Songklanakarin J. Sci. Technol.
46 (6), 539–544, Nov. – Dec. 2024

http://www.sjst.psu.ac.th

Original Article

Piscine intestinal coccidian: Goblet cell response in juvenile Asian sea bass *(Lates calcarifer)* across different age groups

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Received: 15 March 2024; Revised: 26 August 2024; Accepted: 8 October 2024

Abstract

The parasitic protozoan, piscine intestinal coccidia, has been identified as a significant contributor to morbidity and mortality among juvenile Asian sea bass. Despite the fact that this intestinal parasite colonizes the gut and induces damage, the precise innate response of the goblet cell to piscine intestinal coccidia and the role of goblet cell in promoting protozoan infection remain elusive. This study endeavors to elucidate the goblet cell's reaction to this protozoan infection across different age groups of juvenile Asian sea bass. Intestinal samples were collected from forty-four juvenile Asian sea bass, each aged 60 and 90 days, that were infected with piscine intestinal coccidia and were obtained from marine cage farming. These samples were then processed using routine histopathological techniques, including embedding and staining. The goblet cells within the intestinal mucosa were systematically analyzed using histochemistry, Alcian blue staining. Subsequent to the analysis, goblet cells were semi-quantified and compared using statistical methods. The study findings showed a notable rise in goblet cell distribution from the anterior, mid, and posterior regions in two distinct fish groups. Specifically, the 60-day fish exhibited a significantly greater goblet cell hyperplasia compared to the 90-day fish during the infection period ($p<0.05$). This study examines the response of goblet cells in juvenile Asian sea bass models infected with piscine intestinal coccidia, focusing on the potential influence of age on the innate host's protective mechanisms. An interesting finding is that the 60- and 90-day-old Asian sea bass exhibited a goblet cell hyperplasia response, which is associated with coccidiosis infection.

Keywords: Asian sea bass, coccidia, goblet cell, intestine, juvenile

1. Introduction

Lates calcarifer, commonly known as the Asian sea bass or barramundi, holds significant economic importance as a fish species. It exhibits a broad geographic distribution across subtropical and tropical regions, particularly in the Asia-Pacific area, including countries like China, India, Saudi Arabia, and Australia (Roy, 2021; Ye *et al*., 2017). The production of aquaculture by species is a component of global fisheries and aquaculture output, which has exhibited a growth of 45% over the period from 2000 to 2021, reaching a total of 182 million tons (Food and Agriculture Organization of the United Nations [FAO], 2023). Based on intensive farming practices, the prevalence of piscine intestinal coccidia infections poses a heightened risk of illness and mortality, particularly among juvenile Asian sea bass populations (Gibson-Kueh *et al*., 2011; Suyapoh *et al*., 2022, 2024). Suyapoh *et al*. (2024) have further demonstrated that among fish at 60 days and 90 days of age, the younger cohort (60 days old) shows a greater susceptibility to infection and more pronounced intestinal damage compared to their older counterparts (90 days old). Coccidia infection induces a state of persistent inflammation, characterized by direct lysis of host cells and the disruption of gut symbiosis (Lovy & Friend, 2015; Qi *et al*., 2019; Sitjà-Bobadilla, Estensoro & Pérez-Sánchez, 2016). Indeed, infection with piscine intestinal coccidial parasites has been associated with increase in the incidence of the pathological conditions in young Asian sea

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bass (Gibson-Kueh *et al*., 2011; Lovy & Friend, 2015, 2019; Marwan, 2001)

The gastrointestinal tract assumes a crucial role in the processes of nutrient digestion and absorption, as well as the elimination of undigested materials, microorganisms, and their byproducts (Liévin-Le Moal & Servin, 2006). The mucosal lining that encases the gastrointestinal tract serves as the primary barrier of innate host defense, primarily attributed to the secretory factors generated by intestinal goblet cells (Kim & Ho, 2010). Intestinal goblet cells, distributed throughout the intestinal tract as polarized secretory entities, are deemed to play a pivotal protective role through the production and release of diverse mediators, notably mucin (Taupin & Podolsky, 2003). These cells have been implicated in the innate response to various parasitic infections, including helminthic and apicomplexan infections, as part of the host's defense mechanism (Ferenc, 1997; Suyapoh, Tirnitz-Parker, Tangkawattana, Suttiprapa & Sripa, 2021; Suyapoh *et al*., 2021). Moreover, several research investigations have suggested that increased mucus secretion and alterations in mucin composition may exacerbate protozoan infections (Martínez-Ocaña, Maravilla & Olivo-Díaz, 2020; Milgroom, 2013). Nonetheless, the precise response of goblet cells in Asian sea bass infected with piscine intestinal coccidia remains incompletely elucidated.

