DOI: http://dx.doi.org/10.15578/iaj.19.2.2024.111-122 *Indonesian Aquaculture Journal, 19 (2), 2024, 111-122*

Available online at: http://ejournal-balitbang.kkp.go.id/index.php/iaj

GROWTH PERFORMANCE AND INTESTINAL *Aeromonas* spp. OF BONYLIP BARB (*Osteochilus vittatus*) AFTER RECEIVING DIET CONTAINING SALT AND HERBAL CONCOCTION SUPPLEMENTATION

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(Received: March 19, 2024; Final revision: June 30, 2024; Accepted: July 2, 2024)

ABSTRACT

Salt and herbal supplementations in fish feed are expected to promote aquaculture productivity, including for slow-growing fish such as Bonylip barb (Osteochilus vittatus). The objective of this study was to evaluate the growth performance and occurrence of Aeromonas spp. in the intestine of Bonylip barb fed a diet supplemented with salt and herbs. This experiment was conducted using a completely randomized design with four treatments: 1) control, 2) salt 3% supplementation, 3) herbal supplementation, and 4) Salt 3% and herbal supplementation. Growth performance evaluation included weight gain, relative growth rate, and specific growth rate after 30 and 60 days of rearing. Bacterial samples were isolated on trypticase soy agar (TSA) and glutamate starch phenol red agar (GSP) media. Yellow colonies on GSP were counted and confirmed as Aeromonas spp. via molecular identification based on 16S rDNA sequence. Results showed that herbal supplementation, either alone or in combination with 3% salt, insignificantly increased the fish growth performance in the short-term (30 days), but tended to decrease it over the long-term (60 days). Salt supplementation consistently decreased fish growth performance but improved fish survival rates. Aeromonas isolates comprised more than 60 % of the culturable intestinal bacteria in all treatments. Furthermore, herbal supplementation appeared to increase the percentage of Aeromonas spp. in the intestine, with identified species including Aeromonas veronii, Aeromonas caviae, and Aeromonas jandaei.

KEYWORDS: *Osteochilus vittatus*; salt; herbs; growth; *Aeromonas*

INTRODUCTION

Productivity of freshwater aquaculture in Indonesia needs to be increased, particularly for native species such as Bonylip barb (*Osteochilus vittatus*). Freshwater aquaculture has significantly contributed to food security and will continue to play a crucial role in providing reasonable and sustainable sources of protein (Belton *et al.*, 2020; Naylor *et al.*, 2021). Bonylip barb has the potential to be intensively cultivated to support aquaculture production in freshwaters (Aryani *et al.*, 2017; Wongyai *et al.*, 2020). From 2017 to 2021, this aquaculture commodity contributed approximately 32,890 tons per year to Indonesian aquaculture production (FAO, 2024).

The development of aquaculture typically encounters two main challenges, slow growth rate of the fish and diseases (Tran *et al.*, 2017). Therefore, improving growth performance and maintaining fish health at an affordable cost are essential to increasing aquaculture production. These objectives can be achieved by supplementing feed with inexpensive and highly available materials such as salt and herbal concoctions (Putri *et al.*, 2016; Salman, 2009; Singh *et al.*, 2022).

The effect of adding salt or other electrolytes to feed on fish growth and health has been reported in several previous studies and warrants further investigation. Positive impact of salt supplementation on fish growth have been observed at concentrations of 5% in black sea bass *Centropristis striata* (Alam *et al.*, 2015, 2021), and 1.5% in common carp *Cyprinus carpio* (Nasir & Hamed, 2016). Diets supplemented with salt have also been reported to increase the digestibility of macronutrient such as protein in the digestive tract of rainbow trout *Oncorhynchus mykiss* (Nakajima & Sugiura, 2016) and Nile tilapia *Oreochromis niloticus* (Hallali *et al.*, 2018). The ef-

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fect of salt supplementation on fish health could potentially be attributed to modulation of intestinal microbiome. Salt supplementation in fish feed has been shown to influence microbial diversity in the digestive tracts of Nile tilapia *Oreochromis niloticus* (Hallali *et al*., 2018) and seabass *Dicentrarchus labrax* (Sun *et al*., 2013). The diversity and abundance of gut microbiota play a crucial role in fish health by providing protection against pathogenic bacteria (Infante-Villamil *et al*., 2021; Jung-Schroers *et al*., 2018).

Herbal concoctions have been used by fish farmers in Indonesia to improve fish health and growth. Traditionally, herbal concoctions have been used for human health for centuries in Asian countries (Elfahmi *et al*., 2014; Jun *et al*., 2021; Liu, 2021; Rahayu *et al*., 2020). In aquaculture, the use of herbal concoctions shows promises increasing production. Various herbal ingredients such as *Morus alba*, *Curcuma xanthorrhiza*, *Boesenbergia rotunda,* Chinese herbal medicine, *Allium sativum*, *Curcuma longa*, *Curcuma zanthorrhiza*, and *Zingiber officinale* have been reported to enhance growth performance, survival rate, and abundance of beneficial microflora in fish intestines (Cai *et al*., 2023; Fujaya *et al*., 2023; Putri *et al*., 2016; Singh *et al*., 2022; Valenzuela-Gutiérrez *et al*., 2021). The antimicrobial content of these herbal ingredients can also help control pathogenic organisms (Parham *et al*., 2020). As opportunistic pathogen, the members of genus *Aeromonas* consistently pose challenges to freshwater aquaculture worldwide (Fernández-Bravo & Figueras, 2020). Members of the genus *Aeromonas* have been reported to cause outbreaks and significant losses in freshwater aquaculture, especially in Asia (Evan & Putri, 2021; Hoai *et al*., 2019; Llobrera & Gacutan, 1987; Nielsen *et al*., 2001; Rahman *et al*., 2002) and America (Griffin *et al*., 2013; Hazen *et al*., 1978). In Indonesia, diseases caused by *Aeromonas hydrophila* have been documented since 1980 and have spread to regions such as West Java, Central Java, Banten, Jambi and South Kalimantan (Evan & Putri, 2021).

Our previous report indicated possible beneficial effect of salt supplementation at 1-4% on growth performance of Bonylip barp (Syakuri *et al*., 2024). Currently, there is no report available on study of combination of salt and herbal supplementation on fish growth and fish intestinal *Aeromonas* spp. Therefore this study was conducted to evaluate the growth performance and the occurrence of *Aeromonas* spp. in the intestines of Bonylip barb (*O. vittatus*) fed a diet supplemented with salt and herbs.

MATERIALS AND METHODS

Experimental design and treatments

The research carried out an experimental method based on a completely randomized design (CRD) factorial (2 × 2), with four treatments and four replicates for evaluating growth performance, and a single replicate for examining intestinal *Aeromonas*. The first factor was salt supplementation, the second factor was herbal supplementation, and the treatments were as follows: 1) Control (commercial feed without supplementation), 2) Salt supplementation at 3% (w/w), 3) Herbal supplementation at 10 mL.kg^{-1} feed, and 4) Combination of 3% (w/w) salt supplementation and 10 mL.kg^{-1} herbal supplementation.

