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Short Communication

Steroid Hormone Profile and Sperm Quality of Silver Pompano (*Trachinotus blochii*) Fed *Tribullus terrestris* Extract and Gonadotropin Hormone

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Abstract

Male broodstock of silver pompano often exhibit delayed gonadal development, which prolongs the broodstock maintenance period and increases production costs. To address this issue, dietary supplementation with bioactive compounds and hormonal inducers has been explored to stimulate reproductive maturation. Among these, plant-derived extracts such as *Tribulus terrestris* and exogenous hormones like human Chorionic Gonadotropin (hCG) are of interest due to their potential to enhance steroidogenesis and accelerate gonadal development. This study examined the impact of *T. terrestris* extract (ETT) and hCG on the steroid hormone profile, sperm quality, and gonadal histology of male silver pompano. Five treatment groups were established with varying ETT (mg/kg of diet) and hCG (IU/kg of body): T1 (0 + 0), T2 (50 + 0), T3 (250 + 0), T4 (50 + 1000), and T5 (250 + 500), each replicated eight times. Results revealed that ETT at 250 mg/kg (T3) significantly improved reproductive parameters, including absolute weight gain, gonadal development, and semen volume. Histological analyses further indicated advanced stages of gonadal maturation in treated groups, accompanied by increased plasma testosterone levels, which stimulated spermatogenesis and sperm cell formation. Optimal sperm quality characterized by enhanced motility and seminal volume was observed at the T3 dosage, although sperm density showed limited variation. These findings underscore the potential of *T. terrestris* extract as a practical dietary strategy to enhance reproductive efficiency in silver pompano aquaculture, contributing to improved productivity and sustainability. Further studies are recommended to refine dosages and ensure consistent outcomes across production systems.

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1. Introduction

Silver pompano (*Trachinotus blochii*) is a fast-growing marine fish species recognized for its high nutritional value, delicious taste, and strong market demand, both locally and internationally, particularly in countries such as Singapore, Taiwan, China, and Hong Kong (Dawood, 2021; Mansour *et al*., 2022; Ebeneezar *et al*., 2020). With a global market price reaching up to 4.25 USD/kg (Tang *et al*., 2020), the species holds significant economic potential, further evidenced by the steady increase in production in recent years (FAO, 2022). However, the large-scale culture of silver pompano remains hampered by reproductive inefficiencies, particularly related to broodstock management. A reliable supply of fingerlings, crucial for sustainable aquaculture, is contingent on the availability of mature broodstock capable of consistent and high-quality spawning. Yet, silver pompano often exhibit delayed maturation, requiring over two years to reach spawning size (>1.5 kg), with irregular gonadal development observed under captive conditions. Environmental fluctuations, nutritional deficits, and hormonal regulation significantly influence these reproductive limitations (Caldas *et al*., 2021).

Hormonal stimulation has become a common strategy in aquaculture to accelerate reproductive maturation, particularly in male broodstock. Previous research has predominantly examined the isolated effects of hormonal induction, particularly using gonadotropins such as Pregnant Mare Serum Gonadotropin (PMSG) combined with antidopamine (AD), in promoting early reproductive processes like vitellogenesis and gonadal maturation (Hartami *et al*., 2022). Gonadotropins, containing luteinizing hormone (LH) extracted from the pituitary glands of mature fish, are critical in facilitating spermatogenesis and ovulation. In silver pompano (*T. blochii*), studies by (Putra and Raza'i, 2017) and Putra and Raza'i (2020) demonstrated that exogenous hormone applications such as PG600 and hCG, improved gonadosomatic index (GSI) values and accelerated testicular development, with optimal effects observed at PG600 doses of 20 IU/kg. However, the use of synthetic hormones like these presents challenges related to high production costs, environmental concerns, and residual accumulation in aquaculture systems (Maulianawati *et al*., 2021). Consequently, there has been a growing interest in alternative approaches utilizing natural bioactive compounds, particularly plant-derived phytosteroids.

Tribulus terrestris, a medicinal herb rich in steroidal saponins such as protodioscin, has shown potential in enhancing testosterone levels, stimulating spermatogenesis, and promoting reproductive performance across various fish species (Saiyed *et al*., 2016; Shahid *et al*., 2016; Zhu *et al*., 2017; Mansour *et al*., 2022). Despite these promising attributes, research focusing on the application of *T. terrestris* extracts specifically in silver pompano remains scarce. This gap highlights the need for a systematic investigation into the effects of *T. terrestris* on the steroid hormone profile, gonadal histology, and sperm quality in male broodstock of this species. However, current studies have yet to systematically examine the synergistic effects of combining natural phytosteroid sources such as *T. terrestris* extract with gonadotropin hormones on the steroid hormone profile, gonadal histology, and reproductive output in silver pompano. This research, therefore, addresses a significant gap by integrating plant-based bioactive compounds with hormonal induction protocols. The purpose of this study is to explore how the administration of *T. terrestris* extract and gonadotropin hormone influences the reproductive physiology of male silver pompano (*T. blochii*), with a specific focus on the steroid hormone profile. The findings are expected to provide a scientific basis for developing innovative and sustainable broodstock management practices, ultimately supporting the mass production of high-quality seeds in silver pompano aquaculture.

