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ORIGINAL ARTICLE

Biology

Effects of LEDs of different spectra on immunity-related responses in olive flounder *Paralichthys olivaceus* during short starvation

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Abstract
In this study, we investigated the regulation of innate immune responses in olive flounder *Paralichthys olivaceus* under irradiation with various light-emitting diodes (LEDs), blue (460 nm), green (520 nm), and red (630 nm), at two intensities (0.3 and 0.6 W/m^2) for 9 days. To evaluate the effects, we analyzed the levels of melatonin receptor 1 (MTR-1), melatonin, IgM, and lysozyme, which are related to the immunity index. The results showed that melatonin, IgM, and lysozyme significantly decreased during starvation compared to their levels in a normally fed control group. However, the decreases were less pronounced in the green and blue LED-exposed groups than those in the red LED-exposed group during the experimental period. These results confirmed that short-wavelength light (green and blue) is effective in maintaining enhanced immunity in olive flounder.

Keywords IgM · Immunity · Light-emitting diode · Lysozyme · Melatonin · *Paralichthys olivaceus* · Starvation

Introduction

Fish starvation is frequently caused by migratory spawning, shortage of feed due to seasonal changes, and the management of aquaculture breeding, and starvation periods last from a few days to a few months (Cho et al. 2006; Caruso et al. 2010). When fish are under starvation conditions, various biochemical and metabolic activities are induced, related to the energy maintenance in the body for survival (Caruso et al. 2011; Akbary and Jahanbakshi 2016). Therefore, during prolonged starvation periods, the risk of pathogen infection increases because of stress, which induces not only a nutritional imbalance but also cellular immune degradation (Iwama et al. 2006; Caruso et al. 2010).

The optimum water temperature for olive flounder *Paralichthys olivaceus*, which was the experimental organism in this study, is approximately 20 °C, and much damage occurs in hot summers when the water temperature rises above 25 °C. As a solution to this problem, short-term starvation, without supplying feed for a certain period, is considered a realistic approach (Cho et al. 2006; Caruso et al. 2011). In addition, short-term starvation is inevitable in the case of compensatory growth, when fast growth is induced by feeding after restriction of food supply for a certain period of time (Cho et al. 2006). It is also necessary to study the influence of short-term starvation used for compensatory growth on fish immune responses. Although many studies have been carried out on the effects of starvation on the growth and metabolic responses in current aquaculture fish species (De Pedro et al. 2003; Pérez-Jiménez et al. 2007, 2012; Costas et al. 2011), the influence of fish immune responses on starvation has not been adequately studied (Caruso et al. 2011, 2012; Choi et al. 2012).

Immune responses in fish consume much energy and may differ, depending on the nutritional status of fish (Akbary and Jahanbakshi 2016; Eslamloo et al. 2017). In general, melatonin, immunoglobulin M (IgM), and lysozyme serve as immunological indicators in the body (Bowden 2008; Liu et al. 2013).

Melatonin is a neurotransmitter that binds to the melatonin receptor (MT-R), which is a G protein-coupled receptor. Melatonin is known as a strong antioxidant, which directly removes reactive oxygen species in the body and has various physiological functions (Gülçin et al. 2009;

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Carrillo-Vico et al. 2013; Choi et al. 2016a). There are three MT-R subtypes, MTR-1, MTR-2, and MTR-3 (Gülçin et al. 2009; Carrillo-Vico et al. 2013), among which MTR-1 is known to be responsible for physiological functions in fish through sensing environmental changes (Choi et al. 2016b). Furthermore, it has been reported that MTR-1 is expressed in immunocompetent cells and is involved in a specific membrane receptor immune system, so it is a very suitable immunity indicator for cellular immune degradation induced when the fish is exposed to starvation (Carrillo-Vico et al. 2003; Pandi-Perumal et al. 2008).

Meanwhile, lysozyme, which is expressed not only in mammals but also in marine organisms, is known to act on digestive metabolism (Hikima et al. 2003; Liu et al. 2013). In addition, it improves the nutritional status and promotes the growth, while also increasing the efficiency of nutrient utilization through immune responses, which reduce the growth of harmful microorganisms and synthesis of inflammatory cytokines (Hikima et al. 2003; Liu et al. 2013). IgM, an immune antibody initially produced in response to antigenic stimulation, mediates the neutralization and complement activity of infectious viruses as the main component of the humoral and cellular immune systems (Tort et al. 2003; Bowden 2008; Ye et al. 2013).

Recent studies have reported that light at specific wavelengths is effective for reducing stress caused by starvation. Accordingly, light-emitting diodes (LEDs), which can emit light of only specific wavelengths, have drawn attention. LEDs are characterized by not only a low power consumption and high efficiency but also by a longer life than that of fluorescent lamps. Various studies have reported that the use of LEDs with specific wavelengths can reduce fish stresses and increase immunity (Choi et al. 2012; Kim et al. 2014, 2016).

Based on previous research, endocrinological studies are needed to determine whether light of specific wavelengths may exert positive effects on immune responses in fish exposed to a starvation environment. Therefore, this study was conducted to expose the olive flounder, a representative aquaculture fish species, to a starvation environment for a short period of time and evaluate the effects of starvation stress on immune responses. Light of specific wavelengths and intensity was investigated to confirm its effectiveness in decreasing starvation-induced stress and enhancing immunity.

