male progeny without the need for continued hormone treatment. Thus, the integration of molecular sex determination into feminization protocols not only enhances the reliability of sex control strategies but also supports a range of applications in tilapia aquaculture, from efficient broodstock development to sustainable monosex culture systems.

Conclusion

PCR-based sex identification confirmed the successful conversion of genetic males into phenotypic females, validating the reliability of the feminization protocol. Given the saline tolerance of the UPV SpiN tilapia hybrid, this feminization protocol presents a valuable broodstock management strategy for aquaculture in regions affected by seawater rise and salinity intrusion resulting from the effects of climate change. The production of all-female stocks through feminization can help increase fry output, addressing the gradual decline in tilapia production under challenging environmental conditions. Additionally, the protocol shows potential application in YY-male technology. Comprehensive evaluations of growth and reproductive performance in sex-reversed XY females compared to normal XX females are needed to ensure their suitability for sustainable breeding programs in saline-affected areas.

Acknowledgments

We gratefully acknowledge the research funding support of the Department of Science and Technology – Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development. We also thank the individuals involved in the phenotypic sex sorting, particularly John Michael Moleta, Reynaldo Nuñeza, and Ryan Pagsugiron. We also acknowledge the UPV Office of the Vice Chancellor for Research and Extension for providing the APC support grant.

References

Abdel Fattah AF, Ahmed FA, Saleem AY, Mohammed HH, Youssef MI, Said EN (2020) Effect of the different stocking density on behavior, performance and welfare of the Nile tilapia (*Oreochromis niloticus*). Egyptian Journal of Aquatic Biology and Fisheries 24(5): 539–560. https://doi.org/10.21608/ejabf.2020.109369

Alcántar-Vázquez JP, Rueda-Curiel P, Calzada-Ruíz D, Antonio-Estrada C, Moreno-de la Torre R (2015) Feminization of the Nile tilapia *Oreochromis niloticus* by estradiol-17β: Effects on growth, gonadal development and body composition. Hidrobiológica 25(2): 275–283.

Arriesgado DM, Vicente HJ, Vicente DA (2011) Production of male *Oreochromis niloticus* GET Excel tilapia by egg immersion in methyl testosterone hormone. Journal of Environment and Aquatic Resources 2: 21–33. https://doi.org/10.48031/msunjear.2011.02.03

Baroiller JF, Guiguen Y, Fostier A (1999) Endocrine and environmental aspects of sex differentiation in fish. Cellular and Molecular Life Sciences 55(6–7): 910–931. https://doi.org/10.1007/s000180050344

BFAR [Bureau of Fisheries and Aquatic Resources] (2023) Chapter 4: Aquaculture production. In: 2022 Philippine Fisheries Profile. BFAR, Quezon City.

Celik I, Guner Y, Celik P (2011) Effect of orally administered 17α-Methyltestosterone at different doses on the sex reversal of the Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758). Journal of Animal and Veterinary Advances 10(7): 853–857. https://doi.org/10.3923/javaa.2011.853.857

Devlin RH, Nagahama Y (2002) Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. Aquaculture (Amsterdam, Netherlands) 208(3–4): 191–364. https://doi.org/10.1016/S0044-8486(02)00057-1

Eguia RV, Eguia MR (2007) Tilapia broodstock and hatchery management. Aquaculture Extension Manual No. 38. SEAFDEC/AQD, Tigbauan, Iloilo, Philippines.

Fernandez RA (2003) Saline-tolerant tilapia being developed. Philstar. Global. https://www.philstar.com/business/agriculture/2003/09/21/221474/saline-tolerant-tilapia-being-developed

Gennotte V, Mélard C, D'Cotta H, Baroiller JF, Rougeot C (2014) The sensitive period for male-to-female sex reversal begins at the embryonic stage in the Nile tilapia and is associated with the sexual genotype. Molecular Reproduction and Development 81(12): 1146–1158. https://doi.org/10.1002/mrd.22436

George T, Pandian TJ (1996) Hormonal induction of sex reversal and progeny testing in the zebra cichlid *Cichlasoma nigrofasciatum*. Journal of Experimental Zoology 275: 374–382. https://doi.org/10.1002/(SICI)1097-010X(19960801)275:5%3C374::AID-JEZ6%3E3.0.CO;2-M

Guerrero RD III (2019) Farmed tilapia production in the Philippines is declining: What has happened and what can be done. Philippine Journal of Science 148(2): 11–15.

Hekimoğlu MA, Koyakomanda KCK, Fırat MK, Süzer C, Engin S, Sayğı H, Özden O, Güleç F, Saka Ş (2019) Effects of 17β-estradiol hormone on growth and sex differentiation in the Mozambique tilapia (*Oreochromis mossambicus*, Peters, 1852). Aquatic Sciences and Engineering 33(4): 118–123. https://doi.org/10.26650/ASE2018500686

Hoga CA, Almeida FL, Reyes FG (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. https://doi.org/10.1080/19476337.2018.1475423

Huervana FH, Dionela CS, de la Torre ED, del Castillo CS, Traifalgar RF (2022) Utilization of marine diatom *Thalassiosira weissflogii* as a feed additive in seawater-tolerant Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758) strain. Frontiers in Sustainable Food Systems 6: 1052951. https://doi.org/10.3389/fsufs.2022.1052951

