

Effect of Adding Bioactive Compounds from Brown Algae (*sargassum* sp.) in Feed on The Growth Performance of Mahseer Seeds (*Tor soro*)

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ABSTRACT

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This study aims to determine the effect and optimal dose of the addition of brown algae extract on the growth performance of mahseer seeds. The research was conducted in March - May 2024, at the Aquaculture Laboratory building 4 FPIK Unpad. This study uses a Complete Random Design (RAL) experimental method with four treatment and four replicates. The treatment given was (A) 100% feed, (B) feed + 10 g/kg of brown algae extract, (C) feed + 15 g/kg of brown algae extract, and (D) feed + 20 g/kg of brown algae extract. Fish rearing is carried out for 40 days. The parameters observed included survival rate, absolute length (L), absolute weight (W), specific growth rate (SGR), feed conversion ratio (FCR), feed utilization efficiency (EPP) analyzed using ANOVA with a confidence level of 95%. If the difference is real, continue the Duncan test. The water quality were analyzed descriptively. The results showed that the addition of brown algae extract at a dose of 20gr/kg of feed was more optimal than other treatments because it had bioactive compounds. The dose had an effect on the growth of absolute length (1.6 cm), absolute weight (12.63 g), SGR (2.39%), FCR (2.28), and EPP (44%). The water quality value during the maintenance period is still in the optimal range.

INTRODUCTION

Mahseer is a fish that belongs to the Cyprinidae family. This fish is distributed in several regions of Indonesia, on the island of Java (Bogor, Cianjur, Kuningan, Sumedang, Blitar), the island of Sumatra (Lake Toba, Tarutung, Asahan and Aceh), and the island of Kalimantan is found in the Kapuas River and the Barito River (Arifin *et al.* 2019). Its large size with thick and chewy flesh makes it popular for consumption (Muhiardi *et al.* 2021). The selling price reaches Rp250,000 - 500,000/kg (Rumondang 2019). The high market value makes people come to hunt it, which causes the population of this fish to decrease. Its existence in nature for its number and distribution has decreased due to changes in environmental conditions in its natural habitat and overfishing (KKP 2012).

The population of mahseer based on *the IUCN Red List* (2019) is included in the *Least Concern* (LC) category. This is because the mahseer has experienced a population decline due to habitat degradation and high exploitation (Gustiano *et al.* 2013). In line with the opinion of Sikder *et al.* (2012) that the decline in the population of mahseer is due to several things such as land conversion (housing, infrastructure, and industry), water pollution, overfishing and the use of it for traditional ceremonies in several regions in Indonesia. mahseer is considered sacred and has high cultural value, so in North Sumatra it is often used for traditional ceremonies (Rumondang 2019). Some of these reasons can be considered for the importance of cultivating mahseer. The cultivation of mahseer has been successfully carried out as in the broodstock, namely artificial spawning (Frastuti *et al.* 2014), hybridization (Radona *et al.* 2015), and seeding technology (Asih and Setijaningsih 2011). The main obstacle in the cultivation of mahseer is its slow growth, taking about four years from larval to reaching the mother (Radona *et al.* 2015). So an alternative is needed to be able to increase the growth of the mahseer so that the fish can be optimal in their growth. One of the potential natural ingredients for increasing the growth of mahseer is brown algae (*Sargassum* sp.).

Brown algae (*Sargassum* sp.) have a variety of contents. The main content is water content 17.69%, ash 24.51%, fat 0.50%, protein 3.65%, carbohydrates 53.66% and crude fiber 6.52% (Manteu *et al.* 2018). The addition of brown algae to feed can improve growth, feed efficiency, and digestive enzyme activity. Brown algae contains the active ingredient florotanin, which is a phenolic compound that acts as a source of antioxidants to reduce the impact of oxidation (Pakidi and Suwoyo 2017). Other bioactive compounds contained in brown algae include flavonoids, saponins, phenols, steroids, and terpenoids (Putri 2014).

Brown algae can potentially affect the growth of mahseer seeds. Based on this potential, research is needed on the effect of adding bioactive compounds from brown algae extract (*Sargassum* sp.) on feed which is expected to affect the growth performance of mahseer (*Tor soro*) seeds.