Materials and methods

Experimental fish and conditions

Juvenile olive flounders *P. olivaceus* ($n = 160$; length 12.3 ± 0.6 cm; mass 16.2 ± 0.5 g), were purchased from a commercial aquarium (Jeju, Korea) and were allowed to acclimate for 2 weeks in eight 300-l circulation filter tanks in the laboratory.

The fish were divided into control (normal condition) and experimental groups (starvation condition). The fish in the normally fed control group were exposed to white fluorescent light (20 W; Philips, Netherlands), with light intensity at the water surface of approximately 0.96 W/m^2. Fish were fed twice daily at 09:00 and 17:00 with floating commercial feed containing 50% crude protein (KOFEC Feed, Jeonnam, South Korea) at 3% of total body weight.

The fish in the experimental groups were exposed to various spectra of light [white fluorescent light, 0.96 W/m^2; blue (460 nm); green (520 nm); and red (630 nm)] at two different intensities (0.3 W/m^2 and 0.6 W/m^2) for 9 days without feeding (Fig. 1).

The water temperature and photoperiod were 20 ± 1 °C and a 12-h light/dark cycle (lights on at 07:00 and off at 19:00), respectively. The light sources were placed 50 cm above the water surface, and the depth of the water was 50 cm. The irradiance levels at the bottom of the tanks with external light interception were maintained at approximately 0.3 and 0.6 W/m^2 using a spectrometer (MR-16; Rainbow

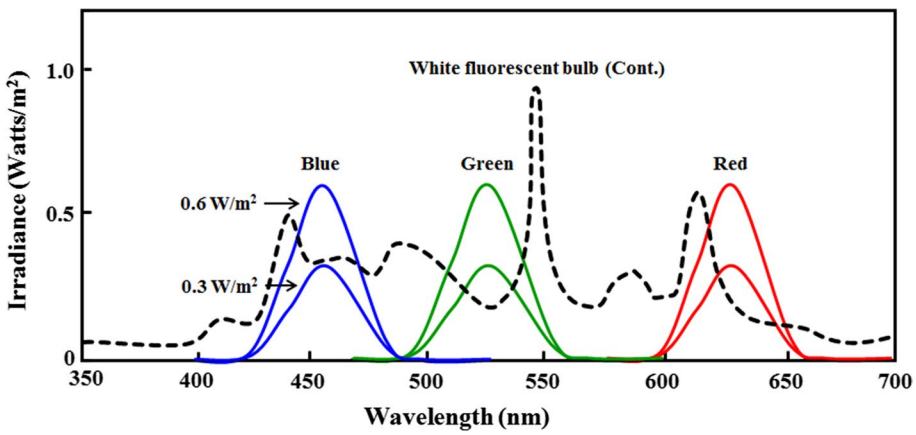

Fig. 1 Spectral profiles of the blue (450 nm), green (520 nm), and red (640 nm) light-emitting diodes (LEDs) and white fluorescent bulb (*Cont.*) used in the present study. Two different intensities (0.3 and 0.6 W/m^2) were used for each LED treatment

Light Technology Co., Ltd., Taoyuan, Taiwan) and a photoradiometer (HD 2102.1; Delta OMH Co., Caselle di Selvazzano, Italy). We sampled 5 fish from each experimental group at 0, 3, 6, and 9 days at 15:00. According to a study by Kim et al. (2007), fish were anesthetized with 400 µg/l 2-phenoxyethanol (Sigma, St. Louis, MO, USA) to minimize the stress prior to blood and tissue collection. Blood was collected rapidly from the caudal vein using a 1-ml syringe coated with heparin. Plasma samples were separated by centrifugation (4 °C, 1000 *g*, for 15 min) and stored at − 80 °C until analysis. The tissues were collected from fish, immediately frozen in liquid nitrogen, and stored at − 80 °C until total RNA was extracted for analysis.

Total RNA extraction and cDNA synthesis

Total RNA (treated by DNase for removing genomic DNA) was extracted from the liver of each sample using TRI Reagent® (Molecular Research Center, Inc., Cincinnati, OH, USA), according to the manufacturer's instructions. The concentration and purity of the RNA samples were determined by spectrophotometer at 260 and 280 nm (BioDrop, Cambridge, UK). Then, 2 µg of total RNA was reverse-transcribed in a total reaction volume of 20 µl using an oligo-$(dT)_{15}$ anchor and M-MLV reverse transcriptase (Promega, Madison, WI, USA), according to the manufacturer's protocol. The resulting cDNA was diluted and stored at 4 °C for use in real-time quantitative polymerase chain reaction (qPCR) analysis.

Real-time quantitative polymerase chain reaction (qPCR)

The qPCR analysis was conducted to determine the relative expression levels of MTR-1, IgM (heavy chain and constant region), and lysozyme using the total RNA extracted from the liver of olive flounder. The qPCR primers were designed using known olive flounder sequences (Table 1). qPCR amplification was performed using a Bio-Rad CFX96™ Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA) and iQ™ SYBR Green Supermix (Bio-Rad) with the following conditions: 0.5 µl of cDNA, 0.26 µM of each primer, 0.2 mM dNTP, SYBR Green, and Taq polymerase in buffer [10 mM Tris–HCl (pH 9.0), 50 mM KCl, 1.4 mM $MgCl_2$, and 20 nM fluorescein] to a total volume of 25 µl. The qPCR process was carried out as follows; one cycle of initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 20 s, and annealing at 55 °C for 20 s; followed by 5 min at 72 °C for the final extension. Each experimental group was run in triplicate to ensure consistency. As an internal control, qPCR analysis for β-actin was performed on the same samples, and all data are expressed as the change with respect to the corresponding β-actin calculated threshold cycle (Ct) level. All analyses were based on the Ct values of the PCR products. The calibrated ΔCt value (ΔΔCt) for each sample and internal controls (β-actin) was calculated [$\Delta\Delta Ct = 2\text{^} - (\Delta Ct_{sample} - \Delta Ct_{internal\ control})$].

