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Effect of Feeding Frequency on Feed Utilization Efficiency and Growth of Asian Seabass (*Lates calcarifer*) Fingerlings

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Abstract

Asian seabass (*Lates calcarifer*) is an economically important euryhaline fish. Feed absorbs 60–70% of the total production costs; therefore, regulating feeding frequency, the amount of feed given to fish daily, is necessary to increase feed utilization efficiency. This study aimed to examine the effects of feeding frequency on the utilization of a phytase enzyme in the feed of Asian seabass fingerlings. The experimental fish used were Asian seabass fingerlings (7.57±0.28 cm in length and 5.31±0.15 g in weight) with a stocking density of one fish/L. This research used an experimental method with a completely randomized design (CRD), which consisted of four treatments and three replicates. The treatments were feeding frequencies of 1 (A), 2 (B), 3 (C), and 4 (D) times a day with the addition of 0.1 g/kg feed of the phytase enzyme. The feeding method used was the relative feeding rate, and the feed dose was 6% of the fish biomass. The results showed that feeding frequency significantly affected total feed consumption (TFC), feed conversion ratio (FCR), feed utilization efficiency (FUE), protein efficiency ratio (PER), and relative growth rate (RGR). However, they had no significant effect on the survival rate (SR) of Asian seabass fingerlings. Treatment C (3 times a day) showed the highest TFC, FCR, FUE, PER, and RGR at 251.85±1.61 g, 1.45±0.03, 69.08 ±1.50%, 1.51±0.03, and 3.90±0.11%/day, respectively. The water quality in the study area was suitable for Asian seabass cultivation. This study concluded that feeding three times a day resulted in the best FUE and RGR of Asian seabass fingerlings.

INTRODUCTION

Asian seabass (*Lates calcarifer*) is a economically crucial marine fish and one of Indonesia's export commodities (Wijayanto *et al.,* 2022). This fish is widely cultivated because it grows relatively quickly, is tolerant of high salinity changes (Hastuti *et al.,* 2020), and has high nutritional value (Tai *et al.,* 2022). Asian seabass has a high market demand of 98.86 tons/year; therefore, Asian seabass hatcheries need to be developed (Saputra and Samara, 2022). This is because hatcheries are an essential activity that is the beginning of cultivation, and they aim to overcome the problem of

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insufficient fingerling availability for aquaculture production needs (Putri *et al.,* 2020).

Feed is an essential component of aquaculture because it plays a role in fish growth (Assan *et al.,* 2021). However, feed costs absorb 60-70% of the total production costs (Tewari and Kaur, 2022; Fava *et al.,* 2022); therefore, efforts are needed to improve feed utilization efficiency by conducting feed management (de Verdal *et al.,* 2017; Gule and Geremew, 2022). Feeding management is one of the efforts made to support the success of aquaculture businesses; with good feeding management, it is hoped that the feed provided can be utilized by fish effectively and efficiently, resulting in optimal fish growth (Munguti *et al.,* 2021). Feeding management must consider several aspects, including the amount, type, and method of feeding (Mulyani *et al.,* 2021). This can also be done with two efforts, including adjusting the feeding frequency (Fitra *et al.,* 2022; Davis and Hardy, 2022) and improving feed quality by adding phytase enzymes to self-made feed made from local plant-based ingredients (Rachmawati *et al.,* 2024). Feeding management requires that the feed given to fish is of the right quality, quantity, and time for successful fish farming (Dewanggani *et al.,* 2021). Feeding at the right time is related to feeding frequency (Hanief *et al.,* 2014). Feeding frequency is the amount of feed given to fish per day (Haetami *et al.,* 2023). Feeding frequency is vital for feed utilization and fish growth efficiency. Overfeeding can cause excessive load on the fish's stomach, resulting in decreased digestive efficiency and feed utilization (Priya *et al.,* 2021), whereas lack of feeding has an impact on inhibited growth and low survival rate (Syakirin *et al.,* 2022).

Feeding frequency is related to the gastric emptying rate because the faster the gastric emptying rate, the higher the feeding frequency, and vice versa (Saputra and Yulianto, 2018; Haetami *et al.,* 2023). The correct feeding frequency helps increase feed utilization efficiency because feeding is performed when the fish are hungry and the stomach is empty, so the fish can be consumed optimally (Ayisi *et al.,* 2021; Eriegha and Ekokotu, 2017). The correct feeding frequency can also reduce feed waste, production costs, and maintain water quality (Abdel-Aziz *et al.,* 2024). Feeding frequency has been studied in several species, including *Trachinotus blochii* (Hamed *et al.,* 2016), *Epinephelus coioides* (Amadou *et al.,* 2019), *Centropomus viridis* (la Parra *et al.,* 2020), and *E. fuscoguttatus-lanceolatus* (Simanjuntak *et al.,* 2022). Research that has been conducted by Hassan *et al.* (2021a) stated that a feeding frequency of 3 times a day using commercial feed without the addition of phytase enzymes resulted in the highest feed utilization and growth in Asian seabass fingerlings as well as research conducted by Yudhiyanto *et al.* (2017), stated that the addition of Natuphos® E 5000 G phytase enzyme at 0.2 g/kg feed increased feed utilization efficiency and growth of Asian seabass fingerlings. However, no information exists on whether self-made feed containing 0.1 g/kg feed of Natuphos® E 10000 G phytase enzyme can affect feeding frequency in Asian seabass fingerlings.

