Supplementation of Gamal leaves flour (*Gliricidia sepium*) in commercial feed on the growth of Nirwana tilapia (*Oreochromis niloticus*) fingerlings

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**Abstract.** This study aimed to examine the effect of Gamal leaves flour supplementation in commercial feed on the growth of Nirwana tilapia (*Oreochromis niloticus*) fingerlings. The experimental design used was a non-factorial completely randomized design consisting of four treatments and four replications. The treatments studied included commercial feed without supplementation of Gamal leaves flour as a control (P0), commercial feed with supplementation of Gamal leaves flour 10% kg⁻¹ feed (P1), commercial feed with supplementation of Gamal leaves flour 15% kg⁻¹ feed (P2), and commercial feed with supplementation of Gamal leaves flour 20% kg⁻¹ feed (P3). Nirwana tilapia fingerlings were stocked at a density of 1 fish L⁻¹. Nirwana tilapia fingerlings size ranges from 2-3 cm. Nirwana tilapia fingerlings maintenance period lasts for 40 days. The Anova test showed that the treatment gave a significant effect on the weight gain, length gain, daily growth rate, and feed conversion ratio (P < 0.05), but did not give a significant effect on the survival rate of Nirwana tilapia fingerlings (P > 0.05). The best dose to increase the growth of Nirwana tilapia fingerlings was commercial feed with supplementation of Gamal leaves flour 10% kg⁻¹ feed.

1. Introduction

In Indonesia, tilapia is classified as one of the leading aquaculture commodities and its production continues to be increased. Tilapia production increases every year with an average increase in the amount of production reaching 31% in 2013-2017. Tilapia production reached 1.15 million tons in 2017.
(up 3.6%) from 2016 (1.14 million tons). Based on the main commodity category of aquaculture, tilapia production ranks second after biofloc catfish [1]. Tilapia production continues to be increased every year to meet the needs of the community and increase the profits of aquaculture farmers. Increased production can be done by using superior tilapia strains in cultivation activities, feeding according to the nutritional needs of fish, and good water quality management. Nirwana tilapia is one of the leading tilapia which is the result of a cross between GIFT tilapia and GET tilapia from the Philippines [2]. Nirwana tilapia has several advantages, namely its high level of tolerance to environmental changes [3], white and thicker flesh [4], and fast growth [5].

The main obstacle in increasing aquaculture production is the relatively high cost of feed production reaching 70%-90% of the total business operating costs [6-8]. Feed is one of the important factors that must be considered in aquaculture because the main energy source for fish comes from the feed. Availability of feed with balanced nutrition will support fish growth, so it can increase aquaculture production to be more optimal [9]. The aquaculture business is very dependent on feed, the price of feed which is relatively expensive can result in increased production costs so that the profits obtained by aquaculture farmers are decreasing [10]. The price of feed is relatively expensive because the raw materials as feed ingredients have a high price in the market (eg fish meal and soybean meal). Therefore, supplementation of alternative raw materials in feed is needed so that production costs are more efficient. The advantages of Gamal leaves are available throughout the year in Aceh and low prices, so it is a consideration to use gamal leaves as an alternative raw material for feed. In addition, gamal leaves still have high nutrition, namely 16.88% protein, 16.97% crude fiber, 10.37 % ash content, 0.20% calcium, 0.40% phosphorus content and 3.01% gross energy [11].

The use of Gamal leaves as raw material for fish feed is still minimal by aquaculture farmers. So far, gamal leaves have been only used as animal feed, so information on the use of Gamal leaves supplementation in commercial feed or substitution in fish feed rations is still very limited. Several research results have been reported related to the use of Gamal leaves in artificial feed rations on the growth of tilapia [4], the application of a combination of Gamal leaves flour and eggs in commercial feed on the feed quality and feed efficiency of Nirwana tilapia fingerlings [12], mixing gamal leaves flour in commercial feed on the growth of gourami [13,14]. The provision of commercial feed formulated with moringa leaves, gamal leaves, and gotu kola leaves has a significant effect on the growth of gourami fish [15]. However, research on the supplementation of Gamal leaves flour in commercial feed on the growth of Nirwana tilapia fingerlings (Oreochromis niloticus) has not been reported. This study aimed to examine the effect of Gamal leaves (Gliricidia sepium) flour supplementation in commercial feed on the growth of Nirwana tilapia fingerlings (Oreochromis niloticus).

