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ORIGINAL ARTICLE

The Potential of Fish Protein Hydrolysate Supplementation in Nile Tilapia Diets: Effects on Growth and Health Performance, Disease Resistance, and Farm Economic Analysis

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Abstract

Fish protein hydrolysate (FPH) has shown immense potential as a dietary protein supplement and immunostimulant in aquaculture, especially in Nile tilapia production. Four isoproteic diets (30% crude protein) were prepared by including FPH at varying percentages (0%, 0.5%, 1%, and 2%). Nile tilapia fed with FPH diets for 90 days, and their growth performance, feed utilization, blood biochemistry, liver and gut morphology, and resistance against *Streptococcus iniae* were investigated. The findings revealed that diets physical attributes such as pellet durability index and water stability were remarkably ($p < 0.05$) varied between experimental diet groups. Furthermore, the test diets were more palatable when FPH was included at 1% and 2%. Fish that were fed with a 2% FPH-treated diet had significantly ($p < 0.05$) greater growth indices than other treatments. Additionally, their feed utilization was significantly ($p < 0.05$) improved. The experimental diets and intestinal total bacteria count (TBC) exhibited a rising trend with FPH levels, where the 2% FPH-treated diet recorded the highest TBC. Neutrophil (10^9/L), lymphocyte (10^9/L), eosinophil (10^9/L), and red blood cell(10^{12}/L) counts were significantly ($p < 0.05$) higher in the 2% FPH-treated group, while the white blood cell (10^9/L), and basophil (10^9/L) counts were not influenced by the FPH inclusion. Moreover, the FPH-treated groups displayed lower creatinine, bilirubin, and urea levels than the control. The histological examination demonstrated that themid-intestine of 2% FPH-fed Nile tilapia had an unbroken epithelial wall, more villi with frequent distribution of goblet cells, wider tunica muscularis, and stronger stratum compactum bonding than other treatments. Additionally, this group exhibited more nuclei and erythrocytes and less vacuolar cytoplasm in liver than their counterparts. Nile tilapia that were given a diet containing 2% FPH had significantly ($p < 0.05$) higher resistance (83.33%) to *S. iniae* during the bacterial challenge test. A significant ($p < 0.05$) enhancement in farm economic efficiency was observed in the higher inclusion of FPH in diets. In summary, 2% FPH supplementation in Nile tilapia diets improved their growth performance, feed utilization, health status, disease resistance, and farm economic efficiency.

Keywords Fish protein hydrolysate · Nile tilapia · Bacterial challenge · Growth performance · Health status · Sustainable aquaculture

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Introduction

Aquaculture is one of the world's most profitable and fast-growing industries that primarily relies on the compound feed, with the key determinant for successful operations being the provision of high-quality fish feed [1]. The feed cost of an aquaculture system accounts for 40 to 70% of the total cost [2–4], particularly protein sources [5, 6]. Currently, fish meal (FM) is regarded as the best animal-based protein for aquafeed. However, the continuous price hike and dwindling supply of this ingredient have forced industrial players to partially or fully replace FM with other protein sources [7, 8]. Furthermore, the recent decline in FM production has prompted researchers to look for alternatives from other animals and plants [9–18]. Protein sources from plant-based ingredients and animal by-products are promising due to their lower cost and abundance [5]. However, the reduced palatability of a plant-based fish feed due to the presence of anti-nutritional compounds, high crude fiber, and other harmful factors adversely impacted fish growth, nutrient intake, and health [18–20]. Therefore, supplementing fish feed with bioactive peptides and free amino acids from animal by-products is pivotal for remarkable improvement of diet palatability, feed utilization, and overall production performance of fish. The fish and seafood processing industries generate a variety of by-products, up to 60% of the total biomass [19–22]. Protease enzymes are useful in transforming these waste materials into valuable compounds, particularly cheaper protein sources such as fish protein hydrolysate (FPH) [23]. The FPH can serve as potential immunostimulant, feed attractant, and palatability enhancer in aqua feed due to the presence of free amino acids and small functional peptides [24, 25]. Small peptides in FPH have antioxidant, anticancer, antihypertensive, immunomodulatory, and antibacterial properties [26, 27].

Other advantage of FPH include excellent water-holding characteristics to improve feed palatability and nutritional intake [28, 29]. For instance, there were no significant adverse effects on *Oreochromis niloticus* feed efficiency and growth performance when fed diet supplemented with 6% protein hydrolysate [30]. Likewise, no adverse effects were observed in Mozambique tilapia growth performance, health status, and gut histology when fed diets supplemented with FPH [31]. Experimental diets containing FPH also reportedly influenced the growth, survival, and health of Wolffish, *Anarhichas minor* [32], Pabda, *Ompok pabda* [33] and Japanese eels, *Anguilla japonica* [34].

Nile tilapia is a commercially valuable aquaculture species owing to its superior growth performance, robustness, and disease tolerance [35–37]. This species also serves as a good research model due to its hardiness and abundance. Nonetheless, Nile tilapia culture encounters several challenges, including the lack of high-quality feed, an inefficient culture system, and disease prevalence [38, 39]. Thus, researchers are identifying bioactive compounds and immunostimulants derived from animal by-products, such as FPH, to address these issues and improve Nile tilapia growth, health, and immunity. Therefore, this study assessed the effects of FPH inclusion at different levels (0%, 0.5%, 1%, and 2%) in the *O. niloticus* diet, particularly their growth performance, feed efficiency, and costs, health status, and disease resistance.

Materials and Methods

Ethical Approval

The experiments were approved by the Animal Ethics Committee of Sylhet Agricultural University, and performed according to the Animal Ethics Procedures and Guidelines of the People's Republic of Bangladesh.

Feed Preparation

Four isoproteic diets (crude protein: 30%) with FPH inclusion at different levels (0%, 0.5%, 1%, and 2%) were prepared in this study, while the control diet was a basal diet with 0% FPH. Commercially available FPH from tuna viscera in liquid form (Symrise Aqua Feed, Specialities Pet Food (226-FR-SPF), France) was purchased and blended with other feed ingredients, including maize, rice polish, de-oiled rice bran, rapeseed, distillery dry grain soluble, soybean meal, full fay soya, corn germ meal, poultry meal, sardine fish oil, soya oil, vitamin and mineral premix and binder (g/100 g). After mixing for 45 min, the mixture was mechanically pelleted using an extruder (2 mm), followed by oven-drying at 80 °C overnight. Subsequently, the pellets were placed in air-tight zipper bags and stored at −20 °C until use. The feed formulation and proximate composition [40] for all experimental diets are detailed in Table 1.

