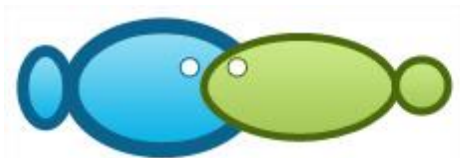

Effects of dietary dopamine on growth, feminization, and gonadal maturity in all-male *Macrobrachium rosenbergii* (De Man, 1879) post-larvae

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Abstract. The production of all-male populations of giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879), is highly desirable to maximize commercial aquaculture yields. A commercially viable approach to achieving this involves generating homogametic neo-females that can be crossed with normal males to produce 100% male progeny. This study evaluated the efficacy of dietary dopamine (DA) supplementation on growth performance, feminization rate, and gonadal development in an exclusively male postlarval (PL) cohort. PLs were fed diets containing 0, 45, 60, 75, and 90 mg DA kg^{-1} for 36 days, followed by a 156-day post-treatment phase. Throughout the trial, physical and chemical water quality parameters were carefully monitored and maintained within optimal ranges to completely preclude potential environmental bias. Dietary DA supplementation induced dose-dependent feminization, achieving a maximum sex reversal rate of 20.8% in the 90 mg kg^{-1} treatment group, compared with 0% in the control group ($p < 0.05$). The administration of DA did not significantly impact somatic growth or survival. Detailed evaluations confirmed that key performance indicators, including final body weight, weight gain, specific growth rate, and feed conversion ratios, remained statistically comparable across the primary treatment phases ($p > 0.05$). Furthermore, histological analysis of near-mature gonads demonstrated that DA-treated neo-females underwent typical oogenesis, possessing mature oocytes and gonadosomatic index (GSI) values (7.27-7.97%) statistically indistinguishable from wild-caught reference females (7.80%). While whole-body tissue accumulation of DA remained largely undetectable, these results confirm that short-lived dietary administration of DA during early PL development effectively generates reproductively viable neo-females without hindering commercial growth metrics. Ultimately, this method serves as a well-tolerated, non-invasive strategy for producing neo-female broodstock, which is essential for maintaining profitable, all-male populations in the aquaculture industry.
Keywords: dopamine, giant freshwater prawn, histological analysis, neo-female, sex reversal.

Introduction. The giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879), is a globally critical aquaculture species with substantial economic importance in Vietnam's Mekong Delta (New 2002; New et al 2010; Day et al 2022). The industry values this species for its rapid growth, short culture cycle, large harvest size, and high profitability (Day et al 2022; Rahman et al 2022). Consequently, global production more than doubled between 2000 and 2020, reaching approximately 294,000 tones (FAO,

2022). This sustained expansion of *M. rosenbergii* farming plays a pivotal role in advancing the socio-economic development of rural aquaculture communities.

Despite its commercial success, pronounced sexual dimorphism remains a major challenge in *M. rosenbergii* aquaculture. Males exhibit significantly faster growth rates and achieve larger harvest sizes than females (Sagi & Aflalo 2005; Ohs et al 2006b). Furthermore, upon reaching sexual maturity, female somatic growth slows considerably as dietary energy is diverted from muscle accretion to reproductive development (Megawati et al 2020; Day et al 2022). Although male populations develop complex social hierarchies with distinct morphotypes (blue claw, orange claw, and small males), all-male stocks consistently generate superior yields, average weights, and profitability compared to mixed-sex or all-female cohorts (Sagi & Aflalo 2005; Rahman et al 2022). Consequently, implementing sex control to establish all-male cultures is highly desirable for optimizing commercial productivity (Sagi et al 1995; Ventura et al 2012).

Traditionally, sex reversal in *M. rosenbergii* is achieved by manipulating the androgenic gland (AG) or associated genes during early development (Sagi & Aflalo, 2005; Ventura et al 2012; Wahl et al 2023). However, hormonal induction has recently emerged as an effective and accessible tool for sex reversal across diverse aquatic taxa (Ohs et al 2006a; Rasheed et al 2023; Wang et al 2025). Administered during critical differentiation windows, these treatments successfully decouple phenotypic expression from underlying genotypic sex (Piferrer 2001; Wang et al 2025). Hormonal monosex production relies on either the direct method, continuous administration to the target culture, or the indirect method, which breeds functionally sex-reversed broodstock (Sagi & Aflalo 2005; Wang et al 2008). For *M. rosenbergii*, the indirect method is the most commercially viable approach. It involves feminizing genetic males (ZZ) into phenotypic "neo-females", which, when mated with normal males (ZZ), yield 100% all-male (ZZ) progeny (Malecha et al 1992; Sagi & Aflalo, 2005). Furthermore, this strategy produces a uniform all-male crop while guaranteeing that prawns harvested for human consumption avoid direct hormonal exposure (Dewi et al 2006; Rasheed et al 2023).

Successfully producing neo-females necessitates hormonal intervention during a specific window of early postlarval (PL) development, before sexual differentiation is permanently established. Although steroid hormones have historically been explored, biogenic amines and neuroregulators, particularly dopamine (DA), have proven highly effective for feminizing crustaceans such as *M. rosenbergii* (Ohs et al 2006a). Male differentiation in this species depends on the AG and its hormone secretion (Sagi & Aflalo 2005; Ohs et al 2006a). Dietary dopamine (DA) functions as a neurotransmitter that disrupts this pathway by simultaneously stimulating gonad-inhibiting hormone (GIH) release from the X-organ-sinus gland complex and inhibiting gonad-stimulating hormone (GSH). This dual action effectively suppresses AG development, thereby halting male differentiation and inducing the development of ovaries and female secondary sexual characteristics (Ohs et al 2006a).

