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Effects of dissolved oxygen and fish size on Nile tilapia, *Oreochromis niloticus* (L.): growth performance, whole-body composition, and innate immunity

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Abstract The present study was carried out to investigate effects of dissolved oxygen (DO) and fish size (FS) on growth, feed utilization, whole-body composition, and innate immunity of Nile tilapia, *Oreochromis niloticus* (L.). The experiment was based on a bifactorial design with three levels of DO (low: 0.1–1.5, medium: 2.5–3.0, and normal: 6.0–6.5 mg/L) and two sizes (3.7 and 12.9 g) within quadruplicates. Fish density was kept at 2.1 g/L, i.e., 50 or 15 fish per 90-L aquarium for small or large fish, respectively. Fish were fed up to satiation twice a day for 12 weeks on a 35 % crude protein diet. After the feeding trial, fish were artificially infected by pathogenic bacteria *Aeromonas hydrophila* for 10 days. It was noticed that DO and FS significantly affected fish growth, feed utilization, whole-body composition, and innate immunity. However, fish growth and feed intake were adversely affected by low DO. Additionally, smaller fish consumed less feed and exhibited better growth than the larger ones. Feed conversion ratio in case of small fish was better than that in case of larger one. Regarding fish body composition, moisture content was affected by FS only, while crude protein, lipid content, and total ash were significantly affected by DO level, FS, and their interaction. It is also noticed that larger fish tolerated low DO better than the small ones where values of nitro blue tetrazolium and lysozyme activity of large fish were better than small one. Additionally, innate immunity increased as DO levels increased. The total fish mortality after 10 days post-challenge was adversely affected by DO, and the highest mortality was observed at low DO in smaller fish, whereas no mortality was observed at normal DO in larger fish. These results indicate that fish growth, feed utilization, and innate immunity were adversely affected by low DO; meanwhile, smaller fish showed better performance than larger ones at normal DO.

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Introduction

Hypoxia is a common phenomenon in stagnant water, where currents and convection do not introduce dissolved oxygen (DO) into water body, especially at night, when plants do not photosynthesize. This means fish live in an environment that often shows great variations in DO levels including hypoxia. Hypoxia may retard fish growth, feed utilization, and hence health status so that fish could utilize several mechanisms to cope a reduction in DO uptake. The different coping styles in individual fish may influence fish health and susceptibility to bacterial infection (MacKenzie et al. 2009; Huntingford et al. 2010), which is one of the limiting factors in fish culture including Nile tilapia. In particular, *Aeromonas hydrophila* is the etiological agent of several diseases and causes mass mortalities in several fish species (Rahman et al. 2001; Li et al. 2006). Fish susceptibility to bacterial infection is associated with size and/or age (Suanyuk et al. 2008; Mian et al. 2009; Zamri-Saad et al. 2010).

The farming of Nile tilapia, *Oreochromis niloticus* (L.), has grown rapidly in the last few decades in Egypt and worldwide (El-Sayed 2006). For fish intensification, DO has to be maintained at levels, which will not affect fish physiology and metabolic activities. Thus, one has to keep in mind that DO requirements depend not only on fish species but also on fish size (FS). With increasing FS during grow-out period, DO requirement increased and DO in ponds water may be insufficient. Therefore, the current study was conducted to investigate the effect of different DO levels on the performance and innate immunity of Nile tilapia with different sizes. In addition, fish susceptibility to experimental *A. hydrophila* infection was evaluated.

Materials and methods

Experimental design

Nile tilapia *O. niloticus* (L.) were collected from nursery ponds of Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abo-Hammad, Sharqia, Egypt. Fish were acclimated for 2 weeks to laboratory conditions. However, fish were kept in indoor fiberglass tanks contained well-aerated tap water through air pump via air stones during which fish were fed twice a day on a 35 % crude protein diet formulated as described by Abdel-Tawwab et al. (2010). After that, fish were graded into the sizes used herein.