Feeding Trial

A group of 1500 juvenile Nile tilapia (average weight: 4.09 ± 0.10 g) were acquired from a local hatchery and acclimated in a hapa (8 ft. length × 6 ft. width × 4 ft. depth) for seven days. During acclimatization, the fish were given a commercial feed (34% crude protein, 6% crude lipid) (ACI Godrej Agrovet Private Limited, Bangladesh) twice daily at 2% body weight. Subsequently, 1200 healthy fish were weighed individually and randomly divided into 12 cages (1 m length × 1 m width × 1.5 m height) at a stocking density of 100 fish/cage. The completely randomized block design comprised of four treatments with three biological replicates each. The feeding trial was conducted for 3 months, where the fish were fed twice daily (9 a.m. and 5 p.m.) *ad libitum.* Meanwhile, the hydrological variables of each cage water were measured weekly throughout the 90 days of experiment according to the standard method. Throughout the experiment, the water quality parameters were maintained at optimum levels including temperature (> 30 °C), water pressure (750.30 to 751.77 mm Hg), dissolved oxygen (4.98 to 5.46 mg/l), conductivity (> 60 S/m), TDS (> 27 mg/l), salinity (0.03 ppt), pH (around 7), ammonia and nitrite (below 0.1 mg/l), and nitrate (0.67 to 0.77 mg/l).

Feed Palatability and Physical Parameter Measurements

The feed palatability and physical parameterswere measured according to an earlier study by Zulhisyam, Kabir [41] with minor modifications. The calculations were as follows:

Table 1 Feed formulation and proximate composition (dry matter basis) of the experimental diets fed to Nile tilapia

Ingredients (g/100 g)	Diets (% FPH)			
	0	0.5	1	2
Maize	27.4	27.4	27.4	27.4
Rice polish	2.5	2.5	2.5	2.5
DORB[1]	9.5	9.5	9.5	9.5
Rapeseed	14.5	14.5	14.5	14.5
DDGS[2]	2.5	2.5	2.5	2.5
SBM[3]	25.0	25.0	25.0	25.0
Full fat soya	12.5	12.5	12.5	12.5
CGM[4]	2.5	2.5	2.5	2.5
Poultry meal	2.5	2.0	1.5	0.5
Sardine fish oil	0.3	0.3	0.3	0.3
Soya oil	0.2	0.2	0.2	0.2
Vitamin and mineral premix[5]	0.3	0.3	0.3	0.3
Binder	0.3	0.3	0.3	0.3
Fish protein hydrolysate[6]	0.0	0.5	1.0	2.0
Total	100	100	100	100
Feed raw material costs (US$/mT)	720.31	725.11	729.91	739.51
Proximate composition (g/100 g)				
Crude protein	30.09	30.29	30.45	30.81
Crude lipid	6.87	6.90	6.99	6.91
Crude ash	10.20	10.45	10.09	10.26
Moisture	10.21	10.09	10.13	10.29
NFE[7]	42.63	42.27	42.34	41.73

[1]*DORB*de-oiled rice bran

[2]*DDGS*distillery dry grain soluble

[3]*SBM*soybean meal

[4]*CGM*corn germ meal

[5]g/kg premix: Vitamin C, KCL, 90; KI, 0.04; $CaHPO_4.2H_2O$, 500; NaCl, 40; $CuSO_4.5H_2O$, 3; $ZnSO_4.7H_2O$, 4; CoO_4, 0.02; $FeSO_4.7H_2O$, 20; $MnSO_4.H_2O$, 3: $CaCo_3$, 215; MgOH, 124: Na_2SeO_3, 0.03; NaF,1; Brand: Bar-Magen

[6]Fish protein hydrolysate: Crude protein, 78.95%; crude lipid, 2.01%; crude ash, 3.13%; moisture, 4.49%

[7]*NFE*Nitrogen free extract

Feed raw material costs (US$/MT): The currency of Taxa- US last update on late December2022

i. Pellet durability index, PDI (%) = (Weight of feed particles remaining on the sieve/ Initial weight of feed particles before being tumbled) × 100
ii. Water stability (%) = (Weight of retained whole feed particles/Initial total weight of feed particles) × 100
iii. Floatability (%) = (Average numbers of floating feed/Average initial numbers of feed) × 100

Calculation of Growth Performance

After concluding the feeding trial, all the experimental fish were fasted for 24 h before being euthanized with MS_{222} to determine individual cage containing fish total biomass. From the group, the Nile tilapia was then randomly selected (25 fishes/cage) and transported to the laboratory for measuring their final weight and total length. The fish were weighed and dissected to remove the viscera, liver, and fat. Each growth parameter was calculated by using the following formulae [33]:

i. Survival rate (%) = (Number of fish survival/Total fish numbers at the beginning of the experiment) × 100
ii. Water stability (%) = (Weight of retained whole feed particles/Initial total weight of feed particles) × 100
iii. Specific growth rate, SGR (%/day) = [(ln (final weight) – ln (Initial weight))/(Days of an experiment)] × 100
iv. Total biomass (TB) gain (Kg) = (Final biomass weight - Initial Biomass weight)
v. Total yield (kg/m^2) = TB gain/cage area
vi. Feed conversion ratio, FCR = Total feed intake/Wet weight gain
vii. Protein efficiency ratio, PER = Live weight gain/Crude protein fed
viii. Hepatosomatic index, HSI (%) = (Weight of liver/Final weight) × 100
ix. Visceral somatic index, VSI (%) = (Weight of viscera/Final weight) × 100
x. Intraperitoneal fat, IPF (%) = (Weight of fat/Final weight) × 100
xi. Condition factor, CF = [Final weight (g)/(Fish total length, $cm)^3$] × 100

Biochemical Composition Analysis

The proximate composition of experimental diets, intestines, livers, and muscle tissues in triplicates were determined according to AOAC [40] method with minor modifications. Shortly, the moisture content was evaluated by oven-drying the feed samples at 105 °C for 24 h, ash by incinerating the diets in a Muffle furnace at 550 °C for 6 h, ether extraction of crude lipid using the Soxhlet apparatus, and Kjeldahl method for crude protein determination (%N × 6.25).

Determination of Total Bacteria in FPH-Included Feed and Fish Intestine

The feed samples (1 g) were first homogenized in 9 ml of sterile saline, followed by serial dilution to 10^{-9}. Subsequently, each sample suspension was pipetted onto the Tryptic Soy Agar (TSA, HiMedia, India). After 48 h of incubation at 37 °C, the visible colonies were counted and quantified as CFU/g (feed or gut) to determine the total bacteria in the feed and intestine samples.

Biochemical Indices and Haematological Assessments

Fish haematology and biochemical parameters were measured following a previous study [42] with minor modifications. First, the fish were sampled randomly (3 fishes/cage), transferred into separate tanks, and fasted for five hours. After anaesthesia, approximately 150

µl of blood was drawn from each treatment fish caudal puncture using 2 ml heparinized syringes and placed in tripotassium ethylenediaminetetraacetic acid ($EDTAK_3$) tubes to prevent coagulation. The blood parameters were then examined using an automatic haematology analyzer (Mythic 18 Vet, USA). Meanwhile, another 400 µl blood samples were centrifuged (3000 rpm, 15 min) to collect the plasma and stored at −20 °C until further analysis. The plasma samples (150 µl) were pipetted into cassettes containing reagents for each biochemical test (IDEXX, USA) and automatically evaluated using the VetTest analyzer (IDEXX, USA). Finally, the globulin content of all samples was obtained by subtracting albumin from the total plasma protein.

