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Effects of oxygen concentration and body weight on maximum feed intake, growth and hematological parameters of Nile tilapia, *Oreochromis niloticus*

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Abstract

Feed intake and satiation in fish are regulated by a number of factors, of which dissolved oxygen concentration (DO) is important. Since fish take up oxygen through the limited gill surface area, all processes that need energy, including food processing, depend on their maximum oxygen uptake capacity. Maximum oxygen uptake capacity relative to body weight in bigger fish is smaller than in smaller fish because the gill surface area is allometrically related to body weight. In this study, effects of DO concentration and body weight on maximum feed intake, growth and hematological parameters of Nile tilapia (*Oreochromis niloticus*) were investigated. Two weight classes of fish (21 g and 147 g) were used. For each class, six tanks were employed of which half were exposed to one of two DO levels (about 3.0 mg/L and 5.6 mg/L). Fish were fed to apparent satiation twice per day with a commercial diet. The results showed that (1) feed intake and growth of the fish at high DO level were significantly higher than at low DO level ($P<0.01$), (2) relative feed intake and growth of small fish were significantly higher than of big fish ($P<0.001$), and (3) fish at low DO level made no hematological adjustments ($P\geq 0.5$). Data suggest that (1) the limitation of the gill surface area results in lower feed intake and growth of fish at low DO concentration than at high DO concentration and (2) the allometric relationship between the gill surface area and body weight results in lower relative feed intake, which in turn results in lower relative growth in big fish than in small fish.

Keywords: Feed intake; Dissolved oxygen; Body size; Hematology; Tilapia; *Oreochromis niloticus*

1. Introduction

Growth prediction, which is crucial for planning of fish production, requires information about feed intake since growth increases with increasing ration (Brett and Groves, 1979; Yoshida and Sakurai, 1984; Klaoudatos and Apostolopoulos, 1986; Cui et al., 1994; Xie et al., 1997). Under *ad libitum* feeding, feed intake prediction still remains a great challenge (Machiels, 1987; van Dam and Pauly, 1995).

Feed intake and satiation in fish are regulated by physiological, social or environmental factors, or by the interaction among them. Despite the recognition of environmental effects on feed intake, dissolved oxygen (DO) concentration has not been investigated systematically as a factor determining feed intake. Fish, like terrestrial animals, need oxygen for maintenance, locomotion, feeding and biosynthesis. In terrestrial animals, oxygen supply from air is hardly ever limiting. Oxygen concentration in water, however, can be 30 times less than in the air. Moreover, oxygen is taken up by fish through the gill surface area, which is limited. Thus, all the processes that need energy, including food processing, depend on maximum oxygen uptake capacity of fish. Pauly (1981) hypothesized that fish stop eating when oxygen supply does not satisfy oxygen demand. He also assumed that maximum oxygen uptake capacity per unit body weight is smaller in big fish than in small fish because gill surface area is allometrically related to body weight, and that therefore maximum feed intake per unit body weight is smaller in big fish than in small fish.

Little experimental work has been done to determine maximum feed intake in fish in relation to DO concentration and body weight. As part of an effort to develop a quantitative model for prediction of fish growth in relation to food quantity and composition and environmental factors (van Dam and

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Pauly, 1995; Tran-Duy et al., 2005), we found fewer than a dozen reports in the literature on experimental work directed specifically at the effect of DO on feed intake, of which more than half lacked replication of treatments or statistical analysis of the results. Herrmann et al. (1962) found that food consumption of juvenile coho salmon (*Oncorhynchus kisutch*) within the weight range of 2–15 g remarkably decreased when DO was reduced from 8 mg L^{-1} to lower than 5 mg L^{-1}. According to Tsadik and Kutty (1987), feed ingestion of Nile tilapia (*Oreochromis niloticus*) of 8.1 g decreased 40% when ambient DO fell from 7.0 mg L^{-1} to 1.5 mg L^{-1}. Systematic studies were done on only a few species, namely turbot (*Scophthalmus maximus*) (Pichavant et al., 2000; Pichavant et al., 2001), channel catfish (*Ictalurus punctatus*) (Buentello et al., 2000) and European sea bass (*Dicentrarchus labrax*) (Thetmeyer et al., 1999; Pichavant et al., 2001).

Under hypoxia, fish can utilize several physiological mechanisms to compensate a reduction in oxygen uptake. For instance, carp (*Cyprinus carpio*) can increase breathing frequency (Lomholt and Johansen, 1979; Glass et al., 1990) and trout (*Oncorhynchus mykiss*) can increase hemoglobin concentration (Soivio et al., 1980). No studies have been done to investigate effect of low DO concentration on hematological parameters of Nile tilapia. Our main objectives were (1) to determine whether maximum feed intake and growth of fish of the same size at low DO concentration are significantly lower than at high DO concentration; (2) to establish a quantitative dataset documenting the effect of DO and body weight on the maximum feed intake of Nile tilapia; and (3) to determine whether Nile tilapia make hematological adjustments at low DO concentrations.

2. Materials and methods

2.1. Fish and rearing conditions

This experiment was conducted at the Faculty of Aquaculture and Fisheries of the Hue University of Agriculture and Forestry in Vietnam. All male tilapia were obtained from a local producer of fingerlings (Cu Chanh fish farm, Huong Thuy, Thua Thien, Vietnam). To obtain two weight classes of fish, fingerlings were purchased at two different dates and reared in 120-L rectangular glass tanks until the start of the experiment. The first group of fish arrived at the university on 7 April 2006 and were kept in six tanks at a density of 134 fish $tank^{-1}$ with an average weight (±SE) of 9.2±0.05 g. The second group arrived on 6 June 2006 and were kept in six other tanks at a density of 117 fish $tank^{-1}$ with an average weight (±SE) of 15.3±0.1 g. All the tanks were connected to a recirculation system equipped with a sedimentation tank (1.6 m^3), a trickling biofilter (2.8 m^3), a sump (2.0 m^3) and pumps. From arrival to the start of the experiment on 20 June 2006 (75 and 14 days for the first and second group, respectively), fish were fed manually twice per day (starting at 8:00 and 16:00) with a commercial diet (Charoen Pokphand floating pellets, 2.5 mm: 17.9 kJ g^{-1}, 94.5% dry matter, 32.0% crude protein, 4.8% crude fat and 10.8% ash on a wet weight basis) using a feeding level of 18 g $kg^{-0.8}$ d^{-1}. During this acclimation period, water flow rate through the tanks was maintained at 6 L min^{-1}, photoperiod at 12 L:12D, pH between 6.92 and 7.86, NO_2–N below 1 mg L^{-1}, NH_3–N below 0.1 mg L^{-1}, water temperature (mean±SD) at 32.3±0.6 °C and DO concentration above 5 mg L^{-1}.