Twenty-four aquaria were set for this experiment, which was based on a bifactorial design with three DO levels: low (1.0–1.5 mg/L), medium (2.5–3.0 mg/L), and normal (6.0–6.5 mg/L) and two fish sizes (3.7 and 12.9 g). Each aquarium set for normal DO level (NDO) was aerated with four air stones, which were inserted at two diagonally opposite corners, while that of medium DO level (MDO) was aerated with two air stones. On the other hand, the aquarium assigned to low DO level (LDO) was aerated with one air stone. Concentrations of DO in all aquaria were measured every 2 h once a month during the experimental period, and the average of dual DO concentrations is plotted in Fig. 1. Aeration is maintained continuously, and DO levels were kept almost stable during the experiment (Fig. 2).

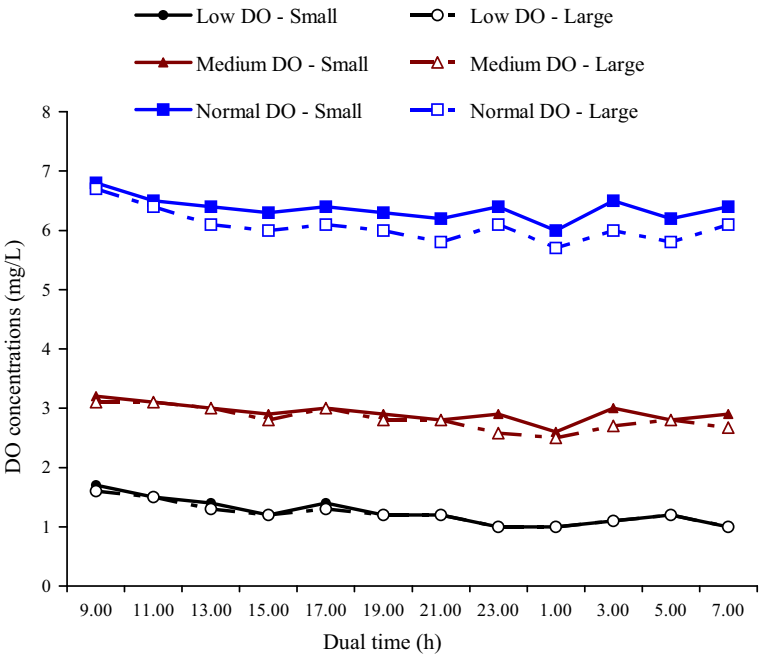

Fig. 1 Dual changes in dissolved oxygen (DO) concentrations (mg/L) of water aquaria stocked with Nile tilapia as affected by different levels of dissolved oxygen and fish size for 12 weeks. Low DO = 1.0–1.5 mg/L, medium DO = 2.5–3.0 mg/L, and normal DO = 6.0–6.5 mg/L. Small = 3.7 g and large = 12.9 g

Under each DO level, fish were randomly distributed into aquaria at a rate of 2.1 g/L (i.e., 50 and 15 fish per 90-L aquarium for small and large fish, respectively) in quadruplicates. Fish were fed up to satiation twice daily at 9:00 and 14:00 h on a 35 % CP diet for 12 weeks. Summation of the given feed during the whole period of the experiment represented the total feed intake. Every 2 weeks, fish in each aquarium were collected, counted, and group-weighed to the nearest 0.1 g. Settled fish wastes were siphoned every day with a three-quarter of aquarium's water, which was replaced by well-aerated dechlorinated tap water from a storage tank. Dead fish were removed and recorded daily. At the end of the experiment, fish were collected, counted, and weighed.

Water quality measurement

Water samples were collected fortnightly at 15 cm depth from each aquarium. Dissolved oxygen and temperature were measured daily in site using a portable DO meter (Jenway, London, UK). The pH values were measured using a Digital Mini-pH Meter (model 55, Fisher Scientific, Denver, USA). The electric water conductivity was measured using a Portable Conductivity Meter (Jenway, London, UK). The unionized ammonia (NH_3) concentration was measured using a Multiparameter Ion Analyzer (HANNA Instruments, Rhodes Island, USA). Total alkalinity and total hardness were measured by titration method according to Boyd (1984).

