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Effect of Stocking Density on Growth, Serum Biochemical Parameters, Digestive Enzymes Activity and Antioxidant Status of Largemouth Bass, *Micropterus salmoides*

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Authors' Contribution
YYW and GCX performed the experiments, analyzed the data, wrote the manuscript. ZJN and NLS prepared all the samples. QJL conducted the feeding trial. PX conceived and designed the project.

Key words
Largemouth bass (*Micropterus salmoides*), Stocking densities, Growth, Digestive enzyme activity, Antioxidant status.

ABSTRACT

A 150 day feeding trial was performed to investigate the effect of stocking densities on growth performance, serum biochemical parameters, digestive enzymes activity and antioxidant status of largemouth bass (*Micropterus salmoides*) reared in an in-pond raceway system (IPRS). Fish (initial average body weight: 35.68±2.12g) were randomly allotted to in-pond raceways (26.2m×5m×2.5m) stocked at two stocking densities (68 and 114 fish/m^3, respectively). Fish were fed twice daily (08:00 and 17:00), and the daily ration feed was 4% of the body weight. No significant differences were observed in growth, digestive enzyme activity and antioxidant status between fish reared at two stocking densities (P>0.05). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), superoxide dismutase (SOD), cortisol and lysozyme in serum showed no significant differences between the two stocking groups (P>0.05). Fish reared at high stocking density had significant lower total protein (TP), cholesterol (TC), triglyceride (TG) and glucose (Glu) content in serum compared with those reared at low density on day 90 and 120 (P<0.05). In conclusion, the present results indicated that the largemouth bass (36-308 g) could be reared at high stocking density without depressed growth and chronic stress in commercial-scale in-pond raceway systems under this experimental conditions.

INTRODUCTION

China is the main producer and consumer of aquaculture products in the world, as it accounted for more than 60% of global aquaculture production, and aquaculture production increased from 34.6 million tons in 2001 to 51.4 million tons in 2016 (China Fishery Statistical Yearbook, 2017). However, in China, the rapid development of aquaculture resulted in many serious problems, including the limitations of finite water and land resources, the deterioration of aquatic environment, the high frequency of diseases, the disordered discharging of breeding wastewater, and the lowered quality and safety of aquatic products. These aquatic environment issues had seriously limited the sustainable development of the aquaculture. To solve these issues, the development of new culture technique with more efficient water and land usage and less environmental impact has become increasingly important.

By the end of the 1990s, researchers in Auburn University developed floating in-pond raceways that could be installed in ponds (Masser, 2004). The original system was successful in research settings, but it had several disadvantages, such as uneven water flow in the raceways and low efficiency of waste removal. Then, partitioned aquaculture system and split-pond system had been developed in the past years, and these systems combine biological, chemical and physical elements into an integrated system that may prove more controllable and efficient than traditional pond culture (Brown *et al*., 2011). In China, we reformed and innovated the in-pond raceways system (IPRS), and the IPRS consisted of culture areas (accounting for 2% to 5% of pond area) and purification areas (accounting for 95% to 98% of pond area). The culture areas consisted of series-connected in-pond raceways, and each raceway consisted of three components: airlift pumps area, fish culture area and waste settling area. Airlift equipment circulated pond water through the culture areas to aerate the water, and a waste collection system was utilized to capture feed remnants and excrement from the end of the raceways. The purification areas were used as waste treatment area, and phytoplankton, filter-feeding fish and shellfish were cultured in this area. Main advantage of IPRS was utilizes

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a minimum of water and land resources, while allowing for greater stocking densities, obtain maximum economic efficiency, and achieve zero discharge of waste water in culture cycle.

Stocking density is one of the most important factors in determining the productivity and profitability of the fish farm. However, high stocking densities can negatively impact on growth, behavior, size heterogeneity, physiological responses, immune functions, intestinal microbiota and disease resistance (Wendelaar-Bonga, 1997; Biswas *et al*., 2006; North *et al*., 2006; Lupatsch *et al*., 2010; Costas *et al*., 2013; Ni *et al*., 2014; Telli *et al*., 2014; Ribeiro *et al*., 2015). For the development of new rearing techniques, it is imperative to ascertain the appropriate stocking density to maximize the fish growth, health and productivity, while simultaneously lowering environmental pollution during the production cycle.

