



Impact of *Withania somnifera* Supplementation on Growth, Reproduction and Hormonal Profile of *Lepidocephalichthys thermalis* in Captivity

Narsingh Kashyap¹, Eswaran Suresh¹, Deepak Agarwal¹,
Ayyathurai Kathirvelpandian², P. Chidambaram³

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ABSTRACT

Background: *Withania somnifera* (Ashwagandha) is a widely known medicinal herb with numerous bioactive compounds that enhance growth, immunity and reproductive performance. This study evaluates the effects of *W. somnifera* ethanolic extract (WSEE) on growth, gonadal maturation, steroid hormone profiling in *Lepidocephalichthys thermalis* under controlled conditions.

Methods: Fish were fed diets supplemented with WSEE at different concentrations (0, 100, 200 and 300 mg/kg) for 70 days. Growth performance, gonadosomatic index (GSI), serum steroid hormone levels and gonadal histology were assessed.

Result: Results demonstrated significant improvements in growth parameters, with the highest weight gain (1.51 ± 0.22 g) and specific growth rate (2.26 ± 0.22) observed in the 300 mg/kg WSEE group. Additionally, FCR improved across treatments, with the lowest value recorded in the 300 mg/kg group (1.08 ± 0.05), indicating enhanced feed utilization efficiency. Steroid hormone analysis revealed a dose-dependent increase in progesterone, estrogen and testosterone levels, with T2 (200 mg/kg) exhibiting the highest concentrations. Histological examination showed enhanced gonadal development, with increased vitellogenic follicles and reduced atretic structures in treated groups. These findings support the use of *W. somnifera* as a sustainable alternative to synthetic hormones in aquaculture reproduction management.

Key words: Gonadal maturation, *Lepidocephalichthys thermalis*, Steroid hormones, *Withania somnifera*.

INTRODUCTION

Lepidocephalichthys thermalis (Indian spiny loach) is a small benthic freshwater fish of the Cobitidae family, commonly found in tropical regions like India and Sri Lanka. It has good market value in Tamil Nadu and is considered a delicacy. *L. thermalis* is increasingly popular in India as a model organism for research and a nutritious food organism. Lack of research in reproductive biology and effective breeding methods have slowed loach farming commercialization. As a nutraceutical, it provides essential nutrients such as proteins, amino acids, fatty acids and vitamins. The growing demand for aquaculture, due to population growth and declining wild fisheries, underscores the need to improve fish reproduction, seed quality and stock health. However, stressors like handling, chemical exposure and synthetic hormones can reduce reproductive efficiency. Their high cost, environmental risks and health concerns have increased interest in sustainable alternatives. Despite their ecological and economic importance, the reproductive biology and maturation processes of *L. thermalis*, remain poorly understood. Furthermore, the absence of standardized hatchery practices and established seed production technologies presents significant constraints to the large-scale commercialization of loach aquaculture. In this context, herbal products have emerged as a promising solution, playing a vital role in aquaculture by act as aphrodisiacs, improving reproductive success, larval quality and while

¹Institute of Fisheries Postgraduate Studies, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Vaniyanchavadi-603 103, Chennai, Tamil Nadu, India.

²ICAR-National Bureau of Fish Genetic Resources, Central Marine Fisheries Research Institute Campus, Kochi-682 001, Kerala, India.

³Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam-611 002, Tamil Nadu, India.

Corresponding Author: Eswaran Suresh, Institute of Fisheries Postgraduate Studies, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Vaniyanchavadi-603 103, Chennai, Tamil Nadu, India. Email: suresh@tnfu.ac.in
ORCID: 0000-0001-5953-6617.

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their rich immune-boosting compounds promote disease resistance and overall fish health (Andriani and Aisyah, 2025). Medicinal plants, rich in bioactive compounds, offer an eco-friendly and cost-effective way to enhance fish health, growth and reproduction. In addition, relevant studies have also been conducted on small commercial fish species, focusing on enhanced gonadosomatic index (GSI) in *Trichopodus trichopterus* (Naji et al., 2014),

reproductive performance in *Danio rerio* (Sarasquete *et al.*, 2018), maturation in *Trichogaster pectoralis* (Jintasataporn *et al.*, 2011) and immunostimulant effects in *Penaeus monodon* and *Betta splendens* (Manilal *et al.*, 2012).

