# Application of Indian nettle (*Acalypha indica*) and mung bean sprouts (*Vigna radiata*) as a source of plant protein to improve gourami (*Osphronemus goramy*) production

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**Abstract**. Indian nettle (*Acalypha indica*) and mung bean sprouts (*Vigna radiata*) are alternatives for soybean meal because of their high protein content. The purpose of this study was to find and analyze the effect of using Indian nettle flour and bean sprouts as a feed substitution of soybean meal on the growth of gourami (*Osphronemus goramy*) fingerlings. Gourami with an average individual length of 7.0±0.24 cm and an average individual weight of 6.04±0.2 g were used. The stocking density was 1 fish $L^{-1}$, in 10 L aquariums, and 42 days of maintenance. The 4 treatments were formulated to have approximately 32% crude protein: A - commercial feed; B - test feed with including 8% Indian nettle flour and 0% mung bean sprout flour; C - test feed with 3% Indian nettle flour and 3% mung bean sprouts flour; D - test feed with 8% mung bean sprouts flour and 0% Indian nettle flour. Observed data include feed consumption level, feed utilization efficiency, feed conversion ratio (FCR), relative growth rate (RGR), survival rate, and water quality. The best feed utilization efficiency was observed in treatment C, 95.92±2.49%, and the best FCR value was also in treatment C, with 1.67±0.03. The treatment with the best growth was treatment C (RGR 6.11±0.07% per day).
**Key Words**: FCR, feed utilization, growth, plant meal.

**Introduction**. *Osphronemus goramy* has a higher economic value compared to Nile tilapia (*Oreochromis niloticus*) and catfish (*Clarias batrachus*). Public interest in gouramy is very high, but farmers cannot maximally capitalize on it due to slow growth (Mudlofar et al 2013). The production of gourami from year to year is increasing. This also increases the need for artificial fish feed (pellets), and, subsequently, for the ingredients in fish feed, like soybean meal or fishmeal, which have decreasing productions. Thus, the price of these ingredients increases. Some raw materials from agricultural and livestock industries such as soybean meal, corn gluten, and bone meal are widely used. Still, the amount is not high. These conditions cause the continuous search for additional ingredients for aquaculture feeds (Sukarman 2011).

Some solutions to replace soybean meal and fishmeal have been found. One alternative is Indian nettle (*Acalypha indica*) flour and mung bean sprouts (*Vigna radiata*). Indian nettle flour and bean sprouts are rich in phosphorus, trace elements, and vitamin B complex. According to Herawati et al (2019), fishmeal contains 66.02% crude protein, 10.82% crude fat and 1.82% ash. The amino acid profile shows 3.44% arginine, 0.49% cystine, 1.42% histidine, 2.64% isoleucine, 3.25% leucine, 4.8% lysine, 1.62% methionine, 2.59% phenylalanine, 2.91% threonine, 0.84% tryptophan, 1.89% tyrosine, and 3.1% valine. The results of proximate analysis of Indian nettle meal show that it contains 19.64% crude protein, 5.07% crude fat, 16.87% ash, while mung bean sprouts contain 30.21% crude protein, 2.29% crude fat, and 5.63% ash (Hernowo et al 2020).

Research on the substitution of soybean meal with Indian nettle flour and bean sprouts in gourami feed has been conducted in several combinations. Li et al (2017) suggested that soybean protein can be replaced by a maximum of 25% of Indian nettle flour and mung bean sprouts without adverse effects on growth, carcass composition, antioxidant enzyme activity, or digestive enzyme activity in carp (*Cyprinus carpio*). This study aims to determine the effect of Indian nettle flour and bean sprouts addition in the feed of gourami fingerlings on feed efficiency and growth.

## Material and Method

***O. gouramy preparation***. Test fish were gourami with an average individual length of 7±0.24 cm and an average individual weight of 6.04±0.2 g. Feeding was conducted 3 times per day *ad libitum*. The fish were cultured with a density 1 fish $L^{-1}$ in the aquariums with volumes of 10 L. The experiment lasted 42 days. This research was conducted at the BPAP Mijen and Aquaculture Laboratory, Faculty of Fisheries and Marine Sciences, Diponegoro University, Indonesia.

