The effect of garlic (*Allium sativum*) supplementation in feed on the growth, survival and profits of Asian seabass (*Lates calcarifer*) cultivation reared in freshwater media

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Abstract. Asian seabass (*Lates calcarifer*) or barramundi is one of the top fish cultivation commodities in Asia, including in Indonesia. In Indonesia, Asian seabass is usually cultivated in the sea and brackish water. However, Asian seabass can also be cultivated in fresh water. To optimize the cultivation of Asian seabass, it is necessary to develop feed supplementation to boost Asian seabass' immune system by using immunostimulants which can reduce the fish mortality rate and increase the profitability. This study was performed to analyse the effect of giving garlic to fish feed on the growth, survival and profitability of the cultivation of Asian seabass reared in fresh water media. The experiment was carried out in 50 days using Asian seabass with an average weight of 4.35 g per fish. The study was designed using a completely randomized design. Fish were reared using fibre ponds with a water volume of 0.5 m^3 and filled with 50 fish per pond. In this study, five treatments with two replications for each treatment were performed. Different contents of garlic were used; 0% in treatment A as control, 0.5% in treatment B, 1.5% in treatment C, 3% in treatment D and 10% in treatment E. The water quality was measured weekly which included the measurements of dissolved oxygen (DO), temperature, salinity and pH. The results of this study indicated that all treatments had significant effect on the survival rate (SR), absolute biomass growth, specific growth rate (SGR), feed conversion ratio (FCR) and benefit cost ratio (BCR). Treatment B appeared to have the best outcomes, with an average SR of 78%, an average SGR of 2.30% per day, an average FCR of 1.44 and an average BCR of 3.88. Therefore, 0.93% garlic concentration is considered the most optimal concentration for SGR, while 0.82% garlic concentration is more optimal for SR.

Key Words: *Allium sativum*, BCR, FCR, SR, SGR, *Lates calcarifer*.

Introduction. Asian seabass (*Lates calcarifer*) or barramundi is one of the leading aquaculture commodities in Indonesia, even in Asia due to high demand and good price (Rajkumar et al 2006; Ghosh 2019; Wijayanto et al 2020a). In Indonesia, Asian seabass are usually reared in floating net cage in the sea and in brackish water ponds (WWF-Indonesia 2015). Asian seabass is a marine-euryhaline fish (Rajkumar et al 2006; Ghosh 2019). Hassan et al (2021) explained that Asian seabass can be reared at waters with salinity ranging from 0 to 56 per mile, including monoculture and polyculture. Likewise, Wijayanto et al (2020a) also proved that Asian seabass can be cultivated in freshwater media. Those results break the barrier that Asian seabass can only be cultivated in area close to the coast. As a follow-up study, it is necessary to increase the immunity of Asian seabass to reduce fish mortality. Immunostimulants can improve the health of fish, including Asian seabass for optimal growth (Xu et al 2020).

In Indonesia, there are many alternative local ingredients that can increase the immunity of cultured fish, including garlic (*Allium sativum*). Garlic contains natural antioxidants such as flavonoids (Yousefi et al 2020). Shang et al (2019) found that garlic contains several bioactive components with anti-inflammatory, anti-bacterial, and anti-fungal properties. The present study analysed the effect of adding garlic to fish feed on the growth, survival and profitability of Asian seabass reared in freshwater media.

Material and Method

Setting. The study was conducted in the laboratory of the Faculty of Fisheries and Marine Sciences, Universitas Diponegoro from December 2021 to January 2022. The experiment was carried out in 50 days.

Materials. The test fish were Asian seabass seedlings with an average size of 4.35 g per fish. Fish were reared in fibre ponds with 0.5 m^3 water and 50 fish per pond. Ten units of fibre pond were used in 5 treatments and 2 replications per treatment.

Feed treatment. The feeding contained 4% of fish biomass (Wijayanto et al 2022) using commercial feed specifically for Asian seabass, containing a minimum of 52% crude protein, 14.5% fat, 3% fibre, and a maximum of 10% moisture. The feed was enriched with grated garlic with different doses to be soaked into the commercial feed. Then, the test feed was aerated to dry. Different doses of garlic were applied; 0% in treatment A as the control group, 0.5% in treatment B, 1.5% in treatment C, 3% in treatment D and 10% in treatment E. Experiments were performed in a completely randomized design, where each pond was not related to another.

