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Physiological effects of various light spectra on oxidative stress by starvation in olive flounder, *Paralichthys olivaceus*

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Abstract

Backgrounds: The present study investigated the effects of different light wavelengths on starvation-induced oxidative stress and apoptosis in olive flounder, *Paralichthys olivaceus*, by using light-emitting diodes (LEDs; blue, green, and red) at two intensities (0.3 and 0.6 W/m^2) for 9 days.
Methods: We measured the expression and concentration of antioxidant enzymes, the levels of H_2O_2, lipid peroxidation (LPO), aspartate aminotransferase (AspAT), alanine aminotransferase (AlaAT) and apoptosis (levels of caspase-3) and DNA damage in olive flounder under experimental conditions.
Results: All parameters except the apaotosis parameter significantly increased by starvation and significantly decreased after exposure to green and blue light compared to white fluorescent light. Likewise, both caspase-3 and DNA damage increased due to starvation-induced oxidative stress, and green wavelength irradiation played a role in preventing this stress.
Conclusion: These results suggest that green and blue wavelengths can inhibit oxidative stress and apoptosis in starved olive flounder and that green light is particularly efficient for this. Furthermore, the irradiation of green wavelength can play a role in protecting against starvation stress.

Keywords: Apoptosis, Light-emitting diodes, Olive flounder, Oxidative stress, Starvation

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Introduction

Fish remain fasting condition for a period of time due to seasonal changes, spawning, migration or environmental problems (such as red tide phenomenon, a regional reduction of food resources)[1]. Starvation is known to cause oxidative stress, to promote the production of reactive oxygen species (ROS), and to accelerate aging and disease processes[2,3]. The decrease in nutrients associated with starvation tends to deplete organ antioxidant stores and increases the generation of ROS, particularly in the liver3.

ROS, such as superoxide ($O2^-$), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), and singlet oxygen ($^1O2$) are produced as normal products of respiration or cellular metabolism processes[4,5]. However, the over-production of ROS because of environmental stresses, including starvation, can increase lipid peroxidation (LPO) and can negatively affect cell viability by causing cell or tissue damage in the form of oxidation of the components of the cell membrane, including lipids, proteins, and DNA, and can lead to an acceleration of cell death (apoptosis)[6-8]. Apoptosis is mediated by proteolytic enzymes called caspases and is characterized by morphological features such as shrinkage of the cell and DNA fragmentation[9]. In particular, caspase-3 plays a central role in the apoptotic process by accompanying biochemical and morphological events such as DNA damage and inflammation response[10-12].

Fish protect themselves from the destructive effects of active ROS with several antioxidant enzymes[13,14] that are reported to play a role in the removal of ROS, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione (GSH)15,16. The basic antioxidant mechanism is SOD converts O_2^- to H_2O_2, and the produced toxic H_2O_2

is decomposed into water and molecular oxygen (O_2) by CAT and GPX[8]. However, it has been reported that these antioxidant enzyme activities may change with exposure to various light wavelengths[17].

Recent studies have investigated the influence of light on the living body by using light-emitting diodes (LEDs) with specific wavelengths and have shown that particular wavelengths of light can affect various physiological responses in fish[17-19]. LEDs have lower power requirements, higher efficiency, and longer life span than do metal halide bulbs and can be used effectively in fish farming[18,20,21].

Recent studies have shown that red wavelength from LEDs can cause oxidative stress and negatively affect physiological functions of fish, whereas short wavelengths such as green light can effectively reduce oxidative stress and enhance immunity[17,22]. Another recent study investigated juvenile rock bream (*Oplegnathus fasciatus*) exposed to the toxic substance bisphenol A under green LED wavelength. The fish increased their antioxidant and immunity levels to protect themselves from toxicity, and oxidative stress was decreased as a result[23]. As well as, LPO and apoptosis were decreased under green LED wavelength in the olive flounder exposed to high temperature[24]. Despite the number of studies about the effects of light exposure, studies of the physiological aspects of the control of starvation-induced stress by the specific wavelength and intensity remain limited.

Olive flounder (*Paralichthys olivaceus*) used in this study is commercially important as a major aquaculture species in East Asia[25]. In addition, this species exposed to various wavelengths of light because it is inhabited at various depths in ecosystems and at shallow depths of less than 1 meter in aquaculture[26,27], as well as is sensitive to light because it does not swim most of the time and lives in one place. Therefore, considering the commercial value and the environmental aspect, it is suggested that this model is suitable for the study of the effect of various wavelengths depending on the starved stress.

We investigated the possibility of controlling oxidative stress and apoptosis using a particular light wavelength and intensity in olive flounder. We used three light wavelengths (blue, green, and red) at two intensities (0.3 and 0.6 W/m^2) in olive flounder starved for up to 9 days to compare levels of oxidative stress and cellular damage and to assess the effectiveness of exposure to specific light wavelengths in combating these effects. Measurements included the changes in antioxidant gene and protein expressions and the concentrations of antioxidant enzymes and the levels of H_2O_2 in the plasma, and LPO in the liver to determine oxidative stress; plasma AspAT and AlaAT levels to

check for starvation-induced liver damage; and terminal transferase dUTP nick end labeling (TUNEL) assays and comet assays to confirm apoptosis and DNA damage.

