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Research Article

Evidences of Gonadal Impairment and Growth Acceleration in *Barbonymus gonionotus* Fed Papaw Seed: Sustainable Green Alternatives for Energy Portioning

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Growth stagnation in early-maturing fish is a concern for higher fish productivity and may be regulated with reproductive arrest using phytochemicals. In this line, the study attempts to improve the growth of silver barb, *Barbonymus gonionotus*, through dietary pawpaw (*Carica papaya*) seed (PS) supplemented diets during a 60-day trial (30-days of feeding PS, followed by a 30-day recovery period). Four levels of PS at 0, 2, 4, and 6 g kg^{-1} diet were added to a commercial feed (32% crude protein) and fed to advanced fingerlings (19.08 ± 0.02 g) at 3% body weight, which forms the experimental groups, designated as control, T1, T2, and T3. Subsequent alterations in growth (% weight gain and specific growth rate), reproduction (gonadosomatic index (GSI) and gonadal histology), and physiological function (haematology and stress biomarkers, viz., cortisol and glucose) were examined at the end of the experiment. Observations noted at the end of the trial showed significantly ($P < 0.05$) enhanced growth in groups fed 2 g kg^{-1}, but not significantly with control. GSI showed an extreme reduction in all groups as the level of supplementation increased with the lowest being noted in T3. Sex change was observed in T2 and T3, wherein only male populations were noticed. Gonadal histology demonstrates tissue-level generation in T1, compared with control. Tissue alteration (atrophy and necrosis) was noticed in the T2 and T3. The haematological profile indicates a gradual decrease in erythrocyte and leucocyte counts, packed cell volume, and haemoglobin in the T2 and T3 groups, compared with the T1 and control groups ($P < 0.05$). Serum biochemistry followed a similar trend. The levels of blood glucose and cortisol were higher in T2 and T3 ($P < 0.05$) than in the control and T1. The overall findings from this study reveal that dietary PS supplementation at 2 g kg^{-1} can enhance the growth of silver barb; however, higher doses suppress both growth and reproductive function, resulting in subsequent disturbances in physiology. Further studies regarding the molecular mechanism of such an outcome warrant immediate investigation.

1. Introduction

In the midst of climate change adaptation and a species diversification program, silver barb (*Barbonymus gonionotus*) has gained importance as a candidate species among aqua-farmers in India. The species belongs to the family Cyprinidae and is distributed in Asian countries, including India, Bangladesh, China, Indonesia, Malaysia, Myanmar, and Thailand. Silver barb is a short-cycle species that can be farmed with low-cost technology and relatively less effort compared with other species, which offers it as a prospective aquaculture candidate among farmers in India. Also, its compatibility in polyculture with the three Indian major carps, *viz.*, *Catla catla*, *Labeo rohita,* and *Cirrhinus mrigala,* has harnessed its importance in aquaculture [1]. It is an early-maturing species, maturing as early as 4–6 months of age [2]. According to Mohanta et al. [3], sexual maturity is attained in 8–10 months in females and 6–8 months in males, and they breed twice a year.

In aquaculture production systems, precocious maturity and prolific breeding behaviours among a few farmed species are major concerns affecting body growth and economic return. Silver barb is no exception to such breeding behaviour, apart from commercial species such as tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*). Such precocious spawning leads to uneven harvesting size, overstocking, resource wastage, and low market price of undersized fish. Additionally, energy costs towards reproductive growth are high in such early-maturing fishes, which could otherwise be redirected for somatic growth using modern technology. An option for mitigating such a bottleneck includes monosex production, sex reversal, manual sexing, and triploidy. However, concerns regarding such approaches are the high cost, time consumption, and issues related to the use of hormones, which disturb the market and export regulations.

The World Health Organization (WHO) encourages the use of potential herbs as alternatives to synthetic hormones as they are less expensive, eco-friendly, available locally, and most importantly biodegradable for commercial aquaculture use [4]. Over the years, research thrust into the use of plant-based phytochemicals as potential drugs to promote the growth and health attributes of several cultured fishes has been well reported. However, the area of research on the ability of phytochemicals to impair the reproductive function of fishes is limited to a few species, such as tilapia. These studies narrate that these potential plant sources possess phytochemicals such as phytoestrogen and phytoandrogens, which are structurally parallel to steroid hormones (17-β estradiol) found in animals [5]. In commercial species such as tilapia, these phytocompounds induce feminization, masculinization, or fertility restriction [6, 7]. One potential plant that is given research priority these days is the pawpaw (*Carica papaya*), a tropical fruit available throughout the year in tropical and subtropical climates. Pawpaw seed contains active ingredients such as caricain, carpasemine, and oleanolic glycoside, which can induce sterility in male rats [8]. Other compounds such as benzyl isothiocyanate, β-sitosterol, and oleanolic glucoside are responsible for the sex reversal and antifertility properties of the plant [7, 9–12].

