Effect of Dietary Lipid on Growth Performance and Body Composition of Thai climbing perch, *Anabas testudineus* (Bloch, 1792)

**ABSTRACT**

An experiment was conducted to investigate the effect of dietary lipid in a practical diet on the growth performance, body composition, and production of Thai climbing perch (*Anabas testudineus*). The rearing was done in six experimental ponds for 100 days (mean area 0.0020 hectares and water depth of 1.5m) at the field laboratory of Department of Fisheries, University of Rajshahi. Feed formulation was performed with basal diet and a different concentration of lipid (soybean oil), considering the nutritional balance of the diets. Three different experimental diets were prepared containing soybean oil 0% (T1), 5% (T2), and 10 % (T3). At the time of rearing experiment, some water quality parameters like temperature, pH, DO and CO2 were monitored. After rearing, growth performance was analyzed. All the water quality parameters were found within suitable limits. The final weight, weight gain, and SGR of fish were found to be significantly higher (*P*<0.05) in the treatment group T3 than in the fish group T2 and T1. The FCR value was significantly lower in T3 (1.83±0.02) than in the T1 (2.43±0.13) fish group. The yield was significantly higher in T3 (7046.48±25.57 kg/ha) fish group than other groups. The body composition of whole fish was significantly different among the treatments. Lipid content was higher in T3 (6.01±0.19) rather than in T2 (5.78±0.17) and T1 (5.05±0.14). Recent findings suggest that incorporating of 10% soybean oil into a typical diet may be optimal for pond-based Thai climbing perch culture.

***Keywords:*** *Climbing perch, Dietary lipid, Soybean oil, Growth performance, proximate composition.*

1. **INTRODUCTION**

The World Aquaculture Production 2022 reached 130.9 million tons and as well, the amount of per capita fish consumption has grown, rising from 9.1 kg in the 1960s to 20.6 kg in 2022 (FAO, 2024). Consequently, the use of fish feed in aquaculture has also been increasing. Dietary lipid plays a significant role in fish nutrition as a source of energy and necessary fatty acids (EFAs) to maintain biological structure and appropriate function of cell membranes (Sargent et al., 1999). It plays crucial roles in fish growth and health by providing essential fatty acids (Lee et al., 2002) and participating in the uptake, transport and metabolism of fat-soluble vitamins and carotenoids (Jiang et al., 2015). In intensive fish farming, a high dietary fat intake is essential because it reduces nitrogenous losses and provides energy for protein sparing (Hardy and Gatlin, 2002; Li et al., 2016). Although it is often recognized that fish prefer to use protein over fats and/or carbohydrates for energy. Fats are also a significant component of fish diets, particularly for carnivorous fish species. Raising dietary fat levels enhances food utilization, but only to a certain extent (Watanabe et al., 1989; Peres & Oliva-Teles, 1999). Fish development, body composition, and health can all be negatively impacted by inappropriate dietary lipid levels (Tucker et al., 1997). Sometimes, a diet high in fat can slow down fish growth by reducing feed consumption (Ellis and Reigh, 1991; Lin and Shiau, 2003). In aquaculture, fish oil is better as a lipid source for fish. However, a rapid increase in global aquafeed production has resulted in a limitation of market availability and an increasing market value of fish oil (FO) (Sarker et al., 2011). The alternatives to FO are vegetable oils, which are rich in polyunsaturated fatty acids (PUFAs) such as linoleate (18:2n-6) and linoleates (LNA, 18:3n-3) but devoid of n-3 HUFAs (Sargent et al., 2002). Freshwater fish species possess the capacity to synthesize n-3 HUFAs from LNA through a pathway involving desaturation and elongation of their respective precursors (Tocher et al., 2002; Zheng et al., 2004). The most used vegetable oils as dietary lipid sources in aquaculture feeds are linseed, soybean, rapeseed, palm, olive, and sunflower oil. Soybean oil is the world's largest source of vegetable oil and contains higher levels of polyunsaturated fatty acids than others, such as rapeseed oil or palm oil, a rich source of vitamin E (a natural antioxidant) and a cholesterol-free oil (Ng et al., 2004; NRC, 1993).