Liver and Mid-intestine Histomorphology

The fish were randomly sampled from each treatment group (9 fish/group) and anaesthetized with MS_{222}at the end of the feeding trial. Their liver and intestine were extracted and preserved in 10% neutral-buffered formalin before being subjected to graded ethanol concentrations (dehydration), wiped in xylene, and embedded in paraffin wax. The paraffin blocks were later sectioned transversely (5–8 µm), mounted on glass slides, and oven-dried at 40 °C overnight, followed by Haematoxyline and Eosin staining. Finally, the histopathological investigation was performed using a light microscope (Leica DMIL-LED, Germany).

S. iniae Infection

Collection and Maintenance of *S. iniae*

Streptococcus iniae was obtained from the Laboratory of Fish Diseases Diagnosis and Pharmacology, Department of Fish Health Management, Sylhet Agricultural University. The subcultures were maintained in nutrient agar slants at 4 °C and later inoculated in Himedia tryptone soy broth (TSB). The culture was kept overnight in an incubator shaker at 37 °C and removed when an optical density (OD) of 0.8 was achieved at 600 nm. Finally, the stock culture was transferred into 1.5% TSB with 20% glyceroland stored at −20°Cfor the next experiment.

S. iniae LD_{50}

The mean lethal dose (LD_{50}) for *O. niloticus* was estimated based on a previous study [43]. Nile tilapia fish were placed in aquaria tanks ($73 \times 35 \times 38$ cm^3), each filled with 70 L water (10 fish/aquarium) with proper aeration, and the experiment was performed in triplicates. Isolates of *S. iniae* were cultured in TSB overnight at 37 °C before the cell suspensions were prepared in phosphate-buffered saline (PBS). Each fingerling in the FPH diet and positive control groups was injected intraperitoneally with 0.1 ml of *S. iniae* (10^4 to 10^9 CFU/ml). Meanwhile, the negative control group was injected with PBS (0.1 ml). The mortality rate was counted daily for ten days, aiming to identify the optimum dosage for challenge study.

Challenge Test

At the end of feeding trial, the fish were sampled randomly from each group (30 fishes/group), including the positive control (0.5% FPH), and then challenged with *S. iniae* (LD_{50}−3.1×10^8 CFU/ml) via intramuscular injection. The negative control (0% FPH) group received an injection of 0.1 ml PBS. Throughout a 10-day bacterial challenge, the fish were inspected three times daily at morning (7:00 a.m.), afternoon (3:00 p.m.), and night (10:00 p.m.) to detect any indications of infection. The numbers of infected fish was noted each day and excluded to calculate the percentage of survival.

Farm Economic Analysis

The cost of raw materials used for each feed formulation in this study was calculated by summing up the prices of ingredients. Subsequently, the farm feed costs (FFC) were determined per unit of produced biomass as the following:

i. FFC (US$/kg) = FCR × raw material cost for the respective diets
ii. Farm revenues (FR) were calculated on an expected farm gate price of US$ 1.582/kg of tilapia: FR (US$/$m^2$) = Total Yield × 1.582
iii. Farm raw margins, FRM (US$/$m^2$): FR – (Total Yield × FFC)
iv. Return on Investments (ROI) (%) = 100 × FPRM/(Total Yield × FFC)

Statistical Analysis

All data collected in this study were first tested for normality. Subsequently, a one-way analysis of variance (ANOVA) was performed using Statistical Package for the Social Sciences (SPSS) version 20.1 (IBM, USA) to determine whether there were significant differences between the control and treatments for all parameters. The data were analyzed by Duncan's testto determine if variance homogeneity could be met. Otherwise, Tamhane's T2 test was employed as the subsequent analysis. The significance level was set at $p < 0.05$ and the results were expressed as mean ± standard deviation (SD).

Results

Physical Characteristics and Palatability of Experimental Diets

The physical characteristics and palatability of each experimental diet are detailed in Table 2. The experimental diet groups showed significant differences ($p < 0.05$) in PDI and water stability. In addition, the PDI of the 2% FPH diet was the lowest but demonstrated significantly higher water stability ($p < 0.05$) than other groups. In contrast, the floatability of diets did not differ significantly ($p > 0.05$) among the treatments. The palatability test of experimental diets demonstrated that the 0% and 0.5% FPH diet groups

Table 2 Physical properties and palatability test of FPH diets

Parameters	Diets (% FPH)			
	0	0.5	1	2
Feed diameter (mm)	1.99 ± 0.03	2.00 ± 0.03	2.03 ± 0.05	2.01 ± 0.02
PDI (%)	99.90 ± 0.05^{ab}	99.93 ± 0.02^{a}	99.84 ± 0.03^{bc}	99.83 ± 0.02^{c}
Floatability (%)	100.00 ± 0.00	99.67 ± 0.58	99.67 ± 0.58	99.67 ± 0.58
Water stability (%)	79.93 ± 0.47^{b}	80.00 ± 0.18^{b}	80.17 ± 0.15^{b}	80.84 ± 0.23^{a}
Palatability	+++	+++	++++	++++

Abbreviation: *PDI* pellet durability index

Results were expressed as mean ± SD. Mean values with various superscript letters represent statistical significance ($p < 0.05$)

+:<25% feed consumption within 5 min, ++: <50% feed consumption within 5 min, +++: <75% feed consumption within 5 min, ++++:<100% feed consumption within 5 min

Table 3 Growth performance and feed utilization parameters of Nile tilapia fed with four experimental diets for 90 days

Parameters	Diets (% FPH)			
	0	0.5	1	2
IW (g)	5.02 ± 0.02	5.04 ± 0.01	5.02 ± 0.03	5.01 ± 0.01
FW (g)	113.15 ± 4.29^{c}	122.63 ± 3.29^{c}	176.51 ± 5.07^{b}	210.4 ± 9.49^{a}
WG (%)	2156.18 ± 82.55^{c}	2336.12 ± 67.54^{c}	3416.54 ± 98.7^{b}	4099.88 ± 197.22^{a}
SGR (%/ day)	3.46 ± 0.04^{d}	3.55 ± 0.03^{c}	3.96 ± 0.03^{b}	4.15 ± 0.05^{a}
TB (g)	10813.57 ± 427.66^{c}	11759.8 ± 329.2^{c}	17148.87 ± 507.08^{b}	20539.13 ± 949.7^{a}
SR (%)	92.67 ± 1.15	95 ± 1.73	96.33 ± 2.89	95.67 ± 2.08
FCR	1.54 ± 0.08^{a}	1.42 ± 0.01^{b}	0.97 ± 0.02^{c}	0.81 ± 0.04^{d}
PER	2.01 ± 0.07^{d}	2.46 ± 0.03^{c}	3.36 ± 0.06^{b}	3.85 ± 0.19^{a}
CF (%)	2.13 ± 0.15^{ab}	2.06 ± 0.1^{b}	2.08 ± 0.15^{b}	2.35 ± 0.06^{a}
HSI (%)	2.47 ± 0.34	3.05 ± 0.55	2.67 ± 0.5	2.33 ± 0.03
IPF (%)	2.23 ± 0.45^{b}	3.12 ± 0.31^{ab}	3.93 ± 0.78^{a}	4.04 ± 0.54^{a}
VSI (%)	2.64 ± 0.36	2.36 ± 0.26	2.49 ± 0.44	2.65 ± 0.33

Abbreviation: *IW* initial weight, *FW* final weight, *WG* weight gain, *SGR* specific growth rate, *TB* total biomass, *SR* survival rate, *FCR* feed conversion ratio, *FCE* feed conversion efficiency, *PER* protein efficiency ratio, *CF* condition factor, *HSI* hepatosomatic index, *IPF* intraperitoneal fat, *VSI* visceral somatic index

Results are expressed as mean ± SD. Mean values with various superscript letters represent statistical significance ($p < 0.05$)

consumed < 75% of the feed within 5 min. Meanwhile, the 1% and 2% FPH diet groups recorded higher consumption at < 100% within the same period.

