Aquaculture Studies, 24(6), AQUAST1974
http://doi.org/10.4194/AQUAST1974

PROOF
RESEARCH PAPER

Zootechnical Performance, Protease Activity and Proximate Composition of Nile Tilapia Fed Diets Containing Fish Silage Produced from Fish Waste

Md. Shahin Parvez[1,*], Bijoy Biswas[1], Sudip Debnath[1,2], Sharmin Aktar[1], Sheikh Mustafizur Rahman[1], Md. Nazmul Ahsan[1]

[1]Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna-9208, Bangladesh
[2]Wageningen University & Research, The Netherlands

How to Cite

Parvez, M.S., Biswas, B., Debnath, S., Aktar, S., Rahman, S.M., Ahsan, M.N. (2024). Zootechnical Performance, Protease Activity and Proximate Composition of Nile Tilapia Fed Diets Containing Fish Silage Produced from Fish Waste. *Aquaculture Studies*, *24(6), AQUAST1974*. http://doi.org/10.4194/AQUAST1974

Article History
Received 04 June 2024
Accepted 17 August 2024
First Online 11 September 2024

Corresponding Author
E-mail: parvez@fmrt.ku.ac.bd

Keywords
Fish Wastes
Feed
Growth
Enzymatic Activity
Proximate Composition

Abstract

The present study was carried out to evaluate the impact of fish silage (FS) on the growth performances, protease enzyme activity and muscle compositions of Nile tilapia. In this regard, FS was produced from the waste parts of carp, pangus and tilapia and five iso-nitrogenous (containing 35% crude protein) diets were formulated in which fishmeal (FM) was used as the primary protein source in the control diet (0% FS, T1). The remaining four diets were formulated by replacing 25% (T2), 50% (T3), 75% (T4), and 100% (T5) FM protein with the FS. These diets were fed to tilapia at 8-6% of their body weight rearing them in glass tanks at the density of 15 fish/tank in triplicates. After fifty-days feeding trial, survival (%) of fish remained unaffected among the dietary treatments. Tilapia fed with 75% FS based diet showed improved growth and lower FCR, whereas those fed a diet with 100% FS exhibited lower growth rates. Notably, protease enzyme activity (mg/ml/min) increased with higher levels of FS inclusion. Increased crude protein content was observed in the fish fed the 75% FS diet. These findings underscore the potential of including FS up to 75% to enhance the growth performance of Nile tilapia.

Introduction

Bangladesh has achieved self-sufficiency in fish production since 2018 with a total current production of 4.76 million metric ton (Parvez et al., 2021; DoF, 2023). Carps (indigenous and exotic), pangus and tilapia are the major aquaculture groups which contributes more than 50% of the total fish production (DoF, 2023). Generally, the major portion of the fish production is locally consumed for meeting protein demand after being processed. A substantial amount of waste is generated during processing in local fish market, home kitchen, processing plant and factories in Bangladesh every year. It has been reported that annually more than 43 thousand tons of seafood waste are generated from the seafood processing plant in Bangladesh (Islam and Peñarubia, 2021). In general, the amount of fish wastes generated during processing ranges from 20 to 80 % depending on the level of processing and type of fish (Ghaly, 2013; Alfio et al., 2021). According to DoF (2023), a significant amount of fish and fishery products (74100 MT) is exported to more than 52 foreign countries after being processed from 107 fish processing plants in Bangladesh. Currently, only a small portion of the waste is used locally for human consumption or as feed for aquaculture species in raw form, and there is no established supply chain or formal marketing system regarding the utilization of these wastes in the country. Unfortunately, the major portion of the wastes is usually discarded in the environment due to the lack of proper

waste management system that resulting environmental pollution, poor resource utilization and economic loss (Islam et al., 2021). The contribution of the fisheries sector would be higher if these wastes could be bio-converted into silage and incorporated it into the fish diet.

Fish feed constitutes a significant portion of cost in aquaculture operations, representing approximately 50-60% of the overall production expenses (Adikari et al., 2017). Among various ingredients used in formulating feed for fish and crustaceans, fish meal (FM) has long been recognized as a high-quality animal protein source due to its balanced nutritive compositions and high palatability (Hardy and Barrows, 2003). However, in recent times, it has become expensive and scarce in the aqua feed industry due to inconsistent supply and unsustainable exploitations (Samaddar et al., 2015; Hussain et al., 2024). Therefore, to ensure the sustainability of aqua feed sector, there is a need to explore nutritionally balanced and economically viable alternative protein sources is warranted. To address this issue, biomass from fish processing wastes (i.e., heads, viscera, bones, fins, scales, appendages etc.) could effectively be utilized as a potential protein source. The waste biomass has been identified as a low-cost nutrient-rich product with the potential to serve as novel proteins, bioactive peptides, enzymes, omega-3-rich oils, minerals, and chitin (Mo et al., 2018; Cretton et al., 2021; Wassef et al., 2023). However, raw fish wastes cannot be directly used as ingredients in fish feed because they are highly perishable items that have potentials for four categories of loss viz., physical, nutritional, quality and market loss. To reduce the loss, therefore, these must be converted into value-added products such as fish silage, and fish protein hydrolysate through proper treatment to ensure nutritional quality (Nor et al., 2011; Saleh et al., 2022).

Fish waste can be inexpensively bio-converted by treating it with chemicals (such as organic or inorganic acids) or by biological means (fermentation using lactic acid bacteria) into a non-homogenous liquid feedstuff called fish silage (FS) (Tatterson, 1982; Vidotti et al., 2002). FS contains a liquid mixture of hydrolyzed proteins, lipids, and minerals, making it a potentially high-quality feed ingredient comparable to FM in terms of nutritional compositions (Stone et al., 1989; Shao et al., 2020). The ensiling treatment process of FS facilitates the production of hydrolyzed proteins that can be easily digested and absorbed by aquatic animals (Arruda et al., 2007; Siddik et al., 2021). Peptides and free amino acids resulting from protein hydrolysis can act as stimulants of nonspecific immunity of fish. Moreover, the chemicals (organic/inorganic acid) added during the ensiling process have been shown to increase disease resistance in tilapia due to its antibacterial properties and preservative nature (Ramli et al., 2005; Toppe et al., 2018; Penarubia et al., 2020). The nutrient content of the FS largely depends on the raw materials used during processing. It has been reported that FS produced from different fish wastes has the potential to substitute FM at different inclusion levels, successful replacements observed in several fishes and crustaceans, including tilapia (Soltan and Tharwat, 2006; Goosen et al., 2016), carps (Haider et al., 2016; Muttharasi et al., 2019), sea bass (Liang et al., 2006; Nor et al., 2011), shrimp (Rodríguez-González et al., 2018; Lobato et al., 2019; Shao et al., 2020), and catfish (Fagbenro et al., 1998; Soltan et al., 2008). The production of FS is a novel concept for animal feed manufacture in Bangladesh and is expected to be a positive development (Islam and Peñarubia, 2021).

In this context, to minimize feeding cost as well as to promote sustainable resource management, the waste generated during processing of major aquaculture species in Bangladesh was used to produce FS and incorporated as an alternative protein source in the diet of Nile tilapia (*Oreochromis niloticus*). Nile tilapia is one of the most cultivated omnivorous fish species globally including Bangladesh due to its broad consumer acceptance and commercial value. Therefore, the present study aimed to assess the impact of FS on zootechnical performances, feed utilization, enzyme activity and body compositions of Nile tilapia.