METHOD

Place and Time of Research

The research was carried out from May to March 2024, located at the Aquaculture Laboratory, Building 4, Faculty of Fisheries and Marine Sciences (FPIK), Padjadjaran University. Preparation of Brown Algae extract (*Sargassum* sp.) has been carried out in previous research at the Aquaculture Laboratory, Building 2, Faculty of Fisheries and Marine Sciences (FPIK), Padjadjaran University.

Tools and Materials

The materials used consisted of 250 mahseer seeds $\pm 9-11$ cm long with a weight ranging from ± 7 g from Mina Kancra Ciburial, Sumedang, West Java. Brown

Algae (*Sargassum* sp.), 70% ethanol, PF-1000 commercial feed, aquades, and binder in the form of progols. The tools used are digital scales with an accuracy of 0.01, blenders, sieves, plastic funnels, 2 L glass jars, Whatman 42 filter paper, measuring cups 1000 ml, rotary vacuum evaporator, 16 aquariums measuring 30x25x20 cm³, fiber tubs, aeration, heaters, mercury thermometers with an accuracy of 0.1°C, DO, pH meter, spray measuring 100 ml, millimeter blocks, and a ruler with a precision of 0.1 cm.

Preparation of Brown Algae Extract (*Sargassum* sp.)

The manufacture of brown algae extract is carried out by washing the brown algae first until clean with running water, then dried in the sun for 3 days. After drying, it is then crushed using a blender to obtain simplisia. The maceration of brown algae is soaked in 70% ethanol solvent for 3x24 hours using room temperature protected from direct light and sunlight. The results of maceration are then filtered with filter paper and stored in a liter jerry can tightly. The algae filtrate is then evaporated with a vacuum rotary evaporator using a temperature of 40°C, then the extract is stored for test preparation on fish (Pratiwy and Pratiwi 2020).

Test Feed Manufacturing

The test feed used was PF-1000 feed with the addition of brown algae extract. The manufacture of test feed is carried out in the following way.

- 1) Separating commercial feed by 1 kg.
- 2) Put the brown algae extract into a spray that has a small amount of water and progol as much as 2% of the feed weight (Rafsyanzani and Hidayatullah 2016). After that, it is shaken until homogeneous and mixed evenly on the entire surface of the feed by the spraying method *Spray*.
- 3) After the solution is sprayed evenly on the feed, it is then dried for ±1 day at room temperature and stored in a dry container that is tightly closed.

Research Methods

The research method was carried out experimentally using a Complete Random Design (RAL) consisting of 4 treatments and 4 replicates.

Treatment A : 100% commercial feed

Treatment B : Commercial feed + 10 g/kg of brown algae extract

Treatment C : Commercial feed + 15 g/kg of brown algae extract

Treatment D : Commercial feed + 20 g/kg of brown algae extract

This research uses a linear model (Hasdar *et al.* 2021) with the following formula.

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Information:

Y_{ij} = Observation results on the i -th observation and the j -th repetition

μ = general average

- τ_i = Influence of treatment to i
 ε_{ij} = influence *Factor Random* i -th treatment and j -th repetition

Observed Parameters

Survival Rate (SR)

According to Effendie (2002), the survival rate can be calculated by the following formula:

$$SR = \frac{N_t}{N_o} \times 100\%$$

Information:

- SR = Survival (%)
 N_t = Number of final fish (tails)
 N_o = Number of initial fish (tails)

Absolute Length Growth

According to Hidayat *et al.* (2013) absolute length growth can be calculated using the formula:

$$\Delta L = L_t - L_o$$

Information:

- ΔL = Growth of absolute length (cm)
 L_t = Length of fish at the end of maintenance (cm)
 L_o = Length of fish at the beginning of rearing (g)

Growth in Absolute Weight

According to Hidayat *et al.* (2013) Absolute weight growth can be calculated using the formula:

$$\Delta W = W_t - W_o$$

Information:

- ΔW = Growth in absolute weight (g)
 W_t = Weight of fish at the end of maintenance (g)
 W_o = Weight of fish at the beginning of rearing (g)

Specific Growth Rate (SGR)

The *Specific Growth Rate (SGR)* is the percentage of average fish weight gain per day that is formulated (Effendie 1997) as follows.

$$SGR = \frac{\ln W_t - \ln W_o}{t} \times 100\%$$

Information:

- SGR = Individual Daily Growth Rate (%/day)
 W_t = Average weight of the test fish at the end of the study (g)
 W_o = Average weight of the test fish at the beginning of the study (g)

T = Length of study (days)

Feed Conversion Ratio

This parameter aims to compare the amount of feed consumed with fish, so as to find out how effective the increase in fish weight after feeding during maintenance and can be seen that the suitability of feed with fish affects the growth of weight of the fish group. effendie formula (1997):

$$FCR = \frac{F}{(W_t + D) - W_o} \times 100\%$$

Information:

FCR = Feed conversion rate

W_o = Weight of the test fish at the beginning of the study (g)

W_t = Weight of the test fish at the end of the study (g)

D = Weight of dead fish (g)

F = Weight of Feed given (g)

Feed Utilization Efficiency

According to (Watanabe 1988) the formula for calculating feed efficiency is:

$$EP = \frac{(B_t + B_d) - B_o}{F} \times 100\%$$

Information:

EP = Feed Efficiency (%)

B_t = Weight of fish at the end of the study (g)

B_o = Weight of fish at the beginning of the study (g)

B_d = Weight of fish that died during the study (g)

F = The amount of feed the fish consumed during the study (g)

Water Quality

Observation of water quality parameters is used as supporting data in determining the optimal conditions for maintaining test fish. The water quality parameters measured include water temperature, *Dissolved Oxygen* (DO), and pH.

Table 1. Observation of Water Quality Parameters

Parameters	Unit	Tool	Observation Time
Temperature	°C	Thermometer	Once a week
Ph	-	pH meter	Once a week
DO	mg/L	DO meter	Once a week

Data Analysis

The survival rate (SR), absolute length growth (L), absolute weight growth (W), specific growth rate (SGR), feed efficiency (EPP), data obtained will be analyzed using the analysis of the F test variety fingerprint (One Way ANOVA / *Analysis of*

Variance). If there is a significant difference, then it is followed by Duncan's multiple distance analysis with a confidence level of 95% (Gaspersz 1995), the water quality is analyzed descriptively.

RESULT AND DISCUSSION

Survival Rate (SR)

The survival rate of mahseer fry kept for 40 days showed that treatment A (Control) had a lower value of compared to treatment B, C and D which reached 100%.

Table 1. Survival Rate of Mahseer Seeds

Treatment Notation	Survival Rate (SR)
A (Control)	96% ± 0.06 ^a
B (Feed + 10 g/kg of brown algae extract)	100% ± 0.00 ^a
C (Feed + 15 g/kg of brown algae extract)	100% ± 0.00 ^a
D (Feed + brown algae extract)	100% ± 0.00 ^a

The results of the analysis of survival parameters in mahseer during 40 days of rearing showed that the addition of brown algae extract to the feed had no real effect on the control treatment. The graduation is of course influenced by biotic and abiotic factors. Biotic factors consist of the age and ability of fish to adapt to their environment. Abiotic factors include the availability of food and the quality of living media or water quality (Irawan *et al.* 2019). In line with Panggabean's *et al.* (2016) Water quality is the main factor determining the percentage of aquaculture fish life graduation because water is the main medium for fish life.

Efforts are made in order to maintain water quality, namely by pouring water every 2 days, using *Heater* In order to stabilize the temperature as well as the oxygen supply that is constantly provided so that the mahseer can survive well. Oxygen is one of the most important factors because, according to Yanuar (2017) Lack of oxygen can reduce the resistance of fish to diseases so that it has the potential to cause death.

Mahseer Seed Growth

The observed growth of mahseer fry included absolute length growth (L), absolute weight growth (W), specific growth rate (SGR), feed conversion ratio (FCR), and feed utilization efficiency (EPP). The following is the data on the growth parameters of mahseer seeds (Table 2).

