Effect of phytase supplementation in *Aspergillus niger*-fermented palm leaf diets on growth performance of *Trichopodus pectoralis* Regan, 1910

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Abstract. This study evaluated the effects of phytase supplementation in diets containing *Aspergillus niger* fermented oil palm leaf meal on the growth performance, feed utilization, protein retention, and survival of snakeskin gourami, *Trichopodus pectoralis* Regan, 1910. A completely randomized design was applied with three dietary treatments containing phytase at 300, 500, and 700 mg kg^{-1}, each with three replicates. Fish (initial weight 5±2 g) were reared for 56 days and fed twice daily at 5% of biomass. Data were analyzed using one-way ANOVA. Phytase supplementation significantly affected absolute growth, relative growth rate, feed conversion ratio (FCR), feed efficiency, and protein retention ($p < 0.05$). The diet supplemented with 700 mg kg^{-1} phytase resulted in the highest absolute growth (4.07 g), relative growth rate (45.84%), feed efficiency (24.20%), and protein retention (24.02%), and the lowest FCR (4.65). Survival rate ranged from 90 to 100% and did not differ significantly among treatments ($p > 0.05$). These results indicate that supplementation of 700 mg kg^{-1} phytase in diets based on fermented oil palm leaf meal improved growth and feed utilization of *T. pectoralis* within the tested range.
Keywords: anti-nutrients, feed utilization, freshwater aquaculture, nutrient digestibility, plant-based aquafeed, protein retention.

Introduction. Aquaculture production is highly dependent on artificial feed, which accounts for 60-70% of total production costs and can reach up to 80% (Silva et al., 2007; Rachmawati et al., 2024). This high cost is mainly caused by the reliance on imported feed ingredients such as fish meal, soybean meal, and corn, combined with declining capture fisheries resources (Akbar et al., 2023). Therefore, the utilization of locally available and low-cost feed ingredients is necessary to reduce production costs and improve the sustainability of aquaculture.

Oil palm leaves are an abundant agricultural by-product with potential as an alternative feed ingredient (Serdiati et al., 2023; Safir et al., 2024). However, their application in aquafeeds is limited by high crude fiber content and low nutrient availability (Haq et al., 2018; Simarmata et al., 2024). Previous studies have demonstrated that fermentation using Aspergillus niger can improve the nutritional quality and digestibility of plant-based feed ingredients, resulting in better growth and feed utilization in several aquatic species (Jannathulla et al., 2018; Dayal et al., 2020).

In addition, dietary phytase supplementation has been widely reported to enhance growth performance and feed efficiency by improving nutrient availability from plant-based diets (Haetami & Abun, 2024; Haetami et al., 2025). Significant positive effects of phytase have been documented in various fish species, including tilapia, milkfish, carp, catfish, pomfret, and snakehead, with optimal doses varying among species (Zulaeha et al., 2015; Fahlevie et al., 2023).

Despite extensive research on fermented plant ingredients and phytase supplementation in aquaculture feeds, information on their combined application in diets for the snakeskin gourami, *Trichopodus pectoralis* Regan, 1910, remains unavailable. In particular, the optimal phytase level in diets containing *A. niger*-fermented oil palm leaf meal for this species has not yet been determined.

Therefore, this study aimed to evaluate the effects of phytase supplementation in diets based on *A. niger*-fermented oil palm leaf meal on growth performance, feed efficiency, protein retention, and survival of *T. pectoralis*. It was hypothesized that increasing dietary phytase levels would significantly improve growth and feed utilization of *T. pectoralis*, with an optimal response at a specific supplementation level.

Material and Method

Place and time of research. This research was conducted in the fish nutrition laboratory of the Faculty of Fisheries and Marine Sciences, Lambung Mangkurat University (ULM), and in Liang Anggang, Banjarbaru, South Kalimantan, Indonesia.

