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Effects of light intensity on growth, immune responses, antioxidant capability and disease resistance of juvenile blunt snout bream *Megalobrama amblycephala*

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ABSTRACT

Light is necessary for many fish species to develop and grow normally since most fishes are visual feeders. However, too intense light may be stressful or even lethal. Thus, this study was conducted to evaluate the effect of light intensity on growth, immune response, antioxidant capability and disease resistance of juvenile blunt snout bream *Megalobrama amblycephala*. Fish (18.04 ± 0.22 g) randomly divided into 5 groups were exposed to a range of light intensities (100, 200, 400, 800 and 1600 lx) in cultures for 8 weeks. After the feeding trial, fish were challenged by *Aeromonas hydrophila* and cumulative mortality was recorded for the next 96 h. The results demonstrated that fish subjected to 400 lx showed the greatest weight gain (125.70 ± 5.29%). Plasma levels of glucose and lactate increased with light intensity rising from 100 lx to 1600 lx while the lowest plasma levels of cortisol was observed at 400 lx group. Post-challenged haemato-immunological parameters (including plasma lysozyme and alternative complement activities, as well as plasma nitric oxide level and globulin contents) improved with light intensity increasing from 100 lx to 400 lx, and then decreased with further increasing light intensity. However, antioxidant biomarkers such as liver catalase and malondialdehyde showed an opposite trend with immune response with the lowest values observed at 400 lx groups. The application of light intensity at 1600 lx significantly lowered liver glutathione activity to 76.78 ± 6.91 μmol g^{-1}. Within a range of light intensity from 100 to 400 lx, no differences were observed in liver total superoxide dismutase and glutathione peroxidase activities while they were significantly higher at 800 and 1600 lx. After challenge, the lowest mortality was observed in fish exposed to 400 lx. It was significantly lower than that of fish exposed to 100 and 1600 lx. The results of the present study indicated that high light intensities (more than 800 lx) not only produced poor growth, but also led to stress response, as might consequently result in the elevated liver oxidation rates and depress immunity of this species. Although no stress response was observed, fish subjected to low light intensities (lower than 400 lx) also showed oxidative stress, immunosuppression and reduced disease resistance. Taken together, the optimal light intensity to enhance growth and boost immunity of this species at juvenile stage is 400 lx.

1. Introduction

Culture management should aim to optimize the farming environment to maximize the growth and welfare of fish. Light is one of the most important culture management factors in that it synchronizes from embryo development to sexual maturation of fish [1,2]. Studies have shown that most fish are visual feeders and need a minimal threshold light intensity to be able to develop and grow normally [3,4]. According to previous studies, insufficient light intensity may lead to poor growth and high mortality of fish [5]. On the other hand, excessively intensive illumination not only requires more electric energy and leads to production costs, but also potentially causes stress or even death for some fish species [4,6]. Considering this, the effect of light intensity has been widely investigated on survival and growth [7], swimming activity and aggression [8,9], skin color [10,11], hatching [12], initiation of

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ecdysis [13], metamorphosis [14,15], physiological hormone [6], metabolism [16] and stress response [17,18] during the last decades.

Blunt snout bream (*Megalobrama amblycephala*) is one of the most important aquaculture species in China due to its delicious taste and high commercial value. In recent years, factories have been established to farm blunt snout breams in inland areas of China. However, aquaculture of this fish has been restrained greatly by growth retardation, immunosuppression and high mortality caused by inappropriate culture management, resulting in great economic loss and energy dissipation [19]. The manipulation of artificial light may lead to an abrupt change to rearing conditions which may result in a stress response compromising fish welfare and growth performances [20]. According to Wang et al. (2013) [18], plasma cortisol concentration of grouper (*Epinephelus coioides*) was significantly higher at both low and high light intensity, indicating that the light intensity was acting as a stressor since plasma cortisol can be seen as a stress-sensitive marker in fish [21]. Besides, chronic stress events may also affect the long-term physiology of fish, suppressing immune function [20]. Roberts (1995) [22] reported that visible light was received by the retina and was then transduced and delivered to the visual cortex, and also via an alternative pathway to the suprachiasmatic nucleus (SCN), which controlled circadian rhythm and neuroendocrine function. Visible light exposure can modulate the pituitary and pineal glands, which leads to the change of neuroendocrine function. In return, these neuroendocrine changes can lead to immune modulation. So far, there is relatively little information on whether or not light intensity could affect the antioxidant defenses of fish. It was reported that various biological and non-biological factors in practical aquaculture might cause oxidative stress of fish [23]. Since light could be one of the stressors for fish, it would affect the physiological state of fish, as might consequently lead to the alteration of oxidative status. In this study, it was hypothesized that light intensity might have an influence on culture performance, stress response and the oxidative stress status during juvenile development in blunt snout bream. In addition, the possibility of reducing disease resistance caused by suppressed immunity and severe oxidative stress was evaluated. Within this context, the effect of five light intensities on the growth performance, stress response and activity of antioxidant enzymes as well as the disease resistance of juvenile blunt snout bream have been studied. The data obtained here may provide some new insights into the immunomodulation of fish via culture management manipulations.