The feed was adjusted to the size of the fish. According to Lestari (2015), razor-sized gourami (7-10 cm) need pellets of 1-2 mm diameter, with a crude protein content between 31-32%. This study used 1.3-1.7 mm diameter pelleted feed, with a protein content of approximately 32%. The treatments in this study consisted in the addition of Indian nettle flour and mung bean sprouts to the artificial feed. The treatments were as follows:

Treatment A: no Indian nettle flour or bean sprouts were added; treatment B: a content of 8% Indian nettle flour was added to the feed; treatment C: 3% Indian nettle flour and 3% mung bean sprouts were added to the feed; treatment D: 8% mung bean sprouts were added to the feed.

Indian nettle and mung bean sprouts were dried in an oven at $50^0$C. The dried material was minced to obtain flour. The flour was weighed according to the specified feed formulation dose. Other ingredients (fishmeal, soy flour, rice bran, wheat flour, fish oil, palm oil, carboxymethyl cellulose - CMC, vitamin and mineral mix) were obtained from the market. The ingredients were mixed evenly until the mix was homogeneous, starting from the smallest weight. The test feed formulations are presented in Table 1.

Protein content was determined by the Kjendahl method (Nx6.25) and lipids were measured as ether extract. Ash was obtained after burning at $600^0$C for 14 h (Laboratory of Animal Feed Nutrition and Food, Faculty of Animal Husbandry and Agriculture, Diponegoro University).

Table 1

Feed formulation and chemical composition of the test feed

| *Ingredients* | *Treatment (%)* | | | |
|---|---|---|---|---|
| | *A* | *B* | *C* | *D* |
| Fish meal | 25 | 25 | 25 | 25 |
| Indian nettle flour | 0 | 8 | 3 | 0 |
| Mung Bean sprouts flour | 0 | 0 | 3 | 8 |
| Soybean flour | 40.8 | 39.1 | 39.8 | 36.2 |
| Bran flour | 24.4 | 18.1 | 18.8 | 20.9 |
| Fish Oil | 2.5 | 2.5 | 3.1 | 2.6 |
| Corn oil | 3.7 | 3.7 | 3.7 | 3.7 |
| Vit-Min mix | 2.8 | 2.8 | 2.8 | 2.8 |
| CMC | 0.8 | 0.8 | 0.8 | 0.8 |
| Total (%) | 100 | 100 | 100 | 100 |
| Crude protein (%) | 31.5 | 32.02 | 32.33 | 32.03 |
| NFE (%) | 28.84 | 28.58 | 28.47 | 28.33 |
| Crude fat (%) | 8.96 | 9.26 | 9.68 | 9.13 |
| Digestible energy (kkal) | 254.92 | 258.51 | 262.79 | 256.92 |
| E/P ratio | 8.09 | 8.07 | 8.13 | 8.02 |

Note: CMC - carboxymethyl cellulose; NFE - nitrogen free extract; E/P - energy per protein.

***Gourami fingerling stock***. The gourami used in this study were obtained from the same broodstock. 40 fingerlings were stocked, 10 for each treatment in 10 L aquariums. Before conducting the research, the gourami were acclimatized first in the new media for 7 days, so that fish were not stressed. The weighing was conducted at the beginning and end of the study, using digital scales to determine the growth rate. The individuals were also periodically counted to determine the survival rate.

***Total feed consumption***. The total value of feed consumption was calculated using the formula of Pereira et al (2007), as follows:

$$Total = F1 - F2$$

Where: total - feed consumption (g); F1 - total feed in the beginning of the experiment (g); F2 - total feed at the end of the experiment (g).

***Relative growth rate (RGR)***. According to Takeuchi (1988), the relative growth rate of fish was calculated using the formula:

$$RGR = (W_t - W_0)/( W_0 \times t) \times 100$$

Where: RGR - relative growth rate (% per day); $W_t$ - total final weight (g); $W_0$ - total initial weight (g); t - days of experiment.

***Survival rate (SR)***. According to Effendie (2002), the survival rate can be calculated using the following formula:

$$SR = Nt/No \times 100$$

Where: SR - survival rate (%); Nt - the number of fish at the end of the observation; No - the number of fish at the beginning of the observation.

***Feed utilization efficiency***. According to Watanabe (1988), the efficiency of feed utilization can be calculated using the following formula:

$$\text{Feed utilization efficiency} = (Wt + D - Wo)/F \times 100$$

Where: Wt - final biomass (g); Wo - initial biomass (g); D - weight of dead fish (g); F - total fish feed administered (g).

