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Research Article

Ameliorative Effects of Different Dietary Levels of Fish Protein Hydrolysate (FPH) on Growth and Reproductive Performance, Feed Stability, Tissues Biochemical Composition, Haematobiochemical Profile, Liver Histology, and Economic Analysis of Pabda (*Ompok pabda*) Broodstock

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This study investigated the impacts of various inclusion levels of dietary potential of fish protein hydrolysate (FPH) on the growth and reproductive performance, biochemical composition, blood parameters, and liver histology of *Ompok pabda* broodstock. About 600 pabda broods (11.00 ± 0.05 g) were distributed into 12 cages and fed twice in a day. For this, four experimental diets (crude protein: 30%; crude lipid: 9%) were prepared by incorporating FPH at different percentages (0%, 5%, 7%, and 9%). The FPH positively impacted ($p < 0.05$) the durability index, water stability, and swelling rates of the experimental diets. Furthermore, significantly higher palatability ($p < 0.05$) was recorded for pabda diets incorporated with 5% and 7% FPH. After 90 days, the growth performance of pabda in final weight, live weight gain, total biomass, specific growth rate, hepatosomatic index, visceral somatic index, and nutrient utilization indices, feed conversion ratio and protein efficiency ratio, was significantly ($p < 0.05$) improved when fed with 7% FPH diet. Additionally, the ovipositor diameter (5.10 ± 0.05 mm), spawning response ($98.48 \pm 2.4\%$), fecundity ($13.28 \pm 0.23 \times 10^4$ eggs/kg), and egg fertilization rate ($87.09\% \pm 0.14\%$) were significantly higher ($p < 0.05$) for the 7% FPH dietary group than other treatments. The fish group that received control diet experienced a marked ($p < 0.05$) reduction in egg hatching rates, coupled with longer ovulation period as compared to FPH-treated groups. Dietary FPH inclusion at different levels also caused notable improvements ($p < 0.05$) in most hematological and serum biochemical indices of pabda broodfish. The 7% FPH group also exhibited enhanced liver health, characterized by superior nuclei, erythrocyte, and cytoplasmic structure and boosted the farm economics efficiency. In summary, 7% dietary FPH is suitable and beneficial for *O. pabda* broodstock development in captivity by improving growth and reproductive performance, overall health, and farm economics.

1. Introduction

Aquaculture has become increasingly critical over the years due to the global decline in seafood supply and rising demand for freshwater fish. For instance, *Ompok pabda*, or the pabda catfish, is gaining popularity as a high-potential aquaculture species due to the fish's unique attributes, including high palatability, lack of intramuscular bones, rapid growth, excellent nutritional profile, and high market price [1]. Nevertheless, optimizing broodstock nutrition remains critical in unlocking the full potential of pabda aquaculture.

Fish meal (FM) is the primary protein source in aquafeeds owing to the high-quality amino acid profile and digestibility. However, FM demand has skyrocketed up to 300% [2], limiting the supply and increasing the costs of this feed ingredient [3]. The high prices and limited availability of FM, coupled with concerns about overfishing, have led the stakeholders to explore alternative and sustainable protein sources [4–6]. Alternative proteins in fish diet ensure long-term sustainability by reducing reliance on wild fish stocks, offering nutrition, and fostering environmentally friendly aquaculture practices. Additionally, researchers are looking to lower feed costs by replacing high-value dietary FM [7–10] with other bioactive substances and immunostimulants to enhance fish's overall productivity and immunity. Numerous scientists opted to substitute FM with secondary alternatives, such as plant-based proteins [5, 11–17], insects [18], and fish by-products [19]. However, their annual production volumes, accessibility, costs, storage capacity, and transport viability pose essential considerations [12].

The aquaculture industry produces significant by-products which constitute up to 50% of the entire fish, ranging from 10% to 90% depending on species and utilization [17, 20–24]. Repurposing aquaculture by-products offers a sustainable waste management approach, including developing feed ingredients such as fish protein hydrolysate (FPH). The FPH is obtained by enzymatically hydrolyzing fish protein, producing a sustainable and nutritionally balanced solution that could replace FM in aquafeed [1]. Furthermore, FPH is nutritious, boasting well-balanced amino acid profiles, excellent digestibility, and nitrogen-containing compounds that aid in the intake and absorption of nutrients by aquaculture species [25]. This feed ingredient also contains free amino acids, nucleotides, and di/tripeptides that function as attractants and enhance feed palatability [26]. Additionally, in vivo studies highlighted the physiological advantages of dietary FPH, including immunomodulatory, antioxidant, antimicrobial, and antihypertensive activities [27, 28]. Considering the issues mentioned above, FPH could be an excellent fit among all the alternatives, contributing to the sustainable utilization of resources while enhancing aquaculture production.

Incorporating FPH in a low FM-based diet could promote growth, feed intake, health status, and immune system of numerous economically valuable fish species such as *Acipenser persicus* L. [29], *Paralichthys olivaceus* [30], *Dicentrarchus labrax* [31], *Scophthalmus maximus* [32], *Chirostoma estor* [33], *Lates calcarifer* [34–37], *Trachionotus blochii* [38, 39], and *O. pabda* [1]. Nonetheless, the literature on how FPH impacts the reproductive and spawning performance of *O. pabda* catfish remains scarce. Therefore, this study investigated the effects of dietary FPH at different levels (0%, 5%, 7%, and 9%) on the growth and reproductive performance and health status of pabda to identify the optimum levels of FPH for the development of quality broodstock in captivity.

2. Materials and Methods

2.1. Collection and Holding of Experimental Brood Fish. The pabda broodfish (11.00 ± 0.05 g) were procured from a commercial fish hatchery. They were acclimatized in a large hapa (10 ft (l) × 8 ft (w) × 6 ft (h)) and fed with a commercial feed (ACI Godrej Agrovet Private Limited, Bangladesh; crude protein: 32%, crude lipid: 6%). After 10 days, approximately 600 pabda catfish were randomly selected, weighed, and distributed into four treatment groups in triplicates (cage size: 1 m (l) × 1 m (w) × 1.5 m (h); stocking density: 50 fish/cage; male-to-female ratio = 1 : 4).

2.2. Diet Preparation and Feeding. The experimental diets (crude protein 30% and crude lipid 9%) were prepared by including FPH at different percentages: 0% (D1), 5% (D2), 7% (D3), and 9% (D4). The D1 group did not receive dietary FPH, serving as the experimental control. First, the experimental diets were prepared by blending commercial liquid FPH (Symrise Aqua-Feed, Specialities Pet Food, 226-FR-SPF, France) with other feed ingredients, including FM, soybean meal, wheat bran, rice bran, palm oil, soybean oil, distillery dry grain soluble, vitamin–mineral premix, and carboxymethyl cellulose. The mixture was extruded (LM40- floating fish feed machine, Henan Lima Machinery Manufacture Co., Ltd., Zhengzhou, China) into 2 mm buoyant pellets, oven-dried at 60°C, and cooled at room temperature. Finally, the pellets were stored at −15°C in airtight containers. The fish were fed with the respective diets at 9:30 am and 5:30 pm daily for 90 days. The experimental diet formulation and proximate analysis [40] are presented in Table 1, while the proximate composition of FM and FPH used in this study can be found in Table 2.

