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ORIGINAL RESEARCH ARTICLE



Growth performance of Thai Pangus (*Pangasianodon hypophthalmus*) using different synthetic amino acids in plant protein based formulated diets

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ABSTRACT

An experiment was carried out for a period of 120 days to evaluate three formulated diets among of which T₁ was formulated based on animal protein, T₂ was partially replaced animal protein with plant protein and adding amino acid whereas the T₃ was formulated with fully plant protein with adding amino acid to assess the growth performance of Thai Pangus, *Pangasianodon hypophthalmus*. Each treatment had three replicates using 9 (nine) mini experimental ponds. The stocking density was 120 fish/decimal. Initially, the fish were fed twice daily at a rate of 5% of their body weight, which was progressively reduced to 2% by the end of the experiment. During the experimental period, the water quality parameters observed (temperature 26.0°C-31.5°C, pH 7.50-8.44, DO 4.30-5.94 mgL⁻¹, ammonia-nitrogen 0.01 to 0.03 mgL⁻¹, and total alkalinity 155-185 mgL⁻¹) were found to be optimal and stable. The mean weight gains (WG) of Thai Pangus were 421.6±4.71 g, 407.34±1.97 g and 345.89±4.44 g for T₁, T₂ and T₃, respectively and significantly (p<0.05) highest WG was obtained in T₁ and T₂ followed by T₃. The specific growth rate (SGR) was found highest in T₂ (2.70±0.03) followed by T₁ (2.53±0.06) and T₃ (2.16±0.04), respectively. The highest feed conversion ratio (FCR) was found in T₁ (1.62±0.03) whereas lowest FCR obtained from T₂ (1.52±0.01) followed by T₃ (1.55±0.02). The protein efficiency ratio (PER) values were ranged between 2.03 and 2.13 and highest was found in T₂ (2.13±0.05). The highest production was attained from T₁ (13557.50±51.60 kg ha⁻¹) followed by T₂ (13227.71±50.72 kg ha⁻¹) and T₃ (11450.60±49.87 kg ha⁻¹), respectively. The findings of this study revealed that, the partial replacement of animal protein with plant protein adding limited amino acid (lysine and methionine) exhibited the best performance on the basis of nutritive value and growth performance.

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INTRODUCTION

The *Pangasius hypophthalmus*, commonly known as Thai Pangus, is renowned in the field of aquaculture for its exceptional growth performance, efficient culture system, remarkable disease resistance, and adaptability to various environmental conditions. These characteristics are shared by other catfish species that are commonly cultured (Begum *et al.*, 2012b; Stick-

ney, 1979). Owing to its impressive growth rate and compelling evidence, the species in question was introduced for aquaculture purposes in Bangladesh in the year 1989 (Sarker, 2000). The observed specimens exhibit favorable body coloration, displaying uniformity in size and demonstrating a robust appetite for feeding. The majority of Thai Pangus farmers heavily depend on a wide range of supplementary feeds for their aquaculture operations. The demand in the production of fresh-

water fish for human consumption has resulted in a heightened need for superior fish feeds of exceptional quality. As per the findings of Zakaria *et al.* (2012) and Guedes *et al.* (2015), it has been established that feed cost is the key factor of operating cost during the production of catfish. As a result, significant effort has been made to concentrate on the quantity and character of feed stuffs to achieve optimum performance of catfish. Consequently, considerable emphasis has been placed on the meticulous evaluation of both the quantity and quality of feed ingredients in order to attain optimal performance outcomes for catfish. The formulation of an appropriate feed is of utmost importance in ensuring the sustained growth of catfish fingerlings. This stimulates the scientific community to explore novel constituents for fish feed that exhibit robust nutritional profiles and encompass essential amino acids crucial for the growth and sustenance of the catfish fingerlings.

