

Original Research Articles

Effects of artificial compound feed, mixed feeding and cannibalism of conspecifics on growth performance, physiological status and metabolic gene expression in juvenile barramundi (*Lates calcarifer*)

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To optimize feeding strategies for juvenile barramundi (*Lates calcarifer*), this study compared the effects of cannibalism-based feeding (CB), formulated feed (FF), and a mixed feeding regime (MIX; formulated feed combined with biological feed) on growth performance, physio-logical responses, and immune-related gene expression. Thirty-six-day-old juveniles were randomly assigned to three treatment groups, with one *L. calcarifer* individual stocked per tank and 15 replicates per treatment, and reared for 20 days. In terms of growth performance, compared with the FF and CB groups, the weight gain rate (WGR: 862.31 ± 346.66) and specific growth rate (SGR: 93.2 ± 42.48) of the MIX group were significantly higher ($p < 0.05$), and the growth performance of the CB group was also better than that of the FF group ($p < 0.05$). In terms of physiological responses, the activities of alkaline phosphatase (AKP), pyruvate kinase (PK), alanine aminotransferase (ALT), and lipid peroxide (LPO) in the MIX group were significantly higher than those in the other two groups, while the activity of catalase (CAT) was significantly lower than that in the other two groups ($p < 0.05$). In terms of immune - related gene expression, the levels of lysosomal protease (cts1a) and the expression of the glycolytic gene eno3 in the CB group were significantly higher than those in the other two groups ($p < 0.05$), while the expression of heat shock protein 90 (hsp90), pro - inflammatory cytokine IL1 β and the activity of superoxide dismutase (SOD) in the FF group were significantly higher than those in the other two groups ($p < 0.05$), indicating enhanced antioxidant and anti - inflammatory capabilities respectively. In conclusion, the MIX treatment promoted superior growth performance; however, the growth response was relatively unstable and was associated with potential risks of liver damage and oxidative stress. Cannibalism-based feeding (CB) may serve as an emergency nutritional strategy, although careful management is required to minimize its negative impacts. In contrast, the formulated feed (FF) treatment resulted in comparatively poor growth performance, with no significant improvement observed ($P > 0.05$). Overall, a mixed feeding strategy supplemented with immune enhancers is recommended for industrial-scale seed production to achieve a better balance between growth performance and fish health.

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1. INTRODUCTION

Cannibalism is a strategy for fish to cope with harsh environments or promote the rapid growth of small individuals.¹ In nature, this strategy can effectively preserve the population's survival under conditions of hunger and food scarcity, and it is easier to prey on conspecifics within the feeding group under high-competition conditions.² At the same time, the occurrence of cannibalism also indicates that the digestive tract of the cannibal has fully developed, enabling it to withstand the struggle of the prey before death and to digest and absorb the complex nutritional components of the whole small fish.³ However, under artificial breeding conditions, where sufficient food can be provided, and food and nutrition are no longer limiting factors for growth, cannibalism still occurs, especially after obvious size differences appear.⁴ So, what differences will feeding artificial feed or live food cause to farmed fish?

Studies have demonstrated that there are notable disparities in nutritional components, palatability, and digestive utilization between artificial food and live food (e.g., small fry).^{5,6} For instance, when juvenile Chinese giant salamanders (*Andrias davidianus*) are fed with artificial feed, the activity of intestinal digestive enzymes (such as trypsin) and the expression levels of genes associated with lipid metabolism (such as fatty acid synthase and carnitine palmitoyltransferase I) were significantly lower compared to those in the live-bait group. Therefore, feeding artificial feed may lead to insufficient nutrient intake in juvenile Chinese giant salamanders, exacerbate growth disparity, and induce cannibalistic behavior.⁵ In addition, changes in the immune status (such as a decrease in lysozyme activity or an up-regulation of inflammatory factor expression) may further exacerbate cannibalistic behavior.⁷ However, under the conditions of artificial breeding, there is insufficient or unclear research on the differences in the effects of cannibalism and feeding on artificial diets on the growth performance, physiology, and biochemistry of *Lates calcarifer*.

The Asian seabass (*Lates calcarifer*) is an important economic fish with fast growth and strong adaptability, and it is widely farmed in Asia and Australia.^{8,9} However, during the fry rearing stage, a widespread and serious phenomenon of cannibalism among Asian seabass larvae and juveniles occurs: larger individuals prey on smaller conspecifics, resulting in a significant decrease in survival rate and seriously affecting farming efficiency.¹⁰ Cannibalism is one of the key bottlenecks in the fry rearing of carnivorous fish, and its occurrence is affected by multiple factors, including food type, size difference, hunger stress, and stocking density.^{11,12} Currently, research on the cannibalistic behavior of Asian seabass primarily focuses on farming management measures (such as graded rearing) and feeding strategies, while the effects of cannibalism on growth, digestive physiology, and immune status remain unclear.