The seed of pawpaw (crude or extract) has been reported to induce reproductive impairment in tilapia [13–15] as a way of controlling prolific breeding in aquaculture. Alternatively, other plants such as China rose (*Hibiscus rosa sinensis*) leaves and *Aloe vera* latex are used to impair reproductive performance in female African catfish, *Clarias gariepinus* [16]. Phytoestrogens that imitate the action of the endogenous fish hormones are regarded as a suitable alternative for 17-methyltestosterone to produce all-male tilapia populations [12]. The impairment of reproductive activities manifests as infertility and majorly affects or reduces fecundity [17]. Mousa et al. [18] and Hossam and Wafaa [19] have used crude extracts of different parts of pawpaw plants to either partially or completely eliminate reproduction in fish.

Considering the positive role of pawpaw seed in reproductive impairment, we speculate that dietary incorporation of it at a considerably low level can manifest better growth in fish. However, dose optimization is essential to avoid any toxic effects that may compromise the physiological attributes of the fish. For instance, Ekanem and Okoronkwo [14] observed histological changes in male *O. niloticus* fed a much higher dietary pawpaw seed meal level (5 or 10 g kg^{-1} diet). Additionally, the discoloration of the liver suggests that pawpaw seeds contain ingredients that can be effective as sterility-inducing agents but can also be damaging at a high dietary level. As the target of reproductive impairment in our study is to promote growth of the fish by supplementing the phytosubstance at an early life stage, we further intend to look into the health and physiological status of the fishes. To the best of our knowledge, there is no reported work investigating the effect of pawpaw seed on the reproductive, growth, and physiological status of silver barb as a measure of the energy trade-off between reproduction and growth. Therefore, the aim of this study is to evaluate the effect of different levels of pawpaw seed on the gonadal architecture, growth, and immune-metabolic profile of silver barb. The outcome of this work will help in accelerating the growth of the fish through reproductive arrest using a sustainable green approach to enhance the overall farm economic returns of aqua-farmers.

2. Materials and Methods

2.1. Experimental Fish and Husbandry. The advanced fingerling of silver barb (avg. body weight = 19.08 ± 0.02 g) was transported from the farm facilities of the institute and acclimatized for 2 weeks at the institute's wet laboratory. During acclimatization, tanks were provided continuous aeration, optimum water quality was maintained, and a commercial diet (32% crude protein) was fed twice daily (2–3% body weight).

2.2. Animal Ethics. The research protocols, animal care, and experimentation standards were approved by the college-level animal ethics committee through application no. CAU-CF/48/IAEC/2018/09a/35. The work accords with the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Government of India.

2.3. Experimental Design. Two hundred forty healthy fishes were randomly selected and distributed into four experimental groups. We used twelve fiberglass reinforced plastic (FRP) circular tanks of 500 L capacity for allotting the four groups, which were performed in triplicates (4 × 3), following a completely randomized design (CRD). Each experimental tank was stocked with 20 fishes. The experimental groups were fed different levels of PS.

2.4. Diet Preparation and Feeding. Fresh PS was collected from ripe fruits from the institute's locality. They were carefully removed, cleaned, and air-dried under room temperature for 5 days. The dried seeds were further ground into a fine powder, weighed, and then stored in dry airtight plastic containers at room temperature. The test diets were prepared from a commercial feed (32% crude protein; CP Aqua, India). The feed was finely ground, sieved (with 0.5 mm sieve), mixed with the PS, and further pelleted using a hand pelletizer. The prepared feeds were designated as control (without PS), T1 (2 g/kg PS), T2 (4 g/kg PS), and T3 (6 g/kg PS), respectively. The prepared feeds were labeled and stored in airtight containers at room temperature. Fishes were fed *ad libitum* during the 60-day experiment, which includes a 30-day feeding trial, followed by a 30-day recovery period.

2.5. Growth and Feed Efficiency. Growth sampling was performed on fortnightly basis to adjust the Fe coding ratio. Growth and feed efficiency parameters were calculated at the end of the experimental period as follows:

$$\begin{aligned}
\text{Growth rate (g/d)} &= \frac{W2 - W1}{\Delta t},\\
\text{Specific growth rate (\%/d)} &= \frac{\log_e(W2) - \log_e(W1)}{\Delta t},\\
\text{Survival rate (\%)} &= \frac{(\text{fish stocked } - \text{mortality})}{\text{fish stocked}} \times 100,\\
\text{Feed conversion ratio (FCR)} &= \frac{\text{total weight of feed consumed}}{\text{weight gained of the fish}},\\
\text{Protein efficiency ratio (PER)} &= \frac{W2 - W1}{\text{protein fed}}.
\end{aligned} \tag{1}$$

2.6. Reproductive Score. The gonadosomatic index (GSI) was calculated based on the formula:

$$\text{Gonado} - \text{somatic index (GSI)} = \frac{\text{weight of gonad}}{W2} \times 100, \tag{2}$$

where W1 = initial weight, W2 = final weight, and Δt = change in time.

