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Dietary Supplementation of Peanut Worm (*Siphonosoma australe-australe*) Meal Enhances Digestive Enzyme Activities, Feed Efficiency, and Growth Performance of the Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

This study evaluates the effects of dietary supplementation with sea peanut worm meal (SPWM; *Siphosoma australe-australe*) on digestive enzyme activities, feed efficiency, and growth performance of the saline Nile tilapia (*Oreochromis niloticus*). Four isonitrogenous and isoenergetic diets containing 0, 1, 3, and 5% SPWM were fed to tilapia (initial weight 6.23 ± 0.86 g) for 42 days under 10ppt salinity conditions. Growth parameters, organosomatic indices, and digestive enzyme activities (amylase, lipase, protease) were assessed. Results showed that fish receiving SPWM exhibited significantly higher final weight, weight gain, specific growth rate, feed efficiency, protein retention, and protein efficiency ratio compared to the control ($P< 0.05$). Optimal responses were generally obtained at 3% inclusion, which produced the lowest feed conversion ratio (1.91 ± 0.04), the highest feed efficiency (52.43 ± 0.99%), and superior protein retention (57.81 ± 1.06%). Survival rate also improved markedly in SPWM groups (90–100%) versus the control (56.7%). Digestive enzyme activities were significantly enhanced at 3% SPWM, with amylase, lipase, and protease activities reaching 9.27 ± 0.52, 0.53 ± 0.04, and 0.88 ± 0.05 IU/mL, respectively, higher than other treatments. Furthermore, hepatosomatic and intestine somatic indices were significantly elevated in all supplemented groups, indicating improved liver metabolism and intestinal development. These findings demonstrate that moderate inclusion of SPWM (3%) provides functional benefits beyond protein supply, enhancing digestive physiology, nutrient utilization, and growth performance of the Nile tilapia.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is one of the most widely farmed aquaculture species worldwide due to its rapid growth rate, tolerance to a broad range of environmental conditions, and relatively low market price (**El-Sayed, 2020**). In recent decades, the expansion of tilapia farming into saline and brackish-water environments has gained increasing popularity as a strategy to diversify culture sites and utilize less productive land for freshwater aquaculture (**Santos *et al*., 2022**). However, adaptation to saline environments can affect various physiological processes in fish, including feed intake, metabolism, and feed efficiency (**Kassim *et al*., 2021**). These conditions necessitate precise feed formulation strategies to ensure optimal nutrient utilization and growth performance of the fish.

Sustainable and cost-effective protein sources for aquafeeds remain a central challenge for intensifying tilapia production (**Gule & Geremew, 2022; Fantatto *et al*., 2024**). Conventional fishmeal is costly and subject to supply limitations, prompting intensive research into alternative protein ingredients including insect meals, single-cell proteins, and underutilized marine invertebrates that can maintain or improve growth, feed conversion, and digestive physiology when formulated appropriately in fish feed (**Aragão *et al*., 2022; Herath, 2022; Jia *et al*., 2022; Rosle *et al*., 2024**). Several recent studies have demonstrated that partial replacement of fishmeal with alternative animal-source meals (e.g., mealworms, black soldier fly larvae) can support good growth while modulating digestive enzyme activities and nutrient retention of fish. These results indicate that novel protein sources may also act as functional ingredients that influence gut physiology and feed efficiency of fish and white shrimp (**Melenchón *et al*., 2022; Sathishkumar *et al*., 2023; Sridharan *et al*., 2023; Mahato *et al*., 2024**). These findings present a significant opportunity for the utilization of locally available, underexplored feed resources such as specific marine invertebrates in the development of functional aquafeeds that can simultaneously promote growth and improve the overall health of farmed fish. One promising feed ingredient for enhancing feed efficiency, digestive enzyme performance, and growth in the Nile tilapia is the sea peanut worm.

The sea peanut worm (*Siphonosoma australe-australe*), belonging to the phylum Sipuncula, is a benthic marine invertebrate commonly found in tropical and subtropical waters, including coastal areas of Indonesia (**Cutmore *et al*., 2018; Bahtiar *et al*., 2024**). Several studies have reported that peanut worms possess a high protein content (over 50% dry weight) with a complete profile of essential amino acids, as well as bioactive components such as collagen and functional peptides (**Vonnahme *et al*., 2020**). These attributes position peanut worm meal not only as an alternative protein source but also as a potential functional feed ingredient capable of influencing fish digestive enzyme performance. The sea peanut worm is recognized as a nutritionally rich marine organism, offering a variety of health-promoting components. Its composition includes abundant macronutrients such as proteins, lipids, and carbohydrates (**Qi *et al*., 2022;**

Leiwakabessy *et al*., 2024). Additionally, it contains essential micronutrients like calcium, phosphorus, and iodine (**Rahayu *et al*., 2019; Silaban & Rieuwpassa, 2019**), as well as magnesium. It also provides a range of vitamins, including B1, B6, B12, and E, essential fatty acids such as arachidonic, linoleic, and linolenic acids, and non-essential fatty acids including myristic, stearic, palmitic, and pentadecanoic acids (**Silaban & Nanlohy, 2011; Leiwakabessy *et al*., 2024**).

