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The Effect of Natural (*Daphnia Sp.)* and Different Synbiotic Feed on Rainbow Celebes (*Marosatherina ladigesi*) Growth Performance and Survival Rate

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ABSTRACT

The Rainbow Celebes fish (*Marosatherina ladigesi*) is a type of freshwater ornamental fish that is endemic to the Maros area in South Sulawesi. However, the species is facing endangerment due to frequent fishing. Therefore, this research aims to determine the effect of conservation efforts by providing good feed such as natural feed, prebiotics, and probiotics to improve the growth performance and survival rate of the Rainbow Celebes fish. The research used 90 tails of the fish which were divided into four categories, namely group A (*artificial feeding*), B (natural food *Daphnia sp.*), C (synbiotic feed and teak leaf extract *Tectona grandis L.*), and D (feeding synbiotic plus seaweed extract *Sargassum sp.*) with a dose of 0.118, 0.120, 0.385, and 0.395gram/day, respectively. The results showed that the highest morphometric was 5.78±0.08mm (length) and 2.70±0.10mm width and the specific growth rate was 2.05±0.04/day. The survival rate was the same in treatments A, B, and D, which was 100%, treatment C was 90%, and the quality of maintenance water was within normal limits. Based on these results, it can be concluded that research with synbiotic feeding and *Sargassum sp.* significantly improved the morphometry, growth performance, and survival rate of rainbow Celebes fish.

Key words: Daphnia sp., Rainbow Celebes Fish, Synbiotic Feed, Sargassum sp., Teak Leaves.

INTRODUCTION

The beseng-beseng fish is an endemic ornamental species found in South Sulawesi. It belongs to the Sulawesi rainbow group, also known as the rainbow Celebes fish. According to Kottelat et al. (1993), this fish originated from the Bantimurung River in Maros Regency and named in homage to where the fish was first discovered. Due to its attractive color and appearance, the beseng-beseng fish is highly sought for ornamental purposes, particularly male species. This has led to the fish having an essential economic value in domestic and international trade. However, this species is only caught in its natural habitat, which is a threat to its population. As a result, it was registered as an endangered fish by the International Union for Conservation of Nature's (IUCN) in 1991. In terms of body morphology, the male fish has a more attractive color pattern and beautiful body shape compared to the female. The fins of male fish are also longer, particularly the second dorsal fin. Maximum

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standard lengths for male and female fish collected are almost the same, measuring 44.1 and 43.8mm respectively. However, female fish have a more comprehensive minimum standard length ranging from 17.7 to 40.2mm, while that of the male is between 26.7 and 37.9mm (Hadiaty 2007).

The rainbow Celebes fish has a transparent body color, therefore, the internal organs are translucent and can be seen clearly, especially the bubbles on the clothes. The dominant color on its body is metallic tosca green, saffron yellow, and black. When viewed from the side, a tint of metallic tosca green is in the middle of the body. At first, it is in form of spots that resemble lines and increasingly widens towards the tail. Dorsal scales with silvery spots distally and all fins have white tips, except the first dorsal fin, which has jet black or gray with saffron tips. It was also reported that the hyaline pectoral fins have a transparent appearance with a yellowish tinge (Hadiaty 2007). Meanwhile, the size of the rainbow Celebes fish depends on the feed and environment, which affect the growth performance.

Previous investigations have shown that providing quality feed can increase the growth performance and survival rate of fish. According to Meyer-Rochow et al. (2021), the quality of feed is not only limited to the value of the nutrients but on the physical properties of the feed, such as its solubility, digestibility, color, smell, taste, and anti-nutrients it contains. One commonly used feed for ornamental fish is synbiotic feed, which applies natural feed, namely *Daphnia sp*. (Djalil et al. 2018).

Several studies on synbiotic have shown many advantages in their use to increase growth rate, feed conversion, and fish body condition (Daniels et al. 2010). Generally, a synbiotic is a nutritional supplement consisting of probiotics and prebiotics that can increase fish growth (Rohani et al. 2022). Probiotic bacteria contained in the feed can maximally increase bacterial activity in the digestion of fish. This will enhance the digestibility of fish in absorbing food extracts and improve growth performance. Meanwhile, prebiotics are materials that cannot be digested by the host but have a beneficial effect on the growth and activity of microflora in the digestion of fish (Davani-Davari et al. 2019). This synbiotic feed can be added with other additives to support the efficacy of the feed, such as *Sargassum sp.* and teak leaf extract.