2. Material and Methods

2.1. Site and Time
This research was conducted from April to May 2019 at the Laboratory of Seed and Fish Feed Production, Politechnic of Indonesian Venezuela (Poliven), Aceh Besar. Feed proximate testing was carried out at the Laboratory of The Center for Testing and Certification of Quality of Goods, Aceh Besar. The experimental design used was a non-factorial completely randomized design consisting of four treatments and four replications. The treatments studied included commercial feed without supplementation of Gamal leaves flour as a control (P0), commercial feed with supplementation of Gamal leaves flour 10% kg⁻¹ feed (P1), commercial feed with supplementation of Gamal leaves flour 15% kg⁻¹ feed (P2), and commercial feed with supplementation of Gamal leaves flour 20% kg⁻¹ feed (P3).

2.2. Preparation and Production of Feed Test
The feed used in this study was commercial pellets with low protein content (28%). The use of low protein feed aims to determine how much the effect of Gamal leaves flour supplementation can increase feed protein. Gamal leaves used in this study were obtained from Bak Dilip Village, Montasik District,
Aceh Besar. Gamal leaves were fermented using the method of Indariyanti and Rakhmawati [16], namely, 100 g of Gamal leaves to put in a heat-resistant plastic and sterilized using an oven at 121°C for 15 minutes. Next, cooled in a plastic container. On the substrate, 10% Aspergillus niger starter was added and the material was stirred until homogeneous, then sterile water was added as much as 70%. The plastic container was covered with plastic wrap, the plastic wrap was pricked with a sterile needle, and incubated for 3 days. The substrate has been fermented then dried and mashed. Gamal leaves are floured using a feed flour machine. The fermented gamal leaves flour is mixed with commercial pellets that have been ground into flour. Ingredients mixed little by little until homogeneous, the dose used is following the research treatment. Next, the CMC binder is added to the material and enough hot water is added until the test material looks blends. Pellet molding is done with a feed press machine. The pellets that have been molded are air-dried. The dried pellets were then put into plastic and labeled based on the treatment.

2.3. Maintenance of Nirwana Tilapia
Nirwana tilapia is the test organism in this study. Nirwana tilapia fingerlings use to have a length range from 2 - 3 cm. Nirwana tilapia fingerlings were obtained from Regional Technical Unit (UPTD) for Freshwater Cultivation Seeds, Batee Iliek, Bireun Regency. Nirwana tilapia fingerling was acclimatized for two days before being used for research. Nirwana tilapia seed stocking density is 1 L⁻¹. Nirwana tilapia fingerlings were reared in two rectangular fiber ponds measuring 2 x 1 m. Each fiber pond is lined with tarpaulin and given a wooden border so that it becomes 8 parts. The total number of containers is 16. The volume of water filled in the container is 40 L. Nirwana tilapia fingerlings maintenance period lasts for 40 days. The frequency of feeding was carried out three times a day at 8 am, 1 pm, and 6 pm (West Indonesian Time). Feed was given as much as 5% of the weight of Nirwana tilapia fingerlings biomass.

2.4. Observed Parameters
Parameters observation was performed by taking 50% samples of total nirwana tilapia fingerlings in each treatment container. Fingerlings weight measurement was performed using a digital scale and length measurement was performed using a ruler. The weight gain and length gain were observed every 10 days. Feed proximate test was performed by taking a feed sample of 50 g in each treatment and then the feed sample was submitted to the SNI standard laboratory. Water quality parameters are measured daily so that the quality of the maintenance media remains appropriate to the maintenance standards of Nirwana tilapia.

2.5. Parameters and Data Analysis

a) Weight Gain (WG)
Weight gain was calculated using the following formula Putra et al. [17]:

\[ WG = Wt - Wo \]

Remarks: \( WG = \) Weight gain (g), \( Wt = \) average body weight of fish at the end of the experiment (g), \( Wo = \) average body weight of fish at the start of the experiment (g)

b) Length Gain (LG)
Length gain was calculated using the following formula Effendie [18]:

\[ LG = Lt - Lo \]

Remarks: \( LG = \) length gain (cm), \( Lt = \) average body length of fish at the end of the experiment (cm), \( Lo = \) average body length of fish at the start of the experiment (cm)

c) Daily Growth Rate (DGR)
The daily growth rate was calculated using the following formula Muchlisin et al. [19]:

\[ DGR = \frac{(W_t - W_0)}{t} \]

Remarks: DGR = daily growth rate (g day\(^{-1}\)), \( W_t \) = average body weight of fish at the end of the experiment (g), \( W_0 \) = average body weight of fish at the start of the experiment (g), \( t \) = duration of the feeding experiment (day)

d) Feed conversion ratio (FCR)
The feed conversion ratio was calculated using the following formula Zonneveld et al. [20]:

\[ FCR = \frac{KP}{\Delta W} \]

Remarks: FCR = Feed conversion ratio, KP = Total amount of feed consumption (g), \( \Delta W \) = Weight gain (g)

e) Survival Rate (SR)
The survival rate was calculated using the following formula Ricker [21]:

\[ SR = \frac{N_t}{N_0} \times 100\% \]

Remarks: SR = Survival rate (%), \( N_t \) = Total of fish at the end of the experiment, \( N_0 \) = Total of fish at the start of the experiment

Data were analyzed using Analysis of Variance (ANOVA). If it has a significant effect it will be followed by Duncan testing at a 95% confidence level. Water quality parameters and feed proximate data were analyzed descriptively.

