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Bactericidal and Immunostimulant Activity of *Scutellaria baicalensis* Georgi in Cobia (*Rachycentrol canadum* L.) Against Photobacteriosis

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Abstract

Photobacteriosis, caused by *Photobacterium damselae* subsp. *piscicida*, is a major bacterial disease affecting cobia aquaculture, leading to high mortality rates and economic losses. This study evaluated the potential of *Scutellaria baicalensis* root extract as a natural antimicrobial and immunostimulant in cobia. A total of 540 cobia (average initial weight: 23.3–26 ± 2 grams) were assigned to six dietary with 0, 0.5, 1, 2, 3, and 5% of *S. baicalensis* root extract and fed for 12 weeks. Parameters measured included growth performance, survival rate, humoral immune responses (myeloperoxidase (MPO), peroxidase, and lysozyme activity), and cytokine-related gene expression (TLR-9, IL-1β, IgM, and TNF-α) in the spleen. Among the supplemented groups, the 1% and 2% doses showed the most significant improvement in immune and growth responses. The 0.5% dose produced moderate effects, while higher concentrations (3% and 5%) showed reduced or plateaued benefits, suggesting potential overstimulation or metabolic burden at higher doses. Additionally, 1% *S. baicalensis* exhibited enhanced immune responses, with increased MPO, lysozyme, and peroxidase activity in cobia. These findings demonstrate the efficacy of 1% *S. baicalensis* as a feed supplement for improving cobia health and immunity in aquaculture, offering a sustainable alternative to antibiotics.

Introduction

Cobia (*Rachycentron canadum* L.), a pelagic fish from the monotypic family Rachycentridae, is found in tropical, subtropical, and mild temperate seas above 20°C. Cobia is a great candidate for mariculture due to its rapid development rate and high market value (Remya et al. 2024; Sakthivel et al. 2019). It has acquired appeal in aquaculture throughout more than 23 nations in the Asian-Pacific area, owing to its excellent nutritional content (Araujo et al. 2022). However, cobia culture faces significant challenges, primarily from disease outbreaks. Farmers may encounter disease symptoms during cultivation, such as abnormal eyes, ulcerated skin, or other external abnormalities. Infections by bacteria, viruses, fungi, or parasites can cause these conditions (Rizky et al. 2017; Sakthivel et al. 2019; Văn Khánh et al. 2022). In addition, fish under biotic stress may exhibit unusual behavior, including decreased feed intake, slow movement, and changed swimming patterns (Sakthivel et al. 2019). This will lead to fish mortality and economic losses.

The most prevalent infection in cobia is caused by *Photobacterium damselae* subsp. *piscicida*, the etiological agent of photobacteriosis/pasteurellosis/ pseudotuberculosis disease (Moi et al. 2017; Aziz and Abdullah 2021). Infected fish exhibit lethargy, loss of balance, increased breathing rates, swim on the surface, and eventually sink before dying, with skin infections as a secondary sign of skin abrasion (Aziz and Abdullah 2021). The condition is distinguished by white granulomatous lesions in internal organs, which indicate the presence of many bacterial cells in the host body, notably in the bloodstream. Thus, it can cause mass

fatality rates of up to 90% and is regarded as one of the most serious bacterial infections in marine culture globally (Moi et al. 2017; Kaygorodova and Matveenko 2023; Morick et al. 2023).

Several management strategies have been implemented to reduce bacterial infections in fish, including the use of plant extracts. *Scutellaria baicalensis* Georgi (Lamiaceae) is one of several medicinal plants that have recently sparked interest in aquaculture due to their significant active components, which have demonstrated increased efficiency in managing fish infections at a low cost, ecological sustainability, and broad-spectrum action (Liao et al. 2022). The extract of this plant has been shown in studies to effectively improve fish growth performance, manage the parasite *Piscicola geometra* infestation (Rizky et al. 2017, 2018), and control several common pathogenic bacteria in aquaculture, including *Aeromonas hydrophila, Edwardsiella tarda, Vibrio alginolyticus*, and *V. harveyi* (Xia et al. 2020; Zaman and Cho 2023).

Since the *Photobacterium* is mainly found in the gut of marine fish, representing a significant portal of entry for this pathogen, it may benefit from using dietary feed to maintain and enhance the barrier integrity of the cobia gut. Building on this background, this study aims to investigate the effects of *S. baicalensis* root water extract on controlling bacterial infections in cobia through oral administration. The research will evaluate fish growth performance, mortality rates, humoral immune responses, and cytokine gene expression in cobia to comprehensively assess the extract's potential as a natural alternative in aquaculture disease management.

Material and Methods

Fish and Experimental Design

This study was conducted at the Tungkang Marine Biotechnology Research Station in Taiwan. A total of 540 cobia (weighing 23.3–26 gr, 10±2 cm length), were used. The fish were kept in 200 L tanks equipped with a continuous flow-through water system and daily siphoning to maintain water quality. During the acclimatization period, the fish were fed a commercial feed twice daily, and 10-20% of the tank water volume was changed daily.

