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Effects of photoperiod on growth performance and melanogenesis pathway for skin pigmentation of Malaysian red tilapia

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Abstract

Farming of red tilapia is increasing rapidly. However, its commercial farming development is challenged by lack of clear information on genetic basis for skin colour and pigmentation differences due to environmental changes. This study investigated the effects of photoperiod (light:dark, L:D) on the growth and skin colour variation of Malaysian red tilapia. A total of 180 fish weighing 150.48 ± 0.44 g were reared under natural photoperiod (13L:11D, control), prolonged lightness (24L:0D) and prolonged darkness (0L:24D) in three replicates for 78 days. The weight gain of fish cultured under both prolonged light and darkness were significantly higher than fish under natural photoperiod. The tyrosinase level in ventral skin was significantly higher for fish cultured under prolonged darkness condition than in the other two photoperiod regimes. Contrary, the cysteine level in the dorsal skin was significantly higher in the fish cultured under natural photoperiod than in prolonged light and darkness. The relative mRNA expressions of SRY-related HMG-Box 10 (*sox 10*), tyrosine (*tyr*), tyrosine-related protein 1 (*tyrp-1*) and solute carrier family 7 member 11 (*slc7a11*) genes were significantly higher in ventral skin of fish under prolonged darkness than the other two photoperiods. This study demonstrates that photoperiod has an impact on melanogenesis and growth of red tilapia. Understanding the effects of photoperiod on genetic basis of red tilapia will help in selective breeding programme of the important economic traits for the development of commercial red tilapia farming.

KEYWORDS

cysteine, growth, melanogenesis, photoperiod, red tilapia, tyrosine

1 | INTRODUCTION

Fish skin colour is among the most important economic traits in aquaculture (Haque et al., 2016; Nüsslein-Volhard & Singh, 2017). Fish display highly diverse and complex skin pigmentation, due to their six different types of chromatophores they possess, which are cyanophores, leucophores, iridophores, erythrophores, xanthophores and melanophores (Cal, Suarez-Bregua, Cerdá-Reverter, Braasch, & Rotllant, 2017; Jiang et al., 2014; Kelsh, 2004). The colour phenotype of different organisms is influenced by the interaction between environmental factors and genetics (Hubbard, Uy, Hauber, Hoekstra, & Safran, 2010). Among the environmental factors, light condition is known to play a major role in pigmentation by affecting melanin concentration and distribution (Pavlidis, Karkana, Fanouraki, & Papandroulakis, 2008; Zhu et al., 2018). Two major types of melanin are known, the eumelanin (black/brown) and pheomelanin (red/yellow) (Bar, Kaddar,

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Velan, & David, 2013), which are the important determinants of colour variations in animals (Morgan, Lo, & Fisher, 2013).

Photoperiod has important impacts on aquatic life (Boeuf & Bail, 1999). It has been used in aquaculture to improve growth (Bani, Tabarsa, Falahatkar, & Banan, 2009; Liu et al., 2015), control reproduction (Rad, Bozao, Ergene, Karahan, & Kurt, 2006; Zhu et al., 2014) and enhance fish coloration for several species (Ali & El-Feky, 2013). Fish exposed to long photoperiod or prolonged light increases locomotion, feed conversion efficiency and feed intake, which may lead to improved growth depending on species (Veras et al., 2013). Also, fish exposed to short photoperiod or prolonged darkness may use more energy for growth rather than spending it in swimming and other metabolic activities (Liu et al., 2015). However, the effect of photoperiod on growth performance is still confusing. Some fish have been reported to display high growth performance in long photoperiod such as Giant gourami, *Trichogaster fasciata* (Bano & Serajuddin, 2018), Gilthead sea bream, *Sparus aurata* L. (Gines, Afonso, Arguello, Zamorano, & Lopez, 2004), Red-eyed orange molly, *Poecilia sphenops* (Zutshi & Singh, 2017) and Topmouth gudgeon, *Pseudoporasbora parva* (Zhu et al., 2014). However, other fish species attain high growth rate in short photoperiods such as Juvenile lenok, *Brachymystax lenok* (Liu et al., 2015) and African catfish *Clarias gariepinus* (Mustapha, Okafor, Olaoti, & Oyelakin, 2012), while exposure to photoperiod did not affect growth performance in other species such as Juvenile halibut, *Hippoglossus hippoglossus* (Hallaråker, Folkvord, & Stefansson, 1995) and miiuy croaker, *Miichthys miiuy* (Shan, Xiao, Huang, & Dou, 2008). These results call for further studies to explore the effects of photoperiods on growth using different fish species.

Red tilapia is commonly known as a hybrid species, which has resulted from continuous selective breeding process. Malaysian red tilapia is believed to be derived from the cross-breeding between male Nile tilapia (*Oreochromis niloticus*) and female Mozambique tilapia (*Oreochromis mossambicus*); however, the genetic background of this species is not well known (Pongthana, Nguyen, & Ponzoni, 2010). It is currently among the most important farmed fish in major aquaculture producing countries such as China, Thailand and Malaysia (He, Wang, Zhu, Dong, & Liu, 2017). Red tilapia are highly preferred for aquaculture due to their fast growth rate, salinity tolerance and attractive red colour resembling marine fish such as red snappers (Haque et al., 2016; Pongthana et al., 2010). However, red tilapia farming is challenged by pigmentation differences and colour variations especially during overwintering (Zhu et al., 2016). Luckily, previous studies established the melanogenesis pathway of Malaysian red tilapia and identified several key genes (such as tyrosinase [*tyr*], tyrosine-related protein 1 [*tyrp-1*], solute carrier family 7 member 11 [*slc7a11*] and SRY-related HMG-Box 10 [*sox 10*]), which are involved in the process of melanin synthesis (Zhu et al., 2016). Moreover, the expression of *slc7a11* and *tyr* genes involved in melanogenesis is re-regulated by nutrition (Wang et al., 2018).