3. Result and Discussion
The results of Analysis of Variance (ANOVA) showed that gamal leaves flour supplementation in commercial feed gave a significant effect on weight gain, length gain, daily growth rate, and feed conversion ratio (\( P<0.05 \)), but did not give a significant effect on survival rate nirwana tilapia fingerlings (\( P>0.05 \)). Based on Table 1, the results of Duncan's advanced test showed that significant differences between treatments on the parameters of weight gain, length gain, daily growth rate, and feed conversion ratio. Treatment of P1 (commercial feed with supplementation of Gamal leaves flour 10 % kg\(^{-1}\) feed), P2 commercial feed with supplementation of Gamal leaves flour 15 % kg\(^{-1}\) feed) and P3 (commercial feed with supplementation of Gamal leaves flour 20 % kg\(^{-1}\) feed) is significantly different from treatment P0 (control). The results of the feed proximate test showed that the protein content was higher in the treatment of Gamal leaves flour supplementation in commercial feed (P1, P2, and P3) than in the control treatment (P0). The results of the feed proximate test at each treatment are presented in Table 2.

**Table 1.** Weight gain, length gain, daily growth rate, feed conversion ratio, and survival rate of Nirwana tilapia fingerlings according to dose levels of Gamal leaves flour in the commercial feed

| Treatment | Parameters | WG (g) | LG (cm) | DGR (g day\(^{-1}\)) | FCR | SR (%) |
|-----------|------------|--------|---------|----------------------|-----|--------|
| P0        |            | 2.65\(^a\) ± 0.21 | 2.13\(^a\) ± 0.09 | 0.06\(^a\) ± 0.005 | 1.69\(^b\) ± 0.23 | 100 ± 0.00 |
| P1        |            | 4.90\(^c\) ± 0.16 | 4.59\(^c\) ± 0.05 | 0.12\(^c\) ± 0.002 | 1.29\(^a\) ± 0.12 | 100 ± 0.00 |
| P2        |            | 4.48\(^c\) ± 0.12 | 3.25\(^c\) ± 0.30 | 0.11\(^c\) ± 0.003 | 1.31\(^a\) ± 0.09 | 100 ± 0.00 |
| P3        |            | 3.71\(^b\) ± 0.34 | 2.64\(^b\) ± 0.21 | 0.09\(^b\) ± 0.002 | 1.57\(^ab\) ± 0.25 | 100 ± 0.00 |

Note: different superscript letters in the same column show significantly different (\( P<0.05 \)).
Water quality parameters during 40 days of maintenance period showed that the temperature was in the range of 25.61-28.67 °C, DO range between 8.31-8.70 mg L\(^{-1}\), and pH ranged between 7.56-8.05. In general, water quality during maintenance is still within the standard range for the growth and survival of nirvana tilapia fingerlings. Water quality data during the maintenance of Nirwana tilapia can be seen in Table 3.

Table 2. Test of feed proximate (%)

| Content           | P0 (%) | P1 (%) | P2 (%) | P3 (%) |
|-------------------|--------|--------|--------|--------|
| Nutrition (%)     |        |        |        |        |
| Protein           | 28.05  | 30.17  | 30.02  | 29.42  |
| Carbohydrate      | 47.7   | 45.6   | 45.39  | 45.52  |
| Fat               | 4.13   | 4.24   | 4.08   | 4.45   |
| Ash level         | 6.28   | 6.45   | 6.62   | 6.39   |
| Crude fiber       | 4.58   | 4.88   | 5.35   | 5.27   |
| Water content     | 9.25   | 8.66   | 8.51   | 8.85   |

Table 3. Parameters of water quality during the maintenance of Nirvana tilapia fingerlings

| Parameter         | P0           | P1           | P2           | P3           |
|-------------------|--------------|--------------|--------------|--------------|
| DO (mg L\(^{-1}\))| 8.31-8.55    | 8.46-8.70    | 8.39-8.74    | 8.48-8.67    |
| pH                | 7.81-8.13    | 7.56-7.87    | 7.66-8.05    | 7.65-7.98    |
| Temperature (°C)  | 25.75-28.67  | 25.61-28.10  | 25.82-28.33  | 25.68-27.29  |

The results of this study showed that the supplementation of Gamal leaves flour in commercial feed gave a significant effect on the growth performance of Nirwana tilapia fingerlings. A similar trend was observed by Nurhayati and Nazlia[3] who stated that the application of fermented gamal leaves flour in feed rations could increase the growth rate of tilapia. Research by Apriani et al. [14] also showed that the addition of fermented gamal leaves flour in commercial feed gave a significant effect on the growth of goramy fingerling. The weight gain, length gain, and daily growth rate from highest to lowest were found in treatments P1, P2, P3, and P0. The parameter of weight gain indicates the consumption of fish on the given feed [22]. If the feed provided can be utilized by fish efficiently can increase its growth.