Sargassum sp. known as a type of seaweed commodity with a quite expensive selling value compared to other seaweed because this type contains high concentration of iodine and phenol's antioxidant. Meanwhile, the active compounds in the phenols that play a role in reducing obesity are flavonoids and tannins. As explained by Nasmia et al. (2022), seaweed is a raw material that can be used as an additional ingredient in fish feed. This is because seaweed contains minerals and nutrients that are needed for fish growth. It is also used for the addition of flavor and aroma to enhance large consumption and reduce feed wastage. In Indonesia, the potential for seaweed has considerable prospects and is one of the export commodities (Peñalver et al. 2020). Therefore, this research aims to determine the effect of natural (*Daphnia Sp*.) and different synbiotic feeds on rainbow Celebes fish (*Marosatherina ladigesi*) growth performance and survival rate.

MATERIALS AND METHODS

Types of Research

This experimental research was carried out using rainbow Celebes fish (*Marosatherina ladigesi*), treated with natural and synbiotic feed. A total of 120 fish samples were used, divided into 4 categories with 30 fish and 3 jars in each treatment group. The fish were kept in 12 jars, each containing 10 fish, with 4 treatment groups and 3 replications. The feed administered consisted of natural *Daphnia sp*., artificial feed, synbiotic feed plus extracts of *Sargassum sp*. And teak leaf extract (*Tectona grandis* L.). The fish were fed three times a day at 08:00, 13:00 and 18:00.

Test Animal Acclimatization

Adaptation was carried out for 30 days and divided into two stages. The first adaptation was conducted for 15 days by giving the natural feed, while the second stage was carried out for another 15 days by treating with feed in 4 different containers consisting of natural *daphnia sp*, teak leaf extract (*Tectona grandis L*.), artificial feed, synbiotic, and *Sargassum sp.* During the second adaptation, feeding alternated between the natural and treated feed. Furthermore, the containers will be engineered to resemble its natural habitat by adding some *algae* and aquatic plants to make the fish feel safer and avoid stress.

Container Preparation and Test Fish

A total of 9 jars with 4-liter capacity were used in the maintenance process. Before their application, the jars were washed with clean water and detergent, rinsed thoroughly, and dried. The rearing water medium was collected in a 100L volume tank that was previously given vital aeration for 24 hours to increase the dissolved oxygen content. Subsequently, clean aerated freshwater was prepared as stock during the maintenance of rainbow Celebes fish.

The maintenance of rainbow Celebes fish was carried out in 3 containers of styrofoam measuring 40x25x30cm^3 and filled with 30 fish of an average weight of 0.59g and 1cm long. The fish were adapted to eating habits for 15 days before being used as test animals. The feed given during the adaptation process was in form of natural food *Daphnia sp*., synbiotic feed and teak leaf extract (*Tectona grandis L.*) and synbiotic feed with the addition of *Sargassum sp*., according to treatment with a frequency of 3 times a day at 08:00, 13:00, and 18:00 regularly. After adaptation, the rainbow Celebes fish (*Marosatherina ladigesi*) was ready to be examined.

Daphnia sp. culture as natural food for Rainbowfish

Daphnia sp. is considered to increase the rate of fish growth. In this research, it was used as natural feed obtained from cultivators located at Jl. Kapapa, Makassar city. At the culture stage of *Daphnia sp*., styrofoam of 40x25x30cm^3 placed in 4 containers were used. The arranged culture container was connected to the aeration hose as a supply of oxygen. Each container was installed with an aeration stone whose function was to supply

oxygen to the culture media and filled with water, followed by the sprinkling of *Daphnia sp*. seeds. Subsequently, nutrients (feed) from fermented rice water using probiotics were given to each culture container twice a day, namely 5mL/container in the morning and evening, respectively. The cultivation of *Daphnia sp.* was carried out for 14 days during the experiment.

Manufacture of Synbiotic Feed Teak Leaf Extract and *Sargassum sp.*

Synbiotic feed is a combination of probiotics and prebiotics, which is beneficial for the health and balance of microorganisms in the digestive tract of fish. The synbiotic feed with the addition of teak leaf extract (*Tectona grandis L*.) used in this research was artificial feed obtained from the Laikang probiotic house assisted by PT. Pertamina Patra Niaga DPPU Hasanuddin.

Teak leaves (*Tectona grandis L*) used as an additive to feed rainbow Celebes fish were taken from Takalar. This was followed by the addition of 7.5% to the synbiotic feed composition, which was usually very good for feeding fish. The sample obtained was cleaned of dirt and mud, dried in the sun until dehydrated, and ground to a powder. Subsequently, the powder form was mixed with synbiotic feed, which contained probiotic and prebiotic ingredients. A total of 3 dosages were given to each treatment with 3 repetitions.

