



Effects of pineapple waste-derived bromelain on digestive enzymes, nutrient utilization, and economics in *Trichopodus pectoralis* Regan, 1910

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Abstract. The use of agro-industrial by-products as functional feed additives represents a promising strategy to enhance sustainability and digestive efficiency in aquaculture. This study investigated the dose-dependent effects of bromelain derived from pineapple processing waste on digestive physiology, nutrient utilization, and economic performance in snakeskin gourami, *Trichopodus pectoralis* Regan, 1910. Fish (95-98 g) were fed diets supplemented with 0, 4, 8, or 12% bromelain for 90 days in a completely randomized design. Growth performance, survival, and feed utilization were not significantly affected by dietary treatments ($p > 0.05$), suggesting that exogenous protease supplementation did not enhance growth under nutritionally adequate conditions. However, bromelain inclusion elicited a pronounced, dose-dependent modulation of digestive enzyme activity. The 8% supplementation significantly enhanced pancreatic protease and amylase activities. This indicates improved digestive capacity and better nutrient assimilation. In contrast, the 12% inclusion level was associated with reduced activity of specific enzymes, suggesting feedback inhibition or enzymatic imbalance at excessive doses. Despite the absence of significant growth responses, a progressive increase in benefit-cost ratio was observed with increasing bromelain inclusion, reflecting improved production efficiency. Variations in muscle composition were primarily attributed to moisture-associated dilution effects rather than reduced protein deposition. Collectively, these findings demonstrate that bromelain derived from pineapple waste functions as a physiologically active feed additive that modulates digestive processes in a dose-dependent manner, with 8% identified as the optimal inclusion level. This study provides mechanistic evidence supporting the valorization of agro-industrial waste as a functional input in sustainable aquaculture systems.

Keywords: aquaculture, bromelain, functional feed additive, pineapple waste, protease supplementation, snakeskin gourami.

Introduction. Aquaculture is rapidly expanding, increasing demand for high-quality diets. Reliance on protein-rich formulations remains costly and environmentally challenging. For numerous aquaculture species, including the Snakeskin gourami, *Trichopodus pectoralis* Regan, 1910, protein demands are comparatively elevated; consequently, augmenting dietary protein concentrations has been demonstrated to improve both growth performance and feed efficiency (Hossain & Jauncey, 1997; Baishya et al., 2012). Conversely, the dependence on high-protein diets, particularly those incorporating fishmeal or high-quality plant proteins, substantially increases production costs and potentially exacerbates environmental issues, such as nutrient loading and the degradation of water quality (Gatlin et al., 2007; Castillo & Gatlin, 2015). These difficulties have spurred investigations into cost-effective and sustainable feeding strategies designed to enhance nutrient utilization efficiency within aquaculture systems. A potentially beneficial method involves the application of exogenous digestive enzymes as functional feed additives. Proteolytic enzymes such as bromelain improve protein

digestion, nutrient absorption, and reduce the adverse effects of anti-nutritional factors in plant-derived ingredients (Bhattacharyya, 2008; Chakraborty et al., 2021). Bromelain, a cysteine protease complex primarily sourced from pineapple (*Ananas comosus*), demonstrates substantial proteolytic activity and possesses supplementary biological characteristics, such as anti-inflammatory and antimicrobial properties (Ketnawa et al., 2012; Santos et al., 2020; Chakraborty et al., 2021).