2.3. Hydrological Variables. The water parameters of the experimental cages, such as temperature, salinity, pH, water pressure, conductivity, total dissolved solids (TDS), and dissolved oxygen (DO), were closely monitored at 3 days intervals and measured using the YSI multiparameter probe (HI 9828, YSI Incorporation, Yellow Spring, USA). In addition, water ammonia, nitrite, and nitrate concentrations were assessed via the HACH test kit (HI 28049, HACH, USA).

2.4. Physical Characteristics and Palatability Assessment of Experimental Feed

2.4.1. Physical Characteristics. The physical attributes of the experimental diets, such as pellet durability index, water stability, floatability, and swelling, were evaluated before the

Table 1: Experimental feed formulation and proximate composition (dry weight basis).

Ingredients (g/100 g)	Diets (FPH (%))			
	D1	D2	D3	D4
Danish fish meal	13.5	8.5	6.5	4.5
FPH[1]	0.0	5.0	7.0	9.0
DDGS[2]	6.5	6.5	6.5	6.5
Rice bran	18.0	18.0	18.0	18.0
Wheat	25.0	25.0	25.0	25.0
Soybean meal	25.0	25.0	25.0	25.0
Soybean oil	3.0	3.0	3.0	3.0
Palm oil	3.0	3.0	3.0	3.0
Vitamin and mineral premix[3]	3.0	3.0	3.0	3.0
CMC[4]	3.0	3.0	3.0	3.0
Total	100.00	100.00	100.00	100.00
Farm raw materials cost (USD/MT)	611.20	620.68	624.47	628.27
Proximate composition				
Crude protein	30.05	30.40	30.65	30.85
Crude lipid	9.29	9.21	9.09	9.01
Crude ash	9.32	9.35	9.38	9.30
Moisture	11.20	11.05	11.00	11.01
NFE[5]	40.14	39.99	39.88	39.83

[1]Fish protein hydrolysate (FPH); [2]distillery dry grain soluble (DDGS); [3]vitamin and mineral premix (g/kg): vitamin A, C, D, E, K, B_1, B_2, B_6, B_{12}, KCl, 90; KI, 0.04; $CaHPO_4{\cdot}2H_2O$, 500; NaCl, 40; $CuSO_4{\cdot}5H_2O$, 3; $ZnSO_4{\cdot}7H_2O$, 4; CoO_4, 121 0.02; $FeSO_4{\cdot}7H_20$, 20; $MnSO_4{\cdot}H_2O$, 3: $CaCO_3$, 215; Mg_2OH, 124: Na_2SeO_3, 0.03; NaF, 1; [4]carboxymethyl cellulose (CMC); and [5]nitrogen free extract (NFE).

Table 2: Proximate analysis (percentage of dry weight basis) of FM and FPH utilized in this study.

Proximate composition	Ingredients	
	Danish fish meal	Fish protein hydrolysate
Crude protein	68.43	77.93
Crude lipid	8.03	2.09
Crude ash	16.93	4.75
Moisture	9.93	5.91

feeding trial as per Zulhisyam et al.'s [41] study with some modifications. The following formulae were employed in the analysis:

(i) Pellet durability index (%) = $100 \times \frac{\text{Weight of pellet remaining on the sieve}}{\text{Total weight of pellets}}$.

(ii) Water stability (%) = $100 \times \frac{\text{Weight of whole feed pellets retained after immersion}}{\text{Feed pellets initial weight}}$.

(iii) Flotability (%) = $100 \times \frac{\text{Mean numbers of floating feed}}{\text{Initial number of floating feed}}$.

(iv) Swelling (%) = $100 \times \frac{\text{Total diameter of swollen feed pellets}}{\text{Total diameter of dry feed pellets}}$.

2.4.2. Palatability Test. The palatability of FPH-treated diets was assessed following the methods outlined by Al-Souti et al. [42] with modifications. Four rectangular glass tanks (80 cm (l) × 40 cm (w) × 40 cm (h)) were filled with water and used to acclimatize randomly selected fish from each experimental group (10 fish/group). After 2 hr, 6 g of each experimental diet were placed in the glass tanks, and the fish were allowed to feed on the pellets for 10 min. Subsequently, the fish were weighed before returning them to their respective groups. The feed residues in the glass tanks were carefully collected from the water surface, oven-dried at 60°C for 8 hr, and weighed. This process was repeated seven times, starting at 9:30 daily. The following formula was employed to compute palatability:

$$\text{Palatability (feed, mg/fish biomass, g)} = \text{Total feed provided (mg)/fish total biomass (g)}. \quad (1)$$

2.5. Growth and Reproductive Performance of Pabda Broodstock. At the end of the feeding trial, all *O. pabda* broodstock were fasted for 24 hr before harvesting. Subsequently, the fish were anesthetized with MS_{222} to determine their total biomass (TB). Ten fish were randomly selected from every cage to record their final body weight and ovipositor diameter individually. Subsequently, the liver, gonad, and viscera were extracted from each fish and weighed individually. The growth, reproductive, and egg quality parameters were calculated using methodologies and formulae outlined by Nandi et al. [6] and Kabir et al. [43]:

(i) Survival rate (%) = $100 \times \frac{\text{Number of survived broodfish}}{\text{Total number of fish at the begining of experiment}}$.

(ii) Live weight gain (%) = $100 \times \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}}$.

(iii) Specific growth rate (%/day) = $100 \times \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Duration of feeding trial}}$.

(iv) Feed conversion ratio (FCR) = $\frac{\text{Total feed fed}}{\text{Live weight gain}}$.

(v) Protein efficiency ratio (PER) = $\frac{\text{Live weight gain}}{\text{Total protein consumed}}$.

(vi) Hepatosomatic index (HSI) = $100 \times \frac{\text{Liver weight}}{\text{Body weight}}$.

(vii) Visceral somatic index (VSI) = $100 \times \frac{\text{Viscera weight}}{\text{Body weight}}$.

(viii) Gonadosomatic index (GSI) = $100 \times \frac{\text{Weight of gonad}}{\text{Body weight of fish}}$.

(ix) Fecundity (eggs · kg^{-1}) = $\frac{\text{Total number of eggs in female ovary}}{\text{Weight of female fish}}$.

(x) Egg fertilization rate (%) = $100 \times \frac{\text{Number of fertilized eggs in subsample}}{\text{Total number of eggs in subsample}}$.

(xi) Spawning response (%) = $100 \times \frac{\text{The number of spawned hormone-injected fish}}{\text{Total number of hormone treated female}}$.

(xii) Hatching rate (%) = $100 \times \frac{\text{Number of hatched eggs}}{\text{Total number of fertilized eggs}}$.

2.6. Assessment of Biochemical Composition. The proximate composition of the experimental diets, ingredients, and fish tissues was determined in triplicates following AOAC (2000) guidelines with modifications. For tissues biochemical analysis,

the liver, intestine, muscle, and oocyte samples were obtained from three randomly selected fish from each cage and stored at −20°C until further examination. The crude protein content was determined using the Kjeldahl technique (calculated as 6.25 × % *N*), while the crude lipid content was assessed using the *n*-hexane extraction method via a Soxhlet apparatus. Meanwhile, the ash content of the respective samples was evaluated by heating them at 550°C for 6 hr in a Muffle furnace, and samples were oven-dried at 105°C for 24 hr to detect the moisture content.