2.2. Experimental procedures

The experiment was conducted as a randomized complete block design with three blocks and four treatment combinations within each block and each tank as an experimental unit. Blocks were used because the tanks were installed linearly in one row with one end more exposed to people passing by and occasional daylight than the other. The four treatment combinations comprised two fish weight classes (small and big) and two DO levels (low and high) which were arranged in a 2×2 factorial: small fish–low DO (SL), small fish–high DO (SH), big fish–low DO (BL) and big fish–high DO (BH). The small and big fish were those obtained on 6 June 2006 and 7 April 2006 (see 2.1), with average weights (±SE) at the end of the acclimation period of 20.00±0.33 and 140.87±1.80 g, respectively. Dissolved oxygen concentrations below 3.5 mg L^{-1} were considered low and above 5.0 mg L^{-1} were considered high.

The same tanks used during the acclimation period were used for the experimental period. After the acclimation period, all fish were taken out of the tanks with the small and big fish separate in different basins. Then, fish were anesthetized with benzocaine (standing stock: 100 g benzocaine dissolved in 1 L ethanol 99.8%; dose: 100 mg benzocaine L^{-1} water), weighed individually and randomly allocated to the tanks corresponding to the weight classes (big fish treatment: 9 fish $tank^{-1}$; small fish treatment: 50 fish $tank^{-1}$). Average initial weights (±SE) of the fish in SL, SH, BL and BH treatments were 21.65±0.71, 21.18±0.15, 148.41±3.38 and 144.74±1.56 g, corresponding to stocking densities (±SE) of 9.01±0.29, 8.80±0.06, 11.13±0.23 and 10.90±0.12 kg m^{-3}, respectively.

During fish allocation, 20 fish from each weight class were randomly sampled for hematological parameters and body composition analysis. One ml of blood was collected from the caudal veins of each fish using a heparinized hypodermic syringe. Directly after collection, each blood sample was placed in a cool 2 ml glass tube containing 3 mg Na_2EDTA, gently mixed and immediately stored at −20 °C for further analysis. After blood sampling, all fish were killed with an overdose of benzocaine (400 mg L^{-1}), cut into small pieces and ground twice using a meat grinder. Three hundred grams of the fish homogenate from each weight class were placed in a tight plastic bottle and immediately stored at −20 °C for further analysis.

Oxygen levels inside the tanks were established by means of aeration and water flow rates through the tanks. Each tank assigned to the high DO level was aerated with two airstones at two diagonally opposite corners. Tanks assigned to the low DO level were not aerated. On the day the fish were allocated to the tanks (20 June), water flow rates were set at 6.6 L min^{-1}. Then, the flow rates in the tanks assigned to the low DO level were gradually reduced to 3.6 L min^{-1} within the next five days (21 to 25 June). As the DO concentrations in the low DO tanks with big fish were about 1 mg L^{-1} higher than in the low DO tanks with small fish between 26 and 30 June, water flow rates in the low DO tanks with big fish were gradually reduced on 1 and 2 July to 2.5 L min^{-1}, at which they were maintained until the end of the experiment. In this way, DO concentrations in the tanks with big and small fish were comparable.

Dissolved oxygen concentrations and temperature at the inlet, inside (central point) and outlet of each tank were measured six times per day, at around 7:30, 9:30, 11:30, 14:00, 15:30 and 17:30 using oxygen meters (WTW 340i; Wissenschaftlich-Technische Werkstätten GmbH, Germany) with membrane-covered galvanic sensors (CellOx® 325). On 30 June, 6 July, 12 July and 18 July, DO concentrations were also measured every 30 min from 8:00 to 9:00 and from 16:00 to 17:00, and every hour from 9:30 to 15:30 and from 18:30 to 23:30; and every 2 h from 0:30 to 6:30 the next day to assess the diurnal variation in DO concentration. The starting day for these intense DO measurements was selected based on the observation that on that day (four days after the *ad libitum* feeding started; see the following paragraph) feed intake of the fish became stable, i.e. the fish had adapted to the *ad libitum* feeding regime. After the starting day, weekly intervals were selected for the intense DO measurements assuming that fish growth would not affect the diurnal pattern of DO variations within a period of less than one week. Total ammonia ($NH_3+NH_4^+$), nitrite and nitrate concentrations were measured twice per day (9:00 and 17:00) at the inlet and outlet of each tank using test kits (Aquamerck; Merck); pH was also measured at the same time and position using a pH meter (MA 130; Mettler Toledo, USA).

Fish were fed manually twice per day (starting at 8:00 and 16:00) with the same diet as used during the acclimation period; each meal lasted for 1 h. From 21 to 25 June, fish were allowed to adapt to new oxygen conditions and were fed at a feeding level of 8 g $kg^{-0.8}$ d^{-1}. From 26 June to 20 July, fish were fed to apparent satiation.

At the end of the experimental period (21 July), fish from each tank were anesthetized with benzocaine (same dose as used for fish allocation) and

Table 1
Mean dissolved oxygen (DO) concentrations (mg L^{-1}) over the *ad libitum* feeding period under the four treatment combinations [a]

	Treatment combination [b]				*P*-value		
	SL	SH	BL	BH	Size	Oxygen	Size × oxygen
Inside tanks	2.78±0.03	5.46±0.06	3.19±0.01	5.81±0.06	<0.001	<0.001	0.539
Outlet of tanks	2.61±0.02	5.28±0.04	2.99±0.01	5.71±0.07	<0.001	<0.001	0.545

[a] Values represent means±SE of three replicates (tanks); each replicate value is the mean of all the data points measured on one tank during the *ad libitum* feeding period.
[b] SL=Small fish–Low DO; SH=Small fish–High DO; BL=Big fish–Low DO; BH=Big fish–High DO. Small and big fish were Nile tilapia *(Oreochromis niloticus*) with individual weights in the range of 21–66 g and of 144–251 g, respectively.

weighed individually. Six fish from each tank were randomly selected for hematological parameter analysis. For each tank with small fish, 10 fish were randomly selected for body composition analysis. For each tank with big fish, all nine fish were used for body composition analysis. Procedures for sample preparation were the same as at the start of the experimental period.

