Int. J. Aquat. Biol. (2018) 6(3): 138-146
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Interaction of fish density and background color effects on growth performance, proximate body composition and skin color of common carp, *Cyprinus carpio*

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Abstract: This study was carried out to evaluate the combined effects of three stocking densities and two tank colors on growth, body composition and skin coloration of common carp (1.41±0.05 g). Fish with low (LD: 20 specimens/tank or 0.70 g/L), medium (MD: 40 specimens/tank or 1.41 g/L) and high (HD: 80 specimens/tank or 2.82 g/L) densities were reared in two tank colors (black and white) for 45 days. At the end of the experiment, density recorded 2.45 g/L and 7.00 g/L at low and high densities treatments, respectively. The final weight and specific growth rate of the fish at LD treatment were significantly higher than those of MD and HD treatments. The highest weight (4.90±0.44 g) and the lowest feed conversion ratio (1.21±0.13) were obtained for the LD fish treatment reared in the black tanks. Rearing density has a significant effect on the fish body total protein content, but the tank color had no effects on this factor. The fish body lipid content in the white tanks and high density was significantly higher than other treatments. Significant interactions between tank color and rearing density were observed for the fish body protein, fiber and dry matter. The fish skin color was considered by three factors: L*, a* and b*. The results showed that black color had a negative effect on the fish skin color indices. Brightness (L*) and yellowness (b*) values of the fish skin in the white tanks were higher than those of the black tanks. In the present study, tank color and rearing density significantly affected growth and feed performance of common carp, while no combined interaction was found between the two factors examined.

Article history:
Received 10 March 2018
Accepted 24 June 2018
Available online 25 June 2018

Keywords:
Culture density
Tank color
Growth
Skin color

Introduction

Common carp, *Cyprinus carpio* L. is one of the most important fish species currently being cultured. It generally inhabits freshwater environments, especially ponds, lakes and rivers, and rarely inhabits brackish-water environments (Barus et al., 2001). In 2015, common carp accounted for the third highest production (4328083 t) among all freshwater species worldwide (FAO, 2017). More than 90% of this production comes from Asia, where *C. carpio* is cultured in earthen ponds, containing a variety of naturally abundant foods supplemented through nutrient fertilization and/or artificial feeds. It is being considered as a potential candidate for commercial aquaculture in Asia and some European countries as it has a very high adaptive capability to both environment and food (Soltani et al., 2010; Manjappa et al., 2011).

An important factor for successful fish production is appropriate stocking density (Rahman et al., 2008). Stocking density directly depends on environmental conditions. It is a key factor in determining productivity and profitability of aquaculture systems production (North et al., 2006; Braun et al., 2010). Environmental stress caused by stocking density can affect digestion and absorption of food, growth rate and nutritional efficiency (North et al., 2006; Abdel-Tawwab, 2012; Abdel-Tawwab et al., 2014). Higher stocking densities were reported to cause chronic stress, which in turn reduced the growth rate due to the reallocation of energy towards activities aimed at restoring homeostasis such as respiration, locomotion, hydromineral regulation, and tissue repair (Biswas et al., 2007; Abdel-Tawwab, 2012; Abdel-Tawwab et al., 2014). Various studies have been carried out about the effects of stocking density on fish growth performance in different species such as Nile tilapia, *Oreochromis niloticus* (Abdel-Tawwab, 2012; Abdel-

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DOI: https://doi.org/10.22034/ijab.v6i3.470

Tawwab et al., 2014), *C. carpio* (Nuwansi et al., 2017), *Rtilus frisii Kutum* (Imanpoor and Abdollahi, 2011), *Oncorhynchus mykiss* (Liu et al, 2016), *Paralichthys olivaceus* (Kang and Kim, 2013) and *Anguilla marmorata* (Tan et al., 2018).