Extensive research has investigated how DA delivery methods and treatment timing affect the success of feminization. DA is typically administered via bio-encapsulation in live *Artemia* nauplii or incorporated into artificial diets using alcohol evaporation; importantly, the exact timing of this PL treatment critically determines sex reversal outcomes (Ohs et al 2006a; Minh et al 2011). A significant constraint in existing research on *M. rosenbergii* feminization is the widespread reliance on mixed-sex experimental cohorts. The lack of a confirmed baseline sex ratio at stocking introduces confounding variables that obscure assessments of hormonal efficacy. This vulnerability to initial population composition is demonstrated by untreated control groups, which display natural sex-ratio fluctuations ranging from 26 to 65% female (Ohs et al 2006a; Rasheed et al 2023). Current literature reveals a notable lack of studies on all-male PL. Using monosex male cohorts facilitates a more rigorous evaluation of feminization rates, as emergent female phenotypes can be directly attributed to the hormonal treatment, effectively eliminating the confounding effects of natural sex-ratio variation.

Previous research indicates that feminization rates in *M. rosenbergii* PL are enhanced by administering higher DA doses over prolonged periods (Ohs et al 2006a; Minh et al 2011). While these foundational studies successfully evaluated macroscopic

metrics, such as growth performance, survival rates (SR), and external sex ratios, they neglected to investigate the underlying histological changes associated with gonadal development (Ohs et al 2006a; Minh et al 2011). Because the structural and cellular transformations within the gonads during DA treatment remain uncharacterized, and previous authors have explicitly called for histological examinations to confirm the presence and developmental stages of internal gonads following treatment (Ohs et al 2006a).

Therefore, this study aims to investigate the impacts of dietary DA fortification on growth metrics, feminization rate, and gonadal development in *M. rosenbergii* all-male PL. By incorporating detailed histological analyses alongside production metrics, this research aims to provide a comprehensive understanding of DA's physiological impacts, ultimately contributing to the optimization of neo-female production for the commercial aquaculture industry.

Material and Method

Ethics statement. This research complied with the national ethical guidelines for animal experimentation in Vietnam. The use of *M. rosenbergii* was authorized by the Animal Ethics Committee of Nong Lam University, Ho Chi Minh City (Approval No. 250618).

Broodstock management and larviculture. The experimental trials were conducted between December 2023 and July 2024 at the Research Center for Aquatic Biotechnology (RECAB) of the Vietnam Academy of Fishery Sciences in Ho Chi Minh City (formerly Research Institute for Aquaculture No. 2). The biological material consisted of all-male PL sourced from neo-female broodstock. These neo-females, which were genotypically male, were produced via functional sex reversal following microsurgical ablation of the AG during the juvenile phase. Upon reaching sexual maturity, the neo-females were crossed with normal males, yielding a progeny population that was 100% genotypically male. Selected broodstock, comprising neo-females and normal males, were maintained in composite tanks integrated with recirculating aquaculture systems (RAS) and continuous aeration. Water temperature was regulated between 28 and 30°C. The feeding protocol consisted of a twice-daily regimen: a morning ration of fresh feed (including marine fish, mollusk tissue, and beef liver) at 5-10% of body weight, followed by an afternoon ration of formulated pellets (38-42% crude protein) at 3-5% of body weight. Gravid females were transferred to specialized hatching tanks until the embryos reached the grey stage. Post-hatching, nauplii were reared in brackish water (salinity 12-14‰) under vigorous aeration and periodic water exchange. Nutritional requirements were met using *Artemia* and stage-specific microencapsulated diets. Following a 25-30-day larval period, metamorphosis into PLs occurred, with the resulting PLs harvested and gradually acclimated to freshwater conditions prior to the initiation of the experimental trial.

Diet preparation. Dopamine hydrochloride (DA; purity ≥ 97.5%, Sigma-Aldrich, St. Louis, MO, USA) was utilized for the experimental treatments. The formulation and proximate composition of the basal diet are provided in Table 1. To ensure uniform dispersion and mitigate hormone leaching during aquatic administration, a specific mixing protocol was employed for the four treatment diets (45, 60, 75, and 90 mg kg^{-1} DA). The required quantities of DA were first completely dissolved in water and subsequently blended into the sieved dry basal ingredients. A gelatin-astaxanthin matrix was integrated into the mixture to serve as a binding agent. The homogenized compound was then processed via cold microextrusion-spheronization to generate pellets in three distinct sizes, accommodating the ontogenetic shifts in the PL feeding capabilities throughout the 36-day trial. A hormone-free control diet was manufactured using the identical procedure.

To ensure the stability of the bioactive compound and prevent UV-induced degradation, the finalized pellets were dried at 40°C for 24 hours and subsequently stored in airtight, light-resistant containers at 4°C. Furthermore, to analytically validate the hormone concentrations and confirm stability throughout the feeding period,

representative feed samples from all groups were collected on days 0, 12, 24, and 36 (n = 1 per group). These samples were immediately preserved at -80°C for subsequent quantification via liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Table 1

Formulation and proximate composition of the experimental diets

Ingredient name	*Ingredient (%)*
Hydrolyzed fish meal	30
Fish meal 65% protein	15
Soy protein concentrated	25
Milk powder	3
Egg powder	6
Wheat flour	10
Dicalcium phosphate	0.7
Vitamin and mineral premix	0.6
Stay C 35%	0.1
Gelatine	3
Fish oil	4
Astaxanthin	0.1
Filler	2.5
Proximate composition	% of dry matter
Crude protein	45.1±1.3
Crude lipid	13.9±1.0