Water quality measurement. To maintain water quality, the water was recirculated using filters consisting of charcoal, coral, sand, and synthetic fibres. The water was changed every 10 days by replacing 30% of the water. Water quality measurements were performed weekly, which included measurements on the dissolved oxygen (DO), temperature, salinity, and pH using a multi-parameter water quality meter (Horiba U-50).

Data analysis. Fish weight was measured every 10 days. Several formulae as proposed by (Ali et al 2016; Hassan et al 2021; Chowdhury et al 2021; Wijayanto et al 2020a; Wijayanto et al 2020b) were used as follows:

$$W = Wt - Wo \quad [1]$$

$$SGR = (Ln\ Wt - Ln\ Wo) / t \quad [2]$$

$$FCR = F / W \quad [3]$$

$$BC = B / C \quad [4]$$

where: W is the absolute growth of Asian seabass biomass (g); Wt is the biomass of Asian seabass in the end of the experiment (t days); Wo is the initial biomass of Asian seabass (g); SGR is the specific growth rate of fish (% day^{-1}); FCR is feed conversion ratio; F is the accumulation of feed fed to Asian seabass (g); B is additional income gained from more optimal Asian seabass growth (IDR); C is the cost for feeding (IDR). The cultivation of Asian seabass is categorized profitable if the BC ratio is greater than 1.0. Analysis of variance and Duncan's tests were carried out to statistically analyse the data.

Results. The results showed that treatment B had the best performance seen from variables SR, W, SGR, FCR and BCR (Table 1). This shows that the results of statistical analysis indicates a significant effect from the treatment of variables SR, W, SGR, FCR and BCR (see Table 2).

Treatment B produced the most optimal SR, W, SGR, FCR and BCR compared to other treatments. Treatment E with the highest garlic concentration resulted in the lowest performance. It implies that the garlic should be added with balance concentration. Excessive dose will actually harm the fish. Treatment B produced a more significant performance compared to control (treatment A) seen from variables W, SR and SGR which indicated that the addition of garlic could improve the business performance of fish farmers. The modelling results (Figures 1 and 2) showed that the garlic concentration of 0.93% was optimal for SGR and 0.82% was optimal for SR. The values were obtained by the derivative of the SGR and SR equations with respect to T equal to zero (dSGR/dT = 0 and dSR/dT = 0).

Table 1

SR, W, SGR, FCR and BCR of Asian seabass

Variables	*A (0%)*		*B (0.5%)*		*C (1.5%)*		*D (3%)*		*E (10%)*	
	A_1	*A_2*	*B_1*	*B_2*	*C_1*	*C_2*	*D_1*	*D_2*	*E_1*	*E_2*
SR (%)	56	58	76	80	62	68	60	60	60	56
Average of SR (%)	57		78		65		60		63	
W_0 (g)	251.83	213.16	216.82	216.56	215.15	211.9	214.14	213.54	212.12	212.1
W_t (g)	544.41	576.22	705.34	665.69	596.81	679.29	567.22	521.30	404.43	420.85
W (g)	292.58	363.06	488.52	449.13	381.66	467.39	353.08	307.76	192.31	208.75
Average of W (g)	327.82		468.82		424.53		330.42		200.53	
SGR (% day^{-1})	1.54	1.99	2.36	2.25	2.04	2.33	1.95	1.79	1.29	1.37
Average of SGR (% day^{-1})	1.77		2.30		2.19		1.87		1.33	
FCR	1.99	1.60	1.43	1.45	1.61	1.41	1.64	1.80	2.63	2.44
Average of FCR	1.79		1.44		1.51		1.72		2.54	
BCR	2.82	3.50	3.91	3.85	3.42	3.90	3.31	3.02	1.93	2.09
Average of BCR	3.16		3.88		3.66		3.16		2.01	

Table 2

Statistical analysis

Variables	*F*	*Sig*	*Duncan test*
W	13.243	0.007**	$E^a < A^b < D^b < C^{b,c} < B^c$
SR	20.639	0.003**	$E^a < A^{a,b} < D^{a,b} < C^b < B^c$
FCR	14.956	0.005**	$E^a > A^b > D^b > C^b > B^b$
SGR	8.903	0.017*	$E^a < A^{a,b} < D^{b,c} < C^{b,c} < B^c$
BCR	12.962	0.008**	$E^a < A^b < D^b < C^b < B^b$

Notes: * means that the treatment has a significant effect with = 5%; ** iIt means that the treatment has a significant effect with = 1%; a, b, and c represent subset groups.