Research materials

1. The materials used in this study consisted of feed ingredients, enzymes, experimental fish, and supporting equipment. The experimental feed was formulated using oil palm leaf meal, fish meal, shrimp head meal, rice bran meal, tapioca flour (as a binder), vitamin mix, mineral mix, and carboxymethyl cellulose (CMC). Phytase enzyme (Notuphos 5000®) was applied at three different dosage levels, namely 300 mg kg^{-1}, 500 mg kg^{-1}, and 700 mg kg^{-1} of feed.
2. The experimental fish used in this study was *T. pectoralis* with an average initial body weight of 5±2 g $fish^{-1}$.
3. The equipment used included laboratory equipment such as digital scales, analytical balances, and an autoclave. Feed processing equipment consisted of a grinder, pellet mold, and drying oven. Water quality measurement tools included a thermometer, pH meter, dissolved oxygen (DO) meter, and water test kits. In addition, maintenance equipment such as aquaria, aeration systems, and feeding tools were also utilized to support the experimental activities.

Preparation of fermented palm leaf flour. The first step in producing fermented palm leaf flour is determining the most effective dose of *A. niger* in reducing the crude fiber content of the palm leaf flour. The *A. niger* dosage was determined based on research by Ramayani et al. (2022), which was 12% of the total amount of palm leaf flour to be fermented.

Feed formulation and manufacturing process. Experimental diets were formulated in pellet form with a crude protein content of 25-30%. Proximate analysis was conducted on all major ingredients (palm leaf meal, fish meal, rice bran meal, and shrimp head meal) before feed formulation.

Table 1

Test feed composition

No	*Ingredients*	*Composition (%)*
1	Palm oil flour	6
2	Bran flour	28
3	Fish meal	30
4	Shrimp head meal	15
5	Vitamin-mineral mix	3
6	Tapioca flour (starch)	17
7	Carboxymethyl cellulose	1
Total		100

The minerals used are a P-free mineral mix containing (g kg^{-1} dry feed): NaCl 0.5; $MgSO_4.7H_2O$ 7.5; KCl 17.53; Fecitrate 1.25; $CaCl_2.2H_2O$ 13.34; filler 30.5 and a trace element mix (0.5 g) consisting of: $ZnSO_4.7H_2O$ 17.365; $MnSO_4$ 8.1; $CuSO_4.5H_2O$ 1.55; KIO_3 0.15; and filler 30.5.

Water changes were performed twice a week, amounting to 10-20% of the total water volume, along with siphoning. Water quality measurements were taken at the beginning and end of the experiment.

Experimental design. The experiment was arranged in a Completely Randomized Design (CRD) with three dietary treatments and three replications per treatment.

Experimental units and replication

- Each experimental unit consisted of one aquarium (60 × 40 × 40 cm) filled with 60 L of freshwater and supplied with continuous aeration. Each aquarium contained 10 fish. Thus, the total number of fish used in the experiment was 90 fish (3 treatments × 3 replications × 10 fish).
- The number of fish per treatment (n per treatment) was 30 fish, and the number of fish per aquarium (n per aquarium) was 10 fish.
- Fish were randomly assigned to aquaria using a random allocation method to ensure unbiased distribution of individuals among treatments.

Fish rearing and feeding. Fish were acclimated for 7 days before the experiment and fed a commercial diet. During the 56-day feeding trial, fish were fed the experimental diets twice daily (08:00 and 16:00) at a feeding rate of 5% of total biomass. Biomass was adjusted every 14 days based on sampling weights. Water quality parameters (temperature, pH, and DO) were monitored daily and maintained within suitable ranges for *T. pectoralis* culture.

Digestibility test. Digestibility evaluation was conducted using *T. pectoralis* because this species shows a strong feeding response, is suitable for fecal collection, and is appropriate for assessing plant-based diets. Previous work on plant-based diets has reported comparable digestive responses in cultured fish (Rachmawati et al., 2024); therefore, the digestibility coefficients were used to support interpretation of the experimental diets. Feces were collected by siphoning one hour after feeding, oven-dried, and analyzed for proximate composition.

Observed parameters. The parameters measured were absolute growth, relative growth rate, feed conversion ratio (FCR), feed efficiency, protein retention, survival rate, blood glucose level, and apparent digestibility coefficients.

Statistical analysis. All data were tested for normality and homogeneity before statistical analysis. Data were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected ($p < 0.05$), Tukey's post-hoc test was applied to determine differences among treatments. Statistical analyses were performed using SPSS.