Feminization experimental design. The trial utilized a single batch of all-male *M. rosenbergii* PL1 with an initial mean body weight of 6.50 mg. A completely randomized experimental design was applied, comprising four hormone dosage treatments (45, 60, 75, and 90 mg DA kg^{-1}) and a control, with four replicates per group. A total of 500 PL1 were randomly allocated into each of the twenty 100-L conical composite tanks. During the 36-day trial, manual feeding occurred four times daily. The baseline feeding rate of 1 g per 500 PL1 was adjusted daily based on the prawns' ad libitum intake. Cannibalism was mitigated by introducing nylon substrates, and continuous aeration ensured dissolved oxygen (DO) >5.0 mg L^{-1}. Tanks were maintained under a natural photoperiod and siphoned every two days to remove feces and unconsumed feed. Water quality was monitored, with temperature, pH, and DO recorded twice daily (07:00 and 16:00 h), and total ammonia nitrogen (TAN) and nitrite assessed once weekly. All parameters were maintained within optimal thresholds for the species (temperature: 28-30°C; pH: 7.3-7.8; DO: >5.0 mg L^{-1}; TAN: 0-0.22 mg L^{-1}; nitrite: 0-1.70 mg L^{-1}).

To evaluate DA accumulation, subsamples of 100, 40, and 20 *M. rosenbergii* per tank were collected on days 12, 24, and 36, respectively. Sampled individuals underwent a 12-hour starvation period to empty their digestive tracts before being frozen at -80°C for later hormone analysis. These specimens were subsequently excluded from survival analyses.

Following the 36-day period, tanks were drained through a sieve to collect all surviving juveniles. Surviving *M. rosenbergii* were counted and weighed to calculate survival rate (SR), final body weight (FBW, W_t), weight gain (WG), and specific growth rate (SGR). Survival and growth metrics were calculated as described by Ricker (1975) as follows:

$$SR(\%) = \frac{N_t}{N_0 - N_s} \times 100$$

$$WG(g\ ind^{-1}) = W_t - W_0$$

$$SGR(\%\ day^{-1}) = \frac{lnW_t - lnW_0}{t} \times 100$$

Metrics were derived using the initial stocking density (N_0), harvest count (N_t), number of sampled *M. rosenbergii* (N_s), initial mean weight (W_0), and trial duration (t = 36 days). The feed conversion ratio (FCR) was calculated as the ratio of total dry feed consumed to total wet biomass gained.

Post-feminization experiment. Following the initial feminization phase, *M. rosenbergii* growth across all experimental groups was evaluated over a 156-day period. All individuals from each 100 L conical tank were restocked into every 3 m³ circular tank, with four replicates per treatment. *M. rosenbergii* was fed to apparent satiation three times daily using a commercial juvenile diet (50% crude protein, 10% crude fat) without DA supplement. The baseline feeding rate was set at 5% of the total tank biomass and adjusted daily based on observed feed consumption. To accurately determine the FCR, daily feed intake was calculated as the difference between the mass of the remaining feed collected after the final daily feeding and the total daily feed allocated.

Husbandry and water management protocols closely followed the previously described procedures, with minor temporal adjustments for the post-treatment phase. Temperature, pH, and dissolved oxygen (DO) were measured twice daily (07:00 and 16:00 h). TAN and nitrite concentrations were assessed weekly. Throughout this post-feminization phase, all environmental parameters remained well within the optimal physiological limits for *M. rosenbergii* (temperature: 30-31°C; pH: 7.1-7.9; DO > 5.0 mg L^{-1}; TAN: 0.00-0.05 mg L^{-1}; nitrite: 0.00-0.12 mg L^{-1}).

On day 62 of the post-feminization period, the sex ratio within each treatment group was evaluated through the macroscopic examination of primary and secondary sexual characteristics (Tombes & Foster 1979; Shen et al 2020). Specifically, following established morphological criteria, male individuals were identified by the presence of male genital pores on the coxopodite of the fifth pair of pereiopods, alongside a distinct, rod-shaped, protruding accessory, the appendix masculina, situated at the inner edge of the second pleopods. Conversely, phenotypic females were identified by the presence of genital pores at the coxopodite of the third pereiopods, a wider distance between the fifth pereiopods, and the complete absence of the male appendage.

Following this comprehensive sex identification, all surviving individuals were counted and weighed to calculate SR, FBW, WG, and SGR, following the previously detailed equations. All identified juvenile males were subsequently removed from the experiment. The remaining phenotypic females were maintained in their respective 3-m³ circular tanks throughout the trial. To assess the efficacy of the treatments, the feminization rate was calculated as the exact proportion of phenotypic females relative to the total surviving population on day 62 (Rasheed et al 2023).

$$\text{Feminization rate}(\%) = \frac{number\ of\ female\ prawn}{total\ number\ of\ sexed\ prawn} \times 100$$

At the conclusion of the 156 days experiment, all remaining female of *M. rosenbergii* were harvested, counted, and weighed to finalize the evaluations of SR, FBW, WG, and SGR.

Gonadal maturity and histological analysis. To determine the gonadosomatic index (GSI) and oocyte diameter, three DA-treated females approaching sexual maturity (Stage III/IV) were randomly sampled from each treatment. The selected prawns were euthanized utilizing a thermal shock method (ice bath). Ovaries were subsequently excised, weighed to calculate the GSI (%) = $[W_{ovary} / W_{body}] \times 100$, and sectioned into 1-2 mm fragments. The non-sampled prawns were retained in the rearing tanks until ovulation was observed. To establish a reliable baseline for GSI comparisons, three wild-caught females of comparable body weight were obtained from the Dong Nai River, Vietnam (10°52'55"N, 106°50'34"E). These reference specimens were maintained under standard broodstock conditions until they achieved full ovarian maturation, providing a control against which the DA-treated cohorts could be evaluated.