In aquaculture, dietary supplementation with bromelain or other proteases has been shown to improve growth performance, feed utilization, and digestive enzyme activity in several species, such as tilapia, grass carp, sterlet, and Pacific white shrimp (Choi et al., 2016; Yuangsoi et al., 2018; Wiszniewski et al., 2019; Klahan et al., 2023). Enzymes derived from agro-industrial waste streams offer significant prospects for sustainable aquaculture and the circular bioeconomy, extending beyond their immediate utility. The pineapple processing industry generates considerable waste, including peels and crowns. These by-products are rich in bromelain and can be used as beneficial feed additives (Ketnawa et al., 2012; Santos et al., 2020). Utilizing such waste materials mitigates environmental impacts while simultaneously fostering cost savings and resource optimization within aquaculture production systems. Despite growing interest in bromelain supplements, its application in *T. pectoralis* aquaculture has received limited attention. This economically important freshwater species in Southeast Asia is commonly cultured under intensive farming conditions. However, problems like poor feed efficiency, digestive issues, and higher death rates have been reported, especially when fish are kept in crowded conditions and fed plant-based diets (Saowakoon et al., 2021; Swain et al., 2022). Exogenous proteases enhance dietary protein hydrolysis, improve nutrient utilization, and increase the efficiency of plant-based aquafeeds (German & Bittong, 2009; Duarte et al., 2015; Maas et al., 2019). Moreover, the efficacy of enzyme supplementation is subject to a variety of influences, encompassing enzyme dosage, dietary composition, the specific fish species, and prevailing physiological conditions. An overabundance of enzyme inclusion can potentially yield reduced benefits or even impede digestive functions, thereby underscoring the necessity of establishing optimal supplementation levels (Lanari et al., 1998; Chen et al., 2024).

Consequently, this study aimed to assess the effects of adding bromelain, derived from pineapple waste, at different amounts, on growth, feed efficiency, digestive enzyme activity, carcass composition, and economic outcomes in *T. pectoralis*. Furthermore, this study aims to provide insights into the potential use of by-products from agriculture and industry as sustainable, functional feed additives in aquaculture.

Material and Method

Preparation of bromelain extract. Bromelain extract was prepared from pineapple (*Ananas comosus*) processing waste, including peels and crowns, collected from local farms in Phetchaburi and Prachuap Khiri Khan provinces, Thailand. The extraction procedure followed the method described by Klahan et al. (2023) with slight modifications. Briefly, raw materials were thoroughly washed with tap water, air-dried, and chopped into small pieces. The samples were then homogenized with distilled water at a ratio of 1:0.5 (w/w) using a commercial blender for 5 min. The homogenate was filtered through cheesecloth and centrifuged at 6,000 rpm for 20 min at 4°C. The supernatant was collected and used as crude bromelain extract. Enzyme activity was determined according to Ketnawa et al. (2012) prior to dietary incorporation.

Experimental diets. A commercial pellet feed containing 32% crude protein was used as the basal diet. Bromelain extract was supplemented at four levels: 0% (control), 4%, 8%, and 12% (w/w). For all treatments, the total liquid volume applied to the feed was standardized at 12% by adjusting with distilled water. The bromelain extract was sprayed onto feed pellets, air-dried, and stored at ambient temperature until use.

Experimental fish and rearing conditions. *T. pectoralis* with an average initial weight of 95-98 g was obtained from a commercial farm in Samut Songkhram Province, Thailand. Fish were acclimated for 7 days in a 2 m³ tank and fed a commercial diet (32% crude protein). After acclimation, fish were randomly distributed into experimental units. The experiment was conducted using a completely randomized design (CRD) with four dietary treatments and three replicates per treatment. Each replicate consisted of five fish stocked in 200 L tanks with continuous aeration. Fish were fed to apparent satiation twice daily (morning and evening) for 90 days. The experiment was carried out at the Aquaculture Program, Faculty of Agricultural Technology, Phetchaburi Rajabhat University, Thailand.

Data collection and growth performance. At the end of the feeding trial, we counted and individually weighed each fish to calculate growth performance, including weight gain, specific growth rate (SGR), survival, and yield. Feed intake was recorded daily, and feed utilization indices, including feed conversion ratio (FCR) and protein efficiency ratio (PER), were calculated. Water quality parameters, including temperature, pH, ammonia, and nitrite, were monitored weekly throughout the experimental period.

Proximate composition and carcass analysis. At the end of the experiment, fish samples were collected for carcass and proximate composition analysis. Edible flesh, bone, and internal organs were separated and weighed to determine carcass composition. Hepatosomatic index (HSI) and gonadosomatic index (GSI) were calculated. Proximate composition of fish muscle, including moisture, crude protein, lipid, ash, and fiber, was analyzed following standard methods of Horwitz & Latimer (2005). Moisture content was determined by oven-drying at 105±2°C to constant weight. Ash content was measured by incineration at 550±25°C. Crude protein was quantified using the Kjeldahl method ($N \times 6.25$), whereas crude lipid was analyzed by Soxhlet extraction with petroleum ether. Crude fiber was determined using the Weende method.

