



# Regulatory roles of Pawpaw (*Carica papaya*) seed extract on growth performance, sexual maturity, and health status with resistance against bacteria and parasites in Nile tilapia (*Oreochromis niloticus*)

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## Abstract

Papaya (*Carica papaya*) is one of the plants that represent a major source of bioactive substances that are cheaper and non-toxic, such as antibiotics. It is globally used as a supplement diet for positive effects in pharmacological activities. In this regard, the current study sheds light on the possible roles of papaya seed methanolic extract (PSE) as a dietary supplement to improve growth performance, sexual maturity, carcass composition, immunity responses, histological structure of gonads, and anti-pathogenic activity (*Aeromonas hydrophila*) at the end of a feeding trial extend for ten days of Nile tilapia, with special reference to its potential effect as resistance against parasitic (*Cichlidogyrus tilapiae*). Four experimental groups with four replications of Nile tilapia fry ( $0.5 \pm 0.1$  g as 20 fish per replication) were fed (0, 0.5, 1.0, and 2.0 g/kg PSE in diets) for 20 weeks. Results showed that fish dietary supplementation with PSE had a significantly positive ( $P < 0.05$ ) effect on growth performance, feed utilization, digestive enzyme, carcass composition, antioxidant, immunity responses, and hematological and biochemical indices, especially PSE 2.0 g/kg than control diet. Furthermore, PSE had a positively affected fish mortality after injection with *A. hydrophila* and in vitro positive efficacy against *C. tilapiae*. Fish fed with dietary PSE increased the sex ratio in favor of male fish by achieving a 77% male phenotype. Conclusively, this study declared that PSE extract, specifically 2 g/kg, could enhance growth and health through the modulation of innate immunity and a positive effect against disease in fish. Additionally, it reduced the reproductive performance of Nile tilapia by reducing GSI, which subsequently affected the gonadal histology leading to infertility.

**Keywords** Anti-parasitic efficiency · Dietary supplements · Medicinal plants · Nile tilapia · Papaya · Phytochemistry · sexual puberty

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## Introduction

Aquaculture development occurs rapidly in order to overcome the rise in the consumption of fish by humans (Radwan, 2022a). As the wildest food-producing sector, aquaculture represents approximately 50 % of fish food worldwide (FAO, 2022). Nile tilapia (*Oreochromis niloticus*) is considered to be the most popular farmed fish group following carp in the world (Magblenou et al., 2019) since it possesses an elevated nutritional and commercial value and can tolerate stressful conditions (Dawood et al., 2020). More specifically, tilapia possesses many advantages, such as being a widespread culture species. Tilapia display general hardiness and high yield potential, can resist diseases and grow on a broad array of regular and economical artificial food regimes, and sexually mature early (2–3 months old) by reaching a body length of about 8–10 cm before reaching marketable size (Abdel-Tawwab et al., 2021; Yadav et al., 2021). Correspondingly, the extension in this species' production requests continuous progress of aqua-feeds through increased efficiency feed supplements, which play important roles in enhancing the growth, health, and immune responses of numerous fish (Hoseinifar et al., 2018). Conclusively, commercial feed prices peaked during the lockdowns, which caused a daily increase in operational costs; especially for small-scale farmers, which would negatively influence their income (Hamid et al., 2022). Hence, farms need to reduce the fish growing period by improving growth that may be useful to the industry of aquaculture, chiefly the small-scale farmers. Nevertheless, there is an increasing demand for the induction of tilapia sterility by using a reliable, consistent, non-chemical, less expensive, and eco-friendly approach that can control tilapia reproduction and manage its aquaculture production (Yadav et al., 2021). Consequently, this could be accomplished by applying appropriate natural substitutions which are biological, decomposable, and inexpensive reproductive inhibitory agents like Pawpaw (*Carica papaya*), which consider one of these dietary supplements and has been importantly used in the aquafeed sector as it contains many rich phytochemicals substance (Azizah and Fasya, 2019). Moreover, Fakoya et al. (2019) indicated that using PSE in Fish principally concentrated on its anti-microbial properties against different diseases in fishes. Additionally, papaya possesses anti-bacterial, antifungal, and anti-fertility properties, used as a medicinal plant to treat several diseases of humans and animals besides a positive effect on sex skew in fishes (Singh et al., 2020). Notably, bacterium and parasitic pathogens cause a disease that can induce septicemia and hemorrhage leading to significant mortality in cultivated Fish (Lu et al., 2021). However, there is a lack of study on the possibility of using papaya seed extract for dietary, reproductive performance, and resistance against diseases in Nile tilapia. Therefore, the current study planned to assay the impact of PSE on growth performance, carcass composition, hematology, blood biochemistry indices, sexual puberty, and gonadal histology with a special reference to boosting Nile tilapia resistance against bacterial and parasitic diseases.

## Materials and methods

### Experimental design and approach

#### Papaya (*C. papaya*)

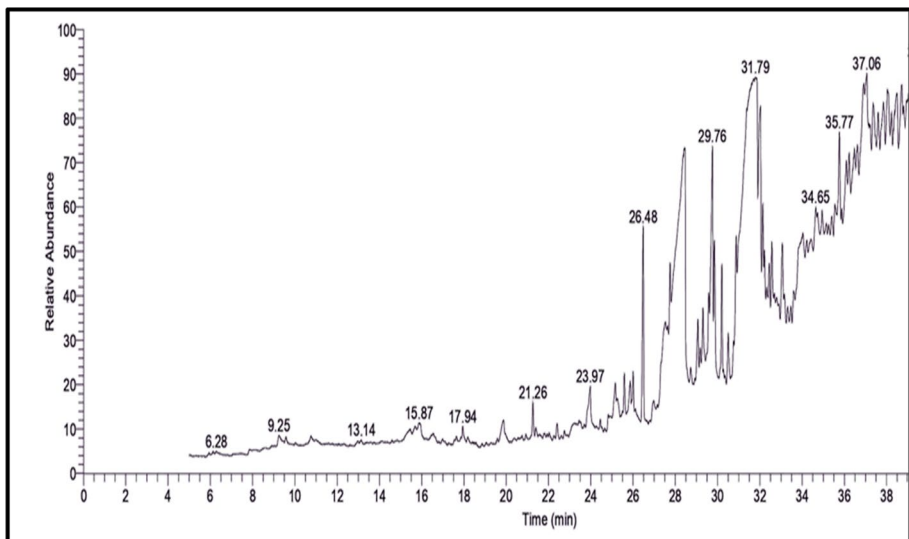
The papaya seeds (PSE) were collected from a private vegetable farm in Putra, then identified through scientists at the Department of Aquaculture, Faculty of Agriculture,

University Putra, Malaysia, at the end of February 2021. Preparation of Papaya seed methanolic extract as described by Ibrahim and Sayed (2021). Furthermore, *P. seed* methanolic extract was kept in the refrigerator in a closed glass flask until used. Based on approaches demonstrated by Radwan, (2022b), gas chromatography-mass spectrometry (GC-MS) was used to examine and identify constituent parts of obtained concentrated PSE seed extracts (Table 2 and Figure 1). The selected varied concentration (0.0,0.5, 1.0, 2.0 g/kg PSE) were selected according to Kareem et al. (2016).