2.3. Analytical procedures

Dry matter content was determined as weight loss after drying the samples for 4–6 h at 103 °C until constant weight (ISO, 1983). Crude protein content was determined using the Kjeldahl method and multiplying nitrogen content by 6.25 (ISO, 1997). Crude fat content was determined after petroleum-ether extraction using a Soxhlett system (ISO, 1999).

Hematological parameters for blood samples analysis were red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). These values were determined using an automated hematology analyzer (XT-1800i; Sysmex Coporation, Japan).

2.4. Data analysis

The present study assessed the maximum feed intake of Nile tilapia in relation to DO level and body weight of the fish. Since fish were fed to satiation from day 6 till day 30 of the experiment (*ad libitum* feeding period, ALFP), all the performance parameters (feed intake, growth rates and FCR) were calculated for this period. Specific growth rates (SGR) were calculated using the following formula (according to Jobling, 1994, chapter nine):

$$\mathrm{SGR}\left(\%\ \mathrm{d}^{-1}\right) = \left(\left(\ln W_{30} - \ln W_6\right)/25\right) \times 100$$

where W_6 and W_{30} are the average fish weight (g) on the day *ad libitum* feeding started (day 6) and at the end of the experimental period (day 30), respectively.

W_6 was estimated based on the average fish weight W_1 (g) on the day the fish were allocated to the tanks (day 1), daily feed intake (g) during the first five days after fish allocation and the overall feed conversion ratio (FCR), which was calculated according to Parker (1987) as

$$\mathrm{FCR} = \mathrm{FI}_{\mathrm{tot}}/\left(W_{30} - W_1\right)$$

where $\mathrm{FI}_{\mathrm{tot}}$ (g) is the total feed intake per fish during the experimental period (day 1 till day 30). This estimation of W_6 was based on the assumption that feed conversion ratios during the first five days and during the ALFP (and therefore during the experimental period) were the same. Growth rates per metabolic weight unit ($\mathrm{GR}_{\mathrm{MBW}}$) were calculated according to Martins et al. (2005) as

$$\mathrm{GR}_{\mathrm{MBW}}\left(\mathrm{g\ kg}^{-0.8}\ \mathrm{d}^{-1}\right) = \left(W_{30} - W_6\right)/\left(W_{\mathrm{mean}}/1000\right)^{0.8}/25$$

where W_{mean} is the geometric mean body weight (g), which was calculated as

$$W_{\mathrm{mean}}(\mathrm{g}) = \sqrt{W_6 \times W_{30}}$$

Feed intake of the fish expressed as a percentage of body weight ($\mathrm{FI}_{\mathrm{perc}}$) and per metabolic weight unit ($\mathrm{FI}_{\mathrm{MBW}}$) were calculated as

$$\mathrm{FI}_{\mathrm{perc}}\left(\%\ \mathrm{d}^{-1}\right) = \mathrm{FI}/W_{\mathrm{mean}} \times 100$$

and

$$\mathrm{FI}_{\mathrm{MBW}}\left(\mathrm{g\ kg}^{-0.8}\ \mathrm{d}^{-1}\right) = \mathrm{FI}/\left(W_{\mathrm{mean}}/1000\right)^{0.8}$$

where FI (g) is the average feed intake per fish over the ALFP per meal or per day (assuming that fish weight is constant from 8:00 to 17:00).

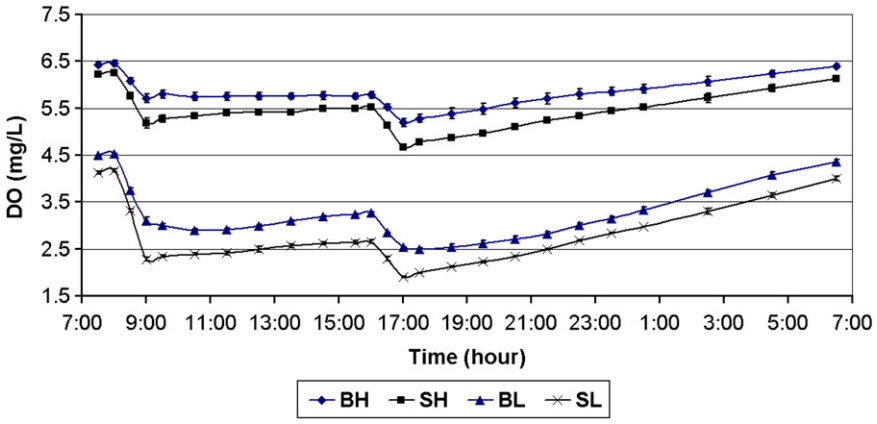

Fig. 1. Diurnal variations of dissolved oxygen (DO) concentrations under the four treatment combinations. Data points represent means of three replicate values; each replicate value is the mean of four data points measured at the same time in four 24-hour periods. Vertical bars represent two times the standard errors. BH=Big fish–High DO; SH=Small fish–High DO; BL=Big fish–Low DO; SL=Small fish–Low DO. Big and small fish were Nile tilapia (*Oreochromis niloticus*) with individual weight in the range of 144–251 g and of 21–66 g, respectively.

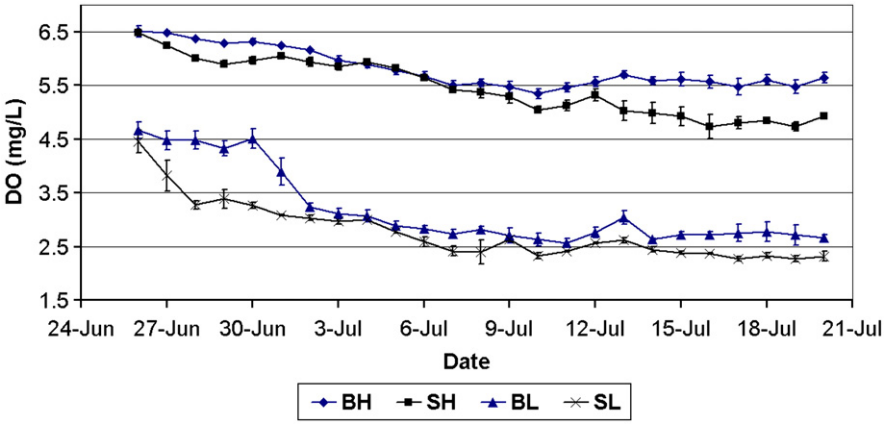

Fig. 2. Variations in dissolved oxygen (DO) concentrations throughout the *ad libitum* feeding period under four treatment combinations. Data points represent means of three replicate values; each replicate value is the mean of six data points measured during each day. Vertical bars represent two times the standard errors. BH = Big fish–High DO; SH = Small fish–High DO; BL = Big fish–Low DO; SL = Small fish–Low DO. Big and small fish were Nile tilapia (*Oreochromis niloticus*) with individual weights in the range of 144–251 g and of 21–66 g, respectively.