Growth Performance and Feed Utilization

Table 3 exhibits the growth and feed utilization performance of the different treatments. Final weight (FW), weight gain (WG), specific growth rate (SGR), total biomass (TB), condition factor (CF), and intraperitoneal fat (IPF) were significantly different ($p < 0.05$)

Table 4 Biochemical composition (% wet weight basis) of intestine, liver, and fish body muscle after supplemented with varying degrees of FPH.

Parameters	Diets (% FPH)			
	0	0.5	1	2
Intestine				
Protein	13.94 ± 0.05^{c}	14.08 ± 0.16^{bc}	14.64 ± 0.44^{b}	15.36 ± 0.44^{a}
Lipid	5.37 ± 0.53^{b}	5.47 ± 0.39^{ab}	6.00 ± 0.09^{ab}	6.08 ± 0.11^{a}
Ash	2.04 ± 0.07^{b}	2.24 ± 0.19^{ab}	2.16 ± 0.29^{ab}	2.62 ± 0.32^{a}
Moisture	77.12 ± 0.86^{a}	76.74 ± 0.92^{a}	75.79 ± 0.63^{ab}	74.74 ± 0.60^{b}
Liver				
Protein	12.57 ± 0.49^{c}	12.88 ± 0.09^{c}	13.73 ± 0.60^{b}	14.83 ± 0.03^{a}
Lipid	9.19 ± 0.07^{c}	10.24 ± 0.50^{b}	10.41 ± 0.55^{ab}	11.20 ± 0.56^{a}
Ash	2.25 ± 0.26^{ab}	2.19 ± 0.17^{b}	2.56 ± 0.22^{ab}	2.60 ± 0.18^{a}
Moisture	73.77 ± 0.42^{a}	73.14 ± 1.00^{a}	71.65 ± 0.75^{b}	69.83 ± 0.85^{c}
Muscle				
Protein	20.43 ± 0.73^{b}	21.59 ± 0.53^{ab}	21.24 ± 0.53^{ab}	22.13 ± 0.89^{a}
Lipid	4.89 ± 0.04^{b}	4.59 ± 0.03^{c}	5.09 ± 0.05^{a}	4.96 ± 0.06^{b}
Ash	2.00 ± 0.02^{a}	2.08 ± 0.07^{a}	2.07 ± 0.12^{a}	2.17 ± 0.28^{a}
Moisture	71.42 ± 0.59^{a}	70.31 ± 0.26^{ab}	69.26 ± 0.75^{b}	68.73 ± 1.51^{b}

Results are expressed as mean ± SD. Mean values with various superscript letters represent statistical significance ($p < 0.05$)

for all treatments. Similarly, feed conversion ratio (FCR) and protein efficiency ratio (PER) differed significantly ($p < 0.05$) between the experimental groups. There was an increasing trend observed in fish FW, WG, SGR, TB, PER, and IPF ($p < 0.05$) with increasing levels of FPH dietary inclusion. Furthermore, the 2% FPH group had a significantly lower FCR ($p < 0.05$) than others.

Biochemical Composition of Intestine, Liver, and Muscle

The biochemical profiles of all treatments are noted in Table 4. There were significant differences ($p < 0.05$) in protein, lipid, and moisture content across all examined organs with varying degrees of FPH supplementation. The protein level was highest in the fish muscle (20.43 ± 0.73 to $22.13 \pm 0.89\%$), followed by the gut (13.94 ± 0.05 to $15.36 \pm 0.44\%$) and liver (12.57 ± 0.49 to $14.83 \pm 0.03\%$). In contrast, the lipid depositions in the gut and liver were substantially greater ($p < 0.05$) in the 2% FPH group compared to other treatments.

Table 5 Total bacterial counts (TBC) in Nile tilapia diets and intestine

Parameters	Diets (% FPH)			
	0	0.5	1	2
TBC (CFU/g feed) × 10^6	4.03 ± 0.15^{d}	5.13 ± 0.21^{c}	8.7 ± 0.20^{b}	10.13 ± 0.15^{a}
TBC (CFU/g intestine) × 10^7	9.00 ± 0.10^{d}	11.90 ± 0.10^{c}	13.07 ± 0.21^{b}	15.53 ± 0.57^{a}

Results are expressed as mean ± SD. Mean values with various superscript letters represent statistical significance ($p < 0.05$)

Furthermore, the muscle lipid content varied significantly ($p < 0.05$) between the groups without any apparent trend. The moisture levels within each tissue exhibited a decreasing pattern with FPH inclusion. Meanwhile, the ash content in the gut and liver varied significantly between the treatments.

Experimental Diet and Fish Gut Total Bacterial Counts (TBC)

Table 5 shows the TBC for the FPH-included experimental diets and Nile tilapia gut in all treatments. There was a significant increasing trend in TBC of the experimental diets and fish intestine ($p < 0.05$) with increasing dietary FPH inclusion, with the highest TBC detected in 2% FPH group. Conversely, the control diet and fish gut recorded the lowest TBC values.

Blood Haematology of Experimental Fish

Table 6 presents the haematological parameters of Nile tilapia. The 2% FPH group recorded the highest NEU, LYM, EOS, and RBC contents compared to other treatment

Table 6 Haematological parameters ofNile tilapia that were fed with diets containing different FPH levels

