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Research article

Histopathological characterization of age-related intestinal lesions and fibrosis in naturally coccidia-infected Asian sea bass *(Lates calcarifer)*

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Abstract

Intestinal coccidiosis is an important protozoan disease in marine teleosts, particularly under aquaculture conditions. In juvenile Asian sea bass (*Lates calcarifer*), infection leads to severe intestinal injury accompanied by inflammation and fibrotic remodeling. This study aimed to characterize inflammatory and fibrotic responses to piscine intestinal coccidia in fish at different developmental stages. A total of 88 intestinal samples from naturally infected juvenile Asian sea bass aged 60 and 90 days (44 samples per group) were collected from marine cage farms in Satun Province, Thailand. Intestinal tissues were processed using standard histological methods, stained with hematoxylin and eosin (H&E) for lesion evaluation, and Picro-Sirius Red for fibrosis assessment. Lesion severity (inflammation, extended inflammation, necrosis, congestion, and desquamation) was graded semi-quantitatively, and fibrotic areas were quantified under polarized light. Statistical comparison between age groups was performed using the Mann–Whitney U test. The 60-day-old fish exhibited significantly higher levels of inflammation ($p = 0.000$), extended inflammation ($p = 0.001$), necrosis ($p = 0.038$), and congestion ($p = 0.000$) compared to the 90-day-old group, whereas desquamation did not differ significantly ($p = 0.685$). In contrast, fibrosis was more pronounced in the 60-day-old fish ($p < 0.05$), indicating stronger fibrotic activity associated with persistent inflammation. Intestinal coccidiosis in juvenile Asian sea bass induces marked inflammatory injury in younger fish and subsequent fibrotic remodeling in older fish. These findings highlight the dynamic progression of intestinal pathology and emphasize the importance of early disease management to minimize long-term intestinal damage in aquaculture systems.

Keywords: Aquaculture pathology, Coccidiosis, Histopathology, Intestinal fibrosis, *Lates calcarifer*.

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INTRODUCTION

Asian sea bass (*Lates calcarifer*) is a commercially important species native to tropical and subtropical regions of Southeast Asia and Oceania (Katersky and Carter, 2007), contributing significantly to food security and aquaculture economies (Islam et al., 2024). Countries such as Malaysia, Indonesia, Taiwan, and Thailand are among the leading producers, with Hong Kong, Singapore, and Australia also maintaining active commercial cultivation (Yusoff, 2014; Alliance, 2016; Islam et al., 2023). Intensive cage farming has enhanced production efficiency but concurrently increased the risk of infectious diseases, notably intestinal coccidiosis in marine-cultured fish (Sornying et al., 2025). Coccidiosis is a major disease affecting juvenile fish, and outbreaks in hatcheries have been associated with severe enteritis and high mortality in Asian sea bass (Suyapoh et al., 2022; Thongrin et al., 2025). The disease is characterized by marked intestinal inflammation, necrosis, congestion, and epithelial desquamation (Dyková and Lom, 2006; Jantrakajorn et al., 2025). Although most infections in wild fish are subclinical, several studies have demonstrated that intestinal coccidia can cause extensive damage to the intestinal wall, extending to the muscular layer (Suyapoh et al., 2022; Suyapoh et al., 2024). Such severe tissue injury necessitates secondary repair through fibrotic remodeling, leading to the development of intestinal fibrosis (Rieder et al., 2007).

Fibrosis represents a critical pathological outcome of chronic inflammation and dysregulated tissue repair (Ku et al., 2024). It arises through complex cellular interactions among epithelial, endothelial, and inflammatory cells that stimulate the activation of myofibroblasts, the principal effectors of extracellular matrix (ECM) deposition (Wang et al., 2021). Persistent fibrosis disrupts organ architecture and function, often leading to irreversible failure (Bai et al., 2019). In fish, hepatic and cardiac fibrosis have been documented in association with stress, environmental factors, and pathogenic insults (Cao et al., 2023). However, the mechanisms linking chronic inflammation and fibrotic remodeling in fish intestinal tissues remain poorly understood. Once initiated, fibrosis may progress independently of the initial inflammatory trigger, underscoring the importance of early detection and intervention (Johnson et al., 2012; Rieder et al., 2012). Previous studies have reported that age-related differences in immune maturity can influence intestinal responses and fibrogenesis in fish (Suyapoh et al., 2024). However, the link between coccidial infection, infection duration, fish age, and fibrotic remodeling remains unclear, particularly in Asian sea bass. Therefore, this study aimed to clarify these relationships.