***Feed conversion ratio (FCR)***. According to Mokoginta et al (1995), the FCR can be calculated using the following formula:

$$FCR = F/(Wt-W0+D)$$

Where: FCR - feed conversion ratio; F - total feed weight (g); Wt - final fish weight (g); W0 - initial fish weight (g); D - dead fish weight (g).

***Water quality parameters***. Water quality data parameters measured include dissolved oxygen (DO), pH, temperature, and ammonia. DO, pH, and temperature were measured using a water quality checker (WQC) and ammonia measurements were carried out in the Aquatic Resource Management Engineering Laboratory, UNDIP. DO, pH, and temperature measurements were carried out every 3 days. Ammonia was measured at the beginning, middle, and end of the study.

***Data analysis***. Data were verified with a normality test, homogeneity test, and additivity test to verify normal, homogeneous and additive properties. Data were analyzed by variance tests (F test) at a 95% confidence level to see its effect. If, in the analysis of variance, a significant difference was obtained ($p<0.05$), then Duncan's multiple region test was performed to determine differences between treatments.

## Results and Discussion

***Feed consumption***. Based on the average results of the total consumption of gourami during the study, the highest value was obtained in treatment C, with a mean of 106.75±4.35 g, followed by treatments A, D and the lowest was treatment B, with averages of 97.25±3.1 g, 94.75±0.96 g, and 90±0.82 g, respectively (Figure 1).

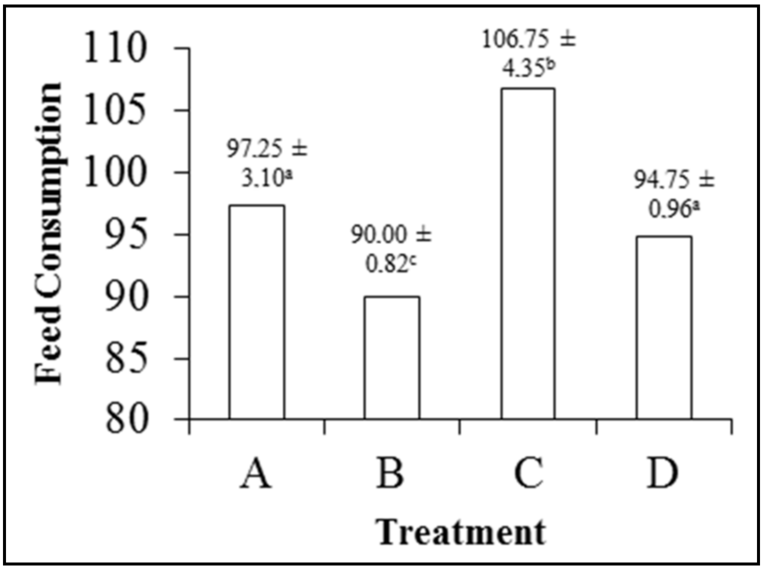


Figure 1. Feed consumption of *Osphronemus goramy* (different letters above bars show significant differences, $p<0.05$).

Treatment C was the best treatment, because during the research, fish had a high appetite. According to Aslamyah & Karim (2012), a good test feed has raw materials with reliable attractants. Another factor that affects fish appetite is water quality because, according to Mulyadi et al (2014), poor water quality can stress the fish, resulting in reduced appetite and disruption of metabolic processes. The temperature can affect fish growth and appetite (Kelabora & Sabariah 2010). High temperatures can reduce DO levels and affect fish appetite.

Treatment B experienced a low level of feed consumption, and there was leftover feed. It resulted in lower fish growth, and water quality pollution. Pinandoyo et al (2014) state that to increase feed consumption, feed utilization efficiency and growth, the time interval of stomach emptying should be considered.

According to Khasani (2013), the feed attraction is significant in the formulation of fish feed. The nutritional quality becomes less effective if the feed does not contain components that can stimulate the fish response to the feed. The size of the total consumption of fish feed is influenced by several factors, including the physical properties of feed such as odor, taste, size, and color (Abidin et al 2015). Other influential factors are water quality parameters.

***Feed utilization efficiency***. Based on the average results of the utilization efficiency of gourami fish feed, the highest value was in treatment C, with a mean of 95.92±2.49%, followed by treatments A, D and the lowest was treatment B, with a mean of 93.75±2.18%, 91.71±1.14%, and 89.58±3.06%, respectively. The efficient utilization of gourami fish feed during the study can be seen in Figure 2.