Materials & Methods

Experimental fish and conditions

We purchased juvenile olive flounders (*P. olivaceus*, $n = 320$, length 12.3 ± 0.6 cm; mass 16.2 ± 0.5 g) from a commercial aquarium (Jeju, Korea), and allowed them to acclimate in eight 300-L circulation filter tanks in the laboratory for two weeks.

The control group was given white fluorescent light (light intensity at the water surface was approximately 0.96 W/m^2; 20 W; PHILIPS, Netherlands) and fed a commercial feed at twice daily (09:00 h and 17:00 h). The experimental groups were divided into groups of 20 fish, which were exposed to either blue (peak at 450 nm), green (520 nm), or red (640 nm) light from LEDs (Daesin LED Co., Kyunggi, Korea), or a white fluorescent light for 9 days without being fed (starvation treatment) (Figure 1). The water temperature, salinity and photoperiod were $20 \pm 1°C$, $32.2 \pm 0.5‰$ and a 12-h light (L): 12-h dark (D) cycle (lights on at 07:00 h; lights off at 19:00 h). The LEDs were placed 50 cm above the water surface and the depth of the water was 50 cm. The irradiance level at the bottom of tanks with external light interception was maintained at approximately 0.3 or 0.6 W/m^2 using spectrometer (MR-16; Rainbow Light Technology Co. Ltd., Taoyuan, Taiwan) and PHOTO-RADIOMETER (HD 2102.1; Delta OMH CO., Caselle di Selvazzano, Italy). We sampled five fish from each experimental group at 14:00 h of 0, 3, 6, and 9 days. The fish were anaesthetized to minimize the stress of collection of blood (1.5 mL/fish) and
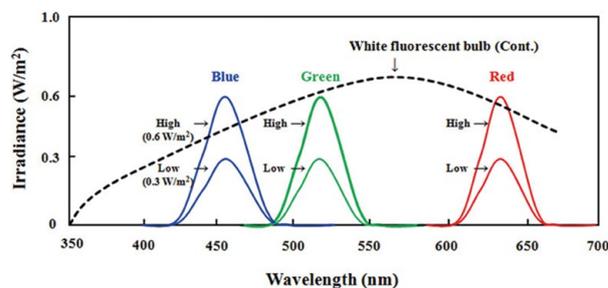
Figure 1. Spectral profiles of light-emitting diodes (LEDs; blue, 450 nm; green, 520 nm; red, 640 nm) and white fluorescent bulb (Cont.) used in the present study. Two different intensities were used (low, 0.3 W/m^2; high, 0.6 W/m^2) for each LED treatment.

liver tissue (1 g/fish) samples and stored at −80°C until for analysis.

Real-time quantitative polymerase chain reaction (qPCR) analysis

Total RNA was extracted from each liver sample (0.05 g/fish) using TRI Reagent® (Molecular Research Center, Inc., Cincinnati, OH, USA), according to the manufacturer's instructions. Subsequently, 2 μg of total RNA was reverse-transcribed in a 20 μL reaction volume, 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, stored at 4°C, and subsequently used for PCR and real-time qPCR analyses.

The qPCR analysis was conducted to determine the relative expression levels of caspase-3 and the antioxidant enzymes SOD and CAT, using the total RNA extracted from the olive flounder livers. The qPCR primers were designed using known olive flounder sequences (Table 1). We conducted the qPCR amplification using a Bio-Rad iCycler iQ multicolor real-time PCR detection system (Bio-Rad, Hercules, CA, USA), and the iQ SYBR green supermix (Bio-Rad, USA), following the manufacturer's instructions. As a control, the β-actin gene was also amplified for each sample, and all data were expressed as the difference with the corresponding calculated β-actin threshold cycle (Ct) levels. The Ct values of the PCR products formed the basis for all analyses. The Ct levels were defined as the PCR cycle in which the fluorescence signal crossed a threshold during the exponential phase of the amplification curve. The calibrated ΔCt value (ΔΔCt) per sample and the internal control (β-actin) were calculated as follows: [ΔΔCt = 2^ − (ΔCtsample − ΔCtinternal control)]. After the PCRs were completed, the qPCR data from three replicate samples were analyzed using Bio-Rad to estimate the transcript copy numbers of each sample.
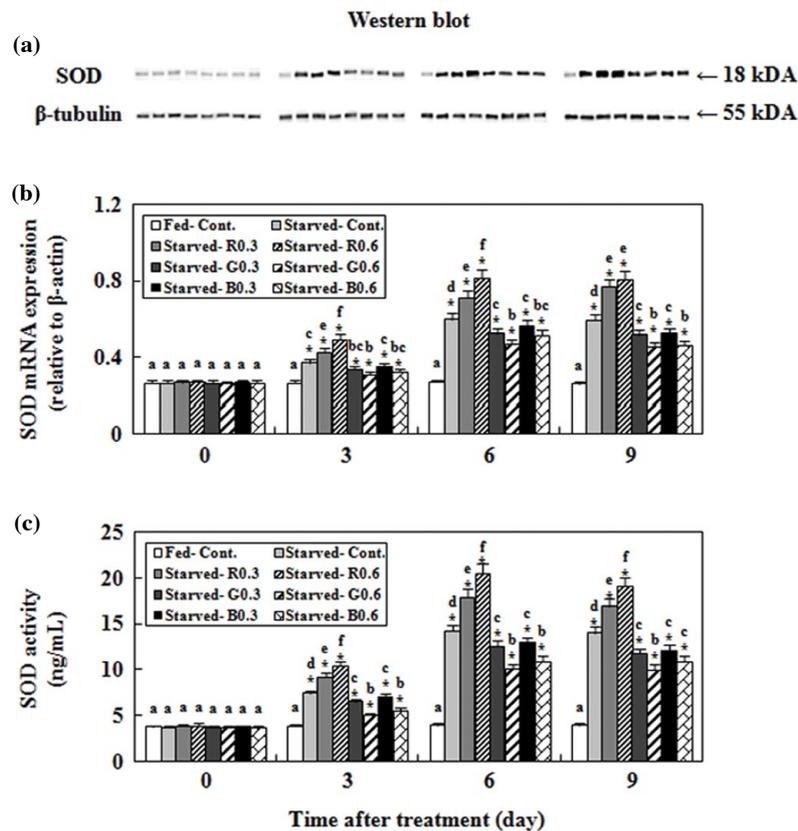
Western blot analysis