In all treatments, water temperature ranged from 26.4 to 27.3 °C, pH ranged from 7.6 to 7.8, conductivity ranged from 363.0 to 403.5 µS/cm, and unionized ammonia

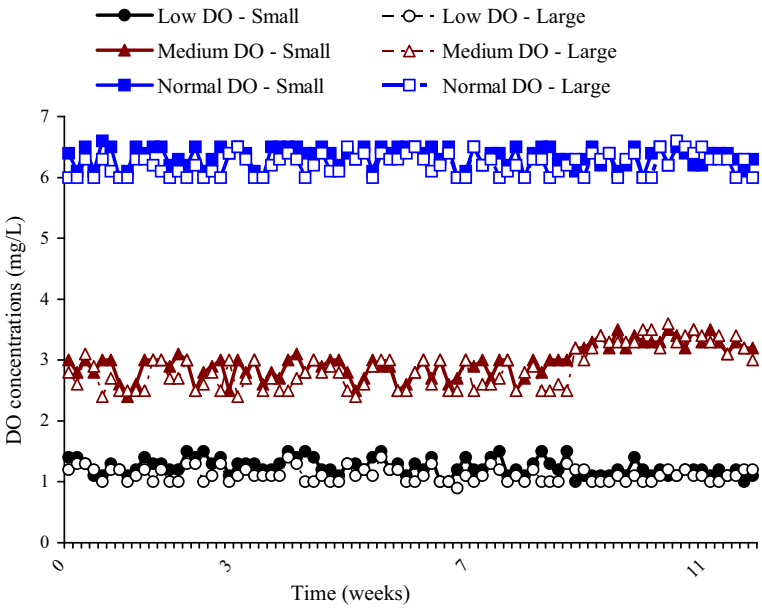

Fig. 2 Changes in dissolved oxygen (DO) concentrations (mg/L) of water aquaria stocked with Nile tilapia as affected by different levels of dissolved oxygen and fish size for 12 weeks. Low DO = 1.0–1.5 mg/L, medium DO = 2.5–3.0 mg/L, and normal DO = 6.0–6.5 mg/L. Small = 3.7 g and large = 12.9 g

concentrations ranged from 0.05 to 0.11 mg/L (Table 1). Total alkalinity and total hardness ranges were 106–136 and 252–258 mg/L as $CaCO_3$, respectively. All the previous water quality parameters are within the acceptable range for fish growth (Boyd 1984).

Growth performance

Fish growth and feed utilization were calculated as follows:

Weight gain (g) = final weight (g) − initial weight (g);
Weight gain % = 100 [final weight (g) − initial weight (g)]/initial weight (g);
Specific growth rate (SGR; 5 %/day) = 100 [Ln final weight (g) − Ln initial weight (g)]/the experimental period (day);
Feed conversion ratio (FCR) = feed intake/weight gain.

Proximate chemical analysis

The proximate chemical analyses of whole fish body were conducted for moisture, crude protein, total lipids, and total ash according to the standard methods of AOAC (1990). Moisture content was estimated by drying the samples to a constant weight at 85 °C in a drying oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA). Nitrogen content was measured using a micro-Kjeldahl apparatus (Labconco, Labconco Corporation, Kansas, Missouri, USA). Total lipid content was determined by ether extraction using a multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 h. Total ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 °C for 6 h.

Table 1 Water quality parameters of aquaria stocked by Nile tilapia as affected by different levels of dissolved oxygen and fish size for 12 weeks

Dissolved oxygen (DO)	Fish size	Temperature (°C)	pH	Unionized ammonia (mg/L)	Conductivity (μs/cm)	Total alkalinity (mg/L)	Total hardness (mg/L)
Low	Small	26.6 ± 0.17	7.6 ± 0.03	0.11 ± 0.045	369.0 ± 5.03	120.0 ± 20.00	252.0 ± 8.00
Low	Large	27.3 ± 0.25	7.6 ± 0.00	0.09 ± 0.02	366.0 ± 14.00	123.3 ± 8.82	253.3 ± 6.67
Medium	Small	27.3 ± 0.07	7.7 ± 0.06	0.11 ± 0.013	375.3 ± 11.57	126.7 ± 12.02	252.0 ± 4.16
Medium	Large	26.5 ± 0.07	7.7 ± 0.03	0.10 ± 0.021	378.7 ± 8.88	136.7 ± 16.67	253.3 ± 2.67
Normal	Small	26.5 ± 0.13	7.7 ± 0.00	0.11 ± 0.014	373.3 ± 11.02	133.3 ± 6.67	258.0 ± 1.16
Normal	Large	26.4 ± 0.15	7.7 ± 0.06	0.08 ± 0.011	378.0 ± 3.00	116.7 ± 8.82	254.7 ± 2.91
Two-way ANOVA		*P* value					
DO		0.050	0.088	0.414	0.578	0.583	0.634
Fish size		0.739	1.000	0.213	0.829	0.664	0.952
DO × fish size		0.799	0.742	0.233	0.913	0.295	0.828

