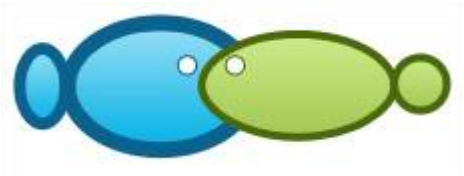

The effect of garlic supplementation on the growth, survival and RCR in cultured TGGG hybrid grouper (♀ tiger grouper × ♂ giant grouper)

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Abstract. This research investigated the impact of supplementing fish feed with garlic on the growth, survival, feed efficiency and profitability in TGGG hybrid grouper culture reared in low salinity media. This research was conducted for 40 days using fish fingerlings with an average weight of 4.34 g (±0.14 g) reared in tanks with water volume 0.4 m^3 containing 28 fish per tank. The experiment consisted of 3 treatments and 3 repetitions; treatment A with 0% garlic supplementation, treatment B with 1% garlic supplementation, and treatment C with 2% garlic supplementation. Multiple parameters were assessed; biomass growth (WGR), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), and revenue cost ratio (RCR). The results showed that treating garlic in feed with the right dosage could improve the performance of TGGG hybrid grouper culture. Treatment A demonstrated superior growth (WGR and SGR) in comparison to treatments B and C. Treatment B exhibited the most favorable outcomes in terms of FCR and RCR. The results of treatment optimization modelling show that the optimal doses are 0.30% for SGR, 0.35% for WGR, 0.59% for FCR, and 0.65% for RCR.
Key Words: *Allium sativum*, FCR, growth, RCR, SR, TGGG hybrid grouper.

Introduction. The cultivation of TGGG hybrid grouper (♀ tiger grouper, *Epinephelus fuscoguttatus* × ♂ giant grouper, *Epinephelus lanceolatus*) has been developing in Indonesia since 2017. Accelerated growth and higher body resistance compared to tiger grouper and humpback grouper (*Chromileptes altivelis*) have prompted fish farmers to transition their focus towards TTGG hybrid grouper cultivation. Swift fish growth guarantees quicker capital turnover (Myoung et al 2013; Koh et al 2016; DJPB 2017; Bulanin et al 2017; Chieng et al 2018; Long et al 2022; Fadli et al 2022). The trajectory of grouper production fluctuated with 14,140 tons in 2015, then 16,141 tons in 2018, and 9,478 tons in 2020. During the Covid 19 pandemic, downturn in global demand for grouper and logistical challenges due to disrupted exports occurred. Grouper fish farming locations in Indonesia are found in many islands, including Sumatra, Riau Islands, Java, Southeast Islands, Maluku, and Bali (DJPB 2017; KKP 2020, 2022).

TGGG hybrid grouper cultivation in Indonesia remains primarily accessible to coastal fish cultivators. The predominant source of grouper aquaculture in Indonesia comes from marine cultivation, contributing to 6,886 tons in 2020 (KKP 2022). Research on the cultivation of TGGG hybrid grouper at low salinity has been developed, with an optimal salinity of 7-8 ppt. This development is expected to enable inland fish farmers to engage in grouper cultivation, capitalizing on the species' lucrative market value (Wijayanto et al 2023a, b). Enhancing the survival and growth rates of TGGG hybrid grouper stands as a pivotal factor for elevating production efficiency and profitability. The prohibition of antibiotic use in fish farming since 2017 (US-FDA 2018) has prompted exploration into alternative methods of boosting fish immune responses, including the use of garlic (*Allium sativum*). Garlic contains natural antioxidants such as flavonoids

while the bioactive components in garlic can act as anti-inflammatory, anti-bacterial and anti-fungal. Garlic in Asia (including Indonesia) is used as a cooking spice and herbal medicine (Shang et al 2019; Yousefi et al 2020; Wijayanto et al 2022). The purpose of this study was to assess the effect of immersing garlic liquid in fish feed on growth, survival, feed efficiency and profitability in TGGG hybrid grouper culture reared in low salinity media.

Material and Method

Location of research. This research was conducted at the Laboratory of the Faculty of Fisheries and Marine Sciences, Universitas Diponegoro (Semarang City, Indonesia).

Time of research. This experimental research was conducted for 40 days from December 2022 to January 2023.

Fish seeds. The TGGG hybrid grouper fingerlings used in this research had an average size of 4.34 g (±0.14 g) and were obtained from marine fish hatcheries in Situbondo Regency (565 km from Semarang City).