Digestive enzyme activity. Digestive organs, including stomach, intestine, pyloric caeca, liver, and pancreas, were dissected and homogenized for enzyme activity analysis. The activities of protease, amylase, and lipase were determined and expressed as U/min/mg protein. Enzyme assays were conducted following standard biochemical procedures as described in previous studies.

Economic analysis. Economic performance was evaluated based on production cost and benefit-cost (B/C) ratio. Production cost per kilogram of fish was calculated from feed cost and total biomass yield, while the B/C ratio was determined as the ratio of total revenue to total production cost.

Statistical analysis. All data were analyzed using one-way analysis of variance (ANOVA) to evaluate differences among treatments. When significant differences were detected, means were compared using Duncan's multiple range test. Statistical significance was set at $p < 0.05$. Data are presented as mean±standard error (SE).

Ethical approval. All experimental procedures involving animals were conducted in accordance with institutional guidelines for the care and use of laboratory animals. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of Phetchaburi Rajabhat University (Approval No. IACUC PBRU 111267007).

Results

Growth performance and yield. Growth performance, survival rate, and yield of *T. pectoralis* fed with dietary bromelain are presented in Table 1. No significant differences ($p > 0.05$) were found among dietary treatment for final weight, weight gain, specific growth rate, or survival rate. The survival rate remained consistently high (93.33-100%) in all treatments, indicating that bromelain supplementation did not adversely affect the

fish health. Although statistical significance was not observed, fish fed the 12% bromelain diet consistently exhibited numerically greater weight gain, SGR, and yield than the other treatments.

Table 1

Growth performance, survival rate, and yield of *Trichopodus pectoralis* fed diets supplemented with different levels of bromelain extract for 90 days

Parameters	Crude bromelain extract supplementation (%)			
	0	4	8	12
Initial weight (g fish ⁻¹)	98.88±21.09 ^a	95.97±5.17 ^a	96.04±16.21 ^a	95.92±15.46 ^a
Final weight (g fish ⁻¹)	121.42±22.03 ^a	115.30±19.75 ^a	117.96±16.15 ^a	119.64±15.45 ^a
Weight gain (g fish ⁻¹)	22.53±5.21 ^a	19.33±4.96 ^a	21.92±2.27 ^a	23.72±2.35 ^a
Percentage of weight gain (%)	23.37±7.26 ^a	20.00±2.86 ^a	23.25±4.60 ^a	25.14±4.73 ^a
Average daily gain (g fish ⁻¹ day ⁻¹)	0.29±0.06 ^a	0.25±0.07 ^a	0.28±0.03 ^a	0.31±0.30 ^a
Specific growth rate (% day ⁻¹)	0.26±0.07 ^a	0.23±0.02 ^a	0.26±0.05 ^a	0.28±0.05 ^a
Survival rate (%)	100.00±0.00 ^a	100.00±0.00 ^a	93.33±11.54 ^a	100.00±0.00 ^a
Yield (g)	471.53±41.63 ^a	576.53±98.75 ^a	539.20±9.33 ^a	598.23±77.26 ^a

Feed utilization and economic performance. Feed utilization parameters, did not differ significantly among treatments ($p > 0.05$) (Table 2). Numerically, fish receiving the 12% bromelain diet showed the lowest FCR and the highest PER, together with the highest benefit-cost ratio, suggesting modest practical advantages despite the absence of statistical significance.

Table 2

Feed utilization efficiency, production cost, and B/C ratio of *Trichopodus pectoralis* fed diets supplemented with different levels of bromelain extract for 90 days