Largemouth bass (*Micropterus salmoides*) is one of the farmed freshwater species and has been widely cultured in China (Chen *et al*., 2012), due to its rapid growth rate, excellent taste, good disease resistance capability and tremendous economic values. Because largemouth bass is a carnivorous fish with gregarious habits, inappropriate stocking densities may cause size heterogeneity and cannibalism, ultimately led to poor farming profits. Therefore, the purpose of this study was to investigate the effect of stocking density on growth performance, serum biochemical parameters, digestive enzyme activities and antioxidant status of largemouth bass reared in IPRS conditions.

MATERIALS AND METHODS

Fish and feeding trial

This feeding trial was conducted in the Yangzhong experimental base, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, China. Two in-pond raceway systems (IPRS) were constructed in a 4.13-ha annular ecological ditch with a mean water depth of 2.5 m.

Largemouth bass were obtained from a local fish farm (Jiangsu, China). Prior to the feeding trial, all fish were reared in the IPRS for 2 weeks to acclimatize to the diets and the experimental conditions. At the start of the feeding trial, fish (initial body weight: 35.68±2.12 g) were stocked under two densities: low density (LD, 68 fish/m^3) and high density (HD, 114 fish/m^3), two replicates per density. The fish were fed with a floating commercial feed (Cargill Feed (Yangjiang) Co., Ltd. Guangdong, China), which contained ≥47% crude protein, ≤5% crude fiber, ≤18% crude ash, ≥5% crude lipids, 0.8-4.0% Ca, ≥0.8% P, 0.5-3.0% sodium chloride, ≥2.5% lysine and ≤13% water. The fish were fed twice daily (08:00 and 17:00), and the daily ration feed was 4% of the body weight. The grain sizes were adjusted based on the fish size. The water temperature, pH, dissolved oxygen (DO), ammonia-N and nitrite nitrogen (Nitrite-N) contents are presented in Table I. The feeding trial lasted for 150 days.

Table I.- Water quality variables measured in the in-pond raceway system.

Water quality	Water inflow	Culture area	Water outflow
DO (mg/L)	3.08-7.44	3.01-7.18	2.71-6.91
Temperature (◦C)	23.9-32.0	24.0-31.8	24.1-31.7
pH	7.73-8.22	7.68-8.27	7.77-8.25
NH_4^+-N (mg/L)	0.18-0.91	0.19-0.86	0.21-0.92
Nitrite-N (mg/L)	0.24-0.43	0.23-0.39	0.40-0.44

Samples collection

Five sampling periods were performed at 30, 60, 90, 120 and 150 days after the start of the feeding trial. Ten fish in each raceway were anaesthetized with 100 mg L^{-1} tricaine methanesulfonate and were individually weighed and body length measured for calculation of condition factor (CF). Blood were collected from the caudal vein using syringe (1 ml) and were transferred to a 1.5 ml centrifuge tube. After centrifugation (4000 rpm for 15 min) at 4°C, the serum was extracted and stored at −80°C for further analyses. After blood collection, liver and viscera were dissected and weighed for calculate hepatosomatic index (HSI) and viscerosomatic index (VSI). Intestine were also dissected used for digestive enzyme assay. Liver were also dissected used for antioxidant enzymes assay. All samples were frozen in liquid nitrogen and then stored at −80°C for further analyses.

Digestive enzyme activity assays

Intestinal and stomach samples were weighed and homogenized in ice-cold 0.86% sterile saline solution (tissue:saline=1:9) using a high speed tissue homogenizer. Then, the homogenates were centrifuged at 3500 rpm for 15 min at 4°C, the resultant supernatants were collected and stored at −80°C for digestive enzyme activity analysis. Activities of pepsin, trypsin, amylase and lipase were measured by the colorimetric method using reagent kits (Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's instructions.