Western blot analysis

Total protein was extracted from the olive flounder brain and liver using a T-PER® tissue protein extraction reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA), following the manufacturer's instructions. Subsequently, 30 µg of protein was loaded per lane onto Mini-PROTEAN® TGX™ gels (Bio-Rad, USA), and a protein ladder (Bio-Rad) was used for reference. Samples were electrophoresed at 180 V, and the gels were immediately transferred to a 0.2-µm polyvinylidene difluoride membrane (Bio-Rad) at 85 V for 3 min using the Trans-Blot® Turbo™ transfer system. The membranes were blocked with 5% skim milk (Difco, France) and dissolved in Tris-buffered saline with Tween 20 (TTBS; pH 7.4) for 45 min and then washed with TTBS. After washing, the membranes were sequentially incubated with polyclonal goat anti MTR-1 primary antibody (NBP1-28,912; dilution 1:4000; Novus biologicals®; approximately 45 kDa) for 2 h, followed by incubation with a horseradish peroxidase-conjugated mouse anti IgG secondary antibody (PMP01; dilution, 1:4000; Bio-Rad) for 60 min. As a loading control, with polyclonal rabbit anti β-tubulin antibody (ab6046; dilution 1:4000; Abcam, Cambridge, UK; approximately 55 kDa) was used. Bands were detected using

Table 1 Primers used for qPCR amplification

Genes (accession no.)	Primer	DNA sequences
MTR-1 (HM107826)	Forward	5′-CCT CAC CTC CAT CTT CCA-3′
	Reverse	5′-ATG TAG CAG TAG CGG TTA ATG-3′
Lysozyme (AB050469)	Forward	5′-GTC TAC GAA CGC TGT GAA TG-3′
	Reverse	5′-TGG CTC TGG TGT TGT AGT-3′
IgM (AB052744)	Forward	5′-GAC TCT GAC TTG CTA TGT GAA-3′
	Reverse	5′-TTG TGG TAT TGA ACT TGT ATC CT-3′
β-actin (HQ386788)	Forward	5′-TGC AGA AGG AGA TCA CAG CC-3′
	Reverse	5′-ACT CCT GCT TGC TGA TCC AC-3′

a sensitive electrochemiluminescence (ECL) system (ECL Advance; GE Healthcare Life Sciences, Uppsala, Sweden) and exposed for 2 min using a Molecular Imager® Chemi-DocTM XRS + System (Bio-Rad, USA). The images were scanned using a high-resolution scanner, and the band densities were estimated using the Image Lab™ software, version 3.0 (Bio-Rad).

Analysis of plasma parameters

Plasma levels of melatonin, IgM, and lysozyme were analyzed using the following immunoassays: quantitative sandwich ELISA kit (MBS013211 for fish melatonin; MBS099538 for fish lysozyme; MyBioSource San Diego, Ca, USA) and competitive ELISA kit (MBS700823 for fish IgM; MyBioSource). Melatonin and lysozyme, which are measured by the sandwich method, are firstly used as capture antibodies for mouse monoclonal antibodies, and then the secondary antibodies analyzed for detection are rat polyclonal antibodies for melatonin and rabbit polyclonal antibodies for lysozyme. The competitive method for IgM first proceeds through coating with the goat anti-rabbit antibody and then rabbit polyclonal antibody is used as the specific antibody. First, for the sandwich method for MTR-1 and lysozyme, 50 µl each of the standard and sample plasma were placed in each well; subsequently, 100 µl HRP-conjugate was added, the well was covered with an adhesive strip, and incubated for 60 min at 37 °C. Following 4 washes, any remaining wash buffer was removed by aspirating or decanting. Subsequently, 50 µl each of substrate A and substrate B were added to each well. These substrate solutions were then incubated for 15 min at 37 °C in the dark, during which time they changed from colorless to dark blue. Following incubation, 50 µl of stop solution was added to each well. Finally, the optical density of each well was determined within 15 min using a microplate reader set to 450 nm. In the competitive method for IgM, 50 µl each of the standard and sample plasma were placed in each well; 50 µl HRP-conjugate and 50 µl antibody were then added to each well. These were mixed and then incubated for 1 h at 37 °C. Each well was aspirated and washed, repeating twice for a total of three washes. Subsequently, 50 µl each of substrate A and substrate B were added to each well, and incubated for 15 min at 37 °C in the dark. Following incubation, 50 µl of stop solution was added to each well. Finally, the optical density of each well was determined within 15 min using a microplate reader set to 450 nm.

Statistical analysis

All data were analyzed using the SPSS statistical package (version 19.0; SPSS, Inc., USA). One-way analysis of variance, followed by Tukey's post hoc test, was used to compare differences between the groups. A value of $p < 0.05$ was considered statistically significant. The data are expressed as the mean ± standard error of the mean (SE).

