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The Effect of Addition of Fermented Gamal Leaf Flour (*Gliricidia sepium*) in Feed on Seed Growth Freshwater Pomfret Fish (*Colossoma macropomum*)

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ABSTRACT

Gamal leaves have good potential to be used as an alternative feed ingredient for herbivorous and omnivorous fish species. The aim of this research is to determine the effect and optimal dose of adding fermented gamal leaf flour as a feed ingredient on the growth of freshwater pomfret seeds (*Colossoma macropomum*). The test fish used in this study were freshwater pomfret fish (*C. macropomum*) with a body length of 6.3–7 cm and weight of 2.6–4.12 g. Experimental fish were maintained at a stocking density of 10 fish/container for 40 days. Feeding is carried out at 07:00 WIB, 12:00 WIB, and 17:00 WIB. The research method used a Completely Randomized Design (CRD) method with six treatments and three replications. Treatment A did not add fermented gamal leaf flour (0%), treatments B, C, D, E, and F added fermented gamal leaf flour 10%, 20%, 30%, 40%, and 50%. The addition of fermented gamal leaf flour in feed had an effect ($P<0.05$) on absolute growth in length and weight, specific growth rate, feed utilization efficiency and feed conversion ratio, but had no effect ($P>0.05$) on the viability of pomfret seeds. tasteless ($P<0.05$). The best growth results were obtained in treatment E (40%), with absolute length and weight growth values of 2.01 cm and 1.81 g, specific growth rate of 4.54%/day, feed conversion ratio of 1.48, and feed utilization efficiency 68.78%.
Keywords: Gamal leaves, growth, feed, fermentation, freshwater pomfret fish

INTRODUCTION

Freshwater pomfret (*Colossoma macropomum*) is one of the fishery products that is expected to be cultivated because it has economic value (Alfiansyah et al., 2010). Due to the increasing public interest in consumed fish, many consumers prefer freshwater pomfret, causing the supply of pomfret to consumers to increase (Directorate General of Aquaculture, 2011). Freshwater pomfret is an omnivorous animal with carnivorous tendencies. At the fry stage, it prefers plankton and aquatic plants (Azam et al., 2010). Feed is a very important element in aquaculture. To ensure the survival of fish, feed must be available on time, tasty to the fish, easy to digest, and free of nutrients (Hidayat et al., 2013). Feed is a major component, accounting for 50-70% of production costs (Babo et al., 2013).

The high price of feed is caused by the high cost of the raw materials used to produce the feed, as 80% of the raw materials are imported (Rambo et al., 2018). One of the ways to improve the efficiency of fish feed utilisation is by adding sulplelmeln nultrisi to fish (Nulgraha et al., 2014). The formulation and moulding of feed in aquaculture must take into account the content of protelin, carbohydrates, fats, minerals, and vitamins (Delvani & Basriati, 2015).

Gamal leaves are plants that have great potential but are rarely used as raw materials for fish feed because the information is still limited (Syaputra et al., 2018). Gamal leaves contain secondary metabolic compounds such as tannins, flavonoids, alkaloids, and saponins that have anti-fungal effects and have a positive effect on growth (Lumowa & Rambitan, 2017). Protelin content of gamal leaves reached 25.7%, lelmak content 1.97%, crude cellulose 23.9%, water content 78.24%, and ash content 7.7% (Herawati & Royani, 2017). Gamal leaves contain high crude fibre, making it difficult for fish to digest their food (Virnanto et al., 2016). Efforts to overcome the high fibre content can be done by fermentation (Utami et al., 2012).

In fermentation, proteins are broken down into amino acids, peptides and other nitrogenous compounds. Some of these compounds can be a source of nutrients that are more easily digested and absorbed by fish, which in turn can accelerate their growth (Utami et al., 2012).

MATERIAL AND METHODS

Time and places

The research was carried out from August to September 2023 at the Fish Hatchery and Breeding Laboratory, Faculty of Marine Affairs and Fisheries, Syiah Kuala University.

Tool and materials

The tools and materials used in this research are 26 litre plastic bucket, aerator, scales, thermometer, ruler, pH meter, DO meter, blender, freshwater pomfret, gamal leaf flour, soybean flour, fish flour, wheat flour, and tapioca flour, bran, refined, vitamins, minerals, fish oil, *Aspergillus niger.*

Research design

The study used the complete randomised design (CRD) method with six treatments and three replicates. The treatment applied to fermented gamal leaves refers to the results of research (Nurhayati & Nazlia, 2019) with treatment modification A: Addition of 0% fermented gamal leaf flour in feed, Treatment B: Addition of 10% fermented gamal leaf flour in feed. Meanwhile, treatments C, D, E and F each added fermented gamal leaf flour in feed by 20%, 30%, 40% and 50%.