However, information on the effects of environmental factors such as photoperiods on growth performance and melanogenesis pathway in red tilapia is currently scarce. This study investigated the effects of photoperiods (light and darkness lengths) on the growth performance and melanogenesis pathway of Malaysian red tilapia. Malaysian hybrid tilapia was chosen based on its growth retardation and pigmentation differences during breeding (Wang et al., 2018; Zhu et al., 2016). We aimed to get information about colour variations of red tilapia during photoperiod changes. The information obtained might be useful in genetic breeding selection programmes for development of commercial red tilapia culture in the future.

2 | MATERIALSANDMETHODS

2.1 | Ethical statement

All experiments in this study were carried out in accordance with the Care and Use of Laboratory Animals formulated by the Ministry of Science and Technology of China. This study was approved by the Bioethical Committee of Freshwater Fisheries Research Center (FFRC) affiliated to the Chinese Academy of Fisheries Sciences (CAFS).

2.2 | Source of Red tilapia and experimental design

The Malaysian red tilapia used in this study were obtained from FFRC. Before the formal experiment, fish were acclimatized for a period of 2 weeks in an indoor re-circulating aquaculture system under natural photoperiod condition. During this period, fish were fed on a floating commercial feed for tilapia (Feed No. 191; Tongwei Co. Ltd.) containing 30% crude protein and 5% crude lipid three times a day. Water temperature in the culture tank was maintained at 28 ± 1°C and dissolved oxygen (DO) was ≥6 mg/L. After the acclimatization period, fish were starved for 24 hr before the start of the experiment.

A total of 180 fish (average weight ± standard error of 150.48 ± 0.44 g) with whole pink coloration on their skin were randomly stocked into nine fibreglass tanks (each tank 20 fish) and cultured by using a flow-through system (diameter 90 cm × depth 80 cm with water volume of 200 L). During the experimental period, the natural photoperiods were between 13:30 and 14 hr of light and 10:30 to 10 hr of darkness (for this case, we used 13L:11D, light:dark). The stocked fish were randomly assigned to the three photoperiods of natural (13L:11D, control), prolonged light for 24 hr (24L:0D) and prolonged darkness for 24 hr (0L:24D) in triplicate. Fluorescent lamps (artificial light frequency—SUNSUN: 220 v/50 Hz, ADO-800W—China) with light intensity of 1,000 lux were placed 30 cm above each light treatment tank in order to supply light. The light treatment tanks were covered on top by using black polythene sheets to avoid light loss. In the dark treatment, fish were cultured in total darkness maintained by covering the tanks with black polythene sheets in order to ensure complete darkness for most of the culture period. However, there were some light emissions during feeding, fish weighing and cleaning of tanks, which were done after every seven days. All fish were measured at the start of the experiment and after every two weeks during the experimental period in order to adjust feeding amounts. Fish were fed at 3% average body weight by using a commercial tilapia feed three times a day at 8:00, 12:00 and 17:00 for 78 days.

2.3 | Determination of survival rate, growth performance and gonadosomatic index

After 78 days of experiment, all the remaining fish in each tank were starved for 24 hr to clear the gut prior to sampling. All fish from each tank were collected by using a scoop net and counted for determination of survival rate (SR) by using the formula:

$$\text{Survival rate}\,(\%) = \left(\frac{\text{Final number of fish}}{\text{Initial number of fish}}\right) \times 100$$

Thereafter, fish from each tank were weighed individually to determine final body weight (FBW), weight gain (WG) and specific growth rate (SGR) by using the following formulae:

$$\text{WG}\,(\%) = \left[\frac{\text{Final weight}\,(\text{g}) - \text{Initial weight}\,(\text{g})}{\text{Initial weight}\,(\text{g})}\right] \times 100$$

$$\text{SGR}\,(\%) = \left[\frac{\ln\left(\text{Final weight}\right) - \ln\left(\text{Initial weight}\right)}{\text{Time}\,(\text{days})}\right] \times 100$$

Three male and female fish from each tank (nine per treatment per each sex) were randomly sampled and anesthetized with tricaine methanesulfonate (MS-222) at 100 mg/L. The sampled fish were weighed, dissected, and their gonads were extracted and weighed for determination of gonadosomatic index (GSI) using the following formula:

$$\text{GSI}\,(\%) = \left(\frac{\text{Gonad weight}\,(\text{g})}{\text{Individual fish weight}\,(\text{g})}\right) \times 100$$

2.4 | Analysis of colour changes

Three fish from each tank (nine per treatment) were randomly sampled, and pictures were taken by using a digital camera (Canon PowerShot G5). Image analysis was performed by using ImageJ Software version 1.52a (National Institutes of Health). The mean colour intensity was calculated on the original image; afterwards, the Red-Green-Black (RGB) obtained was compared among the treatments. The RGB value ranges from 0 (black) to 255 (white), corresponding to lower pigmentation as the value increases (Mezei, Szakács, Dénes, & Jung, 2011).