In nature, light intensity and background color can affect feed detection, feed conversion rate and feeding success of cultured fish (Strand et al., 2007). Therefore, all these factors can affect the fish growth and mortality (Jirsa et al., 2009). The tank color may affect fish growth and body composition (Papoutsoglou et al., 2000; Ginés et al., 2004; Aly et al., 2017), stress reactions (Rotllant et al., 2003) and their skin color (Van der Salm et al., 2004). In the fish body, the α-melanocyte-stimulating hormone and the melanin condensing hormones are the main components of pigmentation (Clement et al., 2005). The pituitary gland releases the alpha-melanocyte hormone. This hormone disperses melanin granules into melanophores, which darkens the skin color. Skin coloration in teleost is under multi-parametric control and a number of external or internal factors such as physical, nutritional, genetic, neuro-hormonal known as influential factors on chromatic state of fish (Fujii, 1993). In addition, fish can alter their coloration in response to environmental conditions, physiological challenges, stressful stimuli (Szisch et al., 2002) and rearing condition (in some fish such as red porgy) (Rotllant et al., 2003). However, fish could adapt to the background color by changing the skin color (Fernandez and Bagnara, 1991; Han et al., 2005).

Although the influence of tank color and rearing density on growth and metabolic activities of cultured fish has been reported in the literatures, the effect of both factors at once has not been studied. Therefore, this study examined the combined effects of rearing density and background color on growth performance, feeding, body composition and skin color of common carp under experimental conditions.

Materials and Methods

Fish and experimental design: Common Carp was obtained from a farm in Gonbad-Kavous, Iran, and transferred to Aquatic Engineering Laboratory of Gonbad-Kavous University, Iran. The fish were acclimatized with laboratory conditions for 12 days. Eight hundred and forty fish with mean initial body weight (±SD) of 1.41±0.05 g were randomly distributed, in six triplicated treatments according to a 2×3 factorial design for 45 days. The fish were reared in tanks with black and white color at three rearing densities of 20 (low density–LD), 40 (medium density–MD) and 80 (high density–HD) fish per tank (the equivalent of 0.70, 1.41 and 2.82 g/L). The fish were fed thrice daily (8:00, 13:00 and 18:00 h) at a rate of 3% of body weight using commercial pellet (40% crude protein, 12% crude lipid, 4.5% crude fiber with a diameter of 1.5 mm) from animal feed and aquaculture of Bezae company, Iran.

Sample collection and analysis: At the end of the feeding experiment, the fish were fasted for 24 hrs, collected, counted, and then total length and final weights were measured in each tank. The fish were profoundly anesthetized with 500 mg/L of clove powder. The growth and feed utilization parameters were calculated as following:

Percentage of weight gain (WG%) = $[(W_t - W_0)/(W_0)] \times 100$

Specific growth rate (SGR; %/day) = $100 \times [(\ln W_t - \ln W_0)/t]$

Feed conversion ratio (FCR) = dry feed intake (g))/wet weight gain (g)

Protein retention efficiency (PER) = weight gain (g)/protein fed (g)

Lipid efficiency ratio (LER) = weight gain (g)/lipid fed (g)

Condition factor (CF) = W_t/L^3

Where Wt (g) is fish body weight at day t and W0 at day 0, t (days) is the duration of experiment, L is total fish length (cm).

Body composition analysis: Seven fish were randomly captured from each tank for measuring the proximate composition of the whole-fish body. Contents of protein, lipid, moisture and ash were analyzed according to the standard methods (AOAC, 1995) and crude protein by analysis of total nitrogen (TN× 6.25) content by the Kjeldahl method (Kjeltec 1030 Auto Analyzer, Tector, Sweden). Crude lipid content was