The experiment was arranged in a completely randomized design (CRD) with six dietary treatments, each replicated three times. Each tank accommodated 30 cobia. The control group received a commercial diet without *S. baicalensis* supplementation, while the other groups received diets supplemented with varying levels of *S. baicalensis*, including 0.5, 1.0, 2.0, 3.0, and 5.0%. The feeding trial was conducted for 12 weeks.

Fish were fed twice daily at 09.00 and 17.00 at a rate of 5% of their body weight. Growth performance (weight and length) was measured every 2 weeks. Blood and organ samples were collected at weeks 2, 4, 6, 8, 10, and 12 for hematological and immunological analyses. After the 12-week feeding period, the fish were subjected to a bacterial challenge test using *Photobacterium damselae* subsp. *piscicida*. Mortality was recorded daily for 30 days post-challenge. Water quality parameters were assessed daily and maintained within the following ranges: temperature (26–32°C), dissolved oxygen (DO) >5 mg/L, salinity 30–34 ppt, pH 7.5–8.5, ammonia <0.5 mg/L, and nitrite <1.0 mg/L, following the established guidelines for cobia rearing (Rajaprabhu et al. 2021).

Plant Extract Preparation and Experimental Feed

The dried root of *S. baicalensis* was obtained from a local market in Neipu, Taiwan, and processed into a fine powder using a mechanical grinder. The resulting root powder was stored in an airtight container for future use. The extraction process involved combining 10 grams of the *S. baicalensis* root powder with 150 ml of distilled water. This mixture was heated to 95°C and then centrifuged at 1000 x g for 10 minutes at 4°C. The resulting clear supernatant was carefully separated and stored at -20°C for later use in the experiment (Zhao et al. 2016).

The commercial diet utilized in this study was obtained from a local fish market and included 45% protein and 8% fat. The supernatant of *S. baicalensis* root water extract was equally mixed with a commercial fish diet using six different concentrations for the treatments. The treated diet was then dried at 60°C for 24 hours to ensure that the extract was fully incorporated.

Photobacterium damselae dubsp. piscicida Culture

The virulent strain of *P. damselae* subsp*. piscicida* was obtained from the Tungkang Marine Biotechnology Research Station, Taiwan. The strain was cultured on Brain Heart Infusion (BHI) agar supplemented with 2% NaCl and incubated at 28°C for 24 hours. The bacterial colonies were then transferred to BHI broth, followed by centrifugation at 1000×g at 4°C for 5 minutes to harvest the bacteria. The pellet was washed twice with phosphate-buffered saline (PBS, pH 7.2). The bacterial concentration was determined using an Eppendorf BioSpectrometer (Germany) and adjusted to an optical density of 1.00 at 600 nm (OD600).

Sample Collection and Analysis

Three randomly selected fish from each tank were sampled at weeks 2, 4, 6, 8, 10, and 12 to collect blood serum for the assessment of humoral immune responses. Approximately 3 mL of blood was drawn from the caudal vein using a heparinized syringe containing 0.5 M EDTA as an anticoagulant. The samples were centrifuged at 400×g for 30 minutes at 20°C to

obtain clear serum. Three random spleen samples were collected from fish in each tank by dissecting them from the caudal vein to the lateral line to assess the cytokine-related gene of cobia. The cytokine-relate gene from the spleen was drawn during the experimental feed in weeks 2, 6, and 8. Spleen tissue samples were placed in 1.5 mL microcentrifuge tubes containing 500 µL of RNA later and stored at -80°C for future analysis (Byadgi et al. 2018).

Fish Growth Performance

Growth performance were assessed at 15-day intervals using equations described by Strand (2005). Feed amounts were adjusted based on fish body weight throughout the experiment. Daily observations included clinical lesions, behavior changes, and mortality. Growth performance parameters were observed in this study following the equation below:

$$SGR = \frac{\text{Ln (Fw(g)) – ln (Iw(g))}}{\text{Total duration of experiment (days)}} x100 \quad (1)$$

Where, SGR is specific growth rate, Fw is final weight, Iw is initial weight.

$$FCR = \frac{\text{Total amount of feed (g)}}{\text{Weight gain (g)}} \quad (2)$$

Where, FCR is feed conversion ration.

$$WGR (\%) = \frac{\text{Fbw(g) – Ibw(g)}}{\text{Ibw(g)}} x100 \quad (3)$$

Where, WGR is weight gain rate, Fbw is final body weight, Iw is initial body weight.

Fish Challenge

After 12 weeks of administering the treatment feed, the remaining fish (n=540) were challenged with 100 µL of a PBS suspension containing *P. damselae* subsp. *piscicida* at a concentration of 3.16×10^5 CFU/mL per gram of body weight via intraperitoneal injection with a predetermined LD50. The challenge dose was determined based on a previously conducted LD_{50} test (unpublished data). Mortality and survival rates were monitored and recorded for up to 30 days post-injection. No further blood or organ sampling was conducted after the challenge, as this phase focused solely on assessing post-infection survival outcomes.