This study investigates the effects of different feeding strategies on the growth performance, digestive and immune enzyme activities, and key gene expression in juvenile barramundi (*Lates calcarifer*), aiming to elucidate how cannibalistic behavior influences the physiological state

and metabolic regulation of predators. Although feed is sufficient under artificial culture conditions, conspecific cannibalism remains frequent, suggesting that this behavior is not solely driven by hunger but is closely associated with nutrient acquisition efficiency, energy metabolism optimization, and physiological regulation. Accordingly, three specific objectives were addressed: (1) to compare growth performance among the artificial feed (FF), cannibalism (CB), and mixed feeding (MIX) groups to evaluate potential growth advantages conferred by cannibalism; (2) to assess digestive, metabolic, and antioxidant enzyme activities in liver and whole-body tissues to determine metabolic load and oxidative stress responses to different nutrient sources; and (3) to analyze the expression of immune- and stress-related genes using qRT-PCR to evaluate the effects of long-term live prey or mixed feeding on immune status and disease resistance. This study is the first to systematically evaluate the physiological consequences of cannibalistic behavior in barramundi under non-hunger conditions. Moving beyond the traditional view of cannibalism as a purely negative loss provides new insights into its ecological and evolutionary significance from a predator-benefit perspective. The findings offer a theoretical basis for optimizing fry-rearing strategies by adjusting feed structure and implementing early feeding interventions, thereby reducing growth heterogeneity and cannibalism rates while improving survival and production efficiency. Moreover, this study provides a valuable reference framework for investigating the mechanisms regulating cannibalism in other carnivorous fish species.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL DESIGN

The juvenile barramundi (*Lates calcarifer*) used in this experiment were obtained from the Hainan Base of the South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The fertilized eggs were self-bred, and the juvenile fish were obtained after hatching and nursing. All the experimental fish were domesticated uniformly and could feed on artificial feed normally. The initial body weight of the experimental fish was (1.07 ± 0.35) g. The animal study was reviewed and approved by the Animal Care and Use Committee of the South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The ethical code was 324QY579, which was approved in April 2024.

Based on the "Haitong Brand" compound feed for marine fish (San Tong Biotechnology (Weifang) Co., Ltd), its raw materials include imported fish meal, shrimp meal, soybean protein, fish oil, flour, vitamins, minerals, etc. The mass fractions of the main nutrients are 49.2% for protein, 9.1% for crude fat, 1.9% for crude fiber, and 15.6% for crude ash in sequence.

At the beginning of the experiment, the fish were 36 days old and had been successfully domesticated to eat artificial feed. The juvenile fish were randomly divided into three groups: the CB group was the cannibalism group, only

fed by bait barramundi (body length: bait barramundi / experimental barramundi $\times 100\% = 40\%-50\%$), each experimental barramundi was fed by 15 bait barramundis every 24 hours; the FF group with only formulated feed, and the MIX group with a mixture of bait barramundi and formulated feed. All formulated feeds were commercial compound feeds (Haitong compound feed for juvenile fish, Santong Bio-engineering (Weifang) Co., Ltd.), and the particle size was selected based on the barramundi's mouth diameter. The experiment was carried out in 16 L tanks, with 8 L of seawater added to each. The water quality conditions in each treatment were consistent with those in the domestication stage. 1 experimental fish was placed in each tank, with 15 tanks per treatment. The experiment was divided into 3 treatments in total. The entire experimental period was 20 days.¹³ All the water tanks are connected to the same recirculating aquaculture system.

In this experiment, the satiation feeding method was adopted. In the CB and MIX treatments, 15 live prey were fed to each tank every 24 hours. Live prey should be starved for eight hours before being fed to predators as food. The mass of 15 live prey was approximately equal to 200% of the predator's body weight. Before placing new live prey each time, the remaining prey from the previous placement was removed, weighed, and the remaining quantity was recorded. The prey was smaller barramundi, and the body length of the prey was 40%-50% of that of the predators. In addition, 10,000 juvenile fish of similar size were selected in advance as potential prey and temporarily kept in a floating bucket. After measuring the wet body weight to estimate the biomass of the prey (OPB, g), the prey were released into the tank for the predators. During the experiment, the number of prey released in each experimental bucket (OPN) was recorded daily, and the body weight of the predators was measured. The FF and MIX treatments were fed with enough feed three times a day (6:00, 14:00, 22:00). The weight of the artificial feed provided each time was approximately equivalent to 40% of the predator's body weight. The aquaculture water was maintained under the following conditions: salinity 32‰, dissolved oxygen > 7.50 mg/L, pH 7.93 ± 0.12 , ammonia nitrogen < 0.10 mg/L, and nitrite nitrogen < 0.05 mg/L. After 20 days of the experiment, five fish were randomly selected, over - anesthetized with MS - 222 (30mg/L), and the whole - fish tissues were quickly collected for enzyme activity determination and RNA extraction. The median lethal concentration of MS - 222 for barramundi was 12.5 mg/L. In this experiment, a concentration of 30 mg/L was used for over - anesthesia. All samples after whole fish homogenization were quickly frozen in liquid nitrogen and then transferred to -80°C for storage for subsequent analysis.

2.2. RESEARCH AND ANALYSIS OF OXIDATION CAPACITY AND METABOLIC ENZYME ACTIVITY

Approximately 0.1 g of tissue was collected from each juvenile barramundi and placed in a 2-mL centrifuge tube. Then, 9 times the volume of 0.86% normal saline solution was added. The tissues were homogenized on ice using a Prima PB100 handheld homogenizer (Gloucester, England).

The homogenate was centrifuged at $3500 \times g$ for 10 min, and the supernatant was collected and stored at -80°C for analysis of antioxidant capacity and metabolic enzyme activity.