Table 2. Mahseer Seed Growth

Variable Value	Treatment			
	A	B	C	D
Absolute Length (cm)	0.61 ± 0.04 ^a	0.89 ± 0.09 ^b	1.13±0.15 ^c	1.60 ± 0.10 ^d
Absolute Weight (g)	7.53 ± 4.88 ^a	9.61 ± 2.83 ^b	11.59 ± 1.33 ^c	12.63 ± 3.38 ^d

Variable Value	Treatment			
	A	B	C	D
SGR (%ind/day)	1.76 ± 0.04 ^a	1.98 ± 0.03 ^b	2.19 ± 0.02 ^b	2.39 ± 0.03 ^c
FCR	2.91 ± 0.19 ^a	2.69 ± 0.16 ^b	2.54 ± 0.10 ^b	2.28 ± 0.15 ^c
EPP (%)	34.42 ± 0.02 ^a	37.28 ± 0.02 ^a	39.38 ± 0.02 ^b	44.01 ± 0.03 ^c

The table above is the result of keeping Mahseer seeds for 40 days. The results will be explained in detail as follows.

Absolute Length Growth (L)

The results of observations carried out for 40 days showed that the absolute growth value of the Mahseer fry varied (Figure 1). The lowest average value is in treatment A, and the highest value is found in treatment D. Results from Statistical Analysis with ANOVA (Analysys of Variance) Test at a confidence level of 95% show that the F value is calculated > F table, which means that the treatment with the addition of brown algae extract In feed, it has an effect on increasing the growth of the length of the Mahseer seeds. Based on the results of the Duncan Test, it was shown that each treatment, namely A, B, C and D treatments, was significantly different from each other. The Duncan test is useful to see the difference between each type of treatment.

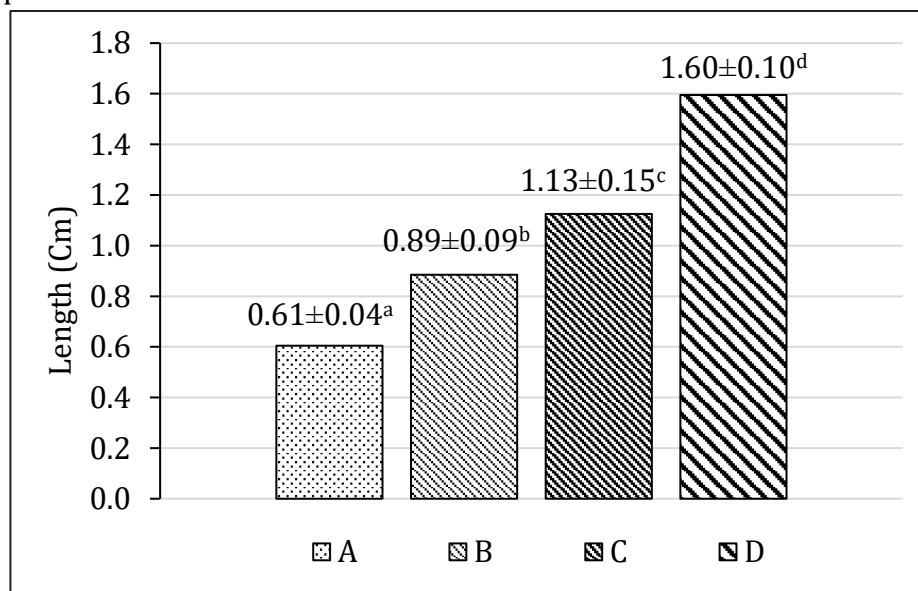


Figure 1. Absolute Length Growth

(A: Control; B: Feed + 10 g/kg of brown algae extract; C: Feed + 15 g/kg of brown algae extract; D: Feed + 20 g/kg of brown algae extract)

The values obtained in treatments B, C and D showed the effect of adding brown algae extract compared to treatment A. An increase in absolute length growth occurred due to an increase in bioactive compounds in fish (Siddik *et al.* 2023). Bioactive compounds that play a role in increasing growth are phenolic compounds (Setiyowati *et al.* 2022). Phenolic compounds have powerful antioxidant abilities,

which help protect fish cells from oxidative damage caused by free radicals. Antioxidants protect cell membranes, proteins, and DNA from damage, which can improve the overall health and metabolic efficiency of fish. This is in accordance with the statement Zeraatpisheh *et al.* (2018) That bioactive compounds from brown algae that function as fish feed supplements can increase growth rate by affecting appetite and increasing feed efficiency.