Cobia Humoral Immune Responses

The total myeloperoxidase (MPO) content in serum was measured using 90 µL of Hanks' Balanced Salt Solution (HBSS) without Ca^{2+} or Mg^{2+} in 96-well plates, following the method of Sahoo et al. (2005). The diluted serum was mixed with 40 µL of 3,3',5,5'-tetramethylbenzidine (TMB) hydrochloride containing H_2O_2 (Amresco). Peroxidase activity was assessed colorimetrically, as described by Quade and Roth (1997). The serum was diluted with 50 µL of Dulbecco's Phosphate-Buffered Saline (DPBS, Invitrogen) and mixed with 10 µL of TMB (Amresco). The reaction was carried out in the dark, and myeloperoxidase activity was recorded at 5-minute intervals at 450 nm using an ELISA reader.

Lysozyme activity was determined using a turbidimetric assay with minor modifications. Fish serum (20 µL) was combined with 180 µL of Micrococcus lysodeikticus suspension (OD 0.5 at 450 nm) in 0.1 M PBS (pH 7.2) at 25°C. OD readings were recorded at 450 nm using a spectrophotometer, 30 seconds and 30 minutes after adding *M. lysodeikticus*. Lysozyme activity was expressed as units per mL of serum, with one unit defined as a reduction of 0.001/min in OD (Byadgi et al. 2018).

Cytokine-related Genes in the Spleen

The expression of immune-related genes, including Toll-like receptor-9 (TLR-9), Interleukin-1β (IL-1β), Immunoglobulin M (IgM), and Tumor Necrosis Factor-α (TNF-α), was analyzed in the spleen of cobia. Total RNA was extracted from spleen tissue using Trizol® reagent (Invitrogen, USA) with minor modifications. RNA concentration was quantified using a UV spectrophotometer (Thermo Fisher Scientific) at 260/280 nm, and integrity was confirmed by visualizing the 28S and 18S ribosomal RNA bands. First-strand complementary DNA (cDNA) was synthesized from 2 µg of total RNA using M-MuLV reverse transcriptase (Lucigen) according to the manufacturer's protocol. The synthesized cDNA was stored at -20°C for subsequent analysis.

Real-time PCR was conducted to analyze the expression of TLR-9A (#KC180322), IL-1β (#AY641829), IgM (#JX025102), and TNF-α (#KX024710.1) using an ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA) (Table 1). Amplifications were performed in a 10 µL reaction volume consisting of 5 µL of SYBR Premix Ex Taq (Tli RNaseH Plus) (Takara Bio Inc.), 50× ROX reference dye, 0.2 µL of each primer, cDNA template, and 3.4 µL of DEPC-treated water. The PCR cycling conditions were performed as specified. Gene expression was quantified using the 2^–ΔΔCt method (Livak and Schmittgen 2001), and results from the treatment groups were compared to those of the control group.

Quantitative gene expression analysis was conducted using a Flex Cycler2 base unit (Germany). The amplification reaction consisted of 5 µL of 10x Taq buffer (Ebio), 1 µL of 10 mM dNTPs (Kappa Biosystems), 1.5 µL of 50 mM $MgCl_2$, 2 µL of cDNA, 5 µL of each primer, 1 µL of Taq enzyme (Ebio), and 29.5 µL of DEPC-treated water, with a total reaction volume of 50 µL. A no-template control (NTC) was included in every qPCR

assay. The qPCR cycling conditions were as follows: initial denaturation at 95ºC for 1 minute, followed by 35 cycles of denaturation at 95ºC for 30 seconds, annealing at 54ºC for 30 seconds, and extension at 72ºC for 1 minute, with a final extension at 72ºC for 5 minutes.

Real-time PCR was performed using the ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems, USA). The reaction mixture included 5 µL of SYBR® Premix Ex Taq™ (Tli RNaseH Plus) (Takara Bio Inc.), 0.2 µL of ROX reference dye (50x), 0.2 µL of each primer, 1 µL of cDNA template, and 3.4 µL of DEPC-treated water, for a total volume of 10 µL. The real-time PCR cycling conditions were as follows: initial denaturation at 95ºC for 1 minute, followed by 40 cycles of denaturation at 95ºC for 15 seconds, and annealing/extension at 60ºC for 60 seconds.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9 software with two-way ANOVA (analysis of variance), and Tukey's test at 0.05 was used to test the level of significance difference between samples. All data are given as means±SD.

