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Evaluation of Production Performance and *Vibrio spp.* Population Dynamics in Cantang Grouper at Different Stocking Densities in a Recirculating Aquaculture System

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Abstract. Cantang grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) is a hybrid fish with high economic value that is widely cultivated intensively using the Recirculating Aquaculture System (RAS). This study aims to (1) evaluate the production performance of cantang grouper at three levels of stocking density, (2) document the population dynamics of presumptive Vibrio spp., and (3) analyze the relationship between Vibrio spp. density and fish growth. The study used a 120-day comparative case study design with three stocking density treatments: 30 heads/m³ (Tank 1), 50 heads/m³ (Tank 2), and 70 heads/m³ (Tank 3). Production performance was evaluated based on specific growth rate and survival rate, while presumptive Vibrio spp. was determined using Thiosulfate Citrate Bile Salts Sucrose media. The relationship between Vibrio spp. density and SGR were analyzed using the Pearson correlation test. The results showed that the best production performance was obtained at a stocking density of 30 heads/m³, while the lowest performance was observed at 70 heads/m³. The proportion of presumptive Vibrio spp. increases with stocking density, from 0.85% in Tank 1 to 2.15% in Tank 3. However, no significant association was found between presumptive *Vibrio spp*. density and decreased SGR ($r = -0.185$; $p = 0.633$; $N = 9$). Decreased production performance at high stocking density is thought to be related to space competition, physiological stress, and accumulation of toxic metabolites, although these mechanisms were not directly measured. Thus, stocking density is the main factor associated with the production performance of cantang grouper in the RAS system, while the increase in the population of presumptive Vibrio spp. plays a secondary role as an indicator of a decline in the quality of the cultivation environment.

Keywords: cantang grouper, production performance, RAS, stocking density, *Vibrio spp*.

I. INTRODUCTION

Aquaculture is a rapidly growing sector of global food production, driven by demand for high-quality seafood. Among the most economically significant species in Indonesia is the cantang grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*), a hybrid resulting from the crossbreeding of tiger grouper (*E. fuscoguttatus*) and giant grouper (*E. lanceolatus*). This hybrid exhibits hybrid vigor, including accelerated growth, improved disease resistance, and greater environmental tolerance compared to its parent species [1]. Its market extends beyond domestic markets to international consumers, making it a flagship commodity in Indonesian marine aquaculture [2].

The Recirculating Aquaculture System (RAS) represents an innovative closed-loop approach in which water is continuously filtered and reused. RAS enables intensive fish cultivation with reduced environmental impact, controlled water quality, and high land-use efficiency [3]. In Indonesia, RAS adoption has grown considerably for high-value species such as the cantang grouper. However, intensive RAS culture at high stocking densities poses challenges, including spatial competition, physiological stress, and the accumulation of metabolic waste products such as ammonia, all of which may compromise fish performance [4].

The negative effect of high stocking density on growth, feed conversion efficiency, and survival has been documented across diverse fish species in RAS, with lower densities consistently yielding better zootechnical outcomes [5]. Larval health management in cantang grouper hatcheries, including feed management, disease control, and water quality maintenance, has been identified as a critical determinant of production success in Indonesian aquaculture facilities [6]. RAS technology has also been developed locally by Indonesian researchers, with the integration of nanobubble systems showing the best grouper nursery production performance at 600 fish/m³, demonstrating the active innovation occurring in the domestic aquaculture sector [7].

Among the biological indicators of RAS environmental quality, *Vibrio spp*. bacteria are of particular significance. Several species, including *V. harveyi*, *V. alginolyticus*, and *V. vulnificus,* are known opportunistic pathogens associated with *Vibrio*sis in grouper, causing skin lesions, hemorrhage, reduced appetite, and, in severe cases, mass mortality [8]. Their population dynamics within RAS are closely tied to environmental conditions, particularly organic matter accumulation driven by stocking density.

