Effects of maggot meal substitution and papain enzyme supplementation on the growth performance of *Puntigrus tetrazona* (Bleeker, 1855)

[1]Agung S. Batubara, [1]Mustaqim Mustaqim, [1]Binari Manurung, [1]Khairiza Lubis, [2]Rahmatsyah Rahmatsyah, [2]Rita Juliani

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, North Sumatra, Indonesia; [2] Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, North Sumatra, Indonesia. Corresponding author: A. S. Batubara, agungsbb@unimed.ac.id

Abstract. Ornamental fish aquaculture requires cost-effective, high-protein feeds. This study evaluated the effects of maggot meal substitution combined with papain enzyme supplementation on the growth performance of Sumatra barb, *Puntigrus tetrazona* (Bleeker, 1855). Fish were reared under four dietary treatments with varying levels of maggot meal and papain supplementation. Growth performance was assessed based on length, weight gain, specific growth rate, survival rate, and feed conversion ratio. The results showed that dietary treatments significantly affected growth performance parameters ($p < 0.05$), except for survival rate ($p > 0.05$). The treatment with 40% maggot meal and 1% papain supplementation produced the best overall growth performance and feed efficiency. These findings suggest that maggot meal combined with papain enzyme can be an effective alternative protein source for improving growth performance in *P. tetrazona* culture.
Keywords: feed conversion ratio, specific growth rate, survival rate, total length, weight gain.

Introduction. The ornamental fish sector is a significant driver of the Indonesian economy, with freshwater species dominating international exports far ahead of marine ornamental fish (Tarihoran et al., 2023). Within this thriving industry, the sumatran barb, *Puntigrus tetrazona* (Bleeker, 1855), stands out as a highly popular native Indonesian species with strong demand among global hobbyists (Batubara et al., 2025). While wild capture has historically supplied the market, increasing constraints on natural stocks have made captive aquaculture essential to meet the rising annual demand for this exotic fish.

The primary bottleneck in expanding *P. tetrazona* aquaculture lies in feed management, where high production costs are heavily driven by a reliance on commercial fishmeal. To enhance aquaculture productivity and lower operating costs, discovering sustainable alternative protein sources is critical. However, many native species like *P. tetrazona* face production limitations and technological constraints, particularly regarding specialized feed formulations that can support optimal growth and development (Lipscomb et al., 2022).

Maggot meal (*Hermetia illucens*) has emerged as a promising candidate to replace expensive fishmeal due to its rich nutritional profile, containing up to 41.1% protein and 25% fat when processed into meal (Mlaga et al., 2022). Despite its benefits, high chitin levels in insect meal can sometimes hinder nutrient absorption. To overcome this limitation, incorporating exogenous enzymes such as papain is a strategic solution. Papain supplementation has been proven to stimulate protease activity, breakdown complex proteins, and maximize feed efficiency across various fish species (Rachmawati et al., 2019; Yadav et al., 2024).

While extensive research on *P. tetrazona* has covered areas such as genetics (Karthikeyan et al., 2024), parasites (Chang et al., 2021; Sugashri et al., 2024), and reproductive biology (Burduhos 2023), its nutritional optimization remains understudied. Specifically, the combined effect of maggot meal replacement and papain enzyme supplementation on the growth performance of *P. tetrazona* has never been evaluated.

Therefore, this study aims to determine the optimal formulation of maggot meal and papain enzyme to maximize the growth performance, survival rate, and feed efficiency of *P. tetrazona*, providing a practical feed technology to boost its aquaculture productivity.

Material and Method. This research was conducted in July-August 2025 at the Biology Laboratory, Universitas Negeri Medan, Indonesia.