Results

Changes in body weight

This study compared the body weights of olive flounders *P. olivaceus* exposed to 9 days in feeding or starvation environments (Table 2). Body weights decreased in all the starvation groups, but not in the normally fed control group. Compared with the initial body weight of 16.2 ± 0.5 g, the normally fed control group increased to 16.6 ± 0.3 g over 9 days. However, the weight of the starved control group decreased to 15.9 ± 0.2 g over 9 days. In the experimental groups irradiated by LEDs, there was no significant difference in final body weight, but the tendency of body weight change was largest in the red LED group and less in the green and blue LED groups.

Table 2 Changes in body weight of fish exposed to 9 days of either feeding or starving

Experimental group & intensity	Initial weight (g/fish)	Final weight (g/fish)
Normally fed control group	16.2 ± 0.5	16.6 ± 0.3
Starvation control group	16.2 ± 0.5	15.9 ± 0.2
Red LED-exposed		
0.3 W/m^2	16.2 ± 0.5	15.9 ± 0.4
0.6 W/m^2	16.2 ± 0.5	15.8 ± 0.3
Green LED-exposed		
0.3 W/m^2	16.2 ± 0.5	16.0 ± 0.4
0.6 W/m^2	16.2 ± 0.5	16.1 ± 0.3
Blue LED-exposed		
0.3 W/m^2	16.2 ± 0.5	15.9 ± 0.3
0.6 W/m^2	16.2 ± 0.5	16.0 ± 0.4

Data presented as mean ± SEM

Changes in MTR-1 mRNA and protein expression, and melatonin plasma levels

This experiment was performed to compare changes in the MTR-1 mRNA and protein expression in the liver and plasma melatonin levels in olive flounder exposed to starvation for 0, 3, 6, and 9 days (Fig. 2). Western blot analysis revealed a protein with specific immunoreactivity, and its mass corresponded to that predicted for MTR-1 (45 kDa; Fig. 2a). The MTR-1 protein band and quantification were significantly decreased in the starvation groups compared with that in the normally fed control group, but were higher in the green and blue LED-exposed groups than in the starvation control group. In particular, at 6 and 9 days, the starvation control and red LED groups showed significantly lower protein band and quantification than those in the normally fed control group, and green and blue LED groups. When the starvation period reached 3 days, MTR-1 mRNA expression and plasma melatonin were significantly decreased in the starvation control group and red LED-exposed group