METHODOLOGY
Ethical Approval

Animals were not harmed or improperly treated during this study. The experimental animals in this study, namely Asian seabass (*L. calcarifer*) fingerlings, were handled appropriately and cultivated under suitable conditions by considering several factors, such as optimal environmental conditions, including water quality, feeding appropriate to the needs of the fish, and sampling was performed carefully. Water for cultivation was maintained under optimal growth conditions. Water quality parameters, including salinity, temperature, pH, and dissolved oxygen, were maintained. The research proposal also underwent a review process and received approval from the supervisory team at the Department of Aquaculture, Faculty of Fisheries and Marine Science, Diponegoro University.

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Place and Time

This research activity was conducted from December 27, 2023, to February 27, 2024, at the Balai Besar Perikanan Budidaya Laut (BBPBL), Pesawaran Regency, Lampung.

Research Materials

The equipments used in this research included container box measuring 48.5×31×27.5 cm with a water volume of 30 L as many as 12 pieces (PT. Sahabat Intim Plasindo, SIP 109 PL CB 30, Indonesia), feed-making machines, which consist of a disk mill (FFC 23+ Engine HONDA GX160 5.5PK), hammer mill (HMR – 50) (Agrowindo, Indonesia), and feed molder (PT. Agro Tunas Teknik, PP 150 ATT, Indonesia), millimeter blocks with an accuracy of 0.01 cm to measure fish length (PT. Sinar Surya Indah Sentosa, Indonesia), air hoses and stones (PT. Tri Putra Aray Axela, Indonesia), siphon hoses, plastic jars for feed containers (PT. Sahabat Intim Plasindo, SIP 610, Indonesia), digital scales with an accuracy of 0.01 g to measure fish weight and feed weight (NCI, KV-DS12-1, China), DO meters to measure dissolved oxygen and temperature (Lutron, DO-5510, Taiwan), pH meters to measure acidity (PT. Jayamas Medica Industri Tbk, OneMed, Indonesia), refractometers to measure salinity (Atago, Japan), and Double Beam UV Vis Spectrophotometer to measure ammonia levels in water (Hitachi, UH5300, Japan). Meanwhile, the materials used in this research included Asian seabass fingerlings with an average length of 7.57±0.28 cm and an average weight of 5.31±0.15 g (BBPBL Lampung, Indonesia), fish feed (UD. Chasanah Rembang, Indonesia), phytase enzyme (PT. BASF, Natuphos® E 10000 G (Indonesia), and marine water (BBPBL Lampung, Indonesia).

Research Design

This research design used an experimental method with a completely randomized design (CRD), which consisted of four treatments and three replications with the following details: treatment A (Feeding frequency once a day, at 8:00 am), treatment B (Feeding frequency twice a day, at 8:00 am and 8:00 pm), treatment C (Feeding frequency three times a day, at 8:00 am, 2:00 pm, and 8:00 pm), and treatment D (Feeding frequency four times a day, at 8:00 am, 12:00 pm, 4:00 pm, and 8:00 pm).

The treatment in this research refers to research conducted by Hassan *et al.* (2021a), who stated that the feeding frequency of 3 times a day has a positive and significant effect on growth, feed utilization, and morphological indices, such as weight gain (WG), feed conversion ratio (FCR), and survival rate (SR) of Asian seabass fingerlings fed commercial feed without phytase enzyme addition. The doses of phytase enzyme used in the research conducted by Yudhiyanto *et al.* (2017), who stated that the best doses of Natuphos® E 5000 G phytase enzyme had a significant effect on digestibility, feed utilization efficiency (FUE), and growth of Asian seabass fingerlings, were 0.2 g/kg feed. The phytase enzyme used in this research was Natuphos® E 10000 G, so the dose of phytase enzyme used in self-made feed was 0.1 g/kg. The observed parameters were total TFC, FCR, FUE, PER, RGR, and SR. At the same time, water quality was carried out as a supporting parameter.

Work Procedure

Preparation

The experimental preparation was divided into three stages: preparation of the experimental diets, preparation of the cultivation containers, and preparation of the experimental fish. The experimental diet preparation begins with a proximate analysis of feed raw materials to determine their nutritional content. The next step is compiling the feed formulation using Microsoft Excel and weighing the raw materials. The raw materials are then mixed from the smallest to the largest amount of composition until homogeneous. Fat sources are added during the feed coating process.

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The feed dough is then molded into pellets according to the required size using a feed molding machine. The last step is drying and packaging the feed. Feed drying aims to reduce the moisture content by aerating at room temperature. The dried feed is packaged and labeled, then stored in a dry room with a wooden pallet floor, and the feed must be protected from sunlight until it is used. The finished Asian seabass fingerlings feed was then analyzed proximate. The feed formulation and the results of the feed proximate analysis are shown in Table 1.

Table 1. Formulation and proximate analysis of Asian seabass fingerlings feed used in the research (in dry weight).

Ingredients	Composition (g/kg feed)	Proximate analysis	
Fish meal	471.27	Protein (%)*	45.56
Shrimp head meal	336.30	Lipid (%)*	10.07
Corn flour	41.69	Crude fiber (%)*	4.67
Soybean meal	69.30	Ash (%)*	10.67
Tapioca flour	51.33	Moisture (%)*	10.64
Fish oil	10.00	NFE (%)	18.39
Corn oil	10.00	Energy (kcal/g)**	423.61
Vitamin mineral premix	10.00	E/P ratio***	9.30
Phytase enzyme	0.10		
Total (g)	1000.00		

Description: *Results of Proximate Analysis of Nutrition and Feed Science Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang (2024); **Calculation based on Digestible Energy (DE) according to Watanabe (1988) with the assumption that 1 g protein is 5.6 kcal/g, 1 g fat is 9.4 kcal/g, and 1 g carbohydrate is 4.1 kcal/g; and *** Energy per protein. According to De Silva (1987), the E/P value for optimal fish growth ranges from 8-12 kcal/g.