Material and Methods

Fish Silage Production

Fish silage was produced using waste of three commercially important group of fish namely carp (three species: *Labeo rohita, Catla catla and Cirrhinus cirrhosus*), pangus (*Pangasianodon hypophthalmus*) and tilapia (*Oreochromis niloticus*). The fish were bought from local markets and their waste parts such as guts, skins, viscera and fins were removed and gathered, combined in equal amounts (carp 1: pangus 1: tilapia 1), and ground into a fine texture using an electric blender. The FS was produced according to the procedures described by Haider et al. (2016) and Goosen et al. (2016). One kilogram of minced raw waste was mixed with 4.5% formic acid (95% concentrated) and stored at 25-30ºC in a plastic bucket for 30 days with regular mixing. A 250 mg of butylated hydroxytoluene (BHT) was added to the silage mixture as an antioxidant to reduce lipid oxidation. The extra oil floating on the upper layer of silage was decanted occasionally. After 30 days, the silage was sun-dried six hours daily for three days followed by drying in an electric oven at 55ºC for 24 hours to produce powder silage. Proximate composition of the silage was determined as described below in the 'chemical composition' section.

Experimental Design and Diet Preparation

The experiment was followed in a completely randomized design, employing five dietary treatments (T) with three replications for each. Five isonitrogenous experimental diets containing 35% crude protein were

formulated in which FM served as the main animal protein source in control diet (T1; 0% FS) due to its high digestibility and balanced amino acids profile. The remaining four diets were formulated by replacing 25% (T2), 50% (T3), 75% (T4), and 100% (T5) FM protein with the FS (as indicated in Table 1). All ingredients other than FS used for the experimental diets (Table 1) were obtained in dry form from local feed ingredient suppliers. Before manufacturing diets, all ingredients were grounded separately into small homogenous particles and proximate compositions (specified in chemical composition sub-section) of representative samples were determined for formulating diets appropriately. Afterwards, the ingredients were mixed at the formulated ratios (Table 1) ensuring proper mixing of all ingredients with water to obtain moist mashes. Then, sinking wet pellets were manufactured from the mashes by passing it manually through a small sieve (2.0 mm diameter) of a hand pellet machine. Finally, the pellets were dried at 50°C in an oven until the moisture content reached around 10-12%.

Stocking and Feeding Regime

The experiment was conducted in fifteen (15) rectangular glass tanks located at the wet laboratory of Khulna University, Bangladesh. Each tank had a 120 L volume (dimension: 27.52×15.75×16.9 inch) containing 90 L usable capacity for water filling which was equipped with an air stone and uninterrupted aeration system for ensuring continuous oxygen supply. A total of 500 tilapia juveniles were obtained from BRAC hatchery Khulna, Bangladesh, and acclimated to the tank unit located at the laboratory for seven days. During acclimation, fish were fed a 32% protein rich commercial feed (Quality feed, Dhaka, Bangladesh). Before stocking the fish in experimental tanks, they were starved for 24 hours to empty their gastrointestinal tract. Visually healthy fish of uniform sizes were then individually weighed to maintain similar tank biomass. A total of 225 tilapia (mean weight 6.89±0.06g) were stochastically distributed into the tanks at a density of 15 fish/tank. The five experimental diets were randomly assigned to tanks in triplicates. The diets were fed to the fish for 50 days at 8% of their body weight for the first 20 days, after which it was reduced to 7% for 15 days and 6% for the last 15 days. The feeding rate and amount was adjusted by sampling at ten days interval considering the satiation levels and feeding behaviour of the fish. The total amount of feed was divided into two equal rations and provided at 09:00 and 18:00.

Water Quality Monitoring

During the growth trial, fifty percent (50%) of water was renewed daily to maintain water quality parameters. The key water quality parameters such as temperature, pH and dissolved oxygen (DO) were checked daily at 8:30, while total ammonia nitrogen (TAN) and nitrite nitrogen (NO_2^-) were measured at every other day. Water temperature was recorded by using a Celsius thermometer, and TAN, NO_2^-, pH and DO were measured by using API test kits (Mars Fishcare North America, Inc., USA).

Sampling Methods and Analytical Procedures

Growth was observed regularly at 10 days interval by collecting all fish from each tank and their live weight (g) was taken by using an electric balance. Before final weighing fish were starved for 24 hours to empty their gastrointestinal tract. Final weight of all fish in each tank

Table 1. Formulations and compositions of experimental diets on dry matter basis

Ingredients (g/100g)	Experimental diets				
	T1	T2	T3	T4	T5
Fish meal[1]	32.11	24.08	16.06	8.03	0.0
Fish Silage[1]	0.0	10.44	20.89	31.33	41.77
Soybean meal[1]	32.11	33.02	33.91	34.85	35.84
Wheat flour[1]	11.31	10.72	10.23	9.56	8.74
Rice polish[1]	9.90	8.46	7.04	5.58	3.94
Corn flour[1]	7.07	5.78	4.37	3.15	2.21
VM premix[2]	2.0	2.0	2.0	2.0	2.0
Soybean oil	3.0	3.0	3.0	3.0	3.0
Binder	1.0	1.0	1.0	1.0	1.0
Di-calcium phosphate	1.0	1.0	1.0	1.0	1.0
Sodium chloride	0.5	0.5	0.5	0.5	0.5
Composition (%)					
Protein	35.01±1.22	34.97±0.20	34.98±0.91	35.10±0.78	35.29±1.28
Lipid	12.85±1.23	14.84±1.72	15.72±1.32	14.63±3.14	15.54±1.15
Ash	11.65±3.96	10.87±2.76	11.86±1.30	12.01±0.60	11.22±1.19
Moisture	10.87±1.25	11.82±2.86	10.67±1.10	11.19±2.83	12.22±2.83

[1]Mean protein content in fish meal: 56.59%, soybean meal; 40.95%, fish silage: 43.50%, wheat flour: 17.12%, rice polish: 12.55% and corn flour: 7.13%.
[2]Vitamin and mineral premix contain (per kg): Vitamin A, 2600000 IU; Vitamin D3, 400000 IU; Vitamin B2, 800 mg; Vitamin K, 800 mg; Vitamin B6, 40000; Vitamin E, 300 IU; Vitamin B12, 3200 mg; L-Lysin, 10000 mg; DL-Methionine, 20000 mg; Calcium chloride, 44000 mg; Calcium, 600000 mg; Phosphorus, 120000 mg; Iron, 8000 mg; Zinc, 16000; Copper 400 mg; Magnesium, 1200 mg; Cobalt, 2400 mg.

was recorded for growth analysis at the end of the experiment, while the number of fishes was counted for determining survival rate. The amount of experimental diet fed to the fish in each tank and weight of fish obtained from the tank was used to calculate FCR. To determine condition factor, weight (g) and length (cm) of individual fish were measured by using a sensitive weighing balance and a measuring board, respectively. Three (03) fish from each tank were randomly selected and sacrificed after euthanizing using 2-phenoxyethanol anesthesia at the rate of 0.3 ml/liter. Immediately the viscera, liver, intestine, and muscle were separated carefully. Liver and viscera were weighed individually for determining hepatosomatic index and viscerosomatic index respectively, while intestines and muscles were mixed separately to prepare pool samples for analyzing protease enzyme activity and proximate compositions respectively.

