The effect of commercial feed enrichment with *Piper betle* leaf extract on the growth and survival rate of tilapia (*Oreochromis niloticus*)

S S Agustina
Aquaculture Study Program, Fisheries Faculty, Muhammadiyah Luwuk University, Luwuk, Central Sulawesi, Indonesia

Email: sri.unismuhlulu@gmail.com

**Abstract.** This research aimed to examine the effect of commercial feed enrichment with *Piper betle* leaf extract on the growth and survival rate of tilapia (*Oreochromis niloticus*). This study used an experimental method with 15-20 g tilapia seed as test organisms at a stocking rate of 150 fish/m². A Completely Randomized Design was used with five treatments and three replicates. The treatments were: A (pelleted feed/control); B (10% *Piper betle* leaf extract/kg feed); treatment C (15% *Piper betle* extract/kg feed); treatment D (20% *Piper betle* leaf extract/kg feed); and treatment E (25% betel leaf extract/kg of feed). The parameters observed during this study were the net increase in biomass and survival rate of the tilapia seed. All data were tabulated, then analysed using an analysis of variance (ANOVA). Water quality parameters in each treatment remained within acceptable limits for tilapia grow-out throughout the study, with pH 6.0–7.96; temperature 27.1 - 34.8 °C; DO 3.7 - 4.8 ppm and 0 ppm ammonia. The *Piper betle* leaf extract concentration in treatment D (20%) gave the best results, with 342.33 ± 10.60 g net increase in tilapia seed biomass and 100% ± 0% survival rate.

1. **Introduction**

Tilapia (*Oreochromis niloticus*) is a freshwater food fish which is widely cultivated and popular with many communities, especially in Indonesia. This is because tilapia has attractive properties such as being easy to breed, tolerant of less than optimal environments, able to live and reproduce in saline water and possessing a generally fast growth response to food [1].

Feed plays an important role in endeavours to increase tilapia productivity [2]. The feed is one of the main supporting factors for improving growth and survival [3]. Therefore, it is important to control the nutrients contained in feed, in order to ensure they meet the needs of the cultured fish. Proper feeding will help prevent tilapia from succumbing to attacks by various diseases, while feed that has a nutritional content meeting the requirements of the fish will accelerate growth and improve survival.

Problems faced by tilapia farmers include the increasing cost of feed and the low quality of some commercial feed. These problems affect the profitability and production yield. One alternative that farmers can implement is to add nutrients to the feed by utilizing locally available traditional medicinal plants that are easily obtained, and that can increase the body's resistance to disease [4]. One of these plants which has the potential to increase the body's resistance to disease *Piper betle* L. The leaves of *Piper betle* L. have anti-parasitic, anti-bacterial, fungicidal, antiseptic and anti-oxidant properties.
properties and they contain bioactive ingredients such as essential oils, saponins, tannins, flavonoids, and alkaloids [5].

It has been found that the concentration of Piper betle L. leaf extract given to tilapia can reduce the intensity of disease from the parasite Trichodina sp. [6]. In addition to decreased severity of infections from Trichodina sp. parasites, Piper betle L. leaf extract can inhibit the development of Trichodina sp. [6]. The stronger the content of phenol compounds in essential oils in the Piper betle L. leaf extract used, the stronger the antioxidant activity to break the crosslinks and break through the cell wall of the parasite. The death of this parasite results from the damage caused to the cell membrane of the protozoa through protein denaturation and the dissolution of fat contained in the cell membrane by the phenol component so that the permeability of the cell membrane is disrupted allowing leakage of cell contents [6]. This research aims to examine the effect of enriching commercial feed through the addition of different concentrations of an extract of Piper betle leaf on the growth and survival rate of tilapia (Oreochromis niloticus).

2. Materials and Methods

This research was conducted from February to June 2018 at the Honduhon Fish Seed Centre (BBI), in Banggai Regency, Central Sulawesi Province, Indonesia. The analysis of chemical compounds in the composition of the Piper betle L. leaf extract used as a treatment was carried out at the Ujung Pandang State Polytechnic laboratory in Makassar, South Sulawesi, Indonesia.