2.7. Sampling Procedure. At the end of the 60-day trial, 20 fishes ($n = 20$) were harvested from each tank using a scoop net. They were anesthetized using clove oil (Merck, Germany) at 50 μg l^{-1}. Blood samples were collected from the caudal peduncle using a 1 ml hypodermal syringe (24-gauge needle) rinsed with 2.7% EDTA solution. For serum, approximately 500 μl of blood samples collected in Eppendorf tubes were centrifuged at 3500 rpm for 5 min at 4°C. The collected serum samples were stored at −20°C, until further processing for serum biochemistry.

2.8. Haematoserological Assays. At the end of the experiment, haematological assays were performed. Sahli's haemometer was used to assess haemoglobin. Pack cell volume (PCV) was assessed by centrifuging whole blood in heparinized microhaematocrit tubes for 3 minutes at 3000 rpm [20]. Total red blood cell (RBC) and white blood cell (WBC) were counted using a haemocytometer and a microscope. For RBC and WBC, blood was diluted in Hayem's solution (Qualigens, India) and Turk's solution, respectively. The determination of glucose in plasma was carried out using a diagnostic kit (Liquizone, Medsource Ozone Biomedicals Pvt. Ltd., India) following the manufacturer's instructions. The serum cortisol was quantified using a commercially available Cortisol ELISA Kit (Catalog No. 500360; Cayman Chemicals, USA). An ELISA plate reader was used to measure absorbance (Biotek India Private Limited). Total protein was determined using a diagnostic kit based on the Biuret method (Liquizone; Medsource Ozone Biomedicals Pvt. Ltd., India) (Strickland et al., 1961). The bromocresol green binding method [21] was used to estimate albumin using an albumin test kit (Qualigens). Serum glutamate pyruvate transaminase (sGPT) and serum glutamate oxaloacetate transaminase (sGOT) levels were measured spectrophotometrically using the Accurex Biomedical Pvt. Ltd., Mumbai, India (diagnostic kit), diagnostic protocol [22].

2.9. Histopathological Study. The histopathological examination of gonads, kidney, and liver tissues was conducted as per Roberts [23]. Samples were removed aseptically and preserved in Bouin's fixative (glacial acetic acid (5%), formaldehyde (9%), and picric acid (0.9%)) and fixed for 24 h. Tissue samples were processed in an Automatic Tissue Processor (Thermo Scientific, Shandon Citadel 2000), followed by paraffin block preparation in Histocentre (Thermo Scientific, Shandon Histocentre 3). The tissue fixed in paraffin blocks was sectioned into 5–8 m thick sections using a Rotary Microtome (Leica, RM2245), stained with haematoxylin and eosin (HiMedia), and mounted with DPX. Image observation was done with Leica DM750 microscope.

2.10. Statistical Analysis. All the data were analyzed using Statistical Package for the Social Sciences (SPSS) version 25 (SPSS, Chicago, IL). A one-way ANOVA was applied to test treatment effects. A significant difference between means was determined by Duncan's multiple range tests. A mixed model analysis of variance (ANOVA) was carried out to examine the impact of different treatments with power analysis to determine the scale of the treatment effects for the individual tanks. Probability levels of 5% were used to find out the significance in all cases, and the analyzed data are presented as mean ± standard error (SE). Normality and homogeneity of variance were checked (Shapiro–Wilk test and Levene's test), and all percentage data were arcsine-transformed. Data are presented as mean ± standard error (SE).

3. Results and Discussion

3.1. Growth and Feed Efficiency. In the present study, PS supplementation influenced the growth of silver barb in a dose-dependent fashion. Figure 1 and Table 1 display the growth pattern and the observed parameters. The percentage BWG was highest in T1 (78.37 ± 1.04) ($p < 0.05$), which was better than the control. The higher supplementation dose of PS lowered BWG, and the lowest was observed in T3 (22.64 ± 3.36). The growth trend showed a comparatively low rate during the initial 30-day (induction period) in PS-fed groups and was much lower than control. This coincides with the low feed intake observed in these groups, supposedly due to the presence of carpasemine, an enzymatic growth inhibitor in PS [24]. After the withdrawal (30-days post-PS feeding), the growth trend noticed a significant growth increment in T1 (2 g/kg), compared with the control. This coincides with the decrease in GSI values and directs our understanding of the energy portioning at this level. In a similar line, Ugonna et al. [25] noticed a substantial improvement in the growth of *O. niloticus* treated with PS, followed by a recovery period. The severe growth reduction in higher doses (4 g/kg and 6 g/kg) may be due to the toxicity induced by excessive levels, which establish a stressful situation in the fish even after the withdrawal period. Earlier work by Aanyu et al. (2020) proclaims that PS possesses phytogenic compounds that have an antagonistic effect on growth response in fish, especially when supplied in higher quantities in fish diets. In terms of feed efficiency, better FCR and PER were noticed in the lowest PS level, parallel to better feed acceptability. This agrees with the earlier report by Farrag et al. [4] in *O. niloticus* fed PS powder at a level of 8 g/kg of diet, beyond which it impacted growth and feed efficiency. The higher dose tolerance noticed by them suggests the tolerance and assimilation capacity among fish species, and therefore, dose optimization needs are further warranted.