Between the years 2003 and 2007, the requirement for freshwater fish production exploded considerably, rising up to 30%. In parallel, during the preceding two decades, there has been a notable surge in fish meal utilization, escalating from 15% to 65% (Tacon and Metian, 2008). As a result of the rise in demand for fishmeal, fish meal prices have grown by more than twice as much as they were in recent years (Ogello *et al.*, 2014). However, it has become vital to look for substitute plant protein sources due to rising fish meal prices and a limited supply. It is crucial to keep in mind that these alternative sources should be capable of maintaining high growth rates and should have balanced dietary components (Tacon and Metian, 2008). The soybean meal (SBM) stands out as a highly accessible and economically viable plant-based protein source, primarily due to its abundant protein content and consistent composition. Moreover, SBM exhibits an optimal amino acid (AA) profile, making it a valuable resource in terms of protein quantity and essential amino acid (EAA) composition (NRC, 2011). The amino acids (AA), specifically methionine, lysine, and cystine, which are rich in sulfur, are found in limited quantities in the context of aquaculture. Additionally, it is worth noting that this particular source also contains a significant amount of endogenous antinutrients, such as phytohaemagglutinin, protease inhibitors (trypsin inhibitors), and anti-vitamins. Thermal processing has the potential to effectively eliminate or deactivate a significant number of these components, as suggested by Tacon (1993). Soybean meal has been extensively investigated in numerous scientific studies as a potential substitute for fish meal (FM) in tilapia aquaculture, exhibiting diverse levels of performance outcomes. The extent to which Soybean Meal (SBM) can serve as a substitute for Fish Meal (FM) in aquaculture diets depends on various factors, including the species and size of the fish, the protein content of the diet, the origin and processing methods employed for SBM, as well as the specific culture systems being utilized. In certain scenarios, the SBM has demonstrated the potential to replace anywhere from 67% to 100% of FM in aquaculture feed formulations. The successful substitution of up to 75% of fishmeal (FM) in test diets for Nile Tilapia fry (Pantha, 1982; Tacon *et al.*, 1983; Jackson *et al.*, 1982) and 67% for hy-

brids Tilapia (Shiau *et al.*, 1989) was achieved through the utilization of prepressed, solvent extracted soybean meal (SBM) with or without methionine supplementation. Based on the findings presented, it is evident that the inclusion of supplemental SBM with the limiting essential amino acid (EAA) may not yield significant advantages for the growth and development of Tilapia. The addition of crystalline essential amino acids (EAA) to Tilapia diets did not yield any significant enhancements in fish performance (Viola and Arieli, 1983; Teshima and Kanazawa, 1988).

According to Lovell (1989), it has been established that fish necessitate a diet that is rich in protein. Protein, as a comprehensive nutrient source, effectively fulfills the body's energy requirements (Keembiyehetty and Gatlin, 1992). Furthermore, the essential amino acids serve as the primary constituents in the dietary requirements of fish. The amino acids exert a notable influence on various aspects of fish physiology, including their developmental processes, reproductive performance, and growth patterns (Wilson, 2003). One of these most limited amino acids in fish diet is lysine (Small and Soares, 2000). Similarly, lysine plays a crucial role as a key nutritional element for various species of finfish, especially in cases where alternative protein sources are employed in lieu of fish meal. Furthermore, extensive research has demonstrated that fish skeletons exhibit the highest concentrations of lysine (Ahmed and Khan, 2004). Moreover, it has been observed that fish subjected to diets lacking essential amino acids exhibit reduced growth and heightened mortality rates (Ketola, 1983). A highly economical approach to modifying essential amino acid (EAA) content within the diet while minimizing changes to macro and micronutrient composition involves the utilization of nutrient-based formulations (Nunes *et al.*, 2014). Based on the findings of the National Research Council (2011), it is recommended that the inclusion of fish in one's diet should range from 0.5-1.5% for methionine and 1.2-3.3% for lysine. The supplementation of plant protein-based diets with these two essential amino acids (EAA) has been shown to effectively conserve other essential amino acids (Kerr and Easter, 1995). Hence, the primary objective of this experiment was to evaluate the growth performance of Thai Pangus (*Pangasius hypophthalmus*) fed with formulated feeds using limiting amino acids.

MATERIALS AND METHODS

The experiment was carried out in the pond complex of Freshwater Station, Bangladesh Fisheries Research Institute (BFRI), Mymensingh for a period of 120 days. Three ponds having 20 decimals in size were selected and then each pond was divided with bamboo banna and filter net to get 3 mini ponds for conducting the experiment. Water depth of each pond was maintained 0.61 to 0.76 m. All the experimental ponds were similar in shape, size, depth, basin configuration type and bottom type including water supply facilities. The ponds were prepared by draining out water and limed with quick lime (CaO) at the rate of 250 kg ha⁻¹ pond before stocking. Each pond was stocked

at 120 fish/decimal. All the stocked fish were of same age group having mean weight of 75.58g. The pellet feeds (1.0-2.0 mm) made with hand pellet machine. The fish was offered the test diets two times daily at the rate of 5-2%. The feeds were dispersed by hand broadcasting over the ponds. Monthly sampling was done to adjust the feeding rate and also to observe the health condition of fish.