Feed Preparation

The basal feed in this study was a commercial fish feed with a protein content of around 39%. This feed is formulated for common freshwater fish seed. The herbal concoction consists of garlic (*Allium sativum*, 2.5%), turmeric (*Curcuma longa*, 5%), ginger (*Curcuma zanthorrhiza*, 5%), red ginger (*Zingiber officinale*, 2.5%), honey (0.5%), brown sugar (5%), anchovies (1.2%), and tap water (78,3%). This herbal concoction was provided by a shrimp farmer in Purworejo. This herbal concoction is commonly applied in shrimp culture in this area. The feed preparation was followed previous relevant work by Hallali *et al.* (2018). The coarse salt was dissolved, filtered (Whatman International, 1001090, England), and the final volume was 100 mL. The herbal mixture was diluted in 100 ml of distilled water. The salt solution and herbal mixture were then sprayed onto 1 kg of feed. The control feed was also sprayed with 100 mL of distilled water. Finally, the feed was air-dried at room temperature.

Fish samples and fish rearing

Healthy fish samples were obtained from a local fish breeder in Purbalingga Regency. The average total length of the fish samples was 7.5 ± 0.5 cm, and the average weight was 4.5 ± 0.9 g. The fish did not exhibit any symptoms of diseases such as red spots, fin necrosis, or wounds on the body surface. Fish juveniles were transported to the research site using standard methods: plastic bags containing a part of water and 2 parts of air.

The fish were reared indoors without sunlight. They were randomly distributed into twenty tanks, each containing 30 L of water, at a density of 10 fish per tank. This setup resulted in five tanks for each treatment, with four tanks used for growth performance evaluation and one tank randomly selected for *Aeromonas* examination. Each fish tank was equipped with a filter composed of Dacron and bio-balls, along with a 2-in-1 pump providing water recirculation and aeration. Fish acclimatization lasted seven days, during which the fish demonstrated normal responses.

The fish were fed at 3% of their body weight, with a feeding frequency of twice per day (in the morning at 06:00-07:00 a.m. and in the evening at 18:00-19:00 p.m.). Every 10 days, approximately 50% of the total water volume was changed, and the filter was cleaned. Water quality parameters including dissolved oxygen (DO), pH, and temperature were measured using a dissolved oxygen meter (Amtast, EC900, China), API freshwater master test kit (Mars Fishcare, 800t, USA), and digital thermometer (Shenzhen Green May Tech, TA298, China) respectively, one day before and one day after each water change.

Growth performance evaluation

Every 10 days, the total weight of fish in each tank was measured and the result was divided by the number of the fish. These were used to calculate weight gain, relative growth rate (RGR), specific growth rate (SGR), and feed conversion ratio (FCR) using commonly used equations (Harpaz *et al.*, 2005; Lugert *et al.*, 2016):

$$\text{Weight gain (g)} = \text{final weight} - \text{initial weight}$$

$$\text{RGR}\ \% = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100$$

$$\text{SGR}\ \%\ \text{per day} = \frac{\text{Ln final weight} - \text{Ln initial weight}}{\text{days}} \times 100$$

$$\text{FCR} = \frac{\text{Total feed (g)}}{\text{Weight gain (g)}}$$

Examination of *Aeromonas spp.*

Three fish samples from each treatment were randomly selected from a specific tank for *Aeromonas* examination on days 10, 20, and 30. The fish was euthanized, dissected, and their digestive tracts were collected. Intestinal samples of three centimeters were taken from the anterior, middle, and posterior sections. These intestinal samples were then pooled, weighed, crushed, and serially diluted (10^{-1}-10^{-5}) using sterile physiological solution (0.9% NaCl). A total of 1 ml of solution from the 10^{-3}-10^{-5} dilution was inoculated onto trypticase soy agar (TSA). After 24 hours of incubation, individual colonies were isolated and cultured on Glutamate Starch Phenol Red Agar (GSP) media. Yellow colonies on GSP media were counted, and the results were used to calculate the percentage of *Aeromonas* spp. Four samples of yellow colonies from each treatment were selected and purified for molecular identification.

Molecular identification of *Aeromonas spp.*

The *Aeromonas* samples were molecularly identified based on 16S rDNA gene sequences. Bacterial DNA extraction was carried out using the Genomic DNA Mini Kit following the manufacturer's instructions (Geneaid, GT300, Taiwan) with slight modifications, including the stages of lysis, DNA binding, DNA washing, and DNA elution. The DNA samples were then used for amplification of the 16S rDNA gene using MyTaq HS Red Mix (Bioline, BIO-25048, USA) with primers UnibacRP1 (5'-CAG GCC TAA CAC ATG CAA GTC-3') and UnibacFD1 (5'-GGG CGG WGT GTA CAA GGC-3') (Marchesi *et al.*, 1998). The PCR steps included pre-denaturation (95°C, 2 min), 40 cycles of denaturation (95°C, 20 s), annealing (54°C, 20 s), and elongation (72°C, 20 s), followed by final elongation (72°C, 5 min) and performed using Primus 25 Thermocycler (Peqlab, 95-4002, Germany). The PCR results were evaluated by DNA electrophoresis (Analytic Jena, 846-025-200, Germany) and visualized using a UV transilluminator (Major Science, MUV26-254, Taiwan). Sequencing of PCR products was performed, and the sequences were used for bacterial identification through BLAST analysis at http://ncbi.nlm.nih.gov (Altschul *et al.*, 1990) and phylogenetic analysis using MEGA 11 software (Tamura *et al.*, 2021).

Data analysis

Growth performance, survival rate, and the percentage of *Aeromonas* were analyzed using two-way analysis of variance (ANOVA) and significant results were followed by the Tukey's test as a post hoc analysis. Results of this analysis were considered significant if the p value was <0.05. All statistical tests were conducted using PAST free software (Hammer *et al.*, 2001).

RESULTS AND DISCUSSION

The salt and herbal supplementation did not significantly influence individual weight gain of the fish ($p>0.05$). The group of fish fed with herbal supplementation had a relatively better weight gain than the control group after 30 days of rearing. However, after 60 days of rearing, the group of fish fed with a diet containing herbal concoction supplementation had a relatively lower growth rate than the control group. The group of fish that received salt supplementation had lower growth than the other groups at both 30 days and 60 days of rearing (Figure 1). The individual weight gain after 30 days was 1.67 ± 0.10 g (control), 1.55 ± 0.13 g (salt 3%), 1.81 ± 0.34 g (herbal concoction), and 1.72 ± 0.06 g (salt 3% & herbal concoction). While the individual weight gain after 60 days was 4.07 ± 0.54 g (control), 3.63 ± 0.29 g (salt 3%), 3.87 ± 0.17 g (herbal concoction), and 3.71 ± 0.14 g (salt 3% & herbal concoction).