The containers, before use, were sterilized first using chlorine at a dose of 100 ppm dissolved in 20 L of water, then rinsed with freshwater until the smell of chlorine disappeared, then dried in the sun to dry. It aims to minimize the presence of bacteria and fungi in the container (Aslamiah *et al.,* 2019). Each container was labeled with the treatment, placed randomly according to a predetermined layout, and equipped with an aeration system. Each container was filled with 20 L of filtered marine water with a stocking density of one fish/L, so the experimental fish in each container was 20 fish.

Preparation of experimental fish begins with the adaptation of Asian seabass fingerlings for 7 days (Hao *et al.,* 2022). It aims to get the Asian seabass fingerlings used to the new environment and the feed given so that the fish are not stressed and minimize mortality at the beginning of the research (Andaru *et al.,* 2018). Fish fingerlings during the adaptation period were fed without the addition of phytase enzyme. Feed is given at a relative feeding rate with a dose of 6% of biomass weight (Hassan *et al.,* 2021b; Vo *et al.,* 2020). The next stage is fish selection. The fish fingerlings to be used must meet the requirements: bright, shiny body color, silvery-white, normal body with complete fin characteristics, disease-free, active movement, and response to feeding (SNI, 2014a). Asian seabass fingerlings were stocked in each container with one fish/L stocking density. Fish fingerlings that have been selected are then fed for one day to eliminate the remnants of feed and metabolism in the body (Rachmawati *et al.,* 2020).

Cultivation

After selection, the length and weight of the Asian seabass fingerlings were measured to determine the average length and weight at the beginning of the research. 240 The stocking density used during the research was one fish/L (Noval *et al.,* 2019). The Asian seabass fingerlings were then cultivated with a cultivation time of 42 days. The feed was pelleted and contained phytase enzyme at 0.1 g/kg with a protein content of 45%. Feeding is carried out using the relative feeding rate method; the feed is given based on the percentage of fish biomass, and the feed dose is 6% (Hassan *et al.,* 2021b). Feeding is based on a predetermined feeding frequency. Feeding frequency is once a day at 08:00 am. Feeding frequency is twice a day at 08:00 am and 08:00 pm. Feeding frequency is three times a day at 08:00 am, 2:00 pm, and 08:00 pm. Feeding frequency is four times a day at 08:00 am, 12:00 pm, 4:00 pm, and 08:00 pm.

Data Collection

Sampling was conducted to evaluate growth performance, and each Asian seabass fingerling was weighed on days 0, 7, 14, 21, 28, and 35 of the experimental period. Weighing of Asian seabass fingerlings was also intended to adjust the amount of feed given based on the average increase in weight and biomass. The mortality of Asian seabass fingerlings was recorded daily to evaluate survival performance during the experiment, and total mortality was calculated at the end of the experiment.

Test Parameters

Total feed consumption (TFC)

Total feed consumption can be calculated using the formula according to Pereira *et al.* (2007) as follows:

$$TFC = F_1 - F_2$$

Where:
TFC = Total Feed Consumption (g)
F1 = Initial feed amount (g)
F2 = Final feed amount (g)

Feed conversion ratio (FCR)

The feed conversion ratio can be calculated using the formula according to Tacon (1987) as follows:

$$FCR = \frac{F}{(W_t + D) - W_0}$$

Where:
FCR = Feed Conversion Ratio
F = Total feed intake (g)
Wt = Final weight (g)
D = Dead fish weight (g)
W_0 = Initial weight (g)

Feed utilization efficiency (FUE)

Feed utilization efficiency can be calculated using the formula according to Tacon (1987) and Zonneveld *et al.* (1991) as follows:

$$FUE = \frac{W_t - W_0}{F} \times 100\%$$

Where:
FUE = Feed Utilization Efficiency (%)
W_t = Final weight (g)
W_0 = Initial weight (g)
F = Total feed intake (g)

Protein efficiency ratio (PER)

Protein efficiency ratio can be calculated using the formula according to Tacon (1987) as follows:

$$PER = \frac{W_t - W_0}{P_i}$$

Where:
PER = Protein Efficiency Ratio
W_t = Final weight (g)
W_0 = Initial weight (g)
Pi = Protein intake (g)

Relative growth rate (RGR)

Relative growth rate can be calculated using the formula according to Zonneveld *et al.* (1991) as follows:

$$RGR = \frac{W_t - W_0}{W_0 \times t} \times 100\%$$

Where:
RGR = Relative Growth Rate (%/day)
W_t = Final weight (g)
W_0 = Initial weight (g)
T = Cultivation time (days)

Survival rate (SR)

The survival rate can be calculated using the formula according to Effendie (1997) as follows:

$$SR = \frac{N_t}{N_0} \times 100\%$$

Where:
SR = Survival Rate (%)
N_t = Number of final fish
N_0 = Number of initial fish

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Water Quality

Water quality measured during the research included salinity, temperature, pH, DO, and ammonia. Salinity, temperature, pH, and DO measurements were taken once a week. In contrast, ammonia measurements were taken 3 times, at the beginning (first week), middle (fourth week), and end (sixth week) of cultivation.

Data Analysis

Data were analyzed using ANOVA, and significant effects ($P < 0.05$) were further explored using Duncan's multiple range test, conducted with the aid of IBM SPSS Version 21 statistical software.

RESULTS AND DISCUSSIONS

The results of the calculation of TFC, FCR, FUE, PER, RGR, and SR of Asian seabass (*L. calcarifer*) fingerlings reared for 42 days are shown in Table 2. The results of statistical tests with ANOVA showed that the treatment of feeding frequency had a significant effect on TFC, FCR, FUE, PER, and RGR, with F counts greater than F tables, but had no significant impact on the SR of Asian seabass (*L. calcarifer*) fingerlings.