Absolute Weight Growth (W)

The results of the study carried out for 40 days on Mahseer fry showed that the weight addition of each treatment varied (Figure 2). The lowest weight gain value was found in treatment A which showed a significant difference from the highest result found in treatment D.

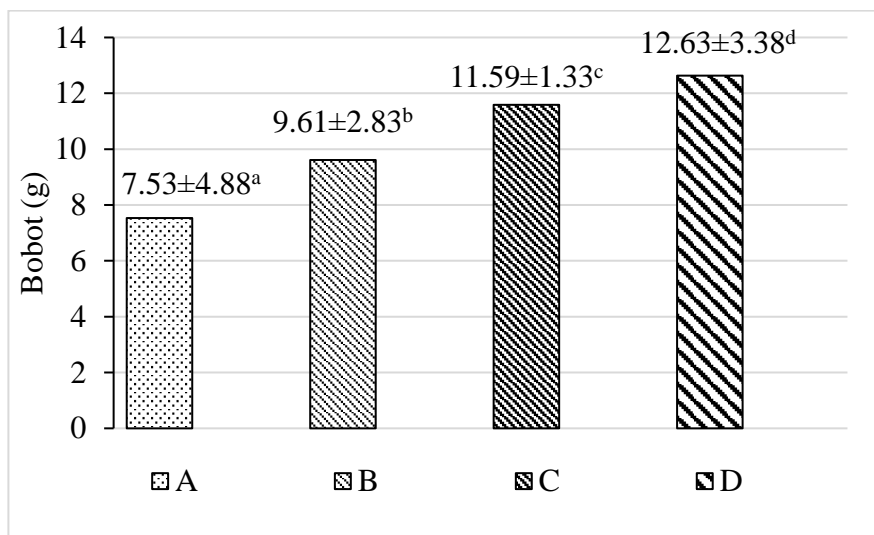


Figure 2. Growth in Absolute Weight

(A: Control; B: Feed + 10 g/kg of brown algae extract; C: Feed + 15 g/kg of brown algae extract; D: Feed + 20 g/kg of brown algae extract)

The results of the Statistical Analysis with the ANOVA (*Analysys of Variance*) Test at a confidence level of 95% showed that the F value calculated > F table, which means that the treatment with the addition of brown algae extract to the feed had an effect on the increase in the final weight of the Mahseer compared to the control treatment.

Similar results were also obtained by Sattanathan *et al.* (2023) in rohu fish (*Labeo Rohita*) family *cypriniade*. The addition of brown algae extract to commercial feed with different doses also gave significantly different results between treatments, the dose of 0 g/kg of feed was the lowest treatment compared to the treatment with a dose of 4 g/kg of feed which resulted in an increase in weight of 3 g so that it became the optimal treatment for weight growth. These results are certainly far different from the research conducted on this Mahseer due to the difference in the doses given. The more doses given, the higher nutrients obtained by the fish.

Figure 2 shows that the addition of 20 g/kg of feed results in a higher weight than the other treatments, which means that treatment D is the most optimal treatment. This is suspected to be due to the high dose of brown algae extract added to the feed. The higher the addition of brown algae extract given, indicating that the absolute weight growth of the Mahseer seeds is better because the nutrients obtained and digested by the fish are more. The addition of brown algae extract to the feed serves as a Feed Supplement which is able to influence the growth and increase the immune system of fish. Feed supplement is an ingredient in the form of a nutrient substance added to feed. Gift Feed Supplement only in small amounts serves to complement and meet nutritional needs, especially important micronutrients (Safitri *et al.* 2020).