Research procedure

Making Gamal Leaf Flour

Gamal leaves are collected and separated from the leaf bones and then washed first with running water and air-dried for approximately 5 days (Rivai, 2010). Next, the gamal leaves were ground with a blender until smooth and sieved to obtain finer flour.

Fermentation of Gamal Leaf Flour

In the fermentation process of Gamal leaf flour, 1500 grams of Gamal leaf flour is put into a heat-resistant container and sterilized in a 121℃ oven for 15 minutes. Then let it cool and put it in a plastic bowl. The next step is to add 10% *Aspergillus Niger* starter to the substrate, stir until homogeneous, and add 70% water. The container was covered with plastic wrap, the plastic was pierced with a needle, and incubated for 3 days (Indariyati & Rakhmawati, 2013). The fermented substrate is dried, ground again, and processed into freshwater pomfret fish feed.

Making Fish Feed

Preparation of fish feed is carried out by preparing raw materials for making fish feed. Next, mix the raw feed ingredients again and stir until smooth while adding enough water. Stir the feed mixture until smooth. Then the feed mixture is printed using a feed molding machine. The next step is to dry the feed in the sun so that it does not become moldy when given to fish or when stored during research.

Tank preparation

The study was conducted using 18 aquarium units equipped with aeration equipment to maintain dissolved oxygen concentration. To prevent the risk of introducing pathogens during the study, the containers used were sterilised. After initially cleaning the containers, fill each container with 10 litres of water.

Fish samples preparation

Freshwater pomfret fry measuring 6.3 to 7 cm and weighing 2.6 to 4.12 g were used as test fish. The test fish were first acclimatised to adapt to the new environment and not stressed. The test fish were placed in test containers with a stocking density of 10 fish/container (Apriani et al., 2019). Body weight and length of the test fish were measured as baseline data.

Fish rearing

Fish samples were reared for 40 days with a feed dosage of 5% biomass and three feedings. Time periode for fish feeding every morning, afternoon and evening was 07:00 am, 12:00 pm, and 17:00 pm. All feed given was adjusted to the size of the fish in each treatment. Furthermore, sampling was carried out at the beginning of rearing and every 10 days during 40 days of rearing.

Parameter observed

Absolute Weight Growth

To calculate absolute weight growth and total length growth acording (Budianto et al., 2019).

$W = W_t - W_0$

Which:

W = total growth (g)
W_t= final weight (g)
W_0= initial weight (g)

Absolute length growth

$GL = L_t - L_0$

Which:

GL = growth length (cm)
L_t = final length (cm)
L_0 = initial length (cm)

Feed efficiency

The amount of feed given during the study and the weight of the fish at the beginning and end of the study provide information on feed efficiency. To calculate feed efficiency, the following formula from (Watanabe, 1988).

$$FE = \frac{(Wt + Wd) - Wo}{F} \times 100\%$$

Which:

FE = feeed efficiency (%)
W_t = final biomass (g)
W_0 = initial biomass (g)
W_d = weight of dead biomass during rearing (g)
F = total feed consumption (g)

Survival Rate

Survival rate compares the number of live fish and the number of fish used during rearing. The survival rate was calculated referring to (Mulchlisin et al., 2016).

$$SR = \frac{Nt}{No} \times 100\ \%$$

Which:

SR= *Survival Rate* (%)
N_t= Number of fish seeds at the beginning of rearing (ind)
N_0= Number of fish fry at the end of rearing

Specific growth rate

The specific growth rate is the rate increase in fish weight per day during the study calculated according to (Gulo et al., 2012).

$$Sgr = \frac{Ln(Wt) - Ln(Wo)}{t} \times 100\ \%$$

Which:

Sgr = specific growth rate (%)
LnW_t = Average weight of fish at the end of rearing (g)
LnW_0= Average weight of fish at the start of rearing (g)
t = rearing time (day).