Three experimental diets (iso-nitrogenous) were formulated to contain 30% crude protein and the diets were used as treatments. The fish meal, mustard oil cake, soybean meal, wheat flour, vitamin and mineral premix were used as a diet ingredients. Limiting amino acids such as lysine and methionine were added in the diets. Diet-1 was prepared based on fish meal protein without adding amino acid and used as control diet. Diet 2 was prepared with the partial replacement of animal protein adding with limiting amino acid and Diet 3 was prepared based on fully plant protein ingredients adding with limiting amino acids. During experimental period water quality parameters such as temperature, pH, dissolved oxygen and transparency

were measured and recorded fortnightly. Growth response was observed monthly for the feed adjustment.

At the onset of the trial, a random sample of fishes was obtained from the stock prior to stocking. Subsequently, at the end of the experiment, a random sample of fish from each treatment was selected and collected for the purpose of observing carcass composition. The carcass compositions were subjected to triplicate analysis following the guidelines outlined in the AOAC (1980) by the Nutrition Laboratory at Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh. The objective was to evaluate the compositions across different treatments and assess any changes that occurred from the initial stage. The assessment of the significance of variation among the treatment means was conducted by the utilization of one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). The formulation of the diets is presented in Table 1. The proximate content of the diets (Diet 1, 2, and 3) was examined and is presented in Table 2.

Table 1. Formulation and proximate composition of the diets (% dry weight basis) for *P. hypophthalmus*.

Diet Ingredients	Diet number		
	1	2	3
Fish meal	19.00	12.00	0.00
Soybean meal	22.80	30.00	40.00
Mustard oil cake	18.00	20.00	27.00
Rice bran	32.00	28.60	22.40
Wheat flour	6.00	6.00	6.00
Binder	2.00	2.00	2.00
Vitamin and mineral premix	0.20	0.20	0.20
Lysine	0.00	0.80	1.60
Methionine	0.00	0.40	0.80
Proximate composition			
Crude protein	30.08	30.04	30.15
Crude lipid	9.75	9.70	9.50
Ash	8.43	8.05	7.88
Fibre	5.84	5.96	5.56
NFE	37.00	36.50	36.06
GE (kJg ⁻¹)	17.04	17.14	17.20

Table 2. Proximate composition (% dry matter basis) of different diets.

Proximate composition	Diet number		
	1	2	3
Crude protein	29.30	29.48	29.14
Crude lipid	8.29	10.67	10.60
Ash	19.02	17.96	11.46
Crude fiber	4.6	4.9	4.1
NFE	18.87	18.76	26.85

RESULTS AND DISCUSSION

The temperature ranged from 26.0-31.5°C, dissolved oxygen (DO) concentration varied from 4.30-5.94 mgL⁻¹, ammonia-nitrogen concentration ranged from 0.01-0.03 mgL⁻¹, total alkalinity ranged from 155-185 mgL⁻¹, and pH ranged from 7.5-8.44. The results of growth performance and dietary utilization has shown in Table 3. The mean initial weight was 75.57±8.38 g, 72.53±11.89g and 78.64±6.08 g reached to a mean final weight 497.17±13.09g, 479.87±13.86 g and 424.53±10.52 g in T₁, T₂ and T₃, respectively. The significantly (p<0.05) highest growth was achieved in T₁ (421.6±4.71) and T₂ (407.34±1.97) followed by T₃ (345.89±4.44). The obtained weight gain of *P. hypophthalmus* in different treatments during the experimental period are graphically shown in Figure 1.

The mean feed conversion ratio (FCR) exhibited variation ranging from 1.52-1.62 across different treatments, as shown in Table 3. The FCR of T₂ was found to be the lowest (1.52±0.01), whereas T₁ showed the highest FCR (1.62±0.03). There was no significant difference (p>0.05) observed in the FCR levels between T₂ and T₃. The experimental results revealed that, the specific growth rate (SGR% day⁻¹) was highest in T₂ (2.70±0.03), followed by T₁ (2.53±0.06) and T₃ (2.16±0.04). The survival rates of fish in different treatments ranged from 91%-93%. There were no significant differences observed in the survival rates. The PER values ranged between 2.03 and 2.13, and there was no statistically significant difference observed among between the treatments (T₂ and T₃) in terms of PER values (p>0.05). The analysis of carcass composition was conducted on the experimental fish for all dietary treatments. The initial and final values of moisture, crude protein, crude lipid and ash are shown in Table 4. In the study, *P. hypophthalmus* was reared for a period of 4 months. The results showed that T₁ had the highest production (13557.50±51.60 kg), followed by T₂ (13227.71±50.72 kg) and T₃ (11450.60±49.87 kg). These differences in production were found to be statistically significant (p<0.05). Additionally, T₂ had the highest specific growth rate (2.70±0.03), followed by T₁ (2.53±0.06) and T₃ (2.16±0.04). These differences in specific growth rate were also found to be statistically significant (p<0.05) (Table 3).