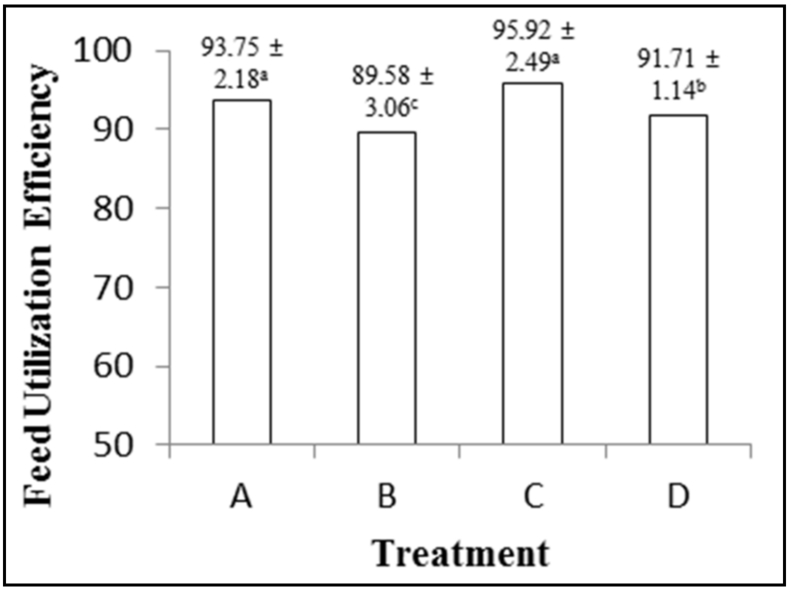


Figure 2. Feed utilization efficiency of *Osphronemus goramy* (different letters above bars show significant differences, $p<0.05$).

The feed utilization efficiency value for each treatment was close to 100%. The efficiency of feed utilization should be as close as possible to 100% (Puspasari et al 2015). According to Maulidin et al (2016), a good feed utilization efficiency value shows that the food consumed has a good quality, so it can be easily digested and utilized efficiently by fish. The efficiency of feed utilization is closely related to the digestibility of the feed. The digestibility of a feed is influenced by several factors, namely the chemical nature of water, water temperature, type of feed, fish size and age, nutrient content of the feed, frequency of feeding and the amount of feed (Yanti et al 2013).

The effectiveness of the utilization of gourami feed is also influenced by the metabolic rate. According to Haetami (2012), feed energy is used by fish in metabolism processes, for growth, and reproduction. Satiated fish will experience a decrease in the body's metabolism rate. The thyroxine hormone can change the pattern of carbohydrate metabolism through increased activity of the amylase enzyme, so that the digestion and absorption of carbohydrates are high (Thalib 2012). Thyroxine also has an effect on increasing the digestive enzymes of protease and lipase, so that fish can stimulate protein digestibility and increase absorption of amino acids and fatty acids through the intestine.

***Feed conversion ratio (FCR)***. The highest FCR value was obtained in treatment C, with a mean of 1.67±0.03, followed by treatments A, D and the lowest was treatment B, with a mean of 1.65±0.1, 1.34±0.05, and 1.29±0.08, respectively (Figure 3).

A good feed quality can also be seen from the FCR value. The FCR value shows the efficient utilization of feed by fish. A lower FCR value shows a more efficient use of the feed (Purnomo et al 2015; Syaputra et al 2018). The FCR results of all treatments during the study were considered good because they were less than 1.5. The results of research conducted by Safir (2012) showed a FCR value of gourami of 1.29-1.67.

***Relative growth rate (RGR)***. The highest value of RGR was in treatment C, with a mean of 3.82±0.07% followed by treatments A, D and the lowest was treatment B, with a mean of 3.37±0.05%, 3,18±0.03%, and 2.92±0.11%, respectively (Figure 4).

Maloho et al (2016) stated that the growth speed depends on the amount of feed consumed, water quality and other factors such as heredity, age, endurance and ability of the fish to use feed. This is supported by Tribina (2012), who stated that, if the need for maintenance exceeds the amount of feed administered, there will be a process of dismantling the energy in the body of the fish itself (catabolism). An adequate amount of feed should be suffice for body growth and daily activities of the fish; excess or lack of feed can result in decreased growth rate.

