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Feed efficiency with the addition of probiotics for asang fish fry, *Osteochilus vittatus*, CV

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ABSTRACT

The purpose of this study was to determine the addition of probiotics in commercial feed to the growth and survival of Asang fish (*Osteochilus vittatus* CV) fry. This study used an experimental method with a completely randomized design (CRD) with 4 treatments and 3 replications. The test fish used were 120 fish with a size ranging from 5-8 cm. The test fish were kept in a waring cage made of a type of paralon frame with a size of 40 x 40 x 45 cm. The treatment in this study was A (without probiotics), treatment B (addition of probiotic 10 ml/kg of feed), treatment C (addition of probiotic 20 ml/kg of feed), and treatment D (addition of probiotics 30 ml/kg of feed). From the results of this study, the highest specific growth rate was found in treatment D (6.66 ± 1.15 g), while the mean survival was 100% for all treatments. The highest FCR of fish fry was found in treatment A namely 1.43 ± 0.22, and the lowest in treatment D namely 1.12 ± 0.21, and for feed efficiency in treatment D, namely 95.24 ± 1.04 %.

Introduction

Asang fish is a freshwater fish that are often discovered in rivers, lakes, or reservoirs, which have a strategic value, as a source of food and income for the rural community and can be cultivated. (Azrita *et al.*, 2014). Furthermore, the species has significant economic value because it is relatively cheap in price and the citizen often like it. In West Sumatra, the selling price ranges from IDR 25,000 per kg (Azrita *et al.*, 2014). Subsequently, the seeds of this species are obtained from wild catches in nature, and therefore the domestication of these fish is needed to avoid extinction. If this fish is successfully domesticated, it is likely to be used as a candidate for cultivation.

Recently, controlled maintenance of the broodfish in a cement tank was carried out. Aferi (2015) reported that broodstock fish (*Osteochillus vittatus, Cyprinidae*) reared in floating net cage habitats had better times and fertility than those raised in concrete tubs. Furthermore, the research by Helwidda (2019) also showed that the hatching of Asang fish eggs could reach 71.11% with larval survival of 91.55%.

Animal feed is important in aquaculture and influences the success of fish farming production activities. To improve the quality of commercial feed, nutritionists add a variety of ingredients, including probiotics, and the addition of probiotics to animals feed has been attempted. Because probiotic The results of previous research also show that the addition of probiotics to commercial feed affects growth and survival, for example, tilapia, *Oreochromis niloticus* (Ulfira, 2018 and Harmilia *et al.*, 2019); depik fish, *Rasbora tawarensis* (Fratiwi *et al.*, 2018); zebrafish, *Danio rerio*, (Rane and Markad, 2013); yellow perch fish, *Perca flavescens*, (Shaheen *et al.*, 2014); catfish, *Clarias gariepinus*, (Wardika *et al.*, 2014; Pratiwi *et al.*, 2020) *Clarias batracus*, (Tarigan *et al.*, 2019); and

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common carp, *Cyprinus carpio*, (Tarigan and Meiyasa, 2019).

Probiotics are live microorganisms in sufficient quantities to provide benefits to the host. Therefore, if probiotics are given into fish feed, it will undoubtedly affect the feed. The bacteria contained in probiotics will decompose the ingredients in the feed into a simple one. According to Widanarni *et al.* (2008), probiotics in feed can increase feed absorption in shrimp so that shrimp growth is maximum, increase protease and amylase enzyme activity (Afrilasari *et al.*, 2016), can improve feed quality, and increase feed utilization in fish (Vershure *et al.*, 2000). The provision of commercial probiotics of the EM-4 type has been tried on several fish such as *Oreochromis niloticus* (Harmilia *et al.*, 2019), *Cyprinus carpio* (Tarigan and Meiyasa, 2019).

Because probiotics can increase feed digestibility in fish, it is necessary to research the addition of probiotics to increase feed efficiency, growth and viability of Asang fish seeds for successful development in aquaculture activities.