Withania somnifera (Ashwagandha) is a key herb in Ayurveda, renowned for its rejuvenating properties that enhance vitality, longevity and overall health (Krishnamurthy and Sarala, 2010). Ashwagandha contains over 35 bioactive compounds, including alkaloids (e.g., isopelletierine, anafierine), steroidal lactones (withanolides, withaferins) and saponins. Various researchers have examined the effects of ashwagandha as dietary supplements on the growth, survival, immunity, health, gonadal maturation and brood stock development of various fish species such as mrigal, tilapia, rohu, pearl spot, common carp, magur and three-spot gourami. (Nyina-Wamwiza *et al.*, 2012; Sivagurunathan and Innocent, 2012; Naji *et al.*, 2014; Mukherjee *et al.*, 2019; Srivastava *et al.*, 2020; Dhas *et al.*, 2015; Singh *et al.*, 2024). Based on this background, this study examined the effects of *W. somnifera* (root) ethanolic extract (WSEE) supplementation at different concentrations on *L. thermalis* gonadal maturation, growth performance and serum steroid levels in the captive condition.

MATERIALS AND METHODS

Preparation of *W. somnifera* extract and formulation of experimental diets

W. somnifera roots were procured from ICAR-CTRI, Tamil Nadu and authenticated (Ref. No.: INSciR/Herbarium/0086). The roots were washed, air-dried and ground into a fine powder, which was stored at 4°C. A 50 g sample was extracted using an 80% ethanol solution in a Soxhlet apparatus (1:10 g/mL) at 60°C for 8-10 hours. The extract was filtered (Whatman No. 42), concentrated via rotary evaporation (65°C, 5-6 hrs) and stored at 4°C in sterile container until needed. For experimental diets, the extract was incorporated at 0, 100, 200 and 300 mg/kg in feed. The feed formulation chart or composition of basal ingredients was given in Table 1. The experiment was conducted at the Institute of Fisheries Post Graduate Studies, TNJFU, Vaniyanchavadi, Chennai during August 2022 to December 2024.

Experimental design

L. thermalis (Indian spiny loach), fingerlings were collected from natural riverine system at Dindigul district, Tamil Nadu. Fish were conditioned for two weeks. During acclimatization period, Feeding was done twice a day (10:00 and 17:00 Hrs). Hand feeding was carried out at ad libitum. The excess feed was removed by siphoning two hours after each feeding session.

The experiment was designed using a 1 × 3 design (completely randomised design), involving a total of 12 experimental troughs, each with a 30-liter capacity. One control and three treatment groups were selected for the 70-day experiment. Each treatment was applied to three

replicate tanks and fish were randomly assigned to minimize bias.

Growth performance

The fish weight was recorded biweekly for each group through random sampling. Average daily growth (ADG), total weight gain (WG), feed conversion ratio (FCR) and specific growth rate (SGR) were calculated using standard formulas:

$$\text{Average daily growth (ADG)} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Experimental days}}$$

$$\text{Total weight gain (TWG)} = \text{Final body weight} - \text{Initial body weight}$$

$$\text{Specific growth rate (SGR)} = \frac{\ln W_t - \ln W_o}{T} \times 100$$

$$\text{FCR} = \frac{\text{Total feed intake (g)}}{\text{Total weight gain (g)}}$$

Estimation of steroid hormones

For the estimation of steroid hormones blood sampling was carried out at the end of trial. Fish from all groups were collected and anesthetized using clove oil. Blood samples were obtained by puncturing the caudal vein with a 1 ml syringe, then stored in blood collection tubes and left undisturbed for 30 minutes (Pedroso *et al.*, 2012). Blood were obtained through a pooled sampling (~5 Adult fish) technique (Jagadeeswaran and Sheehan, 1999). Due to the extremely small size of *L. thermalis*, individual blood collection was not feasible. Hence, pooled sampling (5 fish/sample) was adopted to ensure sufficient serum volume for reliable hormone quantification using Chemiluminescence Immunoassay (CLIA). The samples were centrifuged at 5,000 rpm for 15 minutes in a refrigerated centrifuge set at 4°C. The resulting supernatant (serum) was carefully collected for hormone level analysis using (CLIA) (Chatterjee, 2016).