Means having the same letters in the same column are not significantly different at $P < 0.05$

Low DO = 1.0–1.5 mg/L, medium DO = 2.53.0 mg/L, and normal DO = 6.0–6.5 mg/L

Small = 3.7 g and large = 12.9 g

Innate immunity analysis

The production of oxygen radicals by leukocytes was assayed by the reduction of nitro blue tetrazolium (NBT, Sigma-Aldrich, St. Louis, MO, USA) according to Rook et al. (1985). Absorbance was converted to NBT units based on a standard curve of NBT diformazan per milliliter of blood. Lysozyme activity of fish plasma was determined by turbidometric assays as described by Caruso et al. (2002).

Bacterial challenge test

At the end of the feeding trial, fish at each treatment were randomly divided into two aquaria; 10 fish per 90-L aquaria and were fed a 35 % CP diet up to satiation for 10 days. The challenge experiment was carried out using the pathogenic strain *A. hydrophila* isolated previously in the laboratory of Fish Disease Department, CLAR, Abbassa, Abo-Hammad, Sharqia, Egypt. The first subgroup was challenged with pathogenic *A. hydrophila* using a sublethal dose as described by Reyes-Becerril et al. (2011) where a 0.1-ml dose of 24-h broth from virulent *A. hydrophila* (5×10^5 CFU/mL) was injected interperitoneally (IP). The second subgroup was IP injected with 0.1 ml of saline solution as a control. Two replicates were carried out for each treatment. All subgroups were kept under observation for 10 days to record any abnormal clinical signs and the daily fish mortality.

Statistical analysis

The obtained data were subjected to two-way ANOVA to test the effects of DO concentration and FS for experiment as the two factors simultaneously tested. The Duncan's multiple range test was used as a post hoc test to compare between means at $P \leq 0.05$. The software SPSS version 11.0 (SPSS, Richmond, Virginia, USA) was used as described by Dytham (1999).

Results

Fish growth and feed utilization were significantly affected by DO, FS, and their interaction ($P < 0.05$, Table 2). Fish in all treatments grew gradually as a function of time up to the end of the experiment. Final fish weights in NDO groups were significantly higher than those in LDO and MDO groups ($P < 0.05$). Larger fish showed high final weight and weight gain ($P < 0.05$) as compared with smaller ones; meanwhile, small fish grow faster showing high weight gain % and SGR. In addition, the highest feed intake and the lowest FCR were obtained at NDO at both fish sizes. It was noticed that at LDO, fish consumed low feed (18.5 and 28.4 g feed/fish for smaller and larger fish, respectively). Additionally, the highest FCR was observed at LDO groups (2.01 and 2.22 for smaller and larger fish, respectively). Smaller fish exhibited less FCR values than larger ones. On the other hand, no effect of DO and FS was observed on fish survival ($P > 0.05$), and its range was 96.7–100 % (Table 2).