3.2. Mixed Model Power Analysis. The different biometric and physiological indicators examined in this study underwent a mixed model ANOVA to determine the magnitude of the effect and the potential influence of individual tanks on the overall results. The statistical data, namely, the power ($1 - \beta$) values of the parameters, are shown in Table 2.

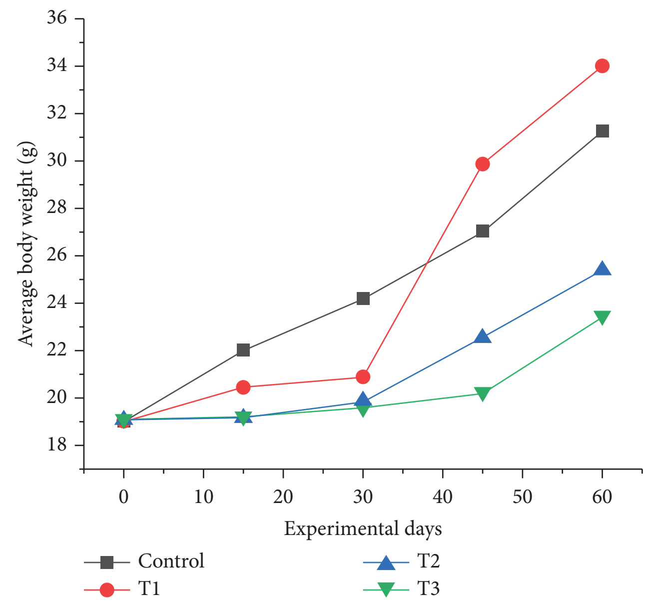

Figure 1: Growth pattern of the different experimental groups fed pawpaw seed powder over the 60-day experiment.

For this investigation, we observed a power value exceeding 0.80 for the treatment groups in all instances, but it dropped below the specified value for the replicates/tanks. The data analysis revealed that the effect of individual tanks did not exert a significant effect on the overall outcome.

3.3. Gonadal Scores and Histology. Earlier studies explain that PS extracts can suppress reproduction by impairing gonadal progression. In this work, male gonadal weight was highest in control, followed by T1 and T2, and lowest in T3 (Figure 2). In males, the GSI value was significantly ($p < 0.05$) lowest in T3 (0.34 ± 0.04 g), followed by T2, T3, and control. Similarly, there is a significant difference in the GSI value of females, and the highest GSI was noticed in control (1.05 ± 0.16 g), followed by T1 (0.28 ± 0.02 g). The significant decrease in GSI indicates a higher level of reproductive degeneration in the fish which explain the negative role of the supplement towards reproductive progression. To support this finding, *Clarias gariepinus* fed PS showed a gradual reduction in ovary weight and the GSI when compared to control [16]. Interestingly, in our study reproductive retardation proportionately improved growth and this is quite possible as the energy drain in maintaining the reproductive process is conserved for somatic gain. It is also reported by Sharma et al. [26] that PS contains phytochemicals such as saponins, alkaloids, terpenoids, and flavonoids, which might have altered the biosynthetic processes underlying the growth and development of the fish. Nevertheless, it would have been appropriate to discuss gonadal degradation after the induction period (30^{th} day); however, due to inappropriate sexual characteristics, this was avoided. Gonad histology (Figure 3) can enhance our knowledge of tissue-level alterations. The testis histology of the control group shows evidence of normality with the presence of primary and secondary spermatocytes with well-defined connective tissues and that

Table 1: Mean growth parameters of *B. gonionotus* in different treatments at the end of the 60-day experiment.