The antioxidant indicators in the tissues mainly included superoxide dismutase (SOD), lipid peroxide (LPO), peroxidase (POD), malondialdehyde (MDA), glutathione reductase (GR), and peroxidase (POD). These antioxidant indicators were measured using commercial detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the kit instructions. The total protein content in the homogenate was normalized and measured using a BCA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

The metabolic enzyme activity indicators mainly included alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (AKP), and acid phosphatase (ACP). These metabolic enzyme activity indicators were measured using commercial detection kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the kit instructions. The total protein content in the homogenate was normalized and measured using a BCA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.3. SAMPLING AND DETERMINATION

The experimental period was 20 days. After the experiment, the predators were anesthetized and weighed. After weighing, the whole fish was ground to measure enzyme activity and genes.

The whole-fish homogenate of barramundi under different feeding strategies was used for RNA extraction. Total RNA was extracted using the Thermo Scientific GeneJET RNA Purification Kit (Waltham, MA, USA). According to the manufacturer's instructions, the integrity, concentration, and purity of RNA were assessed using agarose gel electrophoresis, a Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA), and a 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). Only high-quality RNA ($\text{OD}_{260/280} = 1.8-2.2$, $\text{OD}_{260/230} \geq 2.0$, $28\text{S}:18\text{S} \geq 1.0$, Total RNA $> 10 \mu\text{g}$) could be used for subsequent experiments. $2 \mu\text{L}$ (1000 ng) of total RNA was pipetted, and the first - strand cDNA was 195 synthesized by reverse transcription according to the instructions of the EasyScript All - in - One First - Strand cDNA Synthesis SuperMix for qPCR (One - Step gDNA Removal) kit,¹⁴ which was used as a template for subsequent gene expression determination.

In quantifying immune gene expression at each time point, the CB group's gene expression level was used as a normalization index for relative quantification. Five immune and metabolic single genes were selected for verification by quantitative real-time PCR (qRT-PCR) using a real-time qPCR analysis system (Analytik Jena GmbH, Germany) and SYBR Green (Tiangen Biotech Co., Ltd., China). Specific primers were designed using Primer Premier 5 software (Table 1). The total reaction mixture was $20 \mu\text{L}$, including $10 \mu\text{L}$ of $2 \times \text{RealUniversal PreMix}$, $0.6 \mu\text{L}$ of each primer ($10 \mu\text{M}$), and $2 \mu\text{L}$ of diluted cDNA. First, it was denatured at 95°C for 15 minutes, followed by 40 cycles of amplifica-

Table 1. Sequences of specific primers used for qRT-PCR analysis.

Gene Abbreviation		Primer Sequence (5'-3')	Amplicon Size (bp)	Accession NO	Amplification efficiency (%)
Immune-related genes	<i>C3</i>	F: AAATGCTGCCATCGTTCC R: CCAGTGACCTTCAGACCAAA	175	XM_018679796	98.7
	<i>myD88</i>	F: TCCAACCTTGCCCTCTAAT R: AAGCGTGATACAGAAACC	111	XM_018673801.2	99.3
	<i>IFNγ1</i>	F: TACCAGGAGCAGGACAAGC R: TCGTCAGGCAGCGAACTT	134	NM_001360734	103.4
	<i>pfn6</i>	F: TCCCGTAAGGACCAACAG R: GCACCCAGATAGAGGAAGG	173	XM_018676659.2	101.6
	<i>cts1a</i>	F: CACATCCGTCGGTCTTCT R: GCTCAGCGACACCAGTTT	120	EU143238.1	99.8
	<i>hsp90</i>	F: ACGATGATGAGCAGTATGCC R: CAAACAGGGTGATGGGGTA	210	XM018661637	102.5
Metabolism genes	<i>eno3</i>	F: GCATAGCAAGAGACAGTGC R: GGAGGTGTAGATGTATTGTC	100	KY502198.1	101.9
	<i>ppl</i>	F: ATTCCCCAGGTGATGAGTC R: GCGAATGTGTCGTGTCT	104	XM_018697911.2	98.5
	β -actin	F: AACCAAACGCCCAACAAC R: ATAAGTGAAGCCATGCCAATG	112	XM_018667666	100.6
Stress Response genes	<i>IL1β</i>	F: GACCCTACACCCAGCAAC R: GACGTCCAATGGGCTTCT	181	XR_001963431.1	103.5
	<i>tegt</i>	F: ATTCCCCAGGTGATGAGTC R: GCGAATGTGTCGTGTCT	144	XM_018689545.2	99.6

Note: *C3* (Complement component 3), *myD88* (Myeloid differentiation primary response 88), *IFN γ 1* (Interferon gamma 1), *pfn6* (Profilin 2 domain family member 6), *cts1a* (Cathepsin A), *hsp90* (Heat Shock Protein 90), *eno3* (enolase 3), *ppl* (periplakin), *IL1 β* (Interleukin 1 beta), β -actin (beta-actin), *tegt* (testis enhanced gene transcript).

tion (95 °C, 10 s; 58 °C, 20 s; 72 °C, 30 s). At the end of each qRT-PCR cycle, a melting curve analysis of the primers was performed to ensure that only specific products were obtained and no primer dimers were formed. Each experiment included a no-template control to verify that the PCR reaction mixture was free of contamination. The relative mRNA expression level of the target gene was determined by the $2^{-\Delta\Delta C_t}$ method, with the β -actin gene as the internal reference gene and the CG group at 0 h for normalization. The reaction efficiency was 90-110%, and the Pearson correlation coefficient (R^2) > 0.97.

2.4. STATISTICAL ANALYSIS

All data were organized using Microsoft Excel 2021 and statistically analyzed with SPSS software (Version 26.0). The results are presented as the mean $\pm$ standard deviation (mean $\pm$ SD). One-way analysis of variance (one-way ANOVA) was employed to identify significant differences between groups, and Tukey's test was used for post-hoc multiple comparisons when appropriate. The significance level was set at $p < 0.05$.