2.7. Blood Parameters. At the end of the feeding trial, seven randomly selected broodfish from each cage were anesthetized with MS_{222}, and their blood was collected from the caudal vein using a 1 ml sterile syringe. Approximately 150 μl blood samples from each replication fish were placed in tripotassium ethylenediaminetetraacetic acid (EDTA K_3) tubes and another 500 μl blood samples into cellular serum tubes to facilitate hematological and serum biochemical analyses, respectively. Following this, the processed blood underwent centrifugation for 10 min at 3,000 rpm to achieve serum separation and then evaluated all biochemical parameters in a clinical chemistry analyzer (Beckman Coulter AU680, USA).

On the other hand, red blood cell (RBC), hematocrit (HCT), and hemoglobin (HGB) concentrations were ascertained by the methodology outlined by Blaxhall and Daisley [44]. By using Thoma hemocytometer and Dacie's diluting fluid, the RBC was counted. Similarly, white blood cell (WBC) was counted using a hemocytometer and microscope. The distinct WBC types (neutrophil, lymphocytes, eosinophil, monocytes, and basophil) were recognized and quantified in a blood smear stained using Pappenheim's panchromatic method. Moreover, the HCT levels were determined using a capillary hematocrit tube while HGB concentrations were assessed through spectrophotometry at a wavelength of 540 nm, utilizing cyanomethahemoglobin method. However, other hematological parameters including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined using the formulae of Bain et al. [45]:

(i) $\text{MCV (fl)} = \text{HCT (l/l)}/\text{RBC}(10^{12}/\text{l})$.
(ii) $\text{MCH (pg)} = \text{HGB (g/l)}/\text{RBC}(10^{12}/\text{l})$.
(iii) $\text{MCHC (g/l)} = (\text{HGB (g/l)} \times 1{,}000)/\text{HCT (1/l)}$.

2.8. Liver Histology. Five fish from each cage were randomly selected at the end of the feeding trial and anaesthesized using MS_{222}. The transverse section of their liver tissues was obtained and preserved for 24 hr in a 10% neutral buffered formalin solution. Subsequently, the liver sections were dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin blocks. The tissues were sectioned (5–8 μm) and stained with hematoxylin and eosin (H&E). Once completed, the slides were observed under an electronic light microscope (Leica DMIL-LED, Germany) and visualized using the Cellsens software (Cellsens, Netherlands).

2.9. Economic Analysis. The primary expenditure in fish farming operations, particularly modest farms such as *O. pabda* culture in Bangladesh, is on feed cost. Expenses on raw materials are measured by accumulating the prices of various feed ingredients used to formulate each experimental diet. The following formulae were used to evaluate the farm's economic efficiency as described by Suma et al. [1]:

(i) $\text{Total yield (kg/m}^2) = \text{Biomass gained/cage area}$.
(ii) $\text{Farm feed cost, FFC (USD/kg)} = \text{FCR} \times \text{Cost of raw materials}$.
(iii) Farm revenue was estimated based on an anticipated farm gate price of 3.67 USD/kg of pabda catfish: $\text{FR(USD/m}^2) = \text{Total yield} \times 3.67$.
(iv) $\text{Farm raw margins, FRM (USD/m}^2) = \text{FR} - \text{Total yield} \times \text{FFC}$.
(v) $\text{Return on investment, ROI (\%)} = 100 \times \text{FRM}/(\text{Total yeild} \times \text{FFC})$.

2.10. Statistical Analysis. The quantitative data obtained from this study were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 20.1 for Windows (IBM, USA). The one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was performed to identify differences ($p < 0.05$) between the treatments and control groups. The results were expressed as mean ± standard deviation (SD).

3. Results

3.1. Physical Attributes of Experimental Diets. Table 3 presents the physical properties of experimental diets in this study. The feed diameter and floatability were statistically similar ($p > 0.05$) between groups. Conversely, there were significant differences ($p < 0.05$) in pellet durability index and water stability between the FPH diet and control groups. Figure 1 illustrates the swelling trends of experimental diets at 2, 6, and 10 min. Dietary FPH significantly influenced ($p < 0.05$) the swelling rates of the formulated pellets upon water exposure at different time intervals. Precisely, the D1 (control) (168.93%) and D3 (7% FPH) (166.30%) diets had significantly ($p < 0.05$) higher swelling rates than other diets at 10 min.

3.2. Palatability Assessment of Experimental Diets. The pabda feed intake was observed for 5 min to determine the palatability of the experimental diets. Figure 2 illustrates significant differences ($p < 0.05$) in palatability between the FPH-included feed. Furthermore, the 5% (D2) and 7% (D3) FPH feed were highly palatable to pabda than other diets, recording average values of 14.72 and 15.10 mg feed/g of biomass, respectively.

3.3. Growth and Reproductive Indices of Brood Fish. Pabda growth, feed utilization, and reproductive performance at the end of the feeding trial are presented in Table 4. Fish fed with FPH-included feed exhibited significant improvements ($p < 0.05$) in growth and reproductive performance. The broodstock group fed with the 7% FPH diet demonstrated significantly enhanced ($p < 0.05$) final weight (FW), specific growth rate (SGR, live

Table 3: Physical properties of experimental diets, with and without FPH.

Parameters	Diets (FPH (%))			
	D1 (0)	D2 (5)	D3 (7)	D4 (9)
Feed diameter (mm)	2.02 ± 0.01	2.03 ± 0.01	2.04 ± 0.02	2.04 ± 0.02
PDI (%)	99.76 ± 0.08^{a}	98.18 ± 0.32^{b}	99.62 ± 0.03^{b}	98.35 ± 0.41^{b}
Floatability (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	99.67 ± 0.58
Water stability (%)	78.79 ± 0.05^{c}	79.80 ± 0.10^{a}	78.93 ± 0.04^{b}	79.90 ± 0.05^{a}

Values are expressed as mean ± standard deviation. ER, expansion ratio; PDI, pellet durability index. Distinct superscript letters in each row indicate significant variation ($p < 0.05$).

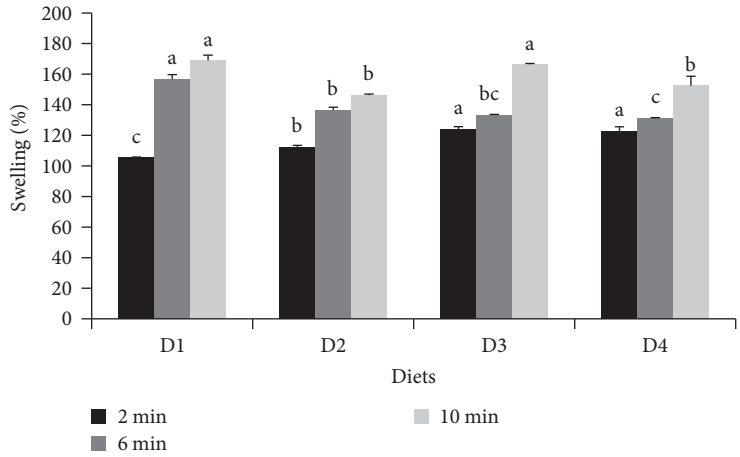

Figure 1: Swelling rates of experimental diets containing FPH at varying levels (0%, 5%, 7%, and 9%) over time (2, 6, and 10 min). The results are expressed as mean ± SD; different superscript letters indicate significant variation ($p < 0.05$).