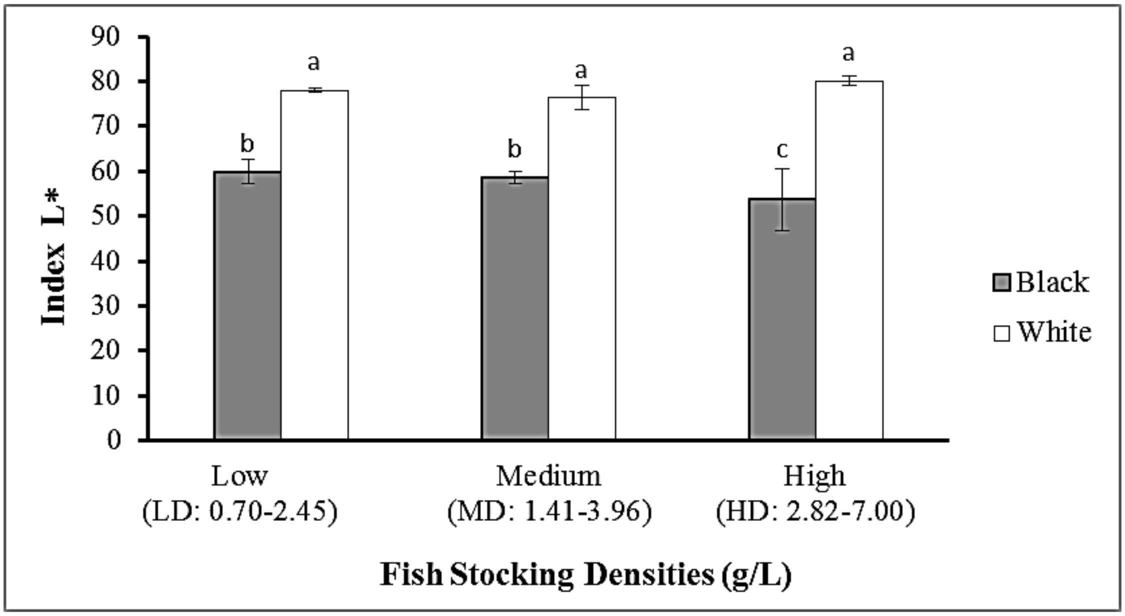

Figure 1. Values of L* in dark and white tanks at three rearing densities (bars assigned with different superscripts are significantly different; n=15; P=0.05).

measured by petroleum ether extraction using the Soxhlet method (model 1043 Extraction Unit; Tecator, Sweden). Moisture content was measured by drying in oven at 105ºC to a constant weight and ash was measured by ignition at 600 ºC in a muffle furnace (Heraeus, Germany) for 24 hrs. Fiber was analyzed using a Fibertec System Tector 1010.

Color analysis: Fish skin color was measured in three fish in each tank using a chromameter WSC-S (SPSIC Inc., Shanghai, P.R. China) equipped with a D65 light source and a 108 observing angle calibrated to black and white standards. The value of L* represents lightness (0 for black and 100 for white), the a* value represents the red/green dimension with positive values for red and negative ones for green and the value of b* represents the yellow/blue dimension with positive values for yellow and negative ones for blue. Colorimetric values of skin color were performed in two sides of each fish body (Han et al., 2005).

Statistical analysis: Normality of data was assessed using Kolmogorov-Smirnov test. Data were analyzed by two-way analysis of variance (ANOVA) with tank color and rearing density as factors using SAS (Statistical Analysis System) program. Each tank was considered as the experimental unit. Where P values were significant (P< 0.05) multiple comparisons were carried out using the Duncan test (Karakatsouli et al., 2010). All values presented in the text and tables are Means±SD.