Parameters	Treatments				
	Control	T1	T2	T3	P value
Initial weight (g)	19.02 ± 0.03	19.07 ± 0.02	19.10 ± 0.02	19.12 ± 0.11	0.69
Final weight (g)	31.27 ± 0.42^{b}	34.02 ± 0.21^{b}	25.41 ± 0.36^{a}	23.45 ± 0.49^{a}	0.00
Body weight gain (%)	64.37 ± 2.33^{b}	78.37 ± 1.04^{c}	33.03 ± 1.63^{bc}	22.64 ± 3.36^{a}	0.00
Specific growth rate (% day^{-1})	0.55 ± 0.01^{c}	0.64 ± 0.01^{c}	0.45 ± 0.02^{b}	0.39 ± 0.04^{a}	0.03
Survival (%)	86.67 ± 1.67^{ab}	91.67 ± 1.67^{b}	81.67 ± 4.41^{a}	80.00 ± 2.89^{a}	0.08
Condition factor	1.14 ± 0.18	1.18 ± 0.10	1.12 ± 0.04	1.00 ± 0.07	0.71
FCR	2.69 ± 0.03^{a}	2.49 ± 0.01^{a}	3.16 ± 0.06^{b}	3.27 ± 0.04^{b}	0.03
PER	0.042 ± 0.001^{a}	0.050 ± 0.003^{b}	0.048 ± 0.005^{b}	0.038 ± 0.001^{a}	0.00

Data are represented as mean ± S.E. ($n = 20$). Different superscripts indicate statistically significant difference ($P < 0.05$) among the experimental groups.

Table 2: Mixed model ANOVA for the test of individual effects considering replicates as individual groups.

Studied parameters	Observed power values (between groups)	Observed power values (between tanks/replicates)
Initial weight	1.000	0.645
Final weight	0.998	0.586
BWG	0.999	0.763
SGR	1.000	0.720
Survival	1.000	0.667
CF	0.875	0.145
FCR	0.945	0.245
PER	0.999	0.111
GSI (male)	0.999	0.435
GSI (female)	1.000	0.125
RBC	1.000	0.342
WBC	1.000	0.723
PCV	0.998	0.234
Haemoglobin	0.899	0.544
Total protein	1.000	0.125
Albumin	1.000	0.177
sGOT	1.000	0.423
sGPT	1.000	0.122
Glucose	0.999	0.345
Cortisol	0.999	0.568

Mixed model analysis of the studied parameters was analyzed taking treatment as a fixed factor, while the tanks as a random factor using SPSS software. Effect size is considered significant at power $(1 - \beta)$ values which falls above 0.80.

of those previously fed 2 g/kg PS showed tissue degeneration, and its inclusion beyond this dose showed significant abnormalities of the gonad. The gonads in the T2 group showed evidence of deformation in the seminiferous tubules and atrophy, while degeneration and necrosis were evident in the spermatozoa present in the ductus deference of the fish in T3. The observed reduction in the size of the gonads indicated a total degeneration of the gonads after being fed with pawpaw seed at a very high dose. Few other plant extracts have been shown to degenerate and alter tissue-level changes as reported by Jegede and Fagbenro [7], wherein degenerative stroma in female *Tilapia zillii* was observed in the groups fed with 2.0 g/kg PS meal. In our study, the supplementation of PS in diets resulted in sex reversal in the entire fish population in treated groups. The ovarian histology of the groups treated beyond 2 g/kg PS could not be obtained due to the absence of females. This further suggests that PS is an efficient sex-reversing compound in silver barb. All these changes in tissue structure may possibly occur due to the presence of endocrine-disrupting compounds (EDCs) in plants such as phytoestrogens, which have negative implications and the ability to impair animal reproduction either by affecting gonad differentiation or by delaying maturation [27]. However, these assumptions need further confirmation, as well as their variability in different fish models. Nevertheless, the current study highlights the potential of using PSM to induce sex reversal in silverfish barbs, thereby promoting enhanced growth. Furthermore, this naturally sourced sex-reversal agent's easy biodegradation makes it a viable alternative to synthetic hormones, the bioaccumulation of which has raised concerns among many scientists.

3.4. Tissue Histology and Function. The liver and kidney are vital organs connected to the metabolic and detoxification processes, and their structural change and function can reveal the physiological stability of the host. In this study, the histological examination of the liver and kidney tissues

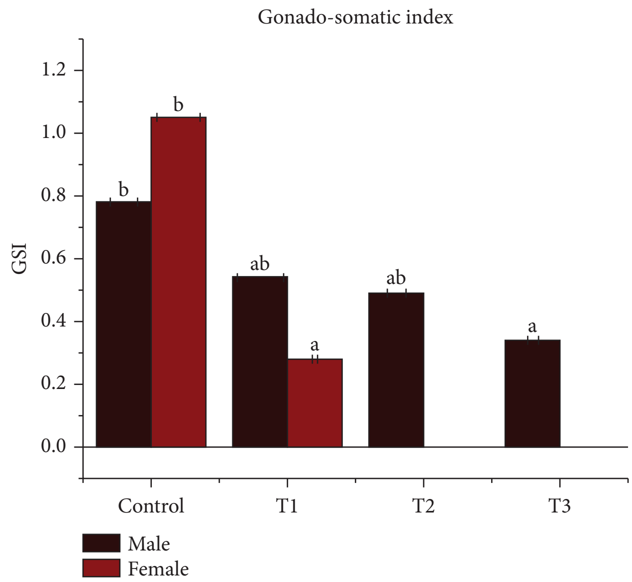

Figure 2: Observed gonadosomatic index (GSI) values in male and female *B. gonionotus* after feeding different doses of pawpaw seed.