The air-breathing fish climbing perch, often known as koi (*A. testudineus*), is one of the most popular fish in Bangladesh due to its flavor and strong market demand (Siddiqua et al., 2000). This species is a useful source of nutrition for the ill and convalescent. It has significant levels of iron and copper, which are important for hemoglobin formation (Saha, 1971). Once upon a time, climbing perch or Koi was very much abundant in almost all freshwater systems of Bangladesh (Mahmood, 2003). The availability of this fish has decreased from natural systems in recent years. However, the Thai Koi is an exotic fish species in Bangladesh that was introduced in 2002 from Thailand after a drastic reduction of popular native koi fish was recognized in the late 1980s (Ahmed et al., 2014). The nice thing about this Thai koi is that its body color is almost identical to 'Deshi Koi' (Torafdar, 2013). Fortunately, artificial propagation procedures have been established to produce seeds of this species (Kohinoor and Zaher, 2006).

Several studies on this species include the stocking density of cage-reared *A. testudineus* (Habib et al., 2015); production of Thai Koi reared in nylon hapas (Hasan et al., 2010); protein requirement (Hossain et al.,2012); Growth and morphological comparison between local and Thai koi (Noor, 2005) have been conducted. However, the effect of dietary lipid in combination with a practical diet on the growth performance and body composition of Thai Koi (*A. testudineus*) has not been studied. To intensify the culture of this species provision of a nutritionally balanced diet is necessary. Thus, the present study aims to investigate the effect of dietary lipid on growth performance and body composition of Thai koi, *A. testudineus*.

1. **MATERIALS AND METHODS**

**2.1. Pond selection and preparation**

The experiment was conducted over 100 days in six experimental ponds (0.0020 ha in area, 1–1.5 m depth) located on the north side of the Fisheries Department at the University of Rajshahi. Before stocking, ponds were dried and cleaned to remove weeds and unwanted aquatic animals. The dried ponds were left exposed to sunlight for several days and then limed at the rate of 1kg/decimal. Five days after liming, ponds were filled up with deep tube well water up to a depth of 1.5m*.* After three days, the ponds were fertilized with cow dung 8 kg/decimal, Urea-150 g/decimal, and TSP-75 g/decimal.TSP was applied after dissolving in plastic buckets for 10 to 12 hours before application. Fertilizers were applied by spreading methods.

**2.2. Experimental Design**

The experiment was conducted in six experimental ponds under three treatments: T1, T2, and T3, each with two replications, treated by three experimental diets at different lipid levels: 0%, 5%, and 10%, respectively. Stocking density remained consistent across all treatments.

**2.3. Experimental diet**

Three experimental diets were formulated by the incorporation of dietary lipids at different levels shown in Table 1. The proximate composition of the experimental diet is shown in Table 2. The soybean oil was used as the lipid source. The lipid content was considered very low, medium, and high in the feed. Diets were prepared using soybean oil at various incorporation levels of 0% denoted ED 0% (T1), 5% soybean oil ED 5% (T2), and 10% soybean oil ED 10% (T3). All the ingredients were well mixed. An adequate amount of water was added to the mixture of feed, and then put into the manually operated pellet machine for the preparation of the pellet. The pellets were dried using sunlight for about 2 days. Then the feeds were cooled down and stored at 4 °C until use.

**Table 1: Percentage composition of different feed ingredients for the formulation of the experimental diet of Thai koi during the study**

|  |  |
| --- | --- |
| **Feed ingredients** | **Treatments** |
| **ED 0%(T1)** | **ED 5% (T2)** | **ED 10% (T3)** |
| Fish meal  | 30 | 30 | 30 |
| Mustard oil cake  | 29 | 29 | 29 |
| Wheat flour  | 04 | 04 | 04 |
| Rice bran  | 05 | 05 | 05 |
| **Lentil bran**  | **30** | **25** | **20** |
| **Soybean oil** | **0** | **5** | **10** |
| Vitamin pre-mixture | 1.4 | 1.4 | 1.4 |
| Chlorine chloride  | 0.5 | 0.5 | 0.5 |
| Vitamin E  | 0.1 | 0.1 | 0.1 |
| Total  | 100.0 | 100.0 | 100.0 |

**Table 2: Proximate composition (% dry basis) of experimental diets**

|  |  |
| --- | --- |
| **Parameters (%)** | **Treatments** |
| **ED 0%** | **ED 5%**  | **ED 10%**  |
|  Moisture  | 13.50 | 12.47 | 12.40 |
| Crude lipid  | 4.64 | 8.47 | 14.52 |
| Crude protein  | 30.50 | 29.90 | 29.07 |
| Ash  | 12.08 | 11.74 | 11.01 |

**2.4. Collection of Fish and Stocking**

The Thai Koi fry was sourced from Faridpur district, Bangladesh, and transported to the experimental area in oxygenated polyethylene bags. Before stocking, the fry was acclimatized in a hapa for seven days and fed a commercial diet. Finally, Thai Koi (*A. testudineus*) fry was stocked at a density of 300 per decimal across all treatments in the afternoon.