Feed Convetion Ratio

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The feed conversion ratio is the ratio of the amount of feed given to produce fish biomass which is calculated according to (Fany, 2020).

$$FCR = \frac{F}{(Wt+D)-W0}$$

Which:

FCR = *Feed Convetion Ratio*
F = total feed used (g)
W_t = final weight of biomass (g)
W_0 = initial weight of biomass (g)
D = fish dead (g)

Water quality measurement

Water quality factors play an important role in supporting the growth and survival of farmed fish. Water quality parameter monitoring is carried out to determine the condition of the water treatment media. The parameters observed were water temperature, oxygen content, and pH.

Data analysis

In this research, analysis of variance (ANOVA) was used for data analysis to determine the effect of treatment. If there is a real effect, then a Duncan test is carried out using the SPSS application to determine the differences between treatments. In addition, water quality data obtained during the research was analyzed descriptively and presented in tabular form (Hanafiah et al., 2013).

RESULT AND DISCUSSION

Result

The addition of fermented gamal leaf flour in feed had a significant effect (P<0.05) on the growth in absolute weight, absolute length, feed conversion ratio, feed efficiency and specific growth rate of Freshwater Pomfret fish (*Colossoma macropomum*). However, it had no significant effect (P>0.05) on survival as shown in (table 1). The best absolute growth in length and weight, feed conversion, feed efficiency and specific growth rate were found in treatment E with respective values of 2.01 cm, 1.81 g, 1.48%, 68.78 and 4.54%, followed by by treatments D, C, B, A. Meanwhile, the lowest absolute growth in length and body weight was obtained in treatment F (50%). Furthermore, further testing showed that there were significant differences between treatments.

Table 1. Data on the results of various treatments for research parameters

Parameters	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F
W_t (g)	$4{,}71\pm0{,}14^{abc}$	$4{,}60\pm0{,}26^{ab}$	$5{,}13\pm0{,}18^{d}$	$4{,}89\pm0{,}13^{bcd}$	$5{,}04\pm0{,}03^{cd}$	$4{,}50\pm0{,}20^{a}$
W_0 (g)	$3{,}38\pm0{,}09^{a}$	$3{,}18\pm0{,}14^{a}$	$3{,}54\pm0{,}13^{a}$	$3{,}20\pm0{,}30^{a}$	$3{,}23\pm0{,}24^{a}$	$3{,}18\pm0{,}13^{a}$
Awt (g)	$1{,}33\pm0{,}05^{a}$	$1{,}42\pm0{,}36^{a}$	$1{,}59\pm0{,}05^{ab}$	$1{,}69\pm0{,}19^{ab}$	$1{,}81\pm0{,}21^{b}$	$1{,}31\pm0{,}08^{a}$
Lwt (cm)	$1{,}35\pm0{,}11^{a}$	$1{,}41\pm0{,}21^{a}$	$1{,}48\pm0{,}16^{ab}$	$1{,}70\pm0{,}15^{b}$	$2{,}01\pm0{,}04^{c}$	$1{,}28\pm0{,}09^{a}$
Sgr (%)	$3{,}19\pm0{,}10^{ab}$	$3{,}56\pm0{,}92^{abc}$	$4{,}04\pm0{,}62^{abc}$	$4{,}23\pm0{,}48^{bc}$	$4{,}54\pm0{,}54^{c}$	$3{,}13\pm0{,}11^{a}$
Fcr	$2{,}73\pm0{,}15^{cd}$	$2{,}30\pm0{,}04^{bc}$	$2{,}21\pm0{,}18^{bc}$	$1{,}92\pm0{,}08^{ab}$	$1{,}48\pm0{,}24^{a}$	$2{,}95\pm0{,}67^{d}$
FE (%)	$36{,}62\pm2{,}02^{a}$	$43{,}40\pm0{,}92^{ab}$	$45{,}42\pm3{,}66^{ab}$	$52{,}06\pm72{,}43^{b}$	$68{,}78\pm12{,}42^{c}$	$35{,}12\pm8{,}02^{a}$
SR (%)	$86{,}66\pm5{,}77^{a}$	$93{,}33\pm5{,}77^{a}$	$86{,}66\pm5{,}77^{a}$	$93{,}33\pm5{,}77^{a}$	$86{,}66\pm11{,}54^{a}$	$83{,}33\pm11{,}54^{a}$

Note: The average values in the same column with different superscripts show a significant difference (P<0.05) and the values with the same superscript in the column show no significant difference (P<0.05).