Parameters	Diets (% FPH)			
	0	0.5	1	2
WBC (10^9/L)	542. 46 ± 0.06	542.49 ± 0.08	542.53 ± 0.01	542.48 ± 0.06
NEU (10^9/L)	301.25 ± 21.43^{b}	250.25 ± 16.55^{c}	337.96 ± 6.67^{a}	355.02 ± 26.33^{a}
LYM (10^9/L)	186.53 ± 7.45^{c}	167.71 ± 9.82^{d}	213.83 ± 4.21^{b}	249.57 ± 29.39^{a}
MON (10^9/L)	23.87 ± 3.03^{a}	20.57 ± 0.48^{ab}	13.66 ± 2.18^{c}	18.05 ± 0.99^{b}
EOS (10^9/L)	4.05 ± 0.27^{a}	1.44 ± 0.26^{b}	1.59 ± 0.08^{b}	3.98 ± 0.82^{a}
BAS (10^9/L)	16.66 ± 0.86	17.43 ± 1.55	17.29 ± 1.47	15.96 ± 1.9
RBC (10^{12}/L)	1.52 ± 0.09^{a}	0.63 ± 0.02^{b}	1.36 ± 0.30^{a}	1.42 ± 0.15^{a}
HGB (g/L)	152.67 ± 9.29^{a}	109.67 ± 7.09^{bc}	123.00 ± 13.53^{b}	100.67 ± 1.53^{c}
HCT (%)	0.31 ± 0.01^{b}	0.37 ± 0.00^{a}	0.24 ± 0.01^{c}	0.13 ± 0.00^{d}
MCV (fL)	201.13 ± 10.05^{a}	168.43 ± 25.57^{b}	193.83 ± 13.90^{ab}	207.13 ± 11.82^{a}
MCH (pg)	77.07 ± 2.20^{b}	76.87 ± 4.85^{b}	92.27 ± 12.21^{a}	91.07 ± 7.49^{ab}
MCHC (g/L)	381.33 ± 11.59^{b}	373.33 ± 24.83^{b}	458.33 ± 13.05^{a}	430.33 ± 5.86^{a}
RDW-CV (fL)	0.16 ± 0.01^{a}	0.13 ± 0.01^{b}	0.16 ± 0.01^{a}	0.17 ± 0.02^{a}
RDW-SD (fL)	154.23 ± 9.73^{ab}	133.50 ± 27.74^{b}	151.47 ± 5.67^{ab}	174.07 ± 23.36^{a}
PLT (10^9/L)	458.67 ± 16.77^{c}	544.33 ± 11.02^{b}	758.67 ± 5.86^{a}	491.00 ± 46.52^{c}
MPV (fL)	7.63 ± 0.15	7.77 ± 0.15	8.00 ± 0.26	7.60 ± 0.30
PDW (%)	17.70 ± 0.17^{b}	18.03 ± 0.15^{a}	17.90 ± 0.20^{ab}	17.70 ± 0.10^{b}
PCT (ml/L)	3.60 ± 0.20^{b}	4.06 ± 0.12^{b}	6.00 ± 0.13^{a}	3.69 ± 0.46^{b}

Abbreviation: *WBC* white blood cell, *NEU* neutrophil, *LYM* lymphocytosis, *MON* monocytes, *EOS* eosinophil, *BAS* basophil, *RBC* red blood cell, *HGB* hemoglobin, *HCT* hematocrit, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *RDW-CV* red cell distribution width-coefficient of variation, *RDW-SD* red cell distribution width-standard deviation, *PLT* platelet, *MPV* mean platelet volume, *PDW* platelet distribution width, *PCT* procalcitonin. Results are expressed as mean ± SD. Mean values with various superscript letters represent statistical significance ($p < 0.05$)

groups. Furthermore, the numerical mean values of haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width-coefficient of variation (RDW-CV), red cell distribution width-coefficient of variation(RDW-SD), platelet (PLT), platelet distribution width (PDW), and procalcitonin (PCT) were significantly ($p < 0.05$) influenced by graded supplementation of dietary FPH, but no particular trend was observed in this study. Nonetheless, the HGB mean value was significantly higher ($p < 0.05$) in the control diet compared to the other treatments.

Plasma Biochemistry of Nile Tilapia Fish

Table 7 demonstrates the plasma biochemical indices for all treatments. There were significant differences ($p < 0.05$) across all groups for the major biochemical parameters of blood (blood glucose, creatinine, bilirubin, serum glutamic pyruvic transaminase (SGPT), urea, serum glutamic oxaloacetic transaminase (SGOT), albumin, alkaline phosphatase, cholesterol, total protein, and globulin) without following any definite trends. The control group had significantly lower ($p < 0.05$) blood glucose, cholesterol, and albumin, while concurrently demonstrating higher levels of SGPT, SGOT, total protein, and globulin as compared to other treatments.

Nile Tilapia Mid-intestine and Liver Histopathology

Figure 1 illustrates the histomorphology of the Nile tilapia mid-intestine that exhibited significant changes in terms of lamina propria, lamina epithelial mucosae, stratum compactum, goblet cells, and tunica muscularis with various percentages of FPH. The fish gut of the 2% FPH group demonstrated an intact epithelial wall and more villi with a high

Table 7 Serum biochemical parameters of Nile tilapia supplemented with different treatment diets

Parameters	Diets (% FPH)			
	0	0.5	1	2
Blood Glucose (mg/dl)	76.00 ± 3.61^{c}	131.33 ± 3.21^{a}	104.00 ± 3.61^{b}	101.00 ± 3.00^{b}
Creatinine (mg/dl)	0.95 ± 0.05^{a}	0.79 ± 0.02^{b}	0.70 ± 0.10^{b}	1.03 ± 0.06^{a}
Bilirubin (mg/dl)	1.08 ± 0.07^{a}	0.98 ± 0.10^{ab}	0.78 ± 0.09^{c}	0.93 ± 0.02^{b}
SGPT (u/l)	46.67 ± 1.53^{a}	18.33 ± 1.53^{c}	30.67 ± 2.08^{b}	34.67 ± 3.51^{b}
Urea (mg/dl)	36.00 ± 1.00^{a}	26.00 ± 2.65^{b}	18.33 ± 3.06^{c}	36.00 ± 3.61^{a}
SGOT (u/l)	50.67 ± 2.52^{a}	22.00 ± 3.00^{c}	38.00 ± 2.65^{b}	39.67 ± 1.53^{b}
Albumin (u/l)	4.10 ± 0.26^{b}	5.07 ± 0.21^{a}	4.50 ± 0.36^{b}	5.07 ± 0.21^{a}
ALKP (u/l)	105.67 ± 6.66^{c}	496.67 ± 40.43^{a}	213.33 ± 20.82^{b}	144.00 ± 3.61^{c}
Cholesterol (mg/dl)	211.67 ± 11.37^{d}	284.00 ± 3.61^{b}	257.33 ± 5.51^{c}	366.33 ± 10.12^{a}
Total protein (g/dl)	9.50 ± 0.20^{a}	8.33 ± 0.35^{c}	8.57 ± 0.21^{bc}	9.00 ± 0.10^{b}
Globulin (g/dl)	4.27 ± 0.25^{a}	3.57 ± 0.15^{c}	3.97 ± 0.21^{ab}	3.70 ± 0.10^{bc}

Abbreviation: *SGPT* serum glutamic pyruvic transaminase, *SGOT* serum glutamic oxaloacetic transaminase, *ALKP* alkaline phosphatase

Results are expressed as mean ± SD. Mean values with various superscript letters represent statistical significance ($p < 0.05$)