**2.5. Feeding of Fish**

The experimental feeds were supplied twice daily (morning 7-8 am and afternoon, 4-5 pm). The fish were initially fed at a rate of 10% of their body weight and the rate was reduced to 5% gradually.

 **2.6. Sampling of Fish**

Fishes were sampled monthly by using a seine net to assess their growth and health condition. At least 10% fish from each pond were taken to assess growth trends. The length and weight of sampled fish were measured using a measuring scale and digital electronic balance (OHAUS, MODEL no CT-1200-5). Fishes were handled carefully to avoid stress during sampling.

 **2.7. Fish Harvesting**

At the end of the experiment, fish were harvested manually after the pond drying from each pond.

**2.8. Water Quality Monitoring**

Some water quality parameters, such as temperature (°C), transparency (cm), pH, dissolved oxygen (mg/l), and alkalinity were measured fortnightly at 8:00-9:00 AM at the pond site to assess the physico-chemical condition of the pond water. The transparency was measured by a Secchi disc of 20 cm diameter. Water temperature was recorded from different layers of the pond by an ordinary Celsius thermometer (0°C to 120°C). pH was measured by a direct reading pH meter (HACH) at the pond site. The dissolved oxygen concentration and alkalinity were determined with the aid of a water quality test kit (HACH kit model FF-2, made in the USA).

 **2.9. Evaluation of growth**

The following parameters were used to evaluate the growth:

 Weight gain (g)=Average final weight– Average initial weight

 Specific growth rate (SGR) (%, bwd-1) = **×**100

 FCR= Feed fed in dry weight $÷$Live weight gain

 Survival rate (%) = × 100

 Yield (kg/ha) = fish biomass at harvest – fish biomass at stock

**2.10. Chemical Analysis of experimental feed and fish**

The chemical composition of feeds and fish bodies, including crude protein, lipid, ash, and moisture content, was analyzed following the methods outlined by the Association of Official Analytical Chemists (AOAC, 2003).

 **2.11. Statistical analysis**

All data were subjected to one-way ANOVA to test the significance of the effect of experimental diets, the growth performance of experimental fish, and water quality parameters. In the case where significant differences occurred (*P* <.05), the means were compared using Duncan’s multiple-range test (Duncan, 1955). The results were calculated and expressed as±SD. This statistical analysis was performed with the computer software SPSS (Statistical Package for Social Sciences, 16) program.

