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Review article

Immunity, feed, and husbandry in fish health management of cultured *Epinephelus fuscoguttatus* with reference to *Epinephelus coioides*

Catherine Cheng Yun Chieng [a], Hassan M. Daud [b], Fatimah M. Yusoff [a], Maha Abdullah [c,] *

[a] Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, Serdang 43400, Malaysia
[b] Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang 43400, Malaysia
[c] Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Malaysia

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ABSTRACT

Groupers are dispersed worldwide in the tropical and subtropical waters. They are prized in the live reef fish trade, making them candidates with high market value and consumer demand. In the Asian-Pacific region, the brown-marbled grouper (*Epinephelus fuscoguttatus*) is widely adapted as an aquaculture species. However, health management remains a major concern in the stressful intensive culture process. The present review discusses techniques and current knowledge in the cultivation of *E. fuscoguttatus* towards healthy fish growth focusing on aspects of immunity, feed, and husbandry. Understanding how the fish immune system responds during infections provides insights into the intricate ways fish resist pathogens. This information is helpful when developing vaccination strategies or immunostimulant compounds to strengthen fish immunity. Feeds that are formulated according to the needs of the fish ensure optimal growth and using suitable alternative ingredients may lower production cost without compromising fish health. Good husbandry practices contribute to a favourable environment for the fish to grow, while interspecific hybridization may be a convenient approach to generate hardier species. Nevertheless, it has been noted that research in *E. fuscoguttatus* is relatively scarce in contrast to the closely-related orange-spotted grouper (*E. coioides*), which is used in the present article as a reference. Ultimately, the identified gaps in knowledge between the two species warrant species-specific research in *E. fuscoguttatus* to promote fish health and ensure continued success in aquaculture.

1. Introduction

Groupers in the family Epinephelidae are formed by 16 genera encompassing 163 species (Craig, Sadovy, & Heemstra, 2011) and are ray-finned, bony fishes distributed worldwide in the tropics and subtropics. They are of economic importance due to their high market value and consumer demand in the live reef fish trade. Global production of grouper has been increasing steadily over the years and the output in 2014 (data include seabasses) was 139 kilotonnes valued at 654 million USD (FAO, 2016).

Despite considerable difficulties in the initial cultivation of groupers involving seed, feed, and diseases, remarkable improvements have since been made in terms of broodstock management and breeding programs, larval rearing, production of hatchery-reared fingerlings, and successful culture to marketable size (Liao, Su, & Chang, 2001; Mat Ali, Om, Idris, Mustafa, & Teoh, 2006; Mustafa, Hajini, Senoo, & Kian, 2015; Sugama et al., 2012). Nonetheless, health management continues to be a major concern in aquaculture as intensive farming and associated overcrowding stress have been linked to mass mortality events and huge economic loss (Ma, Xie, Weng, Zhou, & He, 2012).

In this review, fish health management is discussed from three viewpoints: (1) understanding fish immunity through infection studies and vaccination trials, (2) enhancing fish growth and hardiness through the use of optimal feeds and immunostimulants, and (3) improving culture success through good husbandry practices and interspecific hybridization. Improvements in these aspects ultimately contribute to healthy fish with improved growth. Specific focus is given to *Epinephelus fuscoguttatus* (Forsskål, 1775) (common name: brown-marbled grouper) which is a popular aquaculture candidate in the Indo-Pacific region in Southeast-Asian countries such as China, Indonesia, Malaysia, and Thailand (Rimmer, Phillips, & Yamamoto, 2007).

* Corresponding author. Faculty of Medicine and Health Sciences, Serdang 43400, Malaysia.
E-mail address: maha@upm.edu.my (M. Abdullah).

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However, researches on *E. fuscoguttatus* is relatively scarce and therefore, a closely-related species, *Epinephelus coioides* (Hamilton, 1822) (common name: orange-spotted grouper) is used as a reference. Both *E. fuscoguttatus* and *E. coioides* are placed in the same clade in phylogenetic studies (Ding et al., 2006; Guan et al., 2014; Ma, 2014; Rahim, Esa, & Arshad, 2016) and since *E. coioides* is the most extensively studied *Epinephelus* spp. it could serve as a "model" species. By comparing the two species, gaps in information regarding *E. fuscoguttatus* could be identified and stimulate more research into the deficient topics.

2. Understanding fish immune system: disease and immunological studies

Disease management is an inevitable part of aquaculture. Infectious diseases that frequently break out in farmed groupers are vibriosis caused by *Vibrio* spp., streptococcosis caused by *Streptococcus* spp., sleeping grouper disease caused by iridovirus, viral nervous necrosis caused by betanodavirus, and marine white spot disease caused by *Cryptocaryon irritans*. Antibiotics are widely administered in the face of these diseases, but their excessive use in aquaculture for both prophylactic and therapeutic purposes has led to persistent residues in the water, sediment, and fish products. This spurs the selective pressure for antibiotic-resistant microorganisms, not only in fish, but also consequently in terrestrial animals and humans (Cabello, 2006). Thus, understanding immune response in fish has garnered increasing interest because it is critical in the combat against pathogens without the health risks posed by the use of antibiotics. This section will therefore focus on innate and adaptive defence mechanisms, vaccination methods for disease prevention, targets for therapeutic interventions, and biomarkers for monitoring the immune response.