Histology

The fish were carefully dissected to collect the gonads, which were initially fixed in Bouin's fluid and then stored in 10% formaldehyde. The tissues underwent thorough dehydration using a graded alcohol series, followed by washing in xylene and were subsequently embedded in paraffin wax. Sections of 5-6 µm thickness were cut and cover slips were placed over them. The paraffin was removed using a toluene series. After rehydration, the tissues were stained with hematoxylin and eosin, following the method described by Martoja and Martoja-Pierson (1967).

Estimation of maturation parameter

Fish sampling was conducted at end of trial, during which gonad samples were collected, weighed and preserved in 10% formaldehyde for histological analysis and gonadosomatic index (GSI) studies.

$$\text{Gonadosomatic index (GSI)} = \frac{\text{Total weight of gonad}}{\text{Total weight of fish}} \times 100$$

Statistical analysis

Statistically analyzed was one-way ANOVA followed by Tukey's post hoc test using statistical software SPSS 20.0. The data were represented as mean values $\pm$ standard deviation (SD) of all the three replicates. Prior to performing ANOVA, the data were checked for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. All assumptions for parametric testing were met. The significance level was considered at a value of $p < 0.05$.

RESULTS AND DISCUSSION

Growth performance

The growth performance of *L. thermalis* varied across the experimental diets, the initial weight (IW) of fish across all groups was relatively similar, ranging from 0.37 ± 0.03 g to 0.39 ± 0.04 g. However, the FW increased significantly ($p < 0.05$) with treatment, reaching the highest value in T3 (1.89 ± 0.22 g), followed by T2 (1.83 ± 0.11 g), T1 (1.67 ± 0.20 g) compared to control group (1.32 ± 0.10 g) (Table 2). AWG, weight gain (WG) and SGR showed an increasing trend, with the highest value in T3, followed by T2, T1 and the lowest in the control group in (Table 2). These results clearly demonstrate the growth-promoting potential of *W.*

somnifera ethanolic extract (WSEE), especially at the 300 mg/kg dietary inclusion level (T3) mentioned in Table 2. Similarly, Mukherjee *et al.* (2019) found that the ethanol extract of *W. somnifera* at 0.7 g/kg increased specific weight gain, final weight and daily weight gain in *Oreochromis niloticus*. Medicinal plants like *W. somnifera* are known for their rich phytochemical composition, including bioactive compounds (flavonoids, alkaloids, saponins and glycosides) with antibacterial, antioxidant, anti-inflammatory and immunostimulant properties.

These findings align with previous studies, Srivastava *et al.* (2020) reported similar growth improvements in *Labeo rohita*, To *et al.*, (2023) reported in *Channa striata*, Nguyen (2025) in Loach fish, while Habib *et al.* (2024) observed enhanced growth in *Cyprinus carpio* fingerlings fed *W. somnifera*, Sharma *et al.* (2017) also found positive effects on growth in *Oreochromis niloticus*, respectively. Sivaram *et al.* (2004) also reported significant improvements in WG, SGR and FCR in *Epinephelus tauvina*, supplemented with 100-200 mg/kg of *W. somnifera* extract.

SGR is a critical indicator in aquaculture, as it reflects how efficiently fish convert feed into body mass over time (Lugert *et al.*, 2016, Khan *et al.*, 2021). Similarly, a lower FCR suggests efficient feed utilization, reducing the cost of production and minimizing environmental impacts (Habib *et al.*, 2022). Overall, the present study indicates that dietary supplementation with WSEE, particularly at 300 mg/kg, significantly enhances growth parameters and feed efficiency in *L. thermalis*.