The total protein content of the olive flounder livers was extracted 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 into Mini-PROTEAN® TGX™ gels (Bio-Rad, USA), and a protein ladder (Bio-Rad, USA) was used as a reference. The samples were electrophoresed at 180 V; thereafter, the gels were immediately transferred to a 0.2-μm polyvinylidene difluoride membrane (Bio-Rad, USA) at 85 V for 3 min, using the Trans-Blot® Turbo™ transfer system. Subsequently, the membranes were blocked with 5% milk in Tris-buffered saline buffer (pH 7.4) for 45 min, after which they were washed in the in Tris-buffered saline buffer without milk. Membranes were incubated with SOD antibodies (1:2000 dilution, NBP1-47443, Novus Biologicals, Littleton, CO, USA) and CAT antibodies (1:2000 dilution, sc-58332, Santa Cruz Biotechnology, Dallas, TX, USA), and subsequently incubated with horseradish peroxidase conjugated anti-mouse IgG secondary antibodies (SOD and CAT dilution 1:2000, Bio-Rad, USA) for 60 min. As the internal control, β-tubulin (dilution 1:5000, ab6046, Abcam, Cambridge, UK) was used. Bands were detected using WesternBright™ ECL (Advansta, Menlo Park, CA, USA), and 30 s of exposure with a Molecular Imager® from ChemiDoc™ XRS Systems (Bio-Rad, USA). The membrane images were scanned using a high-resolution scanner, and the band densities were estimated using Image Lab™ Software, version 3.0 (Bio-Rad, USA).

LPO assay

LPO from the olive flounder livers was quantified by measuring levels of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), the degradation products of lipid peroxidation of polyunsaturated fatty acids, according to the manufacturer's instructions (Fish Lipid

Table 1. Primers used for qPCR amplification.

Genes (accession no.)	Primer	DNA sequences
SOD (EF681883)	Forward Reverse	5′-CGT TGG AGA CCT GGG GAA TGT G-3′ 5′-ATC GTC AGC CTT CTC GTGGAT C-3′
CAT (GQ229479)	Forward Reverse	5′-CCA AAC TAC TAT CCC AAC AGC-3′ 5′-CCA CAT CTG GAG ACA CCT T-3′
Caspase-3 (JQ394697)	Forward Reverse	5′-GCA AAT CGC TGG TGG GAA A-3′ 5′-CAT CGT CTA CAC TGT CTG TTT CG-3′
β-actin (HQ386788)	Forward Reverse	5′- GGA CCT GTA TGC CAA CAC TG-3′ 5′- TGA TCT CCT TCT GCA TCC TG -3′

Western blot

(a)

SOD   ← 18 kDA

β-tubulin   ← 55 kDA

(b)

(c)

Time after treatment (day)

Figure 2. Change in SOD protein expression (a), mRNA expression (b), and concentrations (c) during a 9-day starvation period under different wavelengths of light (red (R), green (G), and blue (B) LEDs) at two intensities (0.3 and 0.6 W/m^2), and a white fluorescent bulb (Cont.), as measured by an enzyme-linked immunoassay and quantitative real-time PCR (qPCR). Total liver RNA (2 μg) was reverse-transcribed and amplified. Results are expressed as normalized fold expression levels with respect to the β-actin levels in each sample. Western blot was used to evaluate the expression of SOD (18 kDa) in the livers during starvation; β-tubulin (55 kDa) was used as an internal control. Lowercase letters denote significant differences between groups that received different wavelengths of light within the same starvation period ($P < 0.05$). The asterisk (*) indicate significant differences between different temperatures within the same LED type ($P < 0.05$). All values are represented as means ± SE ($n = 5$).