2.1. Research Materials

The test organisms used in this study were tilapia seed obtained from the Honduhon Fish Seed Centre weighing 15-20 g. The stocking density was ten fish/m³. The experiment units were floating net cages with frames made of flexible coated wire, with a size of 1x1x1 m (1 m³). The base feed used was a Hi-Provite 788-3 floating pellet feed with an advertised composition of 26-28% protein and 4-6% fat and a size of 3.2-4.0 mm produced by PT. Central Proteima Prima TbK.

Piper betle L. leaves were collected and 2,000 g of fresh leaves were weighed then washed with distilled water. The clean leaves were freeze-dried at a temperature of -40°C until dry then ground to powder in a blender and weighed. The betel leaf powder was immersed in 6 L of ethanol 96% solvent for 3x24 hour. The filtrate was obtained through filtration after maceration. The mixture was stirred 12 times for 15 minutes with a resting period of 5 minutes between mixing, then filtered using a funnel and filter paper to separate the filtrate and pulp. The filtrate was then evaporated using a rotary vacuum evaporator so that aliquots of 200 mL of solvent-free concentrated extract were obtained. The resulting extract was used for testing [7].

2.2. Experimental Design

This study used an experimental method with a completely randomized design comprising 5 (five) treatments and 3 (three) replicates. The treatments were different concentrations of Piper betle L. leaf extract added to the feed given to the tilapia: treatment A (pellet feed/control); B (10% Piper betle L. leaf extract/kg of feed); C (15% Piper betle L. leaf extract/kg of feed); D (20% of Piper betle L. leaf extract/kg of feed); and E (25% Piper betle L. leaf extract/kg of feed).

2.3. Research Procedures

The test organisms were acclimated for three days and weighed before being placed into the experimental cages (units) to determine the initial weight of the fish biomass. Piper betle L. leaf extract was sprayed on the pelleted feed using a spray bottle at different concentrations according to each treatment. The feed was then mixed with Piper betle L. leaf extract and dried. Preliminary tests were conducted to determine the concentration range of Piper betle L. leaf extract used in each enrichment treatment. The in-vivo test of Piper betle L. leaf extract in feed given to tilapia was designed to determine the optimal concentration. The tilapia seed were fed at a daily rate of 3% of the total biomass in each unit, with a feeding frequency of 3 (three) times a day, at 08.00, 12.00, and 16.00
local time (GMT+8). The test organisms (tilapia seed) were weighed every two weeks, while survival was observed daily.

2.4. Parameter Analysis

The parameters observed were the net increase in biomass and the survival rate of the tilapia seed. The net increase in biomass of the fish in each unit was calculated using the formula in [8]:

\[ W = W_{t} - W_{o} \]

where:
- \( W \) = Net increase in biomass (g)
- \( W_{t} \) = Fish biomass at the end of the study (g)
- \( W_{o} \) = Fish biomass at the beginning of the study (g)

The survival rate of the test organisms (tilapia seed) was calculated using the formula in [9] as follows:

\[ SR = \frac{N_{t} \cdot N_{o}^{-1}}{1} \times 100\% \]

Where:
- \( SR \) = Survival Rate (%)
- \( N_{t} \) = Number of test fish at the end of the study
- \( N_{o} \) = Number of test fish at the beginning of the study

All data obtained during the study were tabulated and analysed descriptively. An analysis of variance (ANOVA) was performed to test for significant difference between treatments. If there was a significant effect \((p < 0.05)\) a post-hoc least significant difference (LSD) test was applied \((\alpha = 0.05)\) [10].

3. Results

3.1. Effect of *Piper betle* L. leaf extract concentration added to commercial feed on the net increase in tilapia biomass

The commercial feed enriched with addition of betel leaf extract in different concentrations produced different growth increases in the biomass of the tilapia seed (Figure 1 and Table 1). The highest growth in biomass was observed in treatment D (20% leaf extract). However the differences between treatments were not statistically significant (Table 1).