Water quality parameters

The water quality parameters significantly influence the physiology and metabolic rate of fish. The fluctuations in water parameters, including temperature, dissolved oxygen (DO), pH, total alkalinity and total hardness, were analyzed over a 70 days study period (Table 3).

Estimation of steroid hormones

The experimental diets significantly influenced hormone levels in *L. thermalis*. Progesterone significantly ($p < 0.01$) increased across treatment groups, with the highest level observed in T2 (0.220 ± 0.020 ng/mL) followed by T3 (0.180 ± 0.010 ng/mL) and T1 (0.166 ± 0.015 ng/mL) compared to the control (0.120 ± 0.010 ng/mL) (Fig 1). This suggests that WSEE supplementation stimulates progesterone biosynthesis, potentially enhancing reproductive readiness.

Table 1: Composition of basal diet used for experimental feeding of *L. thermalis*.

Basal feed ingredients	Composition %
Fish meal	20
Soybean meal	20
Corn gluten meal	3
Acetes (krill)	4
Prawn head meal	2
Fish oil	3
Soy lecithin	2.5
Wheat flour	12.5
Rice bran	12.5
Broken rice	18.5
CMC binder	1
Vitamin premix	0.5
Mineral premix	0.5

Table 2: Growth performance of *L. thermalis* fed diets with different concentrations of WSEE.

Experimental diet	Initial weight (IW) (gm)	Final weight (FW) (gm)	Average daily weight gain (AWG) (gm)	Feed conversion ratio (FCR)	Weight gain (WG) (gm)	Specific growth rate (SGR)
Control	0.37 ± 0.03	1.32 ± 0.10	0.013 ± 0.12	1.21 ± 0.03	0.94 ± 0.09	1.80 ± 0.12
T1	0.38 ± 0.06	1.67 ± 0.20	0.018 ± 0.16	1.17 ± 0.02	1.29 ± 0.20	2.11 ± 0.19
T2	0.39 ± 0.04	1.83 ± 0.11	0.020 ± 0.18	1.12 ± 0.08	1.43 ± 0.13	2.19 ± 0.19
T3	0.38 ± 0.03	1.89 ± 0.22	0.021 ± 0.13	1.08 ± 0.05	1.51 ± 0.22	2.26 ± 0.22

The growth parameters values were presented as mean $\pm$ SD of three replicate per treatment.

Estrogen levels also exhibited a notable elevation across treatments, peaking in T2 (2.140 ± 0.010 ng/mL) relative to the control (1.713 ± 0.032 ng/mL). The increase in estrogen implies that *W. somnifera* may facilitate ovarian development and maturation by stimulating estrogenic pathways, which is consistent with earlier studies highlighting the phytoestrogenic effects of medicinal plants on aquatic organisms (Singh *et al.*, 2024). Similarly, Kiasalari *et al.* (2009) and Belal *et al.* (2012) demonstrated that herbal supplements could positively regulate hormone synthesis, contributing to enhanced reproductive performance. Another study by Nyina-Wamwiza *et al.* (2012) found that an experimental diet raised 17β -estradiol levels in females (*C. gariepinus*), confirming that plant products improve steroidogenesis.

Testosterone, a key androgen in male fish, plays a critical role in reproductive physiology, including spermatogenesis. In this study, testosterone levels were significantly elevated in T2 (1.636 ± 0.060 ng/mL, $p < 0.05$) compared to the control (1.326 ± 0.110 ng/mL), suggesting a stimulatory effect of *W. somnifera* on androgen production. A comparable study by Gharaei *et al.* (2020) found that extract of *Tribulus terrestris* elevated testosterone levels in zebrafish, a phenomenon ascribed to the existence of estradiol glycosides in the plant. Mansour *et al.* (2018) conducted a similar experiment, revealing that the dietary inclusion of date palm pollen grains (DPPG), tribulus extract

(TE) and ginseng extract (GE), resulted in an 86.27% enhancement of testosterone levels in Nile tilapia. In another important experiment, Mansour *et al.* (2022) found that papaya extract increased testosterone synthesis by activating 17 α -hydroxylase enzymes in female catfish.