Table 2. Average value of the variables obtained during research.

Variables	Treatments			
	A	B	C	D
TFC (g)	200.17 ± 2.85^{d}	232.67 ± 2.56^{c}	251.85 ± 1.61^{a}	245.03 ± 2.02^{b}
FCR	1.62 ± 0.01^{a}	1.57 ± 0.04^{ab}	1.45 ± 0.03^{c}	1.51 ± 0.03^{b}
FUE (%)	61.66 ± 0.28^{c}	63.84 ± 1.61^{bc}	69.08 ± 1.50^{a}	66.17 ± 1.11^{b}
PER	1.35 ± 0.01^{c}	1.40 ± 0.04^{bc}	1.51 ± 0.03^{a}	1.45 ± 0.02^{b}
RGR (%/day)	2.77 ± 0.05^{d}	3.34 ± 0.05^{c}	3.90 ± 0.11^{a}	3.64 ± 0.05^{b}
SR (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Description: Mean±SD values with the same superscript in the same row show the results are not significantly different.

Phytase enzymes include exogenous enzymes and are phosphomonoester hydrolase enzymes beneficial for removing antinutrients such as phytic acid in raw vegetable feed materials (Farahani *et al.,* 2022). Phytic acid needs to be removed because it has several disadvantages, including phytic acid cannot be digested by fish (Begum *et al.,* 2020), reduces the bioavailability of minerals, such as calcium, zinc, iron, and magnesium (de França *et al.,* 2023), reduces nutrient absorption, reduces protein digestibility, increases FCR values, and reduces fish growth (Maas *et al.,* 2020). Adding phytase enzyme at 0.1 g/kg feed into the feed aims to hydrolyze and dephosphorylate phytic acid into inositol and phosphoric acid. This is to Akhtar *et al.*'s (2020) statement that the phytase enzyme can hydrolyze phytic acid into inositol and phosphoric acid. The phytase enzyme can also break and release the bond between phytic acid and mineral complexes so that fish can release and absorb minerals bound to phytate bonds in feed (Norag *et al.,* 2018; de Farias *et al.,* 2020).

Total Feed Consumption

Total feed consumption was calculated during 42 days of cultivation based on the feed consumed by the Asian seabass (*L. calcarifer*) fingerlings. The average TFC value of Asian seabass fingerlings with different feeding frequency treatments can be seen in Figure 1.

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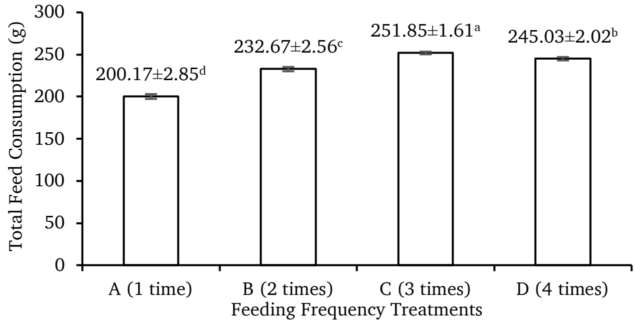

Figure 1. The total feed consumption of Asian seabass (*Lates calcarifer*) during the research.
Description: Different superscript marks show the difference between treatments.

Total feed consumption is the amount of feed consumed by fish during cultivation (Cook *et al.,* 2020; Maharani *et al.,* 2023). The ANOVA test results showed that differences in the feeding frequency significantly affected the TFC of Asian seabass fingerlings. The high value of TFC with a feeding frequency of 3 times a day (treatment C) is thought to be due to the feeding time interval, which is determined by the gastric emptying rate of Asian seabass fingerlings. The rate of gastric emptying of Asian seabass is not known for sure. Still, research conducted by Muhammadar *et al.* (2012), De *et al.* (2014), and Xing *et al.* (2023) showed that the gastric emptying rate of Tiger Grouper (*E. fuscoguttatus*) and Spotted Seabass (*Lateolabrax maculatus*) fed with pellets was 6 hours. Tiger Grouper, Spotted Seabass, and Asian Seabass are carnivorous fish, so it is suspected that the gastric emptying rate of Asian seabass doesn't differ much from the gastric emptying rate of Tiger Grouper and Spotted Seabass.

Feeding frequency is based on the consideration that each type and size of fish has a different time interval for feeding, where the interval between feeding times will depend on the appetite returns (El-Hack *et al.,* 2022). This is because fish will continue to eat available food, subject to the available space in the stomach and the duration it takes to empty its contents. The high TFC value in treatment C with a feeding frequency of 3 times a day is thought to be due to the gastric capacity and gastric emptying rate of Asian seabass fingerlings. This is because the feeding frequency and the amount of feed given are close to the gastric capacity (Kurtoğlu *et al.,* 2019; Salgado-Ismodes *et al.,* 2020), so that the fish will immediately respond to the feed given when the gastric capacity starts to empty and the fish is already hungry (Eriegha and Ekokotu, 2017; Shal *et al.,* 2021). As a result, the feed given can be consumed and digested correctly by the fish (Zidni *et al.,* 2017).

The TFC value increases in line with the increasing value of fish growth. This is supported by the RGR value presented in Table 2. Total feed consumption is related to the growth rate of fish because as fish growth increases, the size of the fish becomes more extensive, and more feed is consumed (Wibowo *et al.,* 2023). For fish that consume feed for their nutritional needs, their growth is more significant because the feed will be utilized for metabolism (Dewanggani *et al.,* 2021; Syamsinar *et al.,* 2023), and then the excess feed is used for growth (Yulindra *et al.,* 2022).