2. Materials and Methods

2.1 Materials

2.1.1 The equipment

The equipment used includes floating net cages measuring 3 x 3 x 3 x 3 m^3, a measuring tape with the accuracy of 1 cm, a digital scale with an accuracy of 0.1 g, parafilm (4 in x 125 ft roll), a 1.5 ml microtube, hemocytometer (Assistent Germany), and a cool box (Lion Star Marina Cooler 6S).

2.1.2 The materials

The materials used includes male silver pompano broodstock candidates with an average length of 46.53 ± 2.40 cm and weight of 1792.88 ± 286.73 g, *T. terrestris* extract (ETT), human Chorionic Gonadotropin (hCG) hormone (Argent Laboratories Inc, Makaty City, Philippines), 96% ethanol (EMSURE #34; ACS, ISO, Reag. Ph Eur MERCK), 10% EDTA anticoagulant, clove oil, 10% buffered formalin, and 70% ethanol.

2.1.3 Ethical approval

This study used male silver pompano (*T. blochii*) broodstock, following standard fish maintenance protocols at the Marine Aquaculture Fisheries Center,

Lampung. The clearance number B.1581/BBPBL/KP.440/6V/2024 was granted by the Research Ethics Committee within the last two years, indicating ethical principles. Fishermen with technical expertise of at least three years and skilled communication skills were part of the research team responsible for ensuring correct broodstock handling and data collection. Ethical standards, biosecurity measures, and best aquaculture practices were observed in all activities under the supervision of the Head of the Work Unit (Ketua Pokja).

2.2 Methods

2.2.1 Experimental design

During the maintenance period, 3 units of floating net cages measuring 3 x 3 x 3 m^3 were used as containers. The method adopted in this study was a completely randomized design (CRD) with 5 treatments of different concentrations of Tribulus extract and gonadotropin hormone in the feed. The study utilized male silver pompano broodstock candidates, with an average length of 46.53 ± 2.40 cm and 1792.88 ± 286.73 g, respectively. These specimens were sourced from the Lampung Marine Aquaculture Center. A total of 40 male broodstock of silver pompano were divided equally into 5 treatments (a combination of *T. terrestris* extract (ETT, mg/kg of diet) and human Chorionic Gonadotropin (hCG, IU/kg of the body): T1 (0 + 0), T2 (50 + 0), T3 (250 + 0), T4 (50 + 1000), and T5 (250 +500), each with eight individuals. The broodstock was fed by Tribulus extract for 30 days, with a feeding regimen consisting of 2 daily feedings, at 08:00 a.m. and another at 02:00 p.m. The amount of feed provided was equivalent to 2% of the total biomass weight of the fish.

2.2.2 Extraction of Tribulus terrestris

The extraction process included mixing 100 g of sieved and weighed *T. terrestris* simplicia with 1 L of 90% ethanol in an Erlenmeyer flask. The mixture was subjected to a hot maceration method at a temperature of 70-80°C for approximately 2 hours with constant stirring until suspended. These steps were modified from Do *et al.* (2013). Subsequently, the solution was cooled, filtered using filter paper, and extracted using a rotary vacuum evaporator. After evaporation, the extract was placed in a dark bottle, sealed with parafilm, and stored in a freezer at -20°C. The *T. terrestris* extract was considered usable after a minimum incubation period of 2 days.

2.2.3 Diet preparation

The feed in this study was a commercial pellet for marine fish with a protein content of 50%. The *T. terrestris* extract was mixed at doses of 50 and 250 mg/kg using 90% ethanol, followed by the addition of 0.50 g/100 ml glycerin to prevent easy dissolution in water (El-Greisy and El-Gamal, 2012). Each 100 ml of the solution was evenly sprayed onto 1 kg of feed, accompanied by air. The drying process was conducted by spreading feed thinly on the surface of a plastic tray and allowing it to air-dry at room temperature for 12 hours or until completely dry and free of alcohol aroma. Subsequently, the feed was stored in a sealed container and kept in a freezer at -20°C.