In Indonesia specifically, pathogenic bacteria, including *Vibrio spp*. isolated from cantang hybrid grouper farms have demonstrated multi-drug antibiotic resistance, highlighting the urgency of developing non-antibiotic biosecurity strategies [9]. Experimental infection studies from East Java, Indonesia, further confirmed that both *V. alginolyticus* and *V. harveyi* induce specific receptor organ responses and adhesion mechanisms in humpback grouper, indicating the need for species-specific understanding of pathogen-host interactions [10]. Moreover, studies conducted in floating net cage systems in the Kepulauan Seribu, Indonesia, detected *Vibrio vulnificus*, *V. alginolyticus*, and *V. parahaemolyticus* in cantang grouper, indicating potential cross-transmission between wild fish and farmed fish and associated histopathological damage to liver and kidney [11].

Despite a growing body of research on the effects of stocking density or Vibrio pathogenesis in isolation, comprehensive studies examining the interrelationship between stocking density and *Vibrio spp*. population dynamics, and production performance of the cantang grouper in RAS remain scarce [12]. To date, no study has simultaneously evaluated production performance and *Vibrio spp*. population dynamics of cantang grouper under commercial-scale RAS conditions across multiple stocking densities. Previous studies have addressed either density effects on growth or *Vibrio* pathogenesis in isolation, but the interrelationship between these factors in an operating commercial RAS remains poorly characterized. This knowledge gap limits the development of evidence-based stocking and biosecurity protocols for cantang grouper production. Furthermore, it is important to note that this study was a comparative observational case study with one tank per density treatment; therefore, the findings should be interpreted as associative rather than causally established. [13]. This study aimed to: (1) evaluate production performance (SGR and survival rate) of cantang grouper under three stocking density treatments, (2) document Vibrio *spp*. Population dynamics, and (3) determine whether a causal relationship exists between *Vibrio spp*. density and fish performance.

II. METHODS

Research Location and Time

The study was conducted at a private cantang grouper aquaculture facility located in Penyabangan Village, Gerokgak Sub-district, Buleleng Regency, Bali, Indonesia. Data collection took place over 120 days from April to July 2025.

Research Design

A comparative observational case study design was employed to describe and explore associations between stocking density treatments, production performance, and presumptive *Vibrio spp*. population dynamics in an operating commercial RAS. The study used three rearing tanks, each representing a different stocking density level (30, 50, and 70 fish/m³), as observation units. Fish growth performance was assessed monthly over 120 days by measuring weight and total length from random samples (n = 50/tank/month). Specific Growth Rate (SGR) and survival rate were calculated as primary performance indicators. Water microbiological quality was assessed by monthly collection of water samples, bacterial enumeration on Marine Agar (total heterotrophic bacteria) and TCBS agar (presumptive *Vibrio spp*.), and calculation of *Vibrio spp*. relative abundance.The relationship between *Vibrio spp*. density and SGR were examined using Pearson correlation analysis. It is important to acknowledge that each stocking density was represented by a single tank (n = 1 per treatment); therefore, true biological replication was absent, and stocking density effects are confounded with potential tank-specific factors. Statistical analyses are presented for descriptive purposes, and findings should not be interpreted as results from a fully replicated controlled experiment.

Population and Sample

Three rearing tanks, each with a volume of 200 m³, were used. Cantang grouper juveniles (15–25 cm body length) were stocked at: Tank 1 was stocked at 30 fish/m³, Tank 2 at 50 fish/m³, and Tank 3 at 70 fish/m³. Fish samples (n = 50/tank) were collected monthly using simple random sampling for growth measurement. Water samples were collected from all three tanks for bacteriological analysis.

Research Variables

The independent variable was stocking density (fish/m³). The dependent variables were SGR (%/day) and survival rate (%). The observational variable was *Vibrio spp*. population dynamics (CFU/ml and relative percentage of total bacteria).

Data Collection

Primary data were obtained through direct field observation and measurement. Parameters recorded included *Vibrio spp*. enumeration, fish growth (weight and length), fish mortality, and physicochemical water quality parameters (DO, temperature, pH, salinity, nitrite, nitrate, ammonia). Secondary data were sourced from published literature, regulatory standards (SNI 8036.2:2014), and institutional records.

RAS Configuration

The RAS used in this study consisted of three concrete rearing tanks (each 200 m³), a drum filter for mechanical filtration of suspended solids, a moving-bed biofilm reactor (MBBR) as the biological filter, a UV sterilization unit, and a protein skimmer. Water was continuously recirculated at approximately 3–5 tank volumes per day. The biofilter was seeded and conditioned for approximately 30 days prior to fish stocking.