Results

Comparing growth and feed indices of common carp reared in black and white tanks with three levels of density (low, moderate and high) are showed in Table 1. The final weight was significantly different among the treatments (P<0.05), the highest was observed in LD (4.90±0.4 g) and lowest in HD (3.50±0.30), regardless of the tank color. At the end of 45 days of experiment, the WG showed a significant difference among the treatments from 136.55±20.88 to 252.76± 29.01. Although the CF was not different among the treatments. The fish reared under black tank at LD had higher SGR compared to the other treatments (P<0.05). FCR was significantly (P<0.05) lower in the LD treatment (1.21±0.13) compared to the HD (2.37±0.33). Statistically, PER and LER was significantly different (P<0.05), so that the highest value was observed in the LD treatment in black tank and the lowest in the HD treatment in both black and white tanks. Growth and feed parameters reduced in the HD treatment in both black and white tank. Independently, tank color and rearing density significantly affected growth and feed performance of common carp, while no combined interaction was

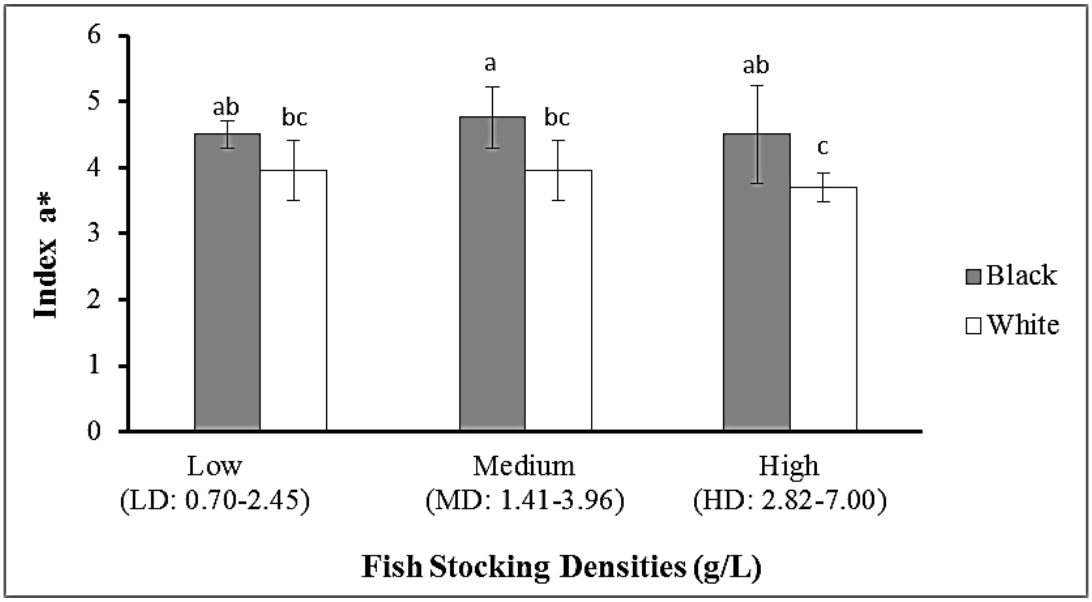

Figure 2. Values of a* in dark and white tanks at three rearing densities (bars assigned with different superscripts are significantly different; n=15; P=0.05).