2.2.4 The induction of gonadotropin hormone

Gonadotropin hormone was induced using the human chorionic gonadotropin (hCG) hormone obtained from Argent. laboratory in Makati City, Philippines. In addition, the hCG hormone was diluted by dissolving one ampoule in 10 ml of 0.9% NaCl (Badran *et al*., 2015). The induction activity was performed on the 28th day of Tribulus treatment. This was accomplished using male silver pompano fish with the criteria of good breeding candidates, which included being healthy, free from defects, and devoid of injuries throughout the body. Each selected fish received a single intramuscular injection of the prescribed hCG hormone dose, administered below the dorsal fin.

2.2.5 Blood collection sampling

A total of 2 randomly selected fish from each treatment group were used for blood sampling on days 0, 7, 21, 28, and 31. Blood was extracted from the caudal vein with a 1 ml syringe and rinsed with 10% EDTA anticoagulant. Before the extraction process, the fish were anesthetized using an anesthesia solution. The collected blood was transferred into a 1.5 ml microtube and stored in a cool box. Subsequently, the blood was centrifuged at a speed of 12,000 rpm for 5 minutes to separate the blood cells from plasma. The obtained supernatant was then transferred back into a microtube and stored at -20°C for hormone-level testing. Testosterone levels in the blood plasma were measured using the enzyme-linked immunosorbent assay (ELISA) method with a commercial kit. The measurement was performed at the Agroindustry and Biomedical Laboratory-Agricultural Production, Puspitek-Serpong.

2.2.6 Histology examination of gonads

Histology analysis of gonads was performed by randomly sampling fish at the beginning (n=4) and end of the experiment (n=2 for each treatment). Anesthesia was induced using a dose of 1 ml/L of clove oil, and the weight of the dead fish was measured. The

gonads were removed, weighed, and fixed in 10% buffered formalin. After 24 hours of post-fixation, they were transferred and preserved in 70% ethanol. The preparation of histology slides in this study was conducted at the Fish Health Laboratory, Bogor Agricultural University, Bogor. Suitably sized pieces from each fixed gonad were processed and stained with hematoxylin and eosin. The developmental stage was classified microscopically following the methodology outlined by Sun *et al.* (2022).

2.2.7 The gonadosomatic index

The GSI was determined during the developmental stage of spermatogenesis on gonadal histology preparations, and it was calculated using the following equation:

GSI = (Gonad weight)/(Body weight including gonads) x 100%.....(i)

2.2.8 Sperm quality

Sperm quality parameters included sperm density, spermatocrit levels, sperm motility, sperm volume, and sperm pH. Sperm density was calculated using a hemacytometer (Assistent Germany).

∑ Total Spermatozoa = (Average at 5 medium box x dilution x 2,5 x 10^5) sel/ml.....(ii)

Spermatocrit levels were calculated by centrifuging sperm fluid samples for 5 minutes at a speed of 8000 rpm.

Spermatocrit Levels = (x (cement liquid solids) (cm))/(y (sperm fluid-solid)(cm)) x 100%.....(iii)

Sperm motility was observed by recording the movement time of sperm until it stopped. Sperm volume was determined by collecting the samples into a microtube before calculating the volume. Meanwhile, sperm pH was observed using a pH indicator.

2.3 Analysis Data

All values were reported as mean ± standard error of the mean (SEM). Multiple comparisons among treatments were conducted using one-way analysis of variance (ANOVA), followed by the Duncan test, provided that the assumptions were met (Kolmogorov-Smirnov one-sample test). Statistical significance was tested at $P < 0.10$, while all analyses and data visualization were performed using SPSS ver. 26 (SPSS Inc., Chicago, IL, USA). The experiment was conducted in the field, where numerous natural variables could not be fully controlled. Setting the significance at $P < 0.10$ allows the study to better capture subtle but potentially meaningful effects under field conditions, while still interpreting results with appropriate caution for future validation.

3. Results and Discussion

3.1 Results

3.1.1 Testosterone level

The changes in testosterone levels in the plasma of male starry pufferfish candidates treated with Tribulus extract and gonadotropin hormone are presented in Figure 1.

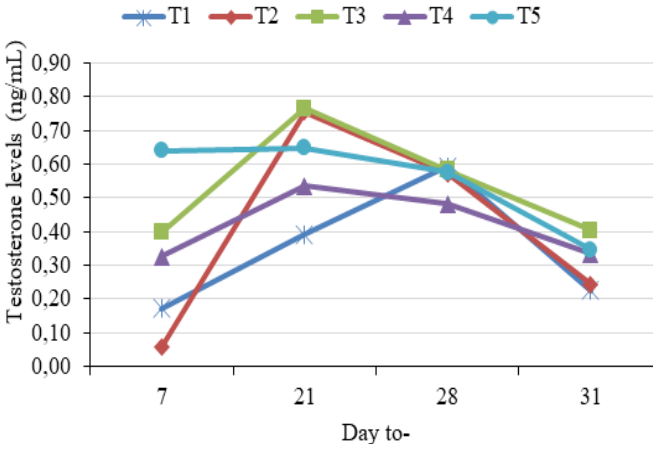

Figure 1. Testosterone level of male candidate broodstock of silver pompano (*Trachinotus blochii*) subjected to the administration of a combination of *Tribulus terrestris* extract (ETT, mg/kg of diet) and human Chorionic Gonadotropin (hCG, IU/kg of the body): T1 (0 + 0), T2 (50 + 0), T3 (250 + 0), T4 (50 + 1000), and T5 (250 + 500).