## Fish management

Fish were procured from a private fish farm that seemed healthy throughout the visual investigation of the area mentioned above, then transported according to the approach mentioned by Radwan (2022c). Fish were placed in fiberglass tanks ( $2 \times 2 \times 2 \text{ m}^3$ ) for 14 days to acclimatize. After acclimatization, a total of 320 sac-larvae with initial weight ( $0.5 \pm 0.1 \text{ g}$ ) of Nile tilapia apportionment as four groups (80 fries) as replicate (20 fries) consistent with formulated diets in the study (Table 1). Then, fish were postured in concrete ponds ( $1.0 \times 1.0 \times 0.90 \text{ m}^3$ ) and fed various formulas twice daily at 9:30 am and 2:30 pm for 20 weeks. Moreover, solid garbage is vacuumed daily, and pools are cleansed and aerated with dechlorinated water (10% change daily). Fish were kept for 20 weeks under a photoperiod of 12 h light:12 h darkness with water temperature ( $27 \pm 2^\circ\text{C}$ ), and other parameters like pH, DO, and total ammonia levels remained constant as described by Boyd and Tucker (2012).

Moreover, at end of the feeding trial, fish fasted for 24 h before sampling, collected from ponds, then washed with water. All fish counted, noted weight and length to calculate growth performance, condition factor, and carcass composition (3 fish/pond) using the previous equations described by Abdel-Tawwab et al. (2021); Bagenal and Tesch (1978), and Thiex et al. (2012) respectively.



**Fig. 1** Investigation of the detected PSE components by GC-MS

**Table 1** Basic dietary intake and nearby chemical composition on a dry matter basis

Ingredient	%	Chemical composition	%
Fish meal (72.0% CP)	9.9	Moisture	7.7
Soy bean meal (48% CP)	42.48	Dry matter	92.3
Yellow corn	20.11	Crude protein	30.14
Wheat flour	5.89	Ether extract	5.43
Wheat bran	14.77	Ash	7.15
Vegetable oil	3.48	Crude fibers	4.84
Cod liver oil	2.14	Gross energy (kcal/kg) <sup>2</sup>	2030.58
Dicalcium phosphate	0.91		
Vitamins and mineral mixture <sup>1</sup>	0.28		
Vitamin C	0.04		
Total	100		

<sup>1</sup>Vitamins and mineral mixture percentage applied as mentioned in Abdel-Tawwab et al. (2021) study

<sup>2</sup>Based on the values of protein, fat, and carbohydrate, which correspond to 23.6, 39.5, and 17.2 KJ/g, respectively, gross energy was computed

### Tilapia sexes and gonadosomatic indices assay

Throughout the current experiment, sexual characteristics of fish maturation were detected based on the size, morphology, colour changes, and behaviour of males (Novelo et al. 2021), fish with one opening on the genital papillae, the appearance of rosy red colour on the chin, the dark black colour of the dorsal and pelvic fins of mature males, as well as courtship activity or pursuit of females. At the end of the experimental trial, samples of males and females were identified in varied groups and weighed at the same maturity period to the nearest 1g. Moreover, gonadosomatic index (GSI) assay anesthetized fish using clove oil (0.25 ml/L H<sub>2</sub>O) with a recorded weight of each fish. Fish gonads were separated after dissection and weighed using an electronic balance. Correspondingly, the gonadosomatic index (GSI) was calculated as the method described by Ahammad et al. (2021).

$$GSI = (G_w/F_w - G_w) \times 100$$

where  $G_w$  is gonad weight, and  $F_w$  is the wet weight of fish.

### Digestive enzymes, haemato-biochemical, and antioxidative indices

The gut sample (3 fish/pond) was homogenized and centrifuged at 8000 rpm for 5 min after being washed with saline solution (0.90%; pH 7.5). For future usage, the supernatant was stored at 80 °C. Protease activity was evaluated after assessing Sigma's non-specific protease activity using casein. In this way, spectrophotometric measurements of lipase and amylase activity were made at A540 and A714, respectively according to Wang et al. (2019).

Collected blood samples (3 fish/pond) from the caudal receptacles at the end of the trial after starved for 24 h (Shah and Altindag, 2004). Blood was drawn using a plastic syringe, first aliquot mixed with EDTA, for hematology all (RBCs, WBCs, Hb, Hct) indices analysis using an automated cell counter (Sino thinker. sk9000, US). While another aliquot of blood for serum collection was separated and stored at -20 °C until