Antioxidant enzymes assays

Liver samples were weighed and homogenized in ice-cold 0.86% sterile saline solution (tissue:saline, 1:9) using a high speed tissue homogenizer. Then, the homogenates

were centrifuged at 3000 rpm for 10 min at 4°C, the resultant supernatants were kept in centrifuge tubes and stored at −80°C for catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) activity and malondialdehyde (MDA) content analysis. The CAT, GSH-Px, SOD activities and the MDA content were measured using reagent kits (Jiancheng Bioengineering Institute, Nanjing, China) via spectrophotometric analysis with a microplate reader (Synergy H1, Bio-Tek Instruments, Inc. USA). The protein concentration of the tissues supernatant were measured by the coomassie brilliant blue method using reagent kits (Jiancheng Bioengineering Institute, Nanjing, China).

Biochemical measurements

Serum biochemical indexes including AST, ALT, ALP, TG, TC, Glu and TP were determined using reagent kits with an automated biochemistry analyzer (BS-400, Mindray Bio Medical Co., Ltd., China). Activities of SOD, lysozyme and cortisol content in the serum were determined using ELISA (BPRO kit; Langton Biotechnology Co., Ltd., Shanghai, China) with a labsystems Multiskan MS (352, Finland).

Statistical analysis

The data from each group were subjected to one-way analysis of variance (ANOVA) to test the effect of stocking density on fish. If significant differences were found ($P<0.05$), Duncan's multiple range test was used to compare the mean values between individual treatment using SAS 9.12 for Windows (Statistical Analysis System Institute, Cary, NC, USA). The data are presented as means ± S.D.

RESULTS

Growth performance

During the entire experimental period, no signs of disease were observed. No significant differences were observed in final body weight, specific growth rate (SGR), weight gain (WG) and body length of largemouth bass reared under different stocking density ($P>0.05$), but fish reared at high stocking density showed poor growth than those reared at low stoking density after 150 days (Table II; Fig. 1). The HSI, VSI and CF are presented in Table III. No significant differences were observed for HSI, VSI and CF of fish reared at both stocking densities ($P>0.05$).

Digestive enzyme activity

No significant difference were observed in digestive enzyme activities between fish reared at different stocking densities ($P>0.05$, Table IV).

Table II.- Effects of stocking densities on growth performance of largemouth bass.

		Density (kg/m^3)	
		LD	HD
Initial body weight (IBW, g)	0 d	36.10±2.71	35.26±3.34
Final body weight (FBW, g)	30 d	64.44±3.21	67.76±7.47
	60 d	124.89±9.78	147.48±12.40
	90 d	229.59±10.76	237.50±14.62
	120 d	327.21±11.56	309.37±11.81
	150 d	314.96±12.14	300.06±15.49
Weight gain (WG, %)	30 d	80.62±8.99	101.11±20.08
	60 d	250.02±27.41	313.33±29.15
	90 d	543.46±30.16	565.63±40.97
	120 d	817.06±32.41	767.08±33.10
	150 d	782.75±34.03	740.98±43.43
Specific growth rate (SGR, %·day^{-1})	30 d	1.94±0.18	2.21±0.34
	60 d	2.05±0.13	2.33±0.13
	90 d	2.06±0.05	2.09±0.07
	120 d	1.84±0.09	1.79±0.10
	150 d	1.45±0.10	1.41±0.12

Data represent as mean±S.D., mean with different superscripts in the same row are significantly different ($P<0.05$). Weight gain (WG, %) = 100 × (FBW-IBW)/ IBW. Specific growth rate (SGR, %·day^{-1}) = (Ln FBW-Ln IBW) × 100/ experimental duration (d).

Table III.- Effects of stocking densities on CF, VSI and HSI of largemouth bass.

		Density (kg/m^3)	
		LD	HD
CF (g/cm^3)	30 d	2.21±0.15	2.24±0.20
	60 d	2.44±0.18	2.55±0.15
	90 d	2.34±0.19	2.29±0.13
	120 d	2.50±0.29	2.44±0.23
HSI (%)	30 d	2.72±0.12	2.96±0.28
	60 d	2.18±0.14	1.95±0.08
	90 d	2.09±0.09	1.99±0.14
	120 d	2.28±0.08	2.33±0.15
VSI (%)	30 d	9.47±0.32	10.25±0.31
	60 d	8.72±0.29	8.02±0.30
	90 d	8.18±0.31	7.91±0.38
	120 d	7.24±0.25	7.54±0.34

Data represent as mean ± S.D., values in the same column with different superscripts are significantly different ($P<0.05$). Condition factor (CF, g cm^{-3}) = 100 × body weight (g)/ (body length, cm)^3. Hepatosomatic index (HSI, %) =100 × (liver weight/whole body weight). Viscerosomatic index (VSI, %) =100 × (viscera weight/whole body weight).