To better elucidate the fibrotic response associated with piscine intestinal coccidiosis, this study investigated the relationship between fish age and intestinal pathology in juvenile Asian sea bass using histochemical approaches. Previous reports demonstrated higher infection intensity and poorer health condition scores in 60-day-old compared to 90-day-old fish, accompanied by marked goblet cell hyperplasia (Suyapoh et al., 2024; Thongrin et al., 2025). Building on these findings, the present study focuses on intestinal fibrosis as a potential contributor to tissue dysfunction in coccidia- infected fish. Understanding the mechanisms and progression of intestinal fibrosis in fish may provide insights into the broader pathogenesis of fibrotic diseases and inform preventive strategies in aquaculture.

This study provides the first histopathological characterization of age-related intestinal fibrosis in naturally coccidia-infected Asian sea bass, offering novel insight into how developmental stage influences the inflammatory-fibrotic continuum in fish aquaculture systems.

MATERIALS AND METHODS

Ethics statement

Juvenile Asian sea bass were obtained from marine aquaculture farms in Satun Province, Thailand, for experimental investigation. All experimental procedures were approved by the Institutional Animal Care and Use Committee, Prince of Songkla University, Thailand (approval no. MHESI 68014/1731, October 07, 2022) and conducted in accordance with the national guidelines for the ethical care and use of animals in scientific research in Thailand.

Experimental design

Eighty-eight intestinal samples were collected from juvenile Asian sea bass aged 60 and 90 days (44 samples per age group) from a marine cage farm in Satun Province, Thailand. Fish were randomly selected from naturally infected populations exhibiting mild to moderate clinical signs, characterized by reduced feeding activity without moribundity, and no fish were excluded based on body size or physical condition. Identification of piscine intestinal coccidia was performed using a Nikon advanced upright microscope equipped with a digital video capture system (ECLIPSE Ni-U; Nikon, Tokyo, Japan), following established protocols for examining parasitic developmental stages (Suyapoh et al., 2022). All morphological identifications were verified by a qualified parasitologist. In addition, all intestinal sections were stained with hematoxylin and eosin (H&E) to evaluate inflammation and associated lesions, and with Picro-Sirius Red to assess fibrotic changes.

Histochemistry and semi-quantitative study of pathology and fibrosis

The intestinal samples were fixed in 10% neutral buffered formalin for 72 hours and subsequently processed using standard histological procedures (Suyapoh et al., 2025). Paraffin-embedded sections were stained with hematoxylin and eosin (H&E) to evaluate tissue architecture and structural integrity. In addition, histochemical staining with Picro-Sirius Red (Abcam, UK) was performed to visualize and quantify fibrotic changes (Suyapoh et al., 2021). Histopathological alterations, including inflammation, extended inflammation, necrosis, congestion, and epithelial desquamation, were assessed using a semi-quantitative grading system based on established criteria (Watakulsin et al., 2023). Lesion severity was categorized as absent (no lesion or ≤1% affected area), mild (2–25%), moderate (26–50%), or severe (>50%). Fibrosis was quantitatively evaluated using ImageJ software (Schneider et al., 2012) by analyzing ten non-overlapping microscopic fields per sample under a light microscope. Lesion scoring was independently performed by two evaluators, and inter-rater reliability was confirmed by consistent scoring results (>90% agreement).

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics® version 23.0 (SPSS Inc., USA). The histological scores of intestinal variables were tested for normality using the Shapiro–Wilk test. As the data did not follow a normal distribution, the Mann–Whitney U test was applied to compare differences between the 60-day and 90-day fish groups. Statistical significance was set at $p < 0.05$.

RESULTS

Intestinal inflammation and lesions

Histopathological examination of the intestines revealed five major types of lesions associated with piscine intestinal coccidiosis: mucosal and submucosal inflammation, extended inflammation, vascular congestion, necrosis, and epithelial desquamation. Mucosal and submucosal inflammation was characterized by diffuse to multifocal infiltration of lymphocytes, macrophages, and eosinophilic granular cells within the lamina propria and submucosal connective tissue (Figure 1a).