Feed Management

Fish were fed a commercial marine pellet feed (crude protein ≥44%, crude fat ≥12%) three times daily (07:00, 12:00, and 17:00) at a feeding rate of approximately 3–5% of estimated body weight per day, adjusted monthly following sampling. Feed conversion ratio (FCR) was not formally recorded during this study, which represents a limitation.

Laboratory media preparation involved: (1) Marine Agar (MA) 11.05 g of MA powder dissolved in 200 mL distilled water, autoclaved at 121°C for 15 minutes, and poured aseptically into Petri dishes for total bacterial enumeration, (2) Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar 8.8 g TCBS and 1.5 g NaCl dissolved in 150 mL distilled water, heated to homogeneity without autoclaving, and poured into Petri dishes for selective *Vibrio spp*. enumeration. All glassware was sterilized in an autoclave at 121°C for 15 minutes prior to use [14].

Water samples (10 mL) were collected from each tank every 30 days. Serial dilutions were prepared (10^{-1} to 10^{-3} for *Vibrio spp*., 10^{-3} to 10^{-5} for total bacteria) [15]. A 0.1 mL aliquot from each dilution was inoculated onto the respective agar plates using the spread plate method and incubated at 30°C for 24 hours. Yellow or green colonies on TCBS were presumptively identified as *Vibrio spp*. based on morphological characteristics, without molecular or biochemical confirmation.

III. RESULTS AND DISCUSSION

Vibrio spp. Population Dynamics in RAS

Vibrio spp. The density in all three tanks fluctuated over the 120-day study period (Table 1). The relative proportion of *Vibrio spp*. as a percentage of total bacteria showed a consistent increasing trend with higher stocking density, averaging 0.85% in Tank 1, 1.38% in Tank 2, and 2.15% in Tank 3.

Table 1.
Mean *Vibrio spp*. Density (CFU/ml) and Percentage across Sampling Periods

Sampling Period	Tank	Total Bacteria (CFU/ml)	*Vibrio spp.* (CFU/ml)	*Vibrio spp.* (%)
Initial	Tank 1	5.1×10^6	6.3×10^4	1.22%
	Tank 2	7.6×10^6	7.5×10^4	0.98%
	Tank 3	4.8×10^6	7.8×10^4	1.63%
Month 1	Tank 1	6.9×10^6	7.8×10^4	1.12%
	Tank 2	4.8×10^6	9.1×10^4	1.90%
	Tank 3	4.5×10^6	9.1×10^4	2.02%
Month 2	Tank 1	8.7×10^6	7.5×10^4	0.86%
	Tank 2	4.3×10^6	6.3×10^4	1.45%
	Tank 3	3.0×10^6	7.4×10^4	2.44%
Month 3	Tank 1	9.2×10^6	2.1×10^4	0.22%
	Tank 2	6.7×10^6	8.2×10^4	1.22%
	Tank 3	3×10^6	7.6×10^4	2.53%

In Tank 1 (30 fish/m³), *Vibrio spp*. density remained relatively controlled (6.3×10^4 to 2.1×10^4 CFU/mL), within the lower range of pathogenicity thresholds reported in the literature [16]. In Tank 3 (70 fish/m³), *Vibrio spp*. density showed a significant increasing trend, reaching 7.6 × 10^4 CFU/mL in the final period, approaching levels capable of activating quorum-sensing-mediated virulence [17]. Importantly, this increase coincided with a general rise in total heterotrophic bacteria, indicating that the enriched organic substrate at high stocking densities facilitated broad-spectrum bacterial proliferation rather than selective *Vibrio spp*. dominance.

Survival Rate

Survival rate differed significantly across treatments (ANOVA: $p = 0.029$). Tank 1 achieved the highest survival (97.5%), followed by Tank 2 (95.0%), with Tank 3 recording the lowest (89.3%) (Table 2).