Experimental media. Fish were reared in 0.4 m^3 water tanks (100 x 100 x 40 cm) filled with 28 individuals per tank. The salinity of the water was gradually reduced from 31 ppt to 5 ppt by adding seawater (initial medium) with fresh water.

Water recirculation. Water recirculation system was employed as the water management method using cotton cloth, synthetic polyester fiber, gravel, and commercial bio-ball as the filters. Fish waste collection was carried out daily. Water quality parameters that included dissolved oxygen (DO), pH, salinity and temperature were measured every 10 days using Horiba U-50.

Test feed. The test fish were fed commercial feed with a minimum crude protein content of 46%. Fish were given 4% of fish biomass per day in the morning, afternoon, and evening. This completely randomized design experiment was conducted using 3 treatments with 3 replications. Fortified feed supplemented with garlic was administered in the treatment, in the form of liquid garlic; 0% (treatment A), 1% (treatment B), and 2% (treatment C). Garlics were crushed and soaked in water. The liquid was then used to soak commercial feed before being aerated to dry.

Data analysis. The progress of fish weight was measured every 10 days. The variables studied in this research include fish growth (WGR or weight growth rate, and SGR or specific growth rate), survival rate (SR), feed convertion ratio (FCR), and revenue cost ratio (RCR) using the following formula (Mapenzi & Mmochi 2016; Long et al 2022; Wijayanto et al 2023a, b):

$$WGR\ (\%) = [(Wt - Wo)/Wo] \times 100\% \quad [1]$$

$$SGR\ (\%) = [(Ln\ Wt - Ln\ Wo) / t] \times 100 \quad [2]$$

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

$$SR\ (\%) = (Nt/No) \times 100 \quad [4]$$

$$RCR = R / C \quad [5]$$

WGR is the rate of weight gain of groupers in %; Wt is the final weight of the grouper in g units on day t; Wo is the initial weight of the grouper in g; SGR is the specific growth rate of grouper in % day^{-1}; Ln is the natural logarithm; FCR is the feed conversion ratio; F is the accumulation of feed in g; SR is the survival rate in %; Nt is the final number of groupers on day t; No is the initial number of groupers; RCR is the revenue cost ratio; R is additional revenue due to growth of grouper fish (IDR); C is the cost of feed (IDR).

Anova test and Duncan's test were conducted as statistical analysis to identify any significant effect on the study variables. The treatment optimization model employed the first derivative procedure.

Results. During the experiment, the fish showed a strong appetite. When given food, the fish eagerly surged to the water's surface, where those from below seized the feed before retreating to the depths. The gradual decrease in salinity from the initial experiment of 31 ppt to 5 ppt did not decrease the appetite of the TGGG hybrid grouper. Detailed results are presented in Table 1 and Figure 1.

Table 1

Research results

Variables	*Treatment A*			*Treatment B*			*Treatment C*		
	A1	*A2*	*A3*	*B1*	*B2*	*B3*	*C1*	*C2*	*C3*
W_o (g)	122.79	120.50	116.19	119.49	129.28	123.58	119.52	120.23	121.80
W_t (g)	618.15	571.07	572.93	617.42	652.32	537.18	552.46	531.48	537.3
Average W_o (g ind^{-1})	4.39	4.30	4.15	4.27	4.62	4.41	4.27	4.29	4.35
Average Wt (g ind^{-1})	22.08	20.40	20.46	22.05	23.30	19.90	19.73	18.98	19.19
N_o (ind)	28	28	28	28	28	28	28	28	28
N_t (ind)	28	28	28	28	28	27	28	28	28
WGR (%)	403.4	373.9	393.1	416.7	404.6	334.7	362.2	342.1	341.1
SGR (%)	3.9	3.8	3.9	4.0	3.9	3.6	3.7	3.6	3.6
SR (%)	100.0	100.0	100.0	100.0	100.0	96.4	100.0	100.0	100.0
FCR	1.051	1.116	1.079	1.007	1.015	1.201	1.108	1.178	1.162
RCR	3.76	3.55	3.67	3.93	3.90	3.30	3.57	3.36	3.41