Results and Discussion

Absolute growth and relative growth. Phytase supplementation significantly improved growth performance of *T. pectoralis* (Table 2). The 700 mg kg^{-1} treatment produced the strongest growth response, indicating that the highest tested phytase level enhanced nutrient availability from the fermented oil palm leaf-based diet.

Table 2

Absolute growth and relative growth of *T. pectoralis*

Treatment	*Absolute growth (g)*	*Relative growth rate (%)*
A (phytase 300 mg kg^{-1})	2.3±0.5	24.78±6.0^{a}
B (phytase 500 mg kg^{-1})	2.6±0.6	27.41±6.6^{a}
C (phytase 700 mg kg^{-1})	4.07±0.7	45.84±8.0^{b}

Note: A - phytase 300 mg kg^{-1}, B - phytase 500 mg kg^{-1}, and C - phytase 700 mg kg^{-1}. Values are presented as mean±SD. Different superscript letters within the same column indicate significant differences ($p < 0.05$; one-way ANOVA followed by Tukey's post-hoc test).

The higher growth response in treatment C demonstrates that increased phytase availability improved the use of nutrients bound in the fermented plant-based feed. The lower responses in treatments A and B suggest that 300-500 mg kg^{-1} phytase was not sufficient to produce the same growth improvement under the present formulation.

Feed conversion and feed efficiency. Increasing phytase supplementation improved feed utilization in *T. pectoralis* (Table 3). Fish fed 700 mg kg^{-1} phytase showed the most efficient conversion of feed into growth, indicating better nutrient digestion and assimilation at the highest tested dose.

Table 3

Feed conversion and feed efficiency of *Trichopodus pectoralis*

Treatment	*Feed conversion ratio (g g^{-1})*	*Feed efficiency (%)*
A (phytase 300 mg kg^{-1})	9.35±1.2^{a}	12.23±2.5^{a}
B (phytase 500 mg kg^{-1})	7.06±1.5^{a}	15.99±3.2^{a}
C (phytase 700 mg kg^{-1})	4.65±1.0^{b}	24.20±3.5^{b}

Note: A - phytase 300 mg kg^{-1}, B - phytase 500 mg kg^{-1}, and C - phytase 700 mg kg^{-1}. Values are presented as mean±SD. Different superscript letters within the same column indicate significant differences ($p < 0.05$; one-way ANOVA followed by Tukey's post-hoc test).

The decrease in FCR and the increase in feed efficiency at 700 mg kg^{-1} indicate that phytase improved feed utilization. This response suggests that phytase supplementation enhanced the release of nutrients from the fermented oil palm leaf-based diet.

Survival rate. Survival remained high in all treatments (Table 4), indicating that the phytase-supplemented diets were tolerated by *T. pectoralis* under the experimental conditions. The absence of a diet-related survival decline suggests that the tested phytase levels did not cause harmful effects.

Table 4

Survival rate of *Trichopodus pectoralis*

Treatment	*Survival Rate (%)*
A (phytase 300 mg kg^{-1})	96.67±1.10^{a}
B (phytase 500 mg kg^{-1})	96.67±1.05^{a}
C (phytase 700 mg kg^{-1})	93.33±1.20^{a}

Note: A - phytase 300 mg kg^{-1}, B - phytase 500 mg kg^{-1}, and C - phytase 700 mg kg^{-1}. Values are presented as mean±SD. Similar superscript letters within the same column indicate no significant differences ($p > 0.05$; one-way ANOVA followed by Tukey's post-hoc test).

Survival data indicated that dietary phytase did not negatively affect fish viability. The limited mortality observed during the trial was more likely associated with handling during periodic weighing than with diet-related stress.

Protein retention. Protein retention increased with phytase supplementation (Table 5), showing that fish receiving higher phytase levels deposited dietary protein more efficiently. The 700 mg kg^{-1} diet produced the best retention response, supporting the role of phytase in improving protein utilization from the fermented plant-based diet.