Wang *et al.* (2022) explained that the growth enhancement process occurs indirectly, the increase is proven by the role of the alkaloids contained which act as immunomodulators. Fish become stronger from environmental stress disorders and diseases, so that the energy used for growth is more efficient and perfect when compared to feed without brown algae (Setiyowati *et al.* 2022). According to (Bindu and Sobha 2004) that the compound Growth Promoter which is found in brown algae plays a role in increasing the nutrition of feed, so that the use of feed can be used for growth.

Specific Growth Rate (SGR)

Based on the results of the study, the specific growth rate or *Specific Growth Rate* (SGR) of Mahseer during the 40-day rearing period can be seen in Figure 3. This parameter explains the percentage of fish growth per day which is observed once every ten days. Treatment A obtained the lowest SGR value and the highest value was found in treatment D.

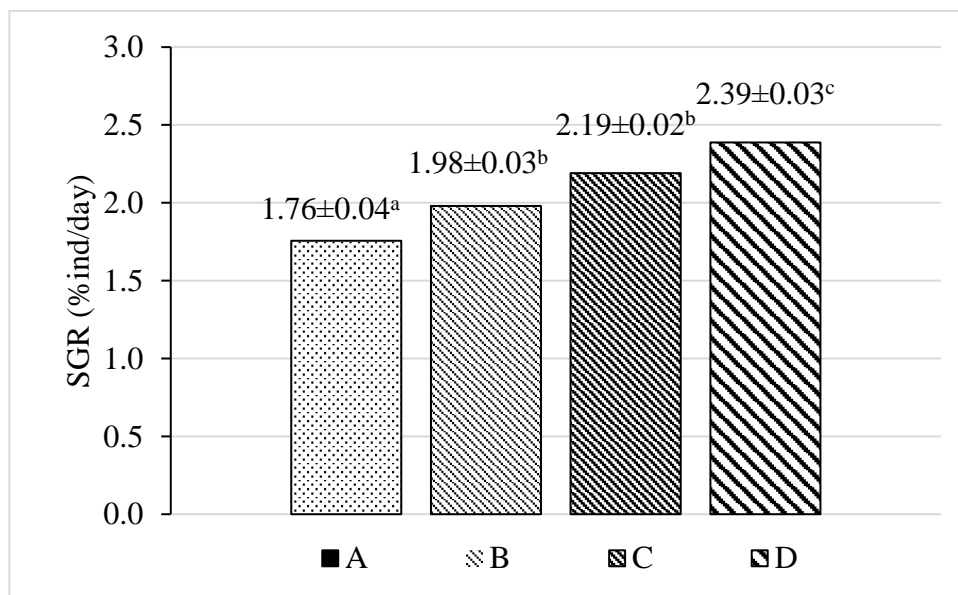


Figure 3. Specific Growth Rate

(A: Control; B: Feed + 10 g/kg of brown algae extract; C: Feed + 15 g/kg of brown algae extract; D: Feed + 20 g/kg of brown algae extract)

The results of statistical analysis with the ANOVA test (*Analysys of Variance*) 95% confidence level is known that $F_{count} > F_{table}$, meaning that the addition of brown algae extract to the feed has a significantly different effect on the specific growth rate of the fish. This shows that during seed maintenance, Mahseer are able to utilize feed with the addition of brown algae extract to grow optimally. The growth of the Mahseer fry can be seen from the increase in body weight and the specific growth rate (SGR) value during the 40-day maintenance period. This can be proven by treatment D (20gr/kg of brown algae extract) which showed a high increase in body weight compared to other treatments, and treatment A (control) provided the lowest weight increase. According to Canosa and Bertucci (2023) The increase in growth rate is affected by the nutrients provided.

The addition of brown algae extract to feed has a mechanism to increase the specific growth rate of fish due to the nutrients it contains. Chakraborty *et al.* (2014) explained that nutrients in the form of bioactive compounds (alkaloids and terpenoids) can affect the digestion process by increasing enzyme activity, increasing nutrient digestibility and feed absorption. This is in accordance with research (Sheikhzadeh *et al.* 2022) in common carp (*Cyprinus carpio*) by adding brown algae extract which explained that the optimal treatment was in the addition of 400 mg/kg of extract which resulted in an SGR value of 1.75%. This can certainly be said to have a lower value compared to the treatment of Mahseer because of the difference in the treatment presented so that the nutrients obtained are different.