Feed preparation. The feed ingredients used included fish meal, maggot meal, vegetable oil, minerals, and papain enzyme. The feed was substituted with the main test component, maggot meal, at varying percentages of 30%, 40%, and 50% (Table 1). The papain enzyme was dissolved in sufficient water, then the feed was soaked for 10 minutes in the enzyme solution to allow absorption, after which it was fed to the fish. Papain enzyme supplementation was given at a rate of 1% per kg of feed (Wiszniewski et al., 2022).
Control = Commercial feed 40% protein
T1 = Maggot meal 30% + Papain enzyme 1%
T2 = Maggot meal 40% + Papain enzyme 1%
T3 = Maggot meal 50% + Papain enzyme 1%

Table 1

Composition of experimental feed ingredients on Sumatran barb

Ingredient (g 1,000 g^{-1} diet)	*Control*	*T1*	*T2*	*T3*
Maggot meal	Commercial feed	300	400	500
Fish meal	Commercial feed	610	510	410
Tapioca meal	Commercial feed	50	50	50
Mineral premix	Commercial feed	10	10	10
Papain enzyme	Commercial feed	10	10	10
Vitamin premix	Commercial feed	10	10	10
Vegetable oil	Commercial feed	10	10	10
Total		1,000	1,000	1,000

Rearing conditions. *P. tetrazona* samples were collected from ornamental fish shops with relatively equal sizes (total length 4.5 cm and body weight 1.4 g). The fish were quarantined in a fiber pool for one day before being tested in a 30 L tank. Ten fish samples were placed in each test tank. Each treatment was replicated three times, with 10 fish per tank. Fish rearing is carried out for 30 days.

Measurement of growth parameters. The growth performance parameters evaluated in this study included total length (TL), weight gain (WG), specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR). Total length was defined as the fish length measured at the end of the experiment. Weight gain was calculated as:

$$WG\ (g) = Wt - W_0$$

Where:
Wt - the final body weight;
W_0 - the initial body weight.

The specific growth rate was calculated according to the following equation:

$$SGR\ (\%\ day^{-1}) = [(\ln Wt - \ln W_0) / \text{feeding duration (days)}] \times 100$$

The survival rate was calculated as:

$$SR (\%) = (\text{number of fish at the end of the experiment/number of fish at the beginning of the experiment}) \times 100$$

The feed conversion ratio was calculated as:

$$FCR = \text{total feed intake (g)} / (Wt - W_0)$$

where total feed intake represents the total amount of feed consumed during the experimental period (Yanti et al., 2022).

Statistical analysis. Measurement data of TL, WG, SGR, SR, and FCR were then statistically analyzed using SPSS ver. 22 with an analysis of variance (ANOVA) approach.

Results and Discussions. Dietary substitution with maggot meal significantly influenced ($p < 0.05$) the TL, WG, SGR, and FCR of *P. tetrazona*. However, no significant difference ($p > 0.05$) was observed for the SR. Overall, the formulation containing 40% maggot meal + 1% papain enzyme (T2) emerged as the optimal treatment, yielding the highest growth performance and feed efficiency compared to the control and other inclusion levels (Table 2).

Table 2

Results of statistical analysis (ANOVA) on Sumatran barb growth parameters

Parameters	*Treatments*				*Sig.*
	Control	*T1*	*T2*	*T3*	
Total length (cm)	4.53 ± 0.17^{ab}	4.45 ± 0.17^{a}	4.94 ± 0.32^{bc}	5.00 ± 0.22^{c}	0.039
Weight gain (g)	0.07 ± 0.05^{a}	0.26 ± 0.12^{ab}	0.71 ± 0.04^{c}	0.62 ± 0.37^{bc}	0.012
Specific growth rate (% days^{-1})	0.14 ± 0.09^{a}	0.65 ± 0.34^{ab}	1.34 ± 0.10^{b}	1.14 ± 0.68^{b}	0.022
Survival rate (%)	96.67±5.77	90.00±10.00	96.67±5.77	86.77±5.77	0.287
Feed conversion ratio	51.17 ± 44.22^{b}	8.93 ± 5.73^{a}	3.05 ± 0.29^{a}	5.31 ± 4.31^{a}	0.048

Note: Different superscripts on the same row indicate significant differences ($p < 0.05$), where T1 = Maggot meal 30% + Papain enzyme 1%, T2 = Maggot meal 40% + Papain enzyme 1%, T3 = Maggot meal 50% + Papain enzyme 1%.