Results and Discussion

Growth Performance and Survival Rate

Figure 1 depicts the growth measures of cobia over 12 weeks, and the data reveal a clear pattern of growth enhancement with *S. baicalensis* extract added to the feed. Over time, all treatments demonstrated a steady rise in fish growth, with 1% *S. baicalensis* producing the highest results. Feed is a fish's principal source of nutrients and energy, which influences its growth, immunological system, and metabolic efficiency (Navaraj Principal et al. 2016). Feed formulations with active chemicals, including *S. baicalensis*, can boost fish growth and immunity (Chmiel and Stompor-Gorący, 2023). *S. baicalensis* includes several bioactive substances that have been found to improve fish performance. The principal active components, baicalin and baicalein, can result in a minor rise in fish-specific

Table 1. Primer sequence (forward and reverse) of target

Gene target	Gene Bank Accession	Product size (bp)	Primer	Sequence (5' - 3')
TLR9	KC180322	160	F	TCTGTTCCATCTTGGCTGTG
			R	CTGGTTTCTGGTGTCAAACA
TNF-α	KX024710	339	F	AACCGGCCTCTACTTTGTCT
			R	CGCCATCACTGCAGGAGACT
IL-1β	AY641829	170	F	CAGGCAGAACAACCACTGAC
			R	TTCCAAGTCCAGTCCTTTGG
IgM	JX025102	182	F	AGACAGCCTGCAGGGAAAAG
			R	TGTTCCTTTCCCCCAGTAGT
β-actin 3	HM754627	160	F	ACAGACTGTTCCTCCTCCCC
			R	AAAATCCTGAGTCAAGCGCC

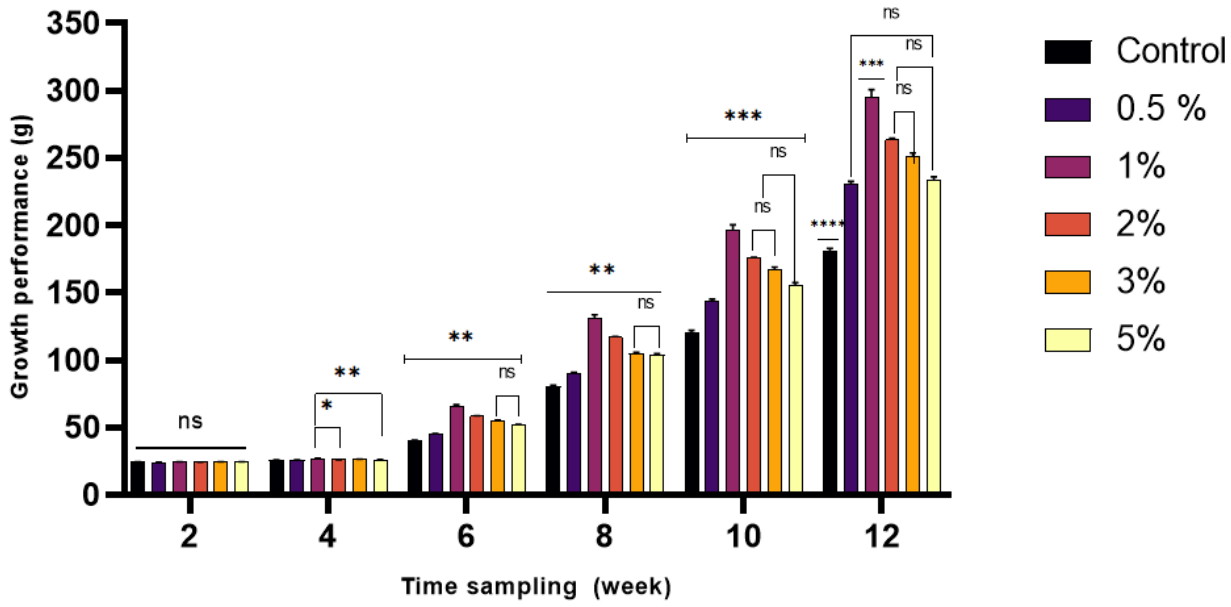

Figure 1. Cobia growth performance (g) after 90 days of culture. Control indicates fish with a regular diet (without *S. baicalensis*).

growth rate and a significant improvement in feed efficiency (Jia et al. 2021).

Table 2 shows that cobia fed with 1% *S. baicalensis* extract had the highest weight gain (270.79 g) and SGR (2.77% BW day^{-1}), outperforming the 2% and 3% treatments, as well as the control (156.06 g; 2.36% BW day^{-1}). All treatments group showed better growth than the control. Similarly, Xia et al. (2021) found that incorporating *S. baicalensis* into fish feed can promote growth and increase the nutritive value of rabbitfish (*Siganus fuscescens*).

Some medicinal plant extracts work as natural immunostimulants, increasing fish immunity and lowering the risk of infections. When fish are healthy, less energy is directed to combat diseases, leaving more energy for growth (Mbokane and Moyo, 2022). Herbal medicines' major function in feeding is to improve flavour, which impacts eating habits, digestive juice generation, and overall feed intake. According to Dauchak and Nikki (2024), olfactory feed components promote fish growth by boosting feed intake, reducing osmotic stress, and improving digestive enzymes, resulting in higher growth and efficiency. In this study, the extract also improved feed efficiency, with all treatments showing a lower FCR (1.40) than the control (1.68), indicating better feed utilisation and potential for sustainable aquaculture.