Table IV.- Effect of stocking densities on digestive enzyme activities of largemouth bass.

	Density	Lipase (U/g prot)		Amylase (U/mg prot)		Protease (U/mg prot)	
		Intestine	Stomach	Intestine	Stomach	Trypsin	Stomach
30d	LD	43.56±7.70	75.25±6.13	0.29±0.01	0.30±0.04	186.61±32.61	18.76±1.14
	HD	44.69±7.89	61.45±12.39	0.35±0.11	0.32±0.01	203.90±52.57	20.14±2.18
60d	LD	31.18±5.24	66.69±8.66	0.34±0.05	0.26±0.03	226.42±16.29	17.03±0.68
	HD	33.02±5.31	59.43±8.34	0.34±0.01	0.33±0.09	177.97±27.29	19.35±3.12
90d	LD	29.24±3.48	59.06±8.78	0.41±0.04	0.25±0.03	188.18±20.43	16.58±1.34
	HD	32.72±7.58	52.50±5.76	0.33±0.04	0.25±0.01	251.31±48.43	15.99±2.26
120d	LD	38.22±2.05	44.81±6.87	0.25±0.03	0.22±0.03	232.41±27.53	13.62±1.34
	HD	43.36±5.56	55.61±8.37	0.27±0.00	0.23±0.02	170.13±25.85	16.46±1.19

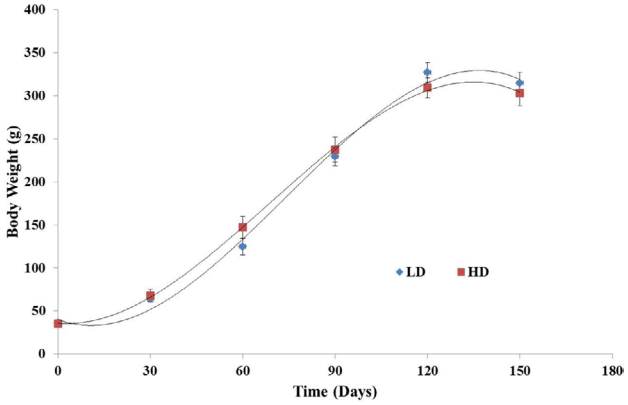

Fig. 1. Growth of the largemouth bass for the two treatments. LD, low density; HD, high density.

Table V.- Effects of stocking densities on SOD, CAT, GSH-Px activities and MDA content in the liver of largemouth bass.

		Density (kg/m^3)	
		LD	HD
CAT (U/mg prot)	30 d	22.73±1.75	30.02±1.96
	60 d	22.76±1.42	20.44±1.36
	90 d	19.39±0.58	17.23±0.89
	120 d	17.87±1.32	15.84±2.26
T-SOD (U/mg prot)	30 d	52.72±2.20	56.69±3.06
	60 d	55.74±1.74	50.80±2.20
	90 d	45.42±1.32	44.05±1.46
	120 d	45.27±0.98	53.00±7.02
MDA (nmol/mg prot)	30 d	1.90±0.16	1.33±0.34
	60 d	1.18±0.28	0.89±0.18
	90 d	1.01±0.15	0.94±0.31
	120 d	1.04±0.21	0.78±0.22
GSH-Px (U/mgprot)	30 d	35.81±3.20	38.45±4.92
	60 d	44.34±4.06	33.50±2.55
	90 d	35.35±3.10	33.68±2.06
	120 d	33.75±4.58	28.36±3.51

Antioxidant enzymes activity

The effect of stocking density on hepatic CAT, GSH-Px, SOD activities and MDA content are shown in Table V. CAT, GSH-Px, SOD activities and MDA content were not different between fish reared in different treatments (P>0.05).