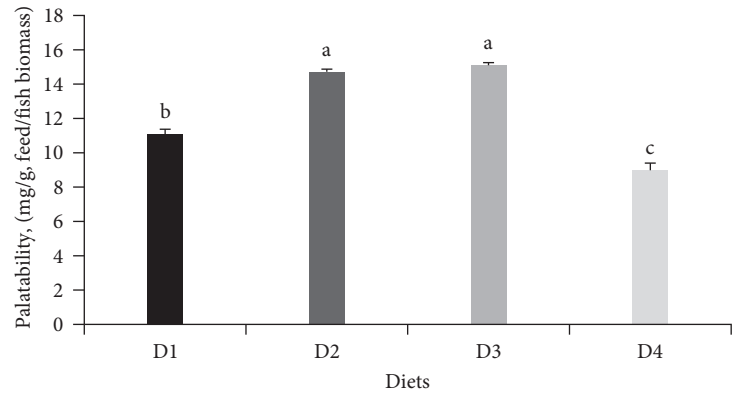

Figure 2: Palatability (feed (mg)/fish biomass, (g)) containing FPH at varying levels (0%, 5%, 7%, and 9%) over time (5 min). The results are expressed as mean ± SD; different superscript letters indicate significant variation ($p < 0.05$).

weight gain (LWG), TB, hepatosomatic index (HSI), and visceral somatic index (VSI) than other treatment groups. Similarly, the feed conversion ratio (FCR) and protein efficiency ratio (PER) were significantly better ($p < 0.05$) in the 7% FPH treatment group. Reproductive and egg quality indicators, including fecundity, egg fertilization rate, spawning response, ovipositor diameter, hatching rate, and ovulation time, were also significantly different ($p < 0.05$) between groups, where the 7% FPH diet demonstrated superior performance. Nevertheless, the dietary FPH did not significantly impact the broodfish survival and GSI ($p > 0.05$).

3.4. Tissues Biochemical Composition of Experimental Brood Fish. The proximate composition of the fish intestine, liver, muscle, and oocytes after the supplementation of dietary FPH is shown in Table 5. There were no significant differences between the groups in intestinal crude protein ($p > 0.05$). In contrast, the crude protein levels in the fish liver and muscle increased significantly ($p < 0.05$) with higher FPH inclusion in their feed. The 7% and 9% FPH groups recorded significant ($p < 0.05$) and highest crude protein in oocytes rather than treatment groups. In addition, the 7% FPH group had significantly ($p < 0.05$) lower crude lipid in their muscle tissues but

Table 4: *Ompok pabda* growth and reproductive performances at the end of the dietary FPH feeding trial.

Parameters	Diets (FPH (%))			
	D1 (0)	D2 (5)	D3 (7)	D4 (9)
IW	11.01 ± 0.01	11.00 ± 0.01	11.01 ± 0.01	10.99 ± 0.03
FW	21.21 ± 0.19^{d}	26.06 ± 0.82^{c}	31.31 ± 0.57^{a}	28.19 ± 0.40^{b}
LWG (%)	92.58 ± 1.77^{d}	136.80 ± 7.32^{c}	184.40 ± 5.20^{a}	156.56 ± 3.08^{b}
SGR (%/day)	0.73 ± 0.01^{d}	0.96 ± 0.04^{c}	1.16 ± 0.02^{a}	1.05 ± 0.01^{b}
TB (g)	509.72 ± 9.45^{d}	752.73 ± 40.72^{c}	$1{,}015.05 \pm 28.59^{a}$	914.46 ± 93.13^{b}
FCR	1.72 ± 0.02^{b}	1.58 ± 0.05^{c}	1.35 ± 0.03^{d}	1.82 ± 0.04^{a}
PER	1.94 ± 0.02^{c}	2.12 ± 0.07^{b}	2.47 ± 0.05^{a}	1.83 ± 0.04^{c}
SR (%)	96.67 ± 1.15	97.33 ± 1.15	98.67 ± 1.15	96.67 ± 1.15
HSI	1.02 ± 0.03^{c}	1.20 ± 0.04^{b}	1.59 ± 0.11^{a}	1.35 ± 0.11^{b}
VSI	1.01 ± 0.05^{c}	1.42 ± 0.09^{bc}	2.02 ± 0.50^{a}	1.61 ± 0.18^{ab}
GSI	16.77 ± 1.67	14.80 ± 1.24	17.34 ± 1.50	15.06 ± 0.89
EFR (%)	67.17 ± 1.40^{d}	76.95 ± 1.63^{c}	87.09 ± 0.14^{a}	84.16 ± 2.22^{b}
Fecundity (eggs/kg) $\times 10^4$	10.51 ± 0.08^{d}	11.47 ± 0.10^{c}	13.28 ± 0.23^{a}	12.85 ± 0.14^{b}
Spawning response (%)	42.71 ± 2.24^{d}	54.94 ± 2.35^{c}	98.48 ± 2.49^{a}	88.11 ± 2.57^{b}
OD (mm)	3.57 ± 0.39^{b}	3.72 ± 0.40^{b}	5.10 ± 0.05^{a}	4.90 ± 0.22^{a}
HR (%)	45.65 ± 1.00^{d}	47.90 ± 0.51^{c}	59.88 ± 0.55^{b}	61.20 ± 0.61^{a}
OT (hr)	29.33 ± 1.53^{a}	24.67 ± 1.15^{b}	19.67 ± 1.15^{c}	21.67 ± 1.53^{c}

Values are expressed as mean $\pm$ SD. IW, initial weight; FW, final weight; LWG, live weight gain; SGR, specific growth rate; TB, total biomass; FCR, feed conversion ratio; PER, protein efficiency ratio; SR, survival rate; HSI, hepatosomatic index; VSI, visceral somatic index; GSI, gonadosomatic index; EFR, egg fertilization rate; OD, ovipositor diameter; HR, hatching rate; and OT, ovulation time. Different superscript letters in each row indicate significant differences ($p < 0.05$).

Table 5: Fish intestine, liver, muscle, and oocytes proximate composition (percentage of wet weight basis) analysis after the FPH diets feeding trial.