3. RESULTS

3.1. GROWTH PERFORMANCE

As shown in Table 2, both the average weight gain rate and the specific growth rate showed the order MIX > CB > FF. That was, the MIX group had the highest growth and the fastest growth rate. The FF group had the least weight gain and the slowest growth rate, followed by the CB group.

However, there was no significant difference in the weight gain rate and specific growth rate between the MIX group, the FF group and the CB group ($P > 0.05$). Feeding solely on artificial feed (FF) resulted in the poorest growth performance. Although cannibalism among conspecifics (CB) was not significantly better than the FF group, it showed higher growth potential.

3.2. EFFECTS OF DIFFERENT BAITS ON THE PHYSIOLOGICAL AND BIOCHEMICAL INDICATORS OF *LATES CALCARIFER*

As shown in Figure 1a, ALT levels in the FF and CB groups were significantly lower ($p < 0.05$) than in the MIX group. The LDH activity was stable in the three groups of experiments (Figure 1b), with no significant differences between groups and small differences within groups, suggesting that none of the three feeding methods would cause significant differences in general cell damage to *Lates calcarifer*. The ACP activity in the CB group was significantly lower than that in the other two groups (Figure 1c).

As shown in Figure 1d, the SOD activity in the CB group was the lowest, significantly lower ($p < 0.05$) than that in the MIX group, and it was stable within the group. The SOD activities in the FF group and the MIX group were the highest, with no significant difference between the two groups, and at the same time, there were relatively large differences within the groups. The CAT activity in the MIX group was much lower than that in the FF group, significantly lower ($p < 0.05$) than that in the CB group (Figure 1e), and the difference within the MIX group was small. The CAT activity values in the FF group and the CB group were relatively high,

Table 2. Effects of different feeding strategies on the growth performance of juvenile *Lates calcarifer* for 20 days

	Initial Weight (g)	Weight Gain Rate (%)	Specific Growth Rate (%/d)
FF	0.83±0.16 ^a	496.68±342.92 ^a	41.89±56.19 ^a
MIX	0.98±0.53 ^a	862.31±346.66 ^a	93.2±42.48 ^a
CB	1.03±0.53 ^a	542.16±61.64 ^a	62.61±38.27 ^a

Note: The same letters represent no significant difference ($p > 0.05$), and different letters indicate a significant difference ($p < 0.05$). The sample size (n) for each group is 5 fish. The data presentation method is mean $\pm$ standard deviation.

with no significant difference between the groups, and the CAT activity values within the groups were unstable. It is worth noting that although the MIX group showed the optimal growth performance, its CAT activities were relatively low, suggesting the possible existence of antioxidant stress; this was confirmed in its subsequent immune gene regulation - the expression of the pro-inflammatory factor *IFN γ 1* in the MIX group increased, which might be a compensatory response of the body to oxidative stress.

The POD activity values showed the order of MIX group > FF group > CB group (Figure 1f), and the differences between the groups were significant ($p < 0.05$). The small error values indicated that the activity values within the groups were stable. The GR activity values showed the order of FF group > MIX group > CB group (Figure 1g), and the differences between MIX group and FF group or between MIX group and CB group were not significant ($P > 0.05$). The small error values indicated that the activity values within the groups were stable, but the GR activity value in the FF group was significantly higher ($p < 0.05$) than that in the CB group.

The MDA content was in the order of FF group > MIX group > CB group (Figure 1h). There was no significant difference between adjacent groups ($P > 0.05$), suggesting that the overall effect was small. The small error values in the MIX group and CB group indicated stable content within the groups. However, the MDA content in the FF group was significantly higher ($p < 0.05$) than that in the CB group. As shown in the Figure 1i, the LPO content was in the order of MIX group > CB group > FF group. There was a significant difference between adjacent groups ($p < 0.05$). The small error values in these groups indicated stable content within the groups.

As shown in Figure 1j, the AKP activity value was in the order of MIX group > FF group > CB group, and there were significant differences between groups ($p < 0.05$), with small fluctuations within each group. The PK activity value was in the order of MIX group > CB group > FF group (Figure 1k), and there were significant differences between groups ($p < 0.05$), with small fluctuations within each group.

3.3. REGULATION OF GENE EXPRESSION: DIFFERENCES IN IMMUNITY, METABOLISM, AND STRESS RESPONSE

To evaluate the effects of diet types on the innate immune capacity of juvenile barramundi, this study measured gene expression levels of *myD88*, a key adapter molecule in the Toll-like receptor signaling pathway, and *C3*, a core component of the complement system. The results showed that

diet types had significant effects on the expression of both immune genes ($p < 0.05$). As shown in Figures 2a and 2b, the expression patterns of *myD88* and *C3* were the highest in the mixed-feeding group (MIX), and their expression levels were significantly higher than those in the formulated feed group (FF) and the cannibalism group (CB). Moreover, the expression levels in the FF group were significantly higher than those in the CB group ($p < 0.05$).

Under different diet feeding conditions, there were significant differences in the expression of immune-related genes in juvenile barramundi (Fig. 2c, 2d and 2e). The expression level of the pro-inflammatory factor *IFN γ 1* was relatively high in the FF and MIX groups (Figure 2c), while it decreased significantly in the CB group. The expression of *pfn6* (Figure 2d), which was related to the cytoskeleton and immune cell migration, was the highest in the FF group, decreased significantly in the MIX group, and was the lowest in the CB group. This result suggests that formulated feed may have a stronger promoting effect on the motility of immune cells in juvenile fish, while feeding solely on small fish carcasses is not conducive to the expression of this gene. In terms of the expression of the lysosomal protease-related gene *cts1a* (Figure 2e), the CB group showed the highest level, significantly higher than the FF and MIX groups.