Disease Control by *Scutellaria baicalensis*

The survival rate data show the effect of *S. baicalensis* supplementation on cobia following bacterial injection. As illustrated in Figure 2, the control group exhibited the sharpest loss in survival, with nearly all individuals dying by day seven. This implies a lack of resistance to the injected pathogen in the absence of

Table 2. Weight gain, specific growth ratio (SGR), feed conversion ratio (FCR), and survival (%) of the healthy cobia-fed diets containing different concentrations of *S. baicalensis* root at the end of the experiment

Treatments	Final body weight (g)	Weight gain (g)	SGR (% BW day^{-1})	FCR (g dry feed/g gain)	Survival (%)
Control	180.61±7.5^{a}	156.06±2.19^{a}	2.22±0.005^{a}	1.68^{b}	100
0.5%	230.6±9.4^{b}	206.34±1.8^{b}	2.50±0.0008^{b}	1.4^{a}	100
1%	295.3±14.6^{c}	270.79±5.5^{c}	2.77±0.023^{a}	1.4^{a}	100
2%	263.46±10.8^{bc}	239.16±1.15^{bc}	2.65±0.001^{ab}	1.4^{a}	100
3%	251.8±10^{bc}	227.38±1.71^{bc}	2.59±0.001^{ab}	1.4^{a}	100
5%	233.67±10.15^{b}	209.37±2.18^{b}	2.51±0.001^{b}	1.5^{ab}	100

Values are expressed as means±standard deviations of three replications. Different superscript letters within the same column indicate significant differences (P<0.05).

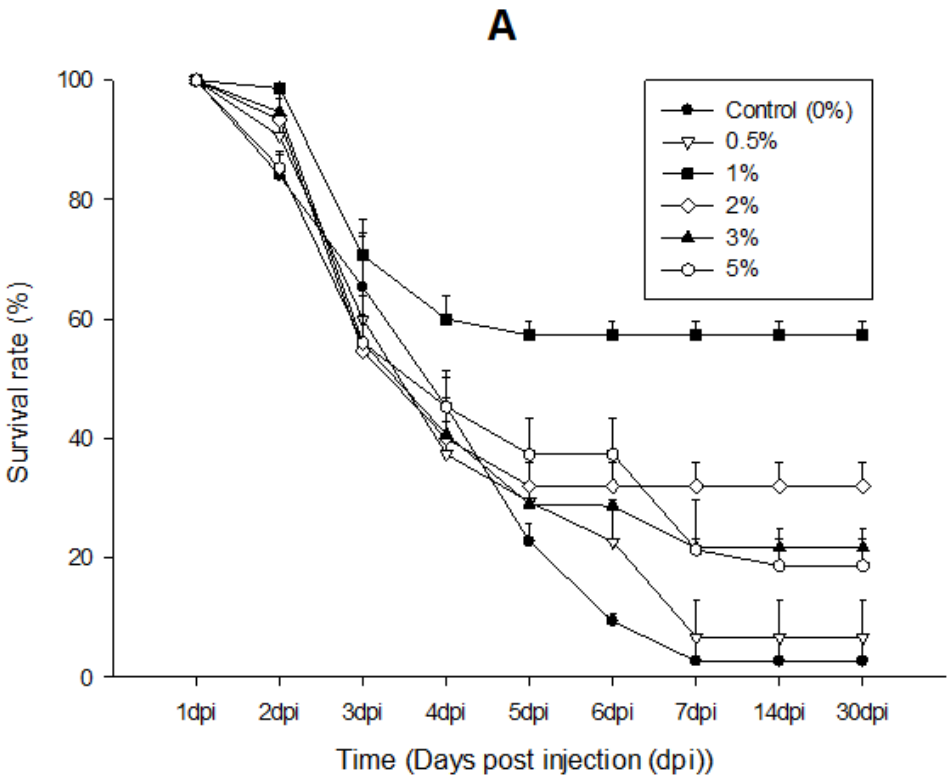

Figure 2. The survival rate of cobia after *Photobacterium damselae* subsp. *pisicicda* injection. Each bar represents the mean value with standard deviation (SD) (P<*0.05*).

supplementation. In contrast, 1% and 2% supplementation yielded the highest survival rates (80–90%), indicating optimal protection. While 0.5% showed moderate improvement, higher doses (3% and 5%) were less effective, suggesting that excessive supplementation may cause immune or metabolic stress.

S. baicalensis root extract has strong antibacterial properties that protect cobia from infection. Natural antibacterials can disrupt cell walls, protein synthesis, and quorum sensing (Dauchak and Nikki, 2024) In this study, 1% supplementation gave the highest post-challenge survival, while higher doses offered no added benefit and slightly reduced survival, highlighting the importance of proper dosage for optimal immune response. Various plant extracts are known for their antibacterial properties and are widely used in aquaculture disease control (Sumathi et al. 2018; Razak et al. 2019; Duyen et al. 2022; Harikrishna et al. 2022; Mohamed 2023). Xia et al. (2020) observed that the leaf and stem extracts of *S. baicalensis* have antibacterial and anti-inflammatory properties against bacterial pathogens in aquaculture. However, the antibacterial action of *S. baicalensis* radix is confined to human pathogens (Zhang and Hu 2015; Yue Zhou et al. 2015). Zhou et al. (2015), noted that flavonoids in the extract, once converted to aglycons by gut bacteria, significantly enhance antibacterial activity.