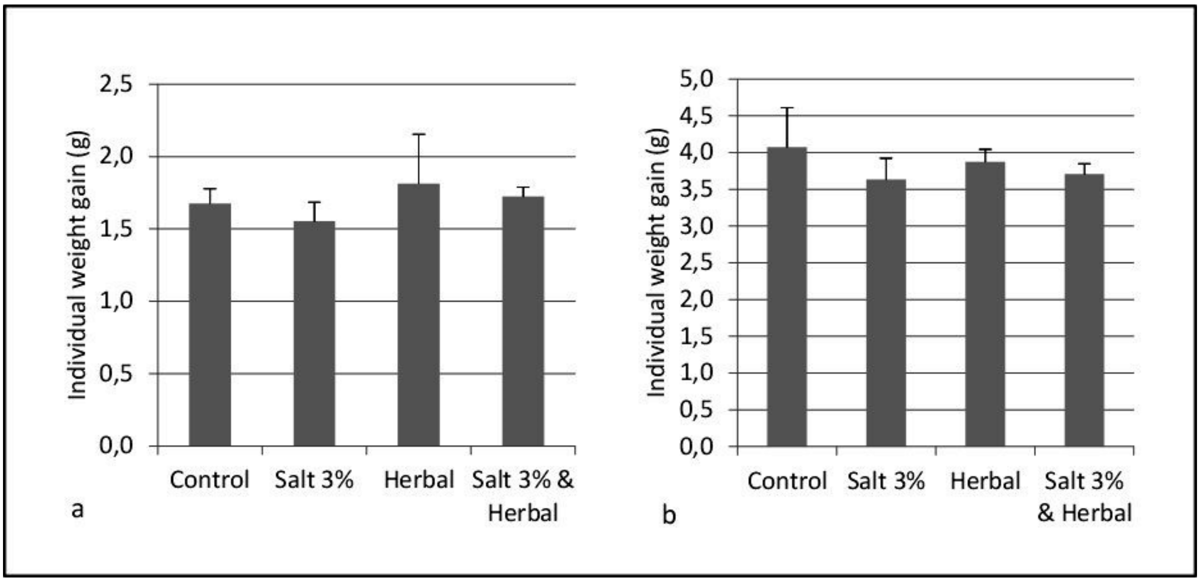

Figure 1. Individual weight gain of Bonylip barb (*Osteochilus vittatus*) after receiving diet containing salt and herbal supplementation for 30 days (a) and 60 days (b). No significant differences were found among treatments.

Growth evaluation based on RGR showed the same pattern compared to growth evaluation based on weight gain. Application of herbal supplementation, especially in combination with salt supplementation, appeared to increase growth performance at 30 days of maintenance, but decreased growth performance at 60 days of maintenance. The use of salt in feed also appeared to reduce the RGR value (Figure 2). The RGR after 30 days was 42 ± 6 % (control), 39 ± 3 % (salt 3%), 45 ± 9 % (herbal concoction), and 43 ± 2 % (salt 3% & herbal concoction). While the RGR after 60 days was 101 ± 10 % (control), 90 ± 2 % (salt 3%), 95 ± 10 % (herbal concoction), and 92 ± 6 % (salt 3% & herbal concoction). However, this difference was not statistically significant ($p > 0.05$).

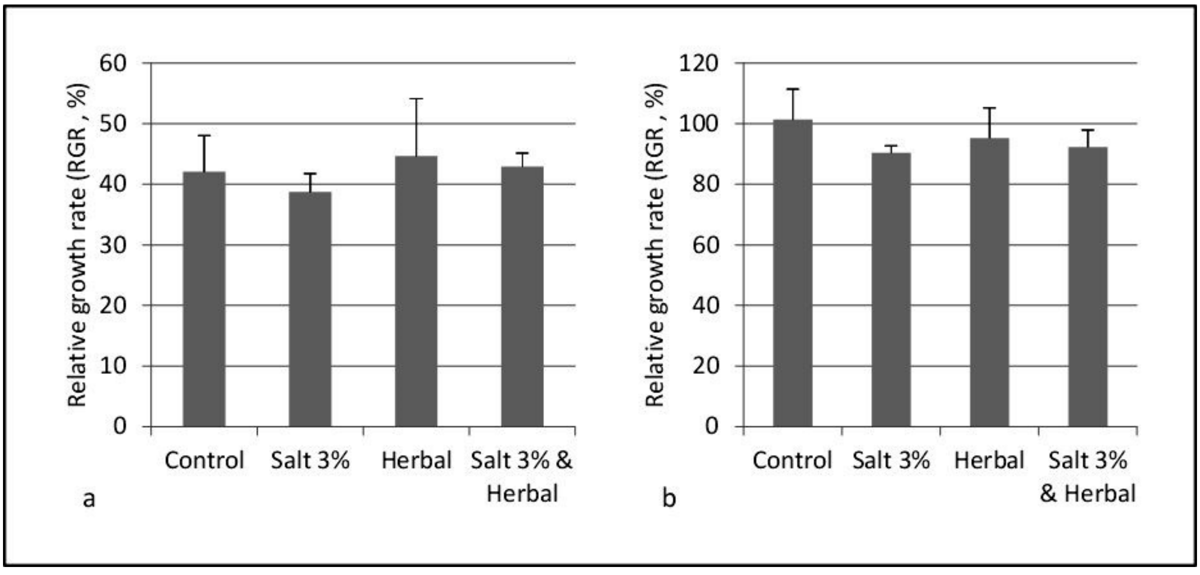

Figure 2. Relative growth rate (RGR) of Bonylip barb (*Osteochilus vittatus*) after receiving diet containing salt and herbal supplementation for 30 days (a) and 60 days (b). No significant differences were found among treatments.

Similarly, specific growth rate (SGR) evaluation showed the same pattern as RGR. There was also no significant difference among treatments ($p>0.05$). The use of herbs as supplementation ingredients for fish feed seemingly increased the specific growth rate after maintenance for 30 days. Prolonged use of herbs caused a decrease in the SGR value (Figure 3). The SGR after 30 days was 1.17 ± 0.14 % per day (control), 1.09 ± 0.07 % per day (salt 3%), 1.22 ± 0.22 % per day (herbal concoction), and 1.19 ± 0.05 % per day (salt 3% & herbal concoction). While the SGR after 60 days was 1.16 ± 0.08 % per day (control), 1.07 ± 0.02 % per day (salt 3%), 1.11 ± 0.08 % per day (herbal concoction), and 1.09 ± 0.05 % per day (salt 3% & herbal concoction).

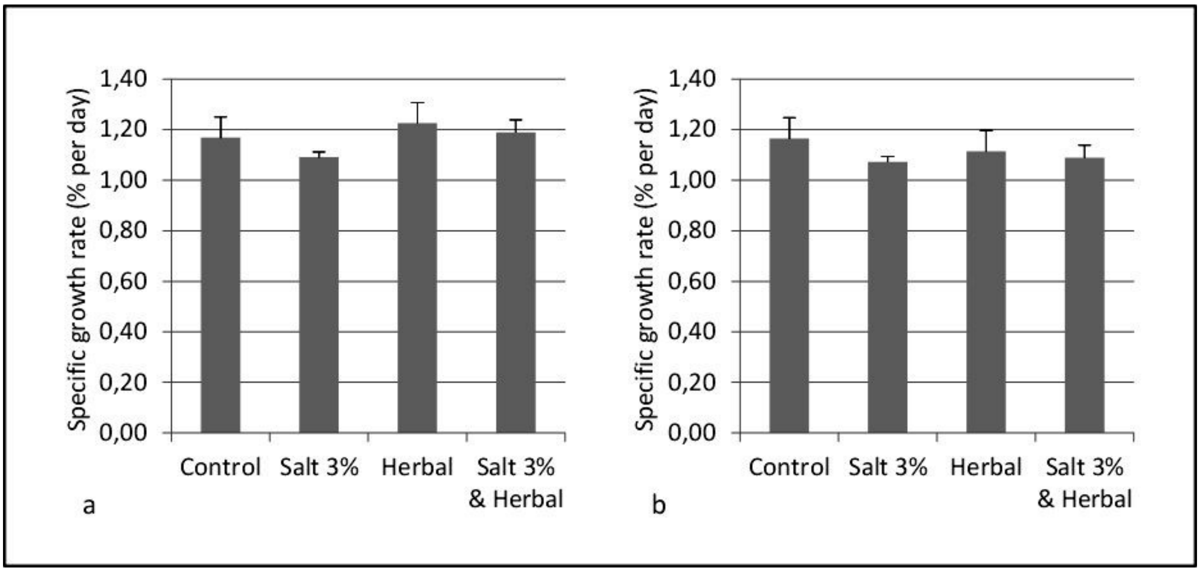

Figure 3. Specific growth rate (SGR) of Bonylip barb (*Osteochilus vittatus*) after receiving diet containing salt and herbal supplementation for 30 days (a) and 60 days (b). No significant differences were found among treatments.