Un-ionized ammonia (NH_3) concentrations were calculated according to Albert (1973) as

$$NH_3 = \left[NH_3 + NH_4^+\right] / \left[1 + 10^{(pKa - pH)}\right]$$

where pKa was calculated based on the equation developed by Emerson et al. (1975) as

$$pKa = 0.09018 + 2729.92/(T + 273)$$

where T is the water temperature (°C).

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc.). The homogeneity of variances of different groups was checked using Levene F test with PROC ANOVA. Since the homogeneity assumption was met for all the observed variables ($P > 0.05$), mean values of DO concentration, growth, feed intake, body composition and hematological parameters were subjected to two-way analysis of variance (ANOVA) using Proc GLM according to the following model:

$$Y_{ijk} = \mu + S_i + O_j + SO_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} represents the kth observation of fish size i and oxygen level j, μ is the overall mean, S_i is the treatment effect of fish size i, O_j is the treatment effect of oxygen level j, SO_{ij} is the interaction between fish size i and oxygen level j, and ε_{ijk} is the error term (SAS Institute Inc., 2004).

Block was excluded from the analysis because there were no effects of block on any of the observed variables. Normal distribution of the residuals obtained from the model was verified using Kolmogorov–Smirnov's test with PROC UNIVARIATE. Except for initial MCH ($P < 0.05$), all the observed variables satisfied the normal distribution assumption ($P > 0.15$). Because logarithmic and square root transformation of the initial MCH did not result in satisfactory assumption, a Kruskal–Wallis one-way ANOVA was performed on this variable with main factor being fish size using PROC NPAR1WAY (SAS Institute Inc., 2004).

Within-day variations in DO concentrations and feed intake (morning versus afternoon feeding) were tested for the effect of fish size, oxygen, time (moment within a day) and their interactions by repeated measures ANOVA using PROC MIXED according to the following model:

$$Y_{ijkm} = \mu + S_i + O_j + SO_{ij} + \varepsilon_{1,ijk} + T_m + ST_{im} + OT_{jm} + SOT_{ijm} + \varepsilon_{2,ijkm}$$

where Y_{ijkm} represents the observation (DO concentration or feed intake) of fish size i, oxygen level j and tank k at time (moment) m, μ is the overall mean, S_i is the treatment effect of fish size i, O_j is the treatment effect of oxygen level j,

Table 2
Feed intake and growth performance of Nile tilapia (*Oreochromis niloticus*) under the four treatment combinations [a]

	Treatment combination [b]				*P*-value		
	SL	SH	BL	BH	Size	Oxygen	Size × oxygen
Ad libitum feeding period (d)	25	25	25	25			
Number. of fish per tank	50	50	9	9			
Number of tanks	3	3	3	3			
Survival (%)	100	100	100	100			
Initial body weight (g)	21.65±0.71	21.18±0.15	148.41±3.38	144.74±1.56	<0.001	0.307	0.424
Final body weight (g)	52.61±1.17	65.02±3.64	223.14±5.88	250.51±7.26	<0.001	0.004	0.176
Feed intake							
Absolute (g fish^{-1} d^{-1})	1.54±0.07	2.15±0.15	4.43±0.09	6.08±0.26	<0.001	<0.001	0.012
FI_{perc}(% d^{-1})	4.58±0.21	5.79±0.22	2.44±0.03	3.19±0.09	<0.001	<0.001	0.187
FI_{MBW} (g kg$^{-0.8}$ d^{-1})	23.23±0.98	29.94±1.29	17.34±0.23	22.90±1.69	<0.001	<0.001	0.537
Growth							
Absolute (g fish^{-1} d^{-1})	1.24±0.07	1.75±0.15	2.99±0.15	4.23±0.23	<0.001	<0.001	0.051
SGR (% d^{-1})	3.55±0.18	4.47±0.23	1.63±0.03	2.19±0.07	<0.001	0.002	0.284
GR_{MBW} (g kg$^{-0.8}$ d^{-1})	18.65±1.02	24.41±1.50	11.68±0.45	15.93±0.64	<0.001	0.001	0.469
FCR	1.25±0.03	1.23±0.03	1.49±0.04	1.44±0.05	<0.001	0.393	0.689

[a] Values represent means±SE over the *ad libitum* feeding period of three replicates (tanks); each replicate value is the mean of the measurements on 50 small fish (those under SL or SH) or 9 big fish (those under BL or BH).

[b] SL = Small fish–Low DO; SH = Small fish–High DO; BL = Big fish–Low DO; BH = Big fish–High DO. Low and high DO were two levels of dissolved oxygen with means around 3.0 and 5.5 mg L^{-1}, respectively.

Table 3
Feed intake of Nile tilapia (*Oreochromis niloticus*) in the morning and afternoon under the four treatment combinations[a]

Treatment combination[b]	SL		SH		BL		BH	
Meal time	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Feed intake								
Absolute (g fish^{-1} d^{-1})	0.79±0.03	0.76±0.03	1.04±0.06	1.11±0.08	2.22±0.08	2.21±0.01	2.91±0.15	3.17±0.12
FI_{perc}(% d^{-1})	2.33±0.10	2.25±0.11	2.79±0.08	2.99±0.14	1.22±0.03	1.22±0.03	1.52±0.05	1.67±0.04
FI_{MBW} (g kg$^{-0.8}$ d^{-1})	11.83±0.50	11.40±0.54	14.46±0.49	15.48±0.82	8.68±0.23	8.66±0.16	10.95±0.41	11.95±0.30

[a] Values represent means±SE over the *ad libitum* feeding period of three replicates (tanks); each replicate value is the mean of the measurements on 50 small fish (those under SL or SH) or 9 big fish (those under BL or BH).
[b] SL=Small fish–Low DO; SH=Small fish–High DO; BL=Big fish–Low DO; BH=Big fish–High DO. Low and high DO were two levels of dissolved oxygen with means around 3.0 and 5.5 mg L^{-1}, respectively.