Interestingly, 11-KT levels showed a variable trend across treatments. While T1 exhibited a decrease (0.720 ± 0.040 ng/mL) compared to the control (0.820 ± 0.034 ng/mL), T2 showed an increase (0.923 ± 0.055 ng/mL), indicating a possible dose-dependent response to WSEE. A similar study by Hassona *et al.* (2020) found that *Tribulus terrestris* increased testosterone and 11-keto testosterone in *Oreochromis niloticus* at higher doses. Given that 11-KT is a potent androgen associated with testicular development and sperm maturation, the observed fluctuations suggest that hormonal modulation by *W. somnifera* may be influenced by both dosage and the physiological condition of the fish. These findings indicate that the experimental diets affect endocrine function by modulating hormone concentrations.

Estimation of maturation parameter

The gonadosomatic index (GSI) exhibited statistically significant increase ($p < 0.05$) trends in both males and females across treatments. In females, GSI showed a significant increasing trend, with the highest value observed in T2 (10.295 ± 0.640), followed by T3 (9.143 ± 0.919) and T1 (8.752 ± 1.457), compared to the control group (Fig 2). In males, GSI displayed variability, peaking in T2 (0.985 ± 0.282), followed by T3 (0.834 ± 0.154) and T1 (0.768 ± 0.108), relative to the control (0.707 ± 0.094) (Fig 2). The GSI serves as a dependable measure of gonadal development and spawning activity in fish. It generally rises as the fish matures, reaching its highest value during the peak reproductive period of the breeding season. The results show a clear increase in GSI in fish fed WSEE diets, indicating a potential impact on reproductive development. GSI was higher in T1, T2 and T3 groups compared to the control, with the strongest effects in T2, followed by T3 and T1. Sarosiek *et al.* (2012) reported a significant increase in the GSI from 0.21 to 3.27 in *Androctonus australis*. This

Table 3: Water quality parameters recorded during the 70-day experimental period for *L. thermalis*.

Water parameter	Recorded
Temperature ($^{\circ}\text{C}$)	29 ± 0.35
pH	8.5 ± 0.15
Total hardness (mg/L)	190 ± 7.90
Total alkalinity (mg/L)	185 ± 8.63
Dissolve oxygen (ppm)	6.04 ± 0.35
Ammonia (mg/L)	0.005 ± 0.002

The water quality parameters values were presented as mean $\pm$ SD of three replicate per treatment.

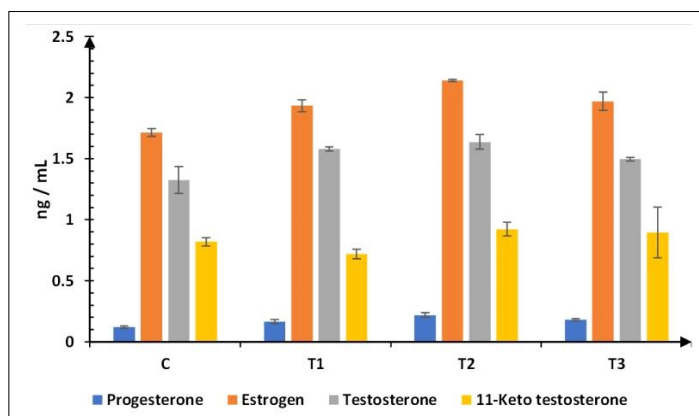


Fig 1: Steroid hormone profiling of *L. thermalis* treated with experimental diets.