Growth Performance, Survival, Feed Utilization and Somatic Index

At the end of the experiment, growth performance indicators, feed utilization as FCR, survival rate (SR), condition factor (CF), and somatic index were determined using the following equations:

Mean final weight, FW (g) = Total weight of fish at harvest (g)/No of fish survived;

Mean weight gain, WG (g) = Final mean weight (g) – Initial mean weight (g);

Weight gain, WG (%) = (Final mean weight (g) – Initial mean weight (g)) × 100/Initial mean weight (g);

Specific growth rate, SGR (%/day) = (ln final weight – ln initial weight)g×100/days;

Survival rate, SR (%) = (Final number of fish harvested/Initial number of fish stocked) × 100;

Feed conversion ratio, FCR = Total weight of feed given (g)/Total wet weight gain of fish (g);

Condition factor, CF = (Final live individual weight (g)/Final individual length^3) × 100;

Hepatosomatic index, HSI (%) = (Liver weight/whole body weight) × 100;

Viscerosomatic index, VSI (%) = (Viscera weight/whole body weight) × 100.

Protease Enzyme Activity

The intestine samples were taken in eppendorf tubes containing 0.1 M Tris-EDTA buffer (pH 8). The samples in the eppendorf tubes placed in an ice-filled container were homogenized using a tissue homogenizer. Then, it was centrifuged at 12000 rpm for 10 minutes at 4ºC for collection of supernatants containing the enzyme. Protease activity was determined using casein as a substrate following the casein digestion method of protease activity as described by Walter (1984). Briefly, aliquots of 200 µl of the sample were mixed with 200 µl soluble casein (Concentration like 0.65% w/v) and incubated for 30 minutes at 35°C by incorporating 200 µl of trichloracetic acid (100 mM) into the mixture. This mixture was then centrifuged at 12000 rpm and 4°C for 10 minutes and the absorbance of the resultant centrifugation was quantified spectrophotometrically at 280 nm using a double beam spectrophotometer (HITACHI, U-2910, Japan). Finally, the protease activity was measured after developing a standard curve of tyrosine where one unit of enzyme activity was defined as the amount of enzyme required to produce 1µg tyrosine for protease in 1 minute at 35°C.

Chemical Composition

Proximate compositions including crude protein, lipid, ash and moisture of all feed ingredients, experimental diets and muscles samples of the fish fed experimental diets were determined according to the methods of Association of Official Analytical Chemists (AOAC, 2012). Moisture and ash were determined gravimetrically by oven drying at 105ºC for 24 hours and by incineration in a muffle furnace at 550ºC for 16 h, respectively. Crude protein (N × 6.25) was determined by the Kjeldahl method after digestion with concentrated H_2SO_4, while crude lipid was determined gravimetrically using chloroform and methanol according to Bligh and Dyer (1959). In addition, amino acid (AA) profile of FM and FS were determined in an amino acid analyzer (LA 8080, Hitachi, Japan). In this regard, a 200 mg of the sample was hydrolyzed in 25 ml of 6 N HCl for 24 hours heated in a sand bath at 110°C. Then it was dried and diluted in 15 ml distilled water, and filtered using 0.45 µm syringe filter. Finally, AA profiles in the sample were determined in the AA analyzer (LA 8080, Hitachi, Japan) equipped with a Hitachi high-performance cation-exchange column at 57°C column temperature according to Isra et al. (2022). The proximate compositions of the experimental diets are presented in Table 1 and the AA profiles of the FM and FS are presented in Table 2.

Statistical Analysis

All the experimental data are presented as means with standard deviation (mean±SD). Treatment wise mean datasets were compared by analysis of variance (ANOVA) using statistical package (SPSS) (version 16.0). Post hoc Duncan's Multiple Range Test was carried out to determine significant differences among the treatment means at $P<0.05$.

Table 2. Amino acid profile (AA) (g/100g) of fishmeal, fish silage and five experimental diets on dry matter basis

Amino acids	Ingredients		Contribution of FM and FS in the experimental diets*				
	FM	FS	T1	T2	T3	T4	T5
EAA							
Arginine	0.53	0.66	0.17	0.20	0.22	0.25	0.28
Histidine	2.35	1.23	0.76	0.70	0.64	0.57	0.51
Isoleucine	2.44	0.81	0.78	0.67	0.56	0.45	0.34
Leucine	1.18	1.86	0.38	0.48	0.58	0.68	0.78
Lysine	1.21	0.80	0.39	0.38	0.36	0.35	0.33
Methionine	0.73	1.05	0.24	0.29	0.34	0.39	0.44
Phenylalanine	5.67	0.66	1.82	1.43	1.05	0.66	0.28
Threonine	3.81	1.22	1.22	1.05	0.87	0.69	0.51
Valine	0.32	4.07	0.10	0.50	0.90	1.30	1.70
NEAA							
Alanine	0.70	0.94	0.22	0.27	0.31	0.35	0.39
Aspartic acid	0.51	1.04	0.16	0.23	0.30	0.37	0.43
Cysteine	6.01	3.94	1.93	1.86	1.79	1.72	1.65
Glutamic acid	7.01	3.95	2.25	2.10	1.95	1.80	1.65
Glycine	0.88	2.46	0.28	0.47	0.65	0.84	1.03
Proline	0.77	1.12	0.25	0.30	0.36	0.41	0.47
Serine	2.25	1.24	0.72	0.67	0.62	0.57	0.52
Tyrosine	2.70	0.18	0.87	0.67	0.47	0.27	0.08

*Values were calculated theoretically using the percentage values of inclusion of FM and FS (without considering the rest of the ingredients), EAA denotes essential amino acid while NEAA denotes non-essential amino acid. All values represent in the above table are presented as mean of two replications.

Results

Water Quality

The key water quality parameters recorded during the experiment exhibited uniformity and consistency, with insignificant variation (P>0.05) among all the treatments. The water quality parameters, including temperature, pH, DO, TAN, and nitrite remained within the ranges of 28 to 32°C, 7.0 to 8.8, 4 to 6 mg/l, 0.5 to 2 mg/l and 0.0 to 0.5 mg/l, respectively (Table 3).

Growth Performances, Survival, Feed Utilization and Somatic Index

Growth performances, survival rate and somatic indexes of tilapia are presented in the Table 4. The initial weight was similar among dietary treatments (P > 0.05). Following the 50-day feeding trial, the inclusion of FS up to 75% had a general impact on the growth performance of the fish. The fish fed with the T4 diet exhibited the highest mean final body weight (FBW), mean weight gain (WG), and specific growth rate (SGR) compared to any other treatment diet (P<0.05). FBW, WG and SGR increased with the increasing replacement level of FM by FS up to its 75% inclusion (T2-T4) compared to control (T1), although there was insignificant difference among the treatments (P>0.05). Diet T5, containing 100% replacement of FM by FS, significantly reduced growth performance of the fish compared to the T4 diet, although the growth reduction was insignificant compared to the other treatments (T1-T3). Similarly, Nile tilapia fed on the T4 diet increased feed efficiency by reducing FCR compared to those fed with any other treatment diets, whereas diet the T5 significantly increased FCR compared to T4 diet.

Dietary treatments of FM replacement by FS had no effect (P>0.05) on the survival rate (SR). The condition factor (CF) of the fish was also not affected (P>0.05) by the diets, ranging between 1.54 and 1.75 across all treatments. However, the hepatosomatic index (HSI) and viscerosomatic index (VSI) of fish were insignificantly increased in the fish fed with T3 and T4 diets as compared to the control diet (Table 4). The HSI for both T3 and T4 diets were considerably greater (P<0.05) than those fed T2 and T5 diets. The VSI for both T3 and T4 diets were considerably greater (P<0.05) than T2 but insignificantly higher than T5.