1. **RESULTS AND DISCUSSION**

 **3.1. Water quality parameters**

A good aquatic environment and increased productivity largely depend on the water's physicochemical state. Data on different water quality parameters are presented in Table 3. There were no significant differences in the water quality parameters among the treatments. The temperature obtained during the study varied from 32.49±0.09 (T2) to 32.55±0.17 (T1) °C. The recent results were similar to Faruk et al., (2018), who found the water temperature ranged from 31.0 to 35.0 °C in the culture pond of Vietnam koi. The recent findings were more or less similar to the findings of Ali et al., (2000); and Kunda et al., (2008). Slightly lower temperatures were recorded by Ali et al., (2004), and Alim, (2005). The water quality parameters of this study followed the parameters found in the experimental production of *Labeo rohita* (Hamilton, 1822) in pond were suitable with the temperature 28.26 to 28.46°C, pH 6.91 to 6.94, DO 5.04 to 5.33 mg/l, CO22.95 to 3.02 mg/L (Sarker et al., 2021), and the temperature 25.23±0.19°C to 29.17±0.09°C, pH 7.10±0.06 to7.43±0.03, DO 5.10±0.06 mg/l to 5.57±0.03mg/l, CO2 3.23±0.03 to 3.25±0.03mg/l were recorded suitable at other study (Sarker et.al., 2020). According to Boyd, (1982) the suitable range of water temperature for fish culture ranged from 26.06 to 31.97°. However, based on the above findings, it can be concluded that the experimental pond water was within the suitable range of fish culture. The mean range of DO was 4.76±0.10 (T3) to 4.94±0.05 (T1) mg/l in the experimental ponds. The dissolved oxygen in the present study was similar to the findings of Ali et al., (2015), who found the dissolved oxygen content in the Vietnamese climbing perch in a cage at the coastal region ranged from 4.30 to 5.30 mg/l.A more or less similar result was reported by Faruk et al., (2018); Wahab et al.,(1995); Zafar et al., (2017) and Shajib et al., (2017). Transparency in fish culture is essential for preserving water quality, providing sufficient light penetration for photosynthesis, and encouraging healthy fish development. In fish culture, it is necessary to maintain a transparency between 30 to 45 cm to ensure a balanced ecosystem for fish life (Boyd, 1998). In the present experiment, the transparency was ranged from 32.18±1.63 (T2) to 33.87±0.93 (T3) cm, which agreed with Wahab et al., (1995) who measured Secchi depth readings between 26 to 50 cm maintaining the right pH prevents harmful substances from affecting fish and their environment. In the present study, the pH was in the alkaline range of 7.64±0.08 (T1) to 7.69±0.03 (T2), which agreed with the findings of Faruk et al., (2018). Total alkalinity levels for natural waters may range from less than 5 mg/l to more than 500 mg/l (Boyd, 1982). Total alkalinity values found in the present study were within the suitable range from 128.69±3.62 (T2) to 130.87±2.25 (T3) mg/l. However, the overall findings indicated that the mean water quality parameters were found in suitable conditions.

**Table 3. Physico-chemical parameters of experimental pond water under three treatments during the study (n=2)**

|  |  |
| --- | --- |
| **Parameters** | **Treatments** |
| **ED 0%** | **ED 5%**  | **ED 10%**  |
| Temperature (°C) | 32.55±0.17a | 32.49±0.09a | 32.54±0.12a |
| Transparency (cm) | 33.46±1.53a | 32.18±1.63a | 33.87±0.93a |
| pH | 7.64±0.08a | 7.69±0.03a | 7.68±0.03a |
| DO (mg/l) | 4.94±0.05a | 4.85±0.06a | 4.76±0.10b |
| Alkalinity (mg/l) | 130.58±1.19a | 128.69±3.62a | 130.87±2.25a |

\* *Mean values in each row with common superscripts have no significant differences (P>.05)*

 **3.2. Growth and production performances of *A. testudineus***

Variations in the mean values of different growth parameters under different treatments during the study period are presented in Table 4. No significant (*P*>.05) variation was recorded in the initial weight of fish among the treatments. The present findings revealed that the highest final weight was observed in T3 (110.52±6.87g) and the lowest in T1 (75.52±8.36g), which was statistically significant (*P*<.05) among the treatments. In the present study, significantly (*P*<.05) higher mean weight gain was observed in T3 (110.17±6.89g) than in T1, (75.16±8.35g) and T2 (95.14±3.22g). Significantly (*P*<0.05) higher SGR (Figure 1) was observed in T3 (5.86) compared to T1 (5.39) and T2 (5.61). In terms of FCR, there was a significant difference among the treatments. The best FCR was observed in T3 (1.83) compared to T1 (2.43) and T2 (2.07). The recent findings were similar to another study by Anwar and Jafri, (1995) who stated that 9% lipid incorporated in the diet of *Heteropneustes fossilis* gave the best performance regarding growth response, food conversion ratio, and specific growth rate. The current findings more or less agreed with the findings of Noor, (2005); Okonji and Okafor, (2013); Hossain et al.,(2012); Hasan et al., (2010), Hossain et al., (2013), and Habib et al., (2015). This is may be due to the fish have utilized the supplied feed enriched with 10% lipid. A quite different result was also found by Ali et al., (2000) who reported that 15% lipid incorporated in the diet of *Oreochromis niloticus* gave the best performance in terms of growth response, food conversion ratio, and protein efficiency may be due to different species.