Humoral Immune Responses

Figure 3 shows that S. baicalensis supplementation significantly increased cobia's humoral immune responses (MPO, peroxidase, lysozyme), with 1% yielding the strongest effect. Higher doses (e.g., 5%) were less effective, suggesting a plateau or negative impact. After bacterial challenge at week 10, the control group showed notably weaker immune responses compared to the supplemented groups. Humoral immunity involves antibodies, complement proteins, and antimicrobial peptides. Neutrophils in fish blood play a key role, with MPO acting as a marker of their activity and a crucial enzyme for fighting infections (Baba et al. 2015; Buchan et al. 2019; Gan et al. 2023). MPO enhances microbicidal function by converting hydrogen peroxide into toxic antimicrobial compounds (Chi et al. 2017). Previously, Gan et al. (2023) reported that pathogen injection significantly reduced MPO expression in *Paralichthys olivaceus* due to pathogen interaction with resident peritoneal cells and susceptible immunological tissue in the peritoneal cavity. This study found that *S. baicalensis* supplementation increased MPO activity in infected cobia, suggesting enhanced immune defense.

MPO cells produce active peroxidase, a heme enzyme that neutralizes ROS and reduces oxidative stress (Buchan et al. 2019; Javed et al. 2020; Andrés et

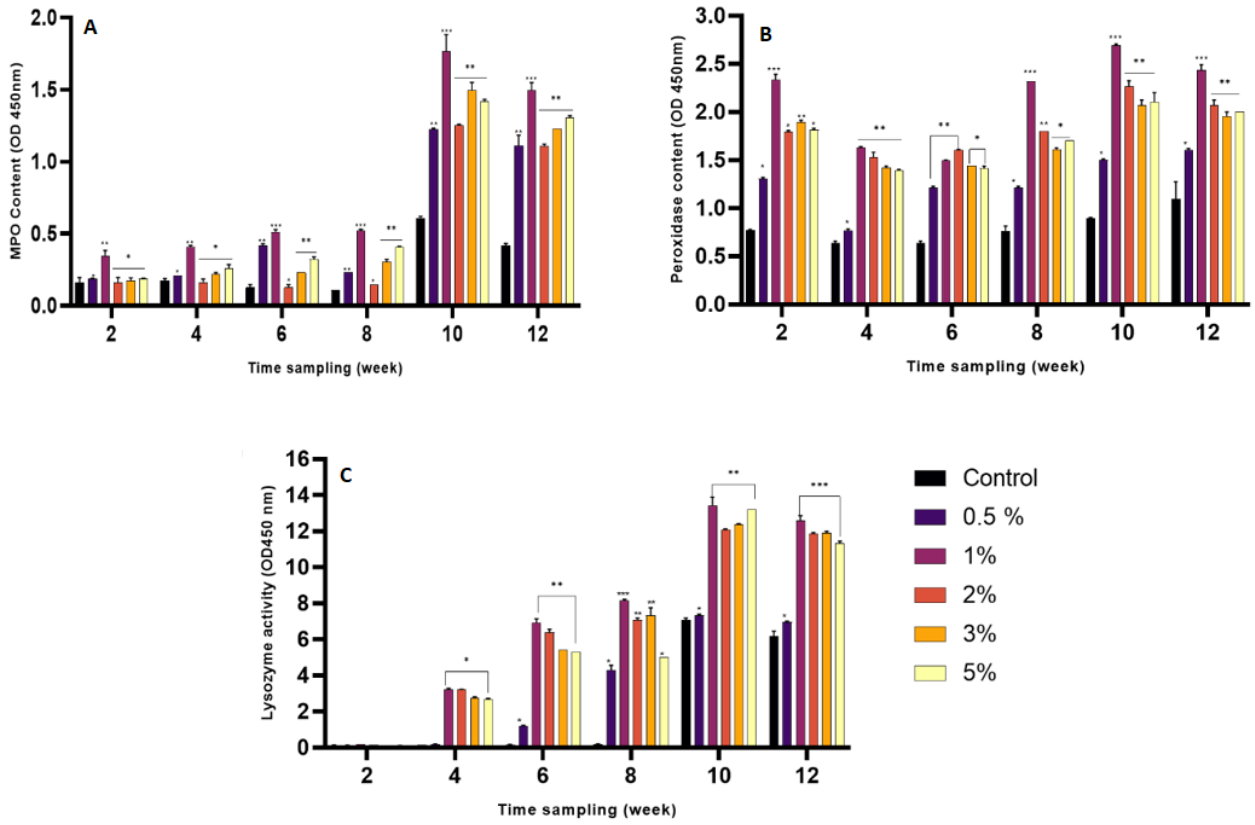

Figure 3. Humoral immune response content in cobia blood serum depends on diets supplemented with *Scutellaria baicalensis* at various doses. A: myeloperoxidase; B: peroxidase activity; C: lysozyme activity. Control indicates infected fish with a regular diet (without *S. baicalensis*). Three infected fish in each treatment were sacrificed at 2, 4, 6, 8, 10, and 12 weeks. Results are presented as mean±SEM. Two-way analysis of variance was used to compare the gene expression levels (* P<0.05, ** P<0.01, *** P<0.001).