Enhancement of digestive enzymes such as protease, amylase, and lipase plays a crucial role in nutrient utilization efficiency and fish growth (**Saleh *et al*., 2021**). Previous studies have shown that supplementation with specific feed ingredients, particularly those rich in animal protein and bioactive compounds, can modulate digestive enzyme activity, improve feed digestibility, and reduce the feed conversion ratio (FCR) of the Nile tilapia (**Sogbesan & Ugwumba, 2008; Magouz *et al*., 2022**). Therefore, incorporating peanut worm meal into feed is expected not only to meet the protein requirements of saline-adapted the Nile tilapia but also to exert beneficial effects on digestive physiology and feed efficiency.

Despite these advancements, limited research has explored the use of peanut worm (*Siphonosoma australe-australe*) meal as a functional ingredient in aquafeeds, particularly for saline tilapia culture. Peanut worms are benthic marine invertebrates with high crude protein content, a well-balanced amino acid profile, and potential bioactive compounds, yet their application in aquaculture diets remains underrepresented in the scientific literature. Moreover, there is a lack of comprehensive studies evaluating its effects on digestive enzyme activities, feed utilization efficiency, and growth performance under saline culture conditions. Therefore, the present study aims to evaluate the effects of dietary peanut worm meal supplementation on digestive enzyme activity, feed efficiency, and growth performance of the Nile tilapia. The findings of this research are expected to contribute to the development of sustainable, functional, and locally sourced feed formulations that enhance aquaculture productivity while reducing reliance on fishmeal.

MATERIALS AND METHODS

This feeding experiment was carried out over a three-month period, from September to November 2024, at the Fish Culture, Breeding, and Production Laboratory, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia. The experimental procedure comprised several stages: Preparation of the saline tilapia and formulated diets; implementation of the feeding trial; assessment of experimental parameters; and subsequent data analysis.

Experimental diets

Four experimental diets were prepared containing 0, 1.0, 3.0, and 5.0% sea peanut worm meal, formulated to be isonitrogenous (40% crude protein) and isoenergetic (4200

cal/g). The protein fraction was derived from fish meal, telescopium muscle meal, shrimp head meal, sea peanut worm meal (SPWM), and soybean meal, while carbohydrate sources included corn meal, fine bran meal, and sago meal. Corn oil and fish oil served as lipid sources, with a premixed vitamin and mineral supplement also incorporated into the diets.

The preparation of the experimental diets followed these steps: first, all ingredients excluding vitamin, mineral, and lipid components were finely ground and sieved through a 60-mesh screen. Second, the weighed ingredients, based on the formulated proportions, were mixed in sequence from the smallest to the largest inclusion rate until homogeneous. The mixture was then combined with the specified amounts of fish and corn oil along with water and was blended thoroughly to ensure even distribution. Third, the dough was pelletized using a pelletizer with 1.0 and 1.5mm die sizes, followed by sun-drying for two to three days. Once dried, the pellets were packed in plastic bags and stored at room temperature until use. The formulation of the experimental diets and the proximate composition results are presented in Table (1).

Table 1. Formulation of experimental feed and proximate analysis results

Feed ingredients	**Experimental feed (g/100 g feed)**			
	0% SPWM	**1%SPWM**	**3%SPWM**	**5%SPWM**
Jackmackerel fish meal	20	20	20	20
Sea peanut worm meal (SPWM)	0	1	3	5
Telescopium muscle meal	16	15	15	15
Shrimp head meal	20	20	20	19
Soybean meal	15	15	15	15
Corn meal	11	11	10	10
Fine bran meal	8	8	7	7
Sago meal	5	5	5	5
Corn oil	1	1	1	1
Fish oil	2	2	2	2
Vitamin and Mineral mix.*	2	2	2	2
Proximate composition **(**%**)**				
Moisture	7.28	6.79	6.05	6.57
Crude protein	39.84	40.44	41.37	40.56
Crude fat	10.07	10.84	10.14	9.91
Crude ash	10.92	11.24	12.59	12.89
Crude fiber	4.11	4.66	4.92	4.29
NFE **	27.78	26.03	24.93	25.78
GE **(**kal/g**)** ***	4243.52	4276.50	4215.97	4185.30

*Vitamin A 12.000.000 IU, Vitamin D3 2.000.000 IU, Vitamin E 8.000 IU, Vitamin K3 2.000 mg, Vitamin B1 2.000 mg, Vitamin B2 5.000 mg, Vitamin B6 500 mg, Vitamin B12 12.000 ug, Vitamin C 25.000 mg, Calcium-D-pantothenate 6.000 mg, Niacin 40.000 mg, Cholin chloride 10.000 mg, Methionine 30.000 mg, Lysine 30.000 mg, Manganese 120.000 mg, Iron 20.000 mg, Iodine 200 mg, Zinc 100.000 mg, Cobalt 200 mg, Copper 4.000 mg.

NFE: nitrogen-free extract was calculated according to the procedure by **Jiang *et al.* (2015); NFE = 100 – **(**protein + fat + ash**)**; and

***GE: gross energy.

Experimental fish and the acclimatization methods of the Nile tilapia

The Nile tilapia used in this study were sourced from a local fish vendor in Cialam Jaya, Konda District, South Konawe Regency. Prior to stocking in the experimental rearing tanks, the fish were acclimated in 0.5-ton capacity fiberglass tanks for two weeks to adapt them to saline conditions. During acclimation, the salinity of the rearing water was gradually increased by 2ppt per day until it reached 10ppt (**Küçük *et al.*, 2013**). Once the target salinity was achieved, the fish were maintained for several additional days to ensure proper adaptation to the saline environment. When the fish exhibited normal behavior and were fully adapted to the 10ppt salinity, they were transferred to the experimental rearing units.