Under different diet feeding conditions, there were significant differences in the expression levels of metabolism- and stress-related genes in juvenile barramundi (Figure 2f, 2g, and 2h). The expression of *hsp90* (Figure 2f), which is related to the molecular chaperone function, was maintained at relatively high levels in the FF and MIX groups, while it decreased significantly in the CB group. This suggests that formulated feed and mixed feed contribute to enhancing the molecular chaperone protection of juvenile fish under stress. The expression trend of the glycolysis-related gene *eno3* (Figure 2g) was the opposite: it was the highest in the CB group, significantly higher than the other two groups ($p < 0.05$), followed by the MIX group, and the lowest in the FF group. There was no significant difference between the MIX group and the FF group ($p > 0.05$). The expression of *ppl* (Figure 2h) was the highest in the FF group, decreased significantly in the MIX group, and was lowest in the CB group.

Under different diet feeding conditions, there were significant differences in the expression levels of the immune-related genes *tegt* and *IL1 β* in juvenile barramundi (Figure 2i and 2j). Among them, the expression level of *tegt* (Figure 2i) was the highest in the FF group, significantly higher than that in the MIX group and the CB group. In terms of

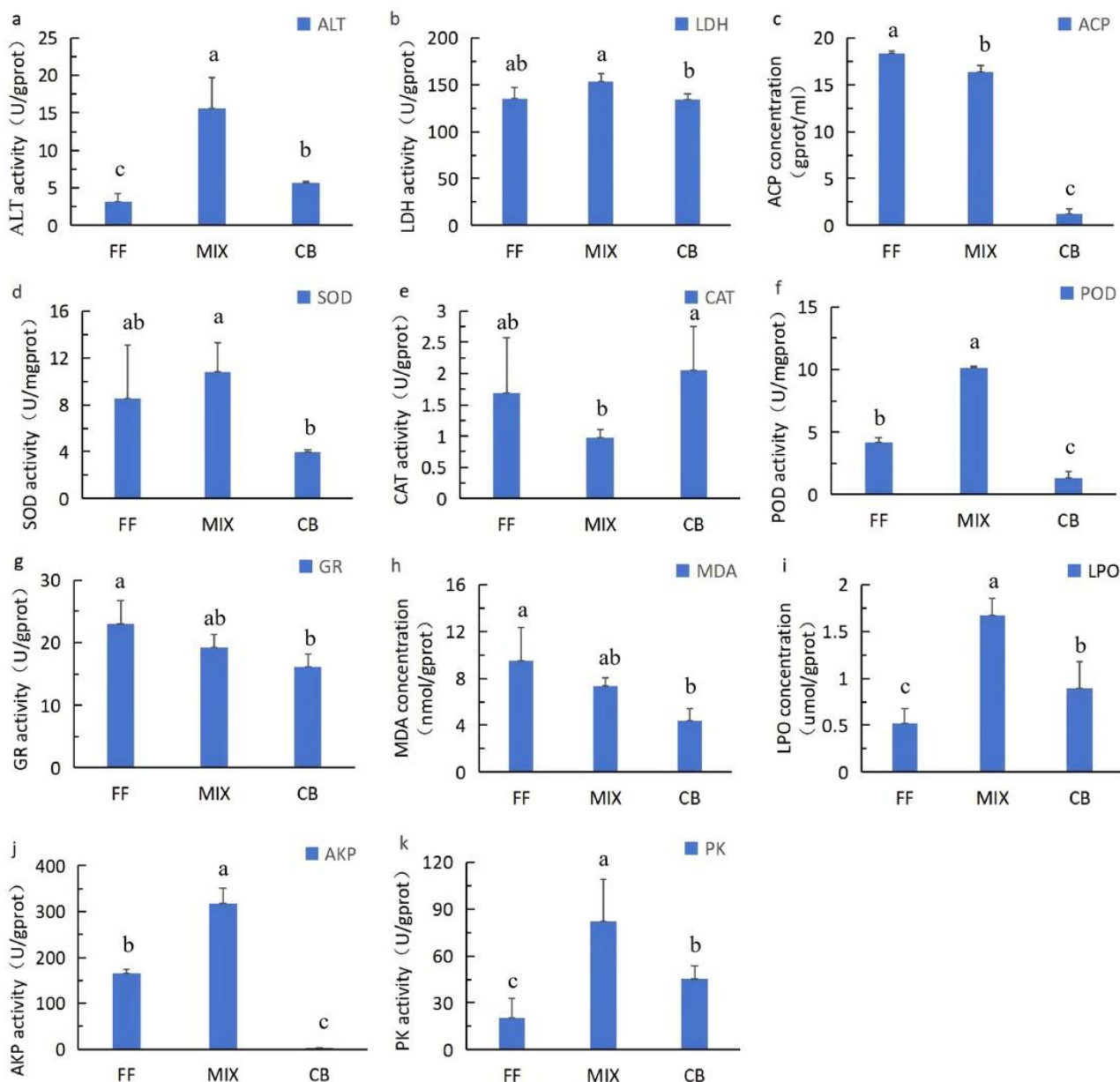


Figure 1. Effects of different baits on the physiological and biochemical indicators of *Lates calcarifer*.