The combination of maggot meal and papain enzyme substantially enhanced the growth of *P. tetrazona*. Treatment T2 achieved the highest WG at 0.71±0.04 g (Figure 1), outperforming the control and T1 (30% maggot meal + 1% papain enzyme), which showed lower, statistically equivalent growth. Interestingly, increasing the maggot meal inclusion beyond 40% (T3: 50% maggot meal + 1% papain enzyme) did not yield further improvements, resulting in a statistically similar WG of 0.62±0.37 g. This indicates a plateau effect where higher inclusion rates do not correlate with proportional growth.

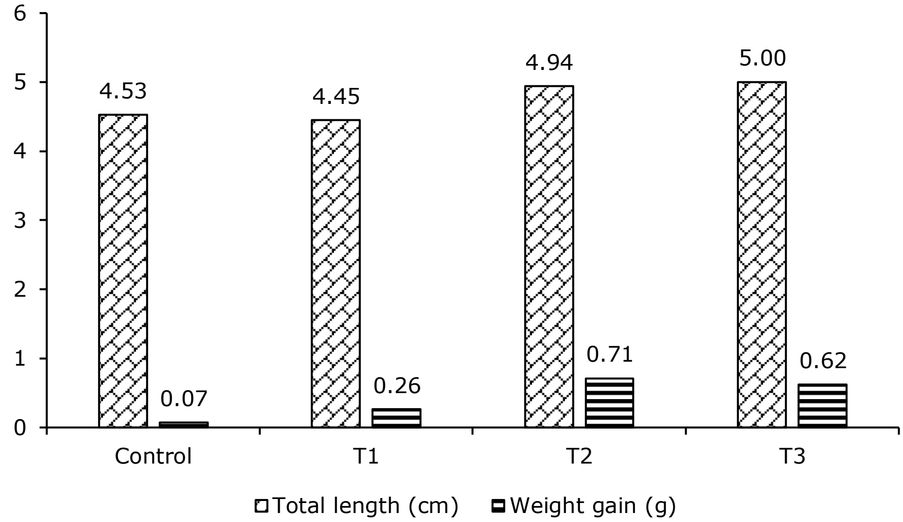

Figure 1. Total length and weight gain of *Puntigrus tetrazona* fed with varying levels of maggot meal and 1% papain enzyme supplementation.

This optimal growth in T2 is driven by the synergistic effect of highly nutritious insect protein and exogenous enzyme supplementation. While maggot meal provides an excellent amino acid profile, its chitin content can often limit nutrient availability. The addition of 1% papain enzyme effectively mitigates this by stimulating endogenous protease activity, breaking down complex proteins into easily absorbable peptides and amino acids (Rachmawati et al., 2019). Consequently, the growth observed in this study surpasses previous benchmarks for *P. tetrazona*, such as Chapman (1997), who reported a WG of only 0.55 g over an 8-week rearing period using a standard 45% protein commercial diet.

The SGR trends mirrored the weight gain patterns, with T2 demonstrating the peak metabolic performance (Figure 2). From a metabolic standpoint, the balanced 40% maggot meal incorporation aligns with the dietary energy-to-protein requirements of *P. tetrazona*. When exogenous papain is introduced, it enhances protein digestibility and elevates digestive enzyme activity in the gut (Yadav et al., 2024). This accelerates nutrient assimilation, allowing more assimilated energy to be channeled toward somatic growth rather than metabolic maintenance. Similar improvements in SGR and protein utilization via papain supplementation have been documented in other commercially important species, such as *Oreochromis niloticus* (Rostika et al., 2018) and *Cyprinus carpio* (Singh et al., 2011).

Interestingly, despite significant growth performance improvements up to treatment T2, the decline or stagnation of growth in treatment T3 (50% maggot meal) indicates a physiological tolerance limit for *P. tetrazona* digestion. This phenomenon is strongly suspected to be related to the accumulation of chitin (the main structural component of the maggot exoskeleton), which increases with increasing substitution doses. Although papain enzyme was added to aid protein hydrolysis, papain is not a specific chitin-degrading enzyme (chitinase). Consequently, the excessive chitin content at a dose of 50% actually begins to act as an antinutritional substance, increasing the viscosity of the chyme in the digestive tract, accelerating gastric emptying, and ultimately reducing the efficiency of core nutrient absorption (Pascon et al., 2025). This bell-shaped growth pattern resulting from the chitin tolerance limit is also frequently observed in other aquaculture commodities when fed excessive insect-based feeds (Hasan et al., 2023).