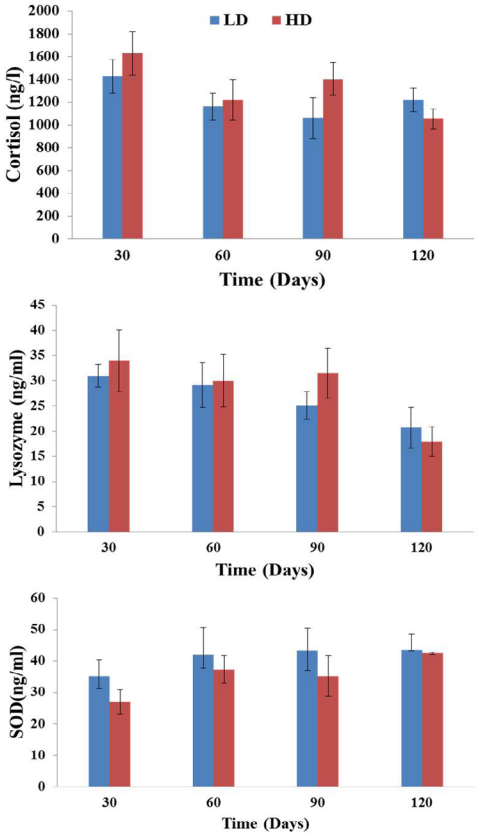

Fig. 2. Effects of stocking densities on serum cortisol, lysozyme and SOD of largemouth bass. No letters denote no significant differences between densities within sampling day. LD, low density; HD, high density.

Table VI.- Effects of stocking densities on serum parameters of largemouth bass.

		Density (kg/m^3)	
		LD	HD
Alanine aminotransferase (ALT, U/L)	30 d	13.02±2.66	14.57±1.40
	60 d	8.38±0.83	9.89±0.76
	90 d	8.20±1.13	9.47±1.36
	120 d	4.88±0.40	4.65±0.68
Aspartate aminotransferase (AST, U/L)	30 d	130.77±12.65	145.75±15.10
	60 d	79.23±4.41	74.28±11.26
	90 d	64.67±9.43	75.51±6.61
	120 d	37.31±4.07	27.73±4.53
Alkaline phosphatase (ALP, U/L)	30 d	80.29±5.29	96.68±7.49
	60 d	165.28±9.38	143.87±8.82
	90 d	135.44±8.74	158.48±8.23
	120 d	109.78±4.65	124.09±10.64
Total protein (TP, g/L)	30 d	34.32±1.75	30.39±2.40
	60 d	42.79±3.22	41.82±3.11
	90 d	40.18±4.18^a	34.57±4.56^b
	120 d	47.41±3.66^a	43.54±3.48^b
Glucose (GLU-HK, mmol/L)	30 d	8.03±0.68	6.05±0.76
	60 d	6.71±0.44	6.08±0.43
	90 d	7.02±0.45	8.67±0.69
	120 d	10.86±0.72^a	8.26±0.39^b
Cholesterol (TC, mmol/L)	30 d	8.50±0.34	8.95±0.94
	60 d	10.22±1.65	9.69±0.62
	90 d	9.48±0.77^a	8.49±0.68^b
	120 d	14.55±0.87^a	11.77±0.89^b
Triglyceride (TG, mmol/L)	30 d	5.32±1.06	5.54±0.63
	60 d	12.11±1.33	11.31±1.02
	90 d	10.34±1.42^a	6.52±0.70^b
	120 d	24.70±2.61^a	17.73±1.72^b

Biochemical measurements

The effect of stocking density on serum biochemical indexes of fish are presented in Table VI. No significant differences were observed in serum ALT, AST and ALP between fish reared at different stocking densities (P>0.05). Fish reared at high stocking density had significantly lower serum TP, TC, TG contents than those reared at low stocking density on day 90 and 120 (P<0.05), while no significant differences were observed on day 30 and 60 (P>0.05). Serum glucose content was significantly lower in fish reared at high stocking density than those reared at low stocking density on day 120 (P<0.05), while no significant differences were observed on day 30, 60 and 90 (P>0.05). The serum cortisol and lysozyme were increased with increasing stocking density, but no significant differences were observed, the opposite trend was found in serum SOD (Fig. 2).