Treatments A, B, and D have lower TFC values than treatment C, with the lowest TFC value found in treatment A, which has a feeding frequency of 1 time a day. This is presumably because the feeding frequency that is not appropriate and doesn't match the gastric emptying rate of Asian seabass fingerlings causes the feed given not to be entirely eaten by the fish so that the feed that has been consumed is not fully utilized by

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the Asian seabass fingerlings for growth and is only excreted as feces (Kim *et al.,* 2021; Haetami *et al.,* 2023).

Feed Conversion Ratio

Based on the research conducted for 42 days, the average FCR value of Asian seabass (*L. calcarifer*) fingerlings with different feeding frequency treatments can be seen in Figure 2.

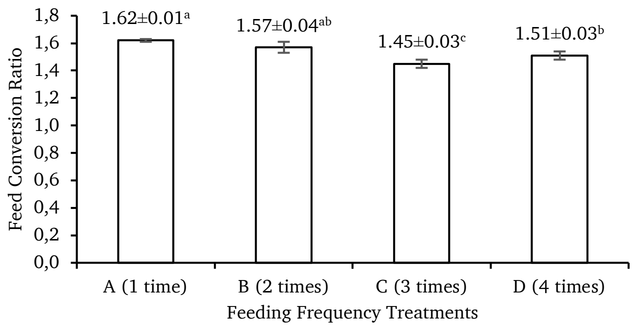

Figure 2. The feed conversion ratio of Asian seabass (*Lates calcarifer*) during the research.
Description: Mean FCR values for each treatment with the same superscript show no significant difference.

Feed conversion ratio is the ratio between the amount of feed given and the weight gain of fish during cultivation (Rodde *et al.,* 2020a). The ANOVA test results showed that the different feeding frequencies significantly affected the FCR of Asian seabass fingerlings. The best FCR value in this research, namely treatment C with a feeding frequency of 3 times a day, amounted to 1.45±0.03, and the highest, namely treatment A with a feeding frequency of 1 time a day, amounted to 1.62±0.01. The best FCR value was indicated by the feeding frequency of 3 times a day (treatment C). This is thought to be because the feeding time interval is based on the gastric emptying rate of the Asian seabass fingerlings, so the feed given will be utilized by the fish optimally. This is to the statement of Hassan *et al.* (2021a) and Stathopoulou *et al.* (2021), which states that feeding 3 times a day has met the nutritional needs of Asian seabass fingerlings and is the rate of gastric emptying can improve growth performance. The low FCR value indicates that the use of feed is more efficient, so the weight of the fish increases, while the higher the FCR value, the efficient the use of feed, so the weight of the fish decreases (Knap and Kause, 2018; Lee *et al.,* 2020). The higher EPP value supports the low FCR value in the 3 times daily feeding compared to the other 3 treatments in Table 2. This is to the statement of Bai *et al.* (2022), which states that the feed conversion ratio is related to feed utilization efficiency because the lower the feed conversion ratio value, the higher the feed utilization efficiency value, and the higher the feed conversion ratio value, the lower the feed utilization efficiency value.

The low FCR value in treatment C indicates that the feed can be digested efficiently because fish utilize it for growth. This is the statement of Idris *et al.* (2023) and Heriansah *et al.* (2022), which states that a low FCR value means that fish digest feed efficiently to support growth. The high FCR value in treatment A with a feeding frequency of 1 time per day is thought to be due to the time of feeding, which is not by the gastric emptying rate of the fish, so the excessive amount of feeding will burden the capacity of the stomach and digestive system of fish so that it can cause decreased feed efficiency and increase the FCR value (Abdel-Aziz *et al.,* 2021; Goodrich *et al.,* 2022). The FCR value in this research ranged from 1.45–1.62 and was still classified as efficient for feed utilization and fish growth. This was reinforced by Mengistu *et al.*

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(2020) and Windarto *et al.* (2023), who state that the FCR value is still classified as efficient if the value is less than 3. However, the best FCR value in treatment C, 1.45±0.03, is optimal for Asian seabass. This is reinforced by Noval *et al.* (2019), which states that the optimal FCR value for Asian seabass ranges from 1.2 to 1.5. The FCR value obtained in this research is better compared to the previous study conducted by Hassan *et al.* (2021a) regarding feeding frequency without the addition of phytase enzyme in Asian seabass fingerlings; FCR values were obtained, which ranged from 1.80–2.60. This is thought to be because adding phytase enzymes can improve feed quality, so fish can more efficiently utilize feed to produce growth, and FCR values will decrease. This is reinforced by Simamora *et al.* (2021), who state that good quality feed can reduce the FCR value because the feed is maximally utilized by fish, so that there is not much wasted feed, and according to Rachmawati *et al.* (2023), which states that the use of phytase enzyme in feed can produce a maximum feed utilization efficiency value and reduce the feed conversion ratio. The addition of phytase enzyme in feed also acts as a supplement to help break down phytic acid, an antinutrient substance in feed, as well as assisting the utilization and absorption of nutrients so that nutrients in feed can be maximally utilized in the fish body (Shahzad *et al.,* 2021).

Feed Utilization Efficiency

Based on the research conducted for 42 days, the average FUE value of Asian seabass (*L. calcarifer*) fingerlings with different feeding frequency treatments can be seen in Figure 3.