Feeding trial

Following the acclimation period, the initial body weight of each Nile tilapia specimen was recorded before randomly distributing the fish into 12 rearing containers (63 × 47 × 35 cm), each containing 80L of filtered water adjusted to a salinity of 10ppt and supplied with continuous aeration. In total, 120 fish with an average initial weight of 6.23 ± 0.86g were stocked at a density of 10 individuals per container. The test diets were provided to apparent satiation three times daily at 08.00 a.m, 12.00 a.m, and 04.00 p.m for a period of 42 days. Feces were removed from the tanks each morning prior to feeding. To sustain optimal water conditions, 30% of the culture water was replaced every five days. Fish were weighed at two-week intervals, specifically on days 0, 14, 28, and 42 using a digital scale with 0.01g precision. The total feed intake of the experimental fish was recorded every 14 days, and any mortality fish was documented for use in calculating feed efficiency and survival rate. Ten fish were collected from each treatment for body carcass proximate analysis and assessment of digestive enzyme activity Water quality variables, including temperature (measured using a thermometer), salinity (refractometer), pH (pH meter PH-009(1)), and dissolved oxygen (PDO-519, Japan), were monitored at regular intervals throughout the experimental period.

Proximate analysis of experimental feeds and body carcass

The proximate composition analysis of the experimental diets and body carcass included the determination of moisture, crude protein, crude lipid, crude ash, and crude fiber contents. Moisture content was assessed using the oven-drying method at 110°C in accordance with SNI 01-2891-1992 (section 5.1). Crude protein was quantified using the Kjedahl method (18-8-31/MU/SMM-SIG). Lipid content was analyzed via Soxhlet extraction following method 18-8-5/MU/SMM/SIG (section 3.2.1). Ash content was determined by incinerating samples in a muffle furnace at 600°C according to SNI 01-2891-1992 (section 6.1). Crude fiber was measured through a gravimetric procedure involving digestion of the sample with concentrated acids and alkalis (18-11-111/MU/SMM-SIG).

Samples preparation for ISI, HSI and enzyme activity assay

The enzyme analysis involved the determination of protease, lipase, and amylase activities in the digestive tract and intestinal tissue of the Nile tilapia at the beginning (day 0) and at the end of the feeding trial (day 42). Sample preparation for enzyme assays was carried out in several stages. First, the hepatopancreas and intestine were carefully removed by dissecting the fish after anesthetization, which was achieved by placing them on ice for approximately 10 minutes. Second, both organs were weighed to calculate the intestinal somatic index and hepatosomatic index. Finally, the tissues were transferred into 1.5mL microtubes pre-filled with 10% formalin solution, then stored in a freezer prior to shipment to the Laboratory of Animal Husbandry, IPB University.

The measurement of digestive enzyme activities (Amylase, Lipase and Protease)

Amylase activity was assessed following the procedure described by **Worthington (1993)**, with slight modifications. In brief, 0.5mL of substrate solution was combined with 0.5mL of the supernatant (sample extract) and incubated in a water bath at 95°C for 3 minutes. Subsequently, 0.5mL of dinitrosalicylic acid (DNSA) reagent was added, and the mixture was further incubated at 95°C for 5 minutes. The absorbance of the resulting solution was then measured at 540nm using a spectrophotometer.

Lipase activity was determined according to the method of **Borlongan (1990)**. A 1mL aliquot of lipase substrate was mixed with 1.5mL of buffer solution containing 0.1M Tris-HCl at pH 8.0. This mixture was combined with 1mL of crude enzyme extract and incubated at 37°C for 6 hours. Prior to terminating the incubation, 3mL of 95% ethanol was added. The liberated fatty acids were quantified by titration with 0.01 N NaOH using 0.9% thymolphthalein in ethanol as an indicator.

Protease activity was analyzed based on the method of **Bergmeyer and Grassi (1983)**. Separate tubes were prepared for samples, standards, and blanks. Each tube received 1mL of 0.05M phosphate buffer (pH 7.0) and 1mL of casein substrate solution (20mg/ mL, pH 7.0). For the sample tubes, 0.2mL of the enzyme extract was added; for the standard tubes, 0.2mL of 5mmol/ L tyrosine standard was used; and for the blank tubes, 0.2mL of distilled water was added. All tubes were incubated at 37°C for 10 minutes, followed by the addition of 2mL of 0.1M trichloroacetic acid (TCA). The blank and standard tubes were then supplemented with 0.2mL of 2mmol/ L $CaCl_2$ solution, whereas the sample tubes received 0.2mL of distilled water. The mixtures were incubated again at 37°C for 10 minutes and centrifuged at 3,500 rpm for 10 minutes. From each supernatant, 1.5mL was collected and mixed with 1mL of Folin–Ciocalteu reagent and 5mL of 0.4 M Na_2CO_3. Absorbance was measured at 578nm using a spectrophotometer.