Parameters	Diets (FPH (%))			
	D1 (0)	D2 (5)	D3 (7)	D4 (9)
Intestine				
Crude protein	16.74 ± 0.12	16.49 ± 1.08	17.22 ± 0.78	18.24 ± 0.48
Crude lipid	11.04 ± 0.84	11.13 ± 0.13	11.31 ± 0.09	10.79 ± 0.30
Ash	2.00 ± 0.01^{b}	2.33 ± 0.13^{ab}	2.40 ± 0.13^{ab}	2.59 ± 0.14^{a}
Moisture	68.09 ± 0.87	67.90 ± 0.88	67.34 ± 1.03	66.77 ± 0.58
Liver				
Crude protein	12.63 ± 0.65^{b}	13.66 ± 0.42^{b}	15.81 ± 0.98^{a}	16.25 ± 0.36^{a}
Crude lipid	11.74 ± 0.44^{a}	11.18 ± 0.99^{ab}	10.57 ± 0.22^{b}	10.13 ± 0.17^{b}
Ash	2.05 ± 0.09^{b}	2.50 ± 0.26^{ab}	2.84 ± 0.27^{a}	2.56 ± 0.29^{ab}
Moisture	71.87 ± 1.54	71.34 ± 1.46	69.41 ± 1.36	69.69 ± 0.97
Muscle				
Crude protein	21.57 ± 0.56^{b}	22.01 ± 1.34^{b}	23.03 ± 0.50^{ab}	23.84 ± 0.70^{a}
Crude lipid	8.13 ± 0.10^{a}	7.18 ± 0.19^{b}	6.84 ± 0.05^{c}	8.17 ± 0.22^{a}
Ash	2.19 ± 0.25^{b}	2.61 ± 0.15^{a}	2.61 ± 0.12^{a}	2.64 ± 0.16^{a}
Moisture	66.67 ± 0.54^{a}	66.10 ± 1.00^{ab}	65.31 ± 0.39^{b}	63.73 ± 0.59^{c}
Oocytes				
Crude protein	17.35 ± 0.11^{b}	17.46 ± 0.23^{b}	18.64 ± 0.08^{a}	18.17 ± 0.61^{a}
Crude lipid	12.68 ± 0.25	12.83 ± 0.40	12.55 ± 0.48	12.74 ± 0.07
Ash	2.46 ± 0.03	2.72 ± 0.27	2.58 ± 0.13	2.63 ± 0.04
Moisture	63.53 ± 0.50^{a}	60.87 ± 1.23^{bc}	61.67 ± 1.51^{ab}	59.35 ± 0.11^{c}

Values are expressed as mean $\pm$ SD. Different superscript letters in each row indicate significant differences ($p < 0.05$).

Table 6: Hematological profiles of pabda supplemented with FPH at varying levels.

Parameters	Diets (FPH (%))			
	D1 (0)	D2 (5)	D3 (7)	D4 (9)
WBC (10^9/l)	542.00 ± 0.48	541.00 ± 0.55	542.00 ± 0.12	542.00 ± 0.14
NEU (%)	30.74 ± 0.76	29.74 ± 0.56	30.16 ± 1.01	30.00 ± 0.81
LYM (%)	15.39 ± 2.34^{b}	15.01 ± 3.90^{b}	20.72 ± 0.60^{a}	19.42 ± 1.99^{ab}
MON (%)	2.00 ± 0.15^{a}	0.83 ± 0.11^{b}	1.80 ± 0.21^{a}	0.73 ± 0.16^{b}
EOS (%)	0.51 ± 0.03^{a}	$0.40 \pm 0.0.08^{b}$	0.56 ± 0.04^{a}	0.37 ± 0.01^{b}
BAS (%)	1.62 ± 0.15^{a}	0.49 ± 0.01^{b}	1.54 ± 0.04^{a}	0.45 ± 0.04^{b}
RBC (10^{12}/l)	2.71 ± 0.15^{c}	5.41 ± 0.02^{b}	6.67 ± 0.40^{a}	2.90 ± 0.05^{c}
HGB (g/l)	77.00 ± 18.35^{a}	19.67 ± 1.53^{c}	25.67 ± 3.06^{c}	45.33 ± 3.06^{b}
HCT (l/l)	0.21 ± 0.03^{b}	0.56 ± 0.05^{a}	0.16 ± 0.04^{b}	0.19 ± 0.03^{b}
MCV (fl)	78.49 ± 5.79^{b}	104.17 ± 7.98^{a}	23.35 ± 4.09^{d}	64.24 ± 9.84^{c}
MCH (pg)	28.32 ± 6.39^{a}	3.64 ± 0.28^{c}	3.86 ± 0.46^{c}	15.61 ± 1.03^{b}
MCHC (g/l)	364.84 ± 103.91^{a}	35.01 ± 3.20^{c}	168.24 ± 35^{b}	203.38 ± 133.98^{b}

Values are expressed as mean ± SD. WBC, white blood cell; NEU, neutrophil; LYM, lymphocytes; EOS, eosinophil; MON, monocytes; BAS, basophil; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; and MCHC, mean corpuscular hemoglobin concentration. Different superscript letters in each row indicate significant differences ($p < 0.05$).

Table 7: Plasma biochemical composition of *O. pabda* supplemented with dietary FPH at varying levels.

Parameters	Diets (FPH (%))			
	D1 (0)	D2 (5)	D3 (7)	D4 (9)
GLU (mg/dl)	66.00 ± 1.00^{c}	99.00 ± 9.64^{c}	148.33 ± 34.03^{b}	221.33 ± 1.53^{a}
CREA (mg/dl)	0.53 ± 0.06	0.70 ± 0.10	0.60 ± 0.43	0.51 ± 0.39
BIL (total) (mg/dl)	0.76 ± 0.02^{a}	0.63 ± 0.03^{b}	0.59 ± 0.04^{b}	0.45 ± 0.04^{c}
SGPT (μ/l)	31.67 ± 1.53^{b}	31.67 ± 1.15^{b}	32.00 ± 1.00^{b}	58.33 ± 12.58^{a}
S. Urea (mg/dl)	16.67 ± 1.15^{c}	18.67 ± 2.08^{bc}	21.67 ± 0.58^{b}	28.67 ± 3.21^{a}
SGOT (μ/l)	36.67 ± 1.52^{b}	61.67 ± 0.58^{a}	36.67 ± 5.13^{b}	62.33 ± 1.53^{a}
ALB (μ/l)	2.63 ± 0.15^{b}	2.53 ± 0.06^{b}	3.43 ± 0.21^{a}	2.80 ± 0.10^{b}
ALKP (μ/l)	185.33 ± 5.51^{a}	84.67 ± 3.51^{c}	65.33 ± 2.31^{d}	118.33 ± 3.79^{b}
CHOL (mg/dl)	163.67 ± 25.70^{c}	275.00 ± 31.22^{a}	255.33 ± 8.96^{ab}	225.00 ± 4.00^{b}
TP (g/dl)	4.44 ± 0.59^{c}	6.27 ± 0.15^{b}	9.25 ± 0.64^{a}	6.91 ± 0.02^{b}
GLOB (g/dl)	2.70 ± 0.01^{c}	2.61 ± 0.43^{c}	6.49 ± 0.40^{a}	3.96 ± 0.02^{b}

Values are expressed as mean ± SD. GLU for blood glucose, CREA for creatinine, BIL for bilirubin, SGPT for serum glutamic pyruvic transaminase, SGOT for serum glutamic oxaloacetic transaminase, ALB for albumin, ALKP for alkaline phosphatase, CHOL for cholesterol, TP for total protein, and GLOB for globulin. Different superscript letters in each row indicate significant differences ($p < 0.05$).

was statistically similar ($p > 0.05$) with other groups in intestinal and oocyte crude lipid contents. Interestingly, the intestinal, liver, and muscle ash contents were significantly ($p < 0.05$) lower in the control group. Conversely, no significant differences ($p > 0.05$) were recorded in oocyte ash content between the fish fed with different experimental diets. Likewise, the intestinal and liver moisture did not differ significantly ($p > 0.05$) between the treatment groups. Finally, the muscle and oocyte moisture levels were significantly different ($p < 0.05$) across the groups, with the lowest value recorded by the control group.