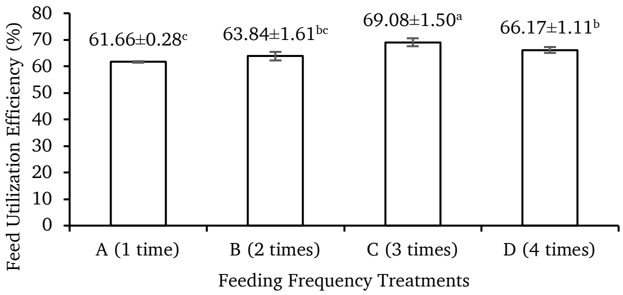

Figure 3. The feed utilization efficiency of Asian seabass (*Lates calcarifer*) during the research.
Description: Mean FUE values for each treatment with the same superscript show no significant difference.

Feed utilization efficiency is a value that shows the percentage of weight gain in a certain period and is related to the amount of feed consumed by fish (Destiyanti *et al.,* 2023; Mamun *et al.,* 2023). The ANOVA test results showed that the different feeding frequencies significantly affected the feed utilization efficiency of Asian seabass fingerlings. The feed utilization efficiency value indicates the amount of feed that fish can utilize efficiently for growth and meat production. This is because the feed utilization efficiency value is directly proportional to fish growth. After all, the higher the feed utilization efficiency value, the higher the fish growth, so the weight of the meat produced is greater (Herawati *et al.,* 2020).

Feed utilization efficiency values ranging from 61.66–69.08% are considered good because the feed is quality. This is reinforced by Craig *et al.* (2017) and Jovantheo *et al.* (2021), who state that feed can be of good quality if the FUE value is more than 50% or close to 100%. The high FUE value in this research was also

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influenced by water temperature. This is by the statement of Lee and Balasubramanian (2023), which states that temperature is a water quality that can affect FUE because temperature affects the rate of gastric emptying, feed consumption, metabolism, and fish behavior. The water temperature during the research, which ranged from 28.40–30.10°C, was considered suitable for Asian seabass cultivation. This is reinforced by Hassan *et al.* (2022a), who stated that the appropriate temperature for Asian seabass cultivation is 26–32°C Asian seabass cultivated with this temperature range can optimize the utilization of nutrients in feed maximally and efficiently, resulting in increased feed efficiency and fish growth (Monzón-Atienza *et al.,* 2023).

The high FUE value with a feeding frequency of 3 times a day is thought to be due to the feeding interval by the gastric emptying rate, so that fish can utilize nutrients in the feed provided efficiently. This is reinforced by Rodde *et al.* (2020b), who stated that the feeding frequency must be by the rate of gastric emptying so that the utilization of nutrients by fish can be maximized so that the FUE value is high and the feeding frequency is the most critical factor in maximizing the FUE value (Oh *et al.,* 2018; Gao *et al.,* 2022). The feeding frequency of 3 times a day provides a high FUE value in Asian seabass fingerlings because the amount and time of feeding are sufficient for the fish's needs, so that fish can utilize the feed given optimally to support their growth (Biswas *et al.,* 2010; Wijayanto *et al.,* 2022).

Protein Efficiency Ratio

Based on the research conducted for 42 days, the average PER value of Asian seabass (*L. calcarifer*) fingerlings with different feeding frequency treatments can be seen in Figure 4.

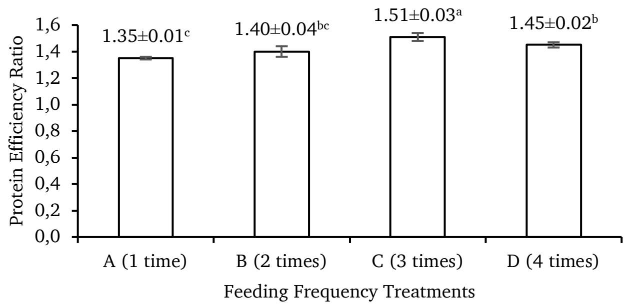

Figure 4. The protein efficiency ratio of Asian seabass (*Lates calcarifer*) was studied during the research.

Description: Mean PER values for each treatment with the same superscript show no significant difference.

The protein efficiency ratio is the protein content in the feed that can be formed into body protein by fish (Anusha *et al.,* 2018; Liu *et al.,* 2021). The protein efficiency ratio value helps determine the amount of protein digested in the fish's body to produce body protein. Protein content in feed is the primary source of fish's energy for growth and maintenance, and repairing damaged body tissues (Li *et al.,* 2023). The ANOVA test results showed that the feeding frequency significantly affected the protein efficiency ratio of Asian seabass fingerlings. The highest PER value was found in treatment C, with a feeding frequency of 3 times a day. The high PER value in treatment C is thought to be due to the feeding frequency of 3 times a day, which produces a high FUE value so that nutrients in the feed, especially protein, can be maximally utilized by fish for growth. This is reinforced by Bake *et al.* (2014), who state that the protein efficiency ratio value is the ratio between the amount of protein consumed

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and the weight produced, so the higher the PER value, the better the protein utilization. The protein efficiency ratio value in treatment C is directly proportional to the growth value produced in the treatment. This is reinforced by Sinaga *et al.* (2021), which states that the more protein fish utilize, the faster fish grow.

The protein efficiency ratio values obtained ranged from 1.35–1.51, higher than the research conducted by Ganzon-Naret (2013) regarding the effect of feeding frequency on Asian seabass fingerlings without the addition of phytase enzyme and obtained protein efficiency ratio values ranging from 0.57–1.42. It is suspected that the addition of phytase enzyme in feed is one of the factors that can increase the protein efficiency ratio value of Asian seabass fingerlings. Phytase enzymes contained in feed can decompose and break the bond between phytic acid and protein and mineral complexes (Norag *et al.,* 2018). Adding phytase enzyme into the feed can also increase the digestibility of feed nutrients, especially protein, so the protein efficiency ratio value and fish growth increase (Goda *et al.,* 2019). The high protein digestibility and fish's ability to digest protein in feed will affect the protein efficiency ratio value (Pinandoyo *et al.,* 2021; Fahlevie *et al.,* 2023).