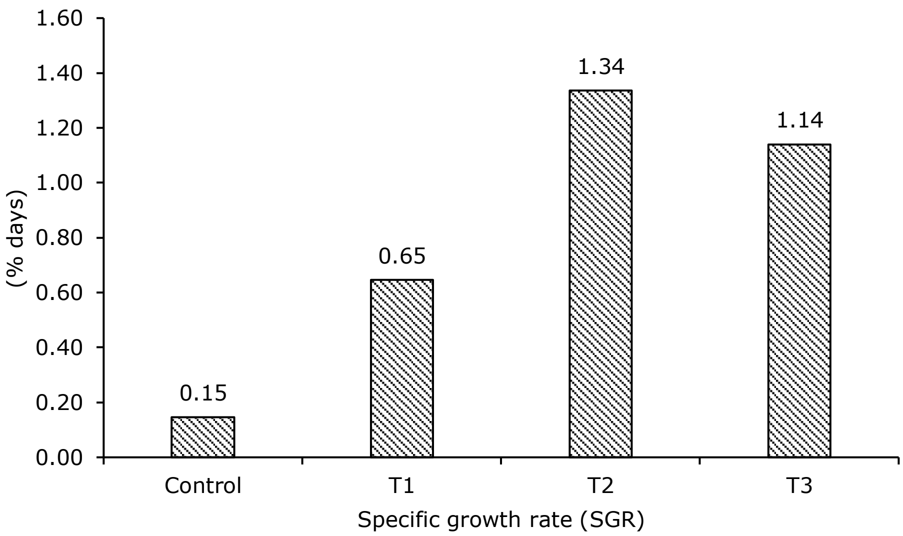

Figure 2. Specific growth rate of *Puntigrus tetrazona* across different dietary treatments, showing peak metabolic growth performance in the T2 formulation (40% maggot meal + 1% papain enzyme)

In contrast to the growth parameters, the survival rate of *P. tetrazona* remained unaffected by the different dietary treatments, showing no significant differences between the maggot-enriches diets and the control group (Figure 3). This lack of divergence suggests that replacing fishmeal with maggot meal up to 50% does not induce toxicity, nutritional deficiencies, or severe physiological stress that would compromise fish viability. The stable SR across all groups implies that the experimental diets met the baseline physiological requirements for the species' survival, and that the rearing environment remained within optimal thresholds throughout the study.

The experimental diets demonstrated superior feed efficiency compared to the commercial control (Figure 4). The lowest, most favorable FCR was achieved by T2 (3.05±0.29), meaning the fish required only 3.05 g of feed to gain 1 g of body weight. In stark contrast, the control group exhibited an extremely high FCR (approximately 51.17), indicating poor nutrient conversion.

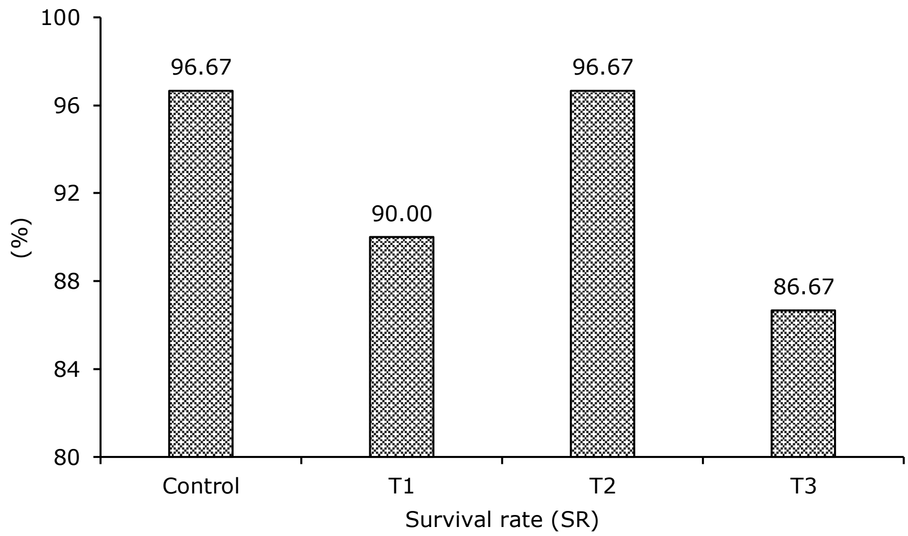

Figure 3. Survival rate of *Puntigrus tetrazona* fed diets with varying inclusion levels of maggot meal and 1% papain enzyme.