For histological evaluation, extracted gonadal tissues were immersed in Davidson's fixative (formulated with 33 mL ethanol 95%, 22 mL formaldehyde 100%, 11.5 mL glacial acetic acid, and 33.5 mL distilled water) at a 1:10 tissue-to-fixative volume ratio for a minimum of two hours. Following fixation, the samples were transitioned into 50% ethanol and processed for manual paraffin embedding according to the established procedures of Bell and Lightner (1988). The paraffin blocks were sectioned at a thickness of 5-6 μm utilizing a VIP 5 Jr microtome (Sakura Finetek, Tokyo, Japan). Sections were subsequently mounted onto glass slides and subjected to a five-minute Hematoxylin and Eosin (H&E) staining protocol using a Shandon Varistain 24-4 automated slide stainer (Thermo Scientific, Waltham, MA, USA). Based on the morphological criteria outlined by Meeratana and Sobhon (2007), oocyte development was categorized into five distinct stages: early previtellogenic (Oc1), late previtellogenic (Oc2), early vitellogenic (Oc3), late vitellogenic (Oc4), and mature oocytes (mOc). Morphometric analysis was conducted under a light microscope (Nikon Eclipse E200MV R, Tokyo, Japan) at magnifications ranging from 10× to 40×. For each defined developmental phase, 30 randomly selected oocytes with intact, clearly visible nuclei were measured using an ocular micrometer. Oocyte dimensions are reported as the mean diameter±standard deviation (SD).

Hormone analysis. To verify the DA concentrations in feed and *M.rosenbergii* tissue, sampling was conducted on days 0, 12, 24, and 36. Briefly, three of 500 PLs at the initial time point and 100, 40, and 20 juveniles from each replicate tank were randomly sampled on days 12, 24, and 36, respectively, immediately euthanized on ice, and whole-body prawns were homogenized. Feed and *M. rosenbergii* tissue samples were snap-frozen in liquid nitrogen and stored at -80°C until analysis. Then, homogenized tissue (0.2 g) or feed (2.0 g) samples were extracted in a solvent mixture containing 5 mL hexane and 20 mL acetonitrile/water (ACN/H_2O, 1:1, v/v, with 0.1% formic acid). The suspensions were vortex-mixed and sonicated for 20 minutes to facilitate complete extraction. Post-centrifugation, 1.0 mL of the lower phase was diluted to 10 mL with the acidified ACN/H_2O solution, vortexed, and filtered through a 0.22 μm PTFE membrane.

The LC-MS/MS analysis was performed at the Center of Analytical Service, Experimentation and Standards, Metrology, Quality of Ho Chi Minh City, Vietnam. Instrumental analysis was conducted using a Thermo TSQ Endura triple quadrupole mass spectrometer with positive electrospray ionization (ESI^+) in multiple reaction monitoring (MRM) mode. Chromatographic separation was achieved on a reversed-phase C8 column (150 × 4.6 mm, 5 μm). A 15-minute mobile phase gradient was applied using water containing 0.1% formic acid (Solvent A) and pure acetonitrile (Solvent B). The gradient started at 10% B, ramped to 100% B at 9 minutes, and was held for 2 minutes before re-equilibration. The MRM transitions utilized for DA quantification were m/z 154.1 to 137.0 and 154.1 to 94.1. The analytical method achieved a limit of detection (LOD) for DA of 1 mg kg^{-1} for feed matrices and 10 μg kg^{-1} for *M. rosenbergii* tissues.

Statistical analysis*.* The initial stocking density was established at 500 PL/tank. During the experiment, 160 *M. rosenbergii* per tank were removed for evaluating DA accumulation on days 12, 24, and 36. This resulted in a final culture density of 340 PL/tank at the end of the trial. To ensure this sampling did not confound the statistical analysis of growth performance, SR was calculated by excluding the intentionally sampled individuals from the mortality count. Additionally, the weight of the sampled prawns was added to the final tank biomass to accurately calculate the FCR. Because the same number of individuals was removed from each replicate tank across all treatments, the reduction in density remained uniform and did not introduce any statistical bias in density-dependent growth.

DA concentrations below the LOD were assigned a value of LOD/2 (US EPA, 2000). Prior to statistical analysis, all data were evaluated for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. To satisfy the assumptions of parametric testing, percentage data were arcsine-square root transformed where necessary, though original unadjusted means are reported for clarity. A one-way repeated-measures ANOVA was used to evaluate the effects of dietary DA

dosage and sampling time on growth indices, FCR, SR, and tissue DA levels, whereas GSI differences were analyzed using a standard one-way ANOVA. Significant differences between group means ($p < 0.05$) were identified using Fisher's Least Significant Difference (LSD) post-hoc test (Gomez & Gomez 1984). These analyses were conducted using IBM SPSS Statistics v22.0 (IBM Corp., Armonk, NY), and results are expressed as mean±standard deviation (SD) or standard error (SE).

The effect of DA supplementation on feminization rates was analyzed employing a binomial generalized linear model (GLM) with a logit link function in R version 4.3.3 (R Core Team, 2023). To resolve the complete separation issue caused by the total absence of females in the control group (0/684), a 0.5 continuity correction was applied to the count data to ensure model stability. Finally, the Holm-Bonferroni method was utilized to adjust p-values and control the family-wise error rate associated with multiple testing (Agresti 2002).

Results

Dietary DA concentrations and tissue bioaccumulation in postlarvae. The analysis of dietary DA concentrations confirmed the relatively successful incorporation of the hormone into the experimental feeds, with detected levels increasing proportionally with the formulated doses (Table 2). Whilst DA concentrations in the control diet were below the LOD, confirming the absence of exogenous hormone in the basal formulation. In contrast, the control diet exhibited no baseline DA concentration. Analysis of *M. rosenbergii* tissues on days 12, 24, and 36 revealed no dose-dependent bioaccumulation of DA across the experimental groups, and concentrations in the treatment groups did not differ significantly from those in the control group throughout the 36-day treatment period ($p > 0.05$).