All fish body constituents were significantly affected by DO, FS, and their interaction except moisture content, which is significantly affected by fish size only ($P < 0.05$, Table 3). Crude protein and total lipids in whole fish body decreased significantly ($P < 0.05$) with decreasing DO levels. Meanwhile, both variables in larger fish were higher than those in smaller one. The highest contents of crude protein (62.6 and 62.0 %) and

Table 2 Growth performance and feed utilization of Nile tilapia as affected by different levels of dissolved oxygen and fish size for 12 weeks ($n = 4$)

Dissolved oxygen (DO)	Fish size	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain %	SGR (% g/day)	Feed intake (g feed/fish)	FCR	Survival (%)
Low	Small	3.7 ± 0.058 b	12.9 ± 0.058 f	9.2 ± 0.115 e	248.6 ± 7.01 c	1.49 ± 0.024 c	18.5 ± 0.219 e	2.01 ± 0.016 b	98.9 ± 1.11
Low	Large	12.8 ± 0.033 a	25.6 ± 0.433 c	12.8 ± 0.404 d	100.0 ± 2.92 f	0.82 ± 0.017 f	28.4 ± 0.493 c	2.22 ± 0.032 a	96.7 ± 3.33
Medium	Small	3.7 ± 0.033 b	16.9 ± 0.173 e	13.2 ± 0.145 d	356.8 ± 2.34 b	1.80 ± 0.006 b	18.9 ± 0.285 e	1.44 ± 0.011 d	96.7 ± 1.92
Medium	Large	12.9 ± 0.058 a	33.5 ± 0.462 b	20.6 ± 0.436 b	159.7 ± 3.16 e	1.14 ± 0.015 e	31.8 ± 0.395 b	1.54 ± 0.046 c	100.0 ± 0.00
Normal	Small	3.7 ± 0.033 b	21.8 ± 0.493 d	18.1 ± 0.467 c	489.2 ± 9.52 a	2.10 ± 0.020 a	21.8 ± 0.788 d	1.20 ± 0.016 e	98.9 ± 1.11
Normal	Large	12.9 ± 0.033 a	37.4 ± 0.635 a	24.5 ± 0.664 a	189.9 ± 5.61 d	1.27 ± 0.023 d	33.5 ± 0.203 a	1.37 ± 0.034 d	100.0 ± 0.00
Two-way ANOVA		*P* value							
DO		0.516	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.619
Fish size		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.603
DO × fish size		0.906	0.002	0.002	0.001	0.001	0.022	0.021	0.294

Means having the same letters in the same column are not significantly different at $P < 0.05$

Low DO = 1.0–1.5 mg/L, medium DO = 2.5–3.0 mg/L, and normal DO = 6.0–6.5 mg/L

Small = 3.7 g and large = 12.9 g

Table 3 Proximate chemical analysis (%; on dry matter basis) of Nile tilapia as affected by different levels of dissolved oxygen and fish size for 12 weeks ($n = 4$)

Dissolved oxygen (DO)	Fish size	Moisture	Crude protein	Total lipid	Total ash
Low	Small	75.8 ± 0.40 a	60.0 ± 0.35 c	15.5 ± 0.40 d	23.4 ± 0.58 a
Low	Large	74.1 ± 0.21 bc	61.5 ± 0.12 b	17.6 ± 0.07 b	19.3 ± 0.16 b
Medium	Small	75.5 ± 0.20 ab	60.7 ± 0.15 c	16.5 ± 0.23c	21.0 ± 0.41 cd
Medium	Large	73.9 ± 0.21 bc	62.0 ± 0.15 ab	18.1 ± 0.15 b	18.4 ± 0.12 d
Normal	Small	74.7 ± 0.50 abc	61.7 ± 0.23 b	16.8 ± 0.26 c	20.0 ± 0.45 bc
Normal	Large	73.3 ± 1.02 c	62.6 ± 0.15 a	18.9 ± 0.12 a	17.1 ± 0.12 e
Two-way ANOVA		*P* value			
DO		0.327	0.0001	0.005	0.0001
Fish size		0.004	0.003	0.0001	0.0001
DO × fish size		0.798	0.0001	0.003	0.001

Means having the same letters in the same column are not significantly different at $P < 0.05$

Low DO = 1.0–1.5 mg/L, medium DO = 2.5–3.0 mg/L, and normal DO = 6.0–6.5 mg/L

Small = 3.7 g and large = 12.9 g

lipids (18.9 and 18.1 %) were observed in larger fish reared at NDO and MDO, respectively. Additionally, total ash contents decreased significantly ($P < 0.05$) by increasing DO level and increasing FS, and the lowest ash content was obtained with larger fish reared at NDO (17.1 %).