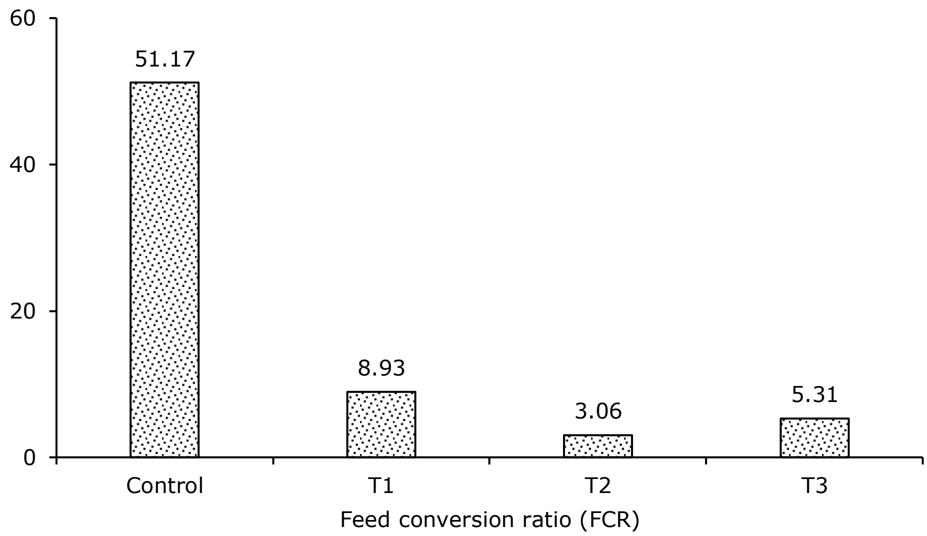

Figure 4. Feed conversion ratio of *Puntigrus tetrazona* across different dietary treatments, highlighting the optimal feed efficiency achieved by the T2 formulation (40% maggot meal + 1% papain enzyme).

This drastic improvement in FCR underscores the economic and practical relevance of the T2 formulation. The addition of papain enzyme maximizes nutrient bioavailability, preventing unabsorbed nutrients from being excreted as waste. As noted by Wiszniewski et al., (2022) in *Acipenser ruthenus*, papain supplementation optimizes feed efficiency by upgrading the functional value of alternative proteins. For aquaculture operations, reducing the FCR from the control levels to 3.05 translates directly into lower feed costs, minimized waste output, and a much more sustainable production cycle for *P. tetrazona*.

The drastic improvement in FCR values in the T2 treatment not only demonstrates the efficiency of the fish's internal metabolism but also has a massive positive impact on the quality management of the rearing media. In the control group, the very high FCR values indicate that most of the consumed feed is not properly absorbed, resulting in it being excreted back into the water in the form of feces and unbound ammonia waste. The addition of the papain enzyme to the T2 formulation successfully breaks down complex protein chains into simpler molecules, minimizing unplanned feed waste, and automatically reducing the accumulation of nitrogen waste in the culture tank (Rachmawati et al., 2020). On a practical scale, this reduction in excretory waste is highly beneficial for ornamental fish cultivation such as *P. tetrazona*, as it can reduce the risk of environmental stress due to ammonia spikes, reduce the frequency of water siphoning, and create a much more economical and environmentally friendly production cycle (Zheng et al., 2020).