Table 2.
Survival Rate of Grouper Cantang Fish

Checking	Tank		
	Tank 1	**Tank 2**	**Tank 3**
Early Month	48	146	363
First Month	38	115	347
Second Month	36	133	382
Third Month	24	106	405
Amount	146	500	1.497
Initial Number of Fish	6.000	10.000	14.000

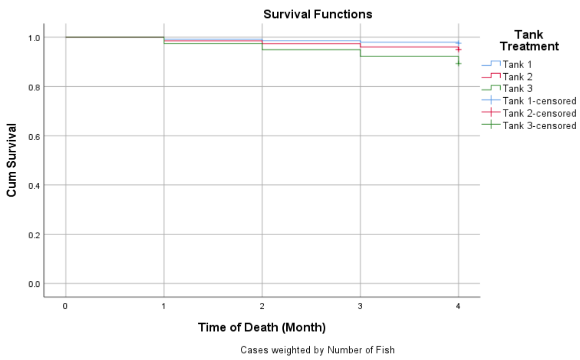

Figure 1. Curve Kaplan-Meier

Kaplan-Meier survival analysis was applied to describe mortality patterns over time. The analysis suggested a gradual, chronic mortality pattern in Tank 3 consistent with long-term overcrowding stress rather than the acute mass mortality characteristic of bacterial infection outbreaks. It should be noted that formal Kaplan-Meier survival curves, hazard estimates, and complete mortality schedules are presented in the supplementary data; censoring in this analysis was applied only to fish removed for sampling.

The relatively high survival rates across all tanks (>89%) reflect the superior hybrid vigor of cantang grouper [18]. Ummah (2019) confirmed that cantang grouper exhibits superior hematological profiles and post-challenge survival compared to its parental species following *Vibrio alginolyticus* infection. The absence of acute mass mortality, even at the highest stocking density, underscores the effectiveness of RAS in maintaining water quality within tolerance limits.

A study on cantang grouper in floating net cages demonstrated that both stocking density and water quality parameters, particularly ammonia and nitrite, exert significant simultaneous effects on cortisol levels, emphasizing the physiological sensitivity of this species to deteriorating rearing conditions [19]. Health status assessment of cantang grouper cultured in floating net cages at Kepulauan Seribu, Indonesia, showed elevated leukocyte counts above normal ranges alongside histopathological damage to liver and kidney, further underscoring the susceptibility of this species to environmental and pathogenic stressors even in established aquaculture systems [20].

Specific Growth Rate (SGR)

Kruskal-Wallis analysis was applied to compare SGR values among individual fish within each tank (n = 50 fish sampled per tank per month), testing for differences in the distribution of individual growth rates across the three tanks ($H = 102.306$; $p < 0.001$). It is important to note that this test examined variation in growth among individual fish within tanks, not replication of the stocking density treatments themselves. Each density level was represented by only one tank. SGR declined with increasing stocking density: 0.48%/day (Tank 1), 0.27%/day (Tank 2), and 0.23%/day (Tank 3). These results indicate that individual fish in Tank 1 grew significantly faster than those in Tanks 2 and 3, but this comparison cannot be attributed solely to stocking density because tank-level confounders cannot be excluded without replicate tanks. The observed inverse pattern between stocking density and SGR is nonetheless consistent with previous findings on intensive marine fish culture, where elevated densities are associated with spatial competition, reduced feed conversion efficiency, and chronic corticosteroid-mediated stress responses that suppress growth.

A comparative study on hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) reared in a recirculating aquaculture system similarly reported superior growth performance and lower *Vibrio* concentrations in fish tissue compared to those reared in pond systems, underscoring the advantage of RAS-based cultivation at controlled stocking densities.

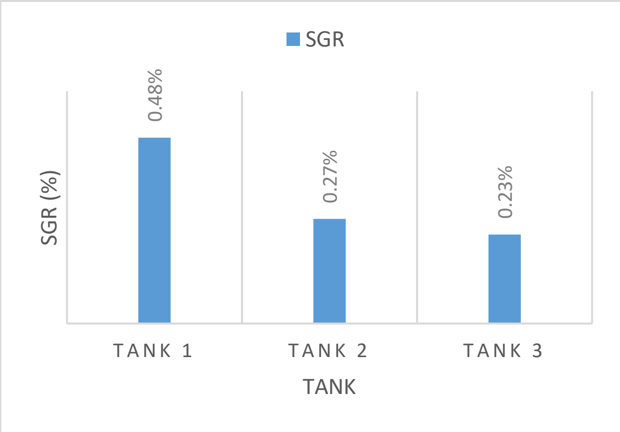

Figure 2. Specific Growth Rate Cantang Grouper

Correlation between Bacterial Density and Growth

Pearson correlation analysis between *Vibrio spp*. density and SGR yielded r = −0.185 (p = 0.633), indicating no statistically significant association within the limitations of the available sample size (N = 9) (Table 3).