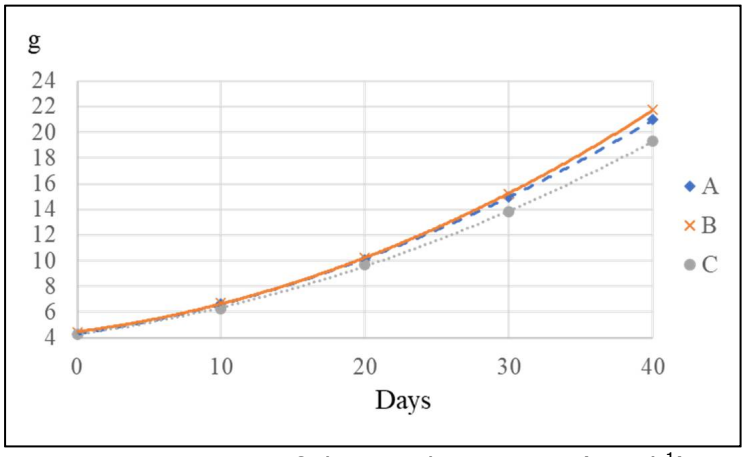

Figure 1. Average fish growth progress (g ind^{-1}).

At the start of the experiment, the average fish weight between treatments was relatively comparable. As the fish matured, a discernible trend emerged: treatments A and B exhibited superior growth compared to treatment C. The statistical analysis results, showcased in Table 2, revealed no significant disparities across treatments for the WGR, SGR, FCR, and RCR variables. An extended study duration might unveil more pronounced treatment effects. The SR in all tanks was consistently high at 100%, except treatment tank B at the 3rd replication (B3). This suggests that feed treatments had negligible impact on the SR variable. In general, treatment A showed the best performance for fish growth for WGR and SGR variables. Meanwhile, the best performance of treatment B was found on FCR and RCR variables. The results of treatment optimization modeling are shown in Figure 2 and Table 3.

The results of the optimization process indicate that the treatment's optimal concentration lies within the range of 0.3% to 0.65% found between treatment A and treatment B. Treatment C at a dose of 2% was capable of reducing the performance of fish farming. Consequently, while garlic holds promise as an immunostimulant ingredient, an excessive dose may impede fish growth. Table 4 outlines the water quality conditions during the study.

Table 2

Statistical analysis

Variables	*Average value*			*F value*	*Sig value*	*Note**
	A	*B*	*C*			
WGR (%)	390.1	385.3	348.5	2.010	0.215	Not significant
SGR (% day^{-1})	3.9	3.8	3.7	1.996	0.217	Not significant
FCR	1.08	1.07	1.15	0.885	0.460	Not significant
RCR	3.66	3.71	3.45	0.885	0.460	Not significant

Note: * at α = 5%.