3.1.2 Gonad histology

Histology results of the male gonad of potential silver pompano broodstock are presented in Figure 2. The duration of maintenance in this study indicates that the tests conducted on prospective male star pomfret candidates, which were treated with *Tribulus terrestris* extract and gonadotropin hormone, exhibited significant development.

3.1.3 Sperm quality

The quality of sperm from male silver pompano prospective parents treated with Tribulus extract and gonadotropin hormone is presented in Table 1. The observed sperm was collected at the beginning and end of the study. The results of the analysis showed that all treatments did not have a significant effect ($P>0.10$) on motility duration, spermatozoa, and sperm density.

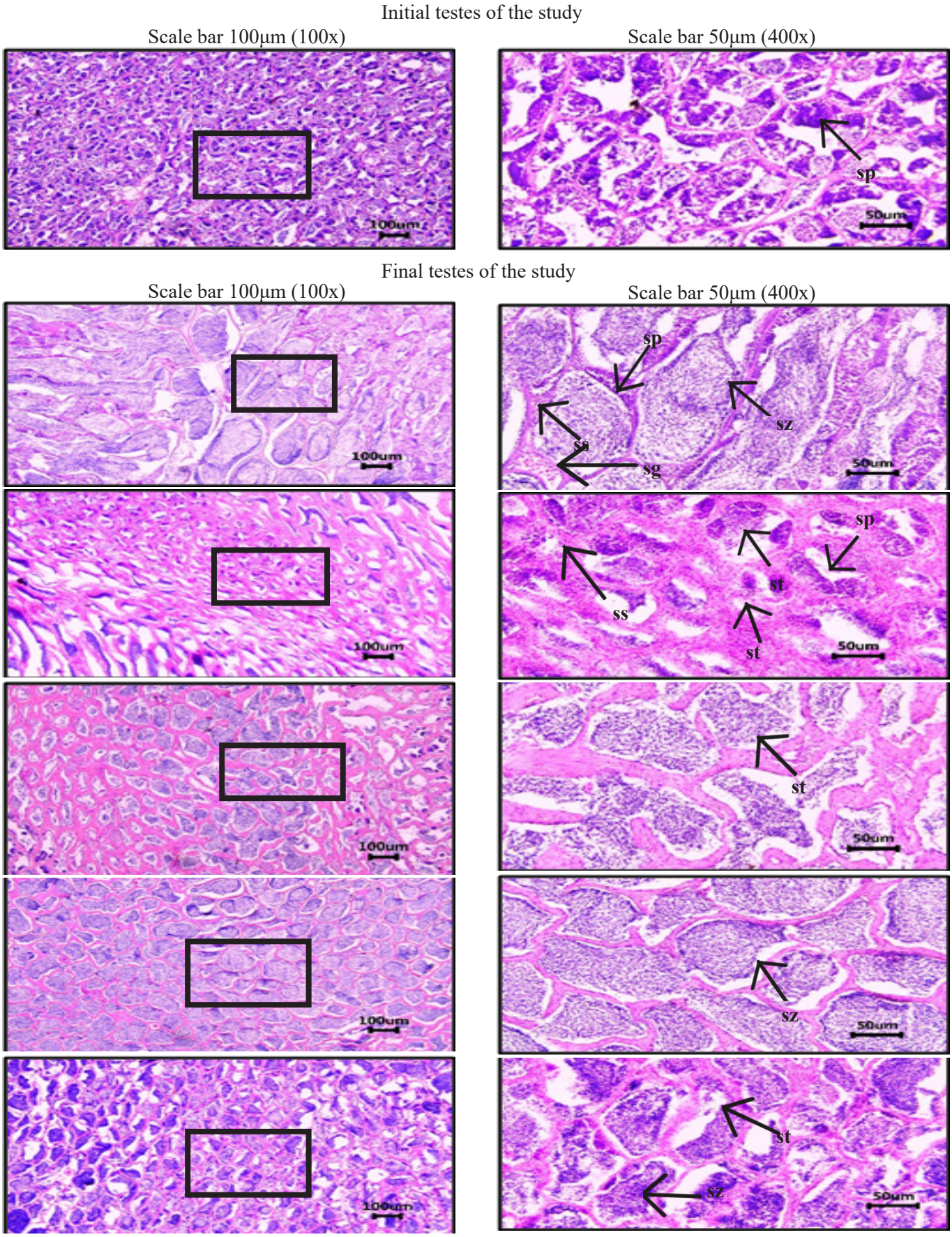

Figure 2. Histology gonad of male candidate broodstock of silver pompano (Trachinotus blochii) subjected to the administration of Tribulus extract and gonadotropin hormone. Spermatogonia (sg), primary spermatocytes (sp), secondary spermatocytes (ss), spermatids (st), and spermatozoa (sz). A-B: initial testes of the study, C-D: final testes of treatment T1 (0 mg/kg feed of Tribullus terrestris extract), E-F: final testes of treatment T2 (50 mg/kg feed of T. terrestris extract), G-H: final testes of treatment T3 (250 mg/kg feed of T. terrestris extract), I-J: final testes of treatment T4 (50 mg/kg feed of T. terrestris extract+ gonadotropin 500 IU/kg of body), K-L: final testes of treatment T5 (250 mg/kg feed of T. terrestris extract+ gonadotropin 1000 IU/kg of body)and Hematoxylin-Eosin staining. Scale bar = 100 and 50 µm (100x and 400x).