Peroxide ELISA Kit, MyBioSource, San Diego, CA, USA). The absorbance was read at 450 nm using a plate reader.

Plasma parameter analysis

Plasma was separated from whole blood through centrifugation (4°C, 10,000 × g, for 5 min). H_2O_2 levels were measured using a modified version of the methods described by Nouroozzadeh et al.[37], and a PeroxiDetect kit (Sigma, USA). Absorbance was read at 560 nm, and the concentration of H_2O_2 was interpolated from a standard curve. The levels of SOD, CAT, and caspase-3 were analyzed using an immunoassay from an ELISA kit (SOD, CSB-E15929fh; CAT, CSB-E15928fh; Cusabio Biotech, Hubei, China; and caspase-3, MBS012786; Mybiosource Inc., USA). In addition, AspAT (EC 2.6.1.1) and AlaAT (EC 2.6.1.2)

levels were measured using a dry multiplayer analytic slide method in a biochemistry auto analyzer (Fuji Dri-Chem 4000; Fujifilm, Tokyo, Japan).

Terminal transferase dUTP nick end labeling (TUNEL) assay

To evaluate the apoptotic response of the fish liver cells to green LED light, we performed the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) technique with a commercially available in situ cell death detection kit (11 684 795 910, Roche Co., Basel, Switzerland). To ensure apoptotic cells would adhere to the slides, the slides were coated with polylysine. The fish liver tissue was washed and fixed with 4% buffered paraformaldehyde, and permeabilized with freshly prepared 0.1% Triton X-100, 0.1% sodium citrate solution. This liver tissue was then incubat-

(a)

Western blot

CAT   ← 64 kDA

β-tubulin   ← 55 kDA

(b)

(c)
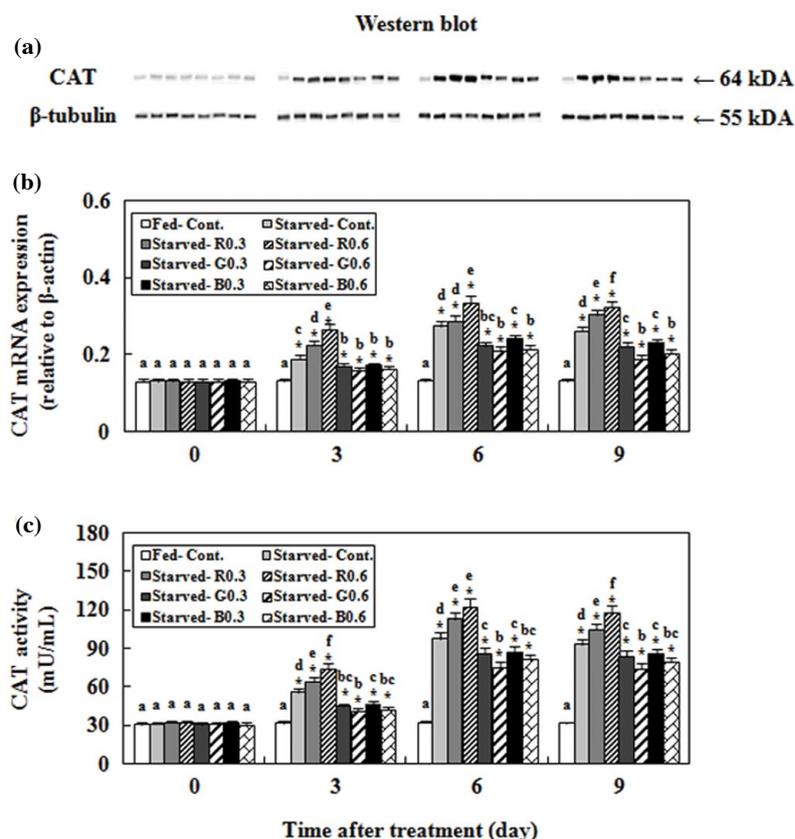
Figure 3. Change in CAT protein expression (a), mRNA expression (b), and concentrations (c) during a 9-day starvation period under different wavelengths of light (red (R), green (G), and blue (B) LEDs) at two intensities (0.3 and 0.6 W/m^2), and a white fluorescent bulb (Cont.), as measured by an enzyme-linked immunoassay and quantitative real-time PCR (qPCR). Total liver RNA (2 μg) was reverse-transcribed and amplified. Results are expressed as normalized fold expression levels with respect to the β-actin levels in each sample. Western blot was used to evaluate the expression of CAT (64 kDa) in the liver during thermal changes; β-tubulin (55 kDa) was used as an internal control. Lowercase letters denote significant differences between groups that received different wavelengths of light within the same starvation period ($P < 0.05$). The asterisk (*) indicate significant differences between different temperatures within the same LED type ($P < 0.05$). All values are represented as means ± SE ($n = 5$).

ed with the TUNEL reaction mixture for 1 h at 37°C in a humidified chamber. The slides were washed three times with phosphate buffered saline (PBS), and the incorporated biotin-dUTP was detected under a fluorescence microscope (excitation filter 465-495 nm; Eclipse C*i*, Nikon Instruments Inc., Tokyo, Japan). For the paraffin-embedded tissue sections, slides were dewaxed and fixed according to standard protocols, and then treated as described above. The green fluorescent cells indicated apoptotic cells.