In this present study, it was observed that the *P. hypophthalmus* in T₁ exhibited the highest increase in weight when provided with a diet consisting entirely of animal protein. This was followed by the fish in T₂, which were fed a diet containing a mixture of animal and plant protein. The observed significant growth of fish in T₁ may be attributed to the modest intake of animal protein. Diet 1 and 3 exhibited protein contents of 29.30% and 29.14%, respectively. Diet 2 showed the superior performance in terms of mineral content, protein quality, and feed when compared to other diets. The potential influence of protein quality and amino acid balance on the enhanced growth performance of fish in T₂ should also be considered. According to Khan (1997), the rate of fish growth can be influenced by factors such as the quantity of crude protein, the presence of other necessary nutrients, and the manner in which the feed is

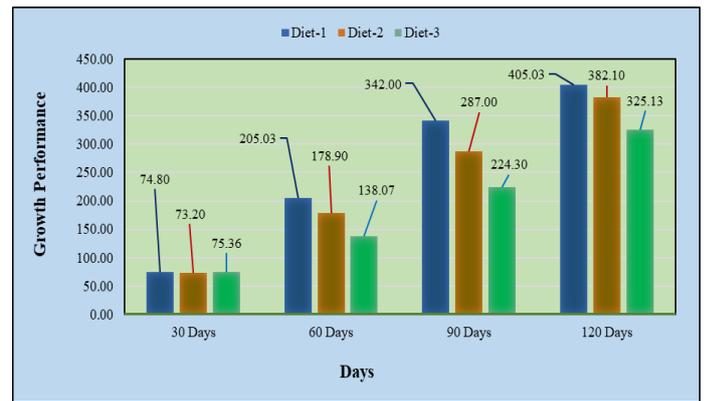


Figure 1. Weight gain of *P. hypophthalmus* in different treatments.

administered. Rahman (1989) also reported that the growth rate of *P. pangasius* exhibited a positive correlation with protein concentration, reaching its peak at a protein content of 40% in the meal. In contrast, Pathmasothy and Jin (1987) asserted that fish exhibited reduced growth rates when provided with pelleted feed containing 22% crude protein, in comparison to those given with feed containing 32% crude protein. Chuapoehek and Pothisoong (1983) suggested that a diet consisting of 25% protein is optimal for the growth and development of *P. sutchi*. In the current study, all treatments involved the administration of a food containing 30% crude protein to the fish.

According to Hillestad (2001), the lowest feed conversion ratio (FCR) is indicative of a high level of food utilization efficiency in formulated feed. However, T₂, which involved the partial replacement of animal protein with plant protein-based feed for the fish, exhibited a much lower feed conversion ratio (FCR) of 1.52. Hillestad (2001) reported that the high energy diet yielded the lowest feed conversion ratio (FCR) and the highest nutrient retention. In a study conducted by Amin et al. (2005), it was observed that the feed conversion ratio (FCR) for *Pangus culture* in eight earthen ponds was determined to be 1.65, a value that exceeds the outcome obtained in the present experiment. According to Razzaque et al. (2008), the specific growth rate (SGR) of *Pangasius pangasius* fed with formulated feed in natural ponds was found to be 0.65% per day, which was comparatively lower than the findings of the current study. Khan et al. (2009) conducted an experiment using 9 ponds over a duration of 135 days to assess the specific growth rate (SGR) under three different treatments (2.46%, 2.62%, and 2.71% day⁻¹). The outcomes of their study were found to be very comparable to the findings of the current experiment. In a study conducted by Pathmasothy and Jin (1987), it was revealed that the utilization of a relatively high protein diet (32% protein) resulted in increased feed conversion ratio (FCR) values ranging from 2.27 to 3.66. The survival rates for T₁ and T₂ were 92% and 93%, respectively. There was no statistically significant difference (p>0.05) observed among the treatments in terms of survival percentage. T₂ exhibited the highest survival percentage at 93%, while T₃ revealed the lowest at 91%. Sayeed et al. (2008) reported that Thai pangus exhibited survival rates ranging from 94% to 97% during an 11-months duration in 9 earthen ponds.