**Table 2** Identification of *P. seel* extract components by GC-MS analysis

No	Compound identification	M.wt	Formula	RT (min)	Area (%)
1	Capric acid	172.26	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	6.48	71.24
2	Oleic acid	282.5	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	8.12	71.22
3	17-Octadecynoic acid	280.45	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	6.51	52.33
4	Tetradecanoic acid	228.37	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	15.28	61.27
5	2-Ethylhex-2-enal	126.20	C <sub>8</sub> H <sub>14</sub> O	7.18	47.33
6	Linoleic acid, methyl ester	294.5	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	16.24	63.38
7	Palmitic acid	256.4	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	18.11	52.71
8	Melezitose	504.4	C <sub>18</sub> H <sub>32</sub> O <sub>16</sub>	9.17	34.25
9	2,4-Di-tert-butylphenol	206.32	C <sub>14</sub> H <sub>22</sub> O	10.28	56.39
10	2-Methoxy-4-vinylphenol	150.17	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	22.23	54.37
11	Caffeic acid	180.16	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	11.37	49.11
12	Chlorogenic acid	354.31	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	16.39	47.39
13	Kaempferol	286.23	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	15.11	32.15
14	Eupatolitin	346.3	C <sub>17</sub> H <sub>14</sub> O <sub>8</sub>	15.78	44.39
15	Phytol	296.53	C <sub>20</sub> H <sub>40</sub> O	12.81	18.04
16	Squalene	410.73	C <sub>30</sub> H <sub>50</sub>	17.58	27.81
17	β-carotene	536.87	C <sub>40</sub> H <sub>56</sub>	20.14	21.15
18	Dihydroactinidiolide	180.24	C <sub>11</sub> H <sub>16</sub> O <sub>2</sub>	8.54	24.28
19	Campesterol	400.68	C <sub>28</sub> H <sub>48</sub> O	26.35	21.28
20	Stigmasterol	412.69	C <sub>29</sub> H <sub>48</sub> O	23.68	41.87
21	Dasycarpidan-1-methanol, acetate (ester)	326.4	C <sub>20</sub> H <sub>26</sub> N <sub>2</sub> O <sub>2</sub>	14.77	42.89
22	β-Sitosterol	414.71	C <sub>29</sub> H <sub>50</sub> O	24.14	39.25
23	Dehydrocarpaine I	476.7	C <sub>28</sub> H <sub>48</sub> N <sub>2</sub> O <sub>4</sub>	19.44	11.78
24	Cystein	121.16	C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub> S	22.36	7.13
25	Valine	117.15	C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>	27.14	11.58
26	Lysergol	254.33	C <sub>16</sub> H <sub>18</sub> N <sub>2</sub> O	17.12	23.11
27	Carpaine	478.7	C <sub>28</sub> H <sub>50</sub> N <sub>2</sub> O <sub>4</sub>	10.44	18.47
28	Retinol	286.00	C <sub>20</sub> H <sub>30</sub> O	32.00	7.18

Molecular weight (M.wt.), retention time (RT).

biochemical analysis, enzymes aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total protein (TP), albumin (ALB), and globulin (GLB) were assessed according to Reitman & Frankel (1957), Henry, (1964), Doumas et al. (1971) respectively. A lysosomal assay (Ellis, 1990) based on small-world lysis (Sigma Chemical Co) was used to measure lysozyme activity in blood, as described by Zahran et al. (2018), with some modifications. Furthermore, based on the manufacturer's (Biodiagnostic, Egypt) instructions, the levels of the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malonaldehyde (MDA) were estimated in tissue using diagnostic reagent kits.

## Bacterium challenge trial

The pathogenic bacterial strain (*Aeromonas hydrophila*) was cultured and then used in the same line using Naiel et al. (2020). Then use the prepared solution to measure the optical density (OD) at 456 nm, corresponding to  $1 \times 10^7$  cells ml<sup>-1</sup>. Correspondingly, after 24 h of the feeding experiment, 40 fish from each group were exposed to the pathogen test. Concurrent with the methods of Zahran et al. (2018), 0.1 ml bacterial suspension was administered intraperitoneally into the fish. Notably, through the challenged period, fish were fed the basal diet (PSE-0), and recorded dead fish daily with removed consecutively from ponds.

The relative live percentage (RLP) was determined using mortality statistics following Amend (1981) equation:

$$\text{RLP} = 1 - [(\text{percentage mortality at treated groups (\%)} / (\text{percentage mortality at un - treated group (\%)})] \times 100$$

## Histological analysis

Histological examination was adopted on the method of Gewaily et al. (2020). Briefly, using clove oil, 0.25 ml per liter of water fish were anesthetized, and then their gonads were removed for histological assay. Ovarian and testicular tissues were fixed in 10% neutral buffered formalin after mincing into small pieces (5 mm) for 24 h. Then, samples were dehydrated at 4 °C by immersing in ascending grades of ethanol series, cleared by methyl benzoate then embedded in paraffin. Hematoxylin and Eosin was used to stain testicular and ovarian sections that were 5 to 8 µm thick. mounted on slides for light-microscope examination (Nikon Phase Contrast Dry, 0.90, Japan).

## Estimation of PSE activity anti-parasitic

The anti-parasitic activity of PSE against the *Cichlidogyrus tilapiae* parasite was prepared and identified in vitro using the Radwan (2022c) method. When the time was set to zero, the tilapia was given three PSE concentrations (0.5, 1.0, and 2 ml/l for 60 min, each reproduced three times). Utilizing a dissecting microscope, parasites were monitored every 10 min and deaths were noted. According to Zhang et al. (2014) adsorption, parasites were considered dead. Finally, Wang et al. (2009) used the following formula to determine the anti-parasitic efficiency for each group:

$$\text{EA} = [M - F] \times 100\% / M$$

where EA: efficacy of anti-parasitic; M: the mean survival number at control in the treatment group, is the average number of survivors(F).

## Statistical analysis

The SPSS program (version 22.0) was used for the statistical analysis. One-way ANOVA was followed by Duncan's test when a significant difference was considered ( $P < 0.05$ ). Mean values were represented as mean  $\pm$  SE, except anti-bacterial values (mean  $\pm$  Sd) (Dytham, 2011).

## Results

### Identified components of PSE extract

Table 2 shows the thorough PSE extract analysis by GC-Mass and demonstrates 28 phytochemical extracts from 13 biochemical groups. Concurrently, extracts were identified as follows: fatty acid nature (capric acid, oleic acid, 17-octadecynoic acid, tetradecanoic acid, 2-ethylhex-2-enal, linoleic acid, methyl ester, palmitic acid), trisaccharide (Melezitose), phenolic (2-methoxy-4-vinylphenol, 2,4-di-tert-butylphenol, caffeic acid, chlorogenic acid), flavonoid (kaempferol, eupatolitin), diterpene (phytol), triterpene (squalene), carotenoid ( $\beta$ -carotene), terpene (dihydroactinidiolide), steroids (campessterol, stigmasterol, dasycarpidan-1-methanol, acetate (ester),  $\beta$ -sitosterol), the organic compound (dehydrocarpaine I), amino acid (cystein, valine), alkaloids (lysergol, carpaine), and vitamin (retinol).