Protease Enzyme Activity

Protease activities (PA) in the intestine of fish fed experimental diets are presented in the Figure 1. In comparison to control diet (T1), intestinal PA increased with the increasing inclusion levels of FS, although the increasing rates were not proportional to inclusion levels. PA of both T4 and T5 diets was significantly higher (P<0.05) than that of other treatments; however, the difference was insignificant in the activity between T4 and T5 treatments (P>0.05).

Proximate Composition of the Fish

After the 50-day feeding trial, crude lipid, ash, and moisture content in the muscle of the fish remained unaffected by the dietary treatments (p > 0.05). There was a significant difference in crude protein content in fish fed different levels of FS diets. When compared to fish fed diets with 25% and 100% FS, fish fed a 75% FS diet had the highest protein levels (Table 5).

Table 3. Key water quality parameters recorded (means±SD) during the experiment

Parameters	T1	T2	T3	T4	T5
Temperature (ºC)	29.31±0.65[a]	29.23±0.79[a]	29.29±0.75[a]	29.17±0.56[a]	29.28±0.61[a*]
pH	8.08±0.18[a]	8.10±0.28[a]	8.13±0.31[a]	8.12±0.25[a]	8.04±0.19[a]
DO (mg/l)	5.08±0.56[a]	4.97±0.62[a]	5.06±0.57[a]	5.01±0.65[a]	5.06±0.72[a]
TAN (mg/l)	0.68±0.51[a]	0.64±0.41[a]	0.69±0.36[a]	0.66±0.35[a]	0.72±0.34[a]
Nitrite (mg/l)	0.05±0.13[a]	0.06±0.13[a]	0.07±0.15[a]	0.06±0.13[a]	0.07±0.14[a]

* Mean values in the same row with different superscripts are significantly different (P<0.05).

Table 4. Growth performances, survival and somatic indexes (means±SD) of Nile tilapia fed five experimental diets with different levels of fish silage. All values represent as mean and SD of three replicates

Parameters	T_1	T_2	T_3	T_4	T_5
IBW (g)	6.89±0.05[a]	6.88±0.05[a]	6.90±0.06[a]	6.87±0.07[a]	6.88±0.10[a]
FBW (g)	21.71±2.66[ab]	21.85±1.99[ab]	23.46±2.58[ab]	24.79±0.69[b]	20.06±1.57[a]
WG (g)	14.82±2.71[ab]	14.97±2.03[ab]	16.56±2.52[ab]	17.92±0.63[b]	13.18±1.64[a]
WG (%)	215.2±40.8[ab]	217.8±30.9[ab]	239.8±34.6[ab]	260.7±7.1[b]	191.9±25.9[a]
SGR (%/d)	2.28±0.27[ab]	2.31±0.20[ab]	2.44±0.20[ab]	2.57±0.04[b]	2.14±0.17[a]
FCR	2.66±0.39[ab]	2.82±0.39[ab]	2.49±0.38[ab]	2.21±0.10[b]	3.04±0.40[a]
SR (%)	95.56±7.70[a]	88.89±7.70[a]	91.11±3.85[a]	93.33±6.67[a]	93.333±6.67[a]
CF	1.60±0.05[a]	1.64±0.06[a]	1.56±0.03[a]	1.60±0.05[a]	1.62±0.11[a]
HSI (%)	2.33±0.39[ab]	2.04±0.36[a]	2.51±0.60[b]	2.52±0.44[b]	2.01±0.42[a]
VSI (%)	8.42±0.90[a]	6.76±0.85[b]	8.55±1.78[a]	8.50±1.09[a]	7.82±0.65[ab]

In the same row with different superscripts are significantly different (P<0.05).IBW=mean initial body weigh; FBW=mean final body weight, WG = weight gain; SGR = specific growth rate; FCR = feed conversion ratio; CF = condition factor; VSI = viscerosomatic index; HSI = hepatosomatic index; SR = Survival rate.

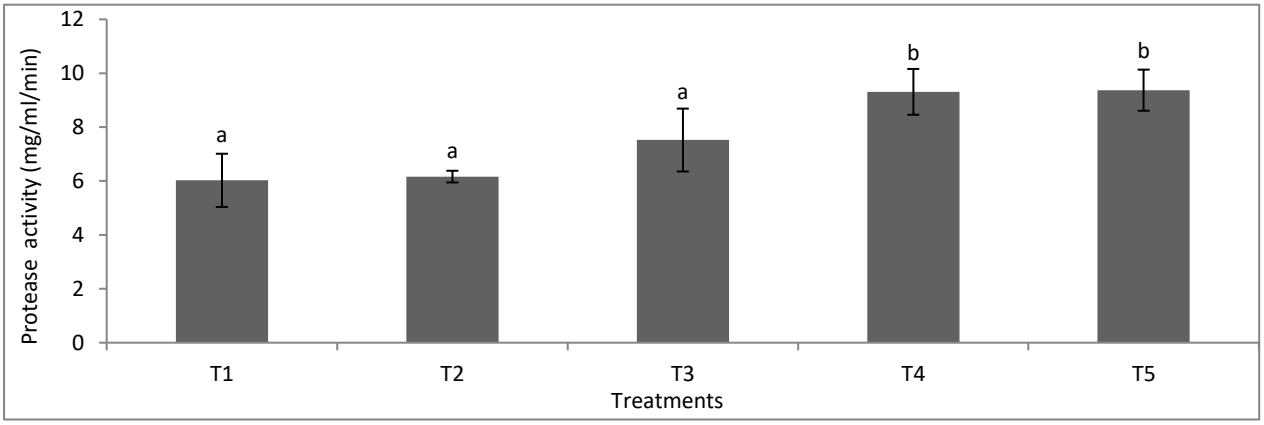

Figure 1. Protease enzyme activity in the intestine of Nile tilapia fed diets with different levels of fish silage. Different letters above bars represent significant difference among dietary treatments

Discussion

This study aimed to assess the potential of incorporating fish silage derived from carp, tilapia and pangus waste into the diet of Nile tilapia. Key water qualities remained consistent among the treatments and were within the optimum ranges required for culturing Nile tilapia as described by El-Hack et al. (2022). The consistent and uniform water quality parameters among treatments revealed that the inclusion of fish wastes and the presence of acid in silage did not adversely impact water quality. Following the harvest, the overall specific growth rates (SGRs) of Nile tilapia fed the treatment diets ranged from 2.14% to 2.57% per day, exceeding the SGR (0.93% per day) reported by Goosen et al. (2016). On the other hand, Soltan and Tharwat (2006) and Madage et al. (2015) reported higher SGR (3.06 and 3.22% per day, respectively) in Nile tilapia fed similar diets compared to those observed in the present study. These discrepancies in SGR among studies may be attributed to variations in basal ingredients in diets, initial fish weight, and rearing conditions.