Comet assay

The comet assay is a relatively simple and sensitive technique for quantitatively measuring DNA damage in eukaryotic cells[28]. Liver cells (1×10^5 cells/mL) were examined using a CometAssay® Reagent kit for

single cell gel electrophoresis assay (Trevigen Inc., Maryland, USA), according to the method described by Singh *et al.*[38], with some modifications. Cells were immobilized in an agarose gel on CometAssay® comet slides and immersed in a freshly prepared alkaline unwinding solution for 20 min. The slides were subsequently electrophoresed at 15 V for 30 min. The samples were stained with SYBR® Green (Trevigen Inc., USA), and kept in the dark for 30 min. They were subsequently read using a fluorescence microscope (Eclipse C*i*, Nikon Instruments Inc., Japan). At least 100 cells from each slide were assessed. For quantitative analysis of the comet assays, we used Comet Assay IV image analysis software (version 4.3.2, Perceptive Instruments Ltd., Bury St. Edmunds, UK) to analyze the comet tail lengths (distance of DNA migration from head), percentage of DNA in the tails (tail inten-

(a)

(b)
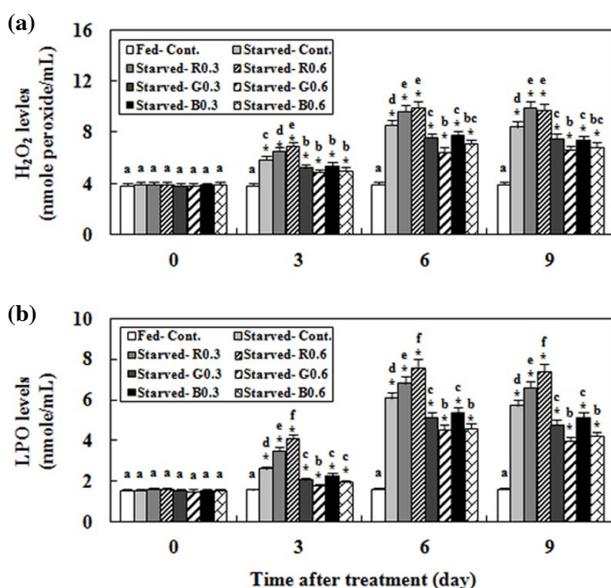
Figure 4. Levels of H_2O_2 in plasma (a) and LPO in liver tissue (b) of olive flounder exposed to red (R), green (G), and blue (B) LEDs at different light intensities (0.3 and 0.6 W/m²) and to a white fluorescent bulb (Cont.), as measured by a microplate reader. Lowercase letters denote significant differences between groups that received different wavelengths of light within the same starvation period ($P < 0.05$). Asterisks (*) indicate significant differences between different starvation period within the same wavelength ($P < 0.05$). All values are means ± SE ($n = 5$).

Figure 5. Levels of plasma AspAT (a) and AlaAT (b) in olive flounder exposed to red (R), green (G), and blue (B) LEDs at different light intensities (0.3 and 0.6 W/m²) and to a white fluorescent bulb (Cont.), as measured by a biochemistry auto analyzer. Lowercase letters denote significant differences between groups that received different wavelengths of light within the same starvation period ($P < 0.05$). Asterisks (*) indicate significant differences between different starvation periods within the same wavelength ($P < 0.05$). All values are represented as means ± SE ($n = 5$).

sity/total intensity in tail), and the tail moments (amount of DNA damage, product of tail length and percentage of DNA in tail).
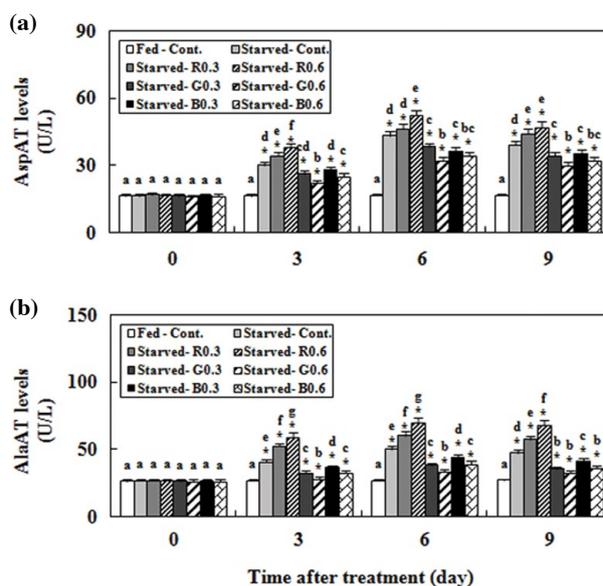
Statistical analysis

All data were analyzed using the SPSS statistical package (version 19.0; SPSS Inc., USA). A two-way ANOVA followed by Tukey's post hoc test was used to compare differences in the data ($P < 0.05$). The values are expressed as the mean ± standard error (SE).