Table 3.
Pearson Correlation between *Vibrio spp*. Density and SGR

		Correlation	
		Density	SGR
Density	Pearson Correlation	1	-.185
	Sig. (2-tailed)		.633
	N	9	9
SGR	Pearson Correlation	-.185	1
	Sig. (2-tailed)	.633	
	N	9	9

Although a weak negative trend was observed, it does not meet the threshold of statistical significance ($p > 0.05$). It is important to note that N = 9 is extremely small, and a non-significant result does not prove the absence of a relationship; the study is likely underpowered to detect weak associations. This finding should therefore be interpreted cautiously: it does not confirm that the observed decline in fish growth performance was not attributable to *Vibrio spp*. pathogenicity, rather, it indicates that no significant association was detected under the conditions and sample size of this study.

The observed reduction in growth at Tank 3 may be associated with: (1) spatial competition and feed competition among densely packed fish, (2) chronic physiological stress characterized by elevated corticosteroid levels suppressing immune function, and (3) accumulation of toxic metabolites, particularly ammonia and nitrite, that reached sublethal yet growth-inhibiting concentrations. This chronic crowding stress mechanism has been documented in rainbow trout reared under high stocking density in mariculture, where elevated corticosteroid levels caused significant reductions in growth performance and disrupted lipid metabolism [21].

Feed access and utilization are further compromised under high density. Research on cantang grouper demonstrated that feeding frequency significantly influences specific growth rate, feed utilization efficiency, and survival rate, highlighting that both stocking management and feeding management must be optimized simultaneously for best production outcomes [22]. The increase in *Vibrio spp*. Density is therefore best interpreted as an intermediate variable, a biological consequence of deteriorating environmental quality, rather than a primary causal agent of reduced production.

Water Quality Parameters

Water quality parameters across all tanks generally met SNI 8036.2:2014 standards for cantang grouper culture throughout the study period (Table 4).

Table 4.
Standard Deviation of Water Quality Parameters

Parameter	Tank 1	Tank 2	Tank 3
DO	8.24 ± 0.93	8.17 ± 1.30	8.10 ± 0.88
Temperature	31.9 ± 0.46	31.3 ± 1.02	31.4 ± 0.76
pH	7.52 ± 0.10	7.67 ± 0.12	7.60 ± 0.12
Salinity	34.0 ± 0.00	34.0 ± 0.00	34.0 ± 0.00
Nitrit	0.11 ± 0.10	0.14 ± 0.14	0.1 ± 0.10
Nitrat	6.14 ± 1.86	5.40 ± 0.44	5.95 ± 0.69
Amonia	0.23 ± 0.15	0.20 ± 0.11	0.21 ± 0.19

Mean DO ranged from 8.10-8.24 mg/L across tanks, mean temperature from 31.3-31.9°C, mean pH from 7.52-7.67, and salinity was stable at 34.00 ± 0.00 ppt across all tanks. Mean ammonia was highest in Tank 1 (0.23 ± 0.15 mg/L) and Tank 3 (0.21 ± 0.19 mg/L), with Tank 3 showing the greatest variability (high SD), reflecting periodic ammonia peaks that approached but did not exceed regulatory limits. Temporal analysis revealed a co-variation between ammonia peaks and elevated presumptive *Vibrio spp*. density in Tank 3, supporting the interpretation that organic accumulation, not direct pathogenic attack, drove bacterial proliferation. Furthermore, a recent RAS-based review noted that diseased systems showed a dominance of *Vibrionales*, whereas healthier systems had more diverse bacterial communities, highlighting the value of routine microbiological monitoring in RAS management [23]. Effective management of ammonia levels through optimized biofilter performance is also a key factor in limiting the conditions that favor pathogen proliferation [24].