Table 5

Protein retention of *Trichopodus pectoralis*

Treatment	*Protein retention (%)*
A (phytase 300 mg kg^{-1})	19.76 ± 1.30^{a}
B (phytase 500 mg kg^{-1})	21.23 ± 1.40^{ab}
C (phytase 700 mg kg^{-1})	24.02 ± 1.50^{b}

Note: A - phytase 300 mg kg^{-1}, B - phytase 500 mg kg^{-1}, and C - phytase 700 mg kg^{-1}. Values are presented as mean±SD. Different superscript letters within the same column indicate significant differences ($p < 0.05$; one-way ANOVA followed by Tukey's post-hoc test).

The protein retention results confirm that the highest phytase level promoted greater dietary protein deposition. The intermediate treatment showed a moderate response, suggesting a dose-related improvement within the tested range.

Feed digestibility. Protein feed digestibility of *T. pectoralis* differed among treatments (Table 6). The 500 and 700 mg kg^{-1} phytase diets showed higher digestibility than the 300 mg kg^{-1} diet, indicating that phytase improved the availability of dietary protein from the fermented oil palm leaf-based feed.

Table 6

Protein feed digestibility of *Trichopodus pectoralis*

Treatment	*Protein feed digestibility (%)*
A (phytase 300 mg kg^{-1})	73.79 ± 2.80^{a}
B (phytase 500 mg kg^{-1})	81.77 ± 2.50^{b}
C (phytase 700 mg kg^{-1})	82.35 ± 2.60^{b}

Note: A - phytase 300 mg kg^{-1}, B - phytase 500 mg kg^{-1}, and C - phytase 700 mg kg^{-1}. Values are presented as mean±SD. Different superscript letters within the same column indicate significant differences ($p < 0.05$; one-way ANOVA followed by Tukey's post-hoc test).

The higher digestibility values in treatments B and C suggest improved hydrolysis of phytate-bound nutrients. This response indicates that supplementation with 300 mg kg^{-1} was more effective in supporting protein digestion under the present diet formulation.

Blood glucose content. Blood glucose was measured before feeding and at 1, 2, 3, 4, 5, 7, and 9 h after feeding to evaluate postprandial energy response in *T. pectoralis* (Table 7).

Table 7

Blood glucose content of *Trichopodus pectoralis*

Treatment	*Blood glucose content (mg dL^{-1})*							
	0	*1*	*2*	*3*	*4*	*5*	*7*	*9*
A1	27	69	79	59	67	52	60	48
A2	37	49	76	51	55	60	51	43
A3	27	48	88	82	65	56	53	37
Average	30.67	55.33	81	64	62.33	56	54.67	42.67
B1	36	93	67	70	60	87	60	35
B2	33	102	67	57	67	75	53	45
B3	29	97	55	51	69	78	63	48
Average	32.67	97.33	63	59.33	65.33	80	58.67	42.66
C1	49	78	73	59	66	62	39	60
C2	29	99	79	68	77	64	48	38
C3	34	89	91	85	67	73	47	36
Average	37.33	88.67	81	70.67	70	66.33	44.67	44.67

Note: A - phytase 300 mg kg^{-1}, B - phytase 500 mg kg^{-1}, and C - phytase 700 mg kg^{-1}.

Blood glucose levels increased after feeding and generally peaked between 1 and 2 h, then decreased toward baseline by 9 h. The 500 and 700 mg kg^{-1} treatments produced higher post-feeding glucose responses than the 300 mg kg^{-1} treatment, suggesting improved carbohydrate utilization and a phytase-related effect on energy metabolism.

Water quality. Water quality parameters were measured at the beginning and end of the experiment to verify that the rearing conditions remained suitable for *T. pectoralis* (Table 8).

Table 8

Water quality parameters measured at the beginning and end of the experiment

Parameter	*Initial*	*Final*	*Reference*
Temperature (°C)	28.4	29.2	Cholik et al., 1986
pH	6.81	6.20-7.50	Cholik et al., 1986; Susanto, 1987
DO (mg L^{-1})	3.0	3.4-3.9	Cholik et al., 1986
NH3 (mg L^{-1})	0.02	0.02-1.98	Wardoyo, 1981

Water temperature, pH, DO, and ammonia remained within ranges that permitted fish survival and growth. These conditions indicate that the observed differences in growth and feed utilization were mainly associated with dietary treatments rather than water-quality stress. These ranges are consistent with freshwater aquaculture water-quality guidance (Wardoyo, 1981; Cholik et al., 1986; Susanto, 1987; Boyd, 1998).