Materials and Methods

Location and time of research

The research was conducted from March-May 2020 at the Integrated Laboratory of the Faculty of Fisheries and Marine Sciences, Bung Hatta University. The material used was a waring cage made of 12 units of pipe framework with a size of 40 x 40 x 45 cm. Meanwhile, probiotics were made manually from natural ingredients and a commercial feed with 39% protein, 5% fat, 6% crude fiber, 12% ash, and 10% water. The design used was a complete randomized design (CRD) with 4 treatments, each was repeated 3 times. The treatments were A. Commercial feed without additional probiotics, B. Addition of probiotics of about 10 ml/kg to the feed, C. Addition of probiotics of about 20 ml/kg to the feed, and D. Addition of probiotics of about 30 ml/kg to the feed. The feed was given to the test fish for 60 days.

Research procedure

Probiotics are made from natural ingredients (100 gr curcuma, 100 gr ginger, 100 gr turmeric, 175 g brown sugar, 195 ml EM-4, 1 liter water, 100 g *Kaempferia galanga*, and 1.8 g yeast tape). All ingredients were grounded using a blender, then brown sugar and 1 liter of water were added. Furthermore, it was stirred again until blended, then EM-4 and yeast tape were added to the solution and stirred again. The mixture was placed in a jerry can be closed tightly to avoid oxygen penetration, and left for 7 days to allow the microbes to multiply. On day 8, depending on the treatment, probiotics can be used. The probiotics were mixed with the feed (PF 1000) by spraying according to the research treatment. The feed sprayed with probiotics was incubated for 24 hours and can therefore be absorbed into the feed. The feed was given 3 times every day at 08.00 a.m, 1.00 p.m, and 6.00 p.m. The amount of feed given was 5% of the biomass weight, and the fish were reared at a density of 10 fish per container with an average weight of 12.51 ± 13.61 gr or a length of 5-8 cm per fish.

The water quality parameters measured were temperature, pH, ammonia, and DO. Temperature and pH measurements were carried out every day, while ammonia and DO were carried out twice during the study, namely at the beginning and the end of the study. Measurements were carried out on each research container. Water quality data is discussed descriptively and supported by the literature

Observed variables

Survival rate (SR)

The survival rate was calculated according to the formula from Effendie (1997):

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

SR = Survival rate (%), Nt = Number of fish at the beginning of the experiment (fish), No = Number of fish at the end of the experiment (fish).

Absolute weight growth asang fish (*Osteochilus vittatus C.V*)

Absolute weight growth was calculated to the formula from Effendie (1997):

$$W = Wt - Wo$$

W = Absolute weight (g), Wt = Final mean weight (g) and Wo = Initial mean weight (g)

Absolute length growth asang fish (*Osteochilus vittatus C.V*)

Absolute length growth was calculated according to the formula from Effendie (1997):

$$Lm = Lt - Lo$$

Lm = Absolute length (mm), Lt = Final mean length (mm) and Lo = Initial mean weight (mm).

Spesific growth rates (SGR)

Specific growth rate (SGR) was calculated according to the formula from Zonneveld *et al.* (1991):

$$SGR = \frac{lnWt - lnWo}{t} X\ 100\ \%$$

SGR = Spesiific growth rate (%/), Wt = Final mean weight (g), Wo = Initial mean weight (g) and t = Culture period (days).

Feed conversion ratio (FCR)

The feed conversion ratio was calculated according to the formula from Mokoginta *et al.,* (1995):

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

where: FCR = feed conversion ratio, F = total weight of feed intake (g), Wt = weight of fish at the end of the rearing period (g), Wo = weight of fish at the beginning of the rearing period (g) and D = weight of dead fish during rearing period (g).