In general, the dietary inclusion of FS had positive effects on the growth performance, survival, feed utilization, somatic health and conditions, protease enzyme activity, and muscle compositions of the fish. The optimal protein requirement for Nile tilapia typically falls between 30% and 35%, with specific concentrations of ten essential amino acids (EAAs) such as 1.79% arginine, 0.84% histidine, 1.33% isoleucine, 1.47% leucine, 2% lysine, 0.98% methionine, 1.51% threonine, 1.23% valine, 1.19% phenylalanine, and 0.28% tryptophan (NRC, 2011; Mai et al., 2022). Analysis of the

Table 5. Proximate composition (means±SD) of Nile tilapia fed five experimental diets with different levels of fish silage. All values represent as mean and SD of three replicates (% of wet weight basis)

Parameters	T1	T2	T3	T4	T5
Crude protein (%)	19.51±0.99^{acd}	19.03±0.56^{ad}	20.16±0.54^{ac}	20.32±0.43^{c}	18.88±0.33^{d}
Crude lipid (%)	4.83±0.51^{a}	4.85±0.79^{a}	4.08±0.65^{a}	4.58±1.28^{a}	3.82±1.17^{a}
Ash (%)	3.35±0.51^{a}	4.10±0.62^{a}	4.43±0.80^{a}	3.93±0.53^{a}	3.51±0.28^{a}
Moisture (%)	70.66±2.01^{a}	71.12±0.86^{a}	69.61±1.72^{a}	70.40±1.59^{a}	72.42±1.50^{a}

* Means±SD in the same row with different superscripts are significantly different (P<0.05).

AA content in FM and FS revealed that tryptophan was not detected in either ingredient. Histidine, isoleucine, lysine, threonine, and phenylalanine concentrations were higher in FM, whereas arginine, leucine, methionine and valine concentrations were higher in FS (Table 2). Therefore, the inclusion of either FM or FS alone could lead to imbalances in EAAs in the final diet. Instead, blending both ingredients ensure a balanced amino acid content in the diet. It is evident that fish growth correlates not only with crude protein level but also with proper proportion of all AAs (Kaushik and Seiliez, 2010). In our study, the inclusion of either 100% FM or 100% FS in the diet did not enhance growth. Instead, better growth was observed when FM was partially replaced by FS, ranging from 25% to 75% in the diets. This improved growth may be attributed, among other factors, to the intake of a relatively balanced blend of AAs from both sources of ingredients.

The growth performance in fish fed with a diet incorporating 75% FS exhibited enhanced outcomes in terms of mean FBW, WG, and SGR compared to other diets. Consistent with our study, several studies have reported improved growth performance of Nile tilapia fed diet with different levels of FS produced through acidic, biological or enzymatic bioconversion method utilizing by-products and wastes from different fish and shrimp species. For instance, Soltan and Tharwat (2006) successfully replaced up to 25% of fish meal (FM) with fermented fish-by-products based silage in Nile tilapia diets, with no significant difference in growth observed up to 25% replacement, but a significant decline thereafter. Following a similar trend, in Red tilapia, acid-based tilapia waste silage effectively replaced up to 50% of dietary FM (Madage et al., 2015). Similar to our findings, studies by El-Hakim et al. (2007) and Cavalheiro et al. (2007) demonstrated improved growth performances in Nile tilapia after the inclusion of 100% acidic FS in the diet, utilizing tilapia and shrimp waste, respectively, valorized into acidic silage as FM substitutes. Furthermore, our findings align with several studies that utilized FS as a protein source in tilapia and other fishes' diet (Plascencia-Jatomea et al., 2002; Liang et al., 2006; Goosen et al., 2016; Gonçalves et al., 2019; Santana et al., 2023a). These studies found that partial or complete replacement of FM with acidic or fermented FS supported satisfactory growth rates and did not significantly affect the growth of reared fish compared to the control (FM) diet.

Similar to growth performances, an improved FCR was noted in the 75% FS diet, while replacing FM with FS up to 75% showed no significant effect on FCR. However, complete replacement (100%) of FM with FS led to an increase in FCR. This indicates a comparable nutrient efficiency among the diets except for the 100% FS diet. Additionally, the 75% FS-based diet demonstrated superior nutrient digestibility and retention capacity compared to others, enabling fish to efficiently utilize nutrients and convert them into weight gain by retaining more nutrients. As observed in the present study, the high inclusion (>50%) FS replacing FM has been reported to increase feed efficiency by improving digestibility and nutrient retention which resulted in decreased FCR and better growth performances in various fish species such as tilapia, *Oreochromis niloticus* (Madage et al., 2015; Montoya-Mejía et al., 2017); rainbow trout, *Oncorhynchus mykiss* (Guzel et al., 2011); tambaqui, *Colossoma macropomum* (Santana et al., 2023a); whiteleg shrimp, *Litopenaeus vannamei* (Shao et al., 2020).

Survival among dietary treatments remained unaffected throughout the feeding trial, which indicates that inclusion of FS up to 100% did not affect fish survival. This might have resulted from the consequence of improved immune stimulating effects of protein hydrolysis products (peptides and free amino acids) in the FS. The finding corroborates several studies that explored wastes-based FS as sources of protein in diets for tilapia (Goosen et al., 2016) and seabass (Cahu, 1999; Liang et al., 2006; Kotzamanis et al., 2007), and generally reported that FS could act as a stimulant of the cellular non-specific immunity, thereby decreasing stress and improving survival. In addition, formic acid, the raw material for FS production, could act as an antimicrobial agent (Ramli et al., 2005) that might result in enhanced disease resistance capacity for improved survival.

The CFs of the fish fed the five diets exhibited no significant differences, ranging from 1.56 to 1.64. The lack of disparity in CFs among the treatments suggests that the fish maintained similar levels of health and physiological conditions during the trial. The CFs obtained in this study are similar to the findings of Mamboya and Githunguri (2005) who recorded CFs ranging from 0.9 to 2.2, with an average value of 1.6 in the wild stock of Nile tilapia from Lake Victoria in Tanzania. A feeding trial of juvenile Nile tilapia in indoor tanks conducted by Zablon et al. (2020) reported the CF ranges were also similar to the CFs observed in this study. Similarity of CF ranges reveals that the fish subjected to the treatment diets exhibited health characteristics comparable to those of wild or captive

stock.

Hepatosomatic index (HSI), a measure of the relative weight of the liver, is a widely used parameter in fish biology as an estimate of the energy status of the fish, while VSI indicates the proportion of muscle tissue relative to visceral tissue in fish (Nunes et al., 2011). The HSI and VSI values of juvenile Nile tilapia observed in this study are similar to other studies found in the fish fed with fish waste silage (Montoya-Mejía et al., 2017; Abdullahi et al., 2023). No significant disparity in HSI among the treatments suggests similar liver sizes and comparable fat storage and energy utilization for cellular metabolic processes. The dietary inclusion of FS neither negatively nor positively influenced the VSI of the fish, which is indicative of a consistent proportion of muscle accumulation relative to viscera in the fish.

In this study, inclusion of FS enhanced protease enzyme activity in tilapia, indicative of improved dietary protein digestion of FS by the assistance of higher protease activity. During digestion, the degradation of dietary protein is facilitated by protease enzymes, activity of which dictates the extent of underlying protein digestibility (Abd Elnabi et al., 2020; Gopalraaj et al., 2024). Due to its hydrolyzed nature, protein derived from FS exhibits higher digestibility potential compared to intact protein such as FM, attributed to the presence of short-chain small peptides and free amino acids (Nørgaard et al., 2015). This inherent property of hydrolyzed protein in FS enhances its bioavailability during digestion, facilitating the complete breakdown of nutrients through the secretion of protease enzymes. Several studies have reported that FS offers enhanced protein digestibility (>80-93%) by stimulating protease activities in various fish species including tilapia (Fagbenro and Jauncey, 1998; Borghesi et al., 2008; Santos et al., 2013), indian major carp (Haider et al., 2017), white shrimp (Shao et al., 2020), tambaqui (Santana et al., 2023b) and pacu (Vidoiti et al., 2002). The increased protease activities observed in this study are likely to improve protein digestibility and absorption, thereby enhancing feed utilization (FCR) and growth performance in fish fed diets containing FS, with the exception of those fed a diet comprising 100% FS.