The salt and herbal supplementation did not significantly influence the feed conversion ratio (FCR) of the fish ($p>0.05$). The control group of fish had relatively better FCR than the group of fish fed with salt and herbal supplementation or the combination. The FCR after 30 days was 2.4 ± 0.1 (control), 2.8 ± 0.1 (salt 3%), 2.6 ± 0.0.2 (herbal concoction), and 2.6 ± 0.2 (salt 3% & herbal concoction). While the FCR after 60 days was 2.4 ± 0.3 (control), 2.7 ± 0.2 (salt 3%), 2.4 ± 0.2 (herbal concoction), and 2.4 ± 0.1 (salt 3% & herbal concoction). The group of fish fed feed containing salt had the highest FCR (Figure 4).

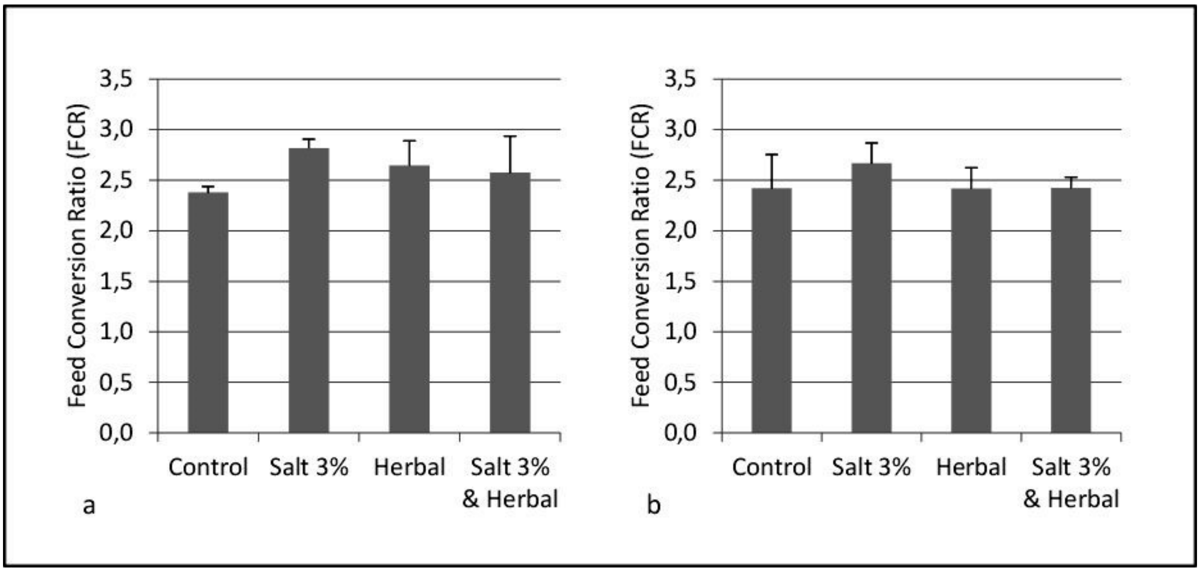

Figure 4. Feed conversion ratio (FCR) of Bonylip barb (*Osteochilus vittatus*) after receiving diet containing salt and herbal supplementation for 30 days (a) and 60 days (b). No significant differences were found among treatments.

The use of salt supplemented feed was apparently beneficial for fish health based on survival rates (Figure 5a). The SR was 95 ± 6 % (control), 100 ± 0 % (salt 3%), 95 ± 6 % (herbal concoction), and 98 ± 5 % (salt 3% & herbal concoction). No mortality occurred in the group of fish fed with salt supplementation for 60 days of rearing. Fish mortalities in the control group and the fish group received herbal supplementation occurred during the first 20 days of rearing. The mortality of the group of fish that received a combination of salt and herbal supplementation occurred after the initial 20 days of rearing (Figure 5b).

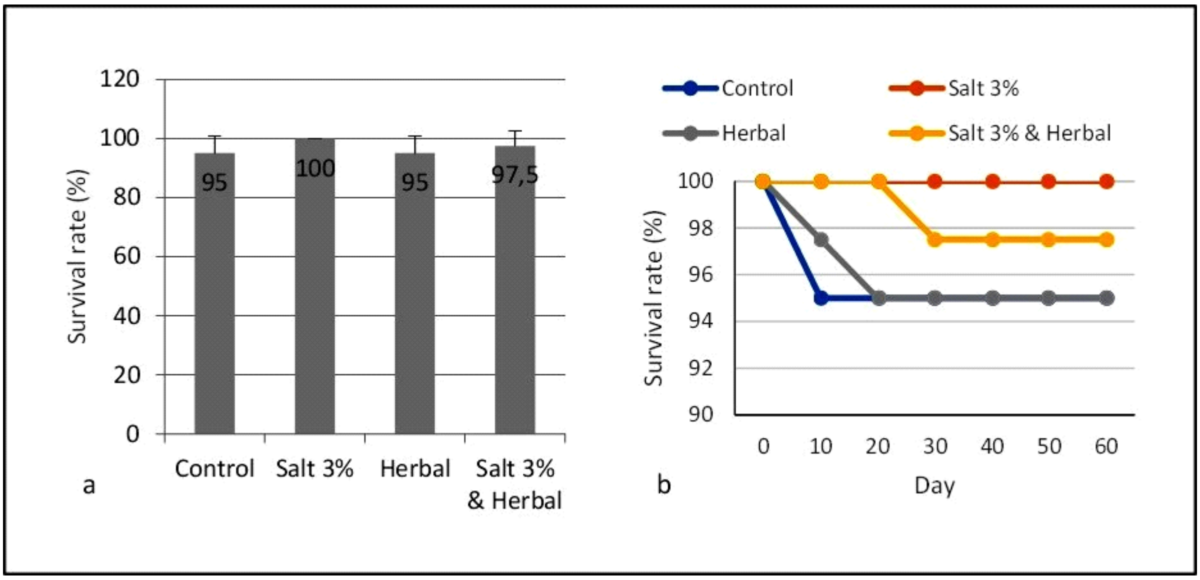

Figure 5. Survival rate of Bonylip barb (*Osteochilus vittatus*) after receiving diet containing salt and herbal supplementation for 60 days in time series (a) and the cumulative data (b). No significant differences were found among treatments.

Aeromonas spp. was found in the intestine of these fish in a large percentage, more than 60% of culturable bacteria in all treatment groups. However it was not significantly difference ($p > 0.05$), the group of fish received herbal supplementation appeared to have a higher percentage of *Aeromonas* spp. (83.3 ± 8.3 %) in comparison to the control group (71.2 ± 25.4 %), salt supplementation group (67.8 ± 31.6 %), and salt-herbal supplementation group (66.8 ± 11.3 %). Salt supplementation appeared to be able to maintain the percentage of *Aeromonas* spp. at the same level as the percentage in the control group (Figure 6).