2.5 | Analysis of cysteine and tyrosine levels

Three fish from each experimental tank were sacrificed as described above for skin samples extraction, from both dorsal and ventral parts by excising and pooling together (Figure S1). We hypothesized that the effect of photoperiod might be more noticeable on the ventral and dorsal parts of the fish. This is because fish colour is naturally bright on ventral part and less or pale on the dorsal part. The extracted skins were stored at −80°C for analysis of melanin components (tyrosine and cysteine) by using enzyme-linked immunosorbent assay (ELISA) kit (Nanjing Jiancheng Institute) according to manufacturer's instructions. The remaining part of the skin was used for mRNA expression analysis of genes as described below.

2.6 | Isolation of RNA, synthesis of cDNA and quantitative real-time PCR

The total RNA was isolated from dorsal and ventral sides of the skin from the three fish per each tank by using the TRIzol method (Invitrogen) by following the manufacturer's protocol. The purity and concentration of RNA were checked by NanoDrop-2000 (Thermo Scientific) and 1% agarose gel electrophoresis. The samples with OD ratios (A260:280) between 1.9 and 2.0 were used for cDNA synthesis by using the Primer-Script RT Master Mix (Takara). The real-time quantitative PCR (RT-qPCR) analysis was performed by using SYBR Premix Ex Taq™ II (Takara) in the CFX96 Real-Time PCR detection system (Bio-Rad). The specific primers for genes analysed were designed based on the original expressed sequence tag (EST) from Malaysian red tilapia transcriptome library (NCBI SRA database SRP076062; Table 1).

Amplifications were conducted in 96-well plate in which each reaction tube had 25 μl solution containing 1.0 μl cDNA template (150 ng/μl), 1.0 μl of each forward and reverse primers (10 μM), 9.5 μl PCR-RNase free water and 12.5 μl 2 × SYBR Premix ExTaq™II (Takara). The RT-qPCR conditions were as follows; 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 63°C for 30 s. Each RT-qPCR was run in three replicate and negative controls (no cDNA) were included. We used β-actin as reference gene due to its specificity, stability and sensitivity results obtained during the preliminary experiment on reference gene validation process. All the primers sequences used for the quantitative real-time PCR (qRT-PCR) analyses of genes are listed in Table 1. The $2^{-\Delta\Delta C_t}$ comparative method (Livak & Schmittgen, 2001) was used to calculate the mRNA expression of *sox 10*, *tyr*, *tyrp-1* and *slc7a11* genes.

2.7 | Analysis of blood biochemical components

Three fish from each tank were randomly selected for blood collection. Blood was taken from the caudal vein by using sterile 1 ml syringes (Klmedical). The blood from each individual fish was transferred into 1.5 ml collection tubes and kept at 4°C for 4 hr in order to allow separation of serum and plasma components. After separation, the tubes containing the blood were centrifuged at 845*g* for 10 min at 4°C, and the serum for each individual fish was shifted into another clean tubes. A total of sixteen serum biochemical components including activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), the contents of albumin (ALB), globulin (GLO), albumin to globulin ratio (A/G), total protein (TP), creatine kinase isoenzyme activity (CK-MB), cholesterol (CHOL), triglycerides (TG), creatine (CREA), potassium ion (K^+), sodium ion (Na^+) chloride ion, (Cl^-) and calcium ion (Ca^{2+}) were detected by using automatic biochemical analyser (Roche Modular P800).

TABLE 1 Primer sequence used for qRT-PCR during the present study

Gene	Primer sequence (5′–3′)	Annealing temperature (°C)	Product length (bp)
SRY-related HMG-Box (*sox 10*)	F: GTTGGCCTCAAGAGCTCAGT R: CTGGGAAGGCCCCATGTAAG	61	121
Tyrosinase (*tyr*)	F: CACTTCAGACGGACTGCGGA R: CCTGCGCACTGACTCTCTGT	61	289
Tyrosinase-related protein (*tyrp-1*)	F: TGCTCCACACCTTTACCGAC R: TAACCGAGGTTTTCAGGGGC	57	181
Solute carries family 7 member 11 (*slc7a11*)	F: GAAGAAGCGTGGTAGGCACT R: TGCTTCAGGATTCCCTTCGG	59	224
β-actin	F: GCCCATCTACGAGGGTTAT R: GCTGTGGTGGTGAAGGAGT	60	115

2.8 | Statistical analyses

Data were tested for normality and homogeneity of variances by using Shapiro–Wilk and Levene's tests respectively. All data were subjected to one-way analysis of variances (ANOVA) to detect significant differences on measured parameters among photoperiod regimes. Specific significant differences among means were determined by Duncan's multiple range tests. All statistical analyses were conducted by using the Statistical Package for the Social Science (SPSS) program for windows (IBM SPSS version 20). Results with $p < .05$ were considered statistically significant different, and the results are expressed as means ± standard error.