Feed efficiency, growth performance and data analysis

Growth performance was assessed based on several parameters, including initial and final body weights, weight gain (WG = Wt − Wo), specific growth rate (SGR = \[Ln

Wt − Ln Wo] / t × 100), feed conversion ratio (FCR = feed intake / \[Wt − Wo]), total feed consumption (TFC = dry feed offered − dry feed remaining), feed efficiency (FE = \[final body weight − initial body weight] / feed intake), protein retention (PR = \[fish body protein at the end − fish body protein at the beginning] / protein consumed during the rearing period), net protein utilization (NPU = WG / total protein intake), protein efficiency ratio (PER = WG / dry weight of protein intake). All growth performance parameters (WG, SGR, FCR, TFC, FE, PR, PER and SR) were measured. Data on growth performance and digestive enzyme activity were analyzed using one-way analysis of variance (ANOVA). Differences among treatments were evaluated using Duncan's multiple range test (DMRT) in SPSS software (version 20.0), with the significance threshold set at $P< 0.05$.

RESULTS

Growth performance and feed efficiency

The effects of dietary SPWM supplementation at levels of 0, 1, 3, and 5% on growth performance parameters, feed utilization indices, and survival rate of the Nile tilapia are detailed in Table (2). The initial body weight of fish was comparable across all treatments, with no significant differences observed. Final body weight increased significantly in fish fed diets containing SPWM compared to the control group. Specifically, fish receiving 1–5% SPWM (16.07±0.68 g –16.74 ± 0.90 g) exhibited higher final weights than the control (13.15 ± 1.61g), with no significant differences among the SPWM-supplemented groups. Weight gain followed a similar trend, where fish in all SPWM treatments (9.97 ± 0.44 g –10.70 ±0.39 g) showed significantly greater increments compared to the control (6.88±1.13 g). No statistical differences were noted among the SPWM groups. Specific growth rate (SGR) was significantly enhanced with increasing levels of SPWM. The control group recorded the lowest SGR (1.64 ±0.10%), while fish fed 3–5% SPWM showed the highest values (2.26±0.01% – 2.27±0.06%), significantly different from the 1% SPWM group (2.15±0.01%).

Table 2. Parameters of growth performance, feed utilization, and survival rate of the saline Nile tilapia

Parameter	**Groups of Treatments**			
	0% SPWM	**1%SPWM**	**3% SPWM**	**5%SPWM**
Initial weight (g)	6.27±0.48	6.10±0.24	6.05±0.20	5.93±0.15
Final weight (g)	13.15±1.61[a]	16.07±0.68[b]	16.74±0.59[b]	16.50±0.90[b]
WG (g)	6.88±1.13[a]	9.97±0.44[b]	10.70±0.39[b]	10.57±0.75[b]

SGR **(%)**	1.64±0.10[a]	2.15±0.01[b]	2.26±0.01[c]	2.27±0.06[c]
TFC **(**g/ind.**)**	20.14±0.05	19.92±0.30	19.93±0.85	20.22±0.32
FCR	2.34±0.10[a]	2.02±0.08[b]	1.91±0.04[b]	1.97±0.04[b]
FE **(%)**	42.74±1.86[a]	49.56±1.94[b]	52.43±0.99[b]	50.69±0.97[b]
PR **(%)**	42.66±6.92[a]	47.80±2.04[a]	57.81±1.06[b]	57.09±3.66[b]
PER	0.80±0.13[a]	1.14±0.05[b]	1.19±0.02[b]	1.20±0.06[b]
Survival rate **(%)**	56.67±5.77[a]	90±10.0[b]	100[b]	100[b]

Note: The values in same rows with different superscript letters indicate significant differences ($P< 0.05$).

Total feed consumption was not significantly affected by dietary treatments, remaining relatively stable across groups (19.92 ± 0.30 –20.22±0.32 g per individual). Feed conversion ratio (FCR) was significantly improved in SPWM-supplemented groups (1.91±0.04 –2.02±0.08) compared to the control (2.34±0.1), with no significant differences among the SPWM treatments. Feed efficiency (FE) mirrored this trend, with significantly higher values in fish fed SPWM (49.56±1.94% –52.43±0.99%) compared with the control (42.74±1.86%). No differences were observed among the SPWM supplemented groups. Protein retention was significantly increased in fish fed 3–5% SPWM (57.09 ±3.66% –57.81±1.06%) compared to both the control and 1% SPWM groups, which showed lower values (42.66±6.92% –47.80±2.04%). Similarly, protein efficiency ratio (PER) was significantly higher in fish fed SPWM diets (1.14±0.05 – 1.20±0.06) compared to the control (0.80±0.13), with no significant differences among the supplemented groups. Survival rate followed a similar trend, with significantly higher survival in all SPWM treatments (90–100%) compared to the control (56.67%). Overall, these findings indicate that dietary inclusion of SPWM at 1–5% enhances growth performance, feed efficiency, and survival of the Nile tilapia, with optimal responses generally observed at 3–5% inclusion levels.

Hepatosomatic index and Intestine somatic index of Nile tilapia

The effects of dietary SPWM supplementation in the diet on the hepatosomatic index (HSI) and intestine somatic index (ISI) of the Nile tilapia are summarized in Table (3). Both parameters serve as important physiological indicators in fish nutrition studies, as HSI reflects hepatic metabolic activity and energy deposition, while ISI represents the development and functional capacity of the digestive tract. Therefore, evaluating these indices provides critical insights into how dietary interventions influence organ development and nutrient utilization efficiency under saline rearing conditions.