Conclusions. The dietary inclusion of 40% maggot meal supplemented with 1% papain enzyme (T2) was the optimal formulation for *P. tetrazona*, significantly improving total length, weight gain, specific growth rate, and feed conversion ratio compared with the commercial control diet, while survival rate remained unaffected. Increasing maggot meal inclusion to 50% did not provide additional growth benefits, suggesting that excessive substitution may reduce nutrient utilization efficiency. These findings indicate that a diet containing 40% maggot meal with 1% papain enzyme represents a promising alternative protein source for ornamental fish culture, with the potential to improve feed utilization and reduce feed costs through enhanced feed efficiency. Future studies should evaluate the long-term economic feasibility of this formulation under commercial farming conditions and investigate its effects on body coloration, reproductive performance, and physiological health.

Acknowledgments. The author would like to thank the Rector of Universitas Negeri Medan for providing financial support for this study. The author also sincerely thanks all individuals and institutions who contributed to this research.

Authors Contributions. Conceptualization: ASB. Methodology: ASB, MM, BM. Formal Analysis: ASB, KL. Investigation: ASB, MM. Data Curation: MM, RR, RJ. Writing – Original Draft: ASB, BM, KL. Writing – Review & Editing: ASB, BM, KL. Supervision: ASB.

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.

Funding. This research was funded by Universitas Negeri Medan through the "Penelitian Produk Terapan" Scheme in 2025 with contract number: 0271/UN33/KPT/2025; 0167/UN33.8/PPKM/PPT/2025.

References

Batubara, A. S., Muliari, M., Manurung, B., Sipahutar, H., Akmal, Y., Irfannur, I., Maulizar, S., Nur, F. M., Ritonga, Y. E., & Lubis, K. (2025). New record of Sumatran barb (*Puntigrus tetrazona*) as an introduced species in Lake Toba, Indonesia: invasiveness risk. *Biosystems Diversity*, *33*(1), e2504.

Burduhos, P. (2023). Reproduction of *Puntigrus tetrazona* (Bleeker, 1855) in aquarium. *Animal Biology and Animal Husbandry*, *15*(2), 81–83.

Chang, R. K., Camus, A. C., Pomaranski, E., Yazdi, Z., & Soto, E. (2021). Pathogenesis of *Erysipelothrix piscisicarius* infection in tiger barbs (*Puntigrus tetrazona*). *Journal of Fish Diseases*, *44*(11), 1681–1688.

Chapman, F. A. (1997). Evaluation of commercially-formulated diets for feeding tiger barb, *Puntius tetrazona*. *Journal of Applied Aquaculture*, *7*(1), 69–74.

Hasan, I., Gai, F., Cirrincione, S., Rimoldi, S., Saroglia, G., & Terova, G. (2023). Chitinase and insect meal in aquaculture nutrition: a comprehensive overview of the latest achievements. *Fishes*, *8*(12), 607.

Karthikeyan, S., Devaa, W., & Ramesh, U. (2024). A study on the genetic diversity of tiger barb (*Puntigrus tetrazona*). *Current Science*, *7*, 777.

Lipscomb, T. N., Durland, D. A., Yanong, R. P., Boldt, N. C., & DiMaggio, M. A. (2022). Investigations into early larval feeding practices for the Tiger Barb *Puntigrus tetrazona*. *North American Journal of Aquaculture*, *84*(1), 53–65.

Mlaga, K. G., Agboka, K., Attivi, K., Tona, K., & Osseyi, E. (2022). Assessment of the chemical characteristics and nutritional quality of meat from broiler chicken fed black soldier fly (*Hermetia illucens*) larvae meal. *Heliyon*, *8*(11), e11718.

Pascon, G., Opere-Akinyi, R., Cardinaletti, G., Daniso, E., Messina, M., & Tulli, F. (2025). Chitin and its effects when included in aquafeed. *Aquaculture International*, *33*(3), 202.

Rachmawati, D., Hutabarat, J., Samidjan, I., & Windarto, S. (2019). The effects of papain enzyme-enrich diet on protease enzyme activities, feed efficiency, and growth of fingerlings of Sangkuriang catfish *Clarias gariepinus* reared in Tarpaulin pool. *AACL Bioflux*, *12*(6), 2177–2187.

Rachmawati, D., Hutabarat, J., Samidjan, I., & Windarto, S. (2020). Utilization of papain as feed additive in the fish feed on activity of digestive enzymes, contents of nutrient and minerals of Sangkuriang catfish (*Clarias gariepinus* var. Sangkuriang). *AACL Bioflux*, *13*(5), 2738–2744.