Table 2

Concentrations of dopamine in feed and *Macrobrachium rosenbergii* postlarval following dietary supplementation with varying DA levels throughout the experimental treatment period

Treatment	*Feed (mg* kg^{-1}*)*	*Postlarvae tissue (μg kg^{-1})*		
		Day 12	*Day 24*	*Day 36*
Control	ND[†]	6.75±3.50[a]	ND[a]	9.98±9.95[a]
45 DA mg kg^{-1}	31.95±2.31[‡]	ND[a]	ND[a]	ND[a]
60 DA mg kg^{-1}	50.88±2.14	ND[a]	ND[a]	ND[a]
75 DA mg kg^{-1}	63.53±3.69	ND[a]	ND[a]	ND[a]
90 DA mg kg^{-1}	77.10±3.93	6.53±3.05[a]	ND[a]	ND[a]

Note: DA: Dopamine; ND: Not detectable; values represent the mean±SD (n = 4). Mean values in the same column with different superscripts differ significantly (results from one-way repeated measure ANOVA and LSD test, $p < 0.05$).

Growth performance, feed efficiency, and survival rates. Dietary DA supplementation did not significantly affect overall growth parameters (FBW, WG, and SGR) across any evaluation period (Table 3). Feed conversion ratios (FCR) and SR were similarly unaffected during the initial 36-day treatment phase ($p > 0.05$). However, significant differences emerged in the post-treatment phases. At Day 98, survival was significantly reduced in the 90 mg kg^{-1} DA group (83.9%) compared to the control (96.5%). By Day 192, the 75 mg kg^{-1} DA female cohort exhibited the most optimal outcomes, demonstrating both the lowest FCR (1.30) and the highest survival rate (67.1%) among the treatments ($p < 0.05$).

Table 3
The growth rates, feed conversion ratio, and survival rates of *Macrobrachium rosenbergii* during dopamine treatment and the post-treatment period

Parameter	*Treatment*	*Day 36*	*Day 98*	*Day 192*
Final body weight (g ind.$^{-1}$)	Control	0.157±0.033$^{† a}$	2.39±0.08^{a}	NA
	45 DA mg kg^{-1}	0.146±0.022^{a}	1.99±0.29^{a}	28.8±3.8^{a}
	60 DA mg kg^{-1}	0.147±0.015^{a}	2.33±0.12^{a}	30.6±1.9^{a}
	75 DA mg kg^{-1}	0.141±0.006^{a}	2.30±0.19^{a}	28.4±3.6^{a}
	90 DA mg kg^{-1}	0.134±0.005^{a}	2.49±0.82^{a}	29.1±2.6^{a}
Weight gain (g ind.$^{-1}$)	Control	0.150±0.033^{a}	2.23±0.10^{a}	NA
	45 DA mg kg^{-1}	0.139±0.022^{a}	1.85±0.30^{a}	18.0±3.4^{a}
	60 DA mg kg^{-1}	0.140±0.015^{a}	2.18±0.12^{a}	18.6±1.6^{a}
	75 DA mg kg^{-1}	0.134±0.006^{a}	2.15±0.18^{a}	16.5±2.6^{a}
	90 DA mg kg^{-1}	0.128±0.005^{a}	2.36±0.82^{a}	17.0±3.2^{a}
Specific growth rate (% day^{-1})	Control	8.80±0.56^{a}	4.42±0.35^{a}	NA
	45 DA mg kg^{-1}	8.62±0.43^{a}	4.22±0.39^{a}	1.53±0.21^{a}
	60 DA mg kg^{-1}	8.65±0.31^{a}	4.46±0.23^{a}	1.47±0.10^{a}
	75 DA mg kg^{-1}	8.54±0.13^{a}	4.50±0.06^{a}	1.35±0.10^{a}
	90 DA mg kg^{-1}	8.41±0.11^{a}	4.66±0.51^{a}	1.37±0.23^{a}
Feed conversion ratio	Control	1.30±0.24^{a}	1.28±0.04^{a}	NA
	45 DA mg kg^{-1}	1.30±0.23^{a}	1.31±0.10^{a}	1.70±0.28^{a}
	60 DA mg kg^{-1}	1.34±0.19^{a}	1.29±0.08^{a}	1.57±0.13^{ab}
	75 DA mg kg^{-1}	1.19±0.07^{a}	1.39±0.26^{a}	1.30±0.13^{b}
	90 DA mg kg^{-1}	1.27±0.16^{a}	1.48±0.17^{a}	1.48±0.14^{ab}
Survival rate (%)	Control	66.8±8.2^{a}	96.5±2.1^{a}	NA
	45 DA mg kg^{-1}	65.1±6.2^{a}	94.9±4.6^{ab}	60.8±6.3^{ab}
	60 DA mg kg^{-1}	61.7±4.5^{a}	88.7±5.1^{ab}	54.8±5.4^{a}
	75 DA mg kg^{-1}	70.3±5.3^{a}	95.9±2.3^{ab}	67.1±5.5^{b}
	90 DA mg kg^{-1}	68.7±7.7^{a}	83.9±17.8^{b}	57.4±11.7^{ab}

Note: values represent the mean±SD (n = 4); DA: Dopamine; NA: not available. Mean values in the same column with different superscripts differ significantly (results from one-way repeated measure ANOVA and LSD test, $p < 0.05$)

***Feminization rate, gonadal maturity, and histological analysis*.** The administration of dietary DA effectively induced feminization within the all-male PL population (Table 4). Feminization rates demonstrated a significant dose-dependent response; notably, the highest DA concentration (90 mg kg^{-1}) altered the phenotypic sex ratio from the 100% male baseline to 79.2% males and 20.8% neo-females (binomial GLM; $p < 0.05$). These findings indicate that dietary DA significantly influences sex differentiation. The control group demonstrated complete masculinization, with a female phenotypic frequency of only 0.29% (0/684 prawns; continuity-corrected to 2/686 females observed).