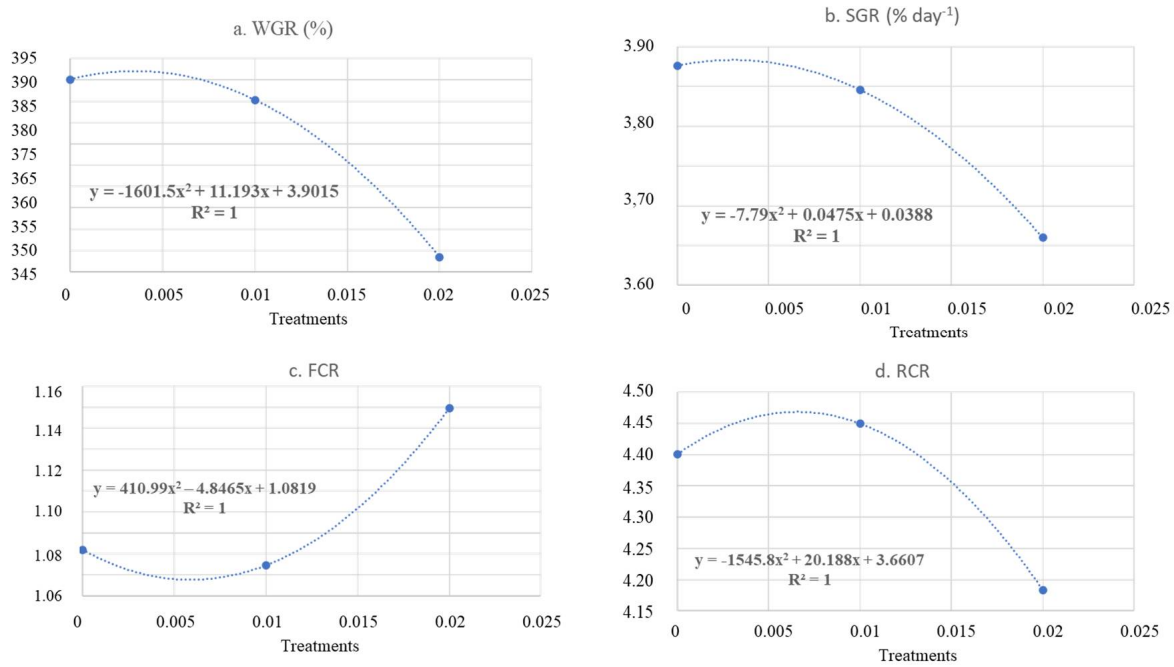

Figure 2. WGR, SGR, FCR and RCR treatment optimization modelling.

Table 3

Optimal treatment estimation

Variables	*Optimal concentration*	*Value estimation*
WGR	0.35%	392.1%
SGR	0.30%	3.9% day^{-1}
FCR	0.59%	1.068
RCR	0.65%	3.73

Table 4

Water quality parameters during the study

Variables	*Treatment A*			*Treatment B*			*Treatment C*		
	A_1	A_2	*A3*	B_1	B_2	B_3	C_1	C_2	C_3
pH	7.8± 0.14	7.9± 0.08	7.9± 0.10	7.8± 0.14	7.8± 0.08	7.8± 0.10	7.8± 0.13	7.8± 0.10	7.8± 0.13
DO (ppm)	5.9± 0.69	5.8± 0.41	5.6± 0.80	5.6± 0.41	5.5± 0.51	5.7± 0.41	5.4± 0.60	5.1± 0.61	5.3± 0.80
T (^{o}C)	24.9± 0.90	25.0± 1.00	25.0± 1.01	24.9± 0.91	24.9± 0.90	24.9± 0.87	25.0± 0.93	25.0± 0.86	25.0± 0.87
IS (ppt)	31	31	31	31	31	31	31	31	31
FS (ppt)	5	5	5	5	5	5	5	5	5

Note: T = temperature; IS = initial salinity; FS = final salinity.

The quality of the water in the experimental media still supports the growth and survival of the TGGG hybrid grouper, particularly in terms of pH and DO levels. Herry et al (2019) stated that the optimal aquatic environment for grouper culture is at a temperature of 29-30^{o}C, pH of 6.5-8.5, and DO of more than 5 ppm. The temperature during the experiment was around 25^{o}C due to the controlled indoor laboratory setting in Indonesia's tropical climate. Despite being slightly below the optimum temperature, the tested fish exhibited robust appetites. The gradual reduction in salinity from 31 ppt to 5 ppt for 40 days of experiment did not decrease the fish appetite. Although grouper is a seawater fish, experiments by Wijayanto et al (2023b) showed that the optimal salinity for cultivating TGGG hybrid grouper is 7-8 ppt.

Discussion. The TGGG hybrid grouper in Indonesia is known as the 'cantang' grouper which is an abbreviation of 'macan' (meaning tiger) grouper and 'kertang' grouper (giant grouper). Among the Serranidae family inhabiting tropical and subtropical waters, including Indonesian waters, the giant grouper (*E. lanceolatus*) stands as the largest species (Myoung et al 2013). Hybridization in fish farming practices is mostly done to produce cultivars with high growth, high resistance, improve meat quality, and higher business profits. The practice of hybridizing TGGG grouper began in 2006 (DJPB 2017; Shapawi et al 2019; Tan 2021; Long et al 2022). Before the TGGG hybrid grouper cultivation developed, the tiger grouper stood as the predominant species raised by Indonesian fish farmers considering the seed availability, selling price, growth, and durability. However, TGGG hybrid grouper is considered to have faster growth than tiger grouper which then made many fish farmers switched to TGGG hybrid grouper.

Fish growth, SR, feed efficiency (FCR) and profit are major consideration among fish farmers. Profit has been the biggest motivation among fish cultivators to cultivate grouper. Meanwhile, growth and SR can influence production which then affect the selling prices and revenue. FCR affects costs which have the largest proportion of variable costs in intensive fish farming. Furthermore, income and costs affect profits (Wijayanto et al 2022, 2023a, 2023b).