![Figure 1. Net increase in tilapia biomass by treatment](image-url)
Table 1. Net increase in tilapia biomass (g) during the study period

| Treatment                        | Net increase in tilapia biomass (g)* | Mean ± SD          |
|----------------------------------|--------------------------------------|--------------------|
| A (0 % *Piper betle* leaf extract) | 289 ± 39.40a                       |                    |
| B (10% *Piper betle* leaf extract) | 311.33 ± 54.37a                     |                    |
| C (15% *Piper betle* leaf extract) | 313.33 ± 43.04a                     |                    |
| D (20% *Piper betle* leaf extract) | 342.33 ± 10.60a                     |                    |
| E (25% *Piper betle* leaf extract) | 295.33 ± 36.69a                     |                    |

*The same superscript letter in the same column indicates no significant difference (P>0.05)

3.2. The effect of concentration of *Piper betle* L. leaf in commercial feed on the survival rate of tilapia

The enrichment of commercial feed through the addition of *Piper betle* L. leaf extract had no effect on tilapia seed survival rate. Survival in all treatments was 100% (Table 2).

Table 2. Survival rate (%) of tilapia seed during the study by treatment

| Treatment                        | Tilapia Survival Rate (%)* | Mean ± SD         |
|----------------------------------|-----------------------------|-------------------|
| A (0 % *Piper betle* leaf extract) | 100 ± 0a                    |                   |
| B (10% *Piper betle* leaf extract) | 100 ± 0a                    |                   |
| C (15% *Piper betle* leaf extract) | 100 ± 0a                    |                   |
| D (20% *Piper betle* leaf extract) | 100 ± 0a                    |                   |
| E (25% *Piper betle* leaf extract) | 100 ± 0a                    |                   |

* The same superscript letter in the same column indicates no significant difference (P>0.05)

3.3. Water Quality

The range of water quality parameters observed during the study are shown by treatment in Table 3.

Table 3. Range of water quality parameters during the study by treatment

| Treatment                        | pH     | Temperature (°C) | DO (ppm) | Ammonia (ppm) |
|----------------------------------|--------|------------------|----------|---------------|
| A (0 % *Piper betle* leaf extract) | 6.00 – 7.96 | 27.1 – 34.7     | 3.7 – 4.6 | 0             |
| B (10% *Piper betle* leaf extract) | 6.05 – 7.96 | 27.1 – 34.8     | 3.7 – 4.8 | 0             |
| C (15% *Piper betle* leaf extract) | 6.01 – 7.96 | 27.1 – 34.7     | 3.7 – 4.6 | 0             |
| D (20% *Piper betle* leaf extract) | 6.00 – 7.96 | 27.1 – 34.7     | 3.7 – 4.7 | 0             |
| E (25% *Piper betle* leaf extract) | 6.03 – 7.96 | 27.1 – 34.7     | 3.7 – 4.8 | 0             |

4. Discussion

Based on Table 1 and Figure 1 the addition of *Piper betle* L. leaf extract to commercial feed (in concentrations of 10%, 15%, 20% and 25% *Piper betle* L. leaf extract per kg of feed) did result in higher average net increases in biomass compared to the control treatment. However the standard deviation values show that within-treatment variation was high, except for Treatment D (concentration of 20%/kg of commercial feed). Despite the lack of statistical significance, the lower variation combined with the highest average value of net biomass increase indicates that this treatment is more beneficial to the tilapia seed compared to the other treatments.

Treatment D (concentration of 20%/kg of commercial feed) could have given the highest net increase in biomass because the use of *Piper betle* L. leaf extract at a concentration of 20% per kg of commercial feed may help boost the immune system of the tilapia and increase fish appetite, with optimal digestion and absorption. Possibly feed containing a higher concentration than 20% of *Piper betle* L. leaf extract might not be so well digested and absorbed. Observations of the behaviour of the
tilapia indicated that the feeding response of tilapia was higher in the *Piper betle* L. leaf extract treatments, especially treatment D, with normal movements and no signs of stress or other unusual symptoms. Because feeding containing *Piper betle* L. leaf extract can improve fish health status, it could indirectly increase the net growth of individual tilapia and thus substantially increase biomass, because of the compounds in the extract which can function as immunostimulants to improve the non-specific defence system of tilapia.