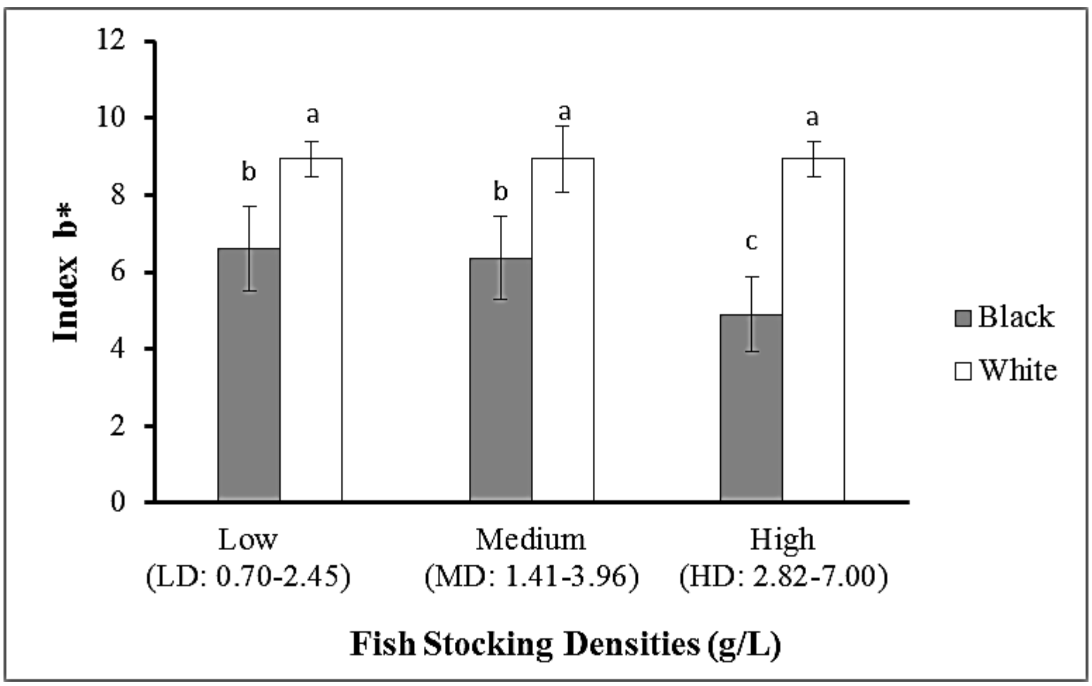

Figure 3. Values of b* in dark and white tanks at three rearing densities (bars assigned with different superscripts are significantly different; n=15; P=0.05).

found between the two factors examined (Table 1).

Table 2 shows the effects of tank color (black and white) and culture density on the fish body compositions. Crude protein was significantly different among the treatments (P<0.05). The crude protein content ranged from 65.76±0.30 to 64.11±0.32%. Fish under in the HD treatment and white tank showed the highest lipid content significantly different compared to the other treatments (P<0.05). Experimental treatments show significant difference in dry matter, fiber and ash (P<0.05). Statistical analysis showed that rearing density had significant effect on the fish body biochemical composition (P<0.05), while the tank color did not significantly affect the body composition (P>0.05). Significant interaction between tank color

Table 1. Growth and feeding performance on common carp reared in two tank colors (black and white) with different densities (Low- LD; Medium- MD; High density- HD) for 45 days.

	Tank Color						*P* value			
	Black			White						
Density	Low	Medium	High	Low	Medium	High	Color	Density	Color × Density	
Final weight (g)	4.90 ± 0.40^{a}	3.96 ± 0.26^{bc}	3.50 ± 0.30^{c}	4.46 ± 0.31^{ab}	3.91 ± 0.25^{bc}	3.59 ± 0.30^{c}	0.391	0.001	0.350	NS
Final length (cm)	6.54 ± 0.24	6.10 ± 0.11	6.90 ± 0.18	6.47 ± 0.19	6.20 ± 0.31	6.07 ± 0.30	0.962	0.018	0.823	NS
WG (%)	252.76 ± 29.01^{a}	193.75 ± 19.37^{bc}	136.55 ± 20.88^{c}	197.96 ± 20.77^{ab}	175.57 ± 18.14^{bc}	162.44 ± 22.29^{bc}	0.308	0.001	0.162	NS
SGR (% $gday^{-1}$)	2.79 ± 0.17^{a}	2.39 ± 0.14^{bc}	1.90 ± 0.19^{c}	2.42 ± 0.15^{ab}	2.24 ± 0.14^{bc}	2.13 ± 0.18^{bc}	0.256	0.001	0.027	S
CF (%)	1.75 ± 0.05	1.75 ± 0.17	1.54 ± 0.06	1.64 ± 0.04	1.65 ± 0.18	1.60 ± 0.11	0.417	0.181	0.433	NS
FCR	1.21 ± 0.13^{c}	1.89 ± 0.19^{ab}	2.37 ± 0.33^{a}	1.44 ± 0.14^{bc}	1.98 ± 0.21^{ab}	2.15 ± 0.28^{a}	0.780	0.001	0.264	NS
PER	0.12 ± 0.010^{a}	0.09 ± 0.006^{bc}	0.08 ± 0.007^{c}	0.11 ± 0.007^{ab}	0.09 ± 0.006^{bc}	0.08 ± 0.007^{c}	0.391	0.001	0.350	NS
LER	0.81 ± 0.06^{a}	0.66 ± 0.04^{bc}	0.58 ± 0.05^{c}	0.74 ± 0.05^{ab}	0.65 ± 0.04^{bc}	0.59 ± 0.05^{c}	0.391	0.001	0.350	NS