Results

Changes in SOD and CAT protein, mRNA expression and concentration

The levels of mRNA and protein expression and the plasma concentration of antioxidant enzymes SOD and CAT were up-regulated during the 9-day starvation period (Figures 2 and 3). Furthermore, the groups exposed to green or blue LEDs had significantly lower (by approximately 1.1- to 1.5-fold) levels of protein and mRNA expression and concentration than the

groups exposed to white fluorescent light (control), especially at higher light intensities. In particular, the green wavelength groups had significantly lower levels of mRNA and protein expression and concentration than the other LED treatment groups. In contrast, the groups with red wavelength light had significantly higher (by approximately 1.1- to 1.4-fold) levels of protein and mRNA expression than control, as well as higher SOD and CAT concentration, which increased with light intensity.

Changes in H_2O_2, LPO, AlaAT, and AspAT levels

As shown in Figure 4, the levels of H_2O_2 in plasma and LPO of liver in starved fish groups significantly higher (by approximately 1.5- to 2.2-fold) than those in fed fish groups. The H_2O_2 and LPO levels in starved fish exposed to green or blue LEDs were significantly lower (~1.3-fold) than those in the control group, and this difference increased with light intensity. However, the H_2O_2 and LPO levels significantly increased (~1.2-fold) in the groups irradiated with red LEDs. The variations of plasma AlaAT and AspAT levels in liver tissue were similar to those of H_2O_2 and LPO levels (Figure 5).

(a)

(b)
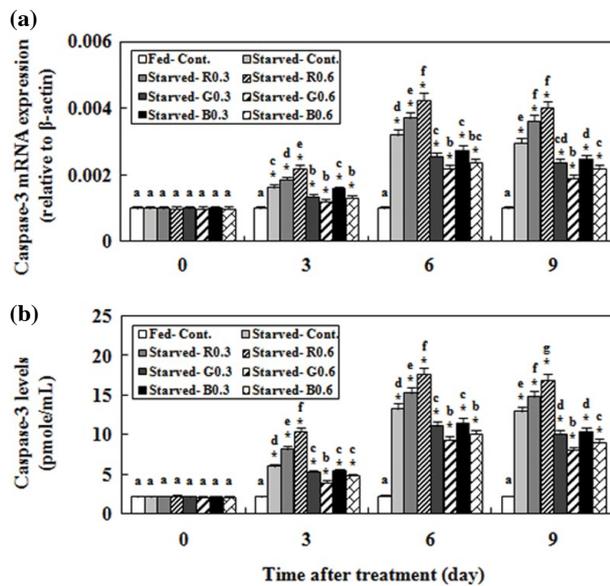
Figure 6. Change in caspase-3 mRNA expression (a), and levels (b) in olive flounder exposed to red (R), green (G), and blue (B) LEDs at different light intensities (0.3 and 0.6 W/ m^2) and to a white fluorescent bulb (Cont.), as measured by an enzyme-linked immunoassay and quantitative real-time PCR (qPCR). Total liver RNA (2 μg) was reverse-transcribed and amplified. Results are expressed as normalized fold expression levels with respect to the β-actin levels in each sample. Lowercase letters denote significant differences between groups that received different wavelengths of light within the same starvation period ($P < 0.05$). Asterisks (*) indicate significant differences between different starvation periods within the same wavelength ($P < 0.05$). All values are means ± SE ($n = 5$).

Changes in caspase-3 levels and mRNA expression

The caspase-3 levels and mRNA expressions in the starvation groups were significantly higher than in the fed groups (Figure 6). Furthermore, the caspase-3 levels and mRNA expression in groups exposed to green or blue LEDs were significantly lower than in control groups with white fluorescent light. Particularly, the caspase-3 levels and mRNA expression in the green light groups were significantly lower (~1.6-fold) than in the groups irradiated with blue or red LEDs. However, the caspase-3 levels and mRNA expression in groups irradiated with red LEDs were higher (~1.7-fold) than in groups irradiated with white fluorescent light during the starvation period.

TUNEL assay

TUNEL assay results are shown in Figure 7. The liver cells in starved fish groups had increased active apoptosis due to starvation for 6 or 9 days, as compared to those in fed fish groups. In contrast, the cells with green LED irradiation had decreased apoptosis cell compared to those with white fluorescent light.

Comet assay

The comet assay is a relatively simple and sensitive technique for quantitatively measuring DNA damage in eukaryotic cells[28]. The liver cells showed normal nuclear DNA in fed groups, but cells with damaged nuclear DNA appeared in starved fish groups (Figure 8a). Furthermore, the cells from starved fish groups had significantly longer tail lengths (distance of DNA migration from head), higher percentages of DNA in tail (tail intensity/total intensity in tail), and higher tail moments (amount of DNA damage, product of tail length and percentage of DNA in tail) than cells in fed-fish groups. However, cells in starved fish irradiated with green wavelength light had decreased tail lengths, percentages of DNA in tail, and tail moments (Figure 8b).