The NBT value and lysozyme activity at NDO in larger fish were highest (0.233 mg/mL and 16.7 unite/mg protein, respectively); meanwhile, their lowest values were obtained at LDO in smaller one (0.156 mg/L and 13.3 unite/mg protein, respectively). Moreover, the cumulative fish mortality post-challenge with *A. hydrophila* increased significantly with time in all treatments up to the 4th day after which no mortalities were observed (Fig. 3). The total fish mortality after 10 days post-challenge was adversely affected by DO, and the highest mortality was observed at LDO in smaller fish (55 %), whereas no mortality was observed at NDO in larger fish (0 %; Table 4).

Discussion

In the current study, low DO level adversely affected fish growth and feed utilization. The low growth obtained at low DO conditions could be explained by the shortage in oxygen availability for fish growth. In this regard, Bergheim et al. (2006) and Duan et al. (2011) reported that fish growth and feed efficiency were affected by DO availability, and fish always showed good feed efficiency when fed at enough DO in water. Abdel-Tawwab et al. (2014) reported that growth of Nile tilapia was significantly retarded at low DO level. The low feed intake and low growth observed in fish at low DO conditions were because fish appetite and digestibility was reduced (Tran-Duy et al. 2012; Gan et al. 2013). Thus, it could be concluded that high growth under normal DO conditions resulted mainly from better feed consumption and nutrient digestibility. Similar results have been obtained from channel catfish, *Ictalurus punctatus* (Buentello et al. 2000); spotted wolfish, *Anarhichas minor* (Foss et al. 2002); Nile tilapia, *O. niloticus* (Tran-Duy et al. 2008, 2012; Abdel-Tawwab et al. 2014); striped bass, *Morone saxatilis* (Brandt et al. 2009); Atlantic halibut,

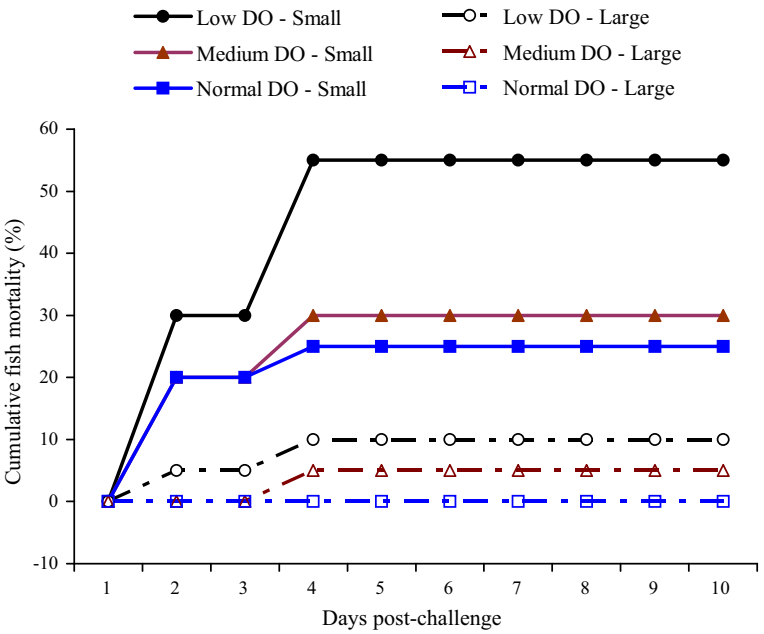

Fig. 3 Cumulative mortality (%) of Nile tilapia as affected by different levels of dissolved oxygen and fish size for 12 weeks. Low DO = 1.0–1.5 mg/L, medium DO = 2.5–3.0 mg/L, and normal DO = 6.0–6.5 mg/L. Small = 3.7 g and large = 12.9 g

Table 4 Immunological parameters of Nile tilapia as affected by different levels of dissolved oxygen and fish size for 12 weeks