DISCUSSION

During the entire production period, water quality parameters in the culture area were as followed: water temperature 24.0-31.8°C, DO 3.01-7.18 mg L^{-1}, pH 7.68-8.27, ammonia nitrogen (NH_4^+-N), 0.19-0.86 mg L^{-1} and nitrite nitrogen (Nitrite-N), 0.23-0.39 mg L^{-1} (Table I). These variables were maintained at acceptable levels for largemouth bass during the production period, and it is speculate that fish had suffered no stress from water quality.

Generally, high stocking density could increase fish production and maximum water utilization, but this can cause crowding stress as it directly influences welfare of cultured fish. In the present study, the fish had better tolerance for cultural conditions and diet without significant effect on mortality in IPRS, and no significant differences were observed in final body weight, WG, SGR, CF, HSI, VSI between fish reared at different stocking densities. Fish reared at high stocking density exhibit a slight change in final body weight, WG and SGR on day 120 and 150. Liu *et al*. (2016) observed no significant difference in growth performance of turbot *Scophthalmus maximus* until 80 days, but fish reared at high density had significant lower body weight and SGR than in medium and low density treatments at day 120. Similar results were also obtained for senegalese sole *Solea senegalensis* (Costas *et al*., 2013; Andrade *et al*., 2015), sea bass *Dicentrarchus labrax* (di Marco *et al*., 2008; Lupatsch *et al*., 2010) and rainbow trout *Oncorhynchus mykiss* (North *et al*., 2006). On the contrary, several studies reported that inappropriate stocking density may impair growth rate, physiological responses and immune competence (Montero *et al*., 1999; Telli *et al*., 2014; Guo *et al*., 2017). It has been stated that high stocking densities may largely responsible for deterioration of water quality through metabolic excretion of fish causing excessively high amount of organic load and ammonia and reduced level of dissolved oxygen, thus affected growth (Biswas *et al*., 2006; North *et al*., 2006). Moreover, the reduced growth in fish reared at high density may be due to lower feed intake and adverse social interactions (Ellis *et al*., 2002; Naderi *et al*., 2017). Interestingly, Papoutsoglou *et al*. (1998) and Millán-Cubillo *et al*. (2016) found that growth increased with increasing stocking density. These variations may be related to differences in fish species, size–physiological

stage, water exchange rate, stocking density levels, social behavior and culture conditions (Papoutsoglou *et al*., 1998; di Marco *et al*., 2008; Millán-Cubillo *et al*., 2016).

The digestive enzymes activities reflect the digestive characteristics and nutritional condition of fish, and these changes would affect digestion and absorption capability, feeding behaviour and ultimately growth and development of fish (Lemieux *et al*., 1999; Dong *et al*., 2018). However, there is little literature regarding the effects of stocking density on digestive enzymes activities. In this study, our results did not show significant differences between two stocking densities in the intestinal amylase, lipase and trypsin activities during the culture period. This indicated that changes in digestive enzyme activities did not occur in largemouth bass when subjected to the high density. Bolasina *et al*. (2006) also found no differences in digestive enzyme activity in larvae Japanese flounder (*Paralichthys olivaceus*), while juveniles flounder had significantly higher trypsin activity in the high density group. On the contrary, Guo *et al*. (2017) and Dong *et al*. (2018) reported that the activities of digestive enzymes tended to decrease at increased stocking densities, especially for fish reared under low DO conditions, and the reduced digestive enzymes activities may be related to lower feed intake and depressed synthesis and secretion of enzyme in stress conditions, and eventually lead to poor growth.