SO_{ij} is the interaction between fish size i and oxygen level j, $\varepsilon_{1,ijk}$ is the error term 1, which represents the random effect of tank k within fish size i and oxygen level j, T_m is the effect of time (moment) m, ST_{im} is the interaction between fish size i and time (moment) m, OT_{jm} is the interaction between oxygen level j and time (moment) m, SOT_{ijm} is the interaction among fish size i, oxygen level j and time (moment) m, and $\varepsilon_{2,ijkm}$ is the error term 2. In this model, effects of fish size, DO level and their interaction were tested against error term 1; effects of time and interactions with time were tested against error term 2 (Kuehl, 2000; SAS Institute Inc., 2004).

Differences among treatment means were considered significant when $P<0.05$ and not significant (no effect) when $P\geq 0.05$. When $0.05\leq P<0.1$, a tendency for significant difference among treatment means was assumed. If significance was detected, multiple comparisons were performed using Tukey adjustment (SAS Institute Inc., 2004).

3. Results

3.1. Dissolved oxygen concentrations and other water quality parameters

Mean DO concentrations inside and at the outlet of the tanks over the ALFP under the four treatment combinations are shown in Table 1. Pair-wise comparisons among these values showed that under the same oxygen treatment, DO concentrations in tanks with big fish were significantly higher than in tanks with small fish ($P<0.001$); however, all the differences were smaller than 0.45 mg L^{-1}. In the same fish weight class, DO concentrations in high DO tanks were significantly higher than in low DO tanks ($P<0.001$); the differences were in the range of 2.62–2.72 mg L^{-1}. There was no interaction effect of fish size and DO level on DO concentrations ($P>0.5$). Because the DO concentrations measured inside and at the outlet of each tank at the same time were highly correlated under all treatment combinations ($r>0.98$) and the difference between them were small (95th percentile of 3231 cases being 0.32 mg L^{-1}), only DO concentrations inside the tanks were used for all the following presentations and analyses.

Analysis of mean DO concentrations at six times per day over the whole ALFP and mean DO concentrations at 25 times in four 24-hour periods (Fig. 1) showed that there were effects of time and interaction effects of time and size and of time and oxygen on the diurnal variations in DO concentrations ($P<0.001$). There was an abrupt change in DO concentrations from the time just before feeding to the time right after feeding (Fig. 1); these changes were significantly larger in tanks with small fish than with big fish at the same DO level ($P<0.05$) and significantly larger in low DO tanks than in high DO tanks in the same weight class ($P<0.001$). Mean DO concentrations in the tanks with small fish showed a declining trend throughout the ALFP while DO concentrations in the tanks with big fish declined during the first 2 weeks and were almost constant during the last week (Fig. 2).

Mean outlet NH_3 concentrations (±SE) over the ALFP under SL, SH, BL and BH treatments were 0.011±0.001, 0.007±0.001, 0.011±0.001 and 0.004±0.001 mg L^{-1}, respectively. NH_3 concentrations in low oxygen tanks were significantly higher than in high oxygen tanks ($P<0.001$). There was no effect of size ($P=0.223$) or interaction effect of size and water flow rate ($P=0.234$) on NH_3 concentrations. Mean outlet NO_2^- concentrations over the ALFP under the four treatment combinations were identical to the inlet NO_2^-, which equaled 0.274±0.194 (SD) mg L^{-1}. Mean outlet NO_3^-concentrations over the ALFP under the four treatment combinations were also identical to the inlet NO_3^-, which equaled 74.5±18.6 (SD) mg L^{-1}. Mean temperature (±SD) of all tanks at both inlet and out let was identical, which equaled 32.7±0.7 °C. Mean outlet pH (±SE) over the ALFP under SL, SH, BL and BH treatments were 7.55±0.06, 7.57±0.06, 7.58±0.05 and 7.60±0.07, respectively. No effect of size ($P=0.651$), water flow rate ($P=0.734$) or interaction effect of size and water flow rate ($P=0.951$) on pH was observed.

3.2. Feed intake and growth

Experimental characteristics, feed intake and growth performance of the fish are shown in Table 2. Mean initial fish weights were significantly different between weight classes ($P<0.001$), but not

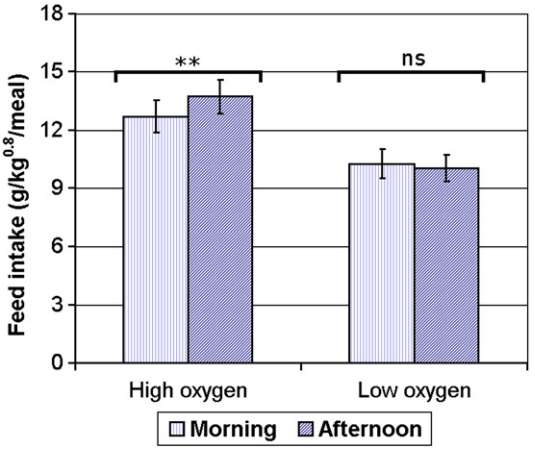

Fig. 3. Mean feed intake of Nile tilapia (*Oreochromis niloticus*) in the morning and afternoon meals at low (3 mg L^{-1}) and high (5.5 mg L^{-1}) dissolved oxygen levels. Bars represent means of six replicates (tanks); each replicate value is the mean of feed intake during the same time (morning or afternoon meal) over the *ad libitum* feeding period. Error bars represent two times the standard errors. ** means "significantly different with $P<0.01$" and ns means "not significantly different with $P>0.05$".