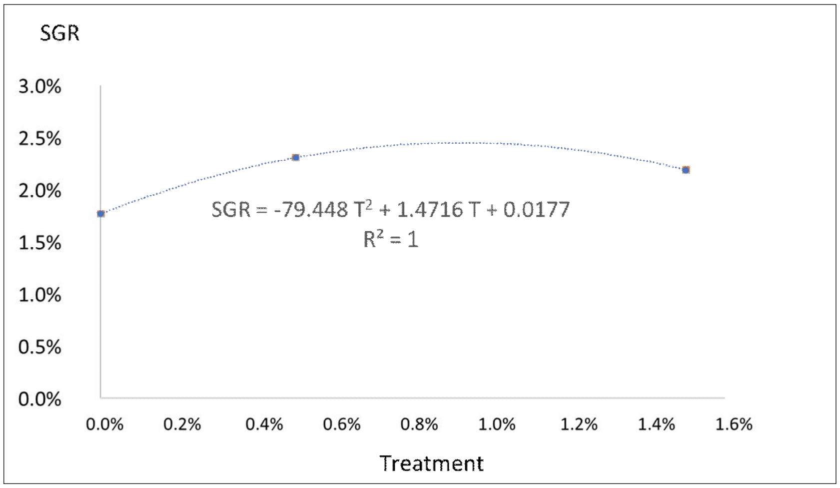

Figure 1. The relation of SGR and garlic supplementation treatment.

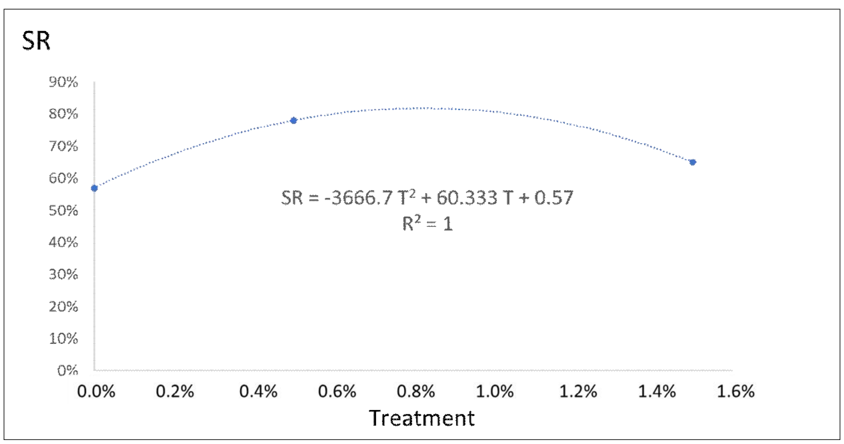

Figure 2. The relation of SR and garlic supplementation treatment.

The modelling results revealed a significant relationship between SR and SGR and W. Higher SR is followed by higher SGR and W values despite R^2 value is moderate:

$$W = -250.334 + 944.585\ SR\ [R^2 = 60\%]$$

$$SGR = -0.003 + 0.035\ SR\ [R^2 = 56\%]$$

Meanwhile, the results of water quality measurement are presented in Table 3. In general, water pH, DO, and temperature relatively supported the Asian seabass cultivation. The salinity was made equal to 0.5 ppt. The present study has proven that Asian seabass can be kept at water with low salinity of 0.5 ppt (fresh water), allowing fish farmers who live far from coastal areas to gain profit from Asian seabass cultivation, even though Wijayanto et al (2020a) stated that a salinity of 5 ppt can provide the optimal results.