Sargassum sp. used as an additive to feed rainbow Celebes fish was obtained from Takalar. This was followed by the addition of 7.5% to the synbiotic feed composition, which is usually very good for feeding fish. *Sargassum sp*. before being processed into flour, the sample was cleaned from garbage and mud and completely dried in the sun. Subsequently, the sargassum was mashed into flour and mixed with synbiotic feed.

The experimental fish that was fed with the natural feed in the first phase were used in the second phase of this research. For the second stage, the fish were given feed based on the treatment method. Subsequently, the treated fish were weighed again using digital scales and the body measurements were determined with a ruler to identify whether there is a change after each adaptation treatment (Jayadi et al. 2020).

Feeding was carried out 3 times a day in the morning, afternoon, and evening. At the beginning of maintenance, initial data were collected in morphometry, growth (initial weight), and population. Meanwhile, growth measurements and the final population were determined after the experiment (Jayadi et al. 2020).

RESULTS AND DISCUSSION

This research aims to determine the effect of *Daphnia sp*., synbiotic feed, teak leaf extract, and *Sargassum sp*. on the growth performance and survival rate of rainbow Celebes fish (*Marosatherina ladigesi).* The fish samples used were randomly selected and fed with *Daphnia sp*. and artificial feed. Subsequently, feed weighing 5% of the total body weight of rainbow Celebes fish was administered. The parameters evaluated were morphometry, survival rate, and water quality parameters. Each parameter was measured twice from the beginning of treatment until the end and the results obtained are expressed below.

Morphology

The morphometry rates of rainbow Celebes fish for 30 days of maintenance with the 4 treatment groups showed different results. It was discovered that the dose of artificial feed has 0.118g, while *Daphnia sp.,* synbiotic and *Sargassum sp.* and synbiotic feed plus teak leaf have 0.120, 0.131 and 0.128g, respectively with feeding 3 times a day at 08:00, 13:00 and 18:00. Furthermore, there was a significant change in the average increase in the length and width of the rainbow Celebes fish in each treatment group. Based on the length and width measurement in Table 1, artificial feed (A), *Daphnia sp.* (B), synbiotic feed with the addition of *Sargassum sp.* (C), and synbiotic feed with the addition of teak leaf extract (D) have values of 3.2±0.17, and 2.0±0.1mm, 4.43±0.35 and 2.30±0.20mm, 5.78±0.8 and 2.70±0.10mm, and 5.6±0.08 and 2.6±0.15mm.

The results of the analysis of variance (ANOVA) showed that different feeds had a significant effect ($P<0.05$) on the morphometry of the fish. Duncan's other test revealed that all treatments differed significantly from one group to another. The morphometry in the synbiotic feed treatment with the addition of *Sargassum sp.* ($P<0.05$) was 5.78mm long and 2.7mm wide, which was higher compared to other groups. This was in line with Sahara et al. (2015) who stated that *Sargassum sp*. had a relatively high nutrient content. Therefore, the best growth rate was obtained in morphometric data of the group treated with synbiotic feed and *Sargassum sp.* This indicated that *Sargassum sp.* is better quality and can be used more efficiently than commercial feed. The fish also obtain nutrients from the feed and *Sargassum sp.,* which maximize their nutritional needs. Morphometric measurements in this research showed that fish fed with a synbiotic combination of *Sargassum sp.* and natural feed had almost the same survival from the start to the end of the experiment. Widanarni et al. (2014) expressed that synbiotic feed is a nutritional supplement that can effectively increase the growth of length and width in fish.

Specific Growth Rate

The specific growth rate of rainbow Celebes fish apart from the aquatic environment is influenced by external factors such as food. In this research, the growth rate was used as a reference to optimize the growth of the further to obtain maximum results. Meanwhile, the results of the specific growth rate including the body weight and

Table 1. Morphometric measurements of rainbow Celebes fish (*Marosatherina ladigesi*) at different feeds.