Table 4
Feminization rate (mean±SE) and 95% confidence interval across different dopamine treatment groups

Treatment	*Females*†	*Total prawn*†	*Feminization rate (%)*‡	*95% CI*‡
Control	0	684	0.29±0.21^{a}	(0.07, 1.15)
45 DA mg kg^{-1}	103	758	13.8±1.2^{b}	(11.5, 16.4)
60 DA mg kg^{-1}	95	662	14.6±1.4^{b}	(12.1, 17.5)
75 DA mg kg^{-1}	98	640	15.5±1.4^{bc}	(12.9, 18.5)
90 DA mg kg^{-1}	92	449	20.8±1.9^{c}	(17.3, 24.7)

Note: DA: Dopamine; †number of females and total prawns of each treatment (a total of four tanks); ‡feminization rate and 95% CI in each group after continuity correction; mean values in the feminization rate column with different superscripts differ significantly (results from a binomial GLM with a logit link function; Holm-Bonferroni method, $p < 0.05$).

Assessments of gonadal maturity indicated normal reproductive development in the resulting neo-females. Notably, the GSI of DA-treated females showed no significant differences compared to normal, wild-caught females (Table 5).

Table 5

The GSI values (%) of dopamine-treated female and wild-caught female *Macrobrachium rosenbergii*

45 DA mg kg^{-1}	*60 DA mg kg^{-1}*	*75 DA mg kg^{-1}*	*90 DA mg kg^{-1}*	*Wild-caught female*
7.27±1.29^a	7.82±1.41^a	7.97±0.56^a	7.67±0.74^a	7.80±0.89^a

DA: Dopamine; Data in the table are presented as mean±SD (n=4 for DA-treated female and n=3 for wild-caught female); Mean values in the same row with different superscript letters differ significantly (results from one-way ANOVA and LSD test, $p < 0.05$).

To verify phenotypic sex differentiation and evaluate gonadal morphological integrity post-treatment, histological examinations were conducted on near-mature *M. rosenbergii* gonads (Figure 1). The structural analysis revealed that neo-females across all DA-treated cohorts experienced typical oogenesis, aligning with the developmental stages outlined by Meeratana & Sobhon (2007). The observed ovarian tissues displayed a continuum of oocyte development, ranging from early previtellogenic phases (Oc1-Oc2), marked by chromatin condensation and basophilic cytoplasm, to vitellogenic phases (Oc3-Oc4), characterized by lipid droplet accumulation and eosinophilic cytoplasm. Furthermore, completely developed ovaries predominantly contained mature oocytes (mOc) characterized by nuclear migration toward the animal pole and dense yolk granules, thereby demonstrating that the hormone-treated prawns successfully attained full reproductive competence.

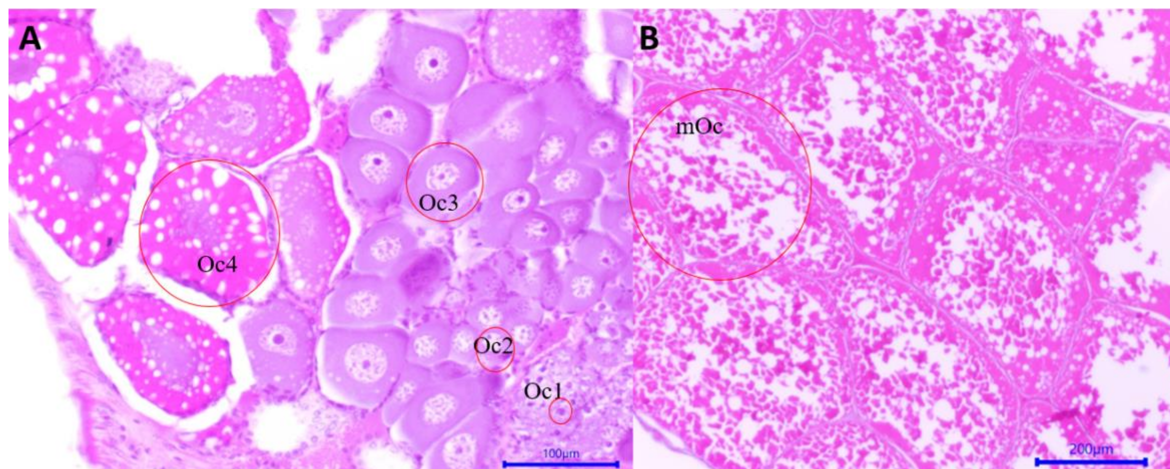

Figure 1. Histological structure of the ovary in dopamine-treated female *Macrobrachium rosenbergii*. (A) Ovary at stage III; (B) Ovary at stage IV; (Oc1: early previtellogenic oocytes; Oc2: late previtellogenic oocytes; Oc3: early vitellogenic oocytes; Oc4: mature vitellogenic oocytes; mOc: fully mature oocytes prior to ovulation.

Table 6 summarizes the measurements of oocyte diameters. A comparative assessment across the various developmental stages revealed morphological traits and growth trends that align with those previously documented for natural females by Meeratana and Sobhon (2007). These results substantiate that the trajectory of ovarian maturation in neo-females closely mirrors that of typical females.