Table 3. Results of hepatosomatic index (HSI) intestine somatic index of the Nile tilapia fed with different levels of SPWM

Parameter	**Groups of treatments**			
	0% SPWM	**1% SPWM**	**3% SPWM**	**5% SPWM**
HSI (%)	1.32±0.11[a]	2.74±0.03[b]	2.80±0.15[b]	2.87±0.1[4b]
ISI (%)	5.75±0.11[a]	8.31±0.13[b]	9.03±1.08[b]	9.00±0.96[b]

Note: The values in same rows with different superscript letters indicate significant differences ($P< 0.05$).

The hepatosomatic index (HSI) of the Nile tilapia showed a clear response to dietary inclusion of SPWM. Fish in the control group (0% SPWM) exhibited the lowest HSI value (1.32 ± 0.11%), which was significantly lower ($P< 0.05$) than all supplemented groups. In contrast, fish fed diets containing 1, 3, and 5% SPWM presented significantly higher and statistically similar HSI values, ranging between 2.74 ± 0.03% and 2.87 ± 0.14%. This indicates that SPWM supplementation, even at the lowest inclusion level, induced a consistent increase in hepatic mass relative to body weight without further incremental changes at higher inclusion levels.

Similarly, the intestine somatic index (ISI) was markedly influenced by SPWM supplementation. The control group recorded the lowest ISI (5.75 ± 0.11%), which was significantly different ($P< 0.05$) from all treatment groups. Diets containing 1–5% SPWM consistently elevated ISI values (8.31±0.13% – 9.03±1.08%), with no significant differences among the supplemented treatments. This suggests that SPWM inclusion promoted intestinal development and mass accretion, with the effect plateauing beyond 1% inclusion.

Digestive enzyme activity of the Nile tilapia

The intestinal digestive enzyme activities of the Nile tilapia in response to dietary supplementation with SPWM in the diet are presented in Table (4). Digestive enzymes such as amylase, lipase, and protease are widely recognized as key physiological indicators of nutrient utilization efficiency in fish, as they directly reflect the capacity of the digestive tract to hydrolyze carbohydrates, lipids, and proteins, respectively. Therefore, monitoring changes in these enzymatic activities provides valuable insights into how dietary interventions modulate digestive physiology and ultimately influence growth performance under saline rearing conditions.

Table 4. Enzyme activity in the intestine of the Nile tilapia at the initial and the end of the experiment

Digestive enzyme activity (IU/ml)	Initial	The end of experiment			
		0% SPWM	1% SPWM	3% SPWM	5% SPWM
Amylase	3.64±0.17	7.34±0.26^{b}	4.67±0.11^{a}	9.27±0.52^{c}	4.60±0.19^{a}
Lipase	0.21±0.02	0.42±0.01^{b}	0.27±0.00^{a}	0.53±0.04^{c}	0.26±0.01^{a}
Protease	0.35±0.02	0.70±0.02^{b}	0.44±0.01^{a}	0.88±0.05^{c}	0.44±0.02^{a}

Note : The values in same rows with different superscript letters indicate significant differences ($P< 0.05$).

The digestive enzyme activities of the Nile tilapia showed clear changes between the beginning and the end of the experiment (Table 4). Amylase activity, which was initially recorded at 3.64±0.17 IU/ml, increased across treatments at the end of the trial. The highest activity was observed in the group receiving 3% SPWM supplementation (9.27±0.52 IU/ml), followed by the 0% control group (7.34±0.26 IU/ml). Both values were significantly higher ($P<0.05$) than those observed in the 1% (4.67±0.11 IU/ml) and 5% (4.60±0.19 IU/ml) SPWM groups, respectively.

A similar pattern was observed for lipase activity. The baseline activity of 0.21±0.02 IU/ml increased at the end of the experiment, with the highest value in the 3% SPWM group (0.53±0.04 IU/ml). This was significantly higher ($P<0.05$) than in the 0% control (0.42±0.01 IU/ml), whereas the 1% (0.27±0.00 IU/ml) and 5% (0.26±0.01 IU/ml) groups showed lower activities, which did not differ significantly from each other.

Protease activity followed the same trend. From an initial level of 0.35±0.02 IU/ml, the activity increased substantially in the 3% SPWM group (0.88±0.05 IU/ml), which was significantly higher than the control (0.70±0.02 IU/ml) and both the 1% (0.44±0.01 IU/ml) and 5% (0.44±0.02 IU/ml) treatments. Again, the statistical notations indicate that treatments with different superscript letters exhibited significant differences, confirming that the 3% SPWM diet promoted superior protease activity compared to other groups. Overall, the findings demonstrate that dietary supplementation with 3% SPWM resulted in the most pronounced enhancement of digestive enzyme activity, particularly amylase, lipase, and protease, whereas lower or higher inclusion levels did not yield comparable benefits.

The activities of digestive enzymes in the Nile tilapia varied considerably between the initial and final phases of the experiment (Table 3). Overall, all enzymes showed an

increase compared to the baseline, although the magnitude of improvement differed depending on the dietary level of SPWM supplementation.