Table 1. Sperm quality of male candidate broodstock of silver pompano (*Trachinotus blochii*) subjected to the administration of *Tribulus* extract and gonadotropin hormone.

Observation data	Treatments	Sperm quality				
		Motility (s)	pH	Spermatocrit (%)	Density (x109 cell/ml)	Volume (ml)
Beginning (H0)	T1	164.25±45.11a	6-7	88.88±2.46 a	151.88±70.08a	0.35±0.22a
	T2	164.25±45.11 a	6-7	88.88±2.46 a	151.88±70.08a	0.35±0.22a
	T3	164.25±45.11 a	6-7	88.88±2.46a	151.88±70.08a	0.35±0.22a
	T4	164.25±45.11 a	6-7	88.88±2.46 a	151.88±70.08a	0.35±0.22a
	T5	164.25±45.11 a	6-7	88.88±2.46a	151.88±70.08a	0.35±0.22a
End (H31)	T1	208.50±102.50 a	6-7	90.94±1.65 a	296.20±13.75a	0.35±0.05b
	T2	272.00±18.00 a	7	91.05±2.17 a	338.75±76.25a	0.35±0.05b
	T3	299.50±15.50 a	6	92.08±1.76 a	387.50±37.50a	1.45±0.05a
	T4	238.67±23.10 a	6	91.36±3.39 a	361.67±19.61a	0.50±0.22b
	T5	228.67±62.94 a	6	91.08±1.74 a	371.67±32.74a	0.53±0.21b

Note: The administration of a combination of *Tribulus terrestris* extract (ETT, mg/kg of diet) and human Chorionic Gonadotropin (hCG, IU/kg of the body): T1 (0 + 0), T2 (50 + 0), T3 (250 + 0), T4 (50 + 1000), and T5 (250 +500). 1000), and T5 (250 +500).

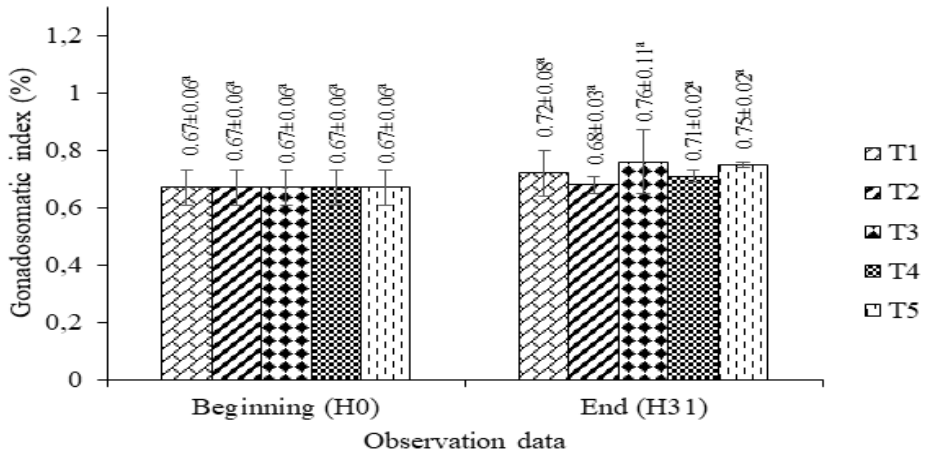

Figure 3. The GSI of male candidate broodstock of silver pompano (*Trachinotus blochii*) subjected to the administration of a combination of *Tribulus terrestris* extract (ETT, mg/kg of diet) and human Chorionic Gonadotropin (hCG, IU/kg of the body): T1 (0 + 0), T2 (50 + 0), T3 (250 + 0), T4 (50 + 1000), and T5 (250 +500). Different superscript letters in the same column showed a significantly different effect of treatment ($p < 0.10$). The values shown are the average and standard error.