### Sexual maturity assay

According to Table 3, the male maturation percentage of Nile tilapia-fed papaya seed extract (PSE) for 3 and 20 weeks was increased significantly than that of fish fed basal diet ( $P < 0.05$ ), particularly with feed rates 0.5% and 1.0%. Conversely, there was a significant ( $P < 0.05$ ) decrease in the percentage of female sexual characteristics in the fish feeding 0.1 and 2.0 PSE g/kg than the control group after 3 and 20 weeks.

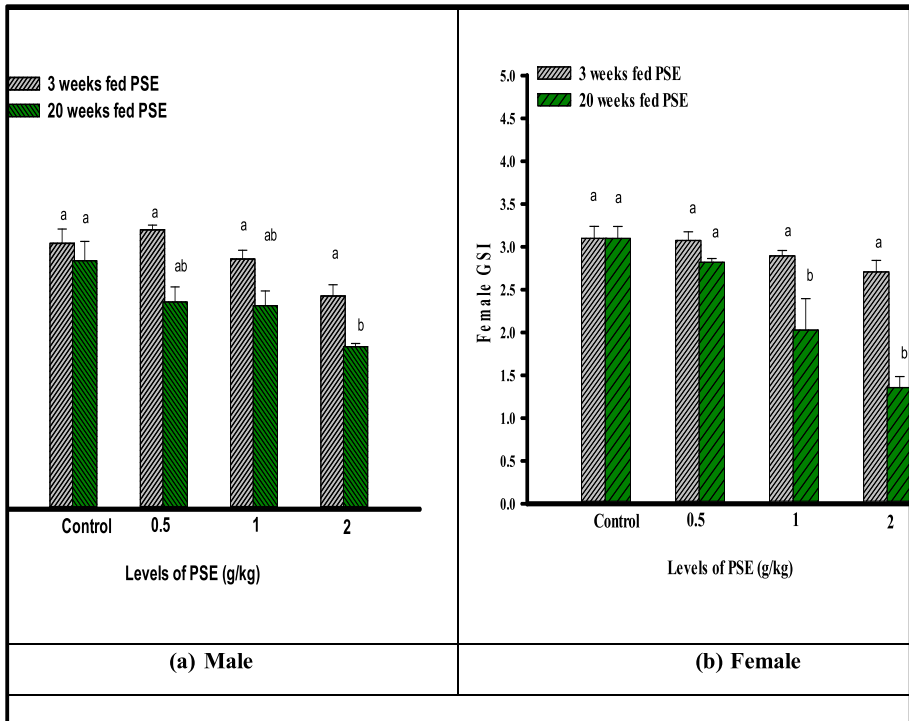
Data declared that gonadosomatic indices mean (GSI) values concerning male and female Nile tilapia (Figs. 1B and 2a) showed significant differences ( $P < 0.05$ ) between the varied levels of PSE than the control group. Moreover, periods of feeding PSE (3 and 20 weeks) showed significant ( $P < 0.05$ ) differences among different groups.

### Growth performance

Table 4 revealed a significantly increased in all growth fish parameters than the control group ( $P < 0.05$ ). Notably, PSE-2 diet exhibited a better performance of growth, followed by PSE-1, then PSE-0.5. Furthermore, the survival rate raised in fish supplemented with PSE than in the control group. In the same line, the highest condition factor value

**Table 3** Percentage of maturing males and females *O. niloticus* fed different diet levels of *P.* seed methanolic extract (PSE) supplemented throughout the time of reaching sexual puberty

Treatments	Sexual characteristics of male %	Sexual characteristics of female %	Time of reaching sexual puberty from starting the experiment (days)
Control	45	55	84
PSE 0.5 (3 weeks)	63	37	70
PSE 1 (3 weeks)	58	42	91
PSE 2 (3 weeks)	51	49	91
PSE 0.5 (20 weeks)	77	23	77
PSE 1 (20 weeks)	65	35	91
PSE 2 (20 weeks)	52.33	47.67	91



**Fig. 2** Histograms illustrating, the gonadosomatic indices mean values ( $M \pm SE$ ) of males (a) and females (b) *O. niloticus* at the end of the experiment among the control group and different fish groups fed on a basal diet supplemented with different doses of papaya seed extract (PSE)

( $2.45 \pm 0.10$ ) was recorded in the PSE-2 group, and the lowest ( $2.12 \pm 0.04$ ) was reported in the control group.

### Whole-body biochemical and digestive enzymes activity

Unlike the control group, all fish dietary PSE supplements had more crude protein (%) and ether extract (%), but they represented less moisture (%) and ash (%). The greatest ( $P < 0.05$ ) crude protein and ether extract values were noticed in the following descending order: PSE-2, PSE-1, PSE-0.5, and PSE-0 groups. The current findings illustrated the highest moisture and ash content recorded in fish in the control group and the lowest was found in PSE -2 treated group (Table 5). Concurrently, Table 6 indicates that fish dietary PSE significantly ( $P < 0.05$ ) increased digestive enzyme activity (lipase, amylase, and protease) more than control fish.



**Table 4** Survival rate, growth, and condition factor of Nile tilapia (*O. niloticus*) fed different diet levels of *P. seed* methanolic extract (PSE) supplemented throughout the experimental period

PSE levels	Final weight (g)	Weight gain (g)	FCO	SGR (% g/day)	SR (%)	CF	Mortality (%)
PSE -0	99.0±2.22 <sup>c</sup>	98.9±2.22 <sup>c</sup>	1.46±0.03 <sup>a</sup>	1.68±0.01 <sup>c</sup>	88.33±1.05 <sup>b</sup>	2.12±0.04 <sup>c</sup>	12.14±1.35 <sup>a</sup>
PSE -0.5	102.8±2.73 <sup>cb</sup>	102.8±2.74 <sup>cb</sup>	1.41±0.04 <sup>ab</sup>	1.69±0.01 <sup>cb</sup>	90.0±0.83 <sup>a</sup>	2.20±0.71 <sup>cb</sup>	9.50±0.73 <sup>b</sup>
PSE -1.0	109.7±4.58 <sup>ab</sup>	109.6±4.58 <sup>ab</sup>	1.33±0.06 <sup>cb</sup>	1.71±0.02 <sup>ab</sup>	90.0±0.83 <sup>a</sup>	2.33±0.09 <sup>ab</sup>	9.51±0.72 <sup>b</sup>
PSE -2.0	115.3±5.08 <sup>a</sup>	115.3±5.08 <sup>a</sup>	1.27±0.06 <sup>c</sup>	1.73±0.02 <sup>a</sup>	91.67±0.83 <sup>a</sup>	2.45±0.10 <sup>a</sup>	8.17±0.55 <sup>c</sup>