On the other hand, based on the results of the protein affinity test, it shows that the higher the dose of gamal leaves in the feed, the higher the protein content (Table 2). The closest analyses observed in this study were for protein. The test results of seven samples of fermented gammal leaf powder, supplemented with (0%, 10%, 20%, 30%, 40%, and 50% gammal leaf powder) in the feed are shown below. Meanwhile, the water quality parameters observed during the research were temperature with a value of

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28.6 - 29.8 °C, where these conditions are still within the optimal threshold for the survival of fish seeds. Meanwhile, the oxygen solubility and water pH values are 5.5 - 5.9 mg.L^{-1} and 7.2 - 7.9, respectively.

Table 2. Proximate Test Results of Gamal Leaves and Test Feed

Samples code	Protein levels (%)
Fermented Gamal Leaves	27.59
A (0%)	29.79
B (10%)	32.76
C (20%)	34.20
D (25%)	34.35
E (30%)	35.93
F (40%)	38.88

DISCUSSION

Absolute Growth

Giving fermented gamal leaf flour in fish feed has a significant effect (P <0.05) on the growth of length and weight of freshwater pomfret fry. Based on Duncan's further test, there is an effect of absolute length growth of freshwater pomfret between treatments (Table 1). The best absolute length and weight growth rate was found in treatment E, namely at a dose of 40% gamal leaves of 2.01 cm and 1.81g, respectively. This is because gamal leaves contain sufficient protein as the main source for fish growth (Putra, 2022). In addition, carbohydrates and fats also affect fish growth (Lasena et al., 2017). The protein content to support the growth of freshwater pomfret seeds is 25% (Kardana et al., 2012).

However, the ability of fish to digest available nutrients is the determinant (Ardita et al., 2015). This is evident in the treatment with a 50% dose of gamal leaf flour addition, which actually gets a low length and weight growth rate. This means that the organism has a maximum limit in digesting the protein in the feed. Some factors that affect the ability to digest feed are caused by internal (genetic, species, fish size) and external factors such as water quality (Effendi, 2002). Excess protein in feed causes fish to use more energy to metabolism and excrete ammonia which is a toxic substance (NRC, 2011). This has an impact on the amount of energy needed for maintenance rather than growth. The addition of gamal leaf flour to the gourami (*Osphronemus gouramy*) ration showed the highest growth at a dose of 10% (Syaputra et al., 2018). The rate of absolute weight gain of fish in this study was higher due to the fermentation process which made it easier for fish to digest feed so that weight gain was more optimal.

Table 3. Results of water quality measurements during the research

Treatment	Result		
	Temperature (°C)	pH	DO (mg.L^{-1})
A (0%)	28.6 - 29.8	7.2 – 7.9	5.6 – 5.8
B (10%)	28.8 - 29.5	7.2 – 7.8	5.6 – 5.9
C (20%)	28.8 - 29.6	7.3 – 7.6	5.5 – 5.8
D (30%)	28.7 - 29.8	7.4 – 7.8	5.6 – 5.8
E (40%)	28.8 - 29.8	7.2 – 7.8	5.6 – 5.8
F (50%)	28.6 - 29.6	7.2 – 7.9	5.5 – 5.9
Standard*	25 - 30	6 - 8	3 - 6

Note. *Kordi (2010)

Specific Growth Rate

The addition of fermented gamal leaf flour to fish feed had a significant effect (P<0.05) on specific growth rate. The highest specific growth rate was found in the 30% fermented gamal leaf meal dose which was 4.54%. The addition of 40% fermented gamal leaf flour affects the specific growth rate of tilapia by 0.7% (Nurhayati & Nazlia, 2019). Meanwhile, the addition of fermented cassava leaf flour to the growth of freshwater pomfret seeds affects the value of the specific growth rate by 3.54% (Nugraha et al., 2022). Fish weight increases if the feed obtained is good and the quality and quantity meet the maintenance requirements (Anti et al., 2018). This shows that feed and fish response also affect the growth rate of fish, feed and the content in it can be optimally utilized by freshwater Bawal fish for growth.

Feed Conversion rate

Feed Conversion Ratio (FCR) is the calculation of the amount of feed consumption into fish body weight. The addition of fermented gamal leaf flour to fish feed has a significant effect (P<0.05) on the FCR of freshwater pomfret fry. Where, treatment E has the lowest or best FCR. This means that freshwater pomfret are able to absorb nutrients in the feed optimally and convert them into meat. The best FCR value in this study is still lower when compared to the research of Nurhayati & Nazlia (2019), with the same dose producing a value of 1.7. Feed containing gamal leaf flour is a good feed because it is utilised and digested well by fish (Apriani et al., 2019). The high and low value of feed conversion can be influenced by several factors, especially the quality and quantity of feed, fish species and fish size (Shimi et al., 2012).