Parameters	Crude bromelain extract supplementation (%)			
	0	4	8	12
Total feed intake (g fish ⁻¹)	63.15±2.62 ^a	56.66±7.16 ^a	66.47±8.45 ^a	63.31±5.76 ^a
Daily feed intake (g fish ⁻¹ day ⁻¹)	0.73±0.12 ^a	0.71±0.17 ^a	0.82±0.26 ^a	0.77±0.13 ^a
Feed intake (g fish ⁻¹ day ⁻¹)	0.83±0.29 ^a	0.74±0.07 ^a	0.86±0.23 ^a	0.82±0.05 ^a
Feed conversion ratio	1.98±0.58 ^a	2.18±0.78 ^a	2.09±0.35 ^a	1.87±0.13 ^a
Protein efficiency ratio	1.16±0.32 ^a	1.10±0.43 ^a	1.06±0.17 ^a	1.17±0.08 ^a
Production cost (Baht Kg ⁻¹)	89.35±8.26 ^a	84.78±4.00 ^a	106.00±24.95 ^a	88.09±8.87 ^a
B/C ratio	1.10±0.19 ^a	1.20±0.07 ^a	1.17±0.16 ^a	1.48±0.25 ^a

The proximate composition of the muscle tissue from each experimental group is presented in Table 3. Analysis of variance indicated significant treatment-related variations ($p < 0.05$) in moisture, crude protein, and ash content. In particular, specimens subjected to the 12% bromelain treatment exhibited the highest tissue hydration levels, alongside a corresponding decrease in crude protein concentration when assessed on a wet-weight basis. Conversely, the 4% and 8% inclusion levels yielded comparatively superior protein deposition, suggesting a threshold effect in nutrient assimilation relative to bromelain concentration. No significant differences ($p > 0.05$) were found in lipid content among treatments. When considered in relation to moisture variation, the observed reduction in protein percentage in the 12% group may reflect a dilution effect associated with increased water content rather than a decrease in absolute protein deposition.

Table 3

Proximate composition of *Trichopodus pectoralis* fed diets supplemented with different levels of bromelain extract for 90 days (dry weight basis)

Parameters (%)	Crude bromelain extract supplementation (%)			
	0	4	8	12
Protein	82.28±3.05 ^b	80.39±0.93 ^a	78.01±1.53 ^a	76.71±1.13 ^b
Fat	13.27±0.84 ^a	13.95±4.19 ^a	15.59±0.57 ^a	14.08±0.93 ^a
Ash	5.29±0.06 ^b	5.70±0.31 ^a	5.54±0.07 ^{ab}	3.77±0.11 ^c
Fiber	0.22±0.01 ^a	0.23±0.03 ^a	0.21±0.02 ^a	0.24±0.01 ^a

Carcass composition and somatic indices are presented in Table 4. Significant differences ($p < 0.05$) were observed in the proportions of edible flesh and the hepatosomatic index (HSI). The fish consuming the 12% bromelain diet demonstrated the highest edible flesh yield and HSI, suggesting a more advantageous allocation of nutrients to muscle and liver tissues. Fish that received bromelain supplementation at 4-12% exhibited increased proportions of bone and viscera; however, no statistically significant differences ($p > 0.05$) were observed in the indices of the stomach and pancreas. The gonadosomatic index (GSI) was significantly greater in the control group relative to the bromelain-supplemented groups ($p < 0.05$), suggesting a potential reallocation of energy from reproductive development to somatic growth in the fish that received supplementation.

Table 4

Carcass composition, hepatosomatic index (HSI), and gonadosomatic index (GSI) of *Trichopodus pectoralis* fed diets supplemented with different levels of bromelain extract for 90 days

Carcass composition (%)	Crude bromelain extract supplementation (%)			
	0	4	8	12
Edible flesh	43.77±1.52 ^b	46.44±0.42 ^{ab}	43.77±2.3 ^b	47.92±1.79 ^a
Bone	31.63±1.58 ^b	34.93±0.38 ^a	34.54±1.49 ^a	33.81±0.45 ^{ab}
Viscera	3.19±0.08 ^b	3.65±0.46 ^{ab}	4.00±0.18 ^a	3.71±0.18 ^{ab}
Stomach	0.52±0.02 ^a	0.53±0.10 ^a	0.44±0.05 ^a	0.54±0.11 ^a
Intestine	1.08±0.04 ^c	1.41±0.01 ^{bc}	1.97±0.35 ^a	1.54±0.13 ^b
Pyloric caeca	0.23±0.00 ^a	0.17±0.04 ^{ab}	0.17±0.07 ^{ab}	0.11±0.02 ^b
Pancreas	0.02±0.02 ^a	0.01±0.01 ^a	0.02±0.01 ^a	0.02±0.00 ^a
Hepatosomatic index (HSI)	1.19±0.01 ^b	1.13±0.06 ^{bc}	0.91±0.16 ^c	1.54±0.21 ^a
Gonadosomatic index (GSI)	0.54±1.14 ^a	0.23±0.02 ^b	0.21±0.02 ^b	0.27±0.03 ^b