Note: Different letters indicate significant differences ($p < 0.05$). The same letters represent no significant difference ($p > 0.05$). The sample size (n) for each group is 5 fish. The data presentation method is mean $\pm$ standard deviation. 1a: ALT activity; 1b: LDH activity; 1c: ACP concentration; 1d: SOD activity; 1e: CAT activity; 1f: POD activity; 1g: GR activity; 1h: MDA concentration; 1i: LPO concentration; 1j: AKP activity; 1k: PK activity; CB: compared cannibalism, FF: formulated feed, MIX: mixed feed (formulated + biological feed).

the expression of the immune-regulatory gene *IL1 β* (Figure 2j), the levels in the FF and CB groups were relatively high, and there was no significant difference between these two groups. In contrast, the MIX group had the lowest level. The MIX group was significantly lower than the other two groups ($p < 0.05$). This shows that feeding with formulated feed or small fish carcasses alone is conducive to the high expression of *IL1 β* , while the expression decreased significantly under the mixed-feed condition.

4. DISCUSSION

This study compared the effects of artificial compound feed (FF), mixed feed (MIX), and cannibalism of conspecifics

(CB) on the growth performance, physiological and biochemical indicators, and gene expression of juvenile barramundi. It revealed the “growth-health” trade-off relationship under different feeding strategies. The core findings and discussions are as follows:

4.1. GROWTH ADVANTAGES AND POTENTIAL RISKS OF MIXED FEEDING (MIX)

The weight gain rate and specific growth rate of the MIX group were significantly higher than those of the FF group and the CB group ($p < 0.05$), indicating that the mixed feeding of “artificial feed + biological feed” can promote the growth of juvenile barramundi. This is consistent with the general conclusion in the larval rearing of marine fish that

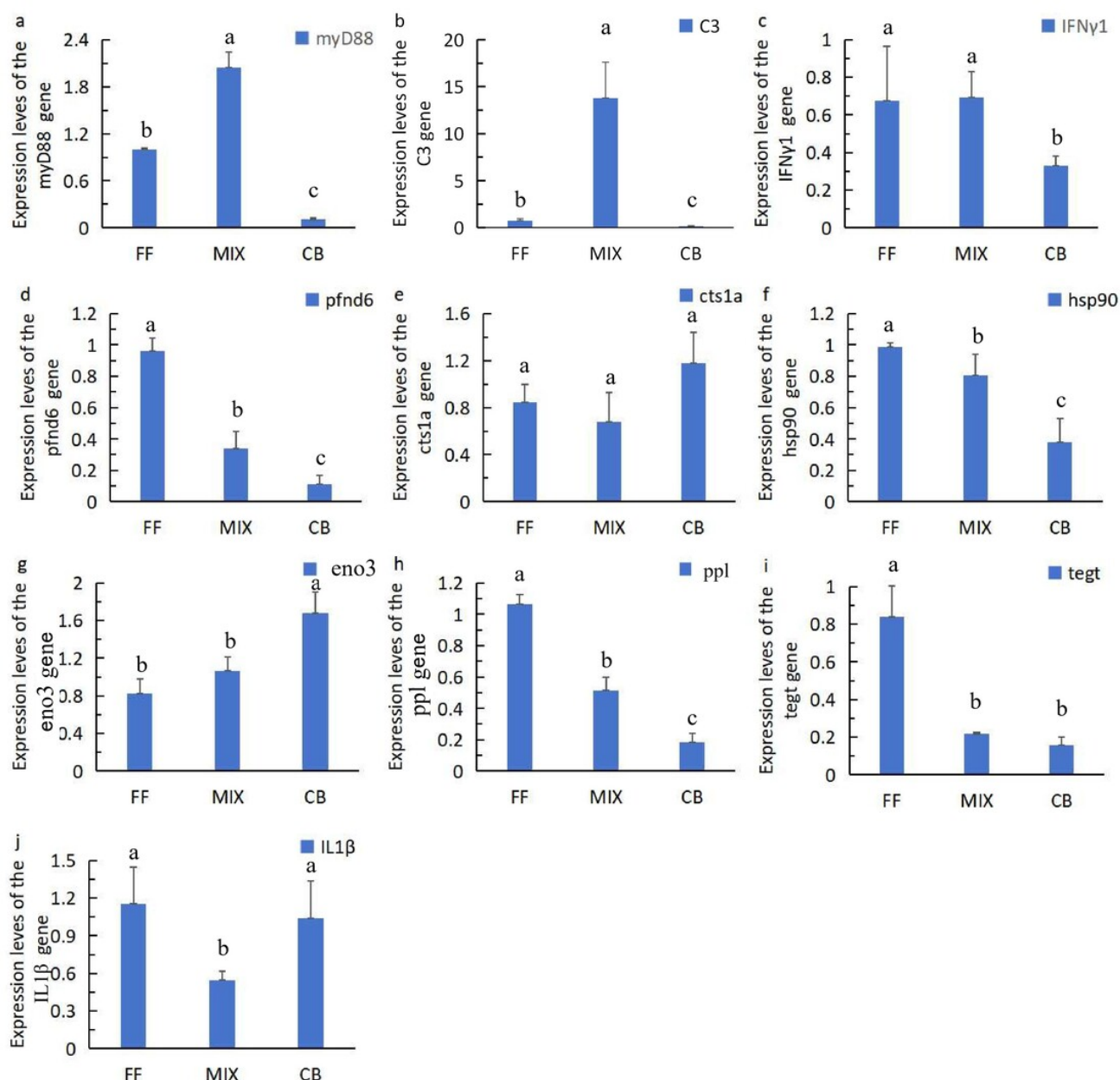


Figure 2. Effects of different baits on the relative gene expression levels of *Lates calcarifer*.