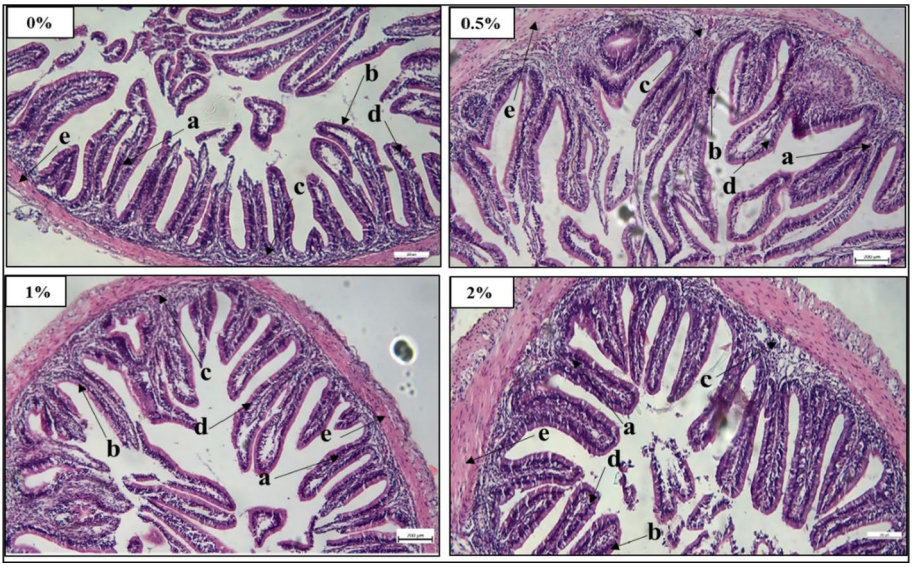

Fig. 1 Histological images of Nile tilapia midgut with different levels of FPH inclusion (0%, 0.5%, 1%, and 2%) under light microscopy (Olympus BX43). The histopathological investigation was performed on the a) lamina propria, b) lamina epithelial mucosae, c) stratum compactum, d) goblet cells, and e) tunica muscularis of all treatments. Magnification: ×10. Scale bar: 200 µm

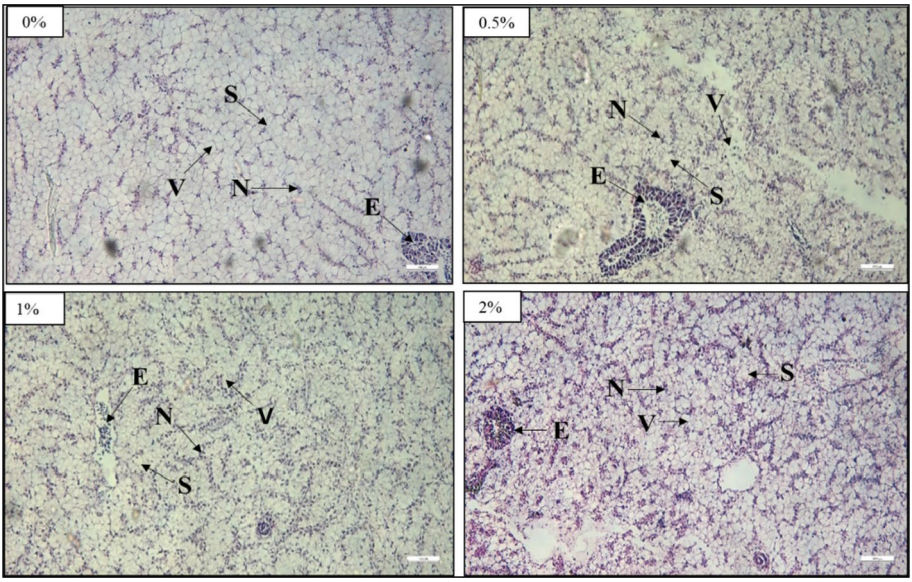

Fig. 2 Histomorphological observation of Nile tilapia liver through various ratios of FPH diets (0%, 0.5%, 1%, and 2%) under light microscopy (Olympus BX43). The liver health of fishes in all treatments was determined based on the morphological alterations in the nucleus (N), sinusoid (S), erythrocytes (E), and vacuole (V). Magnification: ×10. Scale bar: 200 µm

distribution of goblet cells, wider tunica muscularis, and stronger stratum compactum bonding than other groups.

Figure 2 depicts the morphological investigation of *O. niloticus* liver cells. The fish liver experienced substantial changes when fed with FPH diets at different inclusion levels, including alterations in the nucleus, vacuoles, erythrocytes, and sinusoid structures. The number of nuclei and erythrocytes increased, but the vacuoles reduced with increasing FPH levels, particularly in the 2% FPH group.

S. iniae LD_{50} and Challenge

The mean lethal dose (LD_{50}) estimated in *O. niloticus*, according to Reed and Muench [43], was 3.1×10^8 CFU/ml. Kaplan Meyer's analysis revealed substantial differences in percent survival when Nile tilapia fed with graded levels of FPH-containing diets (Fig. 3). The survival of Nile tilapia after being challenged with *S. iniae* was noted in 2% FPH (83.33%), 1% FPH (80%), 0.5% FPH (70%), and 0% FPH (10%). Nevertheless, the survival of fish was not varied significantly ($p > 0.05$) between 1 and 2% FPH group.

Farm Economic Analysis

Table 8 demonstrates that the graded dietary supplementations of FPH improved the economics of Nile tilapia culture by significantly increasing TY and FR(US$/$m^2$) and reducing FFC (US$/kg). Resultantly, FRM (US$/$m^2$) was increased four-fold at 1% dietary FPH supplementation, and the ROI was enhanced almost five-fold at 2% FPH supplementation.

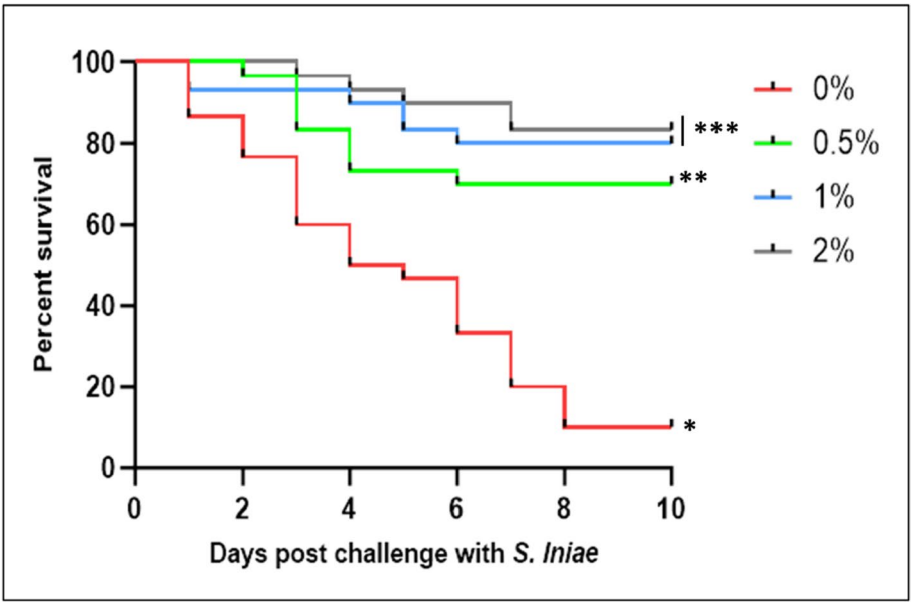

Fig. 3 Kaplan Meyer's percent survival analysis of Nile tilapia upon challenged with *S. iniae* for 10 days. The survival curve shows the outcomes of the *S. iniae* challenge test, with a sample size of $n = 30$ for each treatment. Asterisks *, **, and *** denote significant differences among the various treatments ($p < 0.05$)