Data were represented as mean ±SEM. Different superscript letters are significantly different (ANOVA,  $P < 0.05$ ) in the same column. (PSE) *P. seed* methanolic extract. SGR specific growth rate, FCO feed conversion ratio, SR survival rate, CF condition factor, RPS relative percent survival

**Table 5** Biochemical composition of Nile tilapia (*O. niloticus*) (wet weight basis) fed different diet levels of *P. seed* methanolic extract (PSE) supplemented throughout the experimental period

PSE levels (%)	Moisture (%)	Crude protein (%)	Ether extract (%)	Ash content (%)
PSE-0	74.51±0.52 <sup>a</sup>	14.39±0.63 <sup>d</sup>	5.19±0.82 <sup>c</sup>	6.63±0.37 <sup>a</sup>
PSE-0.5	73.11±0.79 <sup>b</sup>	16.07±0.22 <sup>c</sup>	5.73±0.14 <sup>b</sup>	5.82±0.17 <sup>b</sup>
PSE-1.0	72.54±1.06 <sup>b</sup>	17.15±0.47 <sup>b</sup>	5.84±0.12 <sup>b</sup>	4.26±0.32 <sup>c</sup>
PSE-2.0	70.19±0.72 <sup>c</sup>	19.36±1.11 <sup>a</sup>	6.21±0.08 <sup>a</sup>	3.41±0.28 <sup>d</sup>

Data were expressed as mean ±SE. Different superscript letters are significantly different (ANOVA,  $P < 0.05$ ) in the same column

**Table 6** Intestine activities (amylase, lipase, and protease) of *O. niloticus* (wet weight basis) fed different diet levels of *P. seed* methanolic extract (PSE) supplemented throughout the experimental period

PSE levels (%)	Amylase(U/mg)	Lipase(U/mg)	Protease(U/mg)
PSE-0	19.62±0.85 <sup>d</sup>	22.14±1.04 <sup>d</sup>	14.91±0.71 <sup>d</sup>
PSE-0.5	22.34 ±0.78 <sup>c</sup>	24.11±0.44 <sup>c</sup>	16.28±0.52 <sup>c</sup>
PSE-1.0	25.24±1.32 <sup>b</sup>	27.18±1.40 <sup>b</sup>	19.01±0.72 <sup>b</sup>
PSE-2.0	27.32±0.81 <sup>a</sup>	29.12±1.52 <sup>a</sup>	23.14±1.03 <sup>a</sup>

Data were expressed as means ±SD. Different superscript letters show significant differences ( $p \leq 0.05$ ) in each column

**Table 7** Haemato-biochemical determinants of *O. niloticus* fed different diet levels of *P. seed* methanolic extract (PSE) supplemented throughout the experimental period

Parameter	PSE-0	PSE-0.5	PSE-1.0	PSE-2.0
RBCS ( $\times 10^6$ cell / mm <sup>3</sup> )	1.72±0.18 <sup>c</sup>	1.92±0.22 <sup>bc</sup>	2.04±0.41 <sup>b</sup>	2.76±0.17 <sup>a</sup>
WBCS ( $\times 10^3$ cell / mm <sup>3</sup> )	17.14±0.53 <sup>d</sup>	19.92±0.10 <sup>c</sup>	21.81±0.34 <sup>b</sup>	23.61±0.33 <sup>a</sup>
Hb (g/dl)	7.25±1.15 <sup>d</sup>	8.31±0.45 <sup>c</sup>	10.46±1.07 <sup>b</sup>	13.06±0.83 <sup>a</sup>
Hct (%)	19.45±1.12 <sup>d</sup>	20.44±0.69 <sup>c</sup>	23.11±0.88 <sup>b</sup>	25.62±0.44 <sup>a</sup>
Total protein (g/dl)	2.82±0.53 <sup>d</sup>	3.13±0.42 <sup>c</sup>	3.89±0.27 <sup>b</sup>	4.38±0.58 <sup>a</sup>
ASAT (U/ml)	45.12±2.92 <sup>a</sup>	37.91±1.71 <sup>b</sup>	32.48±1.79 <sup>c</sup>	24.07±0.66 <sup>d</sup>
ALAT (U/ml)	41.44±1.55 <sup>a</sup>	36.11±1.08 <sup>b</sup>	29.28±1.15 <sup>c</sup>	22.14±0.32 <sup>d</sup>
Albumin (g/dl)	1.52±0.22 <sup>d</sup>	1.79±0.16 <sup>c</sup>	1.93±0.31 <sup>b</sup>	2.47±0.14 <sup>a</sup>
Globulin (g/dl)	1.42±0.22 <sup>c</sup>	1.61±0.16 <sup>b</sup>	1.74±0.31 <sup>ba</sup>	2.11±0.21 <sup>a</sup>

Data were expressed as means ±SD. Different superscript letters indicate significant differences ( $P \leq 0.05$ ) in each raw

## Haemato-biochemical, immunity, and antioxidative indices

Fish fed with (PSE) diets throughout 20 weeks showed peaked significantly ( $P < 0.05$ ) in RBCs, WBCs, Hb, Hct, total protein, albumin, and globulin levels than their corresponding in the control group, principally at PSE-1 and PSE-2. Unlike, PSE dietary supplements in Nile tilapia considerably decreased ( $P < 0.05$ ) in ASAT and ALAT activity, particularly in PSE-2 group (Table 7). Overall, the activity of lysozyme and SOD, CAT, and MDA enzymes declared that significant ( $P < 0.05$ ) improvement in fish-supplemented diet PSE than the control group with the highest activity observed in

**Table 8** Antioxidants of *O. niloticus* fed different diet levels of *P.* seed methanolic extract (PSE) supplemented throughout the experimental period