3 | RESULTS

3.1 | Growth performance, survival rate and gonadal maturation

The growth performance and gonadal maturation of Malaysian red tilapia reared under the three photoperiods are summarized in Tables 2 and 3 respectively. The FBW, WG and SGR were significantly higher ($p < .05$) for fish cultured under both prolonged light and darkness than in natural photoperiod. No significant effect was detected in survival rate among the three light regimes ($p > .05$; Table 2). The photoperiod regimes used affected neither the female nor the male GSI ($p > .05$; Table 3).

TABLE 2 Growth performances and survival rate of Malaysian red tilapia cultured in different three photoperiods during the present study

Treatments	Control	Light	Dark
IBW (g)	149.97 ± 0.19^a	150.26 ± 0.06^a	150.48 ± 0.44^a
FBW (g)	231.09 ± 6.71^a	277.93 ± 13.79^b	285.79 ± 13.93^b
WG (%)	54.09 ± 4.58^a	84.87 ± 9.09^b	91 ± 8.22^b
SGR (%)	0.72 ± 0.5^a	1.02 ± 0.8^b	1.08 ± 0.72^b
SR (%)	100 ± 00^a	100 ± 00^a	93.3 ± 4.4^a

Note: Values are means ± standard error for three replicates. Values in the same row with different superscripts are significant different ($p < .05$).
Abbreviations: FBW, final body weight; IBM, initial body weight; SGR, specific growth rate; SR, survival rate; WG, weight gain.

3.2 | Colour observation of fish

At the start of the experiment, all fish used were pink (whole pink body; Figure 1a). After the experiment, the fish reared under the natural photoperiod displayed a pink colour (Figure 1b) as compared to fish under the light condition (Figure 1c), which showed a pale pink body. Under dark photoperiod regime the fish displayed a reddish-pink colour (Figure 1d). The fish reared under prolonged darkness had lower RGB values compared to those cultured under natural photoperiod and prolonged light ($p > .05$; Figure 1e). Similarly, the fish reared under prolonged light had lower RGB values than those raised under natural photoperiod ($p > .05$).

3.3 | Cysteine and tyrosinase levels in fish skin

The results for tyrosinase and cysteine levels in the skin of Malaysian red tilapia under different photoperiod regimes are shown in Table 4. The tyrosinase level in ventral skin was significantly higher ($p < .05$) for fish cultured under prolonged darkness condition than in the other two photoperiod regimes. The tyrosinase level in the dorsal skin of the fish reared under the three photoperiod regimes was not affected ($p > .05$). In the dorsal skin, the cysteine level was significantly higher ($p < .05$) in the fish cultured under natural photoperiod than in prolonged light and prolonged darkness. However, the three photoperiod regimes did not affect the cysteine level in the ventral skin ($p > .05$).

3.4 | The mRNA expression of genes involved in melanogenesis pathway

The photoperiod regimes used affected the mRNA expression of genes in Malaysian red tilapia skin (Figure 2a–d). In the ventral skin, the expression of *sox 10* was significantly higher ($p < .01$) in fish

TABLE 3 Effects of photoperiods on gonadosomatic index (GSI) of Malaysian red tilapia

	Female			Male		
	Fish weight (g)	Ovary weight (g)	GSI	Fish weight (g)	Testes weight (g)	GSI
Control	217.22 ± 13.9	9.66 ± 1.23	4.42 ± 0.41	312.55 ± 8.61^{ab}	1.25 ± 0.10	0.39 ± 0.02
Light	262.55 ± 14.72	11.26 ± 1.80	4.14 ± 0.52	348.44 ± 21.28^{a}	1.33 ± 0.14	0.38 ± 0.02
Dark	229.66 ± 19.66	9.12 ± 1.16	3.93 ± 0.26	296.44 ± 15.5^{b}	1.26 ± 0.22	0.41 ± 0.05

Note: Values are means ± standard error for three replicates. Values in the same column with different superscripts are significant different ($p < .05$). Abbreviation: GSI, gonadosomatic index ($n = 9$).