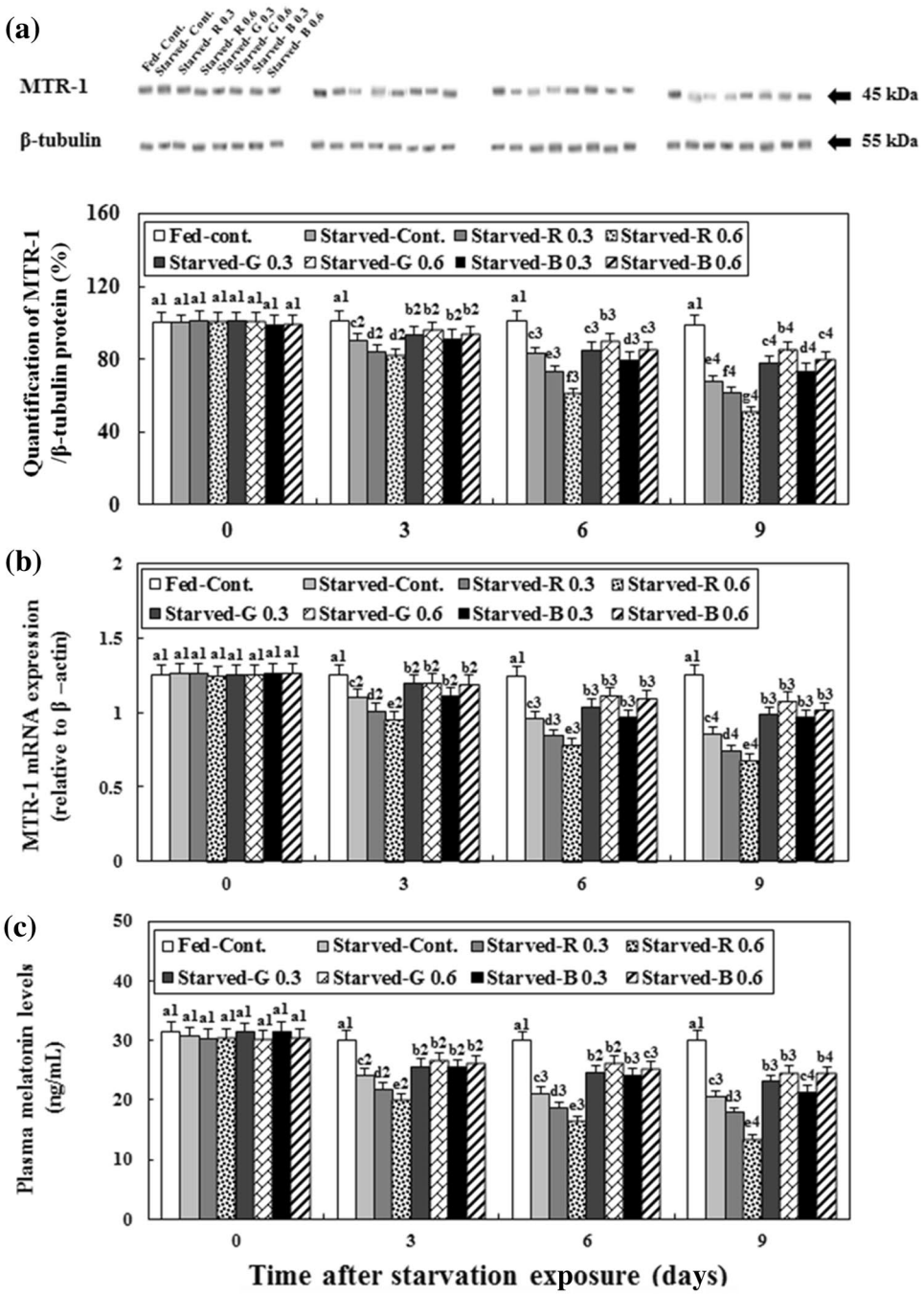

Fig. 2 Changes in western blot quantification and protein of MTR-1 (**a**) and mRNA expression (**b**) and plasma melatonin levels (**c**) in olive flounder exposed to various light sources, including red (*R*), green (*G*), and blue (*B*) LEDs at different light intensities (0.3 and 0.6 W/m^2) and a white fluorescent bulb (*Cont.*). Values with *different numbers* are significantly different ($p < 0.05$) for the same LED spectrum at each starvation period. The *lowercase letters* indicate significant differences ($p < 0.05$) between different LED spectra at the same starvation period. All values are the mean ± SE ($n = 5$)

compared with that in the normally fed control group, but this gradually decreased in the green and blue LED-exposed groups (Fig. 2b, c). At 6 and 9 days of the starvation period, the results of MTR-1 mRNA expression and plasma melatonin showed a similar tendency; however, the differences in the expression were larger than those at 3 days.

Changes in lysozyme mRNA expression and plasma lysozyme levels

On days 3 and 6 of the starvation period, the mRNA expression level of lysozyme in the liver decreased in all experimental groups, except the normally fed control group and the green and blue LED-exposed groups at 0.6 W/m^2 (Fig. 3a). When the starvation period reached 9 days, the expression levels of lysozyme decreased in all experimental groups, except the normally fed control group, unlike those at 3 and 6 days. All plasma lysozyme levels showed the same tendency as mRNA expression levels (Fig. 3b).

Changes in IgM mRNA expression and plasma IgM levels

We also investigated changes in the mRNA expression of IgM in the liver, and the levels of IgM in plasma, following starvation exposure (Fig. 4). On days 3 and 6 of the starvation period, the IgM mRNA expression was the lowest in the red LED group, while there were no significant differences between the groups exposed to light at the other wavelengths. At 9 days, the starvation control and red LED groups showed significantly lower mRNA expression levels than those in the green and blue LED groups. The plasma IgM levels showed similar trends but were significantly higher at 0.6 W/m^2 than at 0.3 W/m^2 in the green and blue LED groups.

Discussion

In this study, olive flounder *P. olivaceus* fish were exposed to short-term starvation, and their immune-related responses were investigated under irradiation with light at various wavelengths (fluorescent, red, green, and blue) and intensities (0.3 and 0.6 W/m^2). To confirm the changes in the immunity of flounders exposed to starvation, we evaluated the MTR-1, IgM, and lysozyme mRNA expression, as well as the melatonin, IgM, and lysozyme concentrations in the plasma, which are mainly used as indicators of immunity.

MTR-1, which is expressed in immunocompetent cells and is related to a specific membrane receptor of the immune system, induces cellular immunity. It decreases when the animal is exposed to a starvation environment (Carrillo-Vico et al. 2003; Pandi-Perumal et al. 2008). As the starvation period elapsed, the mRNA and protein expression of MTR-1 and plasma melatonin levels tended to significantly decrease in all starvation groups. In those exposed to