In general, the growth performance of Nirwana tilapia fingerlings was better in the commercial feed treatment with supplementation of Gamal leaves flour (P1, P2, and P3) compared to the control treatment (P0). This is presumably because the presence of Gamal leaves flour supplementation in commercial feed can increase feed protein. The results of the feed proximate test showed that the protein content of the feed was higher in the treatment of commercial feed with supplementation of Gamal leaves flour (P1, P2, and P3) than the control treatment (P0). The highest feed protein content was obtained in the P1 treatment (30.17%), then the P2 treatment (30.02%) and the P3 treatment (29.42%), while the lowest was obtained in the control treatment (28.05%). Protein is the main source of energy for fish that can be used for growth. The study showed that the higher growth performance was recorded at fish feed with higher protein content, which is an indication that fish growth performance was much affected by the protein content of the feed [23]. The protein content of the feed gave a significant effect on the growth performance of O. niloticus [24]. The optimal dietary protein requirement for O. niloticus is about 30% at the immature gonad growth stage and about 40% at the reproductive stage [25,26]. However, the higher dose of Gamal leaves flour added to commercial feed caused the growth of Nile tilapia fingerlings to decrease (Table 1). This is presumably because the dose of Gamal leaves flour that is too high will result in an increase in crude fiber in the feed so that it can suppress its growth fish. From the results of the proximate test, it was seen that the crude fiber increased with the addition of a dose of Gamal leaves flour in commercial feed. Crude fiber that is too high in feed makes it difficult for
fish to digest the feed given. Crude fiber generally has lignin and cellulose high content and is difficult for fish to digest [27]. The fiber content that is too high in the ingredients used as feed materials can inhibit the growth of fish [28].

The value of Feed Conversion Ratio (FCR) describes the efficiency of the utilization of feed nutrients by fish. The lower FCR value indicates the higher of efficiency feed utilization by fish [29]. The value of the feed conversion ratio was lower in the commercial feed with gamal leaves flour supplementation (P1, P2, and P3 treatment) compared to the control treatment (P0). The lowest feed conversion ratio value was obtained in the P1 treatment (1.29) and the highest was obtained in the control treatment (1.69). Putri et al. [30] stated that the lower FCR value indicates that the fish can utilize feed optimally for growth so that the feed will be absorbed into the body and converted into meat. The lower FCR values in the P1, P2, and P3 treatments compared to P0 were thought to be due to the nutritional content of Gamal leaves which were supplemented in commercial feeds that we're able to be utilized optimally by fish and played a role in complementing commercial feeds in satisfying the nutritional needs of fish. In addition, the use of fermentation technology for gamal leaves flour before it is supplemented in commercial feed makes the feed easier to digest by fish. The fermentation process for vegetable materials needs to be carried out because fish are not able to digest the enzymes cellulose, pectin, beta-glucan, pentose, and xylans so they must be converted into simpler compounds first [31]. The results of this study are following Syahputra et al. [13] which states that giving 10% gamal leaves flour to artificial feed has a lower feed conversion ratio value of goramy than the concentrations of 5% and 15%.

In addition to nutritional factors, the growth performance and survival rate of fish fry are influenced by environmental factors such as water quality. Water quality parameters are very important because they can affect the life and growth of fish [32]. The water quality parameters measured in this study were temperature, DO, and pH. The results of water quality measurements showed that the water quality during the maintenance of Nile tilapia fingerlings was still following quality standards for life. According to Effendi [33], waters designated for fishery purposes should have a dissolved oxygen content of not less than 5 mg L⁻¹. Nirwana tilapia can tolerate the acidity of the waters for optimal life between 5-8.5 [34]. According to BNSI [35], reared fish can grow well in a temperature range of 25-30 °C. The optimal temperature for fish growth ranges from 22-32°C [36,37].

4. Conclusion
Supplementation of Gamal leaves flour in commercial feed gave a significant effect on the weight gain, length gain, daily growth rate, and feed conversion ratio, but did not give a significant effect on the survival rate of Nirwana tilapia fingerlings. The best dose to increase the growth of Nirwana tilapia fingerlings was commercial feed with supplementation of Gamal leaves flour 10% kg⁻¹ feed.

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