Table 3

Water quality of research media

Variables	A (0%)		B (0.5%)		C (1.5%)		D (3%)		E (10%)	
	A_1	A_2	B_1	B_2	C_1	C_2	D_1	D_2	E_1	E_2
Morning										
pH	7.72±0.14	7.59±0.04	7.55±0.11	7.56±0.09	7.58±0.07	7.57±0.05	7.60±0.05	7.52±0.06	7.51±0.06	7.52±0.03
Temperature (°C)	25.53±0.68	25.15±0.56	24.93±0.57	24.85±0.52	24.78±0.56	24.83±0.54	24.80±0.50	24.80±0.45	24.80±0.47	24.85±0.44
DO (ppm)	4.53±0.96	4.38±0.98	4.48±1.10	4.45±0.87	4.40±0.81	4.43±0.84	4.40±0.81	4.40±0.81	4.40±0.81	4.38±0.78
Salinity (ppt)	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0
Afternoon										
pH	7.60±0.15	7.52±0.03	7.49±0.12	7.48±0.11	7.56±0.11	7.49±0.18	7.49±0.13	7.42±0.04	7.44±0.13	7.50±0.07
Temperature (°C)	27.25±1.41	26.43±1.08	26.10±1.04	25.78±0.95	25.58±0.75	25.60±0.76	25.55±0.77	25.53±0.78	25.45±0.70	25.43±0.75
DO (ppm)	5.50±0.50	4.93±0.51	5.23±0.45	5.15±0.54	5.00±0.43	5.13±0.51	5.00±0.50	5.03±0.51	4.95±0.45	4.95±0.39
Salinity (ppt)	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0	0.50±0.0

Discussion. Hassan et al (2021) explained that feeding regime is one of the essential factors in the development of Asian seabass aquaculture because it affects fish growth, feeding effectiveness and fish survival. According to Wijayanto et al (2022), feeding Asian seabass with 3.95% fish biomass per day can produce an optimal SGR. Feed engineering has also been developed by researchers to maintain fish health. The use of additives in fish feed to improve the immune system of fish has been increasingly investigated since the Food and Drug Administration (FDA) banned the use of antibiotics as feed supplements in 2017, including research on the use of garlic containing sulfur. Sulfur is an important element for animal growth. Compounds containing sulfur have anti-oxidant and anti-bacterial effects (Park et al 2021).

The results of this study indicate that the addition of garlic to Asian seabass feed significantly affects the SR, W, SGR, FCR and BC Ratios. Using garlic at optimal concentrations boosts fish health which in turn affects the SR. Fish health also positively affects the fish growth and optimal feed conversion. Furthermore, fish growth, SR and FCR affect the profitability of Asian seabass culture (Wijayanto et al 2020a; Wijayanto et al 2022). There are several benefits of adding garlic into fish feed as it offers anti-oxidant, anti-inflammatory, anti-bacterial, anti-fungal, anti-parasitic, immunomodulatory, anti-cancer properties while it improves the digestive system (Talpur & Ikhwanuddin 2012; Asimi et al 2013; Yang et al 2015; Shang et al 2019; Stipanuk 2020; Yousefi et al 2020; Xu et al 2020; Chang et al 2021; Chowdhury et al 2021). Talpur & Ikhwanuddin (2012) proved that feed added with garlic with a dose of 10 g kg^{-1} significantly increased the survival of Asian seabass to 83.35% compared to control (33.3%) where Asian seabass became more resistant against *Vibrio harveyi* infection. The results of Yousefi et al's (2020) study proved that garlic supplementation of 1 and 1.5% was able to significantly reduce stress, increase plasma antioxidants and immunological parameters of carp. The addition of garlic also applies in chicken farms (Chang et al 2021).