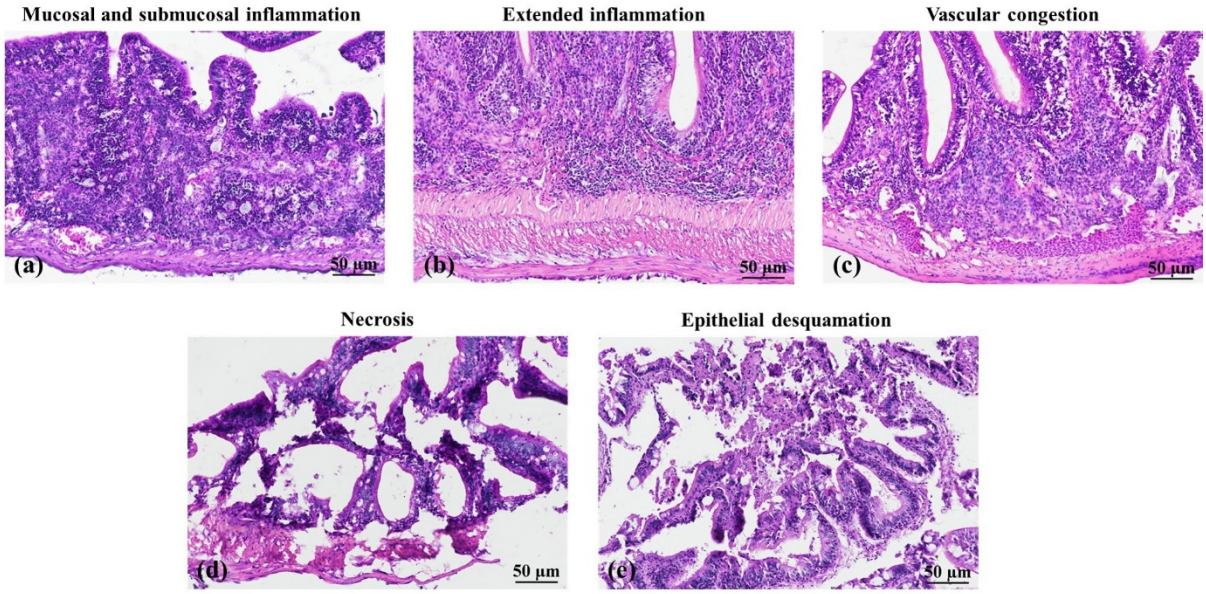

Figure 1 Representative histopathological lesions of the intestine in juvenile Asian sea bass (*Lates calcarifer*) naturally infected with piscine intestinal coccidia. (a) Mucosal and submucosal inflammation showing diffuse infiltration of lymphocytes, macrophages, and eosinophilic granular cells within the lamina propria and submucosa. (b) Extended inflammation with inflammatory cell infiltration extending into the muscularis layer, accompanied by fibroblast proliferation and early fibrotic deposition. (c) Vascular congestion characterized by engorgement of capillaries and venules within the mucosa and submucosa, often associated with hemorrhage and edema. (d) Necrosis showing focal to multifocal epithelial degeneration, nuclear pyknosis, and accumulation of necrotic debris in the intestinal lumen. (e) Epithelial desquamation with partial detachment and sloughing of the mucosal epithelium into the lumen. Hematoxylin and eosin (H&E) stain. (a-e = H&E, original magnification; a-e = ×40, scale bar = 50 µm).

In severely affected areas, inflammatory cells aggregated around parasitic developmental stages embedded in the epithelial lining, often accompanied by mucosal edema and mild distortion of intestinal villi. Extended inflammation was observed when the inflammatory process penetrated beyond the mucosal layer into the muscularis, leading to partial disruption of the muscular structure (Figure 1b). This lesion was commonly accompanied by fibroblast proliferation and mild fibrotic tissue deposition, indicating chronic inflammatory progression. Vascular congestion appeared as engorgement of capillaries and venules within the mucosa and submucosa, frequently associated with hemorrhage and edema in the adjacent tissues. The congested vessels often occurred in regions with dense inflammatory

infiltration, suggesting vascular compromise secondary to inflammation (Figure 1c). Necrosis was identified as focal to multifocal loss of epithelial integrity, cytoplasmic degeneration, and pyknotic nuclei of enterocytes (Figure 1d). In advanced lesions, necrotic debris accumulated in the intestinal lumen, occasionally admixed with parasitic remnants and inflammatory cells. Epithelial desquamation was manifested by partial detachment or sloughing of the superficial epithelium into the intestinal lumen, frequently associated with necrotic or inflamed mucosal regions (Figure 1e).