Dietary bromelain significantly affected digestive enzyme activities in several digestive organs (Table 5; $p < 0.05$). Fish fed the 8% bromelain diet exhibited the highest pancreatic protease and amylase activities, indicating enhanced digestive capacity. In contrast, pancreatic lipase activity declined at the 12% inclusion level, whereas lipase activity in the pyloric caeca increased. These findings demonstrate a dose-dependent modulation of digestive enzyme activity, with the greatest overall response observed at the 8% supplementation level.

Table 5

Specific protease, amylase, and lipase activities in digestive organs of *Trichopodus pectoralis* fed diets supplemented with different levels of bromelain extract for 90 days

Organs	Crude bromelain extract supplementation (%)			
	0	4	8	12
<i>Protease (U min⁻¹ mg⁻¹ protein)</i>				
Stomach	0.02±0.00 ^a	0.03±0.00 ^a	0.01±0.00 ^b	0.02±0.00 ^a
Intestine	0.22±0.0 ^b	0.22±0.0 ^b	0.24±0.00 ^a	0.23±0.00 ^{ab}
Pyloric caeca	1.15±0.04 ^a	0.74±0.04 ^b	0.68±0.33 ^b	0.19±0.02 ^c
Liver	0.03±0.00 ^b	0.05±0.00 ^a	0.01±0.00 ^c	0.01±0.00 ^d
Pancreas	1.16±0.07 ^b	1.80±0.2 ^b	6.93±0.34 ^a	1.34±0.16 ^b
<i>Amylase (U min⁻¹ mg⁻¹ protein)</i>				
Stomach	0.08±0.0 ^b	0.10±0.00 ^a	0.10±0.00 ^a	0.08±0.00 ^b
Intestine	0.03±0.00 ^c	0.05±0.00 ^b	0.05±0.00 ^b	0.06±0.00 ^a
Pyloric caeca	0.27±0.0 ^a	0.23±0.02 ^b	0.26±0.0 ^a	0.17±0.00 ^c
Liver	0.07±0.00 ^{ab}	0.07±0.0 ^b	0.08±0.00 ^a	0.07±0.00 ^{ab}
Pancreas	2.90±0.15 ^c	3.80±0.20 ^b	9.98±0.17 ^a	3.03±0.06 ^c
<i>Lipase (U min⁻¹ mg⁻¹ protein)</i>				
Stomach	0.18±0.0 ^a	0.12±0.0 ^c	0.15±0.0 ^b	0.11±0.00 ^c
Intestine	0.11±0.0 ^a	0.09±0.0 ^b	0.08±0.00 ^b	0.12±0.0 ^a
Pyloric caeca	0.23±0.02 ^b	0.20±0.0 ^{bc}	0.19±0.0 ^c	0.47±0.0 ^a
Liver	0.09±0.00 ^a	0.11±0.0 ^{ab}	0.12±0.0 ^a	0.12±0.0 ^a
Pancreas	0.27±0.0 ^c	0.41±0.08 ^b	1.22±0.08 ^a	0.10±0.03 ^d

Discussion. This study demonstrates that bromelain derived from pineapple waste primarily modulates digestive physiology rather than growth performance in *T. pectoralis* under nutritionally adequate conditions. The absence of significant differences in growth, feed utilization, and survival suggests that endogenous digestive capacity was sufficient to support protein digestion at the dietary protein level used (32%) (Rostika et al., 2018). This is consistent with previous studies showing limited growth responses to exogenous protease supplementation when diets are already highly digestible or nutritionally balanced (Maas et al., 2019; Wiszniewski et al., 2019; Saleh et al., 2022; Thaiso et al., 2024; Souza et al., 2025). Thus, the lack of growth enhancement observed here likely reflects a physiological ceiling rather than a lack of functional efficacy.