Dissolved oxygen (DO)	Fish size	NBT (mg/mL)	Lysozyme (unit/mg protein)	Post-challenge fish mortality (%)
Low	Small	0.156 ± 0.001 d	13.3 ± 0.11 d	55.0 ± 5.0 a
Low	Large	0.163 ± 0.001 d	13.9 ± 0.25 bc	10.0 ± 0.0 c
Medium	Small	0.191 ± 0.001 c	13.6 ± 0.19 cd	30.0 ± 10.0 b
Medium	Large	0.201 ± 0.01 bc	14.4 ± 0.18 b	5.0 ± 0.0 c
Normal	Small	0.216 ± 0.01 b	14.2 ± 0.13 bc	25.0 ± 5.0 b
Normal	Large	0.233 ± 0.001 a	16.7 ± 0.20 a	0.0 ± 0.0 c
Two-way ANOVA		*P* value		
DO		0.0001	0.0001	0.017
Fish size		0.022	0.0001	0.0001
DO × fish size		0.640	0.0001	0.148

Means having the same letters in the same column are not significantly different at $P < 0.05$

Low DO = 1.0–1.5 mg/L, medium DO = 2.5–3.0 mg/L, and normal DO = 6.0–6.5 mg/L

Small = 3.7 g and large = 12.9 g

Hippoglossus hippoglossus (Thorarensen et al. 2010); Japanese flounder, *Paralichthys olivaceus* (Duan et al. (2011); and grass carp, *Ctenopharyngodon idella* (Gan et al. 2013); all these fish experienced reduced feed intake and growth under hypoxic conditions.

The obtained results herein demonstrate that, at normal DO, smaller fish consumed low diet and grew better than that of larger ones. Similarly, Tran-Duy et al. (2008) and Abdel-Tawwab et al. (2010) found that growth of small Nile tilapia was significantly higher than of large fish. The changes in fish growth and feed consumption due to changes in fish size/weight have been postulated by a number of authors. Booth et al. (2008) reported that the feed intake and growth of Australian snapper, *Pagrus auratus,* were significantly affected by fish size. Handeland et al. (2008) found that growth rate, feed intake, feed conversion efficiency, and stomach evacuation rate of Atlantic salmon (*Salmo salar*) were significantly influenced by fish size. Sun and Chen (2014) found that feed consumption, fecal production, nitrogenous excretion, growth rate, and metabolic rate of cobia, *Rachycentron canadum,* were affected significantly by fish size. They also reported that larger cobia had a superior capacity of feed utilization, and energy budgets of cobia were also influenced significantly by fish size.

It is also noticed that larger fish tolerated low DO better than the small ones. These results agreed with Almeida-Val et al. (2000) and Sloman et al. (2006) who found that in the osacar cichlid (*Astronotus ocellatus*) of the Amazon river, small individuals are significantly less hypoxia-tolerant than larger ones. Consequently, they suggested that the relatively low metabolic rate of larger fish could in part explain their higher degree of hypoxia tolerance. Further, fish ability to take up oxygen in hypoxic conditions may be influenced by body size (Nilsson and Östlund-Nilsson 2008).

It is noted that fish survival was not affected by DO, and its range was 96.7–100 %. The low fish mortality confirmed the high tolerance, although fish in LDO groups were sometimes observed performing air gulping, which is a common behavior among tropical freshwater fish. This result may be because Nile tilapia is able to tolerate DO at concentration as low as 1.0 mg/L; below this level, fish may be able to utilize atmospheric oxygen (Ross 2000). Reports on incipient DO concentrations for growth of Nile tilapia showed a range from less than 0.8 to 3 mg/L (Teichert-Coddington and Green 1993).

Fish body composition was affected by many aspects of fish biology and ecology, and it is of special interest in aquaculture because it influences fish appetite, growth, and the efficiency of food utilization. Thus, differences in fish body composition are associated with differences in fish appetite (Bull and Metcalfe 1997; Jobling and Miglavs 1993) and growth (Broekhuizen et al. 1994; Shearer et al. 1997). The present study showed decreased protein and lipid contents in fish body at LDO fish groups. That may be due to the increased energy needed for metabolism to cope with hypoxia stress. In order to adapt to low DO conditions and to maintain overall metabolism, lipid and protein may be used as an energy metabolic substrate causing a depression in lipid and protein contents. On the other hand, increased protein and lipid contents at NDO groups could be explained by lower energy requirements for feed assimilation and different physiological functions. Fish at normal DO concentrations appear to have the ability to decrease the proportions of metabolic energy and energy loss, thus increasing the proportion allocated to growth and lipogenesis (Duan et al. 2011). In this concern, Smith et al. (1996) found that when Crucian carp were exposed to 48-h anoxia, there was more than a 56 % reduction in protein synthesis rate in liver, 52 % in red muscle, and 56 % in white muscle. Gan et al. (2013) reported that protein synthesis of grass carp was inhibited by low DO.