3.1.4 The GSI

The evaluation of the GSI of male silver pompano broodstock subjected to Tribulus extract and gonadotropin hormone treatment is presented in Figure 3.

3.1.5 The absolute weight growth

The examination of the absolute weight growth of male silver pompano broodstock administered Tribulus extract and gonadotropin hormone through feed showed Treatment 3 the highest absolute weight at 343.13 g (Figure 4), followed by T5 (323.57 g), T1 (281.13 g), T2 (226.25 g), and T4 (203.75 g). Based on the analysis of variance, it was observed that the induction of Tribulus extract and gonadotropin hormone had a significant effect ($P<0.10$) on the tested parameter. Furthermore, Duncan's post hoc test showed that T3 and T5 were significantly different from T4 but not significantly different from T1 and T2.

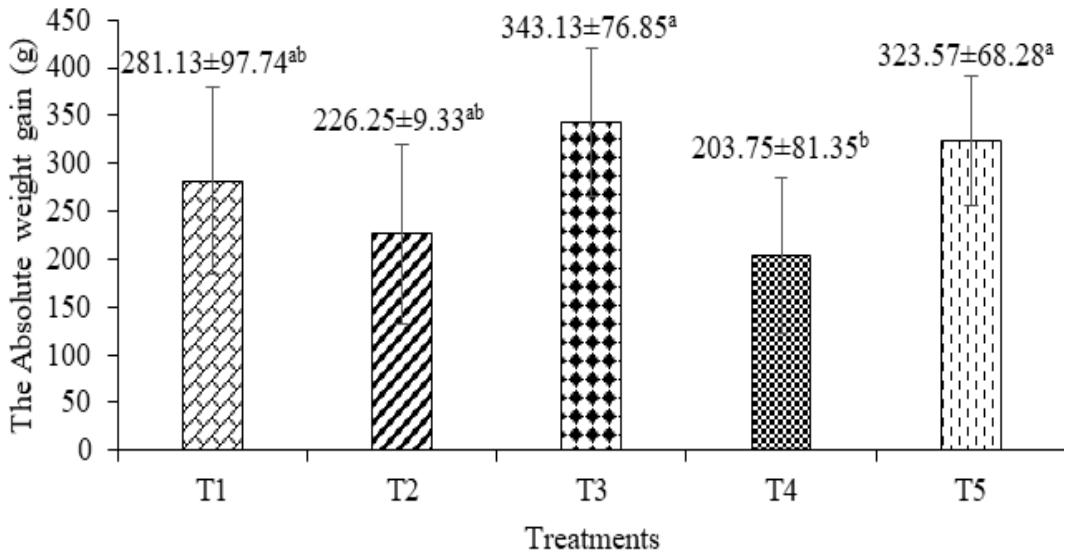

Figure 4. The absolute weight growth of male candidate broodstock of silver pompano (*Trachinotus blochii*) subjected to the administration of a combination of *Tribulus terrestris* extract (ETT, mg/kg of diet) and human Chorionic Gonadotropin (hCG, IU/kg of the body): T1 (0 + 0), T2 (50 + 0), T3 (250 + 0), T4 (50 + 1000), and T5 (250 +500). Different superscript letters in the same column showed a significantly different effect of treatment (P<0.10). The values shown are the average and standard error.

3.2 Discussion

3.2.1 Testosterone level

On the 21st day, plasma testosterone hormone levels of male starry pufferfish candidates showed an increase through the development and maturation of the gonads. The increase in concentration was linked to the presence of protodioscin in *T. terrestris* extract. Protodioscin plays a crucial role in the conversion of testosterone to dihydrotestosterone, a precursor of male sexual characteristic steroid hormones (Santos *et al*., 2019). Additionally, *T. terrestris* contains saponins (Dhas *et al*., 2015), known to stimulate testosterone hormone secretion for gonadal demands (El-Kady *et al*., 2022). Hassona *et al*. (2020) also stated that *T. terrestris* extract in male Nile tilapia can improve testis function, reproductive efficiency, and sperm quality.