Treatment	Length (mm)	Width (mm)
Feed A (Artificial)	3.20 ± 0.17^{a}	2.00 ± 0.10^{a}
Feed B (*Daphnia sp.*)	4.43 ± 0.35^{b}	2.30 ± 0.20^{b}
Feed C (Synbiotic Feed + *Sargassum sp.*)	5.78 ± 0.08^{c}	2.70 ± 0.10^{c}
Feed D (Synbiotic Feed + Teak Leaf Extract)	5.60 ± 0.08^{d}	2.60 ± 0.15^{d}

Different superscript letters in the same column indicate significant ($P<0.05$) differences between treatments.

length of the rainbow Celebes fish fed for 30 days feeding, 3 times a day are presented in Table 2. The results of the highest specific growth rate of rainbow Celebes fish were obtained in the treatment of synbiotic feed with the addition of *Sargassum sp.* (C), namely 2.05±0.04 (%/day).

The lowest specific growth rate was found in the treatment of artificial feeding, namely, 1.41% per day. The group fed with synbiotic and *Sargassum sp.* had the highest growth rate. This was because of the higher amount of protein in synbiotic feed with the addition of *Sargassum sp*. According to Lupatsch et al. (1998), all the energy needed by a fish comes from protein, which is used for the growth and maintenance of the fish body. Maulidiyanti et al. (2015) stated that fish growth is significantly influenced by the quality and the amount of feed given. The amount of feed given can affect the speed of growth, both weight, and length. Fish require quality feed with high nutrition consisting of protein, fat, carbohydrates, and minerals for appropriate growth. Meanwhile, brown algae are one of the most commonly used *Sargassum sp.* as a mixture of feed for fish. Brown algae is a supplement for fish feed to help the growth rate as well as increasing the acceleration of the increase in body length of rainbow Celebes fish (Jayadi et al. 2018). Dewinta et al. (2020) stated that brown algae (*Sargassum sp*.) can be added as feed supplements because they contain rich nutrients such as protein, carbohydrates, crude fiber, fat, and minerals. Based on this research, the feed used as a reference for the specific growth of rainbow Celebes fish in the *Daphnia sp*. reached 1.8% per day. However, the synbiotic feed and *Sargassum sp*. has a better growth rate because the difference from initial to final weight was 2.05% per day. In addition to the high protein content in the feed, S*argassum sp.* can also affect the increase in the growth rate of fish. Fish-fed *Sargassum sp.* produced better growth in rainbow Celebes fish (Astari et al. 2016). This is because the addition of sargassum contains active compounds that can maximize growth in the formation of body tissues (Bhatnagar and Dhillon 2017).

Survival Rates

The survival rate is the ratio of living organisms after a certain period. Fuadi et al. (2021) argued that the survival rate can be used to determine the tolerance and ability of fish. Generally, several internal and external factors influence fish mortality. In the fish body, mortality is affected by differences in age and ability to adapt to the environment. Meanwhile, the external factors include abiotic conditions, competition between species, lack of food, handling, catching, and increasing the number of fish populations in the same space and also heat stress factor (Nasyrah et al. 2019). Based on the research, the lowest survival rate was found in the 90% artificial feed treatment group. This was due to the nutritional content of the feed and the environmental quality. In the *Daphnia sp*. group, the nutrients improved the quality of life of fish. Compared to artificial feed, when the natural feed is not consumed, it does not leave waste that will negatively affect water quality or the living environment. Moreover, synbiotic feeding is a balanced combination of probiotics and prebiotics to support the survival and growth of beneficial bacteria in the digestive tract of living organisms (Cerezuela et al. 2011).

The administration of probiotics and prebiotics can stimulate the growth of beneficial bacteria and improve fish health. Several studies have shown that administering this combination to the host can improve the survival and immune system (Lin et al. 2012). Meanwhile, the survival of fish is determined by several factors, including the ratio of feed, density, and water quality such as temperature, ammonia and nitrite levels, dissolved oxygen, as well as acidity (pH) (Taragusti et al. 2019). Margono et al. (2021) argued that the quality of rearing water can decline rapidly due to the accumulation of feces, leftover feed, and metabolite waste, leading to an increase in water pH and high levels of ammonia. This will make fish become stressed, thereby decreasing the growth rate and enhancing the death risk.

Water Quality

Parameters measured during 30 days of rearing rainbow Celebes fish were temperature, pH, Dissolved Oxygen (DO), and ammonia. Based on the results of water quality measurements (Table 4), it was discovered

Table 2: Specific growth rate of rainbow Celebes fish

Treatment	Specific growth rate (%/day)
Feed A (Artificial)	1.41±0.02a
Feed B (*Daphnia sp*.)	1.80±0.13b
Feed C (Synbiotic Feed + *Sargassum sp*.)	2.05±0.04a
Feed D (Synbiotic Feed + Teak Leaf Extract)	2.02±0.04a

Different letters in the same column indicate significant differences (P<0.05) between treatments.