3.5. Hematological Analysis. Pabda's hematological profile at the end of the FPH diet feeding trial is presented in Table 6. The FPH inclusion in fish feed significantly ($p < 0.05$) impacted most hematological indices, including LYM, MON, EOS, BAS, RBC, HGB, HCT, MCV, MCH, and MCHC, without demonstrating any trend across various treatments. The 7% FPH group exhibited significantly ($p < 0.05$) higher levels of LYM, MON, EOS, BAS, and RBC, with other FPH-included groups but was statistically similar to the control group in MON, EOS, and BAS levels. Other blood parameters such as WBC and NEU levels remained consistent between treatment groups with no significant ($p > 0.05$) variations.

3.6. Plasma Biochemical Analysis. Table 7 demonstrates the plasma biochemical indices of pabda fed with dietary FPH at varying levels. Significant differences ($p < 0.05$) in plasma GLU, BIL, UREA, SGOT, SGOT, ALB, ALKP, CHOL, TP, and GLOB levels were observed between the treatment groups. Precisely, the 7% FPH diet group exhibited significantly ($p < 0.05$) elevated ALB, CHOL, TP, and GLOB levels, but the CHOL level was statistically similar to the 5% FPH group. Meanwhile, the treatment groups had no significant differences ($p > 0.05$) in CREA levels.

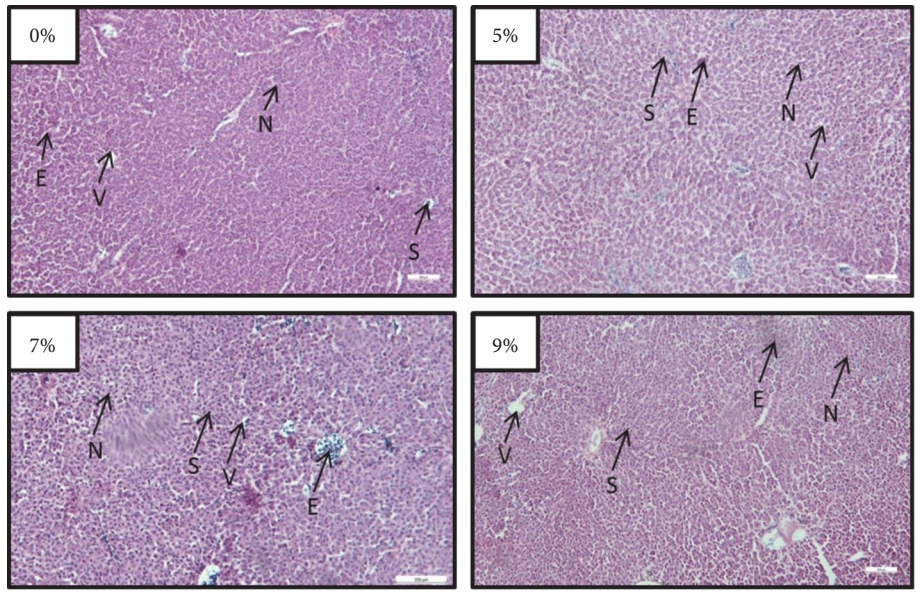

FIGURE 3: Liver histology of pabda broodstock fed with dietary FPH at 0%, 5%, 7%, and 9%, under light microscopy (Olympus BX43) at 10x magnification (scale bar: 200 μm). The black arrows indicate the nucleus (N), vacuole (V), erythrocyte (E), and sinusoid (S) in the fish liver.

TABLE 8: Hydrological parameters of cages inhabited by pabda broodstock supplemented with FPH at varying levels.

Parameters	Diets (FPH (%))			
	D1 (0)	D2 (5)	D3 (7)	D4 (9)
Temperature (°C)	30.91 ± 0.02	30.91 ± 0.02	30.91 ± 0.03	30.87 ± 0.02
Pressure, mm (Hg)	750.39 ± 0.23	750.33 ± 0.21	750.23 ± 0.32	750.23 ± 0.23
DO (mg/l)	5.09 ± 0.02	5.08 ± 0.02	5.08 ± 0.01	5.08 ± 0.02
Conductivity (Siemens/meter)	61.00 ± 0.20	60.13 ± 1.31	60.53 ± 0.87	60.33 ± 0.67
TDS (mg/l)	27.77 ± 0.30	27.68 ± 0.16	27.63 ± 0.04	27.68 ± 0.12
Salinity (mg/l)	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
pH	6.92 ± 0.02	6.92 ± 0.02	6.92 ± 0.01	6.92 ± 0.03
Ammonia (mg/l)	0.08 ± 0.01	0.08 ± 0.01	0.08 ± .01	0.08 ± 0.02
Nitrite (mg/l)	0.08 ± 0.00	0.09 ± 0.01	0.09 ± 0.03	0.09 ± 0.04
Nitrate (mg/l)	0.82 ± 0.01	0.82 ± 0.02	0.80 ± 0.03	0.80 ± 0.04

Values are expressed as mean ± SD. DO, dissolved oxygen; TDS, total dissolved solid. Mean values without superscript letters in each row indicate no significant differences ($p > 0.05$).

3.7. Liver Histomorphology. Histological changes were observed in the liver of pabda broodstock supplemented with dietary FPH at varying levels compared to the control group (Figure 3). The 7% FPH group exhibited enhanced nuclei, erythrocytes, and cytoplasmic organization and reduced cytoplasmic vacuolization than the other treatment groups. Notably, fish fed the basal diet manifested more cytoplasmic and nuclear atrophy and unorganized hepatic cell cords than the FPH-treated groups.

3.8. Water Quality Parameters. The hydroecological parameters of the respective pabda broodstock fed with experimental diets with different FPH inclusion are detailed in Table 8. There were no significant changes ($p > 0.05$) in water temperature, pressure, DO, conductivity, TDS, salinity, pH, ammonia, nitrite, and nitrate concentrations between the cages of all treatment groups.

3.9. Farm Economics. Table 9 demonstrates the farm economic efficiency of *O. pabda* culture when fed with feed containing dietary FPH at different percentages. The TY (kg/m^2), FR (USD/m^2), FRM (USD/m^2), and ROI (%) were significantly different ($p < 0.05$) and highest in the 7% FPH diet group while recording the lowest FFC (USD/kg). Conversely, the 0% and 9% FPH diet treatments recorded significantly higher ($p < 0.05$) FFC than other groups. The TY, FR, FRM, and ROI were also significantly ($p < 0.05$) lower in the control group, but the ROI was statistically similar to the 9% FPH diet group.