Values in a row with different superscripts denote significant difference ($P<0.05$). Each value represents the mean ± SD.

Table 2. The proximate composition (%, on dry matter basis) of the whole body of common carp reared in two tank colors (black and white) with different densities (Low- LD; Medium- MD; High density- HD) for 45 days.

	Tank Color						*P* value			
	Black			White						
Density	Low	Medium	High	Low	Medium	High	Color	Density	Color × Density	
Protein	65.76 ± 0.30^{a}	64.11 ± 0.32^{b}	65.22 ± 0.42^{a}	65.62 ± 1.05^{a}	65.00 ± 0.28^{ab}	64.17 ± 0.32^{b}	0.701	0.005	0.025	S
Lipid	23.55 ± 0.58^{b}	23.82 ± 0.45^{b}	23.40 ± 0.38^{b}	24.04 ± 0.16^{ab}	23.62 ± 0.37^{b}	24.49 ± 0.51^{a}	0.043	0.658	0.069	NS
Fiber	0.92 ± 0.04^{a}	0.93 ± 0.07^{a}	0.51 ± 0.09^{c}	$0.71\pm0.09a^{bc}$	0.67 ± 0.12^{bc}	0.78 ± 0.10^{ab}	0.146	0.013	0.001	S
Ash	9.12 ± 0.46^{b}	10.42 ± 0.60^{a}	10.38 ± 0.68^{a}	9.19 ± 0.31^{b}	10.03 ± 0.30^{ab}	9.95 ± 0.63^{ab}	0.320	0.006	0.663	NS
Dry Matter	25.14 ± 0.24^{bc}	26.95 ± 0.33^{a}	27.33 ± 0.52^{a}	23.76 ± 0.42^{cd}	23.10 ± 0.36^{d}	25.81 ± 0.42^{ab}	0.001	0.001	0.001	S
Computational carbohydrates	0.68 ± 0.09^{a}	0.61 ± 0.05^{ab}	0.48 ± 0.04^{b}	0.59 ± 0.09^{ab}	0.52 ± 0.04^{b}	0.61 ± 0.02^{ab}	0.608	0.075	0.017	NS

Values in a row with different superscripts denote significant difference ($P<0.05$). Each value represents the mean ± SD.

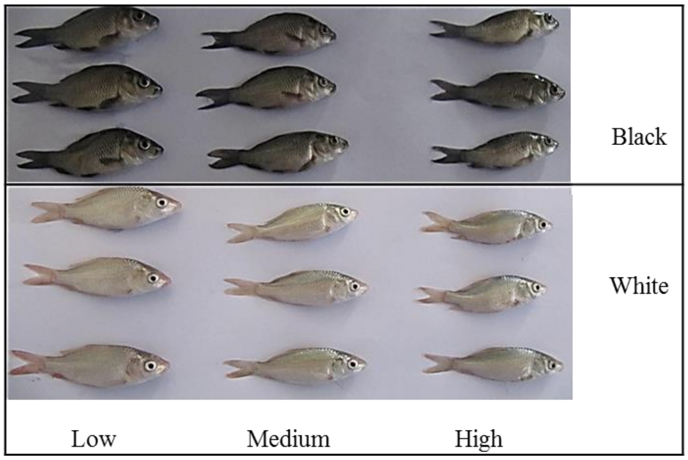

Figure 4. Pictures of common carp skin reared in dark and white tanks with different densities for 45 days.

and density was detected for carcass protein, fiber and dry matter content (Table 2).