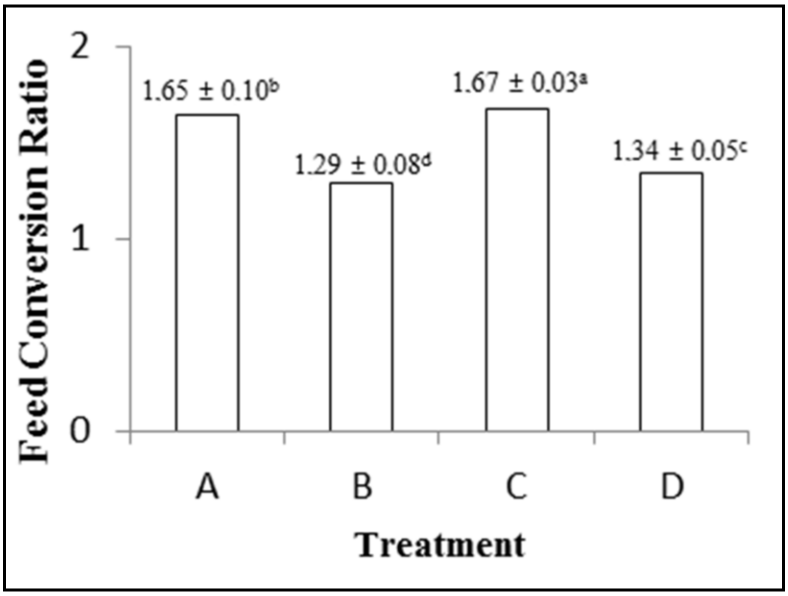


Figure 3. Feed conversion ratio of *Osphronemus goramy* (different letters above bars show significant differences, $p<0.05$).

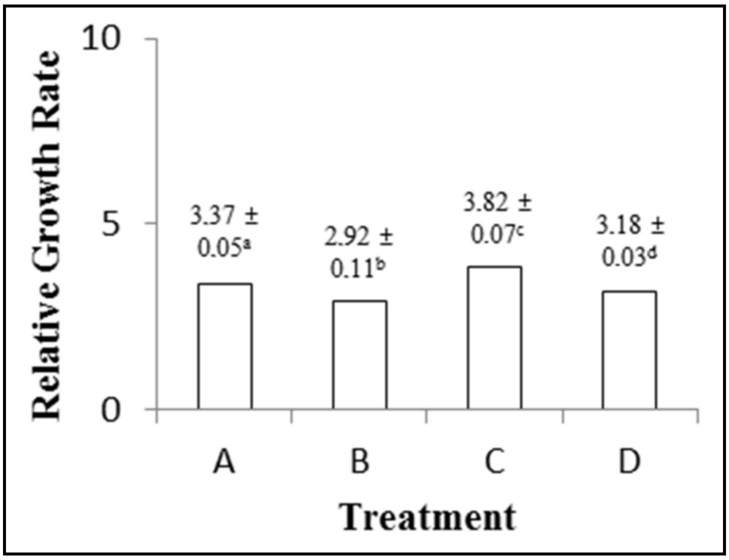


Figure 4. Relative growth rate of *Osphronemus goramy* (different letters above bars show significant differences, $p<0.05$).

***Survival rate (SR)***. Treatment C was the best treatment, with no mortalities, followed by treatments A, D and the lowest was treatment B, with a mean of 95±5.77%, 85±5.77% and 82.5±5.77%, respectively (Figure 6).

The survival rate of gourami showed significant differences between treatments ($p>0.05$). Death of some gourami at the beginning of the experiment is suspected to be due to stress. Stress was caused by moving the fish, uningested feed and weighing. According to Samsundari & Wirawan (2013), the survival of fish is influenced by biotic factors, namely competitors, parasites, population density, and adaptability, and abiotic factors, physical and chemical parameters of the water.

Fish food has an important role in the growth and survival rate of fish. The high survival rate of gourami fingerlings is thought to be due to the fulfillment of nutritional requirements for survival, and suitable environmental maintenance conditions, so that stress was minimum.