Table 3. Mean growth performance and feed utilization of *P. hypophthalmus* fed experimental diets in ponds for 04 months.

Observed components	Diet number		
	1	2	3
Initial weight (g)	75.57±8.38 ^a	72.53±11.89 ^a	78.64±6.08 ^a
Final weight (g)	497.17±13.09 ^a	479.87±13.86 ^{ab}	424.53±10.52 ^c
Weight gain (g)	421.6±4.71 ^a	407.34±1.97 ^{ab}	345.89±4.44 ^c
% Weight gain	562.57±3.21 ^a	574.99±3.09 ^a	441.34±2.23 ^b
Survival rate (%)	92.0±1.13 ^a	93.0±1.09 ^a	91.0±1.03 ^a
Feed conversion ratio (FCR)	1.62±0.03 ^a	1.52±0.01 ^b	1.55±0.02 ^b
Protein efficiency ratio (PER)	2.03±0.09 ^b	2.13±0.05 ^a	2.11±0.06 ^a
Specific growth rate (SGR % day ⁻¹)	2.53±0.06 ^b	2.70±0.03 ^a	2.16±0.04 ^c
Production (kg/ha ⁻¹)	13557.50±51.60 ^a	13227.71±50.72 ^a	11450.60±49.87 ^b

Table 4. Carcass composition (±SD) of experimental fish *P. hypophthalmus* (% dry weight).

Parameters		Dietary Treatments		
		1	2	3
Crude protein	Initial	48.12 ^a	48.12 ^a	48.12 ^a
	Final	49.94±0.02 ^a	50.35±0.04 ^a	48.89±0.01 ^a
Crude lipid	Initial	15.26 ^a	15.26 ^a	15.26 ^a
	Final	20.68±0.03 ^a	19.75±0.01 ^a	19.58±0.04 ^a
Ash	Initial	15.74 ^a	15.74 ^a	15.74 ^a
	Final	7.11±0.02 ^b	7.79±0.03 ^a	6.93±0.04 ^c
Moisture	Initial	80.13 ^a	80.13 ^a	80.13 ^a
	Final	70.91±0.01 ^a	70.25±0.03 ^a	70.13±0.02 ^a

The PER values observed in this study were found to be higher (ranging from 2.03-2.13) compared to the PER values reported by Kamrudin et al. (1987) for *P. sutchi*. Azad et al. (2004) reported that Pangus, as a species, exhibit sensitivity to water quality, therefore emphasizing the criticality of maintaining high water quality levels. Fish growers prioritize the maintenance of water quality parameters within the appropriate range. The water quality parameters, including temperature (ranging from 26.0°C to 31.5°C), pH (ranged from 7.50 to 8.44), dissolved oxygen (ranged from 4.30 to 5.94 mgL⁻¹), ammonia-nitrogen (ranged from 0.01 to 0.03 mgL⁻¹), and total alkalinity (ranged from 155 to 185 mgL⁻¹), were measured in various treatments throughout the experimental period. These parameters were found within the suitable range for fish culture, as indicated by Jhingran (1991). The lipid content of the fish exhibited a notable increase compared to its initial levels, as indicated in Table 4. The lipid content of the feed had a significant influence on the lipid content in the carcass. Specifically, the diet with the highest crude lipid content (20.68%) exhibited the greatest quantity of lipid in the carcass. As has been previously shown (Andrews and Stickney, 1972; Garling and Wilson, 1976), there exists a negative correlation between the lipid and moisture levels.

Conclusion

Based on the findings of this experiment, it was observed that T₂ exhibited significantly higher growth performance, survival rate (%), and overall output, with a yield of 13227.57±50.72 kg ha⁻¹. Therefore, it can be inferred that using limited amino acids (lysine and methionine) as a fish feed additive in *P. hypophthalmus* culture can result in improved feed efficiency and growth

performance. The implementation of this formulated feed will significantly decrease production costs and enhance the profitability of fish farming for both commercial and rural small-scale Thai Pangus farmers. However, it is necessary to evaluate the benefit-cost ratio (BCR) in order to comprehend the sustainability at the farmer's level.

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