The main chemical compounds contained in betel leaves are essential oils, vitamins, organic acids, amino acids, sugar, tannins, fats, starches, and carbohydrates which function as antibacterial, antiseptic, and immunostimulant agents [11]. *Piper betle* L. leaf is a natural remedy that can be use as an antiseptic. *Piper betle* L. leaf contains essential oils, phenyl propane, estragol, chavicol, hydroxychavicol, cavitol, caryophyllene, allylpyrokatekol, cineole, cadinene, tannin, diastase, starch, terpinene, sesquiterpene, and sugar [12].

The mean increase in net tilapia biomass in treatments B and treatment C was lower than in treatment D, probably due to the lower amount of extract mixed with commercial feed which may not have been sufficient to provide optimal benefits in terms of increasing immune system resistance and contributing to growth processes. Conversely, one likely reason for treatment E giving the lowest mean net biomass increase is that the amount of concentrate added was possibly too high compared to the amount of feed, so that excess concentration of *Piper betle* L. leaf extract could directly affect (depress) the appetite of the tilapia seed.

Based on Table 2, the use of commercial feed with or without *Piper betle* L. leaf extract in any of the concentrations used and without the use of *Piper betle* L. leaf extract (control) did not affect the survival rate of the tilapia seed in this study, as all units had a survival rate of 100%. While this does not prove any positive advantage, this result indicates that the addition of *Piper betle* L. leaf extract at concentrations up to 25% does not have a negative effect on the survival rate of tilapia seed.

The 100% survival rate of tilapia in each treatment maintained during the study was likely due to the feeding timing, dose, and frequency provided the nutrition needed by the tilapia for their survival. Survival can be influenced by internal factors such as hereditary traits, disease resistance, and ability to utilize feed. While external factors include inter alia temperature, oxygen, the quality and quantity of feed, and space (optimal density and appropriate size of maintenance units). The intake of energy obtained from feed can be used for the metabolic process, growth, and the development of gonads, and is partially lost through faeces and urine [13].

The results of the analysis of water quality parameters measured in each treatment as outlined in Table 3 show that the pH, temperature, DO, ammonia and salinity ranges are within reasonable limits for the survival and growth of tilapia seed. According to [14], to promote tilapia growth, fluctuations in temperature should not be more than 4°C, while at temperatures below 25°C tilapia will be more susceptible to disease attacks by bacteria. The pH value should be within the range pH 6-8, although tilapia can survive at lower pH values than many fishes. The pH value of waters where tilapia typically live ranges from 6-8.5, but optimal growth occurs at pH 7-8 [14]. The water quality standard for freshwater fish cultivation in Indonesia is within a temperature range of 25-28°C, pH 6.5-9, dissolved oxygen 2-7 ppm, and ammonia <0.1 ppm [15]. The water quality range for tilapia (*Oreochromis niloticus*) according to [16] is 23-34°C, pH 5-8.9, DO 3-7 ppm, and ammonia value <0.3 ppm. Whichever of these ranges is used as a reference, the observed values of all water quality parameters observed throughout the study were within the recommended ranges.

5. Conclusion

Based on the results of this study, the effect of the concentration of *Piper betle* L. leaf extract on commercial feed on the growth and survival of tilapia (*Oreochromis niloticus*) appeared to be positive, despite a lack of statistical significance. Feed enrichment with a concentration of *Piper betle* L. leaf extract corresponding to treatment D (20% of *Piper betle* L. leaf extract/kg of feed) gave the best result, with the highest net increase in tilapia biomass and the lowest variability in growth (342.33 g ± 10.60 g). Survival rate of the tilapia seed was 100% under all treatments. Water quality parameters...
(temperature, pH, DO, and ammonia) in all treatments remained within the optimal ranges for tilapia throughout the study.