Garlic has been widely used as traditional medicine for humans in China, India and Indonesia. Garlic contains several bioactive components (including allicin, alliin, diallyl sulfide, diallyl disulfide, diallyl trisulfide, ajoene, and S-allyl-cysteine) with antioxidant, anti-inflammatory, anti-bacterial, anti-fungal, and anti-cancer properties (Shang et al 2019). Garlic also contains inulin (a natural polysaccharide) that boosts the health, immune function and growth in fish, including Asian seabass (Roberfroid 2005; Reza et al 2009; Ringo et al 2010; Ali et al 2016). Inulin can also improve the immunity of cultured vanamei shrimp (Partida-Arangure et al 2013). In addition, garlic also improves the phagocytic, respiratory, lysozyme, anti-protease and bactericidal activities. Garlic supplementation enhances the levels of serum protein, albumin and globulin, while it reduces serum glucose, lipids, triglycerides and cholesterol in the fish group (Talpur & Ikhwanuddin 2012). Yousefi et al (2020) found that the supplementation of garlic significantly reduced plasma glucose, cortisol and malondialdehyde, alanine transaminase, alkaline phosphatase, aspartate transaminase and glutathione peroxidase, as well as increasing plasma catalase, lysozyme, alternative complement and bactericidal activity, and immunoglobulin levels in common carp (*Cyprinus carpio*). Stress among fish can also be reduced through garlic supplementation. Garlic supplementation of 1% and 1.5% can improve the plasma antioxidants and immunological parameters of carp. Chowdhury et al (2021) also added garlic to fish feed to improve growth, digestion, metabolism, antioxidant enzyme activity and the health status of *Labeo rohita* fry successfully. It was found by Asimi et al (2013) that the combination of phytogenic additives such as turmeric, ginger and garlic can increase the immune system of fish. Some of these additives have pharmacological properties that improve the digestion and metabolism systems which also supply antioxidants that can improve the immunity of both humans and fish. According to Xu et al (2020), garlic can promote growth, stimulate digestion, improve antioxidant function, and hemato-immunology of fish. Some prior studies also confirmed that the addition of garlic can increase the growth of several types of fish, including *Acipenser ruthenus* (Lee et al 2014), *Oncorhynchus mykiss* (Büyükdeveci et al 2018), and *Perca fluviatilis* (Zare et al 2021).

Despite the effectiveness of garlic addition in improving the health of Asian seabass, excessive dose of garlic brings the opposite effect. The treatment E (10%) in this study actually resulted in lower growth than treatment A as control (0%), while the modeling results show that the optimal concentration of garlic is 0.82% for SR and 0.93% for SGR. Although sulfur has anti-biotic, anti-oxidant and anti-bacterial properties, it can also be toxic at high concentrations. Thus, it is necessary to determine the optimal content of garlic in the fish feed to exert a positive effect on the immune response (Kim et al 2015; Park et al 2021). Sulfur (S) is an essential element for the growth of most animals including humans, and it contains mutual compounds found in animal cells. Sulfur compounds have antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. Several studies have proven that sulfur biomolecules in plants and animals play an important role in boosting the immune system (Yang et al 2015; Stipanuk 2020). Blood plasma from cattle fed sulfur-containing feed increases the ratio of polyunsaturated fatty acids to saturated fatty acids (Lee et al 2009; Shin et al 2013). Therefore, sulfur needs to be processed to remove its toxic properties for medicinal use and its optimal amount in food must be determined to exert a positive effect on immunity. Sulfur can be processed through hot smelting of sulfur minerals followed by liquid separation and cooling (Lee et al 2010).

During the experiment, the water quality of the rearing media was relatively supportive for the cultivation of Asian seabass. As stated by WWF Indonesia (2015), the optimal water for Asian seabass cultivation has pH ranging from 7 to 8.5, temperature of 27 to 30°C, and DO greater than 4 ppm. Yudhiyanto et al (2017) also found the optimal water conditions for Asian seabass cultivation had pH from 7 to 8.2, temperature between 26 to 32°C, and DO between 4 to 8 ppm. The growth of aquatic organisms in aquaculture is influenced by many complex factors, including water quality (Wijayanto et al 2020b). Ammonia poisoning is a very serious threat to aquatic organisms. Ammonia toxicity causes growth degradation and decreased fish health. The addition of garlic has also been shown to increase fish immunity and reduce fish stress levels due to poor environmental factors, including high ammonia content in culture media (Yousefi et al 2020).

Conclusions. The results of this study indicate garlic supplementation significantly affected the growth (SGR and W), survival, FCR and BC ratios of Asian seabass reared in freshwater media. Garlic supplementation with a concentration of 0.5% showed the most optimal results, with SR of 78%, SGR of 2.3% per day, FCR of 1.44 and BCR of 3.88. The modelling results showed that the garlic concentration of 0.93% was optimal for SGR, and a concentration of 0.82% was optimal for SR.

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Conflict of interest. The authors declare that there is no conflict of interest.

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