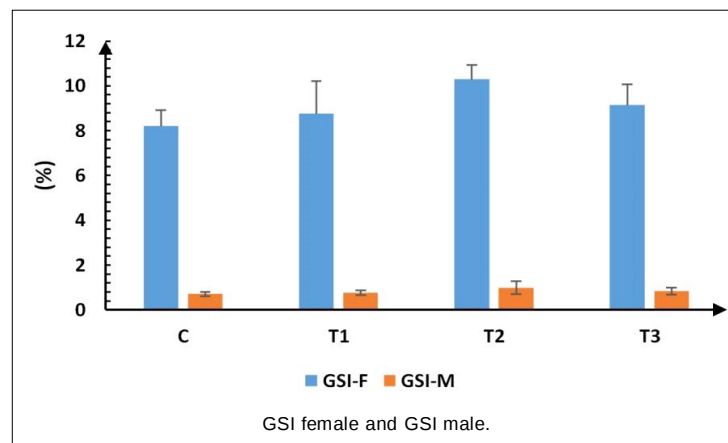


Fig 2: Gonadosomatic index (GSI) of *L. thermalis* treated with experimental diets.

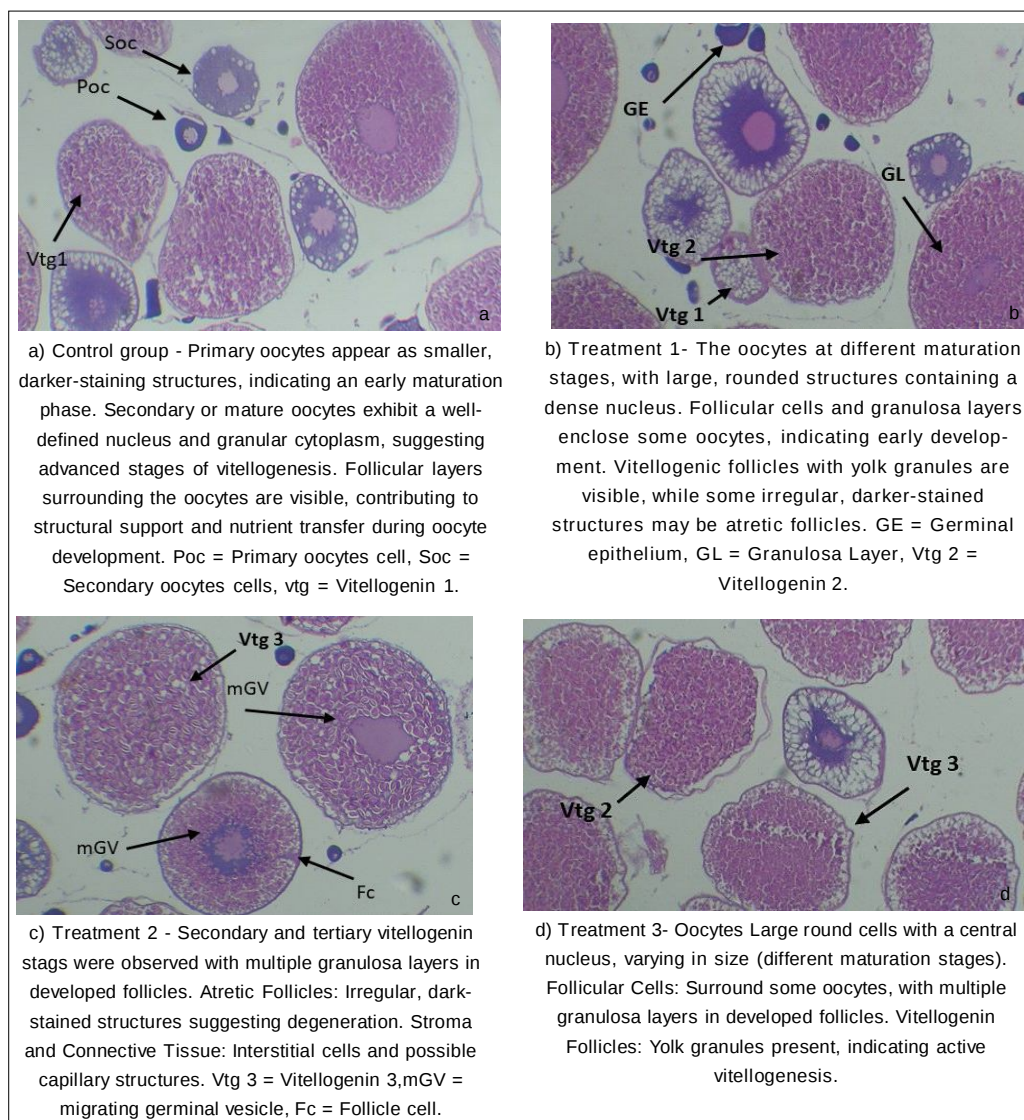


Fig 3: Microscopic observation of fish gonad treated with different WSSE diet.

observation indirectly supports our findings, as it aligns with the idea that herbal maturation diets contribute to an enhanced GSI level, potentially playing a role in reproductive development. WSEE improves GSI because phytoestrogens and steroidal lactones (e.g., withanolides) mimic endogenous reproductive hormones. These compounds may stimulate the hypothalamic-pituitary-gonadal (HPG) axis, increasing LH and FSH production and gonadal growth. The results of this study are consistent with the findings of Dhas *et al.* (2015) observed enhancement in GSI via in *Etroplus suratensis*.

Histology of gonad

Histological studies revealed that the WSEE extract accelerated the maturation process in a shorter period. Consequently, the treatment groups, particularly T2, exhibited a significant difference compared to the control group, where fish matured earlier in (Fig 3). This effect in the test groups may be attributed to an increased level of estrogenic compounds, likely induced by the extract's role in nourishment. Therefore, the use of phytoestrogens such as the WSEE extract before the reproductive season is crucial. Our results are supported by Gholampour *et al.* (2020), who found that ethanolic extract of *Vitex agnus-castus* accelerated maturation in zebrafish. Similarly, Nazari and Roozbehani (2015) reported that *Foeniculum vulgare* (fennel) extract promoted ovum maturity and enhanced growth and gonadal development in *Poecilia reticulata* within a shorter time. Naji *et al.* (2014) reported that flax, pumpkin and silymarin extracts enhanced oocyte formation, diameter and final maturity in *Trichogaster trichopterus*. Similarly, Koca *et al.* (2021) found that *Tribulus terrestris* and *Ferula communis* extracts significantly improved reproductive organs in *Maylandia estherae*, supporting our findings.