DISCUSSION

Growth performance

The present study demonstrated that dietary inclusion of sea peanut worm meal (SPWM) exerted significant effects on the growth performance, feed efficiency, organosomatic indices, and digestive enzyme activities of the Nile tilapia. The observed responses underline the role of SPWM not only as an alternative protein source but also as a functional dietary component capable of enhancing nutrient utilization and digestive physiology under saline rearing conditions.

The enhanced growth parameters (final weight, weight gain, and specific growth rate) observed in the fish fed 1–5% SPWM indicate that this marine invertebrate meal provides a balanced nutrient profile with bioactive components beneficial for tilapia. Improved feed conversion ratio (FCR) and feed efficiency (FE) further confirm that dietary inclusion of SPWM enhanced nutrient utilization. Similar improvements in feed efficiency and protein utilization have been reported in tilapia and other fish species when conventional protein sources were partially replaced with animal-origin ingredients rich in bioactive compounds (**Rahman *et al*., 2020; Bruni *et al*., 2021**).

Fish fed SPWM diets exhibited significantly higher final body weights compared with the control group, with the best performance observed at 3% inclusion. This pattern suggests that SPWM provided a balanced supply of nutrients and bioactive compounds that supported superior tissue accretion. Similar outcomes have been reported when Nile tilapia diets were supplemented with insect meals such as black soldier fly (BSF) larvae or palm weevil larvae, both of which supply high-quality proteins and functional lipids that stimulate growth (**Chinarak *et al*., 2023; Kariuki *et al*., 2024**).

Absolute weight gain followed a similar trend, with maximum values at 3% SPWM inclusion. The enhanced WG may be attributed to the synergistic effect of essential amino acids and functional peptides in SPWM that improved nutrient digestibility and assimilation. Previous studies have shown that alternative animal protein sources such as earthworm meal or BSF meal can similarly stimulate nutrient deposition and enhance growth in tilapia through their bioactive metabolites (**Odedeyi & Ajani, 2020; Wang *et al*., 2024**). The highest specific growth rate was obtained at 3% SPWM, indicating faster nutrient turnover and assimilation. Improved SGR has also been reported when fishmeal was partially replaced with BSF larvae or palm weevil larvae, where optimal inclusion levels provided essential amino acids and lauric acid that enhance nutrient metabolism (**Lu *et al*., 2022; Zhou *et al*., 2024**)

Feed consumption was generally higher in SPWM-fed groups than in the control, with the highest intake recorded in the 5% group. However, the greatest growth

efficiency was achieved at 3% inclusion, indicating that palatability increased with SPWM addition, but nutrient utilization efficiency peaked at moderate inclusion levels. This mirrors earlier findings that inclusion of earthworm or insect meals improves feed attractiveness while the efficiency of conversion depends on optimal dietary levels (**Makkar *et al.*, 2014**). Feed efficiency was maximized in the Nile tilapia fish receiving 3% SPWM. This reflects the combined effects of improved digestive enzyme activity and nutrient retention, ensuring that a greater proportion of consumed feed was converted into biomass. This aligns with earlier studies showing that insect-based proteins improve feed efficiency when balanced appropriately in the diet (**Nogales-Mérida *et al.*, 2019; Bruni *et al.*, 2021**). SPWM supplementation significantly improved FCR, with the lowest values observed at 3% inclusion, comparable to the control diet. This improvement indicates that SPWM enhanced the efficiency with which ingested feed was converted into body mass of the saline Nile tilapia. Comparable effects have been documented with BSF larvae and earthworm meals, where bioactive compounds and favorable amino acid profiles contributed to reduced FCR (**Odedeyi & Ajani, 2020; Eggink *et al.*, 2022**).

Protein retention was highest in the 3% SPWM group, reflecting superior nitrogen utilization. This outcome is consistent with reports that functional ingredients rich in peptides and collagen, such as peanut worms or earthworms, improve protein deposition efficiency by reducing catabolic losses (**Wang *et al.*, 2024**). Similarly, PER values peaked at 3% SPWM inclusion, confirming that dietary protein was most effectively converted into fish biomass at this level. Insect meals such as BSF and palm weevil larvae have demonstrated similar trends in tilapia, where moderate dietary inclusion enhances protein efficiency, while higher inclusion may introduce indigestible fractions such as chitin that limit protein utilization (**Eggink *et al.*, 2022; Barroso *et al.*, 2025**).

Survival remained high across all treatments (≥93%), indicating that SPWM inclusion up to 5% is safe for saline tilapia culture. This is consistent with the safety profiles reported for other unconventional protein sources such as BSF, earthworm, and palm weevil larvae (**Chinarak *et al.*, 2023; Kariuki *et al.*, 2024**). Overall, the findings demonstrate that SPWM supplementation at 3% provides optimal benefits in terms of growth performance, nutrient retention, and digestive efficiency, paralleling the functional benefits reported for other bioactive-rich animal protein sources in aquafeeds.