It was noticed that protein and lipid contents in fish body were significantly affected by fish size where they were lower in small fish than large ones. Several scientific workers have found significant relationships between body composition and body weight (Shearer 1994; Huuskonen et al. 1998; Brigolin et al. 2010). The obtained results may be due to the variation in feed consumed, growth, and physiological functions, which were size-

dependent. The different body composition in different fish sizes was also observed for Nile tilapia (Abdel-Tawwab et al. 2010), for feather back fish (*Notopterus notopterus*; Naeem et al. 2011), and for Northern pike (*Esox lucius*; Salam and Davies 1994). Furthermore, changes in protein and lipid contents in fish body could be linked to changes in their synthesis and/or deposition rate in muscles (Fauconneau 1984; Abdel-Tawwab et al. 2006).

The innate immunity of Nile tilapia reared at different levels of DO and FS was examined by evaluating its resistance to pathogenic bacteria, *A. hydrophila*, NBT, and lysozyme activity. It is known that activities of NBT and lysozyme have important roles in nonspecific immune defense system (Grinde 1989; Ellis 1990). It is noticed that fish resistance to pathogenic *A. hydrophila* infection, NBT, and lysozyme values decreased as DO level decreased, and the innate immunity of smaller fish is less than the larger one. The reduced fish immunity at LDO conditions may be because the innate and adaptive immune responses in fish have been modulated by DO (Cecchini and Saroglia 2002; Ortuno et al. 2002; Cuesta et al. 2003). Fukuda et al. (1997) stated that low DO shortened the incubation period for infection and increased the cumulative mortality in yellowtail jacks, *Seriola lalandi*, challenged with *Enterococcus seriolicida*. Evans et al. (2003) observed that mortality rates increased significantly in Nile tilapia when challenged with *Streptococcus agalactiae* following exposure to low DO. Welker et al. (2007) investigated the effect of sublethal DO exposure on stress and immune responses and susceptibility to *Edwardsiella ictaluri* infection in juvenile channel catfish. They found that total hemolytic complement, bactericidal activities, and antibody response were lower in LDO-exposed channel catfish, indicating that increased susceptibility of channel catfish to *E. ictaluri* may be the result of the immunosuppressive effects of the stress response to LDO. Abdel-Tawwab et al. (2014) found that innate immunity is DO-dependent and fish mortality due to *A. hydrophila* infection was higher at LDO. Further, Cecchini and Saroglia (2002) demonstrated that antibody responses against human γ-gamma globulin in hypoxic European sea bass, *Dicentrarchus labrax*, were weaker than those in hyperoxic European sea bass. Air exposure-induced LDO reduced the respiratory burst in gilthead sea bream, *Sparus auratus* (Ortuno et al. 2002).

It is noticed that fish immunity was higher in larger fish than smaller one, suggesting that fish weight and/or age may be a major factor affecting the bacterial infection and innate immunity in farmed fish. Many studies have described individual variability in disease susceptibility in fish associated with weight and age (Suanyuk et al. 2008; Mian et al. 2009; Zamri-Saad et al. 2010), fish species (Yuasa et al. 1999; Evans et al. 2000), genetic variation, and immune response (Sarder et al. 2001). Similarly, the different coping styles in individual fish may influence the fish and their susceptibility to infection (MacKenzie et al. 2009; Huntingford et al. 2010).

In conclusion, DO and fish size are known to influence fish growth, feed utilization, and innate immunity. Fish should be maintained at adequate DO level to satisfy the functions responsible for improving fish performance and health.

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