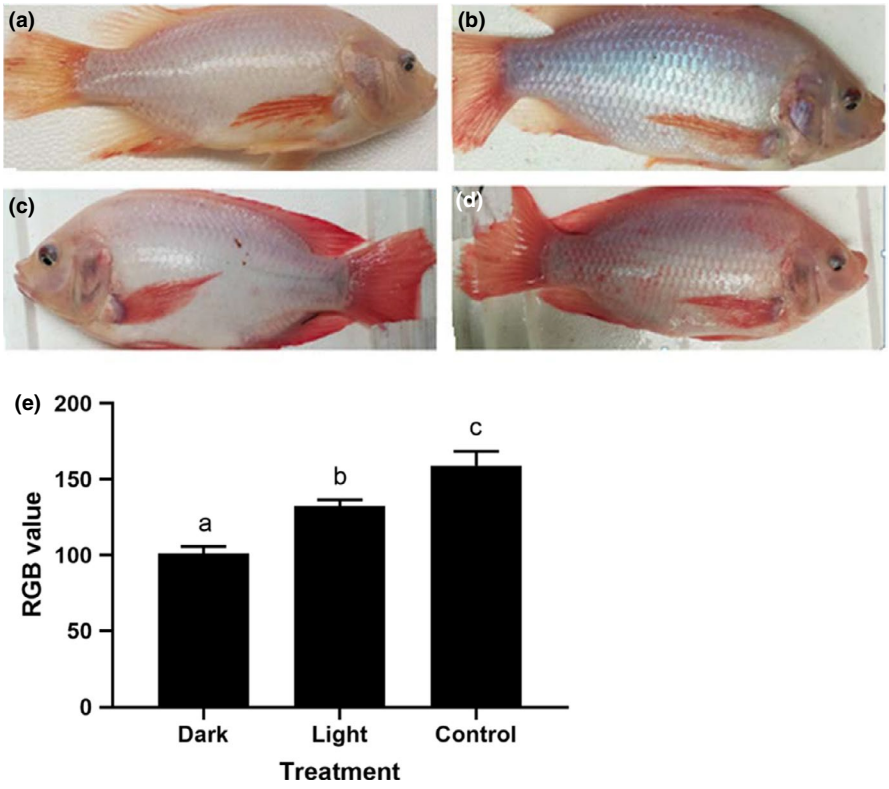


FIGURE 1 Colour variation of Malaysian red tilapia from different photoperiod regimes. (a) Initial colour before experiment; (b) control group (natural photoperiod); (c) prolonged lightness; (d) prolonged darkness; and (e) the RGB values of fish from the three photoperiod treatments. Different letters above bars for each treatment represent statistical difference ($p < .05$) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 4 The concentration of tyrosine and cysteine (ng/L) of Malaysian red tilapia cultured under three photoperiods during the present study

	Ventral skin		Dorsal skin	
Photoperiod	Tyrosinase	Cysteine	Tyrosinase	Cysteine
Control	328.59 ± 10.95^{a}	628.17 ± 16.5^{a}	341.16 ± 10.47^{a}	606.06 ± 18.18^{b}
Light	363.56 ± 12.94^{a}	594.39 ± 42.08^{a}	338.15 ± 10.46^{a}	559.38 ± 7.55^{a}
Dark	438.64 ± 22.03^{b}	684.07 ± 25.6^{a}	335.66 ± 12.97^{a}	547.09 ± 3.19^{a}

Note: Values are presented as mean ± standard error. Means in the same column with different superscripts are significant different ($p < .05$; $n = 9$).

reared under prolonged darkness than prolonged light and natural photoperiod (Figure 2a). Similarly, fish reared under prolonged light had higher ($p < .01$) expression of *Sox 10* mRNA than those reared under natural photoperiod. However, the *Sox 10* mRNA expression in the dorsal skin was not affected ($p > .05$) by the photoperiod regimes used. The mRNA expression of *tyr* gene in the ventral skin was significantly higher ($p < .01$) in prolonged darkness than in prolonged light (Figure 2b). However, no significant differences were obtained in *tyr* gene expression between fish reared under prolonged light and natural photoperiod ($p > .05$). In the dorsal skin, the mRNA expression of *tyr* gene was not influenced by the photoperiods used ($p > .05$). The *tyrp-1* gene displayed a similar pattern to *try* gene (Figure 2c). In the ventral skin, the *tyrp-1* mRNA expression was significantly higher ($p < .05$) for fish reared under prolonged darkness than natural photoperiod and prolonged light photoperiod. In the dorsal skin, no variations in *tyrp-1* mRNA expression were obtained among the three

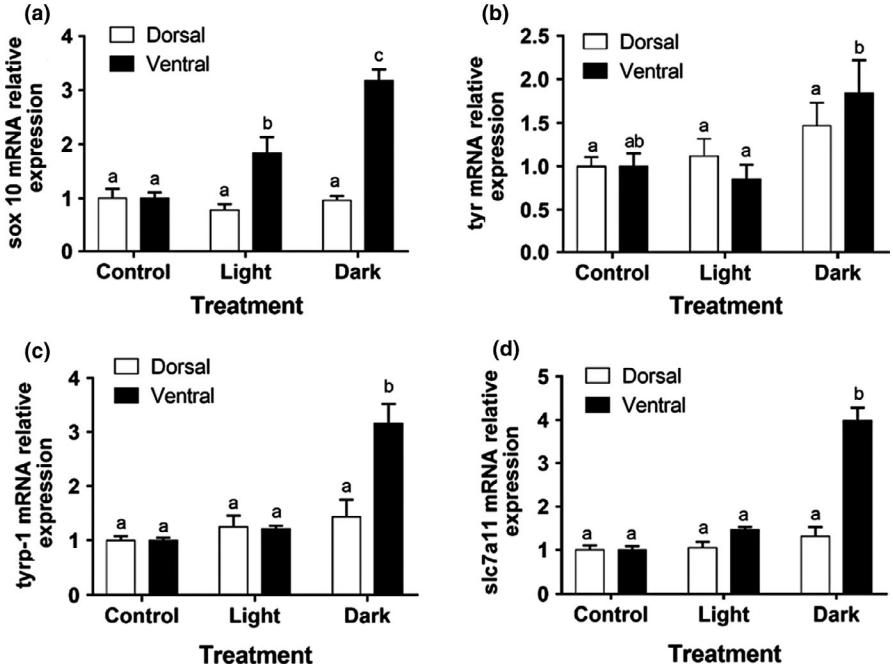


FIGURE 2 Relative mRNA expression of genes in ventral and dorsal skin of Malaysian red tilapia reared under different photoperiods. (a) SRY-related HMG-Box 10 (*sox 10*), (b) tyrosine (*tyr*), (c), tyrosine-related protein 1 (*tyrp-1*) and (d) solute carrier family 7 member 11 (*slc7a11*). Different letters above bars for each treatment represent statistical difference ($p < .05$)