Note: Different letters indicate significant differences ($p < 0.05$). The same letters represent no significant difference ($p > 0.05$). The sample size (n) for each group is 5 fish. The data presentation method is mean $\pm$ standard deviation. 2a: Expression levels of the *myD88* gene; 2b: Expression levels of the *C3* gene; 2c: Expression levels of the *IFN γ 1* gene; 2d: Expression levels of the *pfnd6* gene; 2e: Expression levels of the *cts1a* gene; 2f: Expression levels of the *hsp90* gene; 2g: Expression levels of the *eno3* gene; 2h: Expression levels of the *ppl* gene; 2i: Expression levels of the *tegt* gene; 2j: Expression levels of the *IL1 β* gene; CB: compared cannibalism, FF: formulated feed, MIX: mixed feed (formulated + biological feed).

mixed feeding is required during the transition from biological feed to artificial feed to improve feeding efficiency.¹⁵

Potential mechanism could be related to lipid peroxidation (LPO) which is an intermediate product in the process of lipid peroxidation. Studies have shown that feeding artificial diets can lead to increased peroxides in fish, which is reflected in the increased LPO levels in the measured indicators.¹⁶ In this study, the LPO content was found to follow the pattern: MIX group > FF group > CB group, with significant differences between adjacent groups ($p < 0.05$). This indicates that lipid peroxidation induced by the three diets differs significantly, with the MIX group exhibiting the most active lipid peroxidation. These findings suggest

that mixed feeding is more likely to increase peroxide levels in fish. The MIX group had the highest activities of AKP (substance metabolism) and PK (glycolysis) ($p < 0.05$), suggesting that the mixed feed may accelerate energy conversion and growth by optimizing nutritional balance (such as essential amino acids and unsaturated fatty acids) and digestion utilization rate. This led to the MIX group's fastest growth rate. This is consistent with the view proposed by Chatzifotis et al. (2011)¹⁷ that mixed feed can improve the metabolic enzyme activities of marine fish through nutritional complementarity.

Although the MIX group had the best growth performance, there were the following health risks:

Liver damage risk: Previous studies have indicated that under single feeding regimes, nutritional imbalance in live feeds (e.g., *Artemia*) or the accumulation of anti-nutritional factors in formulated diets may lead to liver damage in fish.¹⁸ In the present study, alanine aminotransferase (ALT) activity was significantly elevated in the MIX group ($p < 0.05$), suggesting that neither exclusive feeding of formulated diets nor cannibalism alone caused hepatic damage in *Lates calcarifer*. In contrast, mixed feeding may increase the risk of liver injury, and whether damage has already occurred remains to be further verified. This is consistent with the result reported by Chatzifotis et al. (2011),¹⁷ that long-term mixed feeding increases the metabolic load on the liver of sea bass.

Weak antioxidant defense: Lushchak (2006) proposed that rapid growth can lead to the accumulation of reactive oxygen species (ROS), such that when the growth rate exceeds a certain threshold, the antioxidant defense system of fish may be temporarily suppressed.¹⁹ In the present study, catalase (CAT) activity in the MIX group was the lowest, while LPO content was significantly higher than that in the FF and CB groups ($p < 0.05$), indicating that fish in the MIX group were subjected to a higher level of oxidative stress. These results are in agreement with the findings of Lushchak.¹⁹

Imbalance of immune homeostasis: Two pro-inflammatory genes (e.g., *myD88* and *C3*) were highly expressed, low expression of one pro-inflammatory gene *IL1 β* ($p < 0.05$). Concurrently, elevated lipid peroxidation (LPO) levels, increased alanine aminotransferase (ALT) activity, and reduced catalase (CAT) activity, suggesting that “rapid growth may be achieved at the expense of immune regulation”. This aligns with the growth-immunity resource allocation trade-off theory in teleost fish.²⁰

4.2. ADAPTIVE CHARACTERISTICS OF CANNIBALISM OF CONSPECIFICS (CB)

Although the weight gain rate and specific growth rate of the CB group were lower than those of the MIX group, they were significantly higher than those of the FF group ($p < 0.05$), indicating that juvenile barramundi can obtain nutrients and maintain a moderate growth rate through cannibalism of conspecifics. The lowest activities of ACP and AKP indicate that the CB group has a relatively low energy utilization capacity, which is consistent with its lower growth rate compared to the mixed-feeding group. This is consistent with barramundi's carnivorous habit in the natural environment. However, its cannibalistic behavior may be affected by factors such as stocking density and individual size difference, and should be avoided through graded culture in actual production.²¹

These responses could be related to physiological and genetic adaptations. The CB group exhibited the highest expression level of *cts1a* (lysosomal protease), suggesting that cannibalism may reduce the risk of pathogen infection by enhancing antigen processing and clearance capacity. This finding is consistent with the observations of Corcos (2015), who reported that cannibalistic behavior in carnivorous fish is often accompanied by the upregulation of immune-related genes.²²

orous fish is often accompanied by the upregulation of immune-related genes.²²

Previous studies have shown that decreased acid phosphatase (ACP) activity in fish is indicative of accelerated erythrocyte lysis or apoptosis.²³ In the present study, ACP activity in the CB group was significantly lower than that in the other two groups, indicating that cannibalism in *Lates calcarifer* induced a similar physiological response under the experimental conditions.