Hunter GA, Solar II, Baker IJ, Donaldson EM (1986) Feminization of coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) by immersion of alevins in a solution of estradiol-17β. Aquaculture (Amsterdam, Netherlands) 53(3–4): 295–302. https://doi.org/10.1016/0044-8486(86)90360-1

Ikhwanuddin M, Bahar H, Ma H, Manan H (2019) Effect of estrogen hormone, 17β-estradiol on feminization of banana shrimp, *Penaeus merguiensis* (de Man, 1888) postlarvae and the identification of the age of external sex differentiation. Aquaculture Reports 13: 100177. https://doi.org/10.1016/j.aqrep.2018.100177

Intoy MM, Traifalgar RF (2021) Feed value of fermented copra meal as a sustainable feed ingredient in the diet of saline-tolerant Nile tilapia *Oreochromis niloticus* (Linnaeus 1758). Journal of Sustainability

Science and Management 16(8): 28–43. https://doi.org/10.46754/jssm.2021.12.003

Jiang D-N, Kuang Z-Y, Yang K-S, Huang Y-Q, Mustapha UF, Guo X-Z, Zheng D-F, Dong Z-D, Shi H-J (2022) Polymorphism in a sex-linked DNA marker located on LG23 in Hainan strain of Nile tilapia (*Oreochromis niloticus*). Journal of the World Aquaculture Society 53(1): 205–223. https://doi.org/10.1111/jwas.12815

Jumah YU, Traifalgar RF, Monteclaro RC, Jumah DS, Mero FF (2016) Influence of hyperosmotic culture conditions on osmoregulatory ions, gill chloride cells and Na^+/K^+-ATPase activity of Nile tilapia, *Oreochromis niloticus*. Aquaculture, Aquarium, Conservation and Legislation 9(3): 498–506.

Kim DS, Cho HJ, Bang IC, Choi GC, Nam YK (2001) Effects of immersion of larvae in oestradiol-17β on feminization, structural changes of gonad and growth performance in the Far Eastern catfish *Silurus asotus* (Linnaeus). Aquaculture Research 32(5): 323–328. https://doi.org/10.1046/j.1365-2109.2001.00559.x

Król J, Pobłocki W, Bockenheimer T, Hliwa P (2014) Effect of diethylstilbestrol (DES) and 17 β-estradiol (E2) on growth, survival and histological structure of the internal organs in juvenile European catfish *Silurus glanis* (L.). Aquaculture International 22: 53–62. https://doi.org/10.1007/s10499-013-9664-3

Ly MA, Ngom A, Fall AB, Diouf O (2021) Broodstock sex ratio improves fry production in Nile tilapia (*Oreochromis niloticus*) in Northern Senegal. International Journal of Advanced Research 9(2): 72–76. https://doi.org/10.21474/IJAR01/12419

Mashaii N, Rajabipour F (2022) A review on the breeding of Nile tilapia, *Oreochromis niloticus* in brackish water hatchery, Iran. International Journal of Aquatic Research and Environmental Studies 2(2): 29–45. https://doi.org/10.52547/injoar.2.2.29

Melard C (1995) Production of a high percentage of male offspring with 17α-ethynylestradiol sex-reversed *Oreochromis aureus*. I. Estrogen sex-reversal and production of F2 pseudofemales. Aquaculture (Amsterdam, Netherlands) 130(1): 25–34. https://doi.org/10.1016/0044-8486(94)00313-D

Mohamed AH, Traifalgar RF, Serrano AE (2013) Maternal size affects fecundity of saline-tolerant tilapia *Oreochromis mossambicus* (Peters) in freshwater tanks. Annals of Biological Research 4(3): 138–142.

Mwainge VM, Ogwai C, Aura CM, Mutie A, Ombwa V, Nyaboke H, Oyier KN, Nyaundi J (2021) An overview of fish disease and parasite occurrence in the cage culture of *Oreochromis niloticus*: A case study in Lake Victoria, Kenya. Aquatic Ecosystem Health and Management 24(1): 43–55. https://doi.org/10.14321/aehm.024.01.08

Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H (2020) The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biology 18(7): e3000410. https://doi.org/10.1371/journal.pbio.3000410

Piferrer F (2001) Endocrine sex control strategies for the feminization of teleost fish. Aquaculture (Amsterdam, Netherlands) 197(1–4): 229–281. https://doi.org/10.1016/S0044-8486(01)00589-0

Setthawong P, Khemthong M, Lertwanakarn T, Surachetpong W (2024) Ultrasonography for non-invasive sex identification and reproductive assessment in Nile tilapia (*Oreochromis niloticus*). Frontiers in Veterinary Science 11: 1467158. https://doi.org/10.3389/fvets.2024.1467158

Supplementary material 1

Sixty-three unique dorsal fin clipping codes generated from selective removal of the first six dorsal spines in UPV SpiN tilapia hybrid (*Oreochromis spilurus* × *O. niloticus*) samples

Authors: Chris Jan P. Dinaga, Ande Bryle N. Genciana, Alan A. Failaman, Maria Alexandra G. Fontanilla, Regie A. Estrada, Emelyn Joy G. Mameloco, Victor Marco Emmanuel N. Ferriols, Rex Ferdinand M. Traifalgar, Sanny David P. Lumayno

Data type: pdf

Explanation note: Each pattern represents a distinct temporary identification code for individual fish.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/aiep.55.161420.suppl1