References

[1] Prabu E, Rajagopalsamy C B T, Ahilan B and Jeevagan, I J M A Renuhadevi M 2019 Tilapia – An Excellent Candidate Species for World Aquaculture: A Review Annu. Res. Rev. Biol. 31 1–14

[2] Henriksson P J G, Tran N, Mohan C V, Chan C Y, Rodriguez U P, Suri S, Mateos L D, Utomo N B P, Hall S and Phillips M J 2017 Indonesian aquaculture futures – Evaluating environmental and socioeconomic potentials and limitations J. Clean. Prod. 162 1482–1490

[3] El-Sayed A-F M and Teshima S 1992 Protein and energy requirements of Nile tilapia, Oreochromis niloticus, fry Aquaculture 103 55–63

[4] Lusiastuti M, Taukhid T, Anggi I and Caruso D 2017 Dry green leaves of Indian almond (Terminalia catappa) to prevent streptococcal infection in juveniles of the Nile tilapia (Oreochromis niloticus) Pathol. Bull. Eur. Assoc. Fish 37 119–25

[5] Hamidah T S, Kumalaningsih I K and Dewi 2014 Pembuatan Ekstrak Oleoresin Daun Sirih Hijau (Piper betle L.) Sebagai Pengawet Alami (Kajian Suhu Dan Lama Waktu Ekstraksi) (Malang: Fakultas Teknologi Pertanian Universitas Brawijaya)

[6] Agustina S S Y M and A A B 2018 Uji Daya Antiparasit Konsentrasi Ekstrak Piper betle L. Terhadap Parasit Trichodina sp. Yang Menginfeksi Benih Ikan Nila (Oreochromis niloticus) Prosiding Seminar Nasional Kelautan Fakultas Teknik dan Ilmu Kelautan Vol 1 No 1 (Universitas Hangtuah) pp C2-9 – C2-16

[7] Innayatullah S 2012 Efek Ektrak Daun Sirih Hijau (Piper betle L.) Terhadap Pertumbuhan Bakteri Staphylococcus aureus (Universitas Islam Negeri Syarif Hidayatullah Jakarta)

[8] Islami E, Yunita, Basuki F and Elfitasari T 2013 Analisa Pertumbuhan Ikan Nila Larasati (Oreochromis niloticus) Yang Dipelihara Pada KJA Wadaslintang Dengan Kepadatan Berbeda J. Aquac. Manag. Technol. 2 115–21

[9] Alfiah A R, Arini E and Elfitasari T 2013 Pengaruh Kepadatan Yang Berbeda Terhadap Kelulushidupan Dan Pertumbuhan Ikan Nila (Oreochromis niloticus) Pada Sistem Resirkulasi Dengan Filter Bioball J. Aquac. Manag. Technol. 2 86–93

[10] Gaspersz V 1995 Teknik Analisis Dalam Penelitian Percobaan Tarsito Bandung (Bandung: Tarsito)

[11] Hamzah H and Muskita W 2010 Pemanfaatan Bubuk Daun Sirih (Piper betle L.) Untuk Meningkatkan Status Kesehatan Ikan Nila Gift (Oreochromis niloticus) J Ris Akuakultur 5 135–41

[12] Widaryati R 2017 Penambahan Ekstrak Jenis Tanaman Herbal yang Berbeda pada Media Pemeliharaan Terhadap Kelangsungan Hidup Benih Ikan Nila (Oreochromis niloticus) J. Ilmu Hewani Trop. 6 52–5

[13] Effendi M I 2002 Biologi Perikanan Bagian II Dinamika Populasi Ikan (Bogor: Fakultas Perikanan Institut Pertanian)

[14] R S 2010 Pembenihan dan Pembesaran Nila (Jakarta: Penebar Swadaya)

[15] Syamsuddin R 2014 Pengelolaan Kualitas Air Teori dan Aplikasi di Sektor Perikanan (Makassar: Pijar Press)

[16] Saparianto C and Susiana R 2011 Kiat Sukses Budidaya Ikan Nila (Yogyakarta: Lily Publisher)