Table 4
Final body composition (% fresh weight) of Nile tilapia (*Oreochromis niloticus*) under the four treatment combinations [a]

	Treatment combination [b]				*P*-value		
	SL	SH	BL	BH	Size	Oxygen	Size × oxygen
Moisture	71.8±0.5	70.9±0.4	70.3±0.7	68.9±0.6	0.011	0.073	0.624
Crude fat	6.9±0.2	8.8±0.6	8.9±0.4	9.1±0.2	0.018	0.029	0.058
Crude protein	15.9±0.3	16.2±0.2	16.0±0.3	16.7±0.1	0.215	0.054	0.370

[a] Values represent means±SE of three replicates (tanks); each replicate value is a measurement on the homogenate of 10 small fish or 9 big fish.
[b] SL=Small fish–Low DO; SH=Small fish–High DO; BL=Big fish–Low DO; BH=Big fish–High DO. Small and big fish were those with individual weights in the range of 21–66 g and of 144–251 g, respectively. Low and high DO were two levels of dissolved oxygen with means around 3.0 and 5.5 mg L^{-1}, respectively.

significantly different between low and high DO tanks ($P=0.307$). Mean final fish weights in high DO tanks, however, were significantly higher than in low DO tanks ($P=0.004$). Effects of fish size were present in all the responses ($P<0.001$). Although absolute feed intake and growth rates were higher in big fish, these values were smaller in big fish than in small fish when expressed as percentage of body weight or per metabolic weight unit. Feed conversion ratios (FCR) of big fish were higher than small fish. Oxygen significantly affected feed intake and growth rates ($P<0.005$) but had no effect on FCR ($P=0.393$). In the same weight class, feed intake and growth rates of fish in high DO tanks were significantly higher than in low DO tanks ($P<0.05$). There were no interaction effects of fish size and oxygen on any of the responses ($P>0.05$), except on the absolute feed intake ($P<0.05$).

Mean feed intake per fish per meal (g $fish^{-1}$ $meal^{-1}$) and feed intake as percentage of body weight per meal (% $meal^{-1}$) and per metabolic weight unit per meal (g $kg^{-0.8}$ $meal^{-1}$) over the ALFP (Table 3) were significantly different between morning and afternoon meals ($P<0.05$). There was an interaction effect of meal time and oxygen ($P<0.01$), but no interaction effect of meal time and fish size ($P>0.05$). Multiple comparisons showed that feed intake in the morning was significantly lower than in the afternoon at high oxygen level ($P<0.01$). At low oxygen level, however, no effect of meal time on feed intake was found ($P>0.5$), although feed intake in the morning was numerically higher than in the afternoon (Fig. 3).

3.3. Body composition

Initial moisture, crude fat and crude protein contents on a fresh weight basis of the small fish were 77.2, 3.9 and 14.4%, respectively, and of the big were 73.2, 8.1 and 14.2%, respectively. Final body composition of the fish is presented in Table 4. Both initial and final body composition showed the following trend: crude fat contents in lighter fish were lower than in heavier fish and the opposite occurred with moisture content. Fish size significantly affected final moisture and crude fat contents ($P<0.02$), but did not affect crude protein content ($P=0.215$). Final crude fat content in fish at low DO level was significantly lower than at high DO level ($P=0.029$). There was a tendency that final moisture content in fish at low DO level was higher than at high DO level ($P=0.073$) and final protein content in fish at low DO level was lower than at high DO level ($P=0.054$). There were no interaction effects of size and oxygen on moisture, crude protein and crude fat contents ($P>0.05$), although a tendency on crude fat content was found ($P=0.058$).

3.4. Hematological parameters

Mean hematological parameters of small fish and big fish at the beginning of the experimental period are given in Table 5. No significant difference in any parameter between small and big fish was observed ($P\geq 0.08$). Mean hematological parameters of fish at the end of the experimental period under the four treatment combinations are given in Table 6. Except for MCV ($P=0.147$), there was an effect of fish size on all the parameters ($P<0.05$), but no effect of oxygen level on any of the parameters ($P>0.5$). As the fish grew bigger, RCB, HGB and HCT became significantly higher ($P<0.001$), while MCH and MCHC became significantly lower ($P<0.05$). There was no interaction effect of fish size and oxygen level on any of the parameters ($P>0.5$).

4. Discussion

4.1. Effects of DO on feed intake and growth

The high and low DO levels in the experiment were created by applying different aeration regimes and by changing the water flow rates. In addition to affecting the DO levels, the contrast in water flow rates might have affected some of the other water quality parameters which influence the feed intake of fish. However, we think that these effects are negligible. Temperature, nitrite and nitrate concentrations and pH were within the optimal ranges for Nile tilapia (Masser et al., 1999; Popma and Masser, 1999; Ross, 2000). Of all the water quality

Table 5
Mean hematological parameters of Nile tilapia (*Oreochromis niloticus*) at the beginning of the experimental period [a]

Fish size	Hematological parameter [b]					
	RBC ($\times 10^{12}$/L)	HGB (g/L)	HCT (L/L)	MCV (fL)	MCH (pg)	MCHC (g/L)
Small	1.878±0.269	76.250±8.012	0.197±0.045	104.330±12.910	40.925±3.217	398.700±62.638
Big	1.853±0.413	79.100±10.223	0.206±0.054	111.470±12.193	44.715±11.724	402.850±96.150
P-value	0.825	0.333	0.568	0.080	0.665 [c]	0.872

[a] Values represent means±SD of 20 replicates (fish).
[b] RBC=red blood cell count; HGB=hemoglobin; HCT=hematocrit; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration.
[c] Result from Kruskal–Wallis one-way analysis of variance (see Materials and methods for details).

Table 6
Mean hematological parameters of Nile tilapia (*Oreochromis niloticus*) at the end of the experimental period under the four treatments combinations [a]

Treatment combination [c]	Hematological parameter [b]					
	RBC ($\times 10^{12}$/L)	HGB (g/L)	HCT (L/L)	MCV (fL)	MCH (pg)	MCHC (g/L)
SL	1.852±0.069	82.111±3.379	0.208±0.002	112.583±5.807	47.806±3.912	426.333±19.390
SH	1.831±0.140	82.944±1.645	0.208±0.022	112.083±3.394	47.333±4.562	434.667±53.431
BL	2.582±0.026	101.278±2.469	0.311±0.007	120.556±3.785	40.261±1.756	336.500±12.366
BH	2.569±0.022	101.333±1.110	0.308±0.013	118.517±4.560	39.417±0.438	338.944±11.212
P-value						
Size	<0.001	<0.001	<0.001	0.147	0.039	0.014
Oxygen	0.840	0.853	0.901	0.784	0.839	0.860
Size × Oxygen	0.957	0.871	0.921	0.868	0.954	0.923

[a] Values represent means±SE of three replicates (tank); each replicate value is the mean of the measurements on six fish.
[b] RBC=red blood cell count; HGB=hemoglobin; HCT=hematocrit; MCV=mean corpuscular volume; MCH=mean corpuscular hemoglobin; MCHC=mean corpuscular hemoglobin concentration;
[c] SL=Small fish–Low DO; SH=Small fish–High DO; BL=Big fish–Low DO; BH=Big fish–High DO. Small and big fish were those with individual weights in the range of 21–66 g and of 144–251 g, respectively. Low and high DO were two levels of dissolved oxygen with means around 3.0 and 5.5 mg L^{-1}, respectively.