The lowest growth with decreased feed utilization in the fish fed 100% FS diet could be attributed to the loss of nutrients by lixiviation. The extent of hydrolyzed protein content in FS may lead to differences in diet quality, resulting from variations in the concentrations of amino acids, dipeptides, tripeptides, oligopeptides, and polypeptides. High levels of amino acids and smaller peptides are typically more soluble, leading to cause lixiviation (Leal et al., 2010). Although the 100% FS-based diet containing excessive amount of hydrolyzed protein obtained the highest protease activity during digestion, feeding fish with the protein in the diet might not be bio-available for absorption and utilization in the body due to lixiviation loss of nutrients that could result in lowest growth performances.

Lipid, ash and moisture in the muscle remained unaffected among dietary treatments, while higher protein content was found in fish fed 75% FS diet and it significantly declined with the lowest value in 100% FS diet. Higher protein content of the fish obtained in this study supported the results of Ramasubburayan et al. (2013) who found increasing level of crude of protein contents in the body-muscle of common carp fed the acid silage of marine fishery waste. Conversely, there were no notable changes in crude lipid, moisture, and ash content among fish fed the experimental diets, and no discernible pattern was observed regarding the inclusion of FS in the diets.

Conclusion

In conclusion, incorporating FS up to 75% in the diet resulted in superior performances in terms of growth, feed utilization, survival, organo-somatic index, protease activity, and muscle composition compared to a higher incorporation level (100%), which adversely affected growth performances of Nile tilapia. The results of this study demonstrate the potential of utilizing FS derived from fish waste, including carp, tilapia, and pangus, in fish diets. This utilization could offer a pathway to promote circular bio-economy practices by enhancing the efficient utilization of these waste resources in Bangladesh.

Ethical Statement

The study was carried out according to the guidelines of animal ethical procedures of the Khulna University.

Funding Information

The present study was carried out with research support from the 'Research and Innovation Centre' of Khulna University, Khulna-9208, Bangladesh (Funding ID: 36/2021).

Author Contribution

MSP: conceptualization, fund acquisition, supervision, original draft manuscript preparation; BB: Execution of experiment and laboratory analysis; SD: Conceptualization, review and editing of draft manuscript; SA: Data interpretation and statistical analyses, methodology; SMR: Methodology, review and editing of draft manuscript; MNA: Overall monitoring of experiment, review and editing of draft manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts or relationships that could have appeared to influence the work reported in this manuscript.

References

Abd Elnabi, H.E., Hassanen, G.D.I., Soltan, M.A., & Dokdok, G.A. (2020). Effect of protease and prebiotic mixtures with free fishmeal diets on physiological responses and histological examinations of the red tilapia, *Oreochromis* sp. *Egyptian Journal of Aquatic Biology & Fisheries, 24,* 361–378. https://doi.org/10.21608/ejabf.2020.82015.

Abdullahi, A.I., Mohammed, T., Haruna, M.Y., & Aliyu, M. (2023). Digestibility, growth and hepatosomatic index of *Oreochromis Niloticus* (Linnaeus, 1758) fed diets containing blanched *Lemna paucicostata* (Hegelm). *International Journal of Oceanography & Aquaculture, 7(3),* 1-9.

Adikari, A., Sundarabarathy, T., Herath, H., Nayananjalie, W., & Adikari, A. (2017). Formulation of artificial feeds for Indian carp (*Catla catla*) fry using aquatic plants (*Ipomea aquatica* and *Hydrilla vercillata*). *International Journal of Scientific Research Publications, 7(7),* 83–89.

Alfio, V.G., Manzo, C., & Micillo, R. (2021). From fish waste to value: an overview of the sustainable recovery of omega-3 for food supplements. *Molecules, 26,* Article 1002. https://doi.org/10.3390/molecules26041002.

AOAC. (2012). *Official methods of analysis of the association of official analytical chemists international* (16th ed.) Association of Official Analytical Chemists, Washington, DC, Arlington, VA.

Arruda, L.F., Borghesi, R., & Oetterer, M. (2007). Use of fish waste as silage: a review. *Brazilian Archives of Biology and Technology, 50*, 879–886. https://doi.org/10.1590/S1516-89132007000500016.

Bligh, E.G. & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37(8),* 911-917.

Borghesi, R., Portz, L., Oetterer, M. & Cyrino, J.E.P. (2008). Apparent digestibility coefficient of protein and amino acids of acid, biological and enzymatic silage for Nile tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition, 14,* 242-248. https://doi.org/10.1111/j.1365- 2095.2007.00523.x.

Cahu, C.L. (1999). Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass (*Dicentrarchus labrax*) larvae. *Aquaculture, 171,* 109–119.

Cavalheiro, J.M.O., de Souza, E.O., & Bora, P.S. (2007). Utilization of shrimp industry waste in the formulation of tilapia (*Oreochromis niloticus* Linnaeus) feed. *Bioresource Technology, 98(3),* 602-606. https://doi.org/10.1016/j.biortech.2006.02.018.

Cretton, M., Malanga, G., Mazzuca-Sobczuk, T., & Mazzuca, M. (2021). Lipid fraction from industrial crustacean waste and its potential as a supplement for the feed industry: a case study in Argentine patagonia. *Waste and Biomass Valorization, 12,* 2311-2319.

DoF. (2023). National fish week 2023 compendium (in Bangla). Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh.

El-Hack, M.E.A., El-Saadony, M.T., Nader, M.M., Salem, H.M., El-Tahan, A.M., Soliman, S.M., & Khafaga, A.F. (2022). Effect of environmental factors on growth performance of Nile tilapia (*Oreochromis niloticus*). *Int J Biometeorol, 66,* 2183–2194. https://doi.org/10.1007/s00484-022-02347-6.

El-Hakim, N.F.A., El-Gendy, M.O.A., & Salem, M.F.I. (2007). Effect of incorporation of fish silage into diets on growth performance and body composition of Nile tilapia (*Oreochromis niloticus*). *Egyptian Journal of Aquatic Biology and Fisheries, 11,* 101–117

Fagbenro, O.A., & Jauncey, K. (1998). Physical and nutritional properties of moist fermented fish silage pellets as a protein supplement for tilapia (*Oreochromis niloticus*). *Animal Feed Science and Technology, 71,* 11–18.

Ghaly, A. (2013). Fish processing wastes as a potential source of proteins, amino acids and oils: a critical review. *Journal of Microbial & Biochemical Technology, 5,* 107–129. https://doi.org/10.4172/1948-5948.1000110.

Gonçalves, A.A., Emerenciano, M.G.C., Ribeiro, F.D.A.S, & Neto, J.D.R.S. (2019). The inclusion of fish silage in *Litopenaeus vannamei* diets and rearing systems (biofloc and clear-water) could affect the shrimp quality during subsequent storage on ice. *Aquaculture, 507,* 493-499. https://doi.org/10.1016/j.aquaculture.2019.04.032.