Table 6

Oocyte diameter (µm) of dopamine-treated female and normal female *Macrobrachium rosenbergii*

Oc1	*Oc2*	*Oc3*	*Oc4*	*mOc*
14.5±1.7†	41.3±6.0	97.6±14.1	223±25	434±35
(11.8-18.1)‡	(30.1-57.9)	(71.0-118)	(171-256)	(379-490)
10-30$^\#$	30-100$^\#$	100-200$^\#$	150-250$^\#$	300-550$^\#$

Note: Oc1: early previtellogenic oocytes; Oc2: late previtellogenic oocytes; Oc3: early vitellogenic oocytes; Oc4: mature vitellogenic oocytes; mOc: fully mature oocytes prior to ovulation; † and ‡ mean±SD and min – max; $^\#$ min - max of normal female prawns (Meeratana & Sobhon, 2007).

Discussion

Levels of DA in feed and postlarvae tissue. The present study aimed to evaluate the bioaccumulation and physiological efficacy of dietary DA in feminizing all-male *M. rosenbergii* PL. Despite the successful and proportional incorporation of DA into the experimental diets, the whole-body tissue analysis revealed an unexpected trend: DA-supplemented PL maintained uniformly low tissue DA levels (not detectable) throughout most of the 36-day trial. Conversely, the untreated control group exhibited relatively higher values, particularly on Day 12 and Day 36.

The consistently low DA levels detected in the treated groups highlight the complexities of oral hormone administration in crustaceans. As previously suggested, dietary administration of hormones faces significant challenges due to the high potential for loss arising from leaching into the water column and from digestive breakdown prior to physiological assimilation (Ohs et al 2006a; Rasheed et al 2023). Our analytical quantification demonstrated no significant accumulation of DA in the tissues following the feeding trial. This lack of bioaccumulation is likely explained by the rapid metabolic clearance of exogenous catecholamines, as proposed by Ohs et al (2006a), the digestive processes of prawns may rapidly alter the chemical structure of DA before it can accumulate in whole-body tissues.

Furthermore, DA is a naturally occurring biogenic amine that functions as a critical neurotransmitter and neuroregulator in the crustacean central nervous system (Ohs et al 2006a; Tinikul et al 2016). Endogenous DA levels are not static; they fluctuate naturally during different developmental stages and gonadal maturation cycles (Tinikul et al 2008; 2011). The relatively elevated and variable DA levels observed in the control group at Day 36 may reflect endogenous DA synthesis triggered by environmental or social stressors. Previous studies have demonstrated that acute stressors can induce rapid neuroendocrine responses in decapods, leading to significant surges in hemolymph DA levels (Li et al 2005; Chang et al 2016). It is highly plausible that continuous exogenous dietary DA administration initiates a negative feedback loop within the neuroendocrine system, suppressing endogenous DA synthesis in the treated groups and maintaining stable basal physiological levels (Aparicio-Simón et al 2010; Chang et al 2016).

A primary limitation in interpreting these bioaccumulation results is the LOD inherent in the LC-MS/MS methodology used. According to the data analysis protocol, analytical values falling below the LOD were assigned a conservative value of LOD/2. The uniform recording of exactly not detectable across multiple sampling points and treatments strongly suggests that these tissue concentrations fell below the assay's detection threshold. Consequently, using whole-body homogenates for DA extraction may have diluted the hormone's localized concentrations, masking subtle differences in bioaccumulation between treatments. Future research evaluating hormonal sex reversal via dietary administration should employ more sensitive analytical techniques with lower detection limits. Additionally, rather than relying on whole-body homogenates, subsequent studies should focus on quantifying DA levels within specific target tissues, such as the central nervous system, eyestalk ganglia, and hemolymph, where biogenic amines are actively synthesized, localized, and transported (Tinikul et al 2011; Chang et al 2016).

Evaluation of dietary DA supplementation on growth indices, feed utilization, and reproductive ontogeny in M. rosenbergii postlarvae. The present study investigated the effects of dietary DA supplementation on the growth performance, survival rate, feed conversion ratio (FCR), feminization rate, and gonadal development of an all-male *M. rosenbergii* PL population. The results indicate that exogenous DA administration successfully induces phenotypic feminization in genotypic males (ZZ) during the early PL stages. Notably, the highest dietary DA concentration (90 mg kg^{-1}) yielded a feminization rate of 20.8%. Moreover, the untreated control group exhibited a 0% female phenotype, thereby validating the all-male status of the initial population. The absence of females in this cohort confirms that the experimental subjects were a consistent monosex male population prior to hormonal administration. Furthermore, the

dietary administration of DA did not significantly affect the somatic growth performance of the prawns. FBW, WG, and SGR were statistically comparable across all treatment groups and the control during the 36-day treatment period and the subsequent 156-day post-treatment phase. SR and FCR also remained largely unaffected during the primary treatment period, confirming that DA can be administered via feed without compromising general husbandry metrics. Importantly, histological evaluations confirmed that the resulting neo-females exhibited normal gonadal maturation and oogenesis, mirroring the reproductive development of wild-caught normal females. Furthermore, throughout the trial, water temperature (28-31°C), pH (7.1-7.9), DO (> 5.0 mg L^{-1}]), TAN (0-0.22 mg L^{-1}), and nitrite (0-1.70 mg L^{-1}) remained highly stable and well within the established optimal ranges for *M. rosenbergii* post-larvae. Because these environmental variables were kept uniform and optimal across all control and treatment tanks, we can confidently rule out environmental stress as a confounding factor.

The induction of feminization via dopaminergic intervention aligns with foundational studies in decapod endocrinology, specifically the work of Ohs et al (2006a). Ohs et al (2006a) demonstrated that dietary administration of DA increased the proportion of phenotypic females in *M. rosenbergii* nursery populations. However, previous investigations typically used mixed-sex cohorts, which introduces confounding variables related to natural sex-ratio fluctuations (which can range from 26 to 65% females in untreated groups (Ohs et al 2006a; Rasheed et al 2023)). By utilizing a confirmed all-male (ZZ) PL cohort generated from neo-female broodstock, the present study clearly attributes the emergence of female phenotypes entirely to the hormonal intervention.