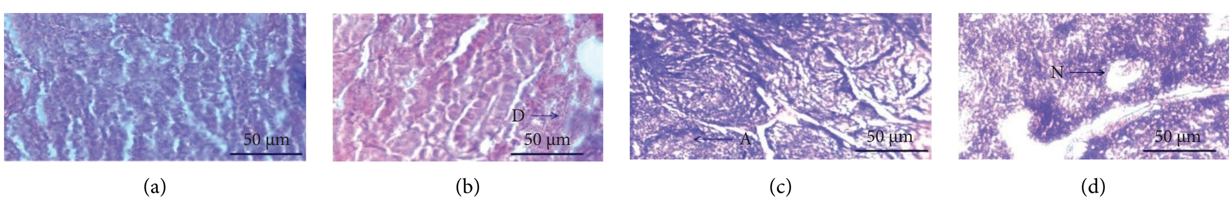

Figure 3: Histology of *B. gonionotus* gonad fed with diet incorporated with papaya seed extract (H&E stain, 40x): (a) normal cells (control); (b) tissue degeneration (D) (T1); (c) atrophy (A) (T2); (d) necrosis (N) (T3).

revealed the significant visible effect of pawpaw seed extract (Figures 4 and 5). In the liver, the treated groups show signs of tissue degeneration, atrophy, and necrosis. These observations mark the toxicity of the PS in excessive levels that consequently alter other hepatocyte functions. Such liver tissue damage accords with earlier reports on the toxicity of plant extracts in the liver tissues of fishes [28]. To check the liver function, we examined the key enzymes, i.e., sGOT and sGPT. Their levels were found to be the highest in T3, whereas T1 displayed significantly ($P < 0.05$) low level among the treatments (Figure 6). These enzymes are regarded as key markers for hepatic impairment. Their elevated levels in higher PS doses are possibly a result of the hepatocyte cell membrane damage or increased permeability that releases these enzymes from the cytosol to the blood stream [29]. The kidney is vital in executing physiological functions such as osmoregulation and removal of contaminants. The feeding of PS caused alterations such as vacuolization and epithelium degeneration in the T1 group, while melanomacrophage aggregate detached epithelial cells from the basal lamina, and hyaline degeneration of tubular epithelium was visible in T2. The cloudy swelling degeneration melanomacrophage aggregate and cellular necrosis was the observed evidences in the highest PS-fed group (T3). The noticed changes suggest the sensitivity of the organ to the supplemented compound.

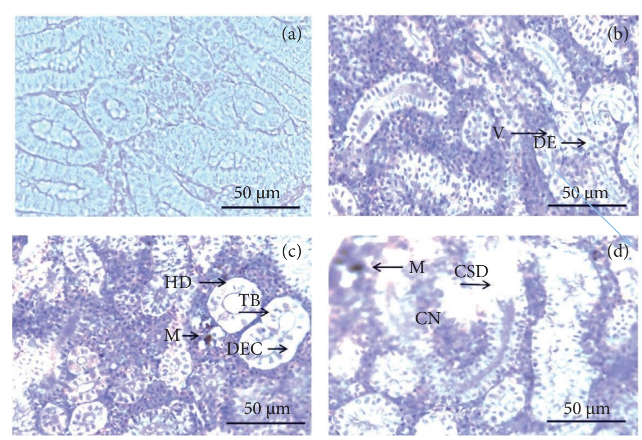

Figure 4: Histopathology of *B. gonionotus* kidney tissue (H&E stain, 40x): (a) control; (b) vacuolization (V) and degenerating epithelium (DE) (T1); (c) melanomacrophage aggregate (M), detached epithelial cells from the basal lamina (DEC), and hyaline degeneration of tubular epithelium (HD) (T2); (d) cloudy swelling degeneration (CSD) melanomacrophage aggregate (M) and cellular necrosis (CN) (T3). Bar scale = 50 μm in 40x.