The comparison between the two age groups revealed that 60-day-old fish exhibited significantly greater intestinal inflammation than the 90-day-old group ($p < 0.000$) (Figure 2a). Extended inflammation, characterized by inflammatory cell infiltration extending into the muscularis layer, was also markedly more severe in the 60-day-old fish ($p < 0.001$) (Figure 2b). Similarly, vascular congestion was significantly increased in the 60-day-old fish, indicating more severe circulatory disturbance within the intestinal wall ($p < 0.000$) (Figure 2c). Necrotic lesions were more prominent in the younger group, showing greater epithelial degeneration and tissue loss compared to the 90-day-old fish ($p = 0.038$) (Figure 2d). In contrast, epithelial desquamation did not differ significantly between the two age groups ($p = 0.685$) (Figure 2e). The corresponding statistical details are presented in Table 1

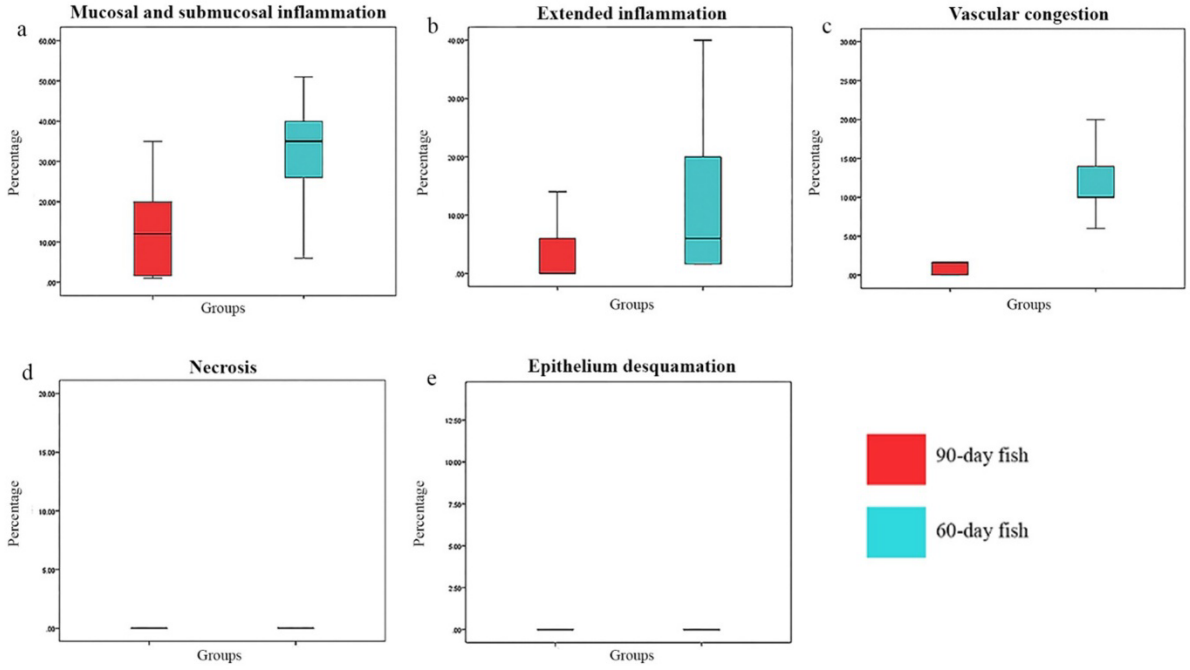

Figure 2 Comparison of histopathological lesion severity between 60-day-old and 90-day-old juvenile Asian sea bass (*Lates calcarifer*) naturally infected with piscine intestinal coccidia. (a) Intestinal inflammation showing significantly higher severity in the 60-day group ($p < 0.000$). (b) Extended inflammation with inflammatory cell infiltration extending into the muscularis layer, more pronounced in 60-day fish ($p < 0.001$). (c) Vascular congestion with engorged capillaries and venules, markedly increased in the 60-day group ($p < 0.000$). (d) Necrosis characterized by epithelial degeneration and tissue loss, significantly greater in younger fish ($p = 0.038$). (e) Epithelial desquamation showing no significant difference between age groups ($p = 0.685$). Data are expressed as median lesion scores; error bars represent interquartile ranges.