Our study observed no significant impacts of DA levels on growth and survival, which is consistent with findings that, when administered within optimal thresholds, hormonal treatments do not invariably stunt somatic growth in crustaceans (Ohs et al 2006a). While some endocrine therapies (such as 17β-estradiol) have historically resulted in marginally elevated mortality rates in *Penaeus* species (Ikhwanuddin et al 2019; Wang et al 2025), the dosages of DA used here (45-90 mg kg^{-1}) were well tolerated.

Furthermore, the histological confirmation of normal oogenesis, progressing from early previtellogenic oocytes (Oc1) to fully mature oocytes (mOc), closely matches the standard ovarian developmental classifications established by Meeratana and Sobhon (2007). The GSI values of the DA-treated neo-females (7.27 to 7.97%) were statistically indistinguishable from those of wild-caught reference females (7.80%), indicating that functional reproductive capacity is preserved. This is a critical validation that addresses a gap identified by Ohs et al (2006a), who noted the need for histological examinations to confirm the internal structural integrity of gonads following DA treatment.

The present study effectively demonstrates that dietary DA induces feminization in *M. rosenbergii* through a transient neuroendocrine cascade rather than through tissue bioaccumulation, a finding that aligns well with the foundational work of Ohs et al (2006a). By acting as a neuroregulator that stimulates GIH release from the X-organ-sinus gland complex while simultaneously inhibiting GSH, DA appears to disrupt the AG and the subsequent production of androgenic gland hormone (AGH). This transient receptor-mediated signaling explains how DA can successfully drive phenotypic sex reversal and arrest testicular development despite its rapid metabolism and clearance, further validating the efficacy of dietary DA administration in manipulating crustacean sex differentiation. This dual neuroendocrine action effectively starves the AG of necessary stimulatory inputs, leading to its atrophy or failure to develop during the critical, labile window of early PL differentiation (Malecha et al 1992; Ohs et al 2006a). In the absence of AG hormone, the default female developmental pathway is initiated, leading to ovarian differentiation and the expression of female secondary sexual characteristics (Sagi & Aflalo, 2005; Ohs et al 2006a). Because the DA treatment is transient and applied only during the early PL phase, it successfully alters the primary sexual differentiation without permanently disrupting the downstream vitellogenic processes required for subsequent oocyte maturation. This explains why the resulting neo-females are capable of undergoing normal exogenous vitellogenesis and accumulating yolk granules (as seen in

the Oc3 and Oc4 stages) in a manner identical to that of genotypic females (Meeratana & Sobhon 2007).

While the study provides robust evidence for DA-induced feminization, another limitation must be candidly addressed. The maximum feminization rate achieved was 20.8% at the highest dietary dose of 90 mg kg^{-1}. Although this represents a statistically significant increase from the control, it falls short of the 100% sex reversal rate required for optimal commercial neo-female broodstock production. This suggests that the dosage threshold, delivery method, or ontogenetic stage of administration (e.g., initiating treatment during the larval rather than PL stages) may require further studies.

While the dietary administration of DA successfully altered the sex differentiation pathway in a dose-dependent manner, it is important to contextualize these results within the broader scope of aquaculture technologies. Despite statistical significance, the biological efficiency remains moderate. From a practical standpoint, the application of dietary DA presents significant advantages for large-scale aquaculture operations. Although the feminization rate achieved (20.8%) may appear modest compared to invasive methods such as surgical ablation or RNA interference (Aflalo et al 2006; Ventura et al 2009), it is highly sufficient for broodstock development. Because a single sex-reversed neo-female (ZZ) can produce tens of thousands of all-male progeny per spawning cycle, hatcheries do not require 100% sex-reversal efficiency to establish a successful breeding program. Furthermore, the dietary administration of a natural biogenic amine such as DA eliminates the need for highly skilled labor, required for micro-injections or surgery, and circumvents the stringent environmental and consumer safety regulations associated with synthetic steroid hormones like 17β-estradiol. Consequently, DA supplementation offers a scalable, cost-effective, and eco-friendly strategy for commercial hatcheries aiming to produce all-male M. rosenbergii seeds.

Conclusions. This study demonstrates that the dietary administration of DA is an effective, non-detrimental strategy for inducing sex reversal in genotypic male *M. rosenbergii* PL. By utilizing a confirmed monosex baseline population, the emergence of female phenotypes could be unequivocally attributed to the hormonal intervention. Crucially, short-lived DA exposure during the labile PL phase did not affect long-term somatic growth, feed efficiency, or SR. Histological and morphometric evaluations confirmed that the DA-treated neo-females exhibited normal reproductive ontogeny, with mature vitellogenic capabilities and GSI metrics directly comparable to those of natural females.

However, while a peak feminization rate of 20.8% was achieved at 90 mg kg^{-1}, this falls short of the 100% efficacy desired for commercial broodstock operations. Furthermore, tracking whole-body hormone bioaccumulation proved challenging due to rapid metabolic clearance and methodological limits of detection. Future research should aim to optimize the timing and duration of DA administration, potentially initiating treatment during the earlier larval stages. Additionally, targeted endocrinological investigations should prioritize quantifying DA at specific bioactive sites, such as the central nervous system and hemolymph, to better elucidate the pharmacokinetics of dietary hormonal feminization in decapods. These findings offer a viable, non-invasive, and well-tolerated protocol for developing the neo-female broodstock required to support commercial all-male aquaculture.

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Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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