Feed efficiency

The efficiency of feed (EP) was calculated according to the formula from Tacon (1987):

$$EP = \frac{Wt-Wo}{F} X\ 100\ \%$$

Data analysis

Data on survival, growth, FCR, and feed efficiency were analyzed using one-way ANOVA $P < 0.05$. Differences between treatments were analyzed using Duncan's Multiple Range Test at $P < 0.05$. The data were analyzed using the SPSS version 20 software program.

Results

Based on the results, it can be seen that the average survival value of Asang fish is 100%, the absolute weight gain ranges from 4.00 gr - 3.08 gr, the absolute length growth of Asang fish seed ranges from 11.43 mm - 4.33 mm, the specific growth rates ranges from 6.66% - 5.13%, FCR of Asang fish seeds ranges from 1.43 to 1.12, and feed efficiency ranges from 95.24% - 76.54%, the details can be seen in Table 1.

The analysis of variance showed that the addition of probiotics to commercial feed had no significant effect ($P > 0.05$) on absolute weight gain, specific growth rate, survival rate and FCR, but significantly different on absolute length growth and feed efficiency for asang fish seeds.

The results of water quality parameters for all treatments, where the temperature ranged from 26 – 29 ° C, pH ranged from 5.3 – 7.6, dissolved oxygen (DO) 5.33 – 6.12, while ammoniac ranged from 0.011 – 0.095. Water quality data are presented in Table 2.

Discussion

The survival of Asang fish seeds is 100%, both for feeding with and without the addition of probiotics. This is thought to be closely related to probiotics, where probiotics can increase stress tolerance (Eissa *et al.,* 2014) and increase immunity (Rane and Markad, 2015). The same results were also reported in silver catfish (Setiawati *et al.*, 2013) and catfish (Afrilasari *et al.*, 2016). The high survival rate of Asang fish seeds was taken into account not only because of the appropriate density and quality of the feed but also because of the texture and smell of the feed with probiotics. They were not different from the feed without probiotics and therefore the feed consumed could be optimally utilized. The quality of the feed is very important in maintaining survival (Bulanin, 2002). The results on the survival of feed containing probiotics are generally high, for example in the case of tilapia in the range of 80 to 98.50% (Arsyad *et al.,* 2015; Ulfira, 2018; Umasugi *et al.*, 2018; Harmilia *et al.,* 2019 and Opiyo *et al.*, 2019), for catfish, generally above 80% (Wardika *et al.*, 2014; Fadholi *et al.,* 2016; Tarigan *et al.,* 2019 and Pratiwi *et al.*, 2020), depik fish seeds 60 -100% (Fratiwi *et al.*, 2018), commond carp 86 - 100% (Tarigan and Meiyasa, 2019), giant gouramy 57.50% (Suminto and Cilmawati, 2015) and silver catfish 51.67 - 73.33% (Khotimah *et al.*, 2017). The differences in the existence and the survival of the fish fed with probiotics are certainly influenced by various factors including stocking density, fish size, and species.

The dosage of probiotics used by researchers also differed, for example referring to the number of bacteria administered and the dose of probiotics (Wardika *et al.*, 2014; Arsyad *et al.*, 2015; Fratiwi *et al.*, 2018; Umasugi *et al.*, 2018; Tarigan *et al.*, 2019 and Xia *et al.,* 2020). Based on the dosage used according to Harmilia *et al.* (2019) it was reported that the best probiotic dose for red tilapia is 11 ml/100 g of feed, while the results of research from Ulfira (2018) reported that the best dose is 20 ml/kg. Although the types of fish are the same, there is a difference in the dosage of probiotics be given. This is due to the size of the fish, the basic ingredients of probiotics, and the type of bacteria provided. Furthermore, the dose of probiotics suitable for goldfish is 15 mL/kg of feed (Tarigan and Meiyasa, 2019), catfish 20 mL/kg of feed (Tarigan *et al.,* 2019), silver catfish (Khotimah *et al.,* 2017), and depik fish (Fratiwi *et al.*, 2018) is 30 ml/kg. Likewise, the results of this research showed that the best dose for Asang fish seeds is 30 ml/kg. In addition, it is believed that high doses of probiotics can provide high fish growth as well. The feed added with probiotics as much as 30 ml/kg can optimally increase the number of beneficial bacteria that enter the digestive tract from the dominant food