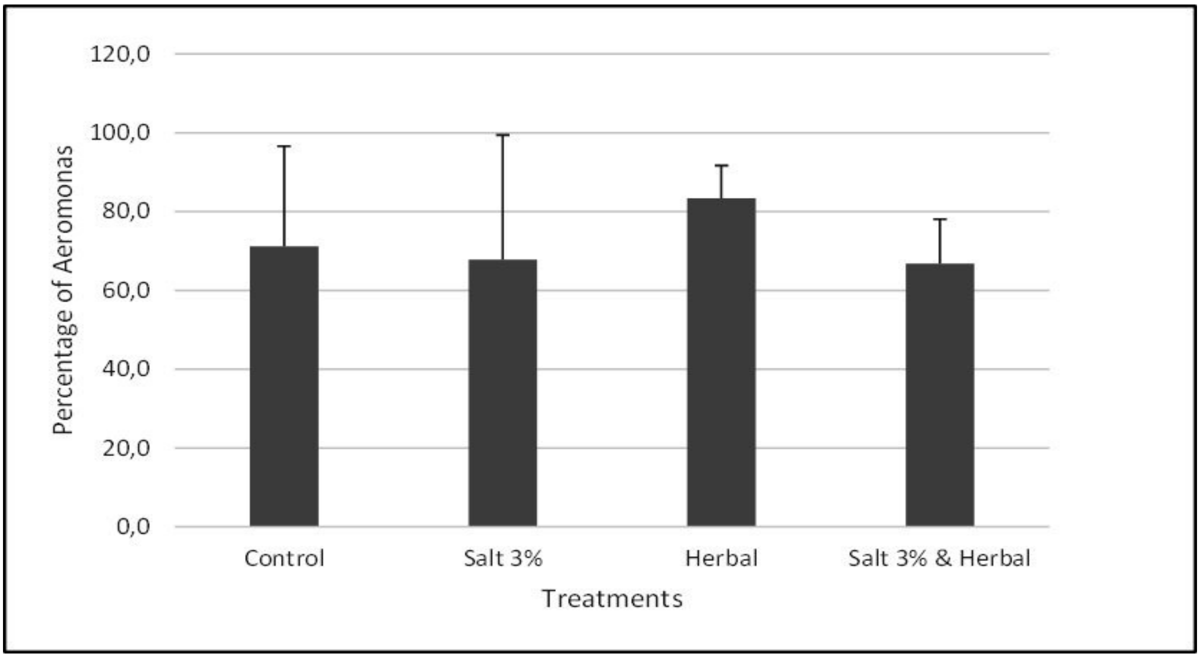

Figure 6. Percentage of *Aeromonas* spp. in the intestine of Bonylip barb (*Osteochilus vittatus*) after receiving diet containing salt and herbal supplementation. No significant differences were found among treatments.

Table 1. BLAST results of 16s rDNA sequences of selected *Aeromonas* isolates from the intestine of Bonylip barb (*Osteochilus vittatus*) after receiving diet containing salt and herbal supplementation for 30 days

Treatments	Number of isolates	Blast result		
		Species	Identity (%)	Acc. Number
• Control	3	*Aeromonas veronii*	99.86	MT345040
	1	*Aeromonas caviae*	100	MG737571
• Salt 3%	4	*Aeromonas veronii*	99.86	MT345040
• Herbal	1	*Aeromonas jandaei*	100	JX426061
	2	*Aeromonas veronii*	99.86	MT345040
	1	*Aeromonas caviae*	100	MG737571
• Salt 3% & Herbal	2	*Aeromonas veronii*	99.86	MT345040
	2	*Aeromonas caviae*	100	MG737571

The results of molecular identification using BLAST analysis and phylogenetic analysis showed that three members of the genus *Aeromonas* found in this study: *Aeromonas veronii, Aeromonas caviae*, and *Aeromonas jandaei* (Table 1 and Figure 7). The bacterial samples from the fish group received salt supplementation consisted of only one species, *Aeromonas veronii*. In contrast, samples of *Aeromonas* from other fish groups comprised two or three different species (Table 1).

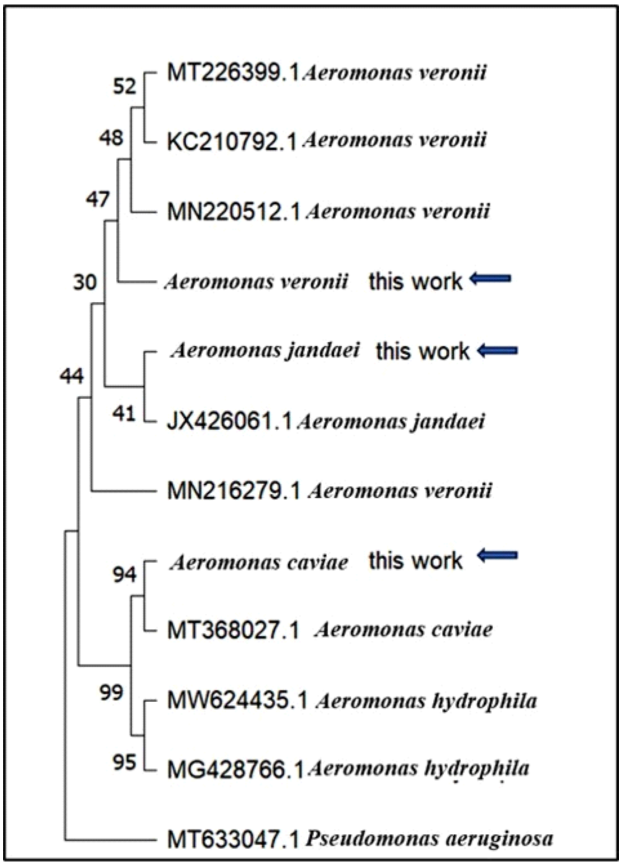

Figure 7. Phylogenetic analysis result based on 16S rDNA gene sequences of selected *Aeromonas* isolates from the intestines of Bonylip barb (*Osteochilus vittatus*) and relevant sequences from genbank. Blue arrows indicate the samples of this work.

In this study, the fish was reared under suitable water conditions based on dissolved oxygen (DO), pH, and water temperature (Table 2). The DO level exceeded the recommended standard of 5 mg/L (Boyd *et al.*, 2008), ensuring optimal oxygenation. The pH of the water remained within the standard range of 6.0-9.0 (Boyd *et al.*, 2008). Additionally, the water temperature was maintained around 28°C. These suitable water quality parameters likely contributed to the high survival rate of Bonylip barb in this study.

Table 2. Dissolved oxygen (DO), pH, and water temperature during the experiment

Treatments	DO (mg/L)	pH	Water Temperature (°C)
Control	6-7.6	7.4-7.8	28.4-28.9
Salt 3%	6.8-7.2	7.4-7.8	28.4-28.9
Herbal	6.0-6.8	7.4-7.8	28.2-28.8
Salt 3% & Herbal	6.2-6.9	7.4-7.8	28.4-28.9

However the fish during the experiment showed a high survival rate, the fish showed a low growth performance and high feed conversion ratio (FCR). The SGR of the fish in this work (~1% per day) was lower than the results of other work of the same species reared in aquaponic system, which showed SGR of around 3% per day (Hadiroseyani *et al*., 2023). Other report also showed SGR of 2.3 % per day of Bonylip barb cultivated in recirculation system (Mulyadi *et al*., 2024). The fish in this work showed higher FCR (2.4-2.8) in comparison to the same fish species reared in aquaponic system and recirculation system which showed FCR of 1.03 and 2.23, respectively. The low growth performance and the high FCR of Bonylip barb in this work might be related with rearing system. In this work, every tank was supported by a 7 watt-pump with output 1000 L water per hour. In one hand, this equipment was maintaining water quality but on the other hand it was causing high water current. Therefore, the fish should spend more energy against water movement resulting in low growth performance and high FCR.