Table 8 Farm economic analysis resulting from Nile tilapia feeding trial after 90 days

Parameters	Diets (% FPH)			
	0	0.5	1	2
TY (Kg/m^2)	10.0 ± 0.2^{d}	11.1 ± 0.2^{c}	16.5 ± 0.6^{b}	19.6 ± 0.7^{a}
FFC (US$/kg)	1.23 ± 0.04^{a}	1.11 ± 0.02^{b}	0.76 ± 0.02^{c}	0.65 ± 0.02^{d}
FR (US$/m^2)	15.79 ± 0.35^{d}	17.63 ± 0.26^{c}	26.12 ± 0.88^{b}	31.06 ± 1.08^{a}
FRM (US$/m^2)	3.50 ± 0.45^{d}	5.22 ± 0.19^{c}	13.60 ± 0.81^{b}	18.32 ± 1.01^{a}
ROI (%)	28.6 ± 4.1^{d}	42.2 ± 2.0^{c}	108.6 ± 5.9^{b}	143.7 ± 7.4^{a}

Abbreviation: *TY* total yield, *FFC* farm feed costs, *FR* farm revenue, *FRM* farm raw margin, *ROI* return on investment. Mean values with various superscript letters represent statistical significance ($p < 0.05$)

Discussion

Fish protein hydrolysate is a highly promising animal-based protein supplement for aqua-feed formulation, which can significantly enhance total fish productivity and health status when used optimally. However, finding the suitable levels of FPH inclusion in diet is vital for achieving sustainable and robust aquaculture growth while minimizing feed cost and ensuring a consistent supply of high-quality fish to satisfy the global protein demand for consumers. Consequently, experimental diets physical, biochemical, and microbiological characteristics and Nile tilapia (*O. niloticus*) growth indices, gut microbiota, health status, and disease resistance against *S. iniae* were examined in this study to gain deeper understanding of this study.

The development and commercialization of any aqua-feed greatly depend on the experimental diets physical characteristics. Pellets with outstanding physical properties ease handling, transportation, feeding, and storage. There were no significant changes in the floatability of the experimental diets in this study attributed to the relatively uniform size of the feed particles. Furthermore, dietary FPH inclusion at different levels minimally impacted the PDI and water stability, which aligned with the findings by Khater, Bahnasawy [44] and Zulhisyam, Kabir [41]. Several studies [41, 44–46] reported that water stability of pellets increased with feed dimensions, while the opposite effect was observed in PDI and floatability [41, 44]. These reports show pellet physical parameters are closely linked to feed diameter. FPH possesses various physical attributes, including excellent solubility, foaming, emulsifying, lipid binding, and water-holding characteristics [28, 29]. The slight variations noted in the physical attributes of the feed could be accredited to these distinct features of FPH.

This study also found that dietary FPH inclusion at 1% and 2% was more palatable to Nile tilapia than control and 0.5% FPH diets. Previously, FPH reportedly improved feed palatability and nutritional absorption in aquaculture species due to the superior chemical properties, including free amino acids, small peptides, and other low molecular weight nitrogenous compounds [28, 29]. Moreover, previous studies reported comparable results in the Asian seabass (*Lates calcarifer*) diet that included 3% tuna viscera hydrolysate [47], 2% fish protein hydrolysate in Striped catfish (*Pangasianodon hypophthalmus*) diet [48] ,and 2% FPH in Pabda (*O. pabda*) diet [33]. In this study, increasing dietary FPH inclusion (0–2%) improved the FCR, FCE, and PER. Additionally, the 2% FPH group exhibited the highest weight gain, SGR, TB, and condition factor. These outcomes indicate that 2% FPH inclusion in diet can significantly promote the growth performance and feed efficiency

compared to other treatments. According to Siddik, Howieson [23], dietary FPH inclusion at recommended levels is a good source of protein, peptides, and amino acids and enhances antioxidant qualities. Large molecules might break down into small peptides and free amino acids via protein hydrolysis, leading to improved diet palatability and digestibility, and thereby impacting fish growth and feed intake. These study findings are consistent with many previous literatures, when fed fish with different levels of FPH [49–58]. Conversely, reduced growth and feed utlization in control fish might be attributed to the decreased bioavailability of free amino acids and peptide molucules.

The Nile tilapia survival rate, HSI, and VSI in this study were not significantly influenced by the dietary FPH, which were consistent with earlier studies in Nile tilapia, *O. niloticus* [59], Barramundi, *Lates calcarifer* [57] and Pompano, *Trachinotus blochii* [60, 61]. Generally, HSI is influenced by glycogen content and fat deposition in liver tissue [62, 63]. The greater IPF score in the 2% FPH treated fish denotes a high-fat deposition in the fish body, enhancing their palatability for consumers.

Graded dietary FPH supplementation significantly affected the biochemical composition of the Nile tilapia gut, liver, and muscle tissues. Protein levels were much higher in the fish muscle than in the gut and liver, which aligned with previous studies [5, 64, 65]. Fish muscle has high and consistent protein content due to the protein mobilization from the liver and gut upon maturation. Protein concentrations in all tissue organs increased with dietary FPH inclusion. These results indicated that FPH may provide essential constituents for protein synthesis, potentially augmenting the overall protein levels in all tissues. Furthermore, lipid accumulation was higher in the liver and lower in the muscle tissues, consistent with Dawood, Koshio [66]. It was explained that lower fat content could increase lipase activity and intestinal morphology, improving fat digestion. Conversely, excessive fat deposition in the liver may lead to hepatic disorders.

The dietary FPH inclusion in this study significantly affected the TB counts in Nile tilapia diets and intestines. The lowest TBC was recorded in the control diet, while TBC was higher in FPH-supplemented diets. This finding was similar to Zulhisyam, Kabir [41], who used probiotic supplements for African catfish diets as coatings. The fish intestinal total bacterial loads also exhibited an upward trend with dietary FPH inclusion. Kotzamanis, Gisbert [67] noted that FPH serves as a medium for bacterial growth, consequently influencing the bacterial numbers. The present outcomes are aligned with an earlier study that incorporated canola protein hydrolysate in Beluga (*Huso huso*) diets [68] and FPH in Pabda (*O. pabda*) diets [33]. Moreover, fish diets containing fermented soy pulp up to 50% demonstrated high bacterial loads in their intestines [5]. Microbiota dominance in fish guts at recommended levels could be essential in nutrient digestion and assimilation, disease resistance, and growth performance.

Haematological indices are vital physical indicators that reflect the overall health and nutritional state of an aquaculture species [55, 69]. In this study, the experimental diets did not influence the WBC counts of Nile tilapia. When Pabda catfish treated with different degrees of FPH showed corresponding result [33]. The 2% FPH fed fish had the remarkably highest RBC contents compared to other treatment fish, which is identical with other reports [68]. The WBC and RBC counts are essential to protect fish against infection and carry oxygen respectively. Furthermore, the 2% FPH group recorded higher NEU, LYM, and EOS levels, indicating an enhancement in the fish immune system. Different dietary FPH influenced the HCT content, which coincided with the study by Ribeiro, Fonseca [70]. Nevertheless, numerous reports have notified the opposite outcomes [5, 71, 72]. HCT content in fish is indicative of the percentage of RBC in the blood, implying oxygen-carrying capacity and general cardiovascular health.