TABLE 5 Effect of photoperiods on serum biochemical components

Biochemical parameter	Control	Light	Dark
ALT (U/L)	13.2 ± 0.7[a]	16.24 ± 1.07[b]	10.76 ± 0.68[a]
AST (U/L)	20.08 ± 1.65[a]	51.41 ± 2.64[b]	17.89 ± 1.26[a]
ALP (U/L)	28.30 ± 1.8[b]	24.70 ± 1.55[b]	19.65 ± 1.69[a]
LDH (U/L)	152.70 ± 9.96[a]	282.50 ± 29.29[b]	101.40 ± 7.08[a]
ALB (g/L)	12.48 ± 0.43[a]	12.12 ± 0.77[a]	12.37 ± 0.53[a]
GLO (g/L)	19.19 ± 0.40[a]	18.65 ± 0.62[a]	18.71 ± 0.94[a]
A/G (g/L)	0.64 ± 0.017[a]	0.62 ± 0.02[a]	0.64 ± 0.017[a]
TP (g/L)	31.67 ± 0.74[a]	31.94 ± 1.36[a]	30.83 ± 1.67[a]
CK-MB (U/L)	811 ± 216.84[ab]	1812 ± 594.14[b]	514 ± 120.52[a]
CHOL (mmo/L)	3.5 ± 0.31[a]	3.4 ± 0.27[a]	3.49 ± 0.33[a]
TG (mmo/L)	2.5 ± 0.32[a]	2.2 ± 0.37[a]	1.99 ± 0.33[a]
CREA (μmo/L)	18.63 ± 1.19[c]	8.3 ± 0.52[a]	11.50 ± 0.84[b]
K^+ (mmo/L)	3.05 ± 0.71[a]	2.24 ± 0.2[a]	2.64 ± 0.29[a]
Na^+ (mmo/L)	173.77 ± 1.21[a]	173.54 ± 1.22[a]	172.94 ± 3.72[a]
Cl^- (mmo/L)	119.28 ± 1.07[a]	128.23 ± 2.18[b]	117.44 ± 4.23[a]
Ca^{2+} (mmo/L)	3.43 ± 0.31[a]	3.54 ± 0.33[a]	3.33 ± 0.28[a]

Note: Values in the same row with different superscripts are significant different ($p < .05$). Values are presented as mean ± standard error.

Abbreviations: A/G, albumin to globulin ratio; ALB, albumin; ALP, alkaline phosphate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ca^{2+}, calcium ion; CHOL, cholesterol; CK-MB, creatine kinase isoenzyme activity; Cl^-, chloride ion; CREA, creatine; GLO, globulin; K^+, potassium ion; LDH, lactate dehydrogenase; Na^+, sodium ion; TG, triglyceride; TP, total protein.

photoperiod regimes used ($p > .05$). Similar to *tyr* and *tyrp-1* genes, the relative mRNA expression of *slc7a11* gene was also significantly higher ($p < .05$) in the ventral skin of fish reared under prolonged darkness than natural photoperiod and prolonged light (Figure 2d). The three photoperiods used did not affect the relative mRNA expression of *slc7a11* gene in the dorsal skin of the fish ($p > .05$).

3.5 | Effects of photoperiods on the blood serum biochemical components

The results showed that the fish reared under prolonged light had higher ALT, AST, ALP and LDH activities than both prolonged darkness and natural photoperiod (Table 5; $p < .05$). Fish raised under prolonged darkness had higher CK-MB activity than the other two photoperiod regimes, while those reared under natural photoperiod had higher CREA content than the other two photoperiod regimes ($p < .05$). No significant differences were obtained in ALB, GLO and A/G contents ($p > .05$). Also, the photoperiod regimes used did not affect the nutritional content indicators in blood serum (TP, CHOL, and TG). Ion parameters/electrolytes such as Cl^- were significantly higher in fish reared under prolonged light than other photoperiods ($p < .05$), while no significant differences were obtained in Na^+, K^+ and Ca^{2+} contents for the three photoperiod regimes used ($p > .05$).

4 | DISCUSSION

In the present study, Malaysian red tilapia reared under both prolonged light and darkness attained high growth rate compared to fish under natural photoperiod. The high growth rate of Malaysian

red tilapia under prolonged light is consistent with results obtained in *O. niloticus* (El-Sayed & Kawanna, 2007; Kawanna, 2004; Rad et al., 2006; Veras et al., 2013). We attribute the high growth for Malaysian red tilapia reared in the prolonged light to an efficient feeding activity during light hours as suggested by Zav'yalov and Lavrovskii (2001). Indeed, Rad et al. (2006) reported that the high growth rate in prolonged light is due to efficiency feed utilization, which enhances somatic growth. Tilapia are diurnal feeders, and they grow better under light condition (Zav'yalov & Lavrovskii, 2001). However, in our study we found higher growth performance in both prolonged darkness and light than natural photoperiod. High growth rate in prolonged darkness might be facilitated by low energy expenditure due to reduced metabolic activities during darkness period (Biswas & Tateuchi, 2002). Tilapia feed during the day (Zav'yalov & Lavrovskii, 2001), and the maximum feeding time is early in the morning at 05:00 a.m. up to 8:00 a.m. with the low feeding rate between 14:00 and 18:00 p.m. (Veras et al., 2013). Therefore, during light conditions, Tilapia use more energy for metabolic activities while in the dark environment they use lower energy (Ginneken et al., 1997). Moreover, high growth rate under darkness might be due to reduced swimming, which allocate more energy for growth (Liu et al., 2015).