3.5. Haematoserological Indicators. Haematological and serum biochemical scores serve as effective health indicators in fish [30]. The haematological parameters, viz., RBC and WBC counts, Hb, and PCV, are depicted in Table 3. Values

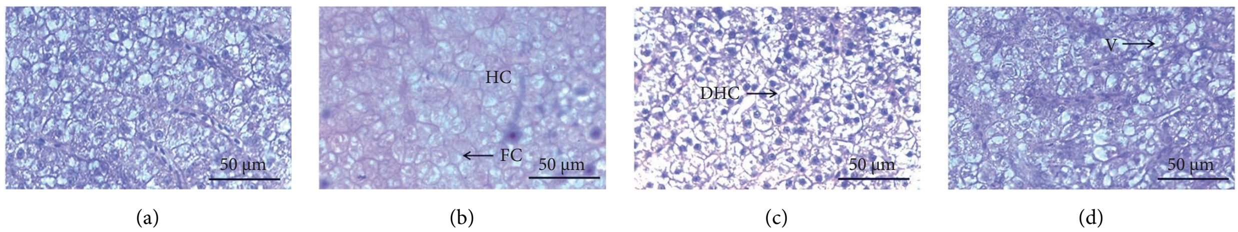

Figure 5: Histopathology of *B. gonionotus* liver (H&E stain, 40x): (a) normal cells (control); (b) hypertrophied cell (HC); focal necrosis (FC) (T1); (c) degeneration of hepatic cell (DHC) (T2); (d) vacuolation (V) (T3). Bar scale = 50 μm in 40x.

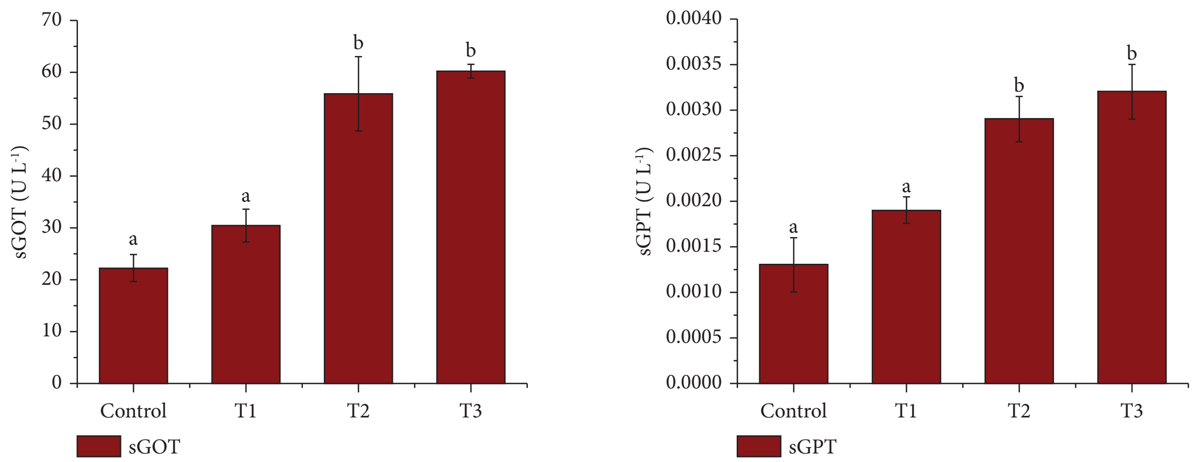

Figure 6: sGOT and sGPT levels in the liver tissue of silver barb (*B. gonionotus*) in different treatments at the end of the 60-day experiment. Different superscripts indicate statistically significant difference ($P < 0.05$) among the experimental groups.

decreased in treatment groups, compared with the control. The RBC and WBC counts in T1, although lower than the control, did not differ significantly ($P < 0.05$). This implies that a higher level of PS induces a negative health effect on silver barb. In one of the related works by De Pedro et al. [31], papaya seed supplied in the diet induced anemic conditions, due to the excessive levels of papaya seed meal. Plasma protein is considered as a reliable indicator of physiological response, which is directly associated with the immunity, disease susceptibility, and health status of fishes [32]. Their levels were significantly high ($P < 0.05$) in control and T1, compared with T2 and T3. In the case of albumin, T1 had the highest level ($P < 0.05$) among the treatments, whereas T3 reported the lowest value among all the treatments. Thus, higher levels of total plasma protein and albumin observed in the present study in the lowest inclusion of pawpaw seed meal extract corroborated a better health status, while at high level it compromised the immune function and probably retarded the growth of the fish.

3.6. Stress Biomarker. The possibility of stress in fish having dietary suppression of physiological duty can be well explained by the level of cortisol and glucose in their serum. Both plasma glucose and cortisol levels followed the same trend among all the treatment groups (Figure 7). T3 was observed to have the highest value, whereas T1 reported the lowest significant value ($P < 0.05$) among all the treatments. Again, control and T1 have approximately the same plasma glucose and cortisol values. In aquatic animals, cortisol is produced from the interrenal tissue upon stimulation from the corticotrophin-releasing factor (CRF) via the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland [33]. Under stress situations, catecholamine hormones such as adrenaline and noradrenaline released from the chromaffin tissue in conjunction with cortisol mobilize and increase glucose synthesis through the process of either glycogenesis or glycogenolysis [34]. Here, the observed increase in glucose and cortisol at a higher inclusion dose of pawpaw seed opined that a higher level of supplementation compromises the physiological function, and the reserved energy is drained towards body equilibrium maintenance via the secretion of glucose to adapt to the stress conditions. The observed lower values of glucose and albumin may possibly explain that the low-dose supplementation did not affect the stress level and silver barb could well maintain its normal function.