Hepatosomatic index (HSI) and intestine somatic index (ISI)

In the present study, HSI values were significantly higher in SPWM-supplemented groups compared with the control, with the most consistent increases observed from 1% inclusion onward. The elevation of HSI indicates enhanced hepatic metabolic activity and energy storage, which are commonly associated with improved nutrient assimilation and deposition. The liver is central to intermediary metabolism, regulating protein turnover, glycogen storage, and lipid metabolism; thus, increased HSI

often reflects heightened nutrient processing capacity in response to improved dietary quality (**Krogdahl *et al*., 2015**). The elevation of HSI in SPWM-supplemented groups suggests enhanced hepatic metabolic activity, which may reflect increased glycogen or lipid storage as a result of improved nutrient intake and assimilation. Previous studies have emphasized that an increase in HSI often indicates a greater metabolic demand on the liver due to higher feed efficiency and protein turnover (**Krogdahl *et al*., 2015; Henry *et al*., 2022**).

Comparable findings have been reported in tilapia fed earthworm meal, where elevated HSI was associated with improved protein metabolism and increased hepatocellular growth, likely due to the presence of bioactive peptides and enzymes such as lumbrokinase that stimulate hepatic function (**Odedeyi & Ajani, 2020; Wang *et al*., 2024**). Similarly, studies on palm-weevil larvae supplementation revealed increases in HSI linked to their high lipid and amino acid content, which may promote energy storage and liver hypertrophy while maintaining healthy physiological status (**Chinarak *et al*., 2023**). Black soldier fly (BSF) larvae meal has also been shown to influence HSI in tilapia; when included at moderate levels, BSF improved liver condition and nutrient storage, whereas excessive inclusion sometimes reduced HSI due to the indigestible chitin fraction (**Eggink *et al*., 2022; O'Neill *et al*., 2025**). These findings suggest that functional metabolites in SPWM including collagen, peptides, and essential fatty acids may act synergistically to promote liver function and nutrient metabolism, consistent with the elevated HSI observed in this trial.The elevation of HSI in SPWM-fed tilapia suggests that liver function and metabolic activity were stimulated. The liver serves as a central hub for nutrient metabolism, regulating glycogen storage, lipid turnover, and protein synthesis. Bioactive peptides derived from SPWM proteins may have promoted hepatocellular growth and enhanced protein anabolism, similar to the hepatotrophic effects of earthworm peptides that have been reported to upregulate protein synthesis and modulate antioxidant status in fish (**Wang *et al*., 2024**). Furthermore, the fatty acid profile of SPWM, which includes arachidonic, linoleic, and linolenic acids (**Leiwakabessy *et al*., 2017; Silaban & Rieuwpassa, 2019**), may have contributed to improved hepatic lipid metabolism, providing energy substrates and structural components for membrane synthesis. These combined effects may explain the increased hepatic mass and metabolic efficiency reflected by the higher HSI values in SPWM-fed tilapia.

ISI values also increased significantly in SPWM-fed tilapia, with the highest values recorded in the 3–5% inclusion groups. This indicates improved intestinal development, which likely translates into greater absorptive capacity and enhanced digestive efficiency. Morphological adaptation of the intestine is considered a hallmark response to functional feed ingredients, reflecting increased villus height, mucosal thickness, or intestinal mass that expand the absorptive surface area (**Nogales-Mérida *et***

***al.*, 2019**). Previous studies support these findings. Tilapia fed earthworm meal exhibited greater intestinal mass and improved gut health, attributed to antimicrobial peptides and secondary metabolites in earthworms that modulate intestinal microbiota and stimulate mucosal growth (**Odedeyi & Ajani, 2020; Wang *et al.*, 2024**). Palm-weevil larvae meal has also been associated with intestinal development, where the high lipid and amino acid content contributed to increased mucosal surface area and nutrient absorption efficiency (**Chinarak *et al.*, 2023**). Likewise, BSF larvae supplementation enhanced ISI and intestinal morphology in tilapia when provided at optimal levels, but higher inclusion sometimes led to a reduced ISI due to the structural barrier posed by chitin (**Nogales-Mérida *et al.*, 2019; Eggink *et al.*, 2022**).

The combined stimulation of liver metabolism and intestinal development by SPWM translated into marked improvements in growth parameters, including final body weight, weight gain, and specific growth rate. The observed increase in protein retention and feed efficiency suggests that SPWM not only provided high-quality nutrients but also acted as a functional ingredient that enhanced nutrient assimilation. This is consistent with earlier reports on black soldier fly (BSF) larvae meal, where secondary metabolites such as lauric acid modulated gut microbiota and improved feed efficiency in tilapia (**Nogales-Mérida *et al.*, 2019; Zhou *et al.*, 2024**). By providing both nutritional and functional benefits, SPWM supports the dual role of novel marine invertebrate proteins in aquaculture feeds supplying essential nutrients while enhancing physiological efficiency.

Digestive enzyme activity

The present study demonstrated that dietary supplementation with peanut worm (*Siphonosoma australe-australe*) meal (SPWM) significantly influenced the activities of key digestive enzymes, including amylase, lipase, and protease, in the saline Nile tilapia (*Oreochromis niloticus*). Among the tested inclusion levels, 3% SPWM consistently yielded the highest enzymatic activities, whereas both lower (1%) and higher (5%) inclusion rates did not produce similar enhancements. This indicates that moderate inclusion levels may provide an optimal balance between nutrient enrichment and physiological regulation of digestive function.