Efficiency of Feed Utilization

The higher the feed conversion value, the better the feed quality because the content in the feed meets the needs of fish (Arisandy, 2016). The ANOVA test results showed that the addition of fermented gamal leaf flour to fish feed had a significant effect (P < 0.05) on the feed utilisation efficiency of freshwater pomfret fry. The best results were found in treatment E (40%) and the lowest results were obtained in the treatment with the highest dose of 50%. Previous research by Aida et al. (2020) reported that the addition of fermented moringa flour to freshwater pomfret feed had an effect of 57.31% on feed conversion efficiency. According to Adelina (2009), the efficiency of using feed supplemented with fermented ingredients is proven to be more easily digested and absorbed by fish. The increase in feed efficiency value indicates that the feed consumed by the test fish is of good quality and can be used efficiently. This is in accordance with the opinion of Rahmawan et al. (2014), which states that high feed efficiency indicates efficient feed use, i.e. only a small portion of the protein is broken down to meet energy needs, and the rest is used for growth.

Survival Rate

The addition of fermented gamal leaf flour to feed did not have a significant effect (P>0.05) on the viability (SR) of freshwater pomfret seeds. The survival rate of freshwater pomfret seeds in this study ranged from 83% to 93%. Although previous research reported that freshwater pomfret (*Colossoma macropomum*) fed fermented lemna flour (*Lemna minor*) did not show a significant effect on the survival of freshwater pomfret seeds (Iske & Putri, 2020).

However, the survival rate in this study was 100%. The survival rate of the freshwater pomfret seeds studied was classified as good. This is in accordance with the statement by Mulyani et al. (2014) which states that survival of 50% or more is considered good and survival of 30-50% is classified as moderate, less than 30% is not suitable for planting activities. The high survival rate of freshwater pomfret seeds shows that freshwater pomfret seeds are able to adapt well to the planting medium and water quality. Survival rates are influenced by food, water quality, especially temperature and oxygen (Noviana et al., 2014).

Water Quality Measurement

The water quality parameters measured in this study were temperature, acidity (pH), and dissolved oxygen (DO). The temperature value in the study was 28.6 - 29.8°C. This value indicates that the temperature during cultivation is still suitable for the growth of freshwater pomfret seeds. This is in accordance with the opinion of Taufiq et al. (2016), which states that the ideal temperature range for freshwater pomfret is 25 - 31°C. Hydrogen power (pH) measurements taken during the study ranged between 7.2 and 7.9. This value is classified as an ideal tolerance for the growth and survival of freshwater pomfret seeds. This is in accordance with the statement of Mahyuddin (2011) that the optimal pH for the growth of freshwater pomfret is between 6.5 and 8.5. The oxygen content measured during the study was between 5.5 and 5.9 mg.l^{-1}. Mahyuddin (2011) states that freshwater pomfret can live in waters with dissolved oxygen levels above 4 mg/L.

This water quality indicates that freshwater pomfret is reared in an appropriate environment for its life. The results of water quality measurements in this study are almost the same when compared to the research of Apriani et al., (2019) Measurement of water quality of freshwater pomfret seeds in this study at a temperature of 26.60°C - 26.70°C, pH 6.83 - 7.04 and DO in the study 5.32 - 5.67 mg.l^{-1}.

DISCUSSION

The addition of fermented Gamal leaf flour in the fish feed significantly affected (P<0.05) growth in absolute weight, absolute length, specific growth rate, feed utilization efficiency, and feed conversion ratio. However, it had no effect (P>0.05) on the survival of freshwater pomfret seeds. The best growth results were obtained in the treatment with the addition of 40% Gamal leaves with absolute length and weight growth values of 2.01 cm and 1.81 g respectively, as well as a specific growth rate of 4.54%/day, feed utilization efficiency of 68.78% and FCR of 1.48.

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AUTHORS’ CONTRIBUTIONS

The contribution of each author is as follows, DS; collected the data, drafted the manuscript, and designed the table as well as the graph. DFP and SN; devised the main conceptual ideas and conducted a critical revision of the article. All authors discussed the results and contributed to the final manuscript.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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