The activity of pyruvate kinase (PK) followed the order MIX > CB > FF (Fig. 1k), with significant differences among groups ($p < 0.05$) and relatively low intra-group variability. The CB group exhibited the lowest glycolytic flux and energy production efficiency, which may be associated with the body composition of the consumed fish prey.²⁴

Cannibalism has been reported to potentially trigger immune enhancement in fish.⁷ However, in the present study, the expression levels of the pro-inflammatory cytokine interferon gamma 1 (*IFN γ 1*) were relatively higher in the FF and MIX groups (Fig. 2c), whereas a significant reduction was observed in the CB group. This result suggests that cannibalism in *Lates calcarifer* may more effectively reduce the inflammatory immune responses in juvenile fish.

It has been reported that teleosts feeding on small fish carcasses may exhibit enhanced lysosomal activity and antigen-processing capacity.²⁵ Consistent with this, the CB group in the present study showed the highest expression of the lysosomal protease-related gene cathepsin L1a (*cts1a*) (Fig. 2e), significantly exceeding that of the FF and MIX groups. This trend indicates that *L. calcarifer* feeding on small fish displays immunophysiological adaptations similar to those observed in the formulated feed and mixed diet groups, whereas the formulated feed and mixed diet groups exhibited weaker responses.

The expression of *eno3*, a gene closely associated with energy metabolism,²⁶ further supported these findings. The glycolysis-related gene enolase 3 (*eno3*) showed the highest expression in the CB group, which was significantly higher than that in the other two groups ($p < 0.05$). This was followed by the MIX group, while the FF group exhibited the lowest expression. No significant difference was observed between the MIX and FF groups ($P > 0.05$). These results indicate that diets dominated by small fish carcasses may strongly induce the expression of energy metabolism-related genes, thereby meeting elevated energy demands. This metabolic response is consistent with the adaptive capacity of carnivorous fish or humans to high-protein diets.^{27,28}

4.3. LIMITATIONS AND OPTIMIZATION DIRECTIONS OF ARTIFICIAL FEED (FF)

The weight gain rate and specific growth rate of the FF group were the lowest ($p < 0.05$), which may be due to the poor palatability of artificial feed, single nutritional components (such as lack of essential fatty acids like DHA and EPA), or the inhibition of digestion and absorption by anti-nutritional factors (such as phytic acid). This is consistent with the conclusion of Le Pape et al. (2015)²⁹ and Melaku et al. (2024)²⁰ in the study of artificial feed for marine fish,

that single feed is likely to cause slow growth of juvenile fish.

Potential advantages: The MDA content in the FF group was significantly higher ($p < 0.05$) than that in the CB group, and higher ($P > 0.05$) than that in the MIX. This indicates that the proportion of artificial feed in the diet can affect MDA production. Specifically, the higher the content of artificial feed in the diet, the higher the amount of MDA produced. This might be caused by the lack of certain elements in the feed.³⁰ Prolyl endopeptidase (*ppl*) and transglutaminase (*tegt*) are important indicators of hepatobiliary injury.³¹ Previous studies have demonstrated that feeding formulated diets can impose metabolic stress on the liver of fish, resulting in elevated activities of *ppl* and *tegt*.^{31,32} The FF group had higher expression levels of SOD activity (antioxidant), *hsp90* (stress protection), and *IL1 β* (pro-inflammatory), indicating its advantages in immune homeostasis and stress adaptation. This suggests that artificial feed can further improve growth performance by adding immune enhancers (such as probiotics and β -glucan) or nutritional fortifiers (such as selenium and vitamin E) while retaining its health advantages.^{33,34}

5. CONCLUSIONS

This study systematically compared the effects of artificial compound feed (FF), mixed feeding (MIX), and cannibalism of conspecifics (CB) on growth performance, physiological status, oxidative stress, immune responses, and metabolic gene expression in juvenile barramundi. A clear growth–health trade-off was observed among the different feeding strategies.

Mixed feeding (MIX) produced the highest growth performance, likely due to improved nutritional complementarity and enhanced energy metabolism. However, this growth advantage was accompanied by increased lipid peroxidation, reduced antioxidant capacity, elevated hepatic enzyme activity, and immune imbalance, indicating higher oxidative stress and potential liver injury. Cannibalism of conspecifics (CB) supported moderate growth and induced distinct adaptive responses, including enhanced lysosomal activity and upregulation of immune- and energy metabolism-related genes, while maintaining relatively low oxidative damage. These responses reflect the carnivorous adaptation of barramundi, although cannibalism is not suitable for practical aquaculture due to management risks. In contrast, exclusive feeding with artificial compound feed (FF) resulted in slower growth but better maintenance of antioxidant defenses, immune homeostasis, and stress resistance, suggesting greater physiological stability.

Overall, no single feeding strategy could simultaneously maximize both growth and health. The residual-feeding strategy that mimics natural conditions resulted in a lower growth rate than the MIX group and showed poorer health performance compared with formulated feed. These findings highlight the importance of developing balanced, stage-specific feeding strategies. Future work should therefore focus on the continued optimization of formulated

feed to support the sustainable growth and health of barramundi aquaculture.

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INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study was reviewed and approved by the Animal Care and Use Committee of the South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The ethical code is 324QY579, which was approved in April 2024.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data supporting the results of this study can be obtained from the corresponding author upon reasonable request.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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