The activities of amylase, lipase, and protease were significantly enhanced in the 3% SPWM group, suggesting that this level of supplementation optimized digestive capacity. Enhanced amylase activity implies improved carbohydrate hydrolysis, whereas higher lipase and protease activities reflect greater efficiency in lipid and protein digestion. Similar patterns have been documented when fish diets were supplemented with alternative animal protein sources containing bioactive peptides and functional compounds that modulate enzyme expression (**Sogbesan & Ugwumba, 2008; Bruni *et al.*, 2021**). The plateau or decline in enzyme activity at 5% SPWM suggests that higher inclusion may exceed the optimal physiological threshold, leading to a reduced enzymatic stimulation. This agrees with the concept of optimal inclusion levels of novel ingredients,

beyond which no further benefits or even negative effects may occur (**Makkar *et al*., 2014**).

Amylase activity increased markedly in tilapia fed the 3% SPWM diet, reaching significantly higher levels than in the control group. Enhanced amylase activity suggests an improved capacity to hydrolyze dietary starch and non-structural carbohydrates, which may contribute to better energy utilization. Previous studies have shown that alternative animal protein sources can stimulate endogenous amylase secretion in tilapia, possibly due to synergistic effects of amino acids and bioactive peptides on pancreatic enzyme regulation (**Krogdahl *et al*., 2015; Yusuf *et al*., 2020; Bruni *et al*., 2021**). The presence of functional nutrients such as essential fatty acids and vitamins in peanut worm meal may also play a role in stimulating carbohydrate metabolism.

Lipase activity exhibited a similar pattern, with the highest value observed in fish receiving 3% SPWM. Elevated lipase activity is directly associated with improved lipid digestion and fatty acid absorption, processes that are critical for membrane biosynthesis and energy metabolism. Comparable findings have been reported in tilapia diets supplemented with insect meal and other marine invertebrates, which were shown to enhance lipase activity and lipid utilization efficiency (**Nogales-Mérida *et al*., 2019; Rahman *et al*., 2020**). The relatively lower lipase activity in the 1% and 5% groups suggests that both under- and over-supplementation may disrupt the optimal physiological stimulation of lipolytic enzymes.

Protease activity followed the same trend, being the highest in fish fed 3% SPWM. The enhanced protease activity indicates a greater efficiency in protein hydrolysis and amino acid assimilation. Given that peanut worm meal contains more than 50% crude protein and a balanced amino acid profile (**Vonnahme *et al*., 2020**), it is plausible that these properties directly contribute to the stimulation of proteolytic enzymes. Higher protease activity has been positively correlated with improved growth and feed efficiency in tilapia and other fish species (**El-Sayed, 2020; Desouky *et al*., 2024**). Therefore, the proteolytic stimulation observed in this study may explain the enhanced feed efficiency and protein retention recorded in fish fed 3% SPWM diets. Moreover, the dose-dependent effects observed here, with optimal enzyme stimulation at 3% SPWM, suggest that careful optimization of inclusion levels is necessary to maximize benefits without causing metabolic imbalance. This aligns with earlier reports where excessive inclusion of alternative protein sources occasionally suppressed enzymatic activity or reduced feed palatability (**Kroeckel *et al*., 2012; Bruni *et al*., 2021**).

The upregulation of digestive enzymes was directly associated with improved outcomes. Fish receiving SPWM, particularly at 3%, exhibited significantly lower feed conversion ratios (FCR) and higher feed efficiency (FE) compared with the control group. Enhanced enzymatic hydrolysis allows for more efficient nutrient assimilation, reducing the quantity of feed required to achieve equivalent weight gain. This aligns with

previous findings where dietary supplementation with alternative protein sources such as earthworm meal (**Sogbesan & Ugwumba, 2008**), sago worm meal (**Suprayudi *et al.*, 2014**), and black soldier fly larvae meal (**Devic *et al.*, 2018**) improved the digestive enzyme activities and subsequently reduced FCR in tilapia. Moreover, higher protease activity in the SPWM-fed groups was paralleled by significantly increased protein retention (PR) and protein efficiency ratio (PER), confirming that SPWM enhances not only nutrient digestion but also the metabolic retention of dietary protein for growth.

Ultimately, these physiological improvements translated into enhanced. Fish fed SPWM diets demonstrated significantly higher final weights, weight gain (WG), and specific growth rates (SGR) compared to controls, with optimal values again observed at 3% supplementation. Enhanced nutrient digestibility and retention through improved enzyme activity provide a mechanistic explanation for the superior growth outcomes. Furthermore, survival rates were significantly higher in SPWM-fed groups, indicating that beyond nutritional support, SPWM may also improve resilience and overall health status under saline conditions. Similar integrative responses linking enzyme activity, feed utilization, and growth have been reported in tilapia and other species fed functional feed ingredients such as insect meals and marine invertebrate proteins (**Nogales-Mérida *et al.*, 2019; Bruni *et al.*, 2021**).

CONCLUSION

The present study demonstrates that dietary inclusion of sea peanut worm meal (SPWM) significantly enhances digestive enzyme activities, feed efficiency, and growth performance of Nile tilapia. Among the tested levels, 3% SPWM supplementation consistently produced the most favorable outcomes, including the highest weight gain, specific growth rate, protein retention, and feed efficiency, while reducing feed conversion ratio. Enhanced enzymatic activity provided a clear mechanistic basis for improved nutrient utilization and growth, indicating that SPWM functions not only as a protein source but also as a functional feed ingredient. Moreover, survival rates were markedly higher in SPWM-fed groups, suggesting additional benefits for health and resilience under saline conditions.

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