Aquaculture 490 (2018) 44–52

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Comparative sensitivity of the hepatopancreas and midgut in the white shrimp *Litopenaeus vannamei* to oxidative stress under cyclic serious/medium hypoxia

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ARTICLE INFO

Keywords:
Litopenaeus vannamei
Hepatopancreas
Midgut
Cyclic serious/medium hypoxia
Oxidative stress
Gene expression

ABSTRACT

Low dissolved oxygen (DO) could cause a stress response in shrimp, the objective of this study was to compare sensitivity of the hepatopancreas and midgut to oxidative stress under low DO. The white shrimp *Litopenaeus vannamei* were reared under conditions of cyclic serious/medium hypoxia (CSMH, 0.8–3.5 mg/L) versus normoxia (N, 6.4–7.5 mg/L) for a 28-day period. Survival and growth performance of shrimp were evaluated, and hypoxia inducible factors 1a (*HIF-1a*) expression, antioxidant responses, apoptosis and histology in the hepatopancreas and midgut were investigated. Antioxidant responses tested included the following: manganese superoxide dismutase, glutathione peroxidases, glutathione-S-transferase, metallothionein and heat shock protein70 expression. Results showed enhanced *HIF-1a* expression and antioxidant responses in the hepatopancreas and midgut during short-term cyclic serious/medium hypoxia (≤7 days), which suggested early adaptive mechanism of shrimp to tolerate low DO and avoid oxidative damage. Meanwhile, *HIF-1a* expression, apoptosis and histopathological lesions were induced earlier in the hepatopancreas than the midgut. Thus, the hepatopancreas could be more sensitive to low DO and its oxidative stress than the midgut. However, long-term (≥14 days) cyclic serious/medium hypoxia could disrupt cellular antioxidant mechanism with depressed antioxidant responses, and then aggravate apoptosis and histopathological lesions in the hepatopancreas and midgut, particularly the hepatopancreas would lose antioxidant ability earlier than the midgut because of higher sensitivity to oxidative stress. Therefore, we have a few insights that it is vital to protect hepatopancreas for controlling shrimp death and growth inhibition under cyclic serious/medium hypoxia.

1. Introduction

Dissolved oxygen (DO) conditions in aquatic systems are rarely stable. In the night, DO concentrations may reach critically low levels due to respiration of organisms and decomposition of accumulated organic matter such as unconsumed feed and feces (Cheng et al., 2003). As such, hypoxia can have severe consequences for marine organisms, even leading to death (Vaquer-Sunyer and Duarte, 2008). This is especially critical in rearing ponds that do not use aerators, where shrimp can be exposed to hypoxia as DO levels drop from 3 mg/L to < 1 mg/L (Chantal et al., 2008). The bottom layer of pond waters, where shrimp spend most of their time, may become even anoxic at night, due to respiration of the organisms and decomposition of accumulated organic matter (Cheng et al., 2003).

Hypoxia inducible factors (HIFs) are a family of highly conserved transcription factors that act as principal regulators of oxygen homeostasis and the adaptive response to hypoxia (Semenza, 1999). Treinin et al. (2003) demonstrated that HIF-1α is a transcription factor that regulates dozens of genes involved in response to hypoxia. These molecular responses then cascade into a series of biochemical and physiological processes (Harris, 2002; Sonanez-Organis et al., 2012), enabling the animal to survive under hypoxic conditions. Previous studies have indicated that hypoxia can induce reactive oxygen species (ROS) in crustaceans (Parrilla-Taylor and Zenteno-Savín, 2011; Sun et al., 2014). Moreover, re-oxygenation could induce higher levels of ROS formation compared to hypoxia (Clanton, 2007), as variations in oxygen levels increase ROS levels (Hermes-Lima and Zenteno-Savın, 2002; Storey, 1996). A well-adapted antioxidant defense system is the most efficient strategy to cope with oxygen variations. This strategy serves to avoid or counteract the generation of oxidative stress and

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https://doi.org/10.1016/j.aquaculture.2018.02.021
Received 27 November 2017; Received in revised form 1 February 2018; Accepted 17 February 2018
Available online 19 February 2018
0044-8486/