The MCV, MCH, and MCHC are closely related to the blood HGB, and these values were remarkably highest in the 2% FPH group, indicating no microcytic anaemia or iron deficiency. Likewise, Kari, Kabir [5] reported similar findings when the FM was substituted with fermented soy pulp at varying degrees. Conversely, Ribeiro, Fonseca [70] observed dietary FPH did not significantly affect these variables. There were also significant differences between the FPH diet groups in terms of RDW, PLT, PDW, and PCT. These variations were also evident when African catfish (*Clarias gariepinus*) was fed with different ratios of fermented soy pulp [5]. The variations of most haematological parameters among the treatments in this study reflect the general physiological and health conditions of Nile tilapia.

According to Chaklader, Howieson [57], fish inner organs, nutritional status, and metabolic activity are related to serum biochemical markers. In this investigation, blood glucose levels in the treatments exhibited significant changes and remained low in the control group. In contrast, earlier studies reported no significant changes in blood glucose when fish were supplemented with dietary protein hydrolysate [59, 71, 72]. Generally, blood sugar acts as an instant source of energy for an aquaculture species. Despite that, high blood sugar in fish indicates the presence of pollutants that render them vulnerable to environmental risks [42].

Bilirubin levels were also different between the FPH-treated and control groups, which aligned with previous studies in fish that were fed with tuna hydrolysate [57, 73]. Excessive plasma bilirubin might have detrimental effects on kidney. Furthermore, fish creatinine and urea levels were highest in control, and 2% FPH diets, but no significant differences were reported in previous investigations [57, 73]. The observed increase in creatinine and urea levels in these diet groups could be due to variations in protein metabolism and renal function. In addition, the 2% FPH groups had significantly higher albumin and cholesterol levels. Conversely, Ribeiro, Fonseca [70] found that Arapaima (*Arapaima gigas*) albumin and cholesterol levels remained constant when supplemented with dietary FPH. The control group recorded higher globulin concentrations than the FPH groups. Nya and Austin [74] reported plasma carriers albumin and globulin serve as markers of a healthy immune system. These variations may be accredited to varying levels of FPH inclusion, potentially influencing immune-related processes in fish. Overall, the changes in biochemical parameters of blood plasma in this study reflect the health of the experimental fish.

Besides being an indicator of fish health, the fish intestine and liver are crucial for digestion and absorption of dietary nutrients. The intestinal histology of Nile tilapia fed with a 2% FPH diet revealed an intact epithelial wall and more villi structure with the frequent distribution of goblet cells, a wider tunica muscularis, and stronger bonding of stratum compactum than the control and other FPH groups. These outcomes suggested that FPH showed promise in modulating gut health and function, supporting enhanced nutrient absorption and structural integrity. In particular, the increased goblet cell numbers is associated with safeguarding gastro-intestinal barriers through the secretion of antimicrobial substances and glycoproteins, providing defense against harmful microbiota [75]. Moreover, the long villi structure in the intestine increases the surface area to improve nutrient absorption [76]. Similar findings were reported in other fish species, including Barramundi (*Lates calcarifer*) [55, 57, 77, 78], Olive flounder (*Paralichthys olivaceus*) [79], Atlantic salmon (*Salmo solar*) [80], and Pabda (*Ompok pabda*) [33] when supplemented with fish or other dietary protein hydrolysates at various percentages. An improved gut health indicates better nutrient utilization by fish [81], evident in the present study. The Nile tilapia feed utilization increased as their gut health

improved. In summary, more villi and a smaller lumen gap in the 2% FPH fish group indicated enhanced gut health and nutrient absorption ability than other treatments.

The number of nuclei, erythrocytes, vacuoles, and sinusoids in liver tissues varied across the FPH-supplemented groups. Fish fed with 2% dietary FPH exhibited better nuclei and cytoplasm structure and fewer vacuoles in their liver, indicating improved liver health than other groups. These outcomes could be attributed to the existence of di and tri-peptides as well as free AAs in FPH, leading to improved nutrient absorption and providing comprehensive support for liver metabolism. Likewise, Suma, Nandi [33] demonstrated similar results in Pabda (*Ompok pabda)* when diets supplemented with FPH at approximately 2 g/100 g. Lesser vacuoles were also evident in Pompano (*Trachinotus blochii*) when supplemented with tuna hydrolysate (60 g/kg) in poultry by-product meal-based diets [60]. Nonetheless, Siddik, Howieson [52]reported that excessive tuna hydrolysate in feed promoted vacuolar cytoplasm, fat deposition, and necrosis in fish hepatic tissues.

Following the bacterial challenge against *S. iniae*, the percent survival of Nile tilapia was markedly enhanced with increasing the FPH supplementation level in diets, with the 2% FPH group showed the highest value (see Fig. 3). In contrast, the lowest ($p < 0.05$) survival was observed in the control group compared to other treatments. These outcomes indicated that the dietary FPH supplementation could improve the resistance against *S. iniae* infection. FPH comprises bioactive peptides and immunostimulatory agents, potentially boosting the generation of immune-related substances like antibodies and cytokines, strengthening defense against infection. In a previous study, survival of juvenile Barramundi (*Lates calcarifer*), following challenge with *S. iniae*, was significantly higher in those fed with 5% and 10% tuna hydrolysate diets compared to the control [53]. Similarly, dietary FPH improved the disease resistance in various fish, including Red seabream (*Pagrus major*) against *Edwardsiella tarda* [49], European seabass (*Dicentrarchus labrax*) against *Vibrio anguillarum* [67], and Pabda (*Ompok pabda*) against *Aeromonas hydrophila* [33].

In summary, this study established the benefits of the dietary application of FPH in Nile tilapia culture. The farm economics were significantly enhanced with improvements in growth rate and feed assimilation compared to the zootechnical data. For instance, the farm raw margin increased by 5.23 and the ROI by 5.03 when the fish SGR increased by 20%, and FCR decreased by 50% in the 2% FPH group.

Conclusion

It is recommended that 2% FPH in *O. niloticus* diets could substantially enhance *O. niloticus* growth performance, feed utilization, health status, gut microbiota, and specific disease resistance. These findings are potentially useful in developing a nutritionally sound and economically feasible feed supplement for Nile tilapia and other freshwater fish production. However, further investigation is necessary into the molecular pathways associated with the growth and immune-related gene expression in vitro condition.

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Author contribution M.A.K wrote the main manuscript. S.K.N., A.Y.S., Z.A.K., L.S.W., A.A.M., P.S., M.H., M.I.K., S.A.M.S., and G.T.I involve in editing and polishing the manuscript.All authors reviewed the manuscript.

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

Declarations

Ethical Approval The experiments were approved by Animal Ethics Committee of Sylhet Agricultural University, and performed according to the Animal Ethics Procedures and Guidelines of the People's Republic of Bangladesh.

Consent to Participate Not applicable.

Consent for Publication Not applicable

Competing Interests The authors declare no competing interests.

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