The present study shows the survival rate of *A. testudinus* was significantly (*P*<0.05) varied between 84.00±1.73 (T1) to 85.67±0.58 (T3). Recent results agreed with Faruk et al., (2018) who reported that the survival rate of *A. testudinus* was 85%, and Noor, (2005) discovered that the survival rate of Vietnam koi was 81.67% during her 50-day experiment with homemade feed. The present findings disagreed with Priya et al., (2005) who found a 100% survival rate with Labeo *catla* by using soybean oil at the rate of 0, 2, 4, 6, and 8% inclusion. This may be due to the intensive care of cultured fish. In the present investigation, total production was significantly (*P*<.05) higher in T3 (7046.48±25.57) than in the other treatments. The result is also more or less agreed with Okonji and Okafor, (2013); Priya et al., (2005), and Habib et al. (2015). In contrast to other treatments, the study unequivocally demonstrated the superior performance of Thai koi fed a diet that included 10% soybean oil.

**Table 4. Growth performance of Thai koi fed with experimental diet after 100 days (n = 2)**

|  |  |
| --- | --- |
| **Parameters** | **Treatments** |
| **ED 0%** | **ED 5%**  | **ED 10%**  |
| Initial weight (g) | 0.35±0.09a | 0.36±0.11a | 0.36±0.07a |
| Final weight (g) | 75.52±8.36c | 95.50±3.21b | 110.52±6.87a |
| Weight gain (g) | 75.16±8.35c | 95.14±3.22b | 110.17±6.89a |
| FCR | 2.43±0.13a | 2.07±0.10b | 1.83±0.02c |
| SGR (% bwd-1) | 5.39±0.32c | 5.61±0.23b | 5.86±0.27a |
| Survival rate (%) | 84.00±1.73b | 85.00±2.00ab | 85.67±0.58a |
| Yield (kg/ha/100 days) | 4678.42±10.77c | 5992.25±6.50b | 7046.48±25.57a |

 \**Mean values in each row with different superscripts have significant differences (P<.05)*

**Figure 1: Comparison between FCR and SGR of experimental fish after 100 days of rearing**

**3.3. Proximate composition analysis**

The proximate composition of the whole fish body in terms of moisture, crude protein, crude lipid, and ash after 100 days of rearing (Table 5) was significantly affected by dietary lipid levels in Thai koi (*P*<.05). The recent study results showed that the moisture content of Thai koi decreased with increasing dietary lipid levels ranging from 69.60±0.32 (T1) to 68.30 ±0.50 (T3). Ding et al. (2010); Shearer, (1994), and Jobling, (2001), found a high negative correlation between the dietary lipid content and the whole-body moisture content. These findings were also noted in the recent study. The current study showed that the body's crude lipid content increased in direct proportion to dietary lipid levels (Figure 2), ranging from 5.05±0.14 (T1) to 6.01±0.19 (T2).

The current study's results were in line with those of Ding et al., (2010); Shearer, (1994), and Jobling (2001), who found a positive correlation between fish's whole-body lipid content and dietary lipid content. In the present study crude protein was higher in T3 (17.61±0.23) with increasing dietary lipids of Thai koi, which is contradictory to Ai et al., (2008), who reported that crude protein in the whole body decreased. Still, lipids in the entire body increased with increasing dietary lipid. These might be attributable to species age, and experimental settings. Investigation of Sotulu, (2010) also indicated the inverse relationship between dietary lipids and body protein content.

**Table 5. Proximate composition of whole fish (%wet basis) after 100 days of rearing (n = 2)**

|  |  |
| --- | --- |
| **Parameters** | **Treatments** |
| **ED 0%** | **ED 5%**  | **ED 10%**  |
| Moisture (%) | 69.60±0.32a | 68.40±0.56b | 68.30±0.50c |
| Crude lipid (%) | 5.05±0.14c | 5.78±0.17b | 6.01±0.19a |
| Crude protein (%) | 16.10±0.35c | 17.02±0.20b | 17.61±0.23a |
| Ash (%) | 7.25±0.14c | 7.80±0.12b | 7.97±0.11a |

 \**Mean values in each row with different superscripts have significant differences* (*P<.05*)

**Figure 2: Comparison between moisture and crude lipid content of fish after 100 days of rearing**

**4. CONCLUSION**

The study demonstrates that incorporating 10% soybean oil into the diet of Thai koi (*A. testudineus*) significantly improves growth performance, feed utilization efficiency, survivability, and overall yield in pond culture. Additionally, body composition analysis revealed higher crude lipid and crude protein deposition with increasing dietary lipids. These findings suggest that a 10% inclusion of soybean oil in a practical diet is optimal for enhancing the productivity and profitability of Thai koi farming in pond-based systems.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**COMPETING INTERESTS**

The authors have declared that no competing interests exist.

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