4. Discussion

The current study explores the potential of dietary FPH at varying levels on the reproductive and spawning performance

Table 9: Economic analysis of *O. pabda* culture when fed with feed containing dietary FPH at different percentages.

Parameters	Diets (FPH (%))			
	D1 (0)	D2 (5)	D3 (7)	D4 (9)
TY (kg/m^2)	0.39 ± 0.01^{d}	0.58 ± 0.03^{c}	0.77 ± 0.02^{a}	0.70 ± 0.07^{b}
FFC (USD/kg)	1.07 ± 0.05^{a}	0.97 ± 0.05^{b}	0.81 ± 0.02^{c}	1.15 ± 0.05^{a}
FR (USD/m^2)	1.43 ± 0.03^{d}	2.11 ± 0.11^{c}	2.85 ± 0.08^{a}	2.56 ± 0.26^{b}
FRM (USD/m^2)	1.01 ± 0.04^{c}	1.55 ± 1.00^{b}	2.22 ± 0.08^{a}	1.76 ± 0.19^{b}
ROI (%)	242.26 ± 15.91^{c}	277.07 ± 20.79^{b}	355.68 ± 13.00^{a}	220.12 ± 11.96^{c}

Values are expressed as mean ± SD. TY, total yield; FFC, farm feed cost; FR, farm revenue; FRM, farm raw margin; and ROI, return on investment. Distinct superscript letters in each row indicate significant variation ($p < 0.05$).

of pabda broodstock culture. The FPH is a valuable protein supplement derived from tuna by-products, potentially enhancing farm productivity and overall fish health. Nonetheless, it is imperative to identify the optimal dosage for FPH administration in fish diets to improve farm economics. Therefore, this study evaluated the physical properties of FPH-included diets and the effects on growth parameters, reproduction, blood indices, liver morphology, and farm economics.

Pellet durability is critical for effective handling, transporting, and pneumatic conveying to minimize dust and fine particle generation [46]. This study findings indicated that FPH supplementation at various levels in pabda diets significantly improved the physical attributes of pellets, such as PDI and water stability, compared to the control diet. The FPH inclusion possibly smoothens the pellet to reduce dust production and enhance binding capacity, owing to the water-binding properties of peptides and amino acids in this feed ingredient. These outcomes were consistent with earlier studies by Kari et al. [4], Khater et al. [46], Syamsu et al. [47], and Suma et al. [1]. Additionally, swelling of pellets is a key characteristic in determining the quality of feeds. In the current study, the experimental diets exhibited a notable difference in swelling rates. Notably, the basal diet without FPH and the 7% FPH diet exhibited higher swelling rates within 10 min. A high swelling rate indicates a robust structure and minimal nutrient loss in pellets [48]. Generally, feed manufacturers aim to strike a balance between PDI, water stability, and swelling percentage to ensure the feed maintains its integrity during storage, transportation, and in aquatic environment. Adjusting formulation, processing conditions, and ingredient selection can help to achieve this balance.

Palatability primarily relies on the nutritional composition and toxic elements of a diet and the nutritional requirements of an aquaculture species [49]. Attractants or stimulants are often included in aquafeed preparation to enhance palatability [42]. This study demonstrated that the 5% and 7% FPH diets resulted in higher palatability than other diets, which could be attributed to the shorter peptides and free AAs in FPH that act as attractants. Likewise, 1% and 2% FPH inclusion improved pellet palatability in a previous study [1]. In another study, protein hydrolysates at 5% resulted in comparable variations in *Salminus brasiliensis* diets [50]. Nonetheless, high FPH inclusion (9%) significantly reduced palatability, potentially caused by AAs toxicity, and a bitter taste of the diet, as reported by Detkamhaeng et al. [51] and Siddik et al. [34, 35].

Dietary FPH inclusion of up to 7% improved the FW, LWG, SGR, TB, HSI, VSI, FCR, and PER compared to the other treatment groups in this study. The overall results indicated that FPH positively impacted the fish growth performance and feed utilization, possibly due to the short-chain peptides, free AAs, bioactive compounds, and flavor of FPH that improved feed digestion and absorption and subsequently feed conversion and growth [1, 35, 52–54]. Earlier studies also reported similar outcomes when an aquaculture species were fed with graded levels of FPH in experimental diets [1, 55–61]. A higher SGR results in greater LWG and FW, subsequently contributing to the growth of TB in fish. Understanding these relationships helps fish farmers assess the growth performance of their fish stocks. The higher SGR and TB in 7% FPH fish indicate better growth rates and potentially more profitable and productive aquaculture operations. Nonetheless, growth performance and feed efficiency significantly declined with the high inclusion of FPH (9%), which could be attributed to AAs imbalance or the bitter taste of pellets, as stated in prior research [30, 32, 34, 39, 61]. Certainly, feed utilization parameters have a direct link to fish growth indices, as well-nourished feed boosts its palatability and digestibility, thereby enhancing feed efficiency and ultimately elevating the fish's overall growth. Furthermore, Wei et al. [60] and Fan et al. [61] reported no significant difference in fish survival rate with different levels of FPH supplementation, consistent with the current study findings.

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Reproductive performance of pabda, such as fecundity, egg fertilization rate, spawning response, and ovipositor diameter, were significantly higher when supplemented with 7% FPH in this study. Moreover, fish that consumed basal diet had significantly lower hatching rate and higher ovulation time as compared to FPH diet treatments. These findings indicate that FPH inclusion possibly facilitated nutrient utilization, highlighting the correlation between fish reproductive and somatic growth. In addition, healthy and well-conditioned fish are more likely to have higher fecundity, better egg quality, and increased hatching success. The existing literature concerning the influence of FPH on the fish's reproductive characteristics is limited, but several studies revealed that different levels of protein supplements in diets significantly improved the reproductive performance of *Hemibagrus nemurus* [62], *Pangasianodon hypophthalmus* [63], and *Pelteobagrus fulvidraco* [64]. In contrast, the GSI of experimental fish was not significantly affected by FPH levels. In summary, studies have shown consistent outcomes when aquaculture species were supplemented with hydrolyzed protein at varying levels [6, 43, 63–66].

Different FPH inclusion levels also significantly affected the fish tissues biochemical composition. Fish muscle possessed higher protein content than liver, intestine, and oocyte tissues in this study, which aligned with previous research conducted by Kabir et al. [43, 63, 66], Akter et al. [67], and Suma et al. [1]. Additionally, Pham et al. [39] discovered that Pompano, *Trachinotus blochii*, fed with tuna viscera hydrolysate diets, resulted in higher muscle protein than the rest of the body. Upon reaching maturity, protein mobilization occurs in the fish body from the oocytes, liver, and intestine to the muscle, thus increasing their muscle protein content compared to other tissues. Furthermore, lipid deposition was relatively lower in the muscle than in other fish tissues, a desirable outcome caused by elevated protein in the muscle that reduces fat level [68]. Moreover, high muscle composition is ideal for consumers, and the fish's biochemical composition is a vital indicator of food quality.