High stocking density is commonly regarded as a chronic stressor in aquaculture, and changes in blood nutrient, corticosteroid hormone and catecholamine levels may be used to reflecting the responses to stressors (Barton and Iwama. 1991; Bolasina *et al*., 2006; Saurabh and Sahoo, 2008). In the present study, no significant differences were observed in serum cortisol and lysozyme level of fish reared at two stocking densities. These results are coincide with that reported in sea bass, rainbow trout, tilapia and senegalese sole (Ellis *et al*., 2002; di Marco *et al*., 2008; Telli *et al*., 2014; Andrade *et al*., 2015; Naderi *et al*., 2017). These results indicated that the fish may accustomed to the prolonged period of crowding stress, and exhibit a slight change or no changes in cortisol level, and these studies shown that plasma cortisol would not be a suitable indicator of chronic stress in fish (North *et al*., 2006; Naderi *et al*., 2017). However, Procarione *et al*. (1999) and Millán-Cubillo *et al*. (2016) observed that cortisol levels decreased with increasing stocking densities, and higher cortisol levels are observed in sea bass (Lupatsch *et al*., 2010), rainbow trout (Yarahmadi *et al*., 2016) and gilthead seabream (Montero *et al*., 1999; Varela *et al*., 2010) reared at higher stocking densities. It is known that high cortisol levels can present negative effect on growth and immunological response (Wendelaar-Bonga, 1997; Barton, 2002). Differences in cortisol level under stress conditions may be ascribed to differences in fish species, feed intake and culture conditions. Previous studies found that when zebrafish *Danio rerio* (Ramsay *et al*., 2006) and sea bass (Lupatsch *et al*., 2010) have suffered acute stress, higher cortisol levels were observed in fasted fish than in fed groups, and these results indicated cortisol level is a suitable acute stress indicator in fish.

In this study, fish reared at high density had significantly lower serum TP, TC, TG, triglyceride content than those reared at low density on day 90 and 120, but no significant differences were found during the early culture stages (day 30 and 60). Similar results were obtained in senegalese sole (Andrade *et al*., 2015), tilapia (Telli *et al*., 2014) and rainbow trout (Naderi *et al*., 2017), this probably due to the stress quickly consumes the energy reserves to maintain glucose within normal limits in chronic stress conditions (Martínez-Porchas *et al*., 2009; Naderi *et al*., 2017).

Oxidative stress is an unavoidable aspect of aerobic life, especially in aquaculture, and the oxidative damage of tissues is directly associated with growth, welfare, health and the quality of final products (Senso *et al*., 2007; Sevcikova *et al*., 2011). Reactive oxygen species (ROS) can increase dramatically under stressful conditions in living organisms. When the production and accumulation of ROS is beyond the preventive and detoxifying capacity of the antioxidant system (Halliwell and Gutteridge, 2015; Bano et al., 2017), ROS can cause oxidation of proteins and lipids, alterations in gene expression, changes in cell redox status and loss of antioxidant enzymes (Valavanidis *et al*., 2006; Sevcikova *et al*., 2011). Liu *et al*. (2016) found that fish held at high stocking densities resulted in remarkably depressed hepatic CAT, SOD and GSH-Px levels on day 120. The decreased activity of these enzymes may indicate a response to the continuous stress of stocking density, and reflect the limited abilities for antioxidant systems to remove these harmful superoxide radicals, and eventually cause oxidative damage in fish (Andrade *et al*., 2015; Costas *et al*., 2013). However, in present study, no significant differences in hepatic CAT, T-SOD and GSH-Px levels were found in fish reared at different stocking densities after 120 days. This finding demonstrated that high stocking density probably not induce oxidative damage in largemouth bass. Similar results were previously observed in senegalese sole (Andrade *et al*., 2015). Lipid peroxidation is one of vital sign of cellular oxidative damage and MDA is an important metabolite derived from lipid peroxidation (Draper and Hadley, 1990; Zuo *et al*., 2013). Previous studies reported that high stocking density induced the production of ROS in fish, and it directly attacked polyunsaturated fatty acids in cell membranes and induce lipid peroxidation (Sahin *et al*., 2014; Andrade

et al., 2015). However, our results showed no significant differences in MDA level at different stocking densities, and this result indicated that high stocking density may not initiate lipid peroxidation in largemouth bass. Similarly, no significant differences in MDA level were observed in senegalese sole (Andrade *et al*., 2015) and turbot (Liu *et al*., 2016) held at different stocking densities.

CONCLUSION

In summary, our results indicated that the largemouth bass (36-308 g) could be cultured at high stocking density without depressed growth and cause chronic stress in commercial-scale in-pond raceway system. Further studies should be designed to study whether higher stocking density affects metabolism, expression of immune and stress related genes and the ability of fish to resist diseases.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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