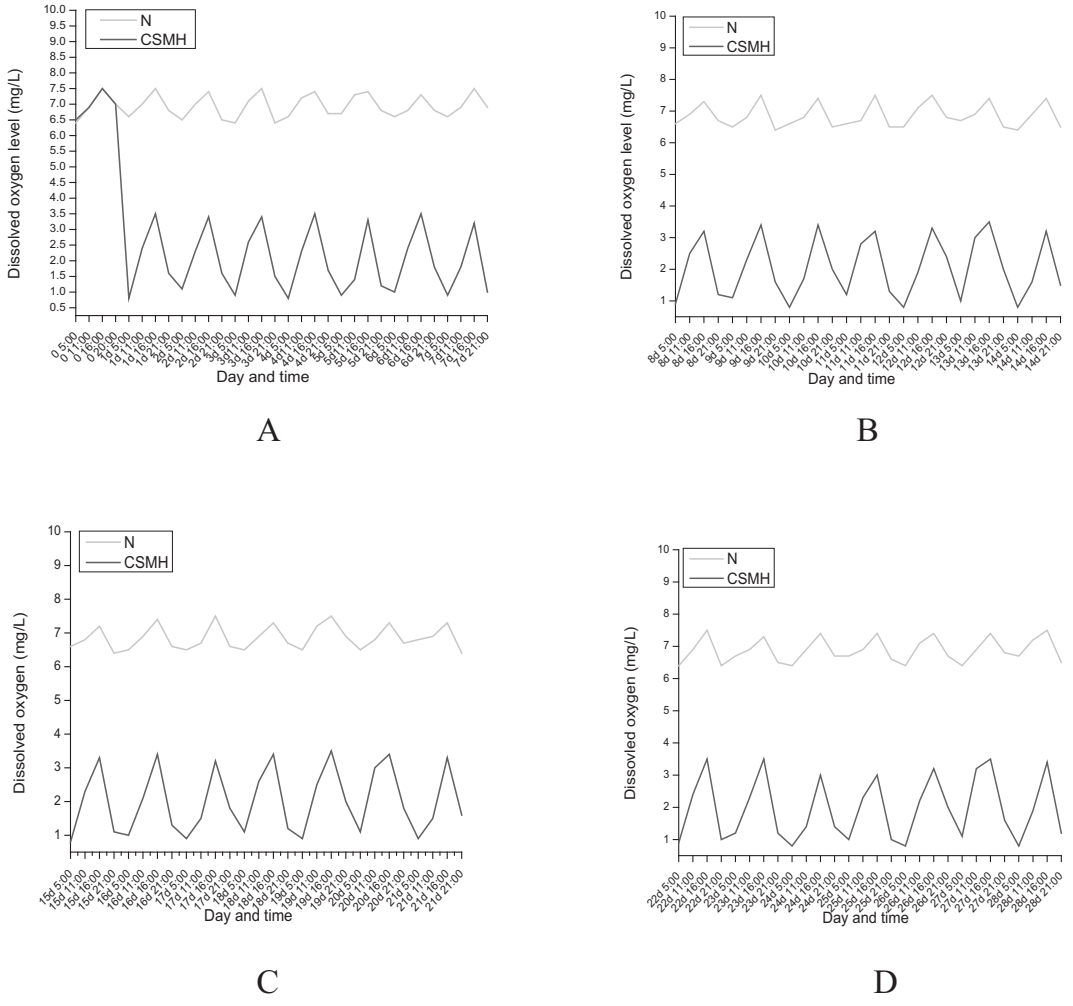

Fig. 1. Dissolved oxygen levels in the seawater of tanks during 0–7 days (A), 8–14 days (B), 15–21 days (C), and 22–28 days (D).

maintain homeostasis in the cell (Aruoma, 1998; Jones, 2006; Sies, 1986).

The superoxide anion (O_2^-) is the first product during the oxidation process, which is known as a respiratory burst (Makino et al., 1986). Manganese superoxide dismutase (MnSOD) catalyzes O_2^- to hydrogen peroxide (H_2O_2) and molecular oxygen (Fridovich, 1995). In the GSH pathway, the reducing power of GSH can be used to convert H_2O_2 to water in a reaction catalyzed by glutathione peroxidases (GPx) or to facilitate the elimination of toxicants and products of oxidative damage. The latter reaction is catalyzed by glutathione-S-transferase (GST) (Armstrong, 1991; Leiers et al., 2003; Zhou et al., 2009). Metallothionein (MT) belongs to a family of small, cysteine-rich, and heat stable proteins involved in the detoxification of heavy metals and protection against oxidative stress (Suzuki and Cherian, 2000). In addition to the cellular mechanism of using the above antioxidant defense system to eliminate ROS, chaperone molecules also assist in this process. For example, heat shock protein70 (Hsp70) functions to prevent both newly synthesized polypeptide chains and assembled subunits from aggregating into nonfunctional structures in order to sustain homeostasis in the cell under stress conditions (Franzellitti and Fabbri, 2005; Hartl et al., 2011). Otherwise, oxidative stress can act synergistically to cause extensive DNA damage leading to apoptosis (Antonova et al., 2009; Ito et al., 2004).

The white shrimp, *Litopenaeus vannamei*, is the major species farmed worldwide in shrimp aquaculture (CFSY, 2011). It has been reported that hypoxia increases oxidative stress in *L. vannamei*, which may significantly jeopardize commercial aquaculture products (Zenteno-Savin, 2005; Zenteno-Savin et al., 2006). Physiologically, both the hepatopancreas and the intestines are key organs involved in digestion, immunity, and detoxification (Robalino et al., 2007; Ruppert et al., 2003; Soonthornchai et al., 2010). Therefore, we evaluated survival and growth performance, and investigated (1) *HIF-1a* expression, (2) *MnSOD*, *GPx*, *GST*, *MT* and *Hsp70* expression, (3) DNA ladder, and (4) histology in the hepatopancreas and midgut of *L. vannamei* reared under conditions of normoxia and cyclic serious/medium hypoxia during a 28-day period. Our study aimed to compare sensitivity of the hepatopancreas and midgut in the *L. vannamei* to oxidative stress under cyclic serious/medium hypoxia, which is necessary to develop an effective strategy for controlling shrimp death.

2. Materials and methods

2.1. Experimental shrimp

One thousand three-hundred and fifty healthy juvenile *L. vannamei* of similar size (mean weight 1.20 ± 0.03 g) were obtained from the Ruizi Seafood Development Co. Ltd. (Qingdao, China). Shrimp were placed in nine 640-L cylindrical tanks equipped with net covers ($N = 150$ per tank). Every 640-L cylindrical tank contained 500-L aerated seawater (DO 6.4–7.5 mg/L). The initial seawater was unfiltered with pH 8.0–8.4, salinity 30–31‰, total ammonia 0.022–0.038 mg/L, nitrite 0.015–0.032 mg/L, and nitrate 0.120–0.205 mg/L at 28–32 °C. Shrimp were acclimated for 2 weeks under a natural photoperiod (12-h light:12-h dark). The shrimp were