The average FCR for treatment B in this investigation stood at 1.07. This implies that a weight gain of 1 gram necessitates the utilization of 1.07 grams of artificial feed. Feed management is crucial as cost the highest among others of around 85.5% (Dennis 2021). The average RCR value for treatment B in this study was 3.71, indicating that every IDR 1 expenditure generates revenue of IDR 3.71. According to DJPB (2017), the RCR of TGGG hybrid grouper cultivation in floating net cages in marine waters was around 1.4. Owners of grouper fish cage enterprises are tasked with effectively overseeing their workforce to ensure efficient feed supply. Notably, not all dispensed fish feed is consumed by the groupers, leading to residual feed contaminating the water and incurring inefficiencies in costs. Water pollution can cause an increase in disease attacks in fish. Leftover feed and faeces can increase the ammonia content in the waters which can lead to fatal ammonia poisoning. Ammonia toxicity causes growth degradation and decreases fish health (Yousefi et al 2020).

The findings of this study underscore that the application of garlic treatment can enhance the performance of the TGGG hybrid grouper cultivation enterprise, provided it is administered at the appropriate dosage. This improvement manifests across various critical aspects, encompassing fish growth (WGR and SGR), feed efficiency (FCR), and the RCR. A dose of 0.65% garlic leads to an estimated RCR value of 3.73, thereby every expenditure of IDR 1 can generates income of IDR 3.73. Garlic contains sulphur which is known as an essential element for animal growth which also has anti-oxidant and anti-bacterial properties that can enhance fish immunity (Park et al 2021). Numerous investigations have corroborated the diverse benefits of garlic, including its role as an antioxidant, anti-inflammatory, antibacterial, antifungal, antiparasitic, immunomodulatory, and potentially anticancer agent, while also aiding digestion (Talpur & Ikhwanuddin 2012; Yang et al 2015; Asimi et al 2013; Shang et al 2019; Stipanuk 2020; Yousefi et al 2020; Xu et al 2020; Chang et al 2021; Chowdhury et al 2021). Garlic is a good herbal medicine for human and fish. Higher resistance against diseases

can affect fish survival, while the energy from feed can be optimized for fish growth, which in turn has a positive impact on fish farmer production and profits.

The utilization of garlic in fish feed has been explored across various fish species, including barramundi (*Lates calcarifer*), common carp (*Cyprinus carpio*), *Labeo rohita, Acipenser ruthenus, Oncorhynchus mykiss*, and *Perca fluviatilis*. At accurate doses, garlic supplementation can elevate serum protein levels and antioxidants, enhance metabolic processes and digestion, as well as bolster fish endurance and growth. Notably, while sulfur functions as an antibiotic, antioxidant, and antibacterial agent, its concentration must be carefully regulated, as excessive levels can prove toxic to fish (Lee et al 2014; Kim et al 2015; Büyükdeveci et al 2018; Zare et al 2021; Chowdhury et al 2021; Park et al 2021; Wijayanto et al 2022).

The water salinity at the end of the experiment was 5 ppt, indicating a strong potential to the development of TGGG hybrid grouper aquaculture away from the coast. Indonesian fish cultivators residing inland often engage in the cultivation of tilapia, catfish, and carp, typically fetching prices below USD 1.9 per kilogram. Whereas, the price of TGGG hybrid grouper can reach USD 6.34 per kilogram, even far higher for TGGG grouper with ornamental features (Wijayanto et al 2022). The hybrid grouper produced by TGGG from Indonesia has been exported to Japan, Hong Kong, China, Malaysia, Singapore, and Taiwan (DJPB 2017). The success of grouper fish farming is also expected to reduce the pressure that exists on the exploitation of grouper fish in nature. Giant grouper and tiger grouper are both under the IUCN Red List of Threatened Species with a vulnerable status (Fennessy et al 2018; Rhodes et al 2018).

Conclusions. The findings of the study underscore the potential enhancement of the TGGG hybrid grouper cultivation enterprise through the judicious inclusion of garlic in feed, provided the correct dosage is employed. This improvement is evident across critical domains encompassing growth, feed efficiency, and profitability. While treatment A exhibited superior growth (WGR and SGR) compared to treatments B and C, statistical significance was not achieved at the α = 5% level. Notably, treatment B demonstrated optimal performance in terms of FCR and RCR, albeit without achieving statistical significance at the α = 5% threshold. The outcomes of the treatment optimization model identify the optimal dosages as follows: 0.30% for SGR, 0.35% for WGR, 0.59% for FCR, and 0.65% for RCR.

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

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