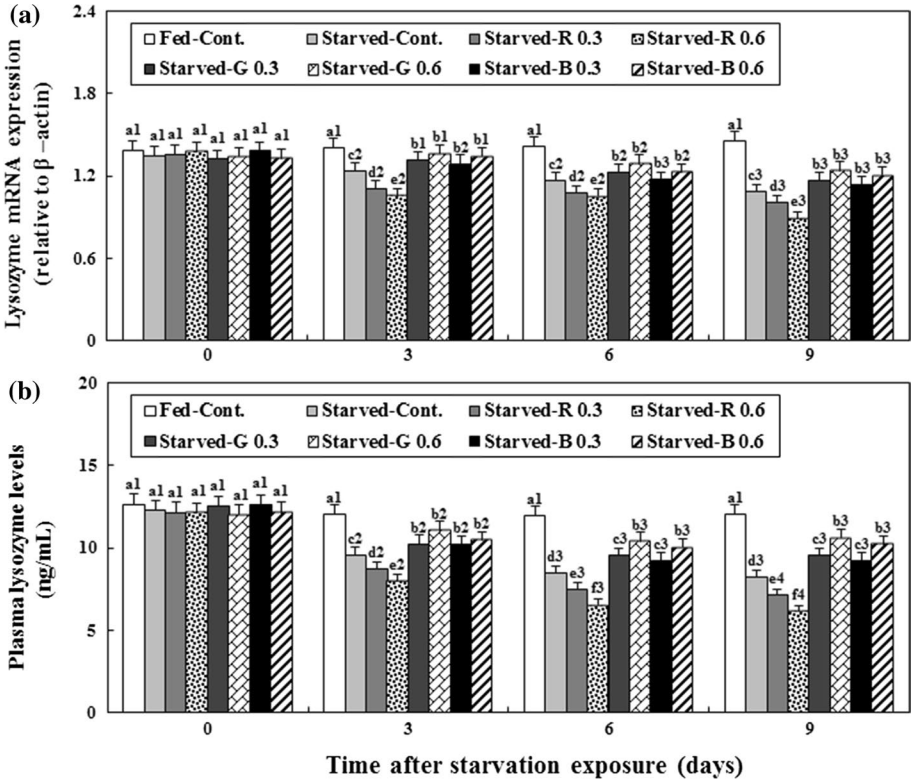

Fig. 3 Changes in lysozyme mRNA expression (**a**) and plasma levels (**b**) in olive flounder exposed to various light sources, including red (*R*), green (*G*), and blue (*B*) LEDs at different light intensities (0.3 and 0.6 W/m^2) and a white fluorescent bulb (*Cont.*). Values with *different numbers* are significantly different ($p < 0.05$) for the same LED spectrum at each starvation period. The *lowercase letters* indicate significant differences ($p < 0.05$) between different LED spectra at the same starvation period. All values are the mean ± SE ($n = 5$)

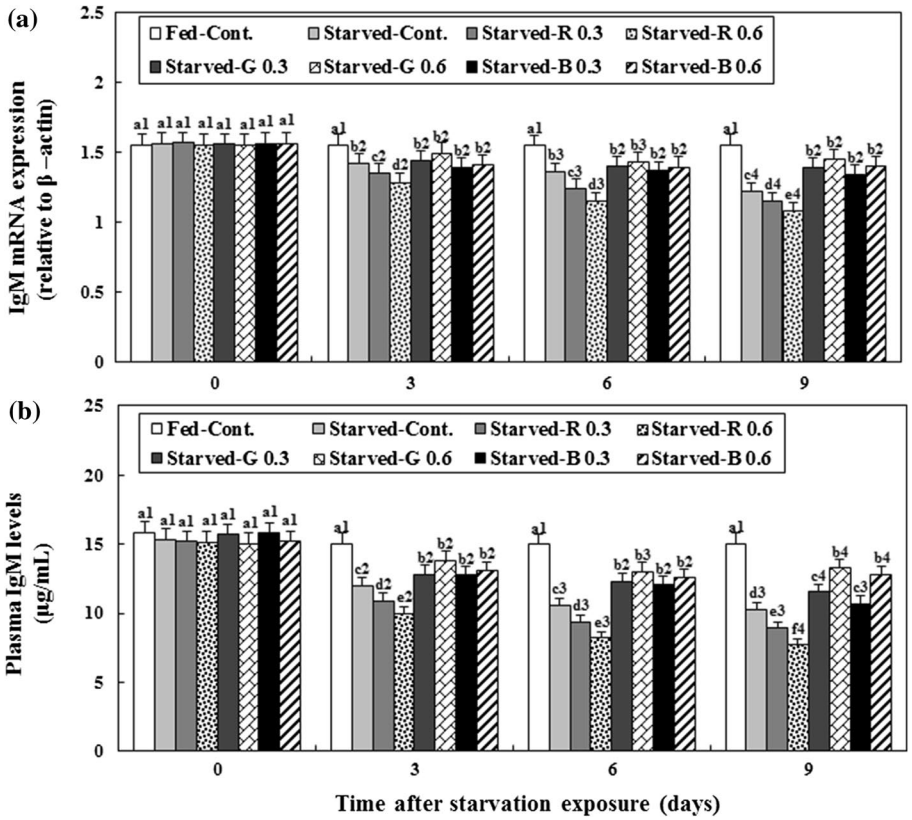

Fig. 4 Changes in IgM mRNA expression (**a**) and plasma levels (**b**) in olive flounder exposed to various light sources, including red (*R*), green (*G*), and blue (*B*) LEDs at different light intensities (0.3 and 0.6 W/m^2) and a white fluorescent bulb (*Cont.*). Values with *different numbers* are significantly different ($p < 0.05$) for the same LED spectrum at each starvation period. The *lowercase letters* indicate significant differences ($p < 0.05$) between different LED spectra at the same starvation period. All values are the mean ± SE ($n = 5$)

short-wavelength light sources such as green and blue LEDs, all of the target immunity indexes were significantly lower than those in the normally fed control group but significantly higher than those in the starvation groups exposed to the red LED. A similar result was reported when the rainbow trout *Oncorhynchus mykiss* was starved for 7 days; the starvation environment acted as a stressor, and the plasma melatonin concentration significantly decreased (Ceinos et al. 2008).

Based on similar studies, we assumed that starvation acts as a stressor, lowering the MTR-1 mRNA expression and plasma melatonin levels and negatively affecting immune activity (Carrillo-Vico et al. 2003, 2013; Pandi-Perumal et al. 2008). However, as the MTR-1 mRNA expression and plasma melatonin concentrations increased upon irradiation using short-wavelength light sources such as green and blue LEDs, it seems that short-wavelength light reduces the stress caused by starvation and thereby increases the fish immunity.