Rostika, R., Sugiyanto, H. N., & Dewanti, L. P. (2018). The effectiveness of crude papain enzyme supplement for tilapia's (*Oreochromis niloticus*) growth at the floating nets of Cirata Reservoir. *IOP Conference Series: Earth and Environmental Science*, *139*(1), 012006.

Singh, P., Maqsood, S., Samoon, M. H., Phulia, V., Danish, M., & Singh, C. R. (2011). Exogenous supplementation of papain as growth promoter in diet of fingerlings of *Cyprinus carpio*. *International Aquatic Research*, *3*(1), 1–9.

Sugashri, D., Devaa, W., & Ramesh, U. (2024). Pathogenic bacterium isolated from the tiger barb (*Puntigrus tetrazona*)-a call for better management of fish farms. *Current Science*, *127*(12), 1379.
Tarihoran, A. D. B., Hubeis, M., Jahroh, S., & Zulbainarni, N. (2023). Competitiveness of and barriers to Indonesia's exports of ornamental fish. *Sustainability*, *15*(11), 8711.
Wiszniewski, G., Jarmołowicz, S., Hassaan, M. S., Soaudy, M. R., Kamaszewski, M., Szudrowicz, H., Terech-Majewska, E., Pajdak-Czaus, J., Wiechetek, W., & Siwicki, A. K. (2022). Beneficial effects of dietary papain supplementation in juvenile sterlet (*Acipenser ruthenus*): growth, intestinal topography, digestive enzymes, antioxidant response, immune response, and response to a challenge test. *Aquaculture Reports*, *22*, 100923.
Yadav, N. K., Sharma, S. K., & Meena, D. K. (2024). Exogenous papain supplementation: impacts on growth, digestibility, digestive enzyme activities and oxidative stress in *Labeo rohita* fingerlings. *Aquaculture Science and Management*, *1*(1), 1–10.
Yanti, R. Z., Muchlisin, Z. A., Nur, F. M., Irham, M., Batubara, A. S., Muhammadar, A. A., & Fadli, N. (2022). Effect of natural feeds on the growth performance, survival rate and feed utilization of the tropical shortfin eel *Anguilla bicolor* McClelland 1844 (Pisces: Anguillidae) Larvae. *Polish Journal of Natural Sciences*, *37*(3), 381–390.
Zheng, C. C., Wu, J. W., Jin, Z. H., Ye, Z. F., Yang, S., Sun, Y. Q., & Fei, H. (2020). Exogenous enzymes as functional additives in finfish aquaculture. *Aquaculture Nutrition*, *26*(2), 213–224.

Received: 01 April 2026. Accepted: 24 May 2026. Published online: 10 August 2026.
Authors:
Agung Setia Batubara (ASB), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Street William Iskandar Ps. V, 20221 North Sumatra, Indonesia, e-mail: agungsbb@unimed.ac.id
Mustaqim Mustaqim (MM), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Street William Iskandar Ps. V, 20221 North Sumatra, Indonesia, e-mail: mustaqim@unimed.ac.id
Binari Manurung (BM), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Street William Iskandar Ps. V, 20221 North Sumatra, Indonesia, e-mail: binarimanurung@unimed.ac.id
Khairiza Lubis (KL), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Street William Iskandar Ps. V, 20221 North Sumatra, Indonesia, e-mail: khairizalubis@unimed.ac.id
Rahmatsyah Rahmatsyah (RR), Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Street William Iskandar Ps. V, 20221 North Sumatra, Indonesia, e-mail: rahmatunimed@gmail.com
Rita Juliani (RJ), Department of Physics, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Street William Iskandar Ps. V, 20221 North Sumatra, Indonesia, e-mail: julianiunimed@gmail.com

How to cite this article:
Batubara, S. B., Mustaqim, M., Manurung, B., Lubis, K., Rahmatsyah, R., & Juliani, R. (2026). Effects of maggot meal substitution and papain enzyme supplementation on the growth performance of *Puntigrus tetrazona* (Bleeker, 1855). *AACL Bioflux*, *19*(4):1812-1819.