In our study, the GSI of red tilapia reared under different photoperiod regimes was not significant different, similar to the results obtained in Nile tilapia by previous authors (Navarro, Sousa, Wlademir, & Bizarro, 2015; Zutshi & Singh, 2017). In contrary, other studies have obtained high GSI and high spawning rate in *O. niloticus* reared under prolonged light (Campos-Mendoza & Mcandrew, 2004; Rad et al., 2006). The results of photoperiods on GSI of tilapia are still confusing and inconsistency. We suggest more studies in order to provide clear reasons. Furthermore, no significant difference in survival rate was obtained in fish reared under different photoperiods, similar to results obtained by Ali and El-Feky (2013) and Zutshi and Singh (2017). Therefore, photoperiod manipulation can be applied in order to improve the growth rate of red tilapia without affecting their survival rate.

Red tilapia are known for their red colour, while the colour for Malaysian red tilapia varies from red to pink, pink/red and red with black spots (Zhu et al., 2016). In this study, all fish used for the experiment were pink at the start (whole pink body), whereas after 78 days of experiment changes in colour based on different photoperiods were observed. Fish reared under natural photoperiod displayed a whole pink colour, while those in the prolonged light condition showed discoloration condition whereby a pink colour turned to pale pink. The lower RGB value obtained for fish reared under darkness environment indicates higher pigmentation compared to fish under light condition. Our observation is in line with the results obtained for peppered shrimp, *Lysmata wurdemani*, which indicated high pigmentation under darkness background (Díaz, Martha, Hernández, Iván, & Rostro, 2018). Fish under darkness had high pigmentation of reddish-pink colour, which is highly preferred by consumers (Haque et al., 2016). The colour of fish is determined by the type of melanin synthesized, whereby a disturbance of the latter due to different environmental factors cause colour defects (Ferreira & Magina, 2013). Prolonged exposure of an organism to a fluorescent light may create potential adverse effects on the health of animals such as extracellular damage (skin; Chang et al., 2015; Gonzalez et al., 2017). Such changes in skin colour due to light were also observed in False Clownfish *Amphiprion ocellaris* (Yasir & Qin, 2010) and Red porgy, *Pagrus pagrus* (Pavlidis et al., 2008). These changes include morphological colour, which occur after long-term adaptation to light or physiological colour changes triggered by short-term stimuli (Sugimoto, 2002).

The content of tyrosinase and cysteine, which are the determinants of fish colour can be affected by several factors such as dietary composition (Wang et al., 2018; Watson, Wayman, Kelley, Feugier, & Biourge, 2018) and environment cues such as light (Gonzalez et al., 2017). In this study, we found that artificial photoperiod manipulation affected the contents of tyrosinase and cysteine. Based on colour observation after the experiment, these results indicate that tyrosinase and cysteine determine the melanin content in fish as suggested by Schwahn, Xu, Herrin, Bales, and Medrano, (2001) and Zhang et al. (2017). Generally, the synthesis of pheomelanin is facilitated by cysteine, which induces red/yellow colour (Jiang et al., 2014; Watson et al., 2018; Zhu et al., 2016). Our results indicate that melanogenesis process was affected by photoperiods whereby fish in prolonged light had a low level of tyrosine but higher RGB, which displayed light colour (pale pink). On the contrary, fish under prolonged darkness had a high level of tyrosinase but lower RGB and they displayed reddish colour.

Understanding the genetic basis of skin colour changes and factors affecting the pigmentation of red tilapia is paramount for the development of its production. Light produces specific wavelength bands, which affect genes and the transcription factors in the skin of fish (Chang et al., 2015; Gonzalez et al., 2017). The effect of light has been studied in model aquatic organisms such as platyfish, *Xiphophorus maculatus*, zebrafish, *Danio rerio* and Medaka, *Oryzias latipes* (Boswell et al., 2018). Skin is the first organ, which receives direct interaction with light in animals. Light stimulates melanin-stimulating hormone (*MSH*) and binds G protein receptor melanocortin receptor (*MC1r*), which upregulate cyclic adenosine monophosphate (*cAMP*) to control melanin synthesis. Under a normal circumstance, after the activation of *MSH* by light, colour synthesis is catalysed by *tyr*, *dct*, *tyrp-1* and silver locus protein homolog (*SILV*), which are regulated by microphthalmia-associated transcription factor (*MITF*). During colour synthesis, tyrosine is oxidized by tyrosinase to form DOPAquinone, which undergoes polymerization to form eumelanin. For pheomelanin synthesis, Agouti-signalling protein precursor (*ASIP*) inversely affects the *Mc1r* and cause downregulation of *cAMP*, *tyrp-1*, *dct* and *tyr*, which consequently lead to DOPAquinone producing pheomelanin. The production of pheomelanin is determined by the presence of cysteine, which is regulated by *xCT* encoded by *slc7a11* gene.