The fish immune system is the basic mechanism involved in the production of immune substances to protect the body from virus infection and stress (Najafi et al. 2015). Among them, the lysozyme activity used as an indicator of immunity varies based on several factors, such as the fish species, nutritional status, sex, and environmental factors (Caruso et al. 2011, 2012). In this study, the lysozyme concentrations tended to significantly decrease in all starvation groups as the process of starvation progressed. However, the lysozyme mRNA expression and plasma concentrations decreased more significantly in the starvation groups exposed to the long-wavelength red LED than did those in the starvation control group. Meanwhile, the lysozyme mRNA expression and plasma concentrations were significantly higher in the starvation groups exposed to short-wavelength light sources such as green and blue LEDs than those in the starvation control group. As shown in similar studies, when tinfoil barb *Barbonymus schwanenfeldii* fish were starved for 2 weeks, the plasma lysozyme concentrations significantly decreased in the starvation groups (Eslamloo et al. 2017). According to a study by Choi et al. (2012), the plasma lysozyme concentrations in cinnamon clownfish *Amphiprion melanopus*, irradiated with fluorescent light and LEDs of different wavelengths (red, green, and blue) for 12 days, significantly decreased in starvation groups. However, on day 6, the lysozyme mRNA expression increased in the starvation groups irradiated using short-wavelength light sources such as green and blue LEDs, and there were no significant differences with the normally fed control group. On days 9 and 12, the lysozyme concentrations in the latter groups were lower than those in the group irradiated with a fluorescent lamp but significantly higher than those in the group irradiated with a long-wavelength red LED (Choi et al. 2012).

In addition, we measured the mRNA expression and plasma concentrations of IgM, which is another index of immunity, to determine whether starvation decreases the fish

immunity. The IgM mRNA expression levels and plasma concentrations showed almost the same trends as those observed for lysozyme, i.e., significantly decreased in all starvation groups; however, higher IgM expression levels and plasma concentrations were observed in the starvation groups irradiated with short-wavelength light sources such as green and blue LEDs compared with those in the starvation groups irradiated with the long-wavelength red LED. Liu et al. (2013) have reported that when catfish *Ictalurus punctatus*, were fasted for 7 days, the IgM mRNA expression decreased in the starvation groups.

Thus, the mRNA expression levels and plasma concentrations of lysozyme and IgM appear to decrease under starvation stress, depending on the fish species and the starvation period. Regardless of the fish species and starvation period, irradiation with short-wavelength light sources such as green and blue LEDs seems to be effective in improving immunity of the fish exposed to a starvation stress environment.

The results of this study showed that the expression of immune-related substances tended to increase when short wavelengths (green and blue LEDs) were irradiated during the starvation period, and the expression of immune-related substances tended to decrease when long-wavelength red LED was irradiated. In addition, it was observed that the effects increase as the intensity of light increases. Kim et al. (2016) reported that antioxidant enzymes in olive flounder exposed to water stress were expressed more at 0.5 W/m^2 than at 0.3 W/m^2. Also, Choi et al. (2016b) showed that the immunopotentiating substance of the rock bream *Oplegnathus fasciatus* exposed to bisphenol A for 5 days was expressed more at 0.5 W/m^2 than 0.3 W/m^2. Therefore, based on previous research, it is considered that the irradiation of short-wavelength light sources such as green and blue LEDs will influence the endocrine system which affects the stress and immune response.

Despite the positive aspect of compensatory growth after short-term fasting, which is used to prevent damage to various aquaculture species at high temperatures, even a short period of fasting is considered to be a stress factor for cultured fish, including flounder. Thus, starvation has been shown to reduce fish immunity, potentially having a negative impact on physiological functions such as stress control and homeostasis. However, it is believed that irradiation with short-wavelength light sources such as green and blue LEDs is effective in reducing the stress caused by starvation and effectively complements the immunity of fish. Future studies should investigate the mechanism of positive effects of fish irradiation with light of specific wavelengths under starvation stress at high summer temperatures, which is important because of global environmental changes.

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References

Akbary P, Jahanbakshi A (2016) Effect of starvation on growth, biochemical, hematological and non-specific immune parameters in two different size groups of grey mullet *Mugil cephalus* (Linnaeus, 1758). Acta Ecol Sin 36:205–211

Bowden TJ (2008) Modulation of the immune system of fish by their environment. Fish Shellfish Immunol 25:373–383

Carrillo-Vico A, Garcia-Perganeda A, Naji L, Calvo JR, Romero MP, Guerrero JM (2003) Expression of membrane and nuclear melatonin receptor mRNA and protein in the mouse immune system. Cell Mol Life Sci 60:2272–2278

Carrillo-Vico A, Lardone PJ, Álvarez-Sánchez N, Rodríguez-Rodríguez A, Guerrero JM (2013) Melatonin: buffering the immune system. Int J Mol Sci 14:8638–8683

Caruso G, Maricchiolo G, Micale V, Genovese L, Caruso R, Denaro MG (2010) Physiological responses to starvation in the European eel *Anguilla anguilla*: effects on haematological, biochemical, non-specific immune parameters and skin structures. Fish Physiol Biochem 36:71–83

Caruso G, Denaro MG, Caruso R, Mancari F, Genovese L, Maricchiolo G (2011) Response to short-term starvation of growth, haematological, biochemical and non-specific immune parameters in European sea bass *Dicentrarchus labrax* and blackspot sea bream *Pagellus bogaraveo*. Mar Environ Res 72:46–52