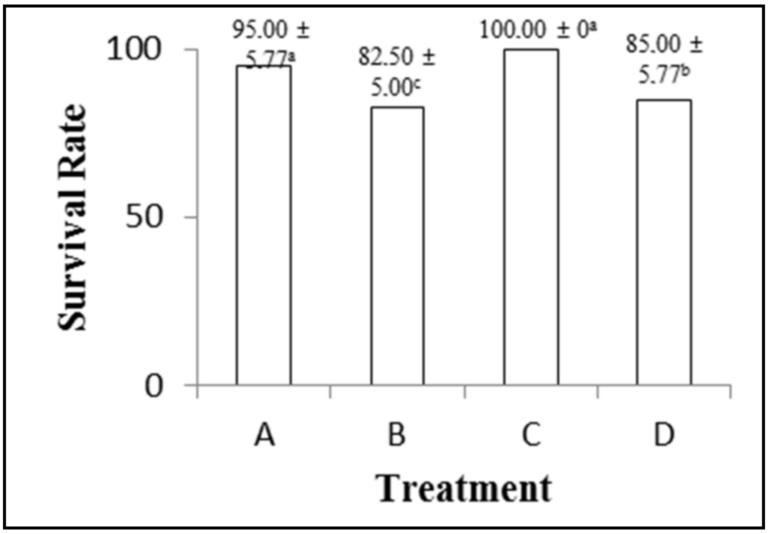


Figure 6. Survival rate of *Osphronemus goramy* (different letters above bars show significant differences, $p<0.05$).

***Water quality***. The measurement results of several water quality parameters including temperature, pH, DO, and ammonia are presented in Table 2.

Table 2

Water quality parameters values

| *Treatment* | *Parameter values* | | | |
|---|---|---|---|---|
| | *Temperature (°C)* | *pH* | *DO (mg $L^{-1}$)* | *$NH_3$ (mg $L^{-1}$)* |
| A | 28.3-29.45 | 7.2-7.9 | 3.8-4.8 | 0.004-0.062 |
| B | 28.5-29.9 | 7.2-8 | 3.8-4.4 | 0.004-0.062 |
| C | 28.2-29.2 | 7.2-7.7 | 3.8-4.6 | 0.004-0.062 |
| D | 28.6-29.6 | 7.2-7.8 | 3.8-4.5 | 0.004-0.062 |
| Optimal range | 28-30[b] | 6.5-9[a] | 3-7[a] | 0-0.12[a] |

Note: DO - dissolved oxygen; a - Nirmala & Rasmawan (2010); b - Oktavianto et al (2014).

The temperature during maintenance was in the optimal range, the normal 25-30°C. Oktavianto et al (2014) stated that low temperatures below or above the normal limits can cause gourami decreased appetite, susceptibility to disease, and even death. Temperature affects the resistance to disease and physiological processes of fish (Bangsa et al 2015).

The pH value in the maintenance media ranged from 6.5 to 8. The pH value was in the normal range. The DO during maintenance was in the range of 3.8-4.5 mg $L^{-1}$. The DO level is in normal limits. According to Nirmala & Rasmawan (2010), DO content for the maintenance of gourami should be between 3-7 mg $L^{-1}$. Gourami has an additional breathing device in the form of a labyrinth that allows it to take oxygen from the air directly, so that it can survive in waters with low DO concentrations (SNI:01-6485.1).

The value of ammonia from fish feces and leftover food was in the range of 0.004-0.062 mg $L^{-1}$. This value is within the tolerance range of gourami. The water was siphoned and changed every 3 days, to remove leftover food and feces that could have increased the value of ammonia. According to Nirmala & Rasmawan (2010), ammonia is toxic when it exceeds 0.2 mg $L^{-1}$. Syahrizal et al (2013) state that ammonia is the end result of protein metabolism. Ammonia in its non-ionized form ($NH_3$) is poisonous to fish even at very low concentrations.

**Conclusions**. The addition of Indian nettle and bean sprouts to feed significantly affected ($p<0.05$) the efficiency of feed utilization, growth, and survival of gourami. The best feed formula with the addition of Indian nettle flour and mung bean sprouts was in treatment C (3% Indian nettle flour and 3% bean sprouts). Treatment C was able to produce a feed consumption level of 106.75±4.35 g and a relative growth rate of 6.11±0.07% per day. Moreover, the efficiency of feed utilization was 95.92±2.49%, the FCR was 1.67±0.03, and the survival rate of fish was 100%.

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