in the digestive environment and therefore inhibit the number of pathogens. In addition, the probiotic bacteria added to the feed support the process of maximum nutrient absorption and can increase weight and length gain. According to Ahmadi's (2012) research, probiotics have microbes that can increase the digestibility of nutrients in feed. According to the research by Xia *et al.* (2020), it was also reported that bacteria in the feed and intestines help accelerate the maximum absorption of nutrients.

Table 1. The results of the data of survival, absolute weight growth, absolute length growth, specific growth rate, FCR, and feed efficiency.

Treatment	Survival rate (%)	Absolute weight growth (gr)	Absolute length growth (mm)	Specific growth rate (%/day)	FCR	Feed efficiency (%)
A (control)	100 ± 0.00 [a]	3.08±0.37 [a]	4.33±0.49 [a]	5.13±0.62 [a]	1.43±0.22 [a]	76.54±1.17 [a]
B (10 ml)	100 ± 0.00 [a]	3.24±0.55 [a]	7.07±0.25 [b]	5.40±0.91 [a]	1.35±0.21 [a]	85.38±3.85 [a]
C (20 ml)	100 ± 0.00 [a]	3.41±0.64 [a]	11.43±0.51 [c]	5.68±1.06 [a]	1.30±0.31 [a]	88.81±2.73 [b]
D (30 ml)	100 ± 0.00 [a]	4.00±0.69 [a]	10.97±0.64 [c]	6.66±1.15 [a]	1.12±0.21 [a]	95.24±1.04 [c]

Description: mean value in the same column with a different superscript sign are significantly different ($p < 0,05$).

Table 2. The water quality during the study

Parameters	Treatments			
	A	B	C	D
Termperature °C	26.5 - 28.0	27.0 - 28.0	26.0 - 27.5	26.5 - 28.0
pH	5.6 - 7.5	5.8 - 7.6	5.5 - 7.4	5.3 - 7.2
DO (mg/L)	5.45 - 6.04	5.33 - 6.12	5.61 - 6.02	5.33 - 6.04
Ammonia (mg/L)	0.012 - 0.082	0.024 - 0.094	0.011 - 0.095	0.018 - 0.080

The absolute weight growth of Asang fish seeds increased from 10 to 30 ml/kg with an increasing number of added probiotic doses. The same results were also reported in depik fish seeds (Fratiwi *et al.*, 2018), tilapia fish seeds (Ulfira, 2018 and Harmilia *et al.*, 2019), commond carp (Tarigan and Meiyasa, 2019), and silver catfish (Khotimah *et al.*, 2017). In the research results of Ulfira (2018), the weight gain of tilapia seeds that received probiotics was between 5.10 g and 7.76 g, red tilapia between 6.26 g and 6.98 g (Harmilia *et al.*, 2019), and depik fish seed ranges from 0.11 gr - 0.41 g (Fratiwi *et al.*, 2018).

Increasing the probiotic dose from 10-30 ml/kg in fish seed resulted in a non-linear increase in length, where the best length growth was at a probiotic dose of 20 ml/kg. Likewise, for silver catfish, long growth increased with a dose of 5 - 10 ml/kg of feed and decreased at a dose of 20 mL/kg of feed, tilapia fish (Opiya *et al.*, 2019). Furthermore, for depik fish seeds, the length growth increases linearly (Fratiwi *et al.*, 2018), tilapia seeds (Ulfira, 2018 and Harmilia *et al.*, 2019), commond carp (Tarigan and Meiyasa, 2019), and catfish (Wardika *et al.*, 2014). Based on the results of Ulfira (2018) research, the length growth of tilapia seeds that received probiotics ranges from 1.75 cm - 2.49 cm, while in the research of Harmilia *et al.* (2019) it ranges from 3.15 cm - 3.59 cm even with the same type of fish, in depik fish seed ranges from 0.29 cm - 0.97 cm (Fratiwi *et al.*, 2018), while in this research, the increase in length growth ranges from 5.33 - 9.43 mm.