parameters measured, only NH_3 concentrations were significantly different between low and high oxygen tanks. The highest difference in NH_3 concentrations among the treatments (0.007 mg L^{-1}) was very small and the highest NH_3 concentration (0.011 mg L^{-1}) was far below the lowest level (0.080 mg L^{-1}) which may affect feed intake of Nile tilapia (Shelton and Popma, 2006). El-Shafai et al. (2004) showed that feed intake of Nile tilapia was not influenced by NH_3–N concentrations within the range of 0.004–0.434 mg L^{-1} (corresponding to 0.005–0.527 mg NH_3 L^{-1}); however, for best growth, they recommended an NH_3–N concentration below 0.10 mg L^{-1} (corresponding to 0.12 mg NH_3 L^{-1}). Muir et al. (2000) even considered an NH_3 level below 0.2 mg L^{-1} ideal for flowing-water tilapia culture. However, Soderberg (2006) doubted that the chronic toxicity level of NH_3 might be lower than the level recommended by Muir et al (2000). Based on studies on channel catfish, rainbow trout (*Oncorhynchus mykiss*) and blue tilapia (*Oreochromis aurea*), he estimated chronic toxicity levels of NH_3 ranging from 0.035 to 0.092 mg L^{-1} for tilapias. The lower bound of this range is higher than the highest NH_3 concentration in the present study.

Carbon dioxide is another factor that may affect feed intake but it was not measured in the experiment. Nile tilapia can tolerate CO_2 concentrations above 20 mg L^{-1} (Wedemeyer, 1996) and CO_2 is unlikely to have an adverse effect on fish in intensive culture systems unless its concentration reaches 100 mg L^{-1} (Balarin and Haller, 1982). In our experiment, CO_2 was unlikely to affect feed intake and growth of the fish because: (1) the densities of the fish in the experiment (lower than 12 kg m^{-3} initially) were much lower than the recommended densities of Nile tilapia in the intensive systems (20–25 kg m^{-3} initially for fish of 20–250 g) (Rakocy, 1989); (2) accumulation of CO_2 in the system was counteracted by letting the water go down through the perforated trays on top of the trickling biofilter and by diffused aeration in the sump (see Huguenin and Colt, 2002; chapter 12); and (3) pH in the experiment was high (around 7.6) and thus toxic gaseous CO_2 must be low. The mole fraction of gaseous CO_2 at pH of 7.6 is only 5.6% (calculated based on Henderson–Hasselbach equation; Boyd, 1990). Therefore, the contrast between the DO treatments in the present study was most likely due to real differences in DO concentrations and not to any other water quality parameters.

The results from the present study clearly showed reduced feed intake and growth of Nile tilapia of average weight of 37 and 190 g at DO concentrations of about 2.8 mg L^{-1} and 3.2 mg L^{-1}, respectively. Some investigators have attempted to define an incipient DO concentration at which feed intake and growth of Nile tilapia starts to decline. Reports on incipient DO concentrations for growth of Nile tilapia showed a range from less than 0.8 to 3 mg L^{-1} (Rappaport et al., 1976; Coche, 1977; Melard and Philippart, 1980; Teichert-Coddington and Green, 1993). The lower extreme value (less than 0.8 mg L^{-1}; Teichert-Coddington and Green, 1993) was obtained from an experiment in which there were no significant differences between the yields of Nile tilapia raised in ponds with two aeration regimes, which were established as follows: pond water samples were taken every hour and DO concentrations measured; if DO concentrations were equal or less than critical levels (10% or 30% of saturation), ponds were aerated for 1 h. The authors suggested that practical threshold of DO for Nile tilapia was not higher than 10% of saturation (0.8 mg L^{-1} at 26 °C; conversion based on Colt (1984)). However, DO variations were not reported and thus actual DO concentrations to which the fish were exposed are unknown.

Based on field observations of Melard and Philippart (1980), where growth rates of Nile tilapia declined at DO concentration of 3 mg L^{-1}, Soderberg (2006) suggested that minimum DO tension for flowing-water tilapia culture should be 60 mm Hg, equivalent to 2.9 mg L^{-1} at 32.7 °C, the average temperate of the present study (conversion based on Colt, 1984). If oxygen supply to fish is limited by diffusion through the gills, it may not be possible to define one single incipient oxygen concentration below which fish growth starts decreasing. The incipient concentration would depend on the balance between oxygen supply to the fish, determined by the oxygen gradient across the gills, the gill surface area (and therefore body weight) and the blood–water distance; and oxygen demand by the fish, determined by the amount and composition of the feed and the activity level of the fish. In the present study, fish were fed to satiation with a high-protein diet. Their requirements for oxygen

may have been higher than fish in natural waters which feed mainly on algae-based materials (Fryer and Iles, 1972; Man and Hodgkiss, 1977; Bowen, 1982; Trewavas, 1983) most likely at a sub-maximum level. For instance, the upper bound of 95% confidence interval of daily feed ingestion of a 200 g Nile tilapia feeding mainly on phytoplankton in Lake George (Uganda) estimated by Moriarty and Moriarty (1973) was about 2.9 g (dry matter), much lower than daily feed intake (4.19 g dry matter) of a 181 g fish (geometric mean) under low oxygen condition in the present study. Thus, under *ad libitum* feeding with a high-protein diet, incipient DO for tilapia may be higher than 3.2 mg L^{-1}, referring to the average DO concentration in tanks with big fish in the present study.

4.2. Effects of body size on feed intake and growth

In accordance with the earlier findings (for examples, see Fänge and Grove (1979) and Jobling (1983)), relative feed intake and growth of small fish were higher than of big fish in the present study. The allometric relationship between growth and body weight in animals has been explained by considering that growth is a result of two counteracting processes: anabolism and catabolism (von Bertalanffy, 1957). Anabolism in a wide range of species has been found to be proportional to a power of body weight smaller than unity (see Glazier, 2005). Catabolism, which occurs in all living cells, was assumed to be directly proportional to the body weight (von Bertalanffy, 1957). Thus, the difference of these two processes must decrease with increasing body weight. The allometric relationship between anabolism and body weight in ectotherms was attributed to the surface area limitation associated with nutrient absorption and gas exchange (Ellenby, 1937; von Bertalanffy, 1957; Whitford and Hutchison, 1967; Hutchison et al., 1968; Ultsch, 1974, 1976) (cited after Glazier, 2005). Pauly (1981) argued that in fish the absorptive area of the gut is unlikely to limit growth for several reasons. Gut surface area is limited only when it is in permanent contact with the ingested food, which is not the case. Also, the relative absorptive area in fish is much more linked to the feeding mode than to growth performance, and long-lasting fat storage in fish allows them to maintain anabolism long after completion of feeding and nutrient absorption, thus making anabolic processes independent of the gut surface. The results from the present experiment provide additional support for the hypothesis that the body size effect acts through the relative gill surface area.