Despite the absence of significant growth responses, bromelain supplementation elicited a clear, dose-dependent modulation of digestive enzyme activity. The 8% inclusion level markedly increased pancreatic protease and amylase activities. This indicates enhanced digestive capacity and improved nutrient hydrolysis (Wu et al., 2024; Wiszniewski et al., 2022). In aquaculture species, enzymatic responses often precede measurable changes in growth, as digestive regulation represents an early adaptive response to dietary inputs (Thaiso et al., 2024). Enzyme modulation may therefore serve as a sensitive indicator of dietary functionality, even when growth remains statistically unchanged under non-limiting nutritional conditions (Souza et al., 2025). The stimulatory effect observed at moderate inclusion levels may result from enhanced substrate hydrolysis (Wu et al., 2024) This may increase the availability of peptides and amino acids that signal pancreatic enzyme synthesis. Similar mechanisms have been reported in fish and other animals, where exogenous proteases complement endogenous enzyme systems and improve nutrient accessibility (Saowakoon et al., 2021; Sharma et al., 2021; Gopalraaj et al., 2022; Liang et al., 2022; Wiszniewski et al., 2022; Wu et al., 2024). In contrast, the decline in specific enzyme activities at the 12% inclusion level suggests an inhibitory effect at excessive bromelain concentrations. This response may reflect feedback inhibition, whereby high exogenous enzyme levels suppress endogenous enzyme secretion, or proteolytic disruption of regulatory proteins. Such dose-dependent responses highlight the importance of optimizing enzyme inclusion levels, as excessive supplementation may reduce functional efficiency (Sánchez-Alcade et al., 2023; Chen et al., 2024; Sholahuddin et al., 2024).

Although growth performance was not significantly improved, the progressive increase in benefit-cost ratio with bromelain supplementation suggests practical

relevance under production conditions. Even modest improvements in feed utilization can yield economic benefits in intensive aquaculture systems, where feed represents the primary operational cost (Gatlin et al., 2007). The divergence between physiological responses (enzyme activity) and production metrics (growth) further supports the interpretation that bromelain enhances digestive efficiency without necessarily accelerating biomass accumulation over the experimental period. At 12% inclusion, higher tissue water content reduced relative muscle protein percentage on a wet-weight basis; however, total protein deposition remained unaffected (Weatherup & McCracken, 1999; Kim et al., 2021; Wang et al., 2025). From a sustainability perspective, bromelain derived from pineapple waste offers a practical means of integrating circular bioeconomy principles into aquaculture nutrition. Agro-industrial by-products are increasingly recognized as cost-effective sources of bioactive compounds that enhance feed functionality while reducing environmental impact (Reguengo et al., 2022; Saini et al., 2025). Compared with purified enzyme additives, the present findings demonstrate that crude extracts from agricultural waste can exert biologically meaningful effects on digestive processes, supporting both resource valorization and sustainable feed development.

Overall, bromelain acts as a dose-dependent feed additive that modulates digestive enzymes. The 8% inclusion level optimizes digestive function, whereas excessive supplementation may induce inhibitory effects. These findings emphasize that enzyme supplementation strategies should be aligned with dietary composition and physiological capacity, rather than assuming linear benefits with increasing dosage.

Conclusions. Dietary bromelain derived from pineapple processing waste effectively modulated digestive physiology in *T. pectoralis* without adversely affecting growth performance or survival. An inclusion level of 8% optimized pancreatic protease and amylase activities, indicating enhanced digestive capacity, whereas excessive supplementation (12%) appeared to reduce enzymatic efficiency, suggesting a dose-dependent regulatory response. Although growth performance and feed utilization were not improved, while 8% inclusion optimized pancreatic enzyme activity, the 12% level showed marginally higher economic returns. However, given the inhibitory effect on enzyme activity at 12%, the 8% inclusion level represents the most balanced recommendation for practical aquaculture applications. These findings demonstrate that pineapple waste-derived bromelain is a promising functional feed additive that supports sustainable aquaculture by enhancing digestive functionality while promoting the circular bioeconomy principles by valorizing agro-industrial by-products.

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Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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