Study limitations

While the results indicate promising effects of *W. somnifera* on growth and reproduction, certain limitations should be acknowledged. Firstly, the small size of *L. thermalis* necessitated the use of pooled blood samples, which may mask individual variability in hormone levels. Secondly, the relatively small number of replicates (n=3) limits the statistical power for some parameters, particularly hormone assays. While normality and variance assumptions were tested, future studies should consider increased replication or use of non-invasive sampling methods. Additionally, the water quality parameters were kept consistent across treatments, but no formal statistical comparison was made due to the controlled experimental conditions.

CONCLUSION

This study highlights the remarkable potential of *W. somnifera* as a natural enhancer of fish growth and reproductive function. The significant improvements in gonadal maturation, steroid hormone levels and feed

conversion efficiency in *L. thermalis* suggest that this medicinal herb could revolutionize fish nutrition and reproductive management. The observed increase in progesterone, estrogen and testosterone levels, along with enhanced gonadal development, indicates that *W. somnifera* actively supports endocrine regulation, leading to improved reproductive success. Beyond its direct physiological benefits, the use of *W. somnifera* offers a sustainable and eco-friendly alternative to synthetic hormones and chemical additives in aquaculture. The findings support further exploration of herbal supplements in aquaculture, with potential to make fish farming more natural and health-focused. Future studies should examine long-term effects, optimal dosages and synergies with other herbs. Addressing the current limitations through long-term trials and refined sampling strategies will enhance the robustness and applicability of these findings.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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