PSE levels (%)	Lysozyme activity ( $\mu\text{g ml}^{-1}$ )	SOD (U g tissue <sup>-1</sup> )	CAT (U g tissue <sup>-1</sup> )	MDA (nmol tissue <sup>-1</sup> )
PSE-0	13.81 $\pm$ 1.12 <sup>d</sup>	1.62 $\pm$ 0.18 <sup>d</sup>	244.66 $\pm$ 13.22 <sup>d</sup>	26.15 $\pm$ 1.44 <sup>d</sup>
PSE-0.5	16.29 $\pm$ 1.74 <sup>c</sup>	1.77 $\pm$ 0.12 <sup>c</sup>	291.14 $\pm$ 8.32 <sup>c</sup>	33.41 $\pm$ 1.61 <sup>c</sup>
PSE-1.0	19.17 $\pm$ 0.44 <sup>b</sup>	2.04 $\pm$ 0.15 <sup>b</sup>	311.17 $\pm$ 11.42 <sup>b</sup>	39.52 $\pm$ 1.31 <sup>b</sup>
PSE-2.0	21.48 $\pm$ 0.51 <sup>a</sup>	2.63 $\pm$ 0.24 <sup>a</sup>	337.92 $\pm$ 9.42 <sup>a</sup>	46.33 $\pm$ 1.15 <sup>a</sup>

Data were represented as mean  $\pm$ SE. Different superscript letters are significantly different (ANOVA,  $P < 0.05$ ) in the same column.

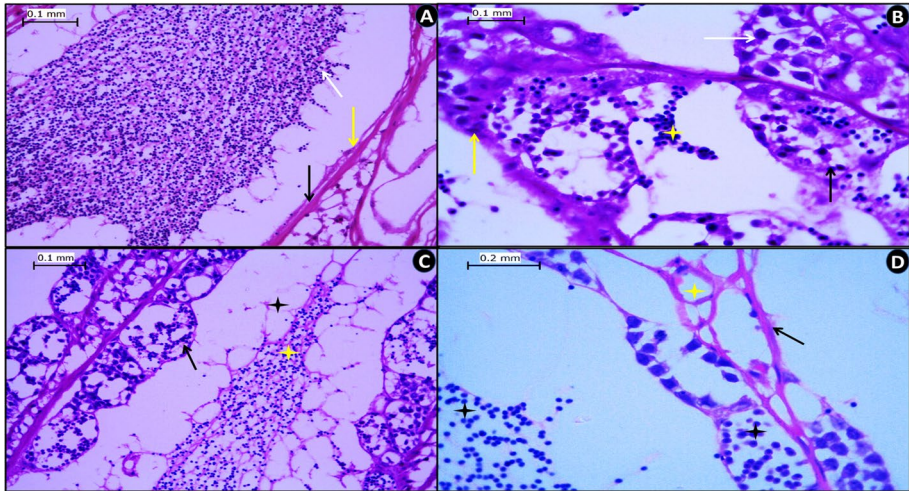
the PSE-2 group (Table 8). Since PSE content increased in the diet, the production of MDA was decreased, as shown in PSM–2 and PSM –1 supplemented diet groups, representing the lowest MDA activity.

## Gonadal histology results

### Clinical signs

In the present study, a visual examination of fish samples fed on different levels of PSE inclusions revealed no morphological abnormalities or surface injuries among experimental fish groups. It is important to mention that the histological sections of the ovary and testis of Nile tilapia fed on the control diet displayed normal tissue architecture. No pathological lesions were observed.

Histological preparations of tilapia testes: the testicular sections of Nile tilapia fed on basal diet indicated normal architecture of testicular tissue with open cyst walls, including spermatozoa (Fig. 3a). Correspondingly, fish fed with PSE 0.5g/kg revealed a relative change of normal testicular tissue architecture as in spermatogenic stages within the seminiferous tubules (Fig. 3b). fish fed on PSE 0.1 g/kg indicated shrinkage of spermatozoa clusters and disintegration of interlobular septa in some regions within the seminiferous tubules (Fig. 3c). Moreover, Fish fed PSE 2.0 g/kg illustrated thickening of the interlobular septa which leads to the formation of an undifferentiated tissue and disintegration of primary spermatocytes, secondary spermatocytes and spermatids in the seminiferous tubules (Fig. 3d). On the other hand, fish ovaries fed on a basal diet showed normal architecture of ripe oocytes (Fig. 4a). Fish fed on PSE 0.5g/kg demonstrated shrinkage and a marked decrease in the size of ripe oocytes, slight degeneration of their cytoplasm, and the wall of some oocytes became very thin (Fig. 4b). Correspondingly, Fish fed on PSE 1.0 and 2.0 g/kg (Figs. 4c and d) showed different oocytes at various stages of oogenesis arranged in strands and separated by ovigerous lamellae. Ripe oocytes revealed histopathological alterations by undergoing different stages of atresia. Some oocytes were corrugated or deflected in others, and the zona radiata layer enlarged among some oocytes. Severe buckling of their oocyte walls as well as degeneration of their cytoplasm were also noted. More specifically, histopathological alterations are more severe and have a higher incidence of atretic oocytes in PSE 2.0 g/kg than in other groups.



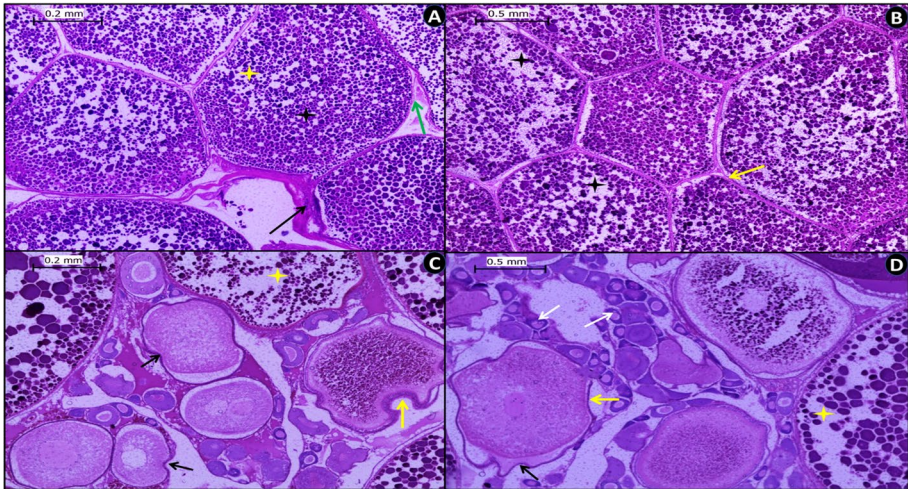
**Fig. 3** Photomicrographs of transverse sections of testis of adult males *O. niloticus* diet PSE-0 showing (A): clusters of spermatozoa (white arrow), Leydig cells (yellow arrow) in the interlobular space composing the walls of the seminiferous lobules (ST) and the interlobular septa (black arrow). **B** *O. niloticus* diet PSE-0.5 showed a fully mature phase of the testis of Nile tilapia with different spermatogenic stages: primary spermatocytes (white arrow), secondary spermatocytes (yellow arrow), spermatids (yellow star) and spermatozoa. **C** *O. niloticus* diet PSE-1.0 showed spermatid (black arrow) and spermatozoa (yellow star) and disintegration of interlobular septa (black star) in the seminiferous tubules. **D** *O. niloticus* diet PSE-2.0 showed thickening of interlobular septa (black arrow) which leads to the presence of undifferentiated tissue (yellow star) and disintegration (black star) of primary spermatocytes. [Specimen fixed in Bouin's fluid and stained with Hematoxylin and Eosin].