Table 3: Effect of pawpaw seed on the haematological parameter of *B. gonionotus* in different treatments at the end of the 60-day experiment (mean ± S.E.).

Parameters	Treatment			
	Control	T1	T2	T3
RBC ($\times 10^6$ cells mm^{-3})	13.86 ± 1.5^b	9.73 ± 1.10^b	8.16 ± 0.33^a	5.41 ± 1.00^a
WBC ($\times 10^3$ cells mm^{-3})	47.83 ± 4.7^b	38.40 ± 4.56^b	27.52 ± 1.8^a	23.73 ± 2.0^a
PCV (%)	50.52 ± 7.95^b	48.93 ± 2.94^b	43.29 ± 3.71^b	37.69 ± 4.91^a
Haemoglobin (%)	0.43 ± 0.02^b	0.44 ± 0.03^b	0.28 ± 0.04^a	0.16 ± 0.03^a
Total protein (g dL^{-1})	3.83 ± 0.30^b	3.23 ± 0.16^b	2.452 ± 0.10^a	2.07 ± 0.19^a
Albumin (g dL^{-1})	2.33 ± 0.08^b	2.36 ± 0.08^b	1.900 ± 0.07^a	1.60 ± 0.14^a

Data are represented as mean ± S.E. ($n = 20$). Different superscripts indicate statistically significant difference ($P < 0.05$) among the experimental groups.

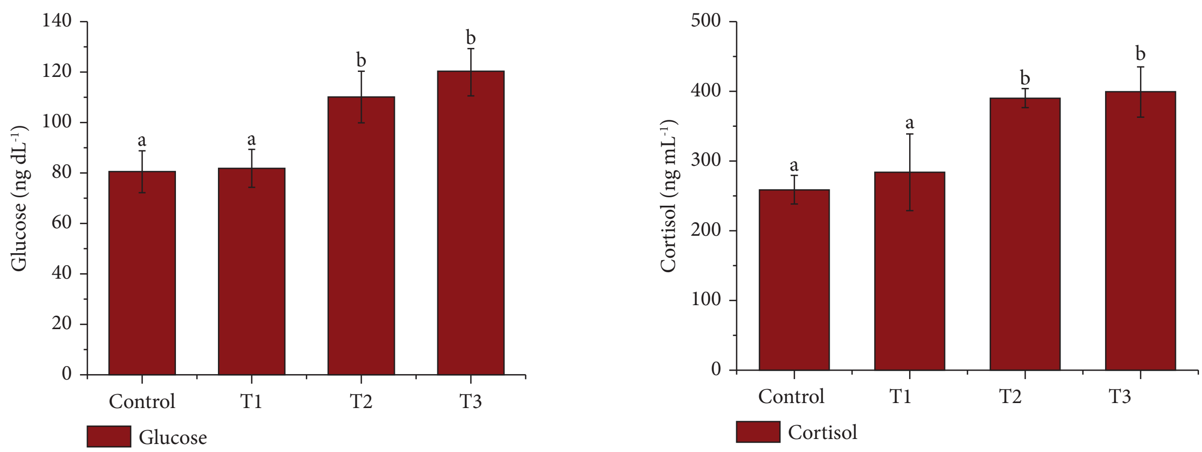

Figure 7: Mean glucose and cortisol levels ± SE of silver barb (*B. gonionotus*) in different treatments at the end of the 60-day experiment. Different superscripts indicate statistically significant difference ($P < 0.05$) among the experimental groups.

4. Conclusion

The present work unpins the potential of dietary pawpaw seed as an alternative to synthetic compounds for effectively impairing the reproductive function of silver barb. The supplied pawpaw seed is further shown to induce toxicity in terms of tissue level and welfare-related imbalances at a higher dose. The overall result implies that a very low level of 2 g/kg was sufficient to induce reproductive arrest and improve growth, without having a negative effect on the welfare of the fish. This approach will help in the energy trade-off between reproduction and growth of the fish and can resolve the early maturity noticed in silver barb.

Data Availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Upendra Suman explored the study, confirmed data, built software programs, and wrote the first draft. Gusheinzed Waikhom designed the study, oversaw it, and evaluated and edited the report. Soibam Khogen Singh created the approach, assisted with visualization and validation, and read and edited the manuscript. Yumnam Abungcha Mangang and Reshmi Debbarma contributed to the visualization and validation, as well as the editing of the text. Pradyut Biswas, Pronob Das, and S.B. Singh curated and validated the data. Upendra Suman and Gusheinzed Waikhom shared equal credit and are co-first authors.

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