Relative Growth Rate

Based on the research conducted for 42 days, the average RGR value of Asian seabass (*L. calcarifer*) fingerlings with different feeding frequency treatments can be seen in Figure 5.

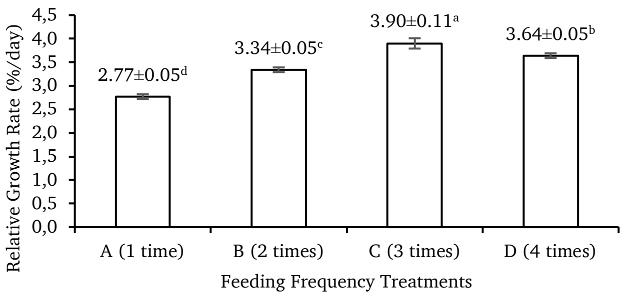

Figure 5. The relative growth rate of Asian seabass (*Lates calcarifer*) during the research.
Description: Different superscript marks show the difference between treatments.

The relative growth rate is the value of fish weight growth in a certain period (Gent, 2017; Ullah *et al.,* 2023). The ANOVA test results showed that the feeding frequency significantly affected the relative growth rate of Asian seabass fingerlings. The highest RGR value was found in the feeding frequency of 3 times daily (treatment C). The high TFC, FUE, and PER values supported the high RGR value in treatment C. Treatment C produces the highest RGR because it has the highest TFC value compared to other treatments, so more nutrients enter the fish body and are used for growth. The relative growth rate value is directly proportional and related to total feed consumption because the growth of cultured fish is influenced by the availability of feed nutrients that meet the needs of fish, so the higher the total feed consumption, the higher the fish growth (Assan *et al.,* 2021; Fabay *et al.,* 2022). The quantity of feed given to Asian seabass fingerlings must also be considered because the amount of feed that suits the needs of the fish will be used for metabolism, body maintenance, and growth (Teles *et al.,* 2019; Zhang *et al.,* 2020).

The high value of relative growth rate in treatment C also increased in line with the increasing values of FUE and PER in the treatment. The increase in relative growth rate value is directly proportional to the feed utilization efficiency value, so if the feed

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utilization efficiency value is high, the relative growth rate value is also high (Dvergedal *et al.,* 2019). Feeding by the gastric emptying rate and meeting fish needs, the feed will be utilized efficiently by fish for growth (Prabu *et al.,* 2017; Langi *et al.,* 2024). The RGR value is also directly proportional to the PER value because the more protein the fish absorbs for growth, the higher the fish's weight gain (Fabay *et al.,* 2022; Liang *et al.,* 2022).

The relative growth rate value obtained ranged from 2.77–3.90%/day, and this value is quite good because fish can utilize the nutritional content of the feed provided, resulting in growth. The growth rate of fish can be accelerated by improving the feed quality (Barani *et al.,* 2019; Kong *et al.,* 2020). Good feed quality will affect its nutritional content. Fish then utilize the nutrient content in the feed for metabolic and growth activities (Lall and Kaushik, 2021). Fish growth rate can also be influenced by feed digestibility because the higher the feed digestibility, the higher the fish growth rate. Several factors can influence feed digestibility, including adding phytase enzymes to feed (Gautier *et al.,* 2018; Rodrigues *et al.,* 2023). The addition of phytase enzyme in feed can increase growth. This is because the phytase enzyme helps hydrolyze the bonds of phytic acid, protein, and complex minerals contained in feed, increase digestibility, and accelerate the absorption of nutrients in feed, especially protein, so that protein can be maximally utilized by fish to support their growth (Rachmawati *et al.,* 2022).

Survival Rate

Based on the results of the research that has been conducted for 42 days, the average SR value of Asian seabass (*L. calcarifer*) fingerlings with different feeding frequency treatments can be seen in Figure 6.

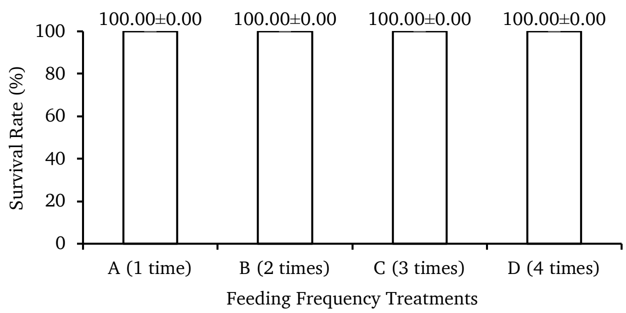

Figure 6. The survival rate of Asian seabass (*Lates calcarifer*) during the research.

There was no death of Asian seabass fingerlings during the research. The data was not tested for normality, homogeneity, or additivity. This is because the SR value of Asian seabass (*L. calcarifer*) fingerlings doesn't meet the requirements for normality, homogeneity, and additivity tests, so SR data doesn't need to be analyzed (ANOVA). The survival rate is the ratio between the number of fish alive at the end of cultivation and the number of fish at the beginning of cultivation, expressed in percent (Anita and Dewi, 2020). It is used as a measure of a fish's ability to survive (Gaffar *et al.,* 2023). The survival rate value is categorized as high if the fish mortality rate is low, while the SR value is categorized as low if the fish mortality rate is high.