On the 28th and 31st days after the administration of gonadotropin hormone, the plasma blood testosterone levels in the prospective broodstock of starry sturgeon experienced a decrease for all treatments. This was with the exception of T1, which showed an increase on the 28th day and a decrease on the 31st day. Li *et al*. (2023a) reported that plasma blood testosterone increases during gonad maturation and decreases during spawning. According to the study by Falahatkar *et al*. (2022), the concentration of testosterone in fish naturally decreases after gonad maturation. Ciji *et al*. (2021) have also conveyed that plasma blood testosterone began to increase during maturation and development and then decreased during adulthood, spawning, and post-spawning stages. Walker (2021), stated that testosterone levels decreased during spermatogenesis, followed by an increase during spermiation. Subsequently, levels weakened after spawning and continued to remain lower during the adult period.

3.2.2 Gonad histology

As the study progressed, the testes of potential male broodstock treated with Tribulus extract and gonadotropin hormone developed. The development of the testes (spermatogenesis) is closely related to the concentration of testosterone in the blood. This hormone functions to stimulate the growth of spermatogonia, the development of primary and secondary spermatocytes, and the differentiation of spermato cytes into sperm. It is important to note that a high er concentration of testosterone in the plasma blood leads to a more mature status of testicular cells. According to the results of the study, the administration of Tribulus extract and gonadotropin hormone caused the gonads of prospective silver pompano parents to develop more. The classification of gonad development and maturity levels Sun *et al*. (2022) showed that the gonads of male prospective silver pompano parents are in the early maturation phase (gonad maturity level II), where the testes are more developed and the connective tissue is less visible. The seminiferous

tubule sacs contain primary spermatocytes. Furthermore, the difference in size between spermatogonia and primary spermatocytes is very small. At the end of the study, male prospective silver pompano parents showed different phases between treatment groups. In treatments T1 and T2, the gonads are categorized as being in the mature phase (gonad maturity level III). This is evident from the reduction in primary spermatocytes, with visible spermatids present within the seminiferous tubules. In treatments T3, T4, and T5, the final maturation phase (gonad maturity level IV) was observed, where the seminiferous tubules were filled with spermatids and spermiogenesis (transformation of spermatids into spermatozoa). At the end of spermatogenesis, spermatozoa were released into the seminiferous lumen. In addition, *T. terrestris* extract also plays a crucial role in the early development of juvenile Nile tilapia and their sex ratio (Sarida *et al*., 2025), as well as the differentiation process. This indicates that, depending on the way it is dosed and applied, some of the active compounds in Tribulus may act as a natural masculinizing agent in fish culture.

3.2.3 Sperm quality

At the beginning of the study, sperm were able to move for only 2.7 minutes. Approaching the end, sperm treated with T3 showed a movement duration exceeding 4 minutes, whereas those exposed to T1 (control) only persisted for 3.4 minutes. Sperm density and spermatozoa level ranged from 151.88-387.50x10^9/ml and 88-91%, respectively. The results of the study showed that the volume of sperm produced is directly proportional to the testis weight. This study showed that the volume of sperm produced increased with an elevation in testis weight. Administration of Tribulus extract and gonadotropin hormone to male silver pompano prospective parents affected semen volume at the end of the experiment ($P<0.10$). The average volume of 1.45 ml from T3 was the highest, while the pH ranged from 6 to 7.

The evaluation of sperm quality in aquaculture fish is necessary to enhance efficiency in egg fertilization, particularly in artificial spawning. One of the crucial parameters for determining sperm quality is motility. Based on observations conducted on male silver pompano candidates, the duration was between 208.50 and 299.50 seconds. This is consistent with the statement by Merino *et al*. (2023) that warm-water fish sperm moves using its tail with a motility time of 0.5 to 1 minute. According to (Abinawanto *et al*., 2023), the length of the sperm tail can determine the activity in movement. The longer the tail, the more active the sperm is in movement (Merino *et al*., 2023).

This study showed no significant influence ($P>0.10$) of all treatments on sperm density. The same results were reported by Swaroop *et al*. (2017), stating that the addition of protodioscin to feed did not have a significant effect on sperm motility and density. Protodioscin is a steroid saponin compound identified in several plant species, particularly in the Tribulus, Trigonella, Dioscorea, and trillium families (Li *et al*., 2023b). According to Falahatkar *et al*. (2022), the hormone dosage used in induction activities determines the quality of the resulting sperm. The observed sperm density in this study ranged from 151.88 to 387.50x10^9 cells/ml. These results were higher than the values reported by Hassona *et al*. (2020), where Tribulus extract yielded sperm density of 220 to 308x10^6 cells/ml.