In this study, the mRNA expression of *sox 10*, *tyr*, *slc7a11* and *tyrp-1* genes in red tilapia was directly affected by light duration, similar to results obtained in *Oreolalax rhodostigmatus* (Zhu et al., 2018). In the ventral skin of Malaysian red tilapia, *sox 10* mRNA expression level was increased in prolonged darkness. The *Sox 10* is a key gene for melanogenesis, which regulates a number of genes such as *tyr*

and *tyrp-1* that are involved in melanin synthesis (Harris, Baxter, Loftus, & Pavan, 2010; Hou, Arnheiter, & Pavan, 2006). The higher expression of *sox 10* gene under prolonged darkness triggered the upregulation of *tyr*, *tyrp-1* and *slc7a11* genes due to its regulatory effects. Thus, the expression of *tyrp-1* and *tyr* genes was also higher in prolonged darkness, which corresponds to lower RGB value and the reddish-pink colour of the fish under dark photoperiod. These results indicate that the upregulation of *sox 10*, *tyr* and *slc7a11* genes under dark photoperiod facilitated pheomelanin reddish-pink colour synthesis. The *Slc7a11* is an important gene in pheomelanin production, and its existence in red tilapia pigmentation has been reported recently (Wang et al., 2018; Zhu et al., 2016).

Although light stimulates colour synthesis, but it may also have some adverse effects on the skin by causing cellular damage, skin cancer and stress, which may downregulate genes involved in melanin synthesis (Chang et al., 2015). Under prolonged light condition, fish were stressed while under dark condition no any negative effects were observed in terms of stress responses (Figure S2). Also, the lower expression levels of *tyr* and *tyrp-1* genes under prolonged light treatment, which was reflected by high RGB and pale pink colour of fish, suggest that the prolonged light possibly caused harmful effects on melanin production. Similar results were obtained in *Carassius auratus* whereby *tyrp-1* gene decreased as the colour changed from grey to red (Zhang et al., 2017). Moreover, the mutation of *tyrp-1* gene in *D. rerio* caused the death of some melanophores (Krauss, Geiger-Rudolph, Koch, Nusslein-Volhard, & Irion, 2014). In general, these results mean that photoperiod has a profound effect on melanogenesis particularly on the ventral side of fish skin due to the role of light in controlling circadian rhythm, which regulates and controls chromophores performance (Yasir & Qin, 2009). To the best of our knowledge, this is the first study to show the effect of artificial photoperiod on the coloration of Malaysian red tilapia.

Changes in concentrations and enzymes activities reflect cell damage and loss of its function (Bernet, Schmidt, & Wahli, 2012). In the present study, the activities of ALT, AST, ALP and LDH increased in fish reared under prolonged light than in other photoperiod regimes, suggesting changes in biological responses. The ALP is an enzyme for catalysing the hydrolysis of phosphate, whereby high concentration indicates hepatic disorder and bone diseases (Adham, Ibrahim, Hamed, & Saleh, 2002). The AST and ALT are both used to evaluate kidney and liver diseases (Shahsavani, Mohri, & Kanani, 2010). Therefore, the high concentrations of ALP, AST and ALT in fish exposed to prolonged light indicate functional disorder in the fish organs. Lactate dehydrogenase (LDH) oxidizes lactate to pyruvate, which is an important enzyme for normal muscle physiology, and the increase in LDH indicates high anaerobic energy production (Limbu, Zhou, Sun, Zhang, & Du, 2018). In general, the high concentrations of these parameters in red tilapia under prolonged light indicate physiological disorders in the fish. Indeed, Carvalho, Mendonça, Costa-Ferreira, and Gonçalves-de-Freitas, (2013) observed high level of aggressiveness in tilapia caused by physiological changes triggered by light. According to Elsbaay, (2013) aggressiveness is a stress indicator.

Generally, this study shows that photoperiod has impacts on growth performance, welfare and pigmentation of Malaysia red tilapia. The results obtained suggest that rearing Malaysia red tilapia under dark condition enhance growth performance and facilitate pigmentation. Photoperiod changes interfere with cysteine and tyrosinase production, which are important components in melanin production. Red/pink colour of Malaysia red tilapia is due to pheomelanin production, which was enhanced by several genes such as *slc7a11*, *tyr* and *sox 10* that are involved in the melanogenesis pathway. Understanding the molecular basis on the effects of photoperiod on red tilapia gives useful information that can be applied in development of selective breeding programme for improvement of the important economic traits (growth and skin colour) in order to develop commercial red tilapia farming. During breeding process, fish capable of withstanding environmental changes and providing positive results will be selected in order to maintain better performing strain. These findings advance our knowledge on genetics and selective breeding for consistent colour production in breeding programmes. We recommend further studies to investigate hormonal aspects in morphological and physiological colour changes in relation to photoperiod effects.

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CONFLICT OF INTEREST

We declare that all authors have no conflict of interest regarding the publication of this manuscript.

AUTHORCONTRIBUTIONS

ZD, WZ, LW and AM designed the study. FS, AM and ZY executed the experiment and collected data. AM, ZY and SML analysed data, and ZD supervised the study. AM and SML wrote the manuscript. All authors edited and approved the final manuscript for submission.

DATAAVAILABILITYSTATEMENT

The data used in this study are available from the first author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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