The tank color significantly affected all body color parameters (Figs. 1, 2, 3). The values of L* in dark tank were significantly ($P<0.05$) lower than white tank. The values of a* in dark tanks were higher than white tanks. The value of b* in common carp skin in white color tanks was significantly higher than that in dark color tanks. The images produced at the end of the experiment indicated that the fish reared in dark tanks were darker than those fish reared in white tanks. Skin color was affected only by tank color (Fig. 4).

Discussion

The results of the present study showed that growth performance was affected only by rearing density being lower in the HD treatment. No significant interaction was detected between tank color and rearing density for growth and feed factors.

It is thought that fish growth is influenced by various environmental factors (i.e. density, nutrition and light) and behavioral (i.e. social interactions, life stage) (Haga et al., 2002; Ashley, 2007). The growth of some fish species is density dependent and there is an inverse relationship between stocking density and individual size of fish produced (Yang et al 2011; Abdel-Tawwab, 2012, 2014). Final weight and SGR were significantly lower in the HD treatment. In this regard, Gang et al. (2010) and Zhu et al. (2011) reported that growth, survival, and food utilization decreased along with increase in rearing density in *Scortum barcoo* and *Acipenser schrenckii*. Using appropriate stocking densities and feeding strategies are two key factors for successful aquaculture management (Kohinoor and Rahman, 2014).

There was a significant difference in FCR among different treatments. In this experiment, FCR was ranged from 1.21±0.13 (dark tank with low density) to 2.37±0.33 (dark tank with high density). In contrast to these results, Oprea et al. (2015) reported that different stocking densities did not negatively influence the fish production (FCR value was 1.41 in density of 30 kg/ha and 1.33 in density of 15 kg/ha. During the present experiment, no significant interaction was observed between the two factors examined for CF, WG and FCR, revealing that when the fish were kept at low density, growth and feed utilization enhanced. Density effect in intensive rearing of carp in tanks has been reported either to agree with the present results (Jha and Barat, 2005) or to suggest even much lower densities (Biswas et al., 2006).

Different factors like feeding components (Ali and Al-Asgah, 2001) and environmental color (Brännäs et al., 2001) and stocking density (Enache et al., 2011) may influence the growth performance of Cyprinidae. Furthermore, under culture conditions, tank color and light intensity can cause stress to fish (Papoutsoglou et al., 2005), resulting in behavioral changes such as swimming performance, activity level and habitat utilization (Mesa and Schreck, 1989). In addition, the tank color can affect fish body composition (Papoutsoglou et al., 2000; Aly et al., 2017). In the present study, black and white tanks did not have any significant effects on the fish body biochemical composition. Significant interaction between tank color and rearing density was detected for carcass protein, fiber and dry matter content. Hassan Akbarian et al. (2012) studied the effect of tank color on growth performance and body composition in common carp and reported that blue tank provided a better condition for growth factors and protein contact compered to white and black tanks. It may be because more physiological compatibility of fish with this color. Contrary to the current research, Karakatsouli et al.

(2007) investigated combined effects of rearing density and tank color on the growth and welfare of juvenile White Sea bream, *Diplodus sargus* L. in a recirculating aquaculture system. The results of their research proved that white and blue tanks could increase the growth factors and protein content and reduces lipid content, because it caused stress reduction, increasing food intake and fish comfort in these tanks. The effect of tank and food color on growth and body composition of grey mullet (*Liza ramada*) was tested. The highest WG and carcass protein content were observed in light tank and the lowest values were obtained in non-colored tank. Therefore, it seems that the fish have different functions based on the conditions of life and adaptation to the specific color (El-Sayed and El-Ghobashy, 2010).