Goosen, N.J, de Wet, L.F., & Görgens, J.F. (2016). Rainbow trout silage as immune stimulant and feed ingredient in diets for Mozambique tilapia (*Oreochromis mossambicus*). *Aquaculture Research, 47,* 329–340. https://doi.org/10.1111/are.12497.

Gopalraaj, J., Velayudhannair, K., Arockiasamy, J.P., & Radhakrishnan, D.K. (2024). The effect of dietary supplementation of proteases on growth, digestive enzymes, oxidative stress, and intestinal morphology in fishes– A review. *Aquaculture International, 32,* 745–765. https://doi.org/10.1007/s10499-023-01191-8.

Guzel, S., Yazlak, H., Gullu, K., & Ozturk, E. (2011). The effect of feed made from fish processing waste silage on the growth of rainbow trout (*Oncorhynchus mykiss*). *African Journal of Biotechnology, 10(25),* 5053-5058. https://www.ajol.info/index.php/ajb/article/view/94191

Haider, M.S., Ali, Z., Abbas, S., Naseem, A., Ahmad, M., Kamal, S., & Afzal, M. (2017). Fatty acid profile and effect of fish fermented silage on digestive enzymes in *Labeo rohita*. *Bioscience Journal, 33(6),* 1562–1571

Haider, M.S., Ashraf, M., Azmat, H., Javid, A., Atique, U., Zia, M., & Iqbal, K.J. (2016). Nutritive evaluation of fish acid silage in *Labeo rohita* fingerlings feed. *Journal of Applied Animal Research, 44(1),* 158–164. https://doi.org/10.1080/09712119.2015.1021811,

Hardy, R.W., & Barrows, F.T. (2003). Diet formulation and manufacture. In: S. Diego, J.E. Halver, & R.W. Hardy (eds), *Fish Nutrition* (pp. 505-600). 3rd edn, Academic Press.

Hussain, S.M., Khurram, F., Naeem, A., Shah, S.Z.H., Sarker, P.K., Naeem, E., Arsalan, M.Z., Riaz, D., Faisal, M., & Amjad, M. (2024). A review on the prospects and potentials of fishmeal replacement with different animal protein sources. *International Aquatic Research, 16,* 7–16. https://doi.org/10.22034/IAR.2024.1996466.1517.

Islam, J., Yap, E.E.S., Krongpong, L., Toppe, J., & Peñarubia, O.R. (2021). Fish waste management – an assessment of the potential production and utilization of fish silage in Bangladesh, Philippines and Thailand. FAO Fisheries and Aquaculture Circular No 1216, Rome. https://doi.org/10.4060/cb3694en.

Islam, M.J., & Peñarubia, O.R. (2021). Seafood waste management status in Bangladesh and potential for silage production. *Sustainability, 13,* Article 2372. https://doi.org/10.3390/ su13042372.

Isra, L., Suraiya, S., Salma, U., & Haq, M. (2022). Nutritional Evaluation and Shelf-Life Study of Mackerel Tuna (*Euthynnus affinis*) Fish Pickle. *Agricultural Research, 11,* 249–257. https://doi.org/10.1007/s40003-021-00559-7.

Kaushik, S.J., & Seiliez, I. (2010). Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs. *Aquaculture Research, 41,* Article 322e332.

Kotzamanis, Y.P., Gisbert, E., Gatesoupe, F.J., Infante, J.Z., & Cahu, C. (2007). Effects of different dietary levels of fish protein hydrolysates on growth, digestive enzymes, gut microbiota, and resistance to *Vibrio anguillarum* in European sea bass (*Dicentrarchus labrax*) larvae. *Comparative Biochemistry and Physiology, 147 (Part A),* 205–214.

Leal, A.L.G., Castro, P.F., Lima, J.P.V., Correia, S.E., & Bezerra, R.S. (2010). Use of shrimp protein hydrolysate in Nile tilapia (*Oreochromis niloticus*, L.) feeds. *Aquaculture International, 18,* 635–646.

Liang, M., Wang, J., Chang, Q., & Mai, K. (2006). Effects of different levels of fish protein hydrolysate in the diet on the nonspecific immunity of Japanese sea bass, *Lateolabrax japonicas* (Cuvieret Valenciennes, 1828). *Aquaculture Research, 37,* 102–106. https://doi.org/10.1111/j.1365-2109.2005.01392.x.

Lobato, O.S.C., de Azevedo Silva Ribeiro, F., Miranda-Baeza, A., & Emerenciano, M.G.C. (2019). Production performance of *Litopenaeus vannamei* (Boone, 1931) fed with different dietary levels of tilapia processing waste silage reared in biofloc system using two carbon sources. *Aquaculture, 501,* 515–518. https://doi.org/10.1016/j.aquaculture.2018.12.006.

Madage, S.S.K., Medis, W.U.D., Sultanbawa, Y. (2015). Fish silage as replacement of fishmeal in red tilapia feeds. *Journal of Applied Aquaculture, 27(2),* 95-106. https://doi.org/10.1080/10454438.2015.1005483.

Mai, K., Xue, M., He, G., Xie, S.Q., & Kaushik, S.K. (2022). Protein and amino acids. In: W. Ronald, S. Hardy & S.J. Kaushik (eds), *Fish Nutrition* (pp. 181-302) 4th edn. Academic Press.

Mamboya, F.A., & Githunguri, C.M. (2005). Growth and condition factor of Nile tilapia (*Oreochromis niloticus* L.) from Mwanza Gulf, Lake Victoria, Tanzania. *African Journal of Aquatic Science, 30(1),* 95-100.

Mo, W.Y., Man, Y.B., & Wong, M.H. (2018). Use of food waste, fish waste and food processing waste for China's aquaculture industry: Needs and challenge. *Sci Total Environ, 613–614,* 635–643. https://doi.org/10.1016/j.scitotenv.2017.08.321.

Montoya-Mejía, M., García-Ulloa, M., Hernández-Llamas, A., Nolasco- Soria, H., & Rodríguez-González, H. (2017). Digestibility, growth, blood chemistry, and enzyme activity of juvenile *Oreochromis niloticus* fed isocaloric diets containing animal and plant byproducts. *Revista Brasileira de Zootecnia, 46(12),* 873-882. http://dx.doi.org/10.1590/S1806-92902017001200001.

Muttharasi, C., Muralisankar, T., Uthayakumar, V., Gayathri, V., Thangal, S.H., & Anandhan, K. (2019). Utilization of marine fisheries wastes for the production of the freshwater fish *Cyprinus carpio*. *Tropical Animal Health Production, 51, 2305–2313.* https://doi.org/10.1007/s11250-019-01938-7.

Nor, N.M., Zakaria, Z., Manaf, M.S.A., & Salleh, M. (2011). The effect of partial replacement of dietary fishmeal with fermented prawn waste liquor on juvenile sea bass growth. *Journal of Applied Aquaculture, 23(1),* 51-57. https://doi.org/10.1080/10454438.2011.549784.

Nørgaard, J.V., Petersen, J.K., Tørring, D.B., Jørgensen, H., & Lærke, H.N. (2015). Chemical composition and standardized ileal digestibility of protein and amino acids from blue mussel, starfish, and fish silage in pigs. *Animal Feed Science and Technology, 205,* 90–97.

NRC. (2011). Nutrient Requirements of Fish and Shrimp. Naitonal Research Council, the National Academies Press, Washington.