### Anti-bacterial and anti-parasitic activity of PSE

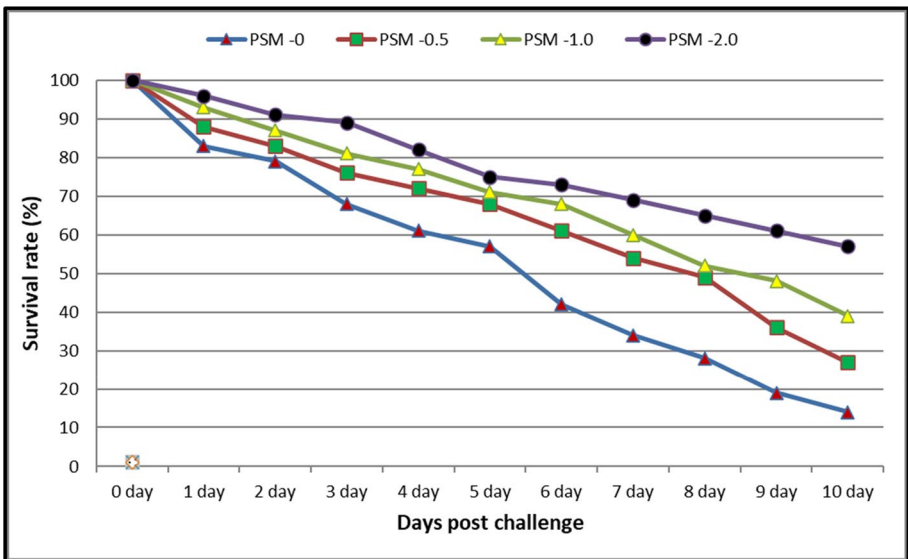
Figure 5, displayed a positive efficiency of PSE against bacterial strain (*Aeromonas hydrophilia*) after 10 days of the bacterial injection, especially at a higher concentration than the control group. Similarly, PSE has a positive impact by reducing *C. tilapiae* parasites' number with increased PSE concentration than the control group. In this context, the dead parasite count was altered from 14% after 40 min to 37% after 60 min in the control group. Conversely, at 2.0 PSE ml/L concentration, the dead parasites (32%) were reported after 10 min and complete parasite death was recorded after 50 min (Table 9). Remarkably, efficiency against monogenean parasites *C. tilapiae* increases with increased levels of PSE.

### Discussion

The present study highlighted evoked medicinal plants (PSE) in improving aquaculture. Results displayed Nile tilapia feeding with additive (PSE) enhanced growth, carcass composition, and health status of fish with resistance against diseases. In this context, Azizah and Fasya (2019) and Hamid et al. (2022) elucidated that PSE plays a vital role as an anti-bacterial which is reflected positively in promoting growth performance, digestibility of feed, absorption of nutrients, and raising health status with resistance diseases of fish due



**Fig. 4** Photomicrographs of transverse sections of the ovary of adult males *O. niloticus* diet PSE-0 showing (A): normal ovary structure as ripe oocytes (yellow star), coated with a follicular epithelial layer (green arrow), vacuoles interfered with yolk globules (black star), Post-ovulatory follicles (black arrow). **B** *O. niloticus* diet PSE-0.5 showed shrinkage of ripe oocytes, slight degeneration (black star) of their cytoplasm and the wall of some oocytes becoming very thin (yellow arrow) in some points. **C** Correspondingly, fish-fed diet PSE-1.0 and 2.0 g/kg showed ripe oocytes which undergo different stages of atresia appeared with an invaginated follicular epithelial layer in some oocytes and retracted, deflected (yellow arrow). **D** Liquification of the nucleus and the severe buckling of the oocyte walls (black arrows) as well as the degeneration of the cytoplasm in the atretic ripe oocytes (yellow star). Atresia of different oocytes at various stages of oogenesis white arrow. [Specimen fixed in Bouin’s fluid and stained with Hematoxylin and Eosin]



**Fig. 5** The relative percentage of the survival rate of Nile tilapia, *O. niloticus*, fed diets comprising different papaya seed extract (PSE) levels during the ten days of post-challenge with *A. hydrophila*. Data were represented as mean  $\pm$ SE



**Table 9** Antiparasitic efficiency of various *P.* seed methanolic extract (PSE) concentrations at different time intervals after treatment (60 min) to *C. tilapiae* (% death)

Time (min)	PSE Levels			
	PSE-0	PSE-0.5	PSE-1.0	PSE-2.0
10	-	11.21±1.73 <sup>c</sup>	20.14±1.11 <sup>b</sup>	32.56±3.58 <sup>a</sup>
20	-	22.34±2.57 <sup>c</sup>	35.88±2.24 <sup>b</sup>	56.13±3.26 <sup>a</sup>
30	-	30.28±3.66 <sup>c</sup>	51.54±3.45 <sup>b</sup>	75.11±3.86 <sup>a</sup>
40	14.41±3.06 <sup>d</sup>	41.37±2.93 <sup>c</sup>	66.51±3.33 <sup>b</sup>	88.14±2.40 <sup>a</sup>
50	28.22±2.54 <sup>d</sup>	2.25 <sup>c</sup> ±51.31	2.75 <sup>b</sup> ±79.40	100.00 <sup>a</sup>
60	37.45±3.11 <sup>d</sup>	62.23±1.49 <sup>c</sup>	1.94 <sup>b</sup> ±88.14	100.00 <sup>a</sup>