Discussion

This study is aim to evaluate the possibility of controlling oxidative stress, apoptosis and DNA damage using a particular light wavelength and intensity in olive flounder.

Our results are similar to those of Morales et al.[29], who reported that common dentex (*Dentex dentex*) starved for 5 weeks showed significantly increased levels of LPO in the liver. Choi et al.[23] reported that bisphenol A induced oxidative stress in juvenile rock bream (*Oplegnathus fasciatus*) and the levels of plasma H_2O_2 as ROS and plasma AspAT and AlaAT concentrations significantly increased. The levels of plasma H_2O_2, AspAT, and AlaAT significantly decreased in bream exposed to green wavelength LEDs compared with white fluorescent light. They suggested, therefore, that green wavelength LEDs can effectively decrease oxidative stress in fish[22]. Our results also show that starvation induces oxidative stress and increases liver damage by promoting the generation of ROS. However, irradiation with short wavelength light, such as green or blue LEDs, can effectively decrease ROS and liver damage and play a role in protecting cells in fish.

Zheng et al.[30] reported that the activity of Cu/Zn-SOD, CAT, and GPX were increased in large yellow croaker (*Pseudosciaena crocea*) starved for 12 days. That is, the generation of ROS increased starvation-induced stress responses, and the concentration of antioxidants Cu/Zn-SOD, CAT, and GPX were increased in order to eliminate ROS. Our results are similar to
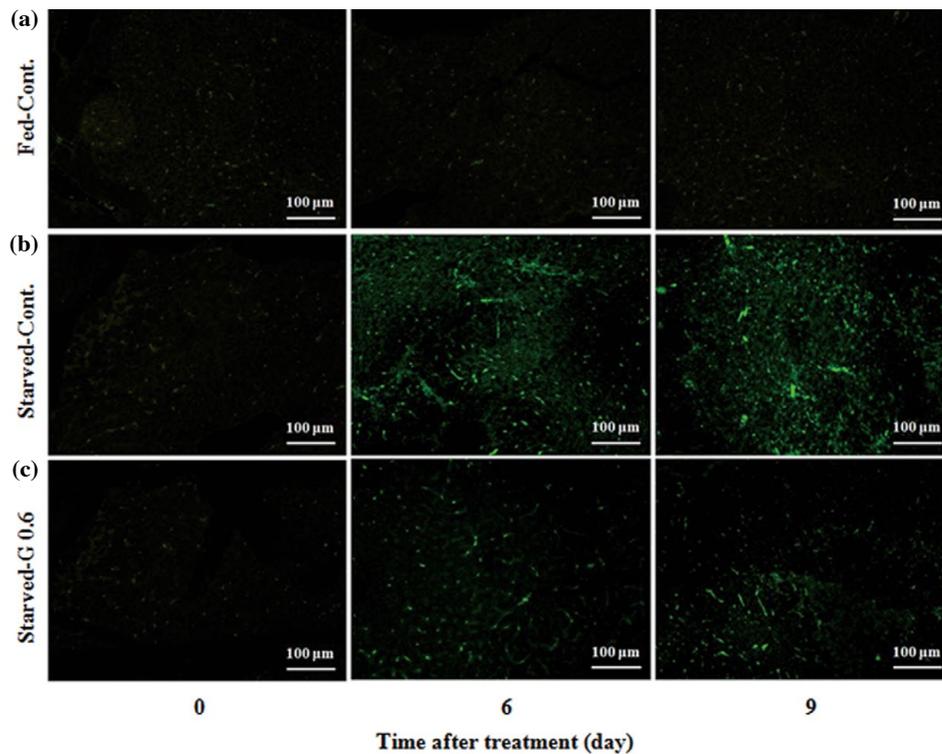
Figure 7. TUNEL detection of liver cell apoptosis of olive flounder under different light conditions for starvation (0, 6, and 9 days) using a white fluorescent bulb (Cont.) (a, Fed; b, Starved), and green LED light of 0.6 W/m^2 (c). Cells were stained with acridine orange and subsequently observed with a fluorescent microscope. The green fluorescent cells are the apoptotic cells. Scale bars = 100 μm.
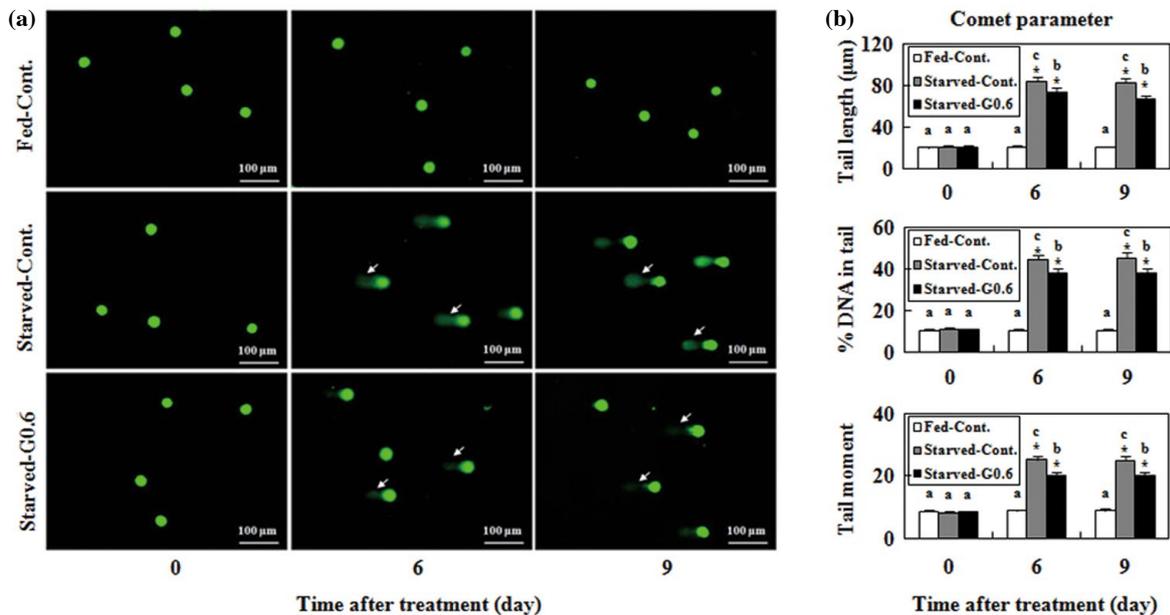
Figure 8. Comet assay images (a) and comet assay parameters (b; tail length, percentage DNA in tail, and tail moment) from starved fish (0, 6, and 9 days), using a white fluorescent bulb (Cont.) or green LED light at 0.6 W/m^2. White arrows indicate the damaged nuclear DNA (DNA breaks) in the liver cells, which were stained with SYBR green. Scale bar = 100 μm. Lowercase letters denote significant differences between groups that received different wavelengths of light within the same starvation period ($P < 0.05$). Asterisks (*) indicate significant differences between different starvation periods within the same wavelength ($P < 0.05$). All values are means ± SE ($n = 5$).