al. 2022). Peroxidase plays a key role in the antioxidant defense system alongside other enzymes like catalase and superoxide dismutase (Sabir et al. 2018). In this study, *S. baicalensis* supplementation enhanced peroxidase activity in infected fish, helping counteract oxidative stress. Baicalin a flavonoid in *S. baicalensis*, is known to boost antioxidant enzymes in serum and liver, supporting fish health and growth (Yefei Zhou, Mao, and Zhou 2019). Lysozyme is a key enzyme in innate immunity, offering protection against infections (Ferraboschi et al. 2021). Thus, enhanced lysozyme activity protects fish from various infections and provides a higher level of disease resistance (Yengkhom et al. 2019). This study showed that *S. baicalensis* supplementation boosts lysozyme production in cobia, enhancing their immune defense. Similar results were reported in catfish and Nile tilapia, where herbal extracts like *Solanum trilobatum*, *Ocimum tenuiflorum*, turmeric, and rosemary improved immune parameters, including phagocytic activity and MPO levels (Subeenabegum and Navaraj 2016; Hassan et al. 2018).

Cytokine-Related Gene Expression

Medicinal plants enhance immunity by stimulating cytokine production (Dauchak and Nikki 2024). Cobia fed with *S. baicalensis*-supplemented diet showed higher expression of cytokine-related genes in the spleen, especially in the 1% group (Figure 4). Expression peaked at week 4, indicating strong immune activation, then slightly declined after week 6—more notably in higher doses—but remained above control levels.

Fish immunity relies heavily on innate responses, which are crucial in early infection stages and primarily involve phagocytes and humoral factors (Sakai et al. 2020). Cytokines are a group of low-molecular-weight proteins that help coordinate the immune response throughout the body. They are often glycosylated and secreted by activated immune cells when prompted by certain pathogens (Binesh and Venkatachalam 2019). Moreover, interleukin is a pro-inflammatory cytokine that promotes innate immunity by activating lymphocytes and phagocytic cells, hence increasing resistance to bacterial infection (Sakai et al. 2020). Interleukins like IL-1β activate lymphocytes and phagocytes, boosting resistance to infectionis. According to Binesh and Venkatachalam (2019), TLRs are non-catalytic, membrane-spanning receptors that recognise PAMPs and begin signalling cascades to enhance innate immunity, culminating in the production of cytokine-related genes such as IL-1β and TNF-α. TNF-α increases granzyme production in nonspecific