Feed Conversion Ratio (FCR)

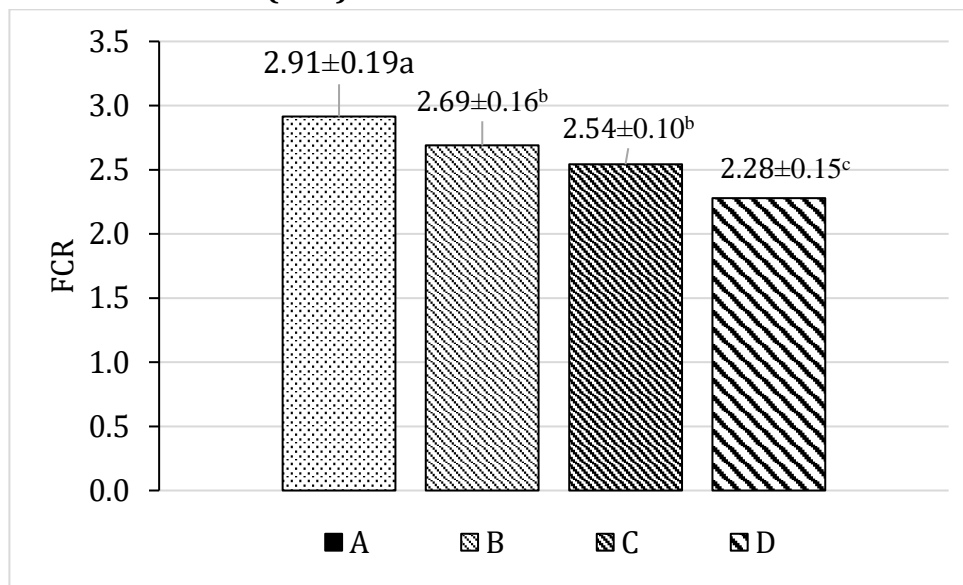


Figure 4. Feed Conversion Ratio

(A: Control; B: Feed + 10 g/kg of brown algae extract; C: Feed + 15 g/kg of brown algae extract; D: Feed + 20 g/kg of brown algae extract)

The results of the FCR of mahseer during the study obtained the lowest score in treatment D followed by treatment C and B. The highest value was obtained for treatment A. FCR of fish in general was 1.5 - 2.5 (Fahrizal and Nasir 2017). The control treatment has a higher value due to the lack of feed utilization. Arief *et al.* (2016) explained that poor feed utilization can result in high FCR values characterized by low growth. This shows that the feed given with the addition of brown algae extract provides a lower feed conversion value compared to the control. The results of low FCR values in the treatment with the addition of brown algae are suspected to be due to the presence of bioactive content that helps protein absorption so that the feed given becomes more effective for growth compared to the treatment without the administration of brown algae extract. The compound that plays this role according to Syakirin *et al.* (2023) is a flavonoid. Flavonoids in brown algae are able to act as probiotic intake in the body of fish which can increase the growth rate of *Lactobacillus* bacteria which are good bacteria for fish. Probiotics from the group of Lactic Acid Bacteria (BAL) such as *the Lactobacillus strain* are superior in stimulating the acceleration of proteolytic enzymes to break down complex proteins into amino acids that are easier to digest by the intestine (Yuriana *et al.* 2017).

Feed Utilization Efficiency (EPP)

Feed efficiency is the use of feed provided can be used well for growth, or in summary, feed that is eaten little, but can be absorbed by the body to the maximum. The value of feed utilization efficiency in the research of Mahseer seeds shows increasing results (Figure 5).