those of previous studies: starvation can increase the concentration of SOD and CAT in olive flounder, but green or blue wavelength irradiation can effectively decrease antioxidant enzyme concentration by reducing oxidative stress induced by starvation.

Our results are also consistent with those of Jiang et al.[31], who reported that juvenile carp (Cyprinus carpio) exposed to 0.6 mg/L copper had oxidative stress, increased DNA damage in muscle cells, increased caspase-3 activity, and increased apoptosis response due to copper toxicity. Wang et al.[32] reported that freshwater crabs (Sinopotamon henanense) exposed to cadmium (7.25, 14.5, 29, 58, or 116 mg/L) for 7 days showed an increased level of oxidative stress with increasing cadmium concentration, with increased caspase-3 and caspase-9 activity. The results of our study are similar to those of previous studies showing that stress, whether from toxins or starvation, may increase the levels of caspase-3. Therefore, the mRNA expression and levels of caspase-3 increase with starvation-induced oxidative stress in olive flounder, but that exposure to green or blue LEDs reduces oxidative stress and inhibits the mRNA expression and levels of caspase-3.

Song et al.[33] reported that mice starved for 72 h had increased apoptosis in gut epithelial cells, and Deponte[34] reported changes in morphological characteristics including cell and nuclear shrinkage and chromatin condensation during the process of apoptosis. However, our results indicate that irradiation with green light can effectively inhibit the apoptosis process induced by starvation, at least in fish.

The findings of this study agree with Nwani et al.[35], who reported that juvenile spotted murrel (Channa punctatus) exposed to glyphosate, an herbicide, exhibited higher oxidative stress factors and had nuclear DNA damage in blood and gill cells. Jung et al.[36] reported that goldfish exposed to ammonia (0.25 and 0.5 mg/L), had nuclear DNA damage in liver cells with increased concentration of ammonia, but groups exposed to green LED irradiation showed less nuclear DNA damage. This is further evidence that green wavelength light can effectively reduce oxidative stress and play a role in decreasing nuclear DNA damage.

Conclusion

In summary, our results suggest that 1) starvation induces oxidative stress in olive flounder, increasing apoptosis and damage to nuclear DNA in liver cells; 2) short-wavelength light, such as that produced by green and blue LEDs, can effectively decrease starvation-induced oxidative stress; and 3) in particular, green light can reduce apoptosis and nuclear DNA damage in cells, and thus, play a role in protecting cells from harmful ROS.

The results of this study are expected to be useful data for reducing the damage caused by starvation in the aquaculture industry and to be used as important data for effective stress control by controlling light wavelength.

Conflict of Interest Cheol Young Choi, Ji Yong Choi, Young Jae Choi & Jin-Hyung Yoo declares that they have no conflict of interest.

Human and animal rights The procedures were carried out in accordance with the Animal Care and Use Guidelines of National Institute of Fisheries Science.

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