Overall, the experimental units maintained comparable water quality, supporting valid comparison among dietary treatments.

Discussion. The improvement in growth performance of *T. pectoralis* fed phytase-supplemented diets is closely related to enhanced nutrient availability and energy utilization. Fish growth primarily depends on protein deposition, which requires sufficient digestible energy from non-protein sources to prevent protein from being catabolized for metabolic needs (National Research Council, 1993). Phytase supplementation reduces phytic acid-protein and phytic acid-mineral complexes, thereby increasing the availability of amino acids and energy-yielding nutrients for tissue synthesis (Vielma et al., 2002; Debnath et al., 2005). This mechanism explains why fish receiving the 700 mg kg^{-1} phytase diet showed superior growth and feed utilization compared with lower doses. This protein-sparing role of dietary energy is consistent with the extra caloric effect reported in carp nutrition (Viola & Rappaport, 1979).

The lower response observed at 500 mg kg^{-1} phytase suggests that this level may have been insufficient to fully hydrolyze phytic acid present in the fermented oil palm leaf-based diet. Plant-based ingredients typically contain high concentrations of phytic acid, and the effectiveness of phytase depends on both substrate concentration and gastrointestinal pH (National Research Council, 1993). Thus, partial degradation of phytate at 500 mg kg^{-1} may have limited improvements in protein and mineral bioavailability, resulting in moderate rather than optimal growth. Similar dose-dependent responses have been reported in milkfish (*Chanos chanos*), where effective phytase levels ranged from 502 to 665 mg kg^{-1} (Rachmawati et al., 2024), and in common carp (*Cyprinus carpio*) (Rachmawati & Samidjan, 2018). These comparisons indicate that phytase requirements vary among species and depend on dietary phytate levels.

Feed efficiency and FCR improved with increasing phytase levels, reflecting better digestion and assimilation of nutrients. Enhanced protein retention in the present study further supports the role of phytase in improving protein utilization by liberating protein bound to phytic acid complexes (Vielma et al., 2002; Debnath et al., 2005). Digestibility values also increased with phytase level, suggesting that fermentation and phytase supplementation jointly improved the availability of nutrients from the oil palm leaf-based diet (National Research Council, 1993; Simarmata et al., 2024). Comparable improvements in feed utilization have also been reported in tilapia receiving plant-derived feed additives (Linayati et al., 2024).

Blood glucose dynamics indicated a postprandial glucose response in phytase-supplemented treatments, suggesting improved carbohydrate utilization and energy partitioning toward growth. Efficient glucose turnover supports anabolic processes such as protein synthesis and tissue formation (Fujaya, 2004). High survival rates and stable water quality conditions further indicate that dietary treatments, rather than environmental stress, were responsible for the observed performance differences.

A limitation of this study is that only three phytase levels (300-700 mg kg^{-1}) were evaluated using a single feed formulation and a relatively short rearing period. Therefore, the superior performance observed at 700 mg kg^{-1} should be interpreted within the tested range and experimental conditions. Future studies should evaluate wider phytase dosage ranges, longer culture periods, and species-specific digestibility assessments to better define phytase requirements for *T. pectoralis* fed fermented oil palm leaf-based diets.

Conclusions. This study demonstrated that phytase supplementation in *A. niger*-fermented oil palm leaf-based diets improved growth performance and feed utilization of *T. pectoralis* within the tested dosage range. Among the treatments, the diet containing 700 mg kg^{-1} phytase produced the best results for growth, feed efficiency, feed conversion, protein retention, digestibility, and blood glucose, while survival rates remained high and water quality stayed within acceptable limits. However, these findings are restricted to the specific feed formulation, rearing period, and phytase levels evaluated in this study; therefore, the superiority of the 700 mg kg^{-1} dose should be interpreted only within the tested range and not as a definitive optimal dose for all culture conditions.

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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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