2.1. Innate immune response against bacteria, virus, and parasites

Studies on bacterial infection in fish are concentrated on pattern recognition receptors (PRRs) that initiate downstream signalling leading to recognition of pathogen-associated molecular patterns (PAMPs) and an immune response against invading pathogens. A number of PRRs from *E. coioides* had been successfully cloned and characterized, including Toll-like receptor (*TLR*)1, *TLR*2 (Wei et al., 2011b), and *TLR*22 (Ding et al., 2012). Activation of these PRRs resulted in production of pro-inflammatory cytokines such as *IL*-1β and *TNF*-α through activation of MAPK signalling cascade via *MyD*88 adaptor. All of these molecules were upregulated in major lymphoid organs such as the spleen and head kidney of *E. coioides* upon infection with *Vibrio alginolyticus* (Ding et al., 2012; Wei et al., 2011b). In addition to extracellular *PRRs*, increased expression of intracellular *PRRs* such as *NOD*1 and *NOD*2 in the spleen (Hou et al., 2012) potentiated the inflammatory response mediated by *IL*-8 via RICK adaptor in *E. coioides*. Other antibacterial factors were also triggered, such as extracellular C-type lysozyme (Wei et al., 2012b), intracellular G-type lysozyme (Wei et al., 2014a), hepcidins (Qu, Chen, Peng, & Wang, 2013; Zhou et al., 2011), complement C8 beta (Luo, Xie, Liu, & Wang, 2015a), and voltage-dependent anion channel 1 (Shi, Zhao, Hong, Chen, & Zhu, 2014).

In addition to pro-inflammatory molecules, *V. alginolyticus* infection in *E. fuscoguttatus* upregulated serum proteins such as apolipoprotein A-I, natural killer cell enhancement factor, and G-type lysozyme (Low, Shamsudin, Chee, Aliyu-Paiko, & Idrus, 2014) which are anti-inflammatory, antioxidant, and antimicrobial, respectively. Interestingly, when fingerlings were infected with *V. parahaemolyticus* (Low, Nor Shamsudin, Abdullah, Chee, & Aliyu-Paiko, 2015b), the resistant fish overexpressed immune-activating molecules such as parvalbumin, alpha-2-macroglobulin, and nattectin. The susceptible fish, that had muscle lesions, overexpressed anti-inflammatory apolipoprotein E. Both resistant and susceptible fish expressed immunoglobulin light chain proteins, albeit with different peptide sequences. These studies highlight the diversity of protein expression in serum which may be useful as markers of disease resistance and susceptibility.

Whereas in *E. coioides* infected with Singapore grouper iridovirus (SGIV), numerous genes were found to be modulated in the spleen (Huang et al., 2011) and head kidney (Wu et al., 2012). The altered genes were cytokine and cytokine receptors, transcription factors, apoptotic factors, as well as those involved in intracellular signalling pathways (*TLR*, *MAPK*, chemokines, retinoic acid-inducible gene 1, and *p*53), cytoskeleton, and metabolism. However, more experimental work is needed to examine the functional expression of individual genes to learn the mechanisms through which host protection is conferred. For instance, thioredoxin (Wei et al., 2012a) and TRP14 (Wei, Ji, Guo, Yan, & Qin, 2013b) were found to be antioxidants which could strengthen viability of host cells under SGIV infection and at the same time inhibit viral transcription. Similarly, *I*κ*B*α orthologues (Gao et al., 2014) and *p*38β (Cai et al., 2011) protected *E. coioides* by suppressing *SGIV* replication, while *p*38β could further restrain host cell apoptosis.

Viral nervous necrosis caused by nodavirus, which is an RNA virus, may elicit different immune mechanisms in the fish than that caused by iridovirus, a DNA virus. One of the upregulated genes in the liver of *E. coioides* after nodavirus and polyI:C (an analogue of double-stranded RNA) infections was *TLR*3 (Lin et al., 2013), which could trigger a downstream signalling cascade involving *TRIF*, *NF*-κ*B* and *IFN* response promoters (Wei, Zhang, Zang, & Qin, 2017), *in vitro* interferon regulatory factor 3 (*IRF*3) (Huang et al., 2015), and interferon-stimulated gene 15 (*ISG*15) (Huang et al., 2013) resulting in an inflammatory response towards the viral stimulation. Type I interferon was also found to localize in the gut (Chen et al., 2014b), implying the involvement of mucosal immunity. However, expression of *TLR*3 was consistently low in fertilized egg until 10 days post-hatching, after which it gradually increased and only reached a significant level in 30-day old larvae (Lin et al., 2013). This observation may explain the particular vulnerability of grouper larvae towards viral diseases.

Unfortunately, viruses are capable of exploiting the host immune system to their benefit. For instance, upregulation of cathepsin B (Wei et al., 2014b) and LITAF (Cai et al., 2013) in the spleen of *E. coioides* after iridovirus infection accelerated both viral replication and virus-induced host cell apoptosis. Similarly, activation of transcription factor c-Jun (Wei, Huang, Huang, & Qin, 2015) and Rab7 (Fu et al., 2014) were shown to participate in viral transcription, transport and assembly of viral genes. In nodaviral infection, heat shock protein 90AB (HSP90AB) (Chen et al., 2010) and heat shock transcription factor 1 (HSF1) (Wang, Chen, & Chen, 2016) increased in *E. coioides* to overcome protein damage caused by inflammation but were instead appropriated by the virus to support its own polypeptide synthesis and cellular translocation. Nodavirus also reduced the levels of SPARC and prevented cells from spreading and adhering, subsequently inhibiting the usual cell repair after viral damage (Chen et al., 2011a). It has been postulated that inhibitors targeting molecules which work in favour of the virus may yield beneficial therapeutic outcomes.

Mucosal immunity was investigated in the skin transcriptome of *E. coioides* infested with *Cryptocaryon irritans* and revealed a multitude of upregulated genes that suggested an activated local immune response (Hu et al., 2017), corroborating and adding to the findings of previous studies (Li, Dan, Zhang, Luo, & Li, 2011a; Li et al., 2011b, 2012, 2014; Mo et al., 2017; Ni et al., 2017). For example, innate factors involved in the acute phase response,

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