The effect of body size on feed intake was also expressed through the difference in DO concentrations between tanks with small and big fish. Because we assumed that oxygen supply to the fish is driven by diffusion and therefore by the oxygen concentration gradient across the fish gills, we tried to maintain equal DO concentrations in the tanks with small and big fish within one DO level by regulating the water flow rates through the tanks. Nevertheless, dissolved oxygen concentrations in tanks with small fish were always slightly lower than in tanks with big fish, although water flow rates through tanks with small fish were either higher than or equal to those in tanks with big fish, and the initial biomass in tanks with small fish was smaller than in tanks with big fish (ca. 1.0 kg vs. 1.3 kg $tank^{-1}$, respectively). Because we could not quantify the amount of the oxygen supply by aeration, oxygen consumption of the fish could not be calculated based on the mass balance principle. However, since tanks with small and big fish received water with the same DO concentration at the inlet and were subject to the same aeration regime, lower DO concentrations in tanks with small fish imply that oxygen consumption relative to body weight in small fish was higher than in big fish. This is in accordance with the earlier studies in which fish metabolism was found to be proportional to a power of body weight smaller than unity (Winberg, 1956; Sauders, 1963; Brett, 1965; Glass, 1969; Brett and Glass, 1973; Andrews and Matsuda, 1975; Hölker; 2003). Because fish were fed to satiation in the present study, higher relative oxygen consumption in small fish than in big fish suggests a higher relative oxygen uptake capacity and therefore higher relative feed intake and growth rate in small fish than in big fish. In the present study, FI_{perc} in small fish was almost double that in big fish at the same DO level.

4.3. Feeding rhythm

Although oxygen concentrations in the morning were higher than in the afternoon, feed intake of the fish under high oxygen treatment was lower in the morning than in the afternoon in the present study. Reports on diurnal feeding rhythms of tilapias in natural waters are mixed (Harbott, 1975; Dewan and Saha, 1979; Haroon et al., 1998) and can hardly be used to draw a conclusion about the biological feeding rhythm of the fish, since feeding activity and feed intake are strongly influenced by environmental factors such as DO, temperature, light and food availability (Kestemont and Baras, 2001; Madrid et al., 2001).

Under controlled conditions, a report on feeding activity of Nile tilapia kept at DO above 80% saturation (6.36 mg L^{-1} at 27 °C) showed that the fish ate mainly during the day time and the cumulative demand for food between 7:00 and 10:00 was lower than between 16:00 and 19:00 (Toguyeni et al., 1997). Another study showed that the filtration rates of Nile tilapia between 6:00 and 12:00 were lower than between 14:00 and 20:00. Mean oxygen concentrations in the morning and afternoon were 4.2 and 3.8 mg L^{-1}, respectively (Turker, 2004). These studies showed the same phenomenon as observed in the present study: Nile tilapia at relatively high DO level (above 3.5 mg L^{-1}) consumed more in the afternoon than in the morning, even when the DO concentration in the afternoon was lower than in the morning. At low DO level (below 3.5 mg L^{-1}), fish in the present study consumed slightly more in the morning (at higher DO concentration) than in the afternoon (at lower DO concentration). This shift in feeding rhythm might be related to the limitation of oxygen uptake of the fish when DO concentration drops below a certain level. From a practical point of view, this phenomenon should be elucidated to optimize feeding strategy.

4.4. Hematological parameters

Common responses of fish under hypoxia to safeguard oxygen uptake include increased gill ventilation (Gerald and

Cech, 1970; Lomholt and Johansen, 1979; Fernandes and Rantin, 1989) and blood oxygen affinity and/or capacity (Wood and Johansen, 1972; Weber et al., 1976; Soivio et al., 1980; Yamamoto et al., 1985; Nikinmaa, 1992; Nikinmaa and Salama, 1998). Among the responses, increase in hemoglobin concentration is considered to be energetically cost-effective (Weber and Jensen, 1988). The release of red blood cells via spleeny contraction is also employed in fish exposed to severe hypoxia (Yamamoto et al., 1985; Jobling, 1994). According to Weber and Jensen (1988), however, erythropoiesis and hemoglobin synthesis require a long time to complete and can only be involved in long-term adaptation. The absence of differences in hematological parameters between fish at low and high DO levels observed in the present study might be due to short-time exposure of the fish to low DO level, which was likely to be far from severe hypoxia for this species. Fernandes and Rantin (1986) found that high number and length of the gill filaments and high frequency of the secondary lamellae in Nile tilapia allow this species to exchange gas very efficiently. Together with high affinity of its hemoglobin to oxygen (Verheyen et al., 1985), Nile tilapia is very tolerant to very low DO concentration (Welcomme, 1969). Chervinski (1982) supposed that tilapias were able to tolerate DO as low as 1 mg L^{-1}. This value is much lower than the DO concentrations in the low DO treatments in the present study (2.78 and 3.19 mg L^{-1}). It is suggested that lower DO concentrations and longer duration are required to study effect of the hypoxia on hematological parameters of Nile tilapia.

5. Conclusions

The results of the present study showed that feed intake and growth of Nile tilapia of average weight of 37 and 190 g at DO concentration of about 3.0 mg L^{-1} were significantly lower than at DO concentration of about 5.6 mg L^{-1}. Feed intake and growth per body weight unit in fish of 37 g were higher than in fish of 190 g at both DO levels (3.0 and 5.6 mg L^{-1}). This body size effect is attributed to differences in the relative gill surface area. Fish at high DO level (5.6 mg L^{-1}) consumed more in the afternoon than in the morning while fish at low DO level (3.0 mg L^{-1}) consumed more in the morning than in the afternoon. This indicates a change in feeding rhythm of the fish when the average DO drops below a certain level. Nile tilapia exposed to DO concentration of 3.0 mg L^{-1} for a duration of 25 days made no hematological adjustments. This might be due to short-time exposure of the fish to this DO level, which is likely to be far from severe hypoxia for this species.

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