Table 1 Semi-quantitative comparison of intestinal lesion severity between 60-day-old (SF) and 90-day-old (BF) juvenile Asian sea bass (*Lates calcarifer*) naturally infected with piscine intestinal coccidia. Lesions evaluated included intestinal inflammation, extended inflammation, necrosis, vascular congestion, and epithelial desquamation. Data are presented as mean rank values, and statistical significance was determined using the Mann–Whitney U test ($p < 0.05$)

Lesions (percentage)	Mean rank of BF	Mean rank of SF	P value
Inflammation	14	31	0.000
Extended inflammation	16.19	26.81	0.001
Necrosis	20.50	24.50	0.038
Congestion	14.75	30.25	0.000
Desquamation	23	22	0.685

Intestinal fibrosis

Intestinal fibrosis is characterized by the excessive deposition of collagen and other extracellular matrix components within the intestinal wall, typically resulting from chronic inflammation and tissue injury. In this study, fibrotic areas appeared as red-stained collagenous tissue under light microscopy following Picro-Sirius Red staining. The distribution pattern of fibrosis revealed that only the 60-day-old fish exhibited fibrous accumulation extending from the submucosa to the muscularis layer, closely associated with regions of active inflammation, whereas no such fibrotic development was observed in the 90-day-old fish (Figure 3a). Quantitative image analysis demonstrated that the mean intensity of fibrosis was significantly higher in the 60-day-old fish compared to the 90-day-old group ($p < 0.000$) (Figure 3b). The corresponding statistical details are presented in Table 2

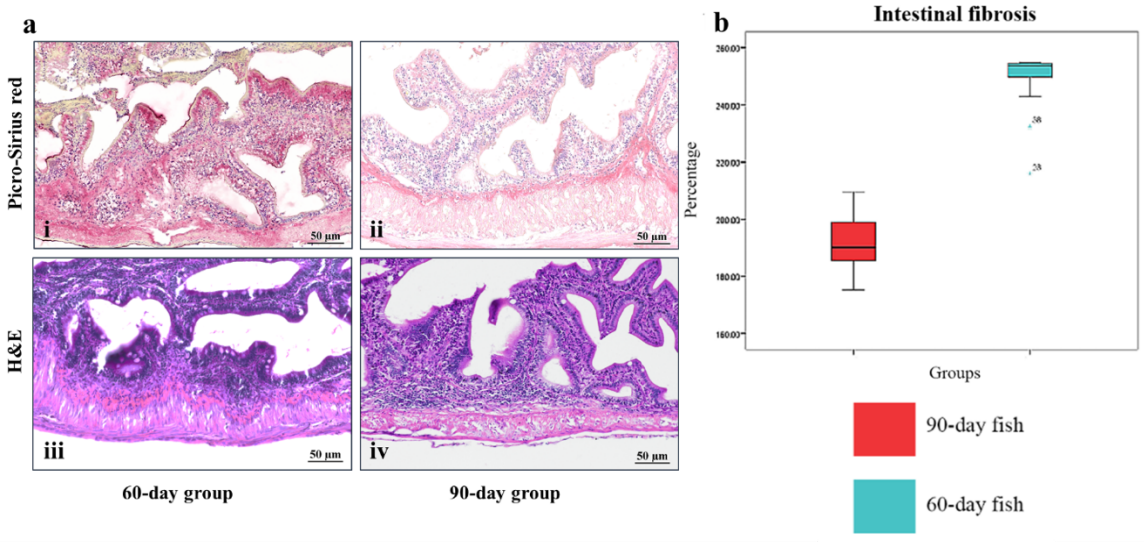

Figure 3 Histopathological and quantitative assessment of intestinal fibrosis in juvenile Asian sea bass (*Lates calcarifer*) naturally infected with piscine intestinal coccidia. (a) Picro-Sirius Red staining showing intense red-stained collagen deposition extending from the submucosa to the muscularis layer in 60-day-old fish, associated with areas of inflammation; no evident fibrosis observed in 90-day-old fish. (b) Quantitative comparison of fibrosis intensity between 60-day-old and 90-day-old fish, showing significantly higher mean fibrotic area in the 60-day group ($p < 0.000$). Scale bars = 50 µm. (ai-ii = Picro-sirius red, aiii-iv = H&E, original magnification; ai-iv = ×40, scale bar = 50 µm).

Table 2 Quantitative comparison of intestinal fibrosis intensity between 60-day-old (SF) and 90-day-old (BF) juvenile Asian sea bass (*Lates calcarifer*) naturally infected with piscine intestinal coccidia. Fibrosis was quantified using ImageJ analysis from Picro-Sirius Red–stained sections, and statistical significance between groups was determined using the Mann–Whitney U test ($p < 0.05$).