To gain a deeper understanding of the goblet cell response to piscine intestinal coccidia infection in juvenile Asian sea bass, this experimental study was designed to investigate the relationship between fish age and goblet cell hyperplasia using histochemical techniques. The findings of this study will contribute to our understanding of the pivotal role of goblet cells in the innate response of the host's defense mechanism against piscine intestinal coccidia infection.

2. Materials and Methods

2.1 Ethics statement

Juvenile Asian seabass were procured from marine farms located in Satun province, Thailand, for the purpose of experimentation. The conduct of all experiments was authorized by the animal ethics committee under the number MHESI 68014/1731, adhering to the guidelines for the ethical care and use of animals in scientific research in Thailand.

2.2 Experimental design

Piscine intestinal coccidia represent a significant disease affecting juvenile fish, while adult fish typically remain asymptomatic. Consequently, juvenile Asian sea bass are particularly susceptible to coccidiosis and its associated mortality. Previous studies have indicated that younger juveniles, specifically those around 60 days old, are more sensitive to this disease. They exhibit higher rates of infection intensity, histopathological damage, free radical production, DNA damage, and cellular apoptosis compared to 90-day-old juveniles. Given the increased susceptibility observed in 60-day-old fish, the authors have concentrated their investigation on this age group to better understand defense responses in this pathophysiological context. Eighty-eight intestinal samples were collected from juvenile Asian sea bass aged 60 and 90 days, with 44 samples from each age group, at a marine cage farm in Satun province, Thailand. The identification of piscine intestinal coccidia was conducted using a Nikon advanced upright microscope with a VDO capture digital camera (ECLIPSE Ni-U) (Nikon, Tokyo, Japan), following established protocols for the examination of parasitic stages (Landsberg & Paperna, 1987; Lovy & Friend, 2015). A parasitologist confirmed all morphological analyses. Additionally, all intestinal samples were stained with Alcian blue for the evaluation of goblet cells.

2.3 Histochemistry and semi-quantitative study of goblet cells

The intestinal samples were fixed in 10% buffered formalin for 72 hours and then processed using routine histological techniques (Tangkawattana *et al*., 2023). Paraffin sections were stained with hematoxylin and eosin (H&E) to evaluate structural histology. Additionally, histochemical staining with Alcian blue (Abcam, UK) was employed to identify mucus-secreting cells (Wendo *et al*., 2022). To assess goblet cell hyperplasia, semi-quantitative grading was used based on previous research (Suyapoh *et al*., 2022; Lvova *et al*., 2012), which initially assesses tissue changes as percentages. These percentage scores are then converted into grading levels, similar to those used in medical histopathological assessments. Ten non-overlapping microscopic fields were examined under a light microscope for each sample. The severity of the lesions was categorized as absent, mild, moderate, or severe, with a clear quantitative proportion: absent (no lesion development or ≤1% lesion development), mild (2–25% lesion development), moderate (26–50% lesion development), and severe (>50% lesion development).

2.4 Statistical analysis

Statistical analysis of the data was conducted using SPSS version 23.0 (SPSS Inc., USA). The Student's t-test was employed to compare the data between the 60-day and 90-day fish groups. Statistical significance was determined at $p < 0.05$.

3. Results

3.1 Coccidial infection

All 88 fish specimens were found to have a positive presence of piscine intestinal coccidia. The coccidia were primarily located in extracellular or subepithelial sites across all sections of the intestine, including the anterior, mid, and posterior regions.

3.2 Goblet cell distribution within the intestinal tract

In both age groups of fish, the intestinal epithelium was composed of absorptive and goblet cells. Goblet cells, which are unicellular intraepithelial mucin-secreting glands, were identified by their narrow bases and wide apex with a cup-like appearance. The cells contained a mucus sac that stained blue with Alcian blue. These cells were embedded in the intestinal mucosa with varying densities. The number of goblet cells progressively increased along the length of the

intestinal tract, with a higher density in the posterior part than in the mid and anterior intestine, as depicted in Figure 1.

3.3 Comparison of goblet cell hyperplasia in 60- and 90-day fish

Goblet cell hyperplasia refers to a condition characterized by an increased count of goblet cells within a given tissue. In this study, we conducted a quantitative analysis to determine the number of goblet cells in intestinal tissues of fish infected with coccidia, comparing specimens from 60-day and 90-day fish. Our findings revealed a significantly higher grade of goblet cells in the 60-day fish compared to the 90-day fish ($p<0.001$), as depicted in Figure 2a-c.