The results showed that the survival rate value of Asian seabass fingerlings in treatments A, B, C, and D was the same, at 100.00%. This indicates no deaths of Asian seabass fingerlings during the research. The survival rate value obtained is classified as good because it is more than 70%. This is reinforced by Bahri *et al.* (2020), which states that the survival rate is categorized as good if the survival rate value is >70%. The

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high survival rate value during the research was influenced by the water quality presented in Table 3. This shows that the water quality during the study was suitable for cultivating Asian seabass fingerlings. This is reinforced by Marimuthu *et al.* (2019) and Shah *et al.* (2022), who state that water quality that is always controlled and managed correctly can affect fish's growth rate and survival rate. High and low survival rates can also be influenced by internal factors, such as genetics, disease resistance, and adaptability, as well as external factors, such as stocking density, stress levels, and water quality (Zhao *et al.,* 2018; Hasibuan *et al.,* 2023).

Water Quality

Based on the research conducted for 42 days, Table 3 shows data on measuring the water quality of Asian seabass (*L. calcarifer*) fingerling cultivation media with different feeding frequency treatments.

Table 3. Results of water quality measurements in the cultivation media of Asian seabass (*Lates calcarifer*) fingerlings during the research.

Variables	Range	Threshold
Salinity (ppt)	32.00–33.00	28.00–33.00*
Temperature (°C)	28.40–30.10	28.00–32.00*
pH	7.62–7.84	7.50–8.50*
Dissolved Oxygen (mg/L)	4.75–5.48	> 4.00*
Ammonia (mg/L)	0.02–0.05	0.3**

Description: * SNI: 6145.3:2014. Ikan Kakap Putih (*Lates calcarifer*, Bloch 1790) Bagian 3: Produksi Induk; and ** KepMen Lingkungan Hidup: No. 51 Tahun 2004. Baku Mutu Air Laut.

Based on the data in the table above, the water quality of Asian seabass fingerlings cultivation is still in a suitable condition to be used as a medium for cultivating Asian seabass fingerlings. This is based on the threshold value of the literature used regarding the optimal water quality conditions for Asian seabass fingerlings cultivation. Water quality is an essential factor that supports the success of aquaculture because water quality affects the growth rate and survival rate of fish (Marimuthu *et al.,* 2019; El-Hack *et al.,* 2022). Salinity is related to fish growth and can affect survival and physiology (Hassan *et al.,* 2022b) and fish osmoregulation (Prananingtyas *et al.,* 2019). The salinity value obtained during the research is optimal and can be tolerated by Asian seabass fingerlings ranging from 32.00–33.00 ppt. This is reinforced by Juharni *et al.* (2022), which states that the stable salinity value for Asian seabass cultivation is 30.00–31.00 ppt. Asian seabass is an euryhaline species with a high tolerance to changes in salinity levels so that it can live in waters with varying salinity (Saghafiankho *et al.,* 2020; Wijayanto *et al.,* 2020).

Temperature affects metabolic rate, growth performance, survival, energy balance, and fish behavior, including movement and feeding (Volkoff and Rønnestad, 2020). The temperature during the research, which ranged from 28.40–30.10°C, was considered optimal because the suitable temperature for Asian seabass cultivation is 26.00–32.00°C (Hassan *et al.,* 2022a), so that cultured Asian seabass can optimize nutrient digestion properly and increase growth and survival (Monzón-Atienza *et al.,* 2023). The pH value during 42 days of cultivation ranged from 7.62 to 7.84. This is in accordance with the optimal pH range for the growth and survival of Asian seabass, which is usually between 7.00 and 8.50 (Wijayanto *et al.,* 2022; Islam *et al.,* 2023). This consistency suggests that the environmental conditions were favorable for the growth, development, and survival rate of Asian seabass.

The Dissolved Oxygen value obtained during the research ranged from 4.75–5.48 mg/L, which is considered optimal and by

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the threshold value of SNI (2014b) for Asian seabass cultivation. Dissolved Oxygen dramatically affects the life of aquatic organisms (Tongmee *et al.,* 2021) because DO content that is within normal levels affects fish growth as well as feed consumption and good nutrient digestibility (Tran-Ngoc *et al.,* 2019; Ali *et al.,* 2022). Similarly, the ammonia levels recorded during the culture period ranged from 0.02–0.05 mg/L, remaining within the optimal ammonia range of 0.3 mg/L for marine water (KepMen LH, 2004). These findings indicate that the culture maintained suitable water quality variables conducive to the healthy growth and survival of Asian seabass throughout the research period.

CONCLUSION

The conclusion obtained from this research is that the feeding frequency with phytase enzyme addition significantly affects feed utilization efficiency and growth, but doesn't significantly affect the survival rate of Asian seabass (*Lates calcarifer*) fingerlings. Feeding frequency 3 times a day gave the best feed utilization efficiency (Total Feed Consumption (TFC) = 251.85±1.61 g, Feed Conversion Ratio (FCR) = 1.45±0.03, Feed Utilization Efficiency (FUE) = 69.08±1.50%, Protein Efficiency Ratio (PER) = 1.51±0.03) and the best growth of Asian seabass fingerlings at 3.90±0.11%/day. Water quality during the research was considered feasible and optimal for cultivating Asian seabass (*L. calcarifer*) fingerlings.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest among them regarding the research, writing, and publication of this manuscript. The manuscript has been approved by all authors for publication, and it is not being submitted to any other journal.

AUTHOR CONTRIBUTION

The contributions of each author are as follows: Dhea Mayang Saputri as the main researcher with contributions in the form of conceptualization, the research design, and conducting research; collected and performed the statistical analysis test parameter data; also drafted and revised the manuscript. Diana Chilmawati and Seto Windarto, as supervisors with contributions in the form of conceptualization of the research design, directed research activities, critically revised the manuscript, and approved the manuscript for final submission. All authors made significant contributions to the publication of this manuscript.

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