Data were represented as mean ±SE. Different superscript letters are significantly different (ANOVA,  $P < 0.05$ ) in the same column

to contain triterpenes, alkaloids, saponin, and flavonoid. Correspondingly, PSE contains papain, which is a potency promoter for fish growth and positively affects body composition with sex-reversed Nile Tilapia (Vij & Prashar, 2015 and Ugonna et al., 2018). In this way, PSE inclusion level applied in diet especially, with increased level was effective to achieve male phenotype, it refers to that PSE was able to skewed sex ratio in the trend of males. Most likely because PSE contains estrogenic phytochemicals that have the potential to be antiestrogenic when present with endogenous estrogens; also, the interplay of endogenous fish hormones with phytoestrogens found in PSE may impact gonadal sex differentiation. These results concurred with the observation of Omeje et al. (2020). Concurrently, Ugonna et al. (2018) stated that masculinization reached over 85% of males in fish groups treated with PSE; they explained that the absence of females in almost treated fish might allow the energy needed for reproduction to be directed toward growth. Interestingly, our results pointed out the positive role of PSE in the improvement activity of the digestive enzymes of Tilapia. This may be due to PSE as continued saponin, polyphenols, and tannins, which enhanced appetite through stimulating digestive enzyme activities and lead to microbial community change in the fish intestine (Rachmawati et al., 2020; Wiszniewski et al., 2022).

Correspondingly, fish fed on PSE for a thorough study period showed a dramatically significant decrease (GSI) in both males and females with increased PSE dietary levels. It reflects the positive role of PSE, a reproductive inhibitor in Nile tilapia. Similar to the present results, Khalil et al. (2014) and Yadav et al. (2021) recorded decreased GSI values of fish-treated groups by increasing the PSE dietary levels. Conversely, Abdelhak et al. (2013) state that there is insignificant differences effect of PSE on the weight of testis; this difference could be due to the variation of conditions, protocols, amount of PSE application rate, etc., or treatment interval between studies.

In the current study, hematological, biochemical, and immunity parameters remarkably improved significantly at varied PSE supplement diets than the control group. This is probably attributed to the increased suggested ability in immunostimulant and protective of PSE as the action of bioactive compounds against various stressors and enhancement hemato-biochemical parameters as explained by Madinah et al. (2015) and Hamid et al. (2022). Similarly, Mansour et al. (2022) explained PSE enhanced immune-modulating activities through varied stress as it is rich in secondary metabolites besides phenolic and flavonoid, which significantly act in hepatoprotective. Concurrently, PSE improved the response of innate immune in Nile Tilapia as the elevated value of serum lysozyme with

promote the ability of fish against various pathogens, as extracts of PSE involved numerous alkaloids, flavonoids, phenolics, terpenoids, and steroids, which stimulate a variety of biological processes, including the activation of the complement system and immunostimulant in cultured Fish (Hamid et al., 2022). Furthermore, the addition of PSE significantly improved oxidative stress through increased antioxidant enzymes, SOD, CAT, and GPx and decreased MDA than control fish that reflect fish physical health. Attractively, phytochemicals present in PSE are considered excellent hydrogen donors as they can interact with stable free radicals by giving them hydrogen atoms and reducing agents, singlet oxygen quenchers, and metal chelators are helpful in converting them into non-radical form molecules (Abouzed et al., 2019 and Mansour et al., 2022).

Results markedly that severe changes in the architecture of the reproductive organs of fish diet at different levels of PSE, especially with an increased level which can predispose to infertility. Besides, the main histopathological alterations in males fed on a diet-supplemented PSE demonstrated shrinkage or disappearance of spermatozoa in some regions, the disintegration of interlobular septa, and autolysis of the seminiferous tubules. This clarified that PSE disruptive effects might be either direct or indirect via the pituitary-gonadal axis or that androgen's effects on the seminiferous tubules could be either direct or indirect because androgen concentrations in tubules must be high for cell development and function. This is concurrent with the results of Udoh et al. (2005), Omeje et al. (2020), and Owolabi and Abdulkareem (2021). Attractively, testicular and ovarian histopathological changes detected lesional changes in male and female fish treated with varied PSM levels through a period of experiment comprising the scarcity of the germinal cells and the spermatozoa, the presence of cysts and a hyaline substance in the interstitial cells in the testis and a decrease in the vitellogenesis in ovaries. Similarly, Magblenou et al. (2019), who observed increased interstitial cells and focal necrosis of testicular tissue and atretic follicles in the ovary of Nile tilapia and histopathological modifications in the testis could clarify the effect of PSE on the testicular parenchyma of tilapia fish, which could confirm the sterilizing effect of the treatment in tilapia males.

Study displayed the efficiency of PSE against *C. tilapiae*, a parasite in vitro, especially at 0.2%. This may be attributed to PSE comprising bioactive components such as polyphenols, terpenoids, saponins, sterols, and alkaloids. Concurrent with the results of Farrag et al. (2013) and Singh et al. (2020). Correspondingly, the characteristic of *Carica papaya* as a phytochemical composition and bioactive compounds might help to clarify the anti-parasitic properties seen in our study, which were attributed to the presence of terpenoids, saponins, alkaloids, and various efficacious components in the powerful methanol extract of PSE (Vij and Prashar 2015).

## Conclusion

In conclusion, the current study elucidated the positive effect of dietary supplementation with PSE on growth, health status, immune response, and resistance against bacterial and parasitic diseases in Nile tilapia. Additionally, PSE can achieve sex reversal in favor of fish males. Moreover, dietary PSE, especially at a level of 2.0 g/kg, is recommended as a natural alternative to sex reversal and is protective against diseases and sustainable aquaculture.

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**Data availability** Data will be made available on request.

**Code availability** Not applicable.

## Declarations

**Ethical statement** All applicable guidelines (international, national, and/or institutional) for the care of fish were closely followed by the authors during the experimental works.

**Consent to participate** All of the authors consent to participate in the study above.

**Consent for publication** Not applicable

**Conflict of interest** The authors declare no competing interests.

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