The specific growth rate of Asang fish seeds increased with increasing doses of probiotics administered with the feed by 10-30 ml/kg. In this research, the specific growth rate of Asang fish seed ranges from 5.13% - 6.66%. The same results were also reported in depik fish seeds (Fratiwi *et al.*, 2018), and commond carp seeds (Tarigan and Meiyasa, 2019). While the results of the research by Fratiwi *et al.* (2018) for depik fish seeds with a dose of 30 ml/kg only range from 1.20% - 1.54%, for commond carp seeds with a dose of 15 ml/kg, the best specific growth rates range from 2.53% - 3, 08% (Tarigan and Meiyasa, 2019).

The FCR of asang fish seeds decreased with increasing doses of good probiotics for 10-30 ml/kg feed and may increase growth. This is because the probiotics in the feed enter the digestive tract to suppress pathogenic bacteria to help the absorption process. This is likely because the feed consumed can be digested and absorbed. The lower FCR value indicates that the feed consumed by the fish can be

used efficiently for growth. The same results also occurred in tilapia seeds (Arsyad *et al.*, 2015), tilapia fish seeds (Ulfira, 2018), red tilapia fish seeds (Harmilia *et al.*, 2019). The FCR of Asang fish seeds increased with increasing doses of good probiotics to feed on 10 to 30 ml/kg with the FCR in the Asang fish seed study, ranging from 1.12 to 1.43. While research (Arsyad *et al.*, 2015) has shown that the FCR of tilapia seeds ranges from 1.25 to 1.32, for tilapia seeds it ranges from 1.3 to 1.7 (Ulfira, 2018), and in red tilapia seeds it ranges from 3.66 to 4.17 (Harmilia *et al.,* 2019).

Asang fish seed feeding efficiency, which increases with increasing doses of good probiotics for feeding 10-30 ml/kg, can increase growth. Probiotics can improve the digestive quality of asang fish so that the fish's body absorbs more feed. The same results also occurred in ranged depik fish seeds (Fratiwi *et al.*, 2018), tilapia fish seeds (Arsyad *et al.*, 2015), and commond carp (Tarigan and Meiyasa, 2019). The feed efficiency of Asang fish seeds increased from 10 to 30 ml/kg with increasing doses of probiotics in the feed, in this research, it ranged from 70.76% - 91.40%. While in the results of previous studies, the feed efficiency of depik fish seeds ranges from 29.87% - 77.83% (Fratiwi *et al.*, 2018), tilapia seeds range from 0.76% - 0.80% (Arsyad *et al.* ., 2015), and commond carp ranges from 47.66% - 72.07% (Tarigan and Meiyasa, 2019).

Based on Table 2, the results of water quality measurements during the research were suitable for the requirements of fish seed cultivation. Furthermore, it is believed that the stable water quality during the study was under the influence of probiotic bacteria. Arief (2008) reported that Bacillus and acetobacter bacteria were effective in improving water quality. Hasan and Banerjee (2020) also stated that probiotics play a role in improving water quality. Several research results on fish rearing with the addition of probiotics, it turns out that water quality parameters during maintenance such as temperature, pH, DO, and ammonia are still in suitable conditions for aquaculture activities (Wardika *et al.,* 2014; Fratiwi *et al.,* 2018 and Tarigan *et al.,* 2019).

Conclusion

The addition of probiotics to commercial feed increases the growth of Asang fish seeds with the best dose of 30 ml/kg feed for absolute weight gain, specific growth rate, FCR, and feed efficiency, while the best probiotic dose for absolute length growth of the seeds is 20 ml/kg.

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