Lesions	Mean rank of BF	Mean rank of SF	P value
Mean intensity of fibrosis	11.5	33.5	0.000

DISCUSSION

Piscine intestinal coccidiosis is recognized as one of the most significant protozoan disease affecting fish populations, including pearl gentian groupers (*Epinephelus lanceolatus* ♂ × *E. fuscoguttatus* ♀) (Sornying et al., 2025), Spotted Scat (Scatophagus argus) (Suyapoh et al., 2025) and Asian sea bass (Suyapoh et al., 2024). High infection rates have been reported in fingerlings reared under intensive marine cage farming systems, where the anterior intestine serves as a major site of infection and tissue injury, often leading to mortality (Suyapoh et al., 2022). The severity of intestinal lesions in this study demonstrated a clear relationship with fish age. Consistent with previous findings, 60-day-old fish exhibited a higher infection intensity, poorer health assessment index (HAI) scores, and more pronounced pathological alterations than 90-day-old fish (Suyapoh et al., 2024). Younger fish also showed greater goblet cell hyperplasia, indicating a heightened mucosal response to infection (Thongrin et al., 2025). These observations suggest that early-stage fish possess a more reactive yet less regulated immune system, which may predispose them to severe intestinal injury. Comparable findings have been observed in *Epinephelus fuscoguttatus* (tiger grouper), where intestinal immune structures are established as early as 30 days post-hatch, but functional maturity—and thus more effective immune regulation—develops later (Firdaus-Nawi et al., 2013). Hence, the younger group's heightened susceptibility may reflect immature or exaggerated inflammatory responses during coccidial infection.

The common pathological manifestations of piscine intestinal coccidiosis include mucosal and submucosal inflammation, necrosis, vascular congestion, and epithelial desquamation (Dyková and Lom 2006; Jantrakajorn et al., 2025). In the present study, inflammation and extended inflammation were the most prominent lesions, consistent with the role of macrophages and other immune cells in promoting wound healing during acute injury (Djudjaj and Boor, 2019). Our results align with these earlier reports, confirming that piscine intestinal coccidiosis induces acute to subacute inflammatory reactions that vary in severity depending on developmental stage. Fibrosis, the excessive accumulation of extracellular matrix (ECM) components, frequently arises from chronic or repetitive inflammation (Yoshimura, 2024). Similar associations between persistent inflammation and fibrosis have been described in mammalian models of ulcerative colitis, where the severity of inflammation correlates with the degree of submucosal fibrosis and thickening of the muscularis mucosae (Gordon et al., 2018). In Asian sea bass, oxidative stress and the overproduction of reactive nitrogen species during infection have been shown to exacerbate mucosal injury and inflammatory responses, particularly in younger fish (Suyapoh et al., 2024). Together, these findings support the concept that sustained inflammatory stimuli can promote fibrogenic remodeling within the intestinal wall. Fibrosis was predominantly observed in 60-day-old fish, extending from the submucosa to the muscularis layer,

corresponding with persistent inflammation. In contrast, 90-day-old fish showed partial recovery and reduced fibrotic deposition.

Although the present study utilized naturally infected fish to reflect field conditions, this approach inherently limited control over parasite burden among individuals. Furthermore, sampling from a single aquaculture pond may constrain broader epidemiological interpretation. Parasite quantification and molecular identification were not performed due to technical limitations associated with naturally infected samples. Nonetheless, these factors do not undermine the histopathological patterns consistently observed in this study. Future investigations employing controlled experimental infections, quantitative parasite assessment, multi-site sampling, and molecular confirmation would provide greater resolution regarding species-specific pathogenicity and disease mechanisms.

CONCLUSIONS

Overall, our findings highlight the dual pathological consequences of piscine intestinal coccidiosis—acute inflammation and progressive fibrosis—which are key drivers of intestinal tissue remodeling and functional impairment in young fish. This study provides foundational evidence linking age-dependent immune responses to the development of intestinal fibrosis in coccidia-infected Asian sea bass, underscoring the need for early intervention and improved management strategies in aquaculture systems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

T.T.: Conceptualization; Validation; Formal analysis; Investigation; Writing—original draft preparation; Writing—review and editing; Supervision.
S.M.: Methodology; Writing—review and editing.
N.K.: Methodology; Writing—review and editing.
P.S.: Conceptualization; Methodology; Validation; Formal analysis; Investigation; Resources; Writing—original draft preparation; Supervision.

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