The background color of the living media is an important factor in determining the skin color (Papoutsoglou et al., 2000). Body colors and patterns are determined by the distribution, density and aggregation state of different chromatophores in the integument. In fish, colors are produced by light absorption of pigments contained in the chromatosomes of melanophores, erythrophores and xanthophores and cyanophores, as well as by reflection from purine crystals, which, depending on their spatial organisation in refractosomes or leucosomes, produce the metallic iridescence of iridophores or the whitish hue of leucophores (Goda and Fujii, 1995; Leclercq et al., 2010). Fish can synthesize eumelanin from tyrosine and pteridine pigments from GTP (Maan and Sefc, 2013).

In the present study, it was found that fish in white tank have suitable color status, and their light color makes them marketable. Fish color changes are the result of adaptation to the environment, which is related to physiological changes (Van der Salm et al., 2004).

Acknowledgments

This research was conducted with the support of Gonbad-Kavoush University.

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Int. J. Aquat. Biol. (2017) 6(3): 138-146
E-ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

چکیده فارسی

اثر متقابل تراکم و رنگ مخزن به عملکرد رشد، تغذیه، ترکیبات بدن و رنگ پوست ماهی کپور معمولی (*Cyprinus carpio*)

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چکیده:

این مطالعه به‌منظور بررسی اثر ترکیبی سه تراکم ذخیره‌سازی و دو رنگ مخزن بر رشد، ترکیبات بدن و تغییر رنگ پوست ماهی کپور معمولی (۰/۰۵± ۱/۴۱ گرم) انجام شد. ماهیان با تراکم کم (LD: ۲۰ ماهی در هر مخزن یا ۰/۷۰ گرم در لیتر)، متوسط (MD: ۴۰ ماهی در هر مخزن یا ۱/۴۱ گرم در لیتر) و زیاد (HD: ۸۰ ماهی در هر مخزن یا ۲/۸۲ گرم در لیتر) در دو مخزن رنگی (سیاه و سفید) به‌مدت ۴۵ روز پرورش داده شد. در پایان آزمایش، تراکم ذخیره‌سازی در تیمارهای تراکم کم و زیاد به‌ترتیب ۲/۴۵ گرم در لیتر و ۷/۰۰ گرم در لیتر به‌ثبت رسید. وزن نهایی و نرخ رشد ویژه در تیمار LD به‌طور معنی‌داری بیشتر از تیمار تراکم MD و HD بود. بیشترین میزان وزن (۰/۴۴± ۴/۹۰ گرم) و کمترین میزان ضریب تبدیل غذایی (۰/۱۳± ۱/۲۱) برای ماهیان پرورش یافته تحت مخازن سیاه در LD به‌دست آمد. تراکم تاثیر قابل توجهی بر محتوای پروتئین کل بدن دارد، اما رنگ مخزن مقدار این عامل را تغییر نمی دهد. مقدار چربی بدن در مخزن سفید با تراکم بالا به‌طور معنی‌داری بیشتر از سایر تیمارها بود. اثر متقابل بین تراکم و رنگ مخزن، در مقدار پروتئین لاشه، فیبر و ماده خشک به‌دست آمد. رنگ پوست توسط سه فاکتور *L، *a و*b سنجیده شد. نتایج نشان داد که رنگ سیاه بر شاخص‌های رنگی تأثیر منفی دارد. مقدار روشنایی (*L) و زردی (*b) پوست ماهی در مخازن سفید بیشتر از مخازن سیاه بود. در این مطالعه، رنگ مخزن و تراکم رشد به طور قابل توجهی بر رشد و کارایی تغذیه ماهی کپور معمولی تأثیر گذاشته است، در حالیکه هیچگونه اثر متقابلی میان این دو عامل وجود ندارد.

کلمات کلیدی: تراکم پرورش، رنگ مخزن، رشد، رنگ پوست.