The application of salt supplementation to fish feed in this study insignificantly reduced the growth performance and increase the FCR of Bonylip barb. This result is not consistent with our previous report which showed that diet supplemented with salt at 1-4% tended to increase growth performance and decrease FCR of Bonylip barp (Syakuri *et al*., 2024). Other studies showed also inconsistency effect of salt supplementation on fish growth and FCR. A report showed increase of growth of tilapia (Cnaani *et al*., 2010), however, other report showed a contrary result on the same species (de Aguiar *et al*., 2020). Other report showed that carp *Cyprinus carpio* received 1.5% salt supplementation had the highest growth rate and the lowest FCR; and those received 2% salt supplementation had the lowest growth rate and the highest FCR (Nasir & Hamed, 2016). The decrease in fish growth rate due to salt supplementation may occur due to changes in feed composition, including protein content. The changes of feed composition occurred because a portion of feed was replaced by a portion of salt. This is supported by previous report showing the supplementation of salt to the fish feed was reduced the protein content of the fish feed (Hallali *et al*., 2018). The commercial feed sprayed with salt solution (5%) showed a lower protein content (27.25%) in comparison to the initial commercial feed (30.43%) (Hallali *et al*., 2018).

Even though salt supplementation insignificantly reduced growth performance of Bonylip barb, however, it has the highest survival rate. In this work, the dead fish showed abdominal dropsy (data not shown) that might indicate *Aeromonas* infection. Therefore, no mortality of the fish receiving salt-supplemented diet could be related to the effect of salt to the *Aeromonas* members that were abundance in the intestine of the fish. Salt concentration was reported to decrease the virulence factor of *Aeromonas* (John et al., 2019). Additionally, salt supplementation also could increase fish health through influencing microbial balance in the intestine (Hallali *et al*., 2018; Sun *et al*., 2013) and altering ionic balances (Tavares Dias, 2022). This result was in accordance with previously published results that showed increased survival of fish fed with salt supplementation. Against nitrite toxicity, the supplementation of salt had a positive effect on the resistance of channel catfish *Ictalurus punctatus* (Welker *et al*., 2012). In addition, Black Sea bass *Centropristis striata* fed with 2.5-7.5% added salt performed higher resistance to extreme hypo salinity conditions by showing higher survival rate (Alam *et al*., 2021). The positive effect of salt supplementation on survival rate of several fish such as *Oreochromis mossambicus*, *Oreochromis aureus* × *Oreochromis niloticus* hybrid, *Pterophyllum scalare*, and *Betta splendes* was also summarized (Tavares Dias, 2022).

The results of this work indicate that herbal supplementation should be carried out in the short term, less than 30 days. Long-term application of herbal supplementation has been proven to inhibit growth of the Bonylip barb in this work. In addition, the application of herbal supplementation also reduces the survival rate of fish. This might be related to the increase in the percentage of *Aeromonas* spp. in the fish intestine. The increase of *Aeromonas* spp. was probably caused by the nutritious ingredients including brown sugar in the concoction and unspecific antibacterial effects of the herbal ingredients. Sugar could serve as source of carbon for the growth of the bacteria which was reported to increase in response to the increase of nutrients in the water (Zhang *et al*., 2020). In addition to the nutrient's availability, the growth of the bacteria was related with water quality factors such as dissolved oxygen and water temperature (Li *et al.*, 2020a). Finally, it is generally known that herbal materials contain antibacterial compound (Parham *et al.*, 2020). The antibacterial properties of herbal materials are not specific for certain bacteria and dose-dependent (Kot *et al.*, 2019). The result of this work indicate that herbal supplementation did not inhibit the *Aeromonas* spp. but it might inhibit other intestinal bacteria, and as a consequence, the percentage of *Aeromonas* spp. was increase.

The result of this work showed that *Aeromonas* spp. dominated the culturable bacteria in the intestine of Bonylip barb and it comprised of three species, namely *Aeromonas veronii*, *Aeromonas caviae*, and *Aeromonas jandaei*. The genus *Aeromonas* is commonly known as a widely distributed bacteria in aquatic environments (Fernández-Bravo & Figueras, 2020). In addition to *Aeromonas hydrophila* as the well-known causative agent of motile aeromonads septicemia, these three bacteria were reported as pathogenic bacteria in fish (Dong *et al.*, 2017; Fernández-Bravo & Figueras, 2020; Li *et al.*, 2020b; Sharma *et al.*, 2022). However, during this work there was no clinical signs related with the infection of these bacteria. This indicates that *Aeromonas* spp., found in this work might be part of non-pathogenic strains of the bacteria.

In this study, there was no significant interaction between salt and herbal supplementation in all parameters ($p > 0.05$). However, the fish group receiving diet supplemented with combination of salt and herbs showed growth performance, FCR, and survival rate in between the group receiving salt supplementation and the group receiving herbal supplementation. In addition, the percentage of *Aeromonas* spp. in intestine of the Bonylip barp fed with diet supplemented with salt and herbs was closely similar to that of the fish fed with diet supplemented with salt. Further study is needed to carefully evaluate the use of salt and herbal supplementation in aquaculture.

CONCLUSIONS

Supplementation with salt and herbal concoction, either alone or in combination, did not significantly influence the growth performance and intestinal occurrence of *Aeromonas* spp. in the Bonylip barb. Interestingly, there was no mortality in the fish group receiving salt supplementation, suggesting a potential beneficial effect of this treatment. Additionally, members of the genus *Aeromonas* were found to be dominant bacteria among culturable species in the fish intestines, molecularly identified as *Aeromonas veronii*, *Aeromonas caviae*, and *Aeromonas jandaei*.

ACKNOWLEDGMENTS

This research was made possible by financial support from Jenderal Soedirman University through the Institute for Research and Community Service, under Institutional Research Scheme (B/3567/UN23). Special thanks for technical assistances are extended to Emyl, Nisa, Keisya, Ratika, Awan, Syafrie, Mevira, Sevia, Ronald, and Novia. Finally, we express our gratitude to M. Maulana Iqbal for providing the herbal concoctions.

REFERENCES

Alam, M. S., Watanabe, W. O., & Carroll, P. M. (2021). Evaluation of salt-incorporated diets on growth, body composition and plasma electrolytes of black sea bass *Centropristis striata* reared in a semi-pilot scale low salinity recirculating aquaculture system. *Aquaculture, 533,* 736102. https://doi.org/https://doi.org/10.1016/j.aquaculture.2020.736102

Alam, M. S., Watanabe, W. O., Myers, A. R., Rezek, T. C., Carroll, P. M., & Skrabal, S. A. (2015). Effects of dietary salt supplementation on growth, body composition, tissue electrolytes, and gill and intestinal Na^+/K^+ ATPase activities of black sea bass reared at low salinity. *Aquaculture*, *446*, 250–258. https://doi.org/https://doi.org/10.1016/j.aquaculture.2015.05.001

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Aryani, N., Mardiah, A., Azrita, & Syandri, H. (2017). Influence of different stocking densities on growth, feed efficiency and carcass composition of Bonylip barb (*Osteochilus vittatus* Cyprinidae) Fingerlings. *Pakistan Journal of Biological Sciences/ : PJBS*, *20*(10), 489–497. https://doi.org/10.3923/pjbs.2017.489.497

Belton, B., Little, D. C., Zhang, W., Edwards, P., Skladany, M., & Thilsted, S. H. (2020). Farming fish in the sea will not nourish the world. *Nature Communications*, *11*(1), 5804. https://doi.org/10.1038/s41467-020-19679-9

Boyd, C., Lim, C., Queiroz, J., Salie, K., de Wet, L., & McNevin, A. (2008). Best management practices for responsible aquaculture. Aquaculture Collaborative Research Support Program, Oregon State University, Corvallis, Oregon. 47 pp.