Nunes, C., Silva, A., Soares, E., & Ganias, K. (2011). The use of hepatic and somatic indices and histological information to characterize the reproductive dynamics of Atlantic Sardine, *Sardina pilchardus* from the Portuguese coast. *Marine and Coastal Fisheries, 3(1),* 127–144.

Parvez, M.S., Sarower, M.G., Hasan, M.M., Debnath, S., Aktar, S., & Ahsan, M.N. (2021). Monitoring of genetic variation in mono-sex tilapia stocks using randomly amplified polymorphic DNA markers in some private hatcheries of Bangladesh. *Egyptian Journal of Aquatic Biology & Fisheries, 25(1),* 1017-1030. https://doi.org/10.21608/EJABF.2021.154322.

Penarubia, O.R., Toppe, J., & James, D. (2020). Fish Waste Management: Turning Waste into Healthy Feed with Antimicrobial Properties. Asian Fisheries Science, 33(1), 11–15. https://doi.org/10.33997/j.afs.2020.33.S1.002

Plascencia-Jatomea, M., Olvera-Novoa, M.A., Arredondo-Figueroa, J.L., Hall, G.M., & Shirai, K. (2002). Feasibility of fishmeal replacement by shrimp head silage protein hydrolysate in Nile tilapia (*Oreochromis niloticus* L) diets. *Journal of the Science of Food and Agriculture, 82(7),* 753-759. https://doi.org/10.1002/jsfa.1092.

Ramasubburayan, R., Iyapparaj, P., Subhashini, K.J., Chandran, M.N., Palavesam, A., & Immanuel, G. (2013). Characterization and nutritional quality of formic acid silage developed from marine fishery waste and their potential utilization as feed stuff for common carp *Cyprinus carpio* fingerlings. *Turkish Journal of Fisheries and Aquatic Sciences, 13,* 281-289. https://doi.org/10.4194/1303-2712-v13_2_10.

Ramli, N., Heindl, U., & Sunanto, S. (2005). Effects of potassium-diformate on growth performance of tilapia challenged with *Vibrio anguillarum*. In: World Aquaculture Society Conference. Abstract, CD-Rom, Bali, Indonesia.

Rodríguez-González, H., López-Aguilar, M.R., Fonseca-Madrigal, J., Martínez-Palacios, C.A., & García-Ulloa, M. (2018). Use of a mixture of vegetal (*Jatropha curcas*) and animal (fish silage) by-products as protein source in shrimp (*Litopenaeus vannamei*) diets. *Revist Bras Zootec, 47*, Article e20170165. https://doi.org/10.1590/rbz4720170165.

Saleh, N.E., Wassef, E.A., & Abdel-Mohsen, H.H. (2022). Sustainable fish and seafood production and processing. In: C. Galanakis (Ed), *Sustainable fish production and processing* (9:259-291). Academic Press, Cambridge. https://doi.org/10.1016/B978-0-12-824296-4.00002-5.

Samaddar, A., Kaviraj, A., & Saha, S. (2015). Utilization of fermented animal by-product blend as fishmeal replacer in the diet of *Labeo rohita*. *Aquaculture Reports, 1,* 28-36. https://doi.org/10.1016/j.aqrep.2015.03.004.

Santana, T.M., Dantas, F.M., Armando, G., Prestes, A.G,.Jerônimo, G.T., Costa, J.I., Monteiro dos Santos, D.K., Pastrana, Y.M., Yamamoto, F.Y., & Gonçalves, L.U. (2023a). Evaluating fermented fish viscera silage in diets for *Colossoma macropomum* and its effects on physical quality of pellets, production performance, health parameters, and economic analysis. Preprint (Version 1) available at Research Square

https://doi.org/10.21203/rs.3.rs-3158273/v1

Santana, T.M., Dantas, F.M., Monteiro Dos Santos, D.K., Kojima, J.T., Pastrana, Y.M., De Jesus, R.S, & Gonçalves, L.U. (2023b). Fish viscera silage: production, characterization, and digestibility of nutrients and energy for Tambaqui juveniles. *Fishes, 8,* Article 111.

Santos, J.F., Castro, P.F., Leal, A.L.G., Junior, A.C.V.F., Lemos, D., Carvalho Jr, L.B., & Bezerra, R.S. (2013). Digestive enzyme activity in juvenile Nile tilapia (*Oreochromis niloticus*, L) submitted to different dietary levels of shrimp protein hydrolysate. *Aquaculture International, 21,* 563–577. https://doi.org/10.1007/s10499-012-9589-2.

Shao, J., Wang, L., Shao, X., & Liu, M. (2020). Dietary different replacement levels of fishmeal by fish silage could influence growth of *Litopenaeus vannamei* by regulating mTOR at transcriptional level. *Frontiers in Physiology, 11,* Article 359. https://doi.org/10.3389/fphys.2020.00359.

Siddik, M.A., Howieson, J., Fotedar, R., & Partridge, G.J. (2021). Enzymatic fish protein hydrolysates in finfish aquaculture: a review. *Reviews in Aquaculture, 13,* 406–430. https://doi.org/10.1111/raq.12481.

Soltan, M.A., Hanafy, M.A., & Wafa, M.I.A. (2008). An evaluation of fermented silage made from fish by-products as a feed ingredient for African catfish (*Clarias gariepinus*). *Global Veterinaria, 2,* 80–86.

Soltan, M.A., & Tharwat, A.A. (2006). Use of fish silage for partial or complete replacement of fish meal in diets of Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*). *Egyptian Journal of Nutrition and Feeds, 9(2),* 299-314.

Stone, F.E., Hardy, R.W., Shearer, K.D., & Scott, T.M. (1988). Utilization of fish silage by rainbow trout (*Salmo gairdneri*). *Aquaculture, 76(1-2),* 109-118. https://doi.org/10.1016/0044-8486(89)90255-X.

Tatterson, I.N. (1982). Fish silage—preparation, properties and uses. *Animal Feed Science and Technology, 7,* 153–159. https://doi.org/10.1016/0377-8401(82)90050-5.

Toppe, J., Olsen, R.L., Peñarubia, O.R., & James, D.G. (2018). Production and utilization of fish silage: a manual on how to turn fish waste into profit and a valuable feed ingredient or fertilizer. Food and Agricultural Organization, Rome, Italy.

Vidotti, R.M., Carneiro, D.J., & Viegas, E.M.M. (2002). Acid and fermented silage characterization and determination of apparent digestibility coefficient of crude protein for Pacu *Piaractus mesopotamicus*. *Journal of the World Aquaculture Society, 33,* 57-62.

Walter, H.E. (1984). Probionases: Methods with haemoglobin, casein and azocoll as substrates. In: H.U. Bergmeyer (Ed), *Methods of Enzymatic Analysis,* (pp. 270-277). Verlag Chemic, Weinheim.

Wassef, E., Saleh, N., Abde-Meguid, E.N., & Abdel-Mohsen, H.H. (2023). Utilization of fish waste biomass as a fishmeal alternative in European seabass (*Dicentrarchus labrax*) diets: effects on immuno-competence and liver and intestinal histomorphology. *International Aquatic Research, 15(2),* 145-161. https://doi.org/10.22034/iar.2023.1968087.1333.

Zablon, W.O., Getabu, A., Ogello, E.O., Omweno, J.O., & Outa, N. (2020). Condition factor of Nile tilapia, O*reochromis niloticus* fry under the influence of different protein levels in a biofloc system. *International Journal of Advanced Research, 8(12),* 25-34. http://dx.doi.org/10.21474/IJAR01/12122.