2. Materials and methods

2.1. Experimental fish and feeding trial

Blunt snout bream juveniles were obtained from the Fish Hatchery of Yangzhou (Jiangsu, China). The experiment was performed in a recirculating aquaculture system in the laboratory. After a two-week acclimation period, 300 healthy fish (average weight ± SE = 18.04 ± 0.22 g) were randomly distributed into fifteen 100-L tanks (20 fish/tank). The experiment was carried out with five groups for light intensity treatments at 100 lx, 200 lx, 400 lx, 800 lx and 1600 lx for eight weeks. Triplicate tanks were used for each treatment. Artificial light was placed 50 cm above the water surface with two 14 W fluorescent light tubes, which have a broad spectrum between 450 and 670 nm. The different light intensity was achieved by disconnecting one of the two light tubes and covering the fluorescent tube by shade cloth. Each aquarium was covered by black plastic cloth to shadow the tanks from additional lights. Light intensities were measured at the center and edge of water surface with a model T-10A illuminance meter (Konica Minolta Inc., Japan). The light was provided at 0600–1800 (12 h light: 12 h dark) every day controlled by digital timer. Fish were hand-fed to satiation three times daily (0800, 1200 and 1600) with a commercial fish diet containing 30% crude protein. The water outlet from each tank was passed through a sieve to collect uneaten feed till 30 min after meal termination. The uneaten feed was dried at 65 °C overnight, weighed and subtracted from the amount offered to estimate feed consumed. Feed intake was recorded daily. During the rearing period, the water temperature ranged from 28 °C to 30 °C, pH fluctuated between 7.2 and 7.5, dissolved oxygen was kept approximately at 5.0 mg L^{-1}, total ammonia nitrogen was lower than 0.1 mg L^{-1} and nitrite was less than 0.01 mg L^{-1}.

2.2. Sampling and analysis

2.2.1. Sampling

At the end of the feeding trial, fish were starved for 24 h before sampling. All individuals from each replication were quickly removed and anesthetized in diluted MS-222 (tricaine methanesulfonate, Sigma, USA) at the concentration of 100 mg L^{-1}. Total number and weight of fish in each tank were determined for the calculation of growth performance. Five fish were randomly selected from each tank and blood sample was quickly drawn from the caudal vein using heparinized plastic syringes. One part of the blood sample was transferred immediately to heparinized capillary tubes for the determination of total blood leucocyte and erythrocyte. The remaining blood sample was separated by centrifuged, and the supernatant was pooled and stored at −70 °C for subsequent analysis. Individual livers were washed thoroughly with chilled saline (0.89 g NaCl L^{-1}), then dried quickly over a piece of filter paper and stored at −70 °C for subsequent analysis.

2.2.2. Calculation of growth performance

The fish were weighed individually before and after the eight-week feeding trial. For each treatment, all fish were determined to quantify the percent of weight gain, special growth rate, feed intake and feed conversion ratio. The growth parameters were calculated as follows:

$$\text{Initial weight(g)} = \text{total initial weight/total fish number.}$$

$$\text{Final weight(g)} = \text{total final weight/total fish number.}$$

$$\text{Weight gain(WG, \%)} = 100 \times (\text{mean final weight} - \text{mean initial weight}).$$

$$\text{Special growth rate}\left(\text{SGR, \% d}^{-1}\right) = 100 \times [(\text{Ln final weight} - \text{Ln initial weight})/\text{rearing days}].$$

$$\text{Feed intake}\left(\text{g fish}^{-1}\right) = \text{total feed intake of each tank/total number of fish in each tank.}$$

$$\text{Feed conversion ratio(FCR)} = \text{total feed intake/total weight gain.}$$

2.2.3. Measure of plasma stress parameters

Plasma cortisol concentration was determined by a validated radioimmunoassay (RIA) method for fish according to Winberg and Lepage (1998) [24] using a commercial ^{125}Iodine cortisol RIA kit (ref. no. KD005-0049) produced by Beijing North Institute of