Caruso G, Denaro MG, Caruso R, Genovese L, Mancari F, Maricchiolo G (2012) Short fasting and refeeding in red porgy (*Pagrus pagrus*, Linnaeus 1758): response of some haematological, biochemical and non specific immune parameters. Mar Environ Res 81:18–25

Ceinos RM, Polakof S, Illamola AR, Soengas JL, Míguez JM (2008) Food deprivation and refeeding effects on pineal indoles metabolism and melatonin synthesis in the rainbow trout *Oncorhynchus mykiss*. Gen Comp Endocrinol 156:410–417

Cho SH, Lee S, Park BH, Ji SC (2006) Compensatory growth of juvenile olive flounder *Paralichthys olivaceus* L., and changes in proximate composition and body condition indexes during fasting and after refeeding in summer season. J World Aquacult Soc 37:168–174

Choi CY, Shin HS, Choi YJ, Kim NN, Lee J, Kil GS (2012) Effect of LED light spectra on starvation-induced oxidative stress in the cinnamon clownfish *Amphiprion melanopus*. Comp Biochem Physiol Part A 163:357–363

Choi JY, Kim NN, Choi YJ, Park MS, Choi CY (2016a) Differential daily rhythms of melatonin in the pineal gland and gut of goldfish *Carassius auratus* in response to light. Biol Rhythm Res 47:145–161

Choi JY, Kim TW, Choi YJ, Kim NN, Oh S-Y, Choi CY (2016b) Effects of various LED light spectra on antioxidant and immune response in juvenile rock bream *Oplegnathus fasciatus* exposed to bisphenol A. Environ Toxicol Pharmacol 45:140–149

Costas B, Aragão C, Ruiz-Jarabo I, Vargas-Chacoff L, Jesús Arjona F, Dinis MT, Mancera JM, Conceição LEC (2011) Feed deprivation in Senegalese sole (*Solea senegalensis Kaup*, 1858) juveniles: effects on blood plasma metabolites and free amino acid levels. Fish Physiol Biochem 37:495–504

De Pedro N, Delgado MJ, Gancedo B, Alonso-Bedate M (2003) Changes in glucose, glycogen, thyroid activity and hypothalamic catecholamines in tench by starvation and re-feeding. J Comp Physiol 173(B):475–481

Eslamloo K, Morshedi V, Azodi M, Akhavan SR (2017) Effect of starvation on some immunological and biochemical parameters

in tinfoil barb *Barbonymus schwanenfeldii*. J Appl Anim Res 45:173–178

Gülçin İ, Beydemir Ş, Hisar O, Köksal E, Reiter RJ (2009) Melatonin administration increases antioxidant enzymes activities and reduces lipid peroxidation in the rainbow trout (*Oncorhynchus mykiss*, Walbaum) erythrocytes. Turk J Vet Anim Sci 33:241–245

Hikima S, Hirono I, Aoki T (2003) The lysozyme gene in fish. In: Aquatic Genomics, pp 301–309

Iwama GK, Afonso LOB, Vijayan MM (2006) Stress in fishes. The physiology of fishes. CRC Press, Boca Raton, pp 319–342

Kim J-H, Kim HC, Noh JK, Lee J-H, Kang J-H, Kim K-K (2007) Growth and sex ratio of juvenile olive flounder *Paralichthys olivaceus* at different size-ranks. J Fish Sci Technol 10:191–195

Kim NN, Choi YJ, Shin HS, Lim JR, Han JM, Cho JH, Choi CY (2014) The effect of LED light spectra on antioxidant system by thermal stress in goldfish *Carassius auratus*. Mol Cell Toxicol 10:47–58

Kim B-S, Jung SJ, Choi YJ, Kim NN, Choi CY, Kim J-W (2016) Effects of different light wavelengths from LEDs on oxidative stress and apoptosis in olive flounder *Paralichthys olivaceus* at high water temperatures. Fish Shellfish Immunol 55:460–468

Liu L, Li C, Su B, Beck BH, Peatman E (2013) Short-term feed deprivation alters immune status of surface mucosa in channel catfish *Ictalurus punctatus*. PLoS One 8(9):e74581. https://doi.org/10.1371/journal.pone.0074581

Najafi A, Salati AP, Yavari V, Asadi F (2015) Effects of short-term fasting and refeeding on some hematological and immune parameters in *Mesopotamichthys sharpeyi* fingerlings. Iran J Sci Technol Special issue 39:383–389

Pandi-Perumal SR, Trakht I, Srinivasan V, Spence DW, Maestroni GJM, Zisapel N, Cardinali DP (2008) Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. Prog Neurobiol 85:335–353

Perez-Jimenez A, Cardenete G, Hidalgo MC, Garcia-Alcazar A, Abellan E, Morales AE (2012) Metabolic adjustments of *Dentex dentex* to prolonged starvation and re-feeding. Fish Physiol Biochem 38:1145–1157

Pérez-Jiménez A, Guedes MJ, Morales AE, Oliva-Teles A (2007) Metabolic responses to short starvation and refeeding in *Dicentrarchus labrax*. Effect of dietary composition. Aquaculture 265:325–335

Tort L, Balasch JC, Mackenzie S (2003) Fish immune system. A crossroads between innate and adaptive responses. Immunologia 22:277–286

Ye J, Kaattari IM, Ma C, Kaattari S (2013) The teleost humoral immune response. Fish Shellfish Immunol 35:1719–1728