Cai, J., Yang, Z., Huang, Y., Jian, J., & Tang, J. (2023). Effects of Chinese herbal medicines on growth performance, intestinal flora, immunity and serum metabolites of hybrid grouper (*Epinephelus fuscoguttatus*@& × *Epinephelus lanceolatu*B&). *Fish & Shellfish Immunology*, *140*, 108946. https://doi.org/https://doi.org/10.1016/j.fsi.2023.108946

Cnaani, A., Barki, A., Slosman, T., Scharcanski, A., Milstein, A., & Harpaz, S. (2010). Dietary salt supplement increases the growth rate in freshwater cultured tilapia hybrids. *Aquaculture Research*, *41*(10), 1545–1548. https://doi.org/https://doi.org/10.1111/j.1365-2109.2009.02438.x

de Aguiar, N. C., Dias, P. S., Balen, R. E., Bombardelli, R. A., Colpini, L., & Meurer, F. (2020). Dietary sodium chloride effect in Nile tilapia fed with fish meal-free diets. *Spanish Journal of Agricultural Research*, *18*(3), e0610.

Dong, H. T., Techatanakitarnan, C., Jindakittikul, P., Thaiprayoon, A., Taengphu, S., Charoensapsri, W., Khunrae, P., Rattanarojpong, T., & Senapin, S. (2017). *Aeromonas jandaei* and *Aeromonas veronii* caused disease and mortality in Nile tilapia, *Oreochromis niloticus* (L.). *Journal of Fish Diseases*, *40*(10), 1395–1403. https://doi.org/10.1111/jfd.12617

Elfahmi, Woerdenbag, H. J., & Kayser, O. (2014). Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. *Journal of Herbal Medicine*, *4*(2), 51–73. https://doi.org/https://doi.org/10.1016/j.hermed.2014.01.002

Evan, Y., & Putri, N. E. (2021). Status of aquatic animal health in Indonesia. In F. A. Aya, L. D. de la Pena, N. D. Salayo, & E. A. Tendencia (Ed.), *Proceedings of the International Workshop on the Promotion of Sustainable Aquaculture, Aquatic Animal Health, and Resource Enhancement in Southeast Asian* (hal. 138–149). Aquaculture Department, Southeast Asian Fisheries Development Center.

FAO. (2024). *FAO Fisheries and Aquaculture - Fishery Statistical Collections - Global Aquaculture Production. In: FAO Fisheries and Aquaculture Division [online]. Rome. [Cited Pebruary 2024].* https://www.fao.org/fishery/en/collection/aquaculture

Fernández-Bravo, A., & Figueras, M. J. (2020). An update on the genus *Aeromonas*: taxonomy , epidemiology , and pathogenicity. *Microorganisms*, *129*, 3–6.

Fujaya, Y., Hidayani, A. A., Sari, D. K., Aslamyah, S., Rukminasari, N., Muthalib, A., Cristianto, S., Defista, E., Fazhan, H., & Waiho, K. (2023). The optimal dosage of fermented herbal extract on growth and feed efficiency of Nile tilapia (*Oreochromis niloticus*). *Tropical Life Sciences Research*, *34*(2), 39–56. https://doi.org/10.21315/tlsr2023.34.2.3

Griffin, M. J., Goodwin, A. E., Merry, G. E., Liles, M. R., Williams, M. A., Ware, C., & Waldbieser, G. C. (2013). Rapid quantitative detection of *Aeromonas hydrophila* strains associated with disease outbreaks in catfish aquaculture. *Journal of Veterinary Diagnostic Investigation*, *25*(4), 473–481. https://doi.org/10.1177/1040638713494210

Hadiroseyani, Y., Diatin, I., Faozar, M. F., & Vinasyiam, A. (2023). Nursery of bonylip barb fish *Osteochilus hasselti* in an aquaponics system. *Jurnal Akuakultur Indonesia*, *22*(1), 55–65. https://doi.org/10.19027/jai.22.1.55-65

Hallali, E., Kokou, F., Chourasia, T. K., Nitzan, T., Con, P., Harpaz, S., Mizrahi, I., & Cnaani, A. (2018). Dietary salt levels affect digestibility, intestinal gene expression, and the microbiome, in Nile Tilapia (*Oreochromis niloticus*). *PLOS ONE*, *13*(8), e0202351. https://doi.org/10.1371/journal.pone.0202351

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, *4*(1).

Harpaz, S., Hakim, Y., Slosman, T., & Eroldogan, O. T. (2005). Effects of adding salt to the diet of Asian sea bass *Lates calcarifer* reared in fresh or salt water recirculating tanks, on growth and brush border enzyme activity. *Aquaculture*, *248*(1), 315–324. https://doi.org/https://doi.org/10.1016/j.aquaculture.2005.03.007

Hazen, T. C., Fliermans, C. B., Hirsch, R. P., & Esch, G. W. (1978). Prevalence and distribution of *Aeromonas hydrophila* in the United States. *Applied and Environmental Microbiology*, *36*(5), 731–738. https://doi.org/10.1128/aem.36.5.731-738.1978

Hoai, T. D., Trang, T. T., Van Tuyen, N., Giang, N. T. H., & Van Van, K. (2019). *Aeromonas veronii* caused disease and mortality in channel catfish in Vietnam. *Aquaculture*, *513*, 734425. https://doi.org/10.1016/j.aquaculture.2019.734425

Infante-Villamil, S., Huerlimann, R., & Jerry, D. R. (2021). Microbiome diversity and dysbiosis in aquaculture. *Reviews in Aquaculture*, *13*(2), 1077–1096. https://doi.org/https://doi.org/10.1111/raq.12513

John, N., Vidyalakshmi, V. B., & Hatha, A. A. M. (2019). Effect of pH and salinity on the production of extracellular virulence factors by Aeromonas from food sources. *Journal of Food Science*, *84*(8), 2250–2255. https://doi.org/10.1111/1750-3841.14729

Jun, P., Rahmat, E., Han, C.-H., Yang, C., & Kang, Y. (2021). Traditional Chinese medicine and traditional Indonesian medicine: a comparative review of herbal medicines restricted in pregnancy. *Chinese Journal of Integrative Medicine*, *27*(10), 794–800. https://doi.org/10.1007/s11655-021-3487-7

Jung-Schroers, V., Adamek, M., Harris, S., Syakuri, H., Jung, A., Irnazarow, I., & Steinhagen, D. (2018). Response of the intestinal mucosal barrier of carp (*Cyprinus carpio*) to a bacterial challenge by *Aeromonas hydrophila* intubation after feeding with beta-1,3/1,6-glucan. *Journal of Fish Diseases*, *41*(7), 1077–1092. https://doi.org/10.1111/jfd.12799

Kot, B., Kwiatek, K., Janiuk, J., Witeska, M., & Pêkala-Safiñska, A. (2019). Antibacterial activity of commercial phytochemicals against Aeromonas species isolated from fish. *Pathogens*, *8*(3), 1–12. https://doi.org/10.3390/pathogens8030142

Li, X. M., Zhu, Y. J., Ringø, E., & Yang, D. G. (2020a). Prevalence of *Aeromonas hydrophila* and *Pseudomonas fluorescens* and factors influencing them in different freshwater fish ponds. *Iranian Journal of Fisheries Sciences*, *19*(1), 111–124. https://doi.org/10.22092/ijfs.2019.120174