The measurement of spermatic hematocrit levels, which can be used as an indicator of sperm viscosity, did not show any significant effect ($P>0.10$). According to Gamblin *et al*. (2020), high value shows that the semen is viscous, resulting in a higher concentration of spermatozoa compared to the seminal fluid. Spermatic hematocrit level in this study ranged from 88 to 91%. The%, and the higher the value, the greater the concentration of the semen (Aydin *et al*., 2022; Akbari *et al*., 2024). Consequently, it can be inferred that semen density and the essential substances for sperm motility are abundant.

This study showed that the volume of sperm produced increased with an elevation in testis weight. It is important to note that fish sperm given Tribulus extract at a dose of 250 mg/kg of feed had the highest volume value compared to other treatments ($P<0.10$). According to Knowles *et al*. (2022), testosterone and 11-ketotestosterone cause spermatogenesis and spermiogenesis in fish.

The pH of sperm also influences the quality of sperm and egg fertilization. In this study, the pH of sperm from male silver pompano candidates treated with Tribulus extract and gonadotropin hormone ranged from 6 to 7. This was slightly lower than the values observed in marine fish of the Salmonidae family, which typically ranged from 7.5 to 8.5 (Dziewulska *et al*., 2008). The pH level of sperm affects the physiological condition of fish. Bacteria can alter the pH and metabolic products of plasma and also affect sperm function. Therefore, an acidic environment due to pathological conditions can decrease sperm function. It is important to note that fish sperm should be in an appropriate environmental condition to function properly in the reproductive process. Large fluctuations in pH or water quality can affect fertilization.

3.2.4 The GSI

The GSI for each treatment showed an in-

crease compared to when the study commenced. These values with Tribulus extract and gonadotropin hormone showed no significant difference ($P>0.10$), ranging from 0.67% to 0.76%. According to Hassona *et al.* (2020), the GSI for Nile tilapia given Tribulus extract was between 0.41% and 0.44 %, while for silver pompano induced PMSG hormone, it was 0.6% (Handrianto *et al*., 2017). Based on the study by Honji *et al.* (2022), this parameter has become a standard protocol in selecting fish for the reproductive process. The GSI value can also be used as an estimation for gonad maturity and spawning in many species. It will continue to increase as the gonad matures and will reach its maximum value during the peak period of maturity (Sultana *et al*., 2023).

3.2.5 The absolute weight growth

The study results showed that the administration of Tribulus extract and gonadotropin hormone affects the absolute weight growth of male starry tilapia broodstock ($P<0.10$), indicating a positive impact on body weight gain. According to Ahmed *et al.* (2022) optimal fish growth occurs when nutritional and energy needs are met. The treatment with Tribulus extract led to increased body weight, presumably due to its anabolic properties, enhancing the appetite of male starry tilapia broodstock. The correlation between body weight and gonad maturation is evident, as highlighted by Homrum *et al.* (2022). It influenced the energy expenditure in gamete cell formation for subsequent broodstock and consequently impacted the weight gain in fish (Saha *et al*., 2022). This finding aligns with the research conducted by Hassona *et al.* (2020), who demonstrated that the use of 750 mg ETT/kg feed resulted in the growth-affecting androgenic effects of *T. terrestris* extract.

4. Conclusion

The administration of *T. terrestris* extract and hCG notably enhanced plasma testosterone levels, driving spermatogenesis and testicular development in male silver pompano. These hormonal and structural improvements led to better sperm motility and seminal volume, with the optimal reproductive outcomes achieved at a dosage of 250 mg/kg of *T. terrestris* extract. These findings provide a practical and efficient solution for aquaculture sectors, as improving gonadal maturation and sperm quality in broodstock can enhance breeding success. Incorporating this supplementation strategy into aquaculture systems could boost productivity and sustainability, paving the way for more reliable and efficient fish farming practices. Further research to optimize dosages can ensure consistent and reproducible benefits. The administration of *T. terrestris* extract and hCG significantly influenced hormonal profiles by increasing plasma testosterone levels, which stimulated the process of spermatogenesis. This hormonal shift was associated with notable changes in gonadal histological structure, indicating enhanced testicular development. These structural and functional improvements contributed to better sperm quality, as reflected in motility and seminal volume. These findings highlight the potential of hormonal supplementation to improve reproductive performance in silver pompano broodstock. Broader application of this strategy in aquaculture systems may support more sustainable and efficient breeding practices. Further studies are recommended to evaluate long-term impacts and optimal implementation protocols.

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Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author as follow, AZH; collected the data, analisys data, drafted the manuscript, and designed the figures. MS; devised the main conceptual ideas and critical revision of the article. YTA, GNS, AND AS; All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

Declaration of Artificial Intelligence (AI)

The author(s) affirm that no artificial intelligence (AI) tools, services, or technologies were employed in the creation, editing, or refinement of this manuscript. All content presented is the result of the independent intellectual efforts of the author(s), ensuring originality and integrity.

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