4. Discussion

Coccidia is a pathogenic parasite known to cause coccidiosis, a leading cause of parasitic illness and mortality in both freshwater and marine fish globally (Bamidele, Abayomi, Iyabo & Giwa, 2019; Golomazou & Panagiotis, 2020; Lovy & Friend, 2015; Molnár & Ogawa, 2000; Pasnik, Smith & Lindsay, 2005). Clinical signs of intestinal coccidiosis include inactivity, starvation, malnutrition, mucoid feces, abdominal distention, and retarded growth (Gibson-Kueh *et al*., 2011; Suyapoh *et al*., 2024). The severity of illness and mortality is influenced by factors such as fish susceptibility, culture conditions, coccidial species, degree of parasitic infection, concomitant bacterial infection, and especially fish age and size (Certad *et al*., 2015; Couso-Pérez, Ares-Mazás & Gómez-Couso, 2022; Golomazou & Panagiotis, 2020; Sitjà-Bobadilla, Padrós, Aguilera & Alvarez-Pellitero, 2005; Steinhagen, Oesterreich & Körting, 1997; Suyapoh *et al*., 2022, 2024). Within intestinal tissue, the innate protective function of the host is predominantly mediated by the mucus-secreting response orchestrated by goblet cells (Taupin & Podolsky, 2003). This secretion, rich in glycoproteins, constitutes a critical component of the host's primary defense, facilitating the clearance of intestinal contents and providing protection against a spectrum of infectious agents commonly associated with parasitic infestations (Ferenc, 1997; Kim & Khan, 2013; Suyapoh *et al*., 2021a, 2021b). In the gastrointestinal tract of Asian sea bass, goblet cells are consistently distributed from the esophagus through the stomach, cecum, intestine, and extending to the rectum (Purushothaman *et al*., 2016). Within the intestinal tissue, the greatest concentration of these cells is notably observed in the posterior region, followed by the mid and proximal regions (Purushothaman *et al*., 2016). Our findings align with the observations reported in prior research.

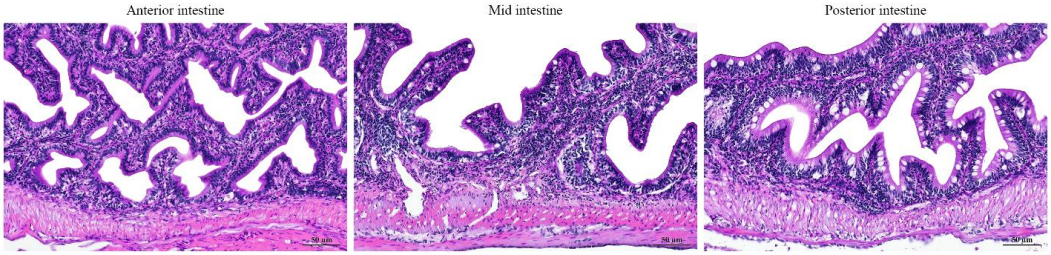

Figure 1. Distribution of goblet cells at intestinal tissue of the juvenile Asian sea bass infected with piscine intestinal coccidia. The proximal segment exhibited a reduced density of goblet cells, whereas the mid and posterior segments displayed elevated goblet cell densities, respectively. (Stained with hematoxylin and eosin, original magnification = ×40, scale bar depicts 50 µm)

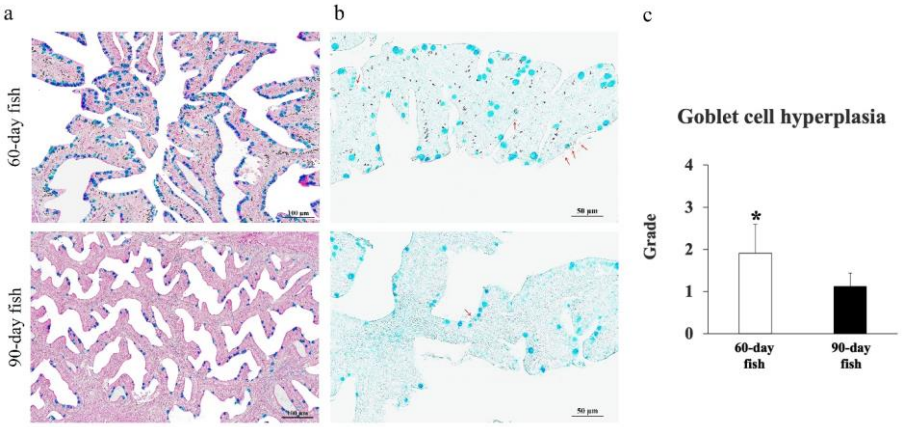

Figure 2. Changes in goblet cell numbers in the intestine of juvenile Asian sea bass infected with piscine intestinal coccidia. (a) Photomicrographs reveal a higher degree of goblet cell hyperplasia in 60-day fish compared to 90-day fish. (b) The higher degree of goblet cell hyperplasia observed in 60-day-old fish, compared to 90-day-old fish infected with coccidia, is highlighted by the red arrows. (c) A comparative analysis of goblet cell hyperplasia grades demonstrated a statistically significant increase in 60-day fish compared to 90-day fish. Values are means ± SD. *Significant at $P \leq 0.05$. (a=Alcian blue staining combined with eosin counterstaining; b= Alcian blue staining, original magnification in a= ×2, scale bar depicts 100 µm; in b= ×40 magnification, scale bar depicts 50 µm.)