The nutritional composition of the liver and oocytes is a critical determinant of egg quality during the spawning season, as oocytes must fulfill the dietary requirements for larval and embryonic growth [69, 70]. Lipid and protein deposition in oocytes was notably higher than in the liver, indicating the transfer of protein and lipid from the liver to the oocytes during fish sexual maturation and gonadal development. Furthermore, the elevated lipid levels in oocytes serve as the primary energy source from the larval stage to maturity [71]. These findings were supported by Abidin et al. [72] and Kabir et al. [63, 66]. In essence, the biochemical composition of fish tissues is of interest for evaluating their nutritional value for human consumption.

Hematological parameters are essential indicators of the physiological state and general well-being of an aquaculture species [36, 73, 74]. In this study, the WBC and NEU levels were not influenced by different percentages of dietary FPH inclusion, indicating better health. When *O. pabda was* treated with 0%, 0.5%, 1%, and 2% FPH diets, there was an insignificant change in the WBC count but a notable difference in the NEU level [1]. Meanwhile, MON, EOS, and BAS levels significantly varied between treatment groups in this study, where the highest values were recorded by the 0% and 7% FPH diet groups. Monocytes are responsible for pathogen phagocytosis and assist in immune response, eosinophil modulates inflammation, and basophil contributes to allergic reactions in fish. Earlier studies also reported similar outcomes with various levels of FPH in *Arapaima gigas* [75], *Penaeus monodon* [76], and *O. pabda* [1].

At 7% FPH graded level, pabda demonstrated significantly higher RBC counts than other treatment groups, consistent with the findings of previous studies by Ebrahimnezhadarabi et al. [77], Nandi et al. [6], Rahman et al. [78], and Suma et al. [1]. The RBC is crucial for oxygen transport throughout the body, ensuring proper tissue function. In addition, earlier studies discovered that various levels of protein hydrolysate supplementation in fish diets affect the HGB, HCT, MCV, MCH, and MCHC levels, similar to the current study findings [75, 76, 78–80]. These are essential parameters in an aquaculture species as indicators of health and physiological status. The variations in hematological parameters observed in pabda are valuable indicators of their overall health and illustrate how nutrition impacts their physiological status.

The plasma biochemical analysis offers information regarding the metabolic and physiological interactions that occur within an organism. In the present study, different FPH levels substantially impacted the serum biochemical parameters of pabda, except for creatinine. The blood sugar level rose as the FPH levels increased, reaching their highest point at a 9% FPH level. Blood sugar is an important biomarker of contaminants that is solely responsible for stress in fish [3], indicating potential environmental stressors. Other studies have also reported similar findings. For instance, fingerlings of *Heteropneustes fossilis* fed with diets containing 0%, 1%, 3%, and 5% spirulina exhibited a consistent upward trend in GLU levels [78]. Meanwhile, *O. pabda* catfish subjected to FPH treatments ranging from 0% to 2% exhibited comparable trends [1].

Pabda broodstocks supplemented with 7% FPH also exhibited high TP, ALB, and GLOB levels, potentially attributed to free AAs, di- and tri-peptide molecules in FPH that facilitate digestion and nutrient absorption. Earlier reports have also revealed similar experimental outcomes [6, 37, 59, 78, 80, 81]. Elevated TP level reflects stronger innate immunity in an aquaculture species [82], while ALB and GLOB are indicators of fish health and function as carriers in the bloodstream [83]. Liver health indicators, SGPT and SGOT, were highest in the 9% FPH diet group, suggesting liver tissue damage [1, 84, 85]. Urea, cholesterol, alkaline phosphatase, and bilirubin were significantly

different between the treatment groups with no discernible pattern, consistent with Suma et al. [1], Chaklader et al. [37], and Adegbesan and Abdulraheem [86]. Hematological and serum biochemical indices are interrelated and collectively provide insights into the health, physiological condition, organ function, nutritional status, and stress response in fish.

Rašković et al. [87] and Lozano et al. [88] highlighted the importance of the liver in nutrient digestion, posing as a key indicator of a fish's nutritional status. Histological analysis of this study shows that 7% FPH in the pabda diet significantly enhanced their liver health. This improvement was characterized by increased nuclei and erythrocytes and reduced cytoplasmic vacuoles. These findings suggest improved nutrient digestion and absorption, enhanced cellular functions and protein synthesis, and reduced capacity for lipid or glycogen storage. Furthermore, the enhancements are potentially contributed by the good nutritional profile, anti-inflammatory properties associated with FPH, and optimal inclusion levels to achieve desirable outcomes in pabda culture. Suma et al. [1] previously reported that FPH dietary inclusion at 2% improved pabda liver health, indicated by enhanced nuclei and erythrocyte counts and the lack of vacuolar cytoplasm. Similarly, rainbow trout feed supplemented with 0.1% grape seed extract reduces the possibility of hepatic disorder by lowering the fat deposition within the organ, as demonstrated by Terzi et al. [89]. Nevertheless, high FPH inclusion (9%) resulted in enlarged liver cells in pabda, suggesting liver damage or hypertrophy as adverse effects of excessive FPH on fish health. This finding might be accredited due to elevated levels of SGPT and SGOT in serum of this fish group. SGPT and SGOT are liver enzymes and their elevated levels signal that the hepatic cells are undergoing damage or death, and thus the enzymes are being released into the bloodstream, causing their levels to rise. Likewise, Siddik et al. [35], Chaklader et al. [85], and Pham et al. [38] also reported similar outcomes in their studies.

Water quality parameters strongly influence fish growth and reproductive development [6, 43, 78, 90, 91]. In this research, the water quality indices were to maintain the optimal health of pabda, as recommended by Suma et al. [1], Bhatnagar and Singh [92], and Ekubo and Abowei [93]. These findings are potentially attributed to the experiment setting in the same water body. In aquaculture systems, several water quality parameters, including temperature, DO, pH, ammonia, nitrite, and nitrate, are crucial for maintaining a healthy environment for successful reproduction and spawning of broodfish.

The economic analysis of FPH incorporation at varying levels in pabda diets revealed accelerated fish growth, improved feed utilization, and significant enhancements in farm economic outcomes that were characterized by higher ROI and lower FFC compared to earlier report [1]. Overall, lowering feed costs without compromising the quality and productivity of farming operations is essential for improving return and ensuring the long-term sustainability of aquaculture practices.

In general, although the findings of this study provide valuable insights into the practical benefits of FPH inclusion in fish diets, further research specifically targeting molecular mechanisms through studies on gene expression, transcriptomics, metabolic analyses, and immunological investigations could offer a more comprehensive understanding of how FPH exerts its effects on *O. pabda* broodstock at a molecular level.

5. Conclusion

Incorporating FPH at different percentages as FM replacement in pabda diets enhanced their growth performance, reproductive outcomes, hemato-biochemical profiles, liver morphology, and farm economics. Precisely, dietary FPH at approximately 7% could benefit the growth and reproductive performance, health status, and cost-effectiveness in Pabda broodstock culture.

Data Availability

The data that supported the findings of this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflicts of interest.

Authors' Contributions

N.H.S. and S.K.N. contributed to original manuscript writing, formal analysis, and writing. A.Y.S. and M.S.H. contributed to writing—review and editing. Z.A.K., L.S.W., M.H., M.I.K., and P.S. contributed to writing—review and editing and funding. M.A.K. contributed to conceptualization, project administration, supervision, writing—review and editing, and funding.

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