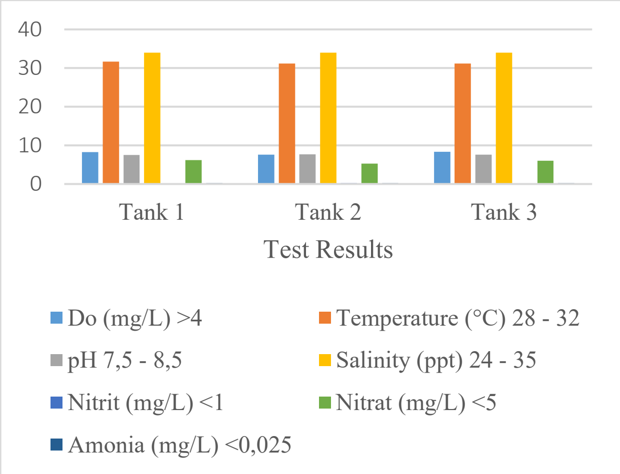

Figure 3. Water Quality

Implications for RAS Management

This study demonstrates that the RAS technology used was effective in preventing acute environmental collapse and mass mortality, even at elevated stocking densities. However, the data from Tank 3 indicate that the biofilter was operating at or near its maximum capacity (biofilter breakthrough), as evidenced by fluctuating ammonia concentrations and rising total bacterial loads. This finding suggests that a stocking density of 30 fish/m³ represents the optimal operational range for this particular RAS configuration, balancing productivity with system stability. This observation aligns with findings in European sea bass, where elevated stocking density was shown to negatively impact growth performance, feed utilization, and hematological parameters, with zeolite supplementation partially mitigating these adverse effects through improved ammonia removal [25].

Studies on *Vibrio spp.* dynamics in aquaculture media in Indonesia have shown that bacterial abundance in culture water fluctuates significantly over the rearing period, with sharp increases correlating with elevated organic matter accumulation towards the end of the culture cycle, a pattern analogous to the Tank 3 dynamics observed in the current study [26]. Furthermore, the integration of macroalgae such as *Gracilaria sp.* into aquaculture pond systems has been shown to reduce *Vibrio spp*. abundance through water quality improvement is a promising complementary strategy applicable to RAS management [27].

From a biosecurity perspective, monitoring *Vibrio spp*. Density serves as a sensitive early-warning indicator of biofilter stress and declining environmental quality rather than as a direct predictor of disease outbreak. Managers should implement enhanced monitoring frequencies (weekly or post-feeding) and consider preemptive probiotics or biofilm management strategies when *Vibrio spp*. relative abundance approaches 2% of total heterotrophic bacteria.

Continuous water quality monitoring in RAS, including parameters such as total ammonia nitrogen, nitrite, and dissolved oxygen, is essential for maintaining system stability and preventing cascade effects on fish health [8]. Natural plant-based treatments have also shown efficacy as alternatives, for example, immersion therapy using *Muntingia calabura* leaf extract successfully reduced *V. alginolyticus* infection in cantang grouper and improved hematological parameters and survival rates [28]. Furthermore, field studies in Indonesia have confirmed that the combined effects of stocking density and water quality, particularly ammonia and nitrite, significantly elevate blood glucose and cortisol levels in cantang grouper, with water quality factors explaining over 50% of glucose variance, reinforcing the need for integrated monitoring protocols in commercial farms [19].

IV. CONCLUSION

This study shows that stocking density affects the production performance of cantang grouper in a recirculating aquaculture system. The stocking density of 30 heads/m³ produces the best specific growth rate and survival rate, so that it has the potential to be the optimal operational density under the conditions of this study. An increase in stocking density is associated with a higher proportion of presumptive Vibrio *spp*., indicating a decline in the quality of the cultivation environment. However, no significant association was found between presumptive Vibrio *spp*. density and SGR decrease. Thus, the increase in *Vibrio spp*. population. reflects more of a response to changes in environmental conditions due to high stocking density than it is the main cause of the decline in fish performance.

Future research should incorporate: (1) true experimental replication with a minimum of three replicate tanks per density treatment and random allocation of fish, (2) higher-resolution microbial monitoring including species-level identification via biochemical testing or molecular methods, (3) direct measurement of physiological stress indicators (cortisol, glucose, haematological parameters), (4) feed conversion ratio recording, and (5) histopathological validation to further disentangle stress-mediated versus infection-mediated mortality mechanisms.

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