Li, T., Raza, S. H. A., Yang, B., Sun, Y., Wang, G., Sun, W., Qian, A., Wang, C., Kang, Y., & Shan, X. (2020b). *Aeromonas veronii* infection in commercial freshwater fish: a potential threat to public health. *Animals*, *10*(4), 608. https://doi.org/10.3390/ani10040608

Liu, C. (2021). Overview on development of ASEAN traditional and herbal medicines. *Chinese Herbal Medicines*, *13*(4), 441–450. https://doi.org/https://doi.org/10.1016/j.chmed.2021.09.002

Llobrera, A. T., & Gacutan, R. Q. (1987). *Aeromonas hydrophila* associated with ulcerative disease epizootic in Laguna de Bay, Philippines. *Aquaculture*, *67*(3–4), 273–278. https://doi.org/10.1016/0044-8486(87)90211-0

Lugert, V., Thaller, G., Tetens, J., Schulz, C., & Krieter, J. (2016). A review on fish growth calculation: multiple functions in fish production and their specific application. *Reviews in Aquaculture*, *8*(1), 30–42. https://doi.org/10.1111/raq.12071

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J., & Wade, W. G. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Applied and Environmental Microbiology*, *64*(2), 795–799. https://doi.org/10.1128/aem.64.2.795-799.1998

Mulyadi., Pamukas, N. A., & Claudya, I. D. (2024). Impact of varied grotop booster doses in feed on the growth and survival of *Osteochilus hasselti* in a recirculation system. *AACL Bioflux*, *17*(1), 166–172.

Nakajima, M., & Sugiura, S. (2016). Effects of dietary NaCl on the in vivo apparent absorption of dietary nutrients determined in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *460*, 1–7. https://doi.org/https://doi.org/10.1016/j.aquaculture.2016.04.003

Nasir, N., & Hamed, Q. (2016). Growth development of young common carp *Cyprinus carpio* through dietary sodium chloride supplementation. *Mesopotamia Environmental Journal*, *2*(2), 12–18.

Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., & Shumway, S. E. (2021). A 20-year retrospective review of global aquaculture. *Nature*, *591*(March), 551–563. https://doi.org/10.1038/s41586-021-03308-6

Nielsen, M. E., Høi, L., Schmidt, A. S., Qian, D., Shimada, T., Shen, J. Y., & Larsen, J. L. (2001). Is *Aeromonas hydrophila* the dominant motile Aeromonas species that causes disease outbreaks in aquaculture production in the Zhejiang Province of China/ ? *Diseases of Aquatic Organisms*, *46*, 23–29. https://doi.org/10.3354/dao046023

Parham, S., Kharazi, A. Z., Bakhsheshi-Rad, H. R., Nur, H., Ismail, A. F., Sharif, S., RamaKrishna, S., & Berto, F. (2020). Antioxidant, antimicrobial and antiviral properties of herbal materials. *Antioxidants 9*(12), 1309. https://doi.org/10.3390/antiox9121309

Putri, I. W., Setiawati, M., & Jusadi, D. (2016). Digestive enzymes and growth performance of common carp, *Cyprinus carpio* Linnaeus , 1758 with additional of turmeric meal, *Curcuma longa* Linn. in the diet. *Jurnal Iktiologi Indonesia*, *17*(1), 11–20. https://doi.org/10.32491/jii.v17i1.21

Rahayu, Y. Y. S., Araki, T., & Rosleine, D. (2020). Factors affecting the use of herbal medicines in the universal health coverage system in Indonesia. *Journal of Ethnopharmacology*, *260*, 112974. https://doi.org/https://doi.org/10.1016/j.jep.2020.112974

Rahman, M., Colque-Navarro, P., Kühn, I., Huys, G., Swings, J., & Möllby, R. (2002). Identification and characterization of pathogenic *Aeromonas veronii* biovar sobria associated with epizootic ulcerative syndrome in fish in Bangladesh. *Applied and Environmental Microbiology*, *68*(2), 650–655. https://doi.org/10.1128/AEM.68.2.650-655.2002

Salman, N. (2009). Effect of dietary salt on feeding, digestion, growth and osmoregulation in teleost fish. In *Essential reviews in experimental biology* (hal. 109–150). Society for Experimental Biology Press.

Sharma, A., Chanu, T. I., Nayak, S. K., Jahageerdar, S., & Krishna, G. (2022). Pathogenesis of *Aeromonas caviae* in *Clarias magur*. *Microbial Pathogenesis*, *169*, 105662. https://doi.org/https://doi.org/10.1016/j.micpath.2022.105662

Singh, M. K., Borah, D., Dutta, M. P., Gogoi, S., Saikia, C., Sonowal, S., & Manhai, S. K. (2022). A review on immunostimulatory and antioxidant potential of herbs, *Curcuma longa* L., *Camellia sinensis* L., *Zingiber officinale* and *Allium sativum* Linn. in fish health/ : a sustainable approach for a healthy aquaculture. *Ecology, Enviroment, and Conservation*, *28*(3).

Sun, H., Jami, E., Harpaz, S., & Mizrahi, I. (2013). Involvement of dietary salt in shaping bacterial communities in European sea bass (*Dicentrarchus labrax*). *Scientific Reports*, *3*(1), 1558. https://doi.org/10.1038/srep01558

Syakuri, H., Pramono, T. B., Ekasanti, A., Nugrayani, D., & Listiowati, E. (2024). Growth Performance of Bonylip barb (*Osteochilus Vittatus*) Fed with Salt Supplementation. *Sainteks*, 21(1), 1-13. https://doi.org/10.30595/sainteks.v21i1.21069

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Tavares Dias, M. (2022). Toxicity, physiological, histopathological, handling, growth and antiparasitic effects of the sodium chloride (salt) in the freshwater fish aquaculture. *Aquaculture Research*, *53*(3), 715–734. https://doi.org/10.1111/are.15616

Tran, N., Rodriguez, U.-P., Chan, C. Y., Phillips, M. J., Mohan, C. V., Henriksson, P. J. G., Koeshendrajana, S., Suri, S., & Hall, S. (2017). Indonesian aquaculture futures: an analysis of fish supply and demand in Indonesia to 2030 and role of aquaculture using the Asia Fish model. *Marine Policy*, *79*, 25–32. https://doi.org/https://doi.org/10.1016/j.marpol.2017.02.002

Valenzuela-Gutiérrez, R., Lago-Lestón, A., Vargas-Albores, F., Cicala, F., & Martínez-Porchas, M. (2021). Exploring the garlic (*Allium sativum*) properties for fish aquaculture. *Fish Physiology and Biochemistry*, *47*(4), 1179–1198. https://doi.org/10.1007/s10695-021-00952-7

Welker, T. L., Lim, C., Yildirim-Aksoy, M., & Klesius, P. H. (2012). Susceptibility of Nile tilapia (*Oreochromis niloticus*) fed with dietary sodium chloride to nitrite toxicity. *Aquaculture International*, *20*(1), 159–176. https://doi.org/10.1007/s10499-011-9449-5

Wongyai, N., Jutagate, A., Grudpan, C., & Jutagate, T. (2020). Condition index, reproduction and feeding of three non-obligatory riverine Mekong Cyprinids in different environments. *Tropical Life Sciences Research*, *31*(2), 159–173. https://doi.org/10.21315/tlsr2020.31.2.8

Zhang, D., Xu, D.-H., Shoemaker, C. A., & Beck, B. H. (2020). The severity of motile *Aeromonas septicemia* caused by virulent *Aeromonas hydrophila* in channel catfish is influenced by nutrients and microbes in water. *Aquaculture*, *519*, 734898. https://doi.org/https://doi.org/10.1016/j.aquaculture.2019.734898