In this investigation, histological sections and semi-quantitative histochemical analyses were employed to examine the goblet cell responses to piscine intestinal coccidia infection across various age groups of juvenile Asian sea bass. As anticipated, a higher degree of goblet cell hyperplasia was observed in younger juvenile fish (60-day-old fish), whereas older fish (90-day-old fish) exhibited a diminished level of this cellular response. This pattern aligns with findings in teleosts, where goblet cell hyperplasia is a common feature of non-specific immune responses to gastrointestinal parasites (Souza, Santos & Chagas, 2019). Comparative studies in mammalian models, such as laboratory mice, have similarly shown that parasitic infections can increase goblet cell numbers; however, the relationship between age and hyperplasia in these models is less consistent (Birchenough *et al*., 2015; Finkelman *et al*., 2004). This highlights that goblet cell hyperplasia is a conserved physiological response across different species, potentially indicating a broader biological significance. Moreover, it is important to note that hyperplasia of goblet cells is often reversible. As shown in various studies, the number of goblet cells can return to baseline levels once the underlying stressor or pathogenic condition is alleviated (Jackson, 2001). Thus, while the observed hyperplasia in 60-day-old fish could reflect a transient physiological adaptation, the potential for normalization underscores the dynamic nature of this response.

Goblet cells primarily function to secrete mucus, which is essential for protecting and lubricating surfaces (Yang & Yu, 2021). However, several studies have suggested that increased mucus secretion and changes in mucin composition might exacerbate protozoan infections (Martínez-Ocaña, Maravilla & Olivo-Díaz, 2020; Milgroom, 2013). The involvement of mucin in the pathogenesis of protozoan diseases is linked to their role in adhesion and colonization at the portal of entry (Martínez-Ocaña *et al*., 2020). In small homoxenous coccidia such as *Cryptosporidium*, two types of proteins, namely mucin-like glycoproteins and thrombospondin-related adhesive proteins (TRAPs), are implicated in adhesion to the mucus layer (Wanyiri & Ward, 2006). Additionally, the P23 protein, a surface glycoprotein found on sporozoites and merozoites of *Cryptosporidium*, is known to participate in attachment and invasion (Bouzid, Hunter, Chalmers & Tyler, 2013). Furthermore, reports indicate the facilitation of adherence to host mucin via specific proteins in other human and animal protozoa (Aguirre García, Gutiérrez-Kobeh & López Vancell, 2015; Chatterjee *et al*., 2010).

Overall, these findings suggest a potential involvement of goblet cell proliferation in young juvenile coccidiosis fish, which may could contribute to the pathogenesis of piscine intestinal coccidia infection in Asian sea bass. Moreover, this finding may underscore a potential physiological adaptation or response to environmental stressors or nutritional factors that warrants further investigation. The elevated goblet cell hyperplasia observed in the 60-day-old fish could be linked to specific management practices, such as variations in fish-rearing techniques or dietary formulations. For instance, certain rearing conditions and feed compositions have been shown to influence the expression and proliferation of goblet cells (Alesci *et al.*, 2022). Additionally, nutritional stressors such as starvation have been documented to induce hyperplasia of goblet cells in various fish species (Berillis & Mente, 2017). This suggests that goblet cell hyperplasia might serve as a compensatory mechanism in response to suboptimal feeding conditions or other stressors.

However, we will address these limitations in the conclusions section of our study and suggest directions for future research to improve the understanding of goblet cell function and its role in the pathology of piscine intestinal coccidiosis.

5. Conclusions

This study observed varying responses of goblet cells in young Asian sea bass infected with piscine intestinal coccidia. Specifically, younger fish exhibited a greater increase in the number of goblet cells. However, further trials are needed to investigate the mucosal capabilities and immune responses to better understand mucosal immunity.

Acknowledgements

We would like to express our gratitude to Assistant Professor Dr. Watcharapol Suyapoh for his valuable suggestions and insightful comments. This research received financial support from the Fundamental Fund for Lecturers at the Faculty of Veterinary Science, Prince of Songkla University, Thailand (Grant/Award Number: VETPSU 01012564).

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