Table 3: Survival rate of rainbow Celebes fish

Treatment	Survival rate (%/day)
Feed A (Artificial)	90±0
Feed B (*Daphnia sp*.)	100±0
Feed C (Synbiotic Feed + *Sargassum sp*.)	100±0
Feed D (Synbiotic Feed + Teak Leaf Extract)	100±0

Survival rate did not differ (P<0.05) between treatments.

Table 4: Results of water quality measurements on rainbow Celebes fish rearing media

Parameter	Value Range				Tolerance Range	Reference
	A	B	C	D		
Temperature (C)	25-27	25-27	25-27	25-28	25-29	Nasyrah *et al.* (2019)
DO (ppm)	5.1-6.4	5.1-6.4	5.1-6.4	5.1-6.4	6.1-9.5	Nasyrah *et al.* (2019)
pH	6.9-7.9	6.9-7.9	6.9-7.9	6.9-7.8	7-8	Nasyrah *et al.* (2019)
Ammonia(ppm)	0.008-0.013	0.009-0.012	0.008-0.012	0.009-0.012	<0.02	Effendi (2003)

Measurement of Temperature (C), Dissolved oxygen (DO), pH, and Ammonia (ppm). (A): Artificial feed, (B): Natural feed (*Daphnia sp*.), and (C): Synbiotic feed and extracts of *Sargassum sp.* (D) Synbiotic feed and teak leaf extract.

that the values obtained were still within the acceptable range for the growth of the fish. Since rainbow Celebes fish lives in freshwater, hence, water quality must be maintained at optimum condition.

Water quality plays a vital role because the entire life cycle is maintained in freshwater media. In addition to the water being clear and free of pollution, the water used must meet the physical and chemical properties standards. The physical and chemical properties of freshwater including temperature, turbidity, DO content, and pH must be suitable for fish farming to support growth (Koniyo 2020). The survival of rainbow Celebes fish can be considered good, when it is above 70% because these fish can adapt to their environment with this water quality (Prajayati et al. 2020). According to Rozi et al. (2019), low water quality can make fish susceptible to stress and digestive disorders, as well as intestinal function and performance. Generally, the pH value indicates the intensity of acids and bases in the water and affects the ability of fish to produce. In this research, the pH value obtained was between 6.9-7.9, which was still in good condition. This followed a report by Mellisa et al. (2018) who stated that freshwater fish can only live at a pH of 6.8-8.0. According to Harmon (2009), an unbalanced pH can cause fish to stress easily and catch diseases more quickly, thereby reducing growth performance.

According to Koniyo (2020), the DO ranges from 5.1-6.4, and a balanced DO is 5-8mg/L. When oxygen is not balanced, it can cause fish stress due to a lack of adequate oxygen supply, leading to death. This is because the body's tissues cannot bind oxygen in the blood.

Fish water temperature with an average value of 25-29°C will significantly affect the activity and appetite of fish (Fekri et al. 2018). At a temperature of 24°C, the activity and appetite will begin to decrease, and the fish starts to freeze to death at 12°C. When the temperature exceeds 35°C, the fish will be stressed and have difficulty breathing due to increased oxygen consumption, while oxygen solubility decreases. Furthermore, frequent temperature changes will affect the growth of phytoplankton and organisms in the waters (Trombetta et al. 2019). In addition to temperature conditions, pH, DO, and ammonia levels which is toxic in a unionized form also affect maintenance. The value of ammonia observed in this research was 0.009-0.012, which was <0.02ppm, therefore, water is in good condition as stated by Hefni (2003). According to Ip and Chew (2010), ammonia is the end product of fish metabolism and can be increased by excessive feed residue. Although fish usually adapt to ammonia conditions, sudden changes can cause damage to the gill tissue.

Conclusion

Based on the results of this research, it can be concluded that the best feeding order for rainbow Celebes fish (*Marosatherina ladigesi*) is synbiotic feed and *Sargassum sp.* extract. This feeding combination significantly improved the fish's growth performance and survival rate, as well as morphometry, compared to the other treatments. Specifically, the highest growth rate and survival rate were observed in the synbiotic and *Sargassum sp*. treatment group, followed by the synbiotic feed and teak leaf extract (*Tectona grandis L.*), *Daphnia sp*., and artificial feed groups. These results suggest that synbiotic feed combined with *Sargassum sp*. extract can be an effective conservation effort in improving the growth performance and survival rate of rainbow Celebes fish.

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