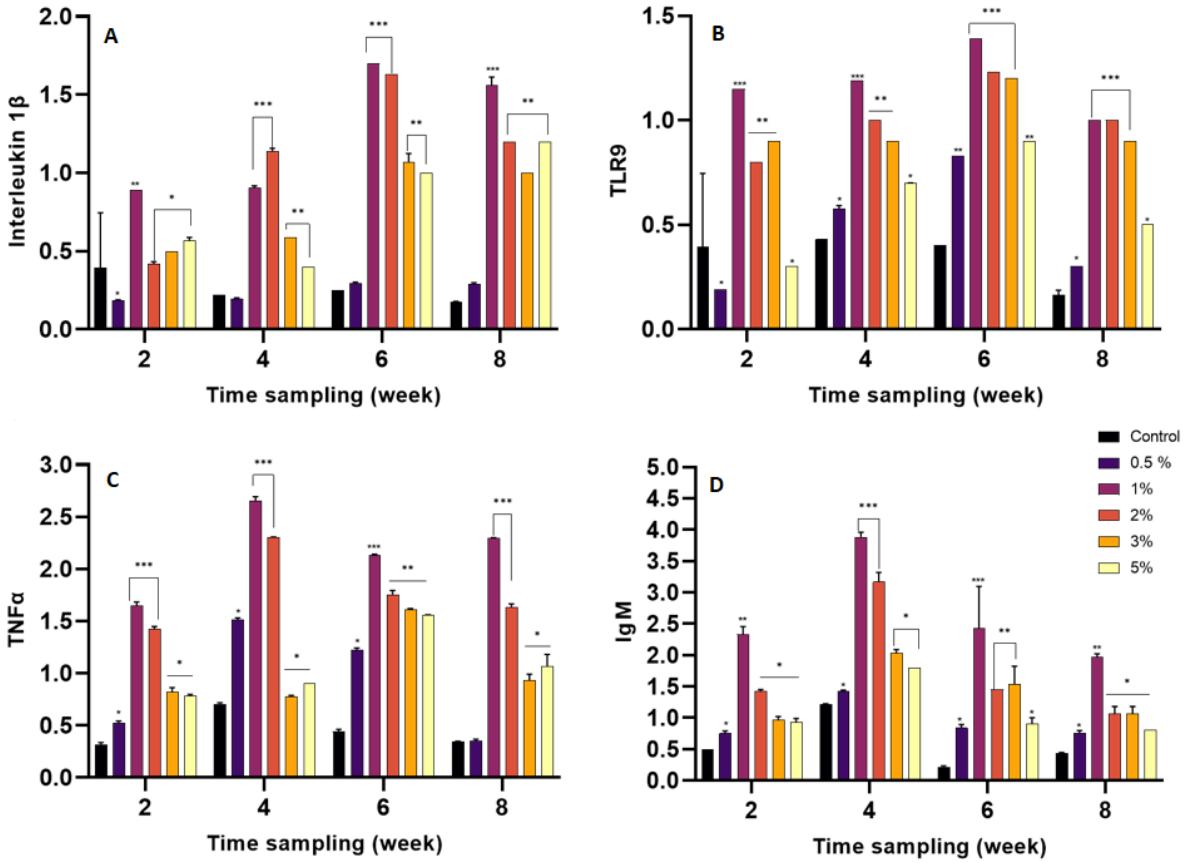

Figure 4. The expression of cytokine-related genes in the cobia spleen was determined using QRT-PCR. A: interleukin-β(IL-1β); B: toll-like receptor-9 (TLR9); C: tumor necrosis factor-α (TNF-α); and D: immunoglobulin M (IgM). The fold change comparison to the control was presented as the expression levels were normalized by β-actin. Control indicates infected fish with a regular diet (without *S. baicalensis*). The results are presented as mean+SEM. Two-way analysis of variance was used to compare the gene expression levels (* P<0.05, ** P<0.01, *** P<0.001).

cytotoxic cells, shielding them against activation-induced death (Sakai et al. 2020). TNF-α, another key cytokine, promotes inflammation, enhances phagocyte activity, supports macrophage survival, and restricts bacterial growth (Zou and Secombes 2016). Together, IL-1β and TNF-α play vital roles in cobia's immune defense against bacterial infections.

Plant extracts can stimulate the growth of immune-related organs, like the thymus and spleen (Jiang et al. 2021). Cytokine-related genes expression reflects immune status and response. In this study, cobia fed 1% S. baicalensis showed the highest up-regulation of TLR-9A, IL-1β, IgM, and TNF-α in the spleen, followed by the 2% group, indicating strong immune activation. Compounds in *S. baicalensis* have been shown to enhance enzyme activity (e.g., superoxide dismutase, catalase), reducing oxidative stress and improving immunity (Guo et al. 2023). IgM which helps block pathogen entry via mucus barriers, is highly expressed in the spleen—a key immune organ and blood filterlimits pathogen entrance through the innate immune system's physical barriers (mucus) (Mokhtar et al. 2023; Tran et al. 2020).

In the current work, we discovered that oral treatment of 1% *S. baicalensis* extract to cobia efficiently improves growth performance, increases antibacterial activity, and boosts immunological response. This is inextricably linked to the bioactive components of *S. baicalensis* radix, which have therapeutic potential. *S. baicalensis* offers strong anti-inflammatory, antibacterial, antioxidant, and immunomodulatory benefits to cobia fish against bacterial infections. Thus, the use of this plant extract in fish feed might be regarded as an effective and efficient disease control strategy.

Conclusion

This study has demonstrated that dietary supplementation with a 1% concentration of *S. baicalensis* root water extract significantly enhances fish growth performance and reduces the feed conversion rate. A 1% *S. baicalensis* root water extract diet improves disease resistance against *Photobacterium damselae* subsp. *piscicida* in cobia. This study discovered the potential of *S. baicalensis* in managing bacterial infections and enhancing the growth performance of cobia. It can also be applied in the cobia culture industry as an immunostimulant with excellent efficacy, low cost, and ease of practice. However, future studies are needed to determine which bioactive compound of the *S. baicalensis* root water extract is responsible for the activity.

Ethical Statement

This study was conducted following ethical guidelines for animal research and aquaculture practices. All experimental procedures involving fish (Rachycentron canadum) were reviewed and approved by the Animal Ethics Committee of Tungkang Aquaculture Research Center for Animal Biologics under. The handling, feeding, and bacterial challenge experiments were conducted in accordance with the guidelines for the care and use of laboratory animals and followed the principles outlined in the ARRIVE guidelines.

No human subjects were involved in this study. The research adhered to the ethical principles of scientific integrity and animal welfare, minimizing fish suffering and ensuring humane treatment throughout the experiment.

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Author Contribution

First Author: Conceptualization, Experimental design, laboratory analysis, methodology, data collection, formal analysis, writing—original draft preparation.

Second Author : Data curation, manuscript revision, writing—review and editing

Third Author : Investigation, manuscript revision, writing—review and editing

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Conflict of Interest

The authors declare no conflict of interest.

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