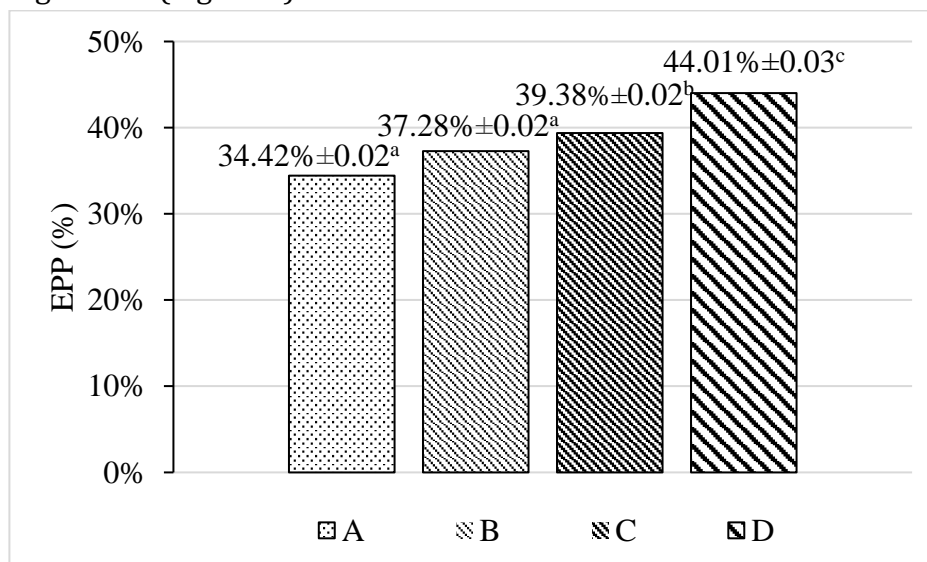


Figure 5. Feed Utilization Efficiency

(A: Control; B: Commercial feed + 10 g/kg of brown algae extract; C: Commercial feed + 15 g/kg of brown algae extract; D: Commercial feed + 20 g/kg of brown algae extract)

The results of the study conducted for 40 days showed that the average value of feed efficiency varied quite variously, ranging from 34 - 44%. The lowest score was obtained in treatment A, while the highest score was obtained by treatment D. This result actually explains that the feed given is still inefficient. But for the size of the seeds that are still in the adaptation stage, the value in this range can be said to be quite good (Sunarto and Sabariah, 2009). This is in accordance with the results of Andriyani's (1997) research on jelawat fish which said that the average value of feed efficiency during the research period (8 weeks) ranged from 38.40 - 62.49%.

The factor of high and low feed efficiency values apart from the internal characteristics of Mahseer, is also due to the influence of the bioactive compounds given. Bioactive compounds that play a role in increasing the value of feed efficiency are terpenoids, flavonoids and alkaloids. These three compounds can stimulate appetite improvement in fish, so that fish can digest food to be used more optimally in their digestive system. Terpenoids are odorous compounds with their isolation results, namely essential oils. On the other hand, essential oils have the property of stimulating hepatocytes to increase bile production and increase bile secretion, acting on the pancreas to increase fish appetite (Wijayakusuma 2003). Flavonoids and alcoloids in feed can be antagonistic to pathogenic bacteria so that the fish's digestive tract is better at digesting and absorbing feed nutrients (Syakirin *et al.* 2022).

CONCLUSION

Based on the research that has been conducted, it can be concluded as follows.

1. Based on the research that has been conducted, it can be concluded that the addition of bioactive compounds from brown algae extract has a real influence on improving the growth performance of mahseer (*Tor soro*) seeds. This is evidenced by the increase in absolute length growth value (L) of 1.6 cm, absolute weight (W) of 12.63 g, specific growth rate (SGR) of 2.39%, feed conversion ratio (FCR) of 2.28 and feed utilization efficiency (EPP) of 44%.
2. The optimal dose for the growth of mahseer is 20 g/kg of feed.

SUGGESTION

Based on the research that has been carried out, the following can be suggested.

1. Brown algae extract with a dose of 20gr/kg in feed can be used as a feed supplement to increase the growth rate of Mahseer seeds.
2. It is necessary to conduct other tests both in the form of doses and types of fish being studied.

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