Growth performance, hematological and biochemical responses of African catfish (*Clarias gariepinus*) reared at different stocking densities

Wei Dai¹, Xiaomei Wang¹*, Yongjun Guo¹, Qian Wang¹ and Jinhong Ma²

¹Department of Fisheries Science, Tianjin Key Laboratory of Aqua-Ecology and Aquaculture, Tianjin Agricultural University, Tianjin 300384, P. R. China.
²Deren Aquaculture Center, Tianjin 300380, P. R. China.

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African catfish (30.71 ± 0.89 g) were stocked at four different densities of 35, 65, 95 and 125 kg m⁻³ in 120 L aerated tanks for 60 days, with one and a half tanks of water being renewed daily. The effects of stocking density were examined in growth performance and welfare indicators, such as hematological and biochemical indices. Growth decreased with the increasing stocking density, and there is no significant difference between 35 and 65 kg m⁻³ treatments. Hematological and biochemical indices, including hemoglobin (Hb), erythrocyte sedimentation rate (ESR), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) values, total protein (TP), glucose and total cholesterol (TC) levels, aspartate transaminase (AST), alkaline phosphatase (AP) and lactate dehydrogenase (LDH) activities, serum ion levels and pH, were not affected by the stocking density. Consistent with the result of growth, no significant difference in red blood cell (RBC) count and Hematocrit (Hct) values, alanine transaminase (ALT) activity, albumin (Alb) and triglyceride (TG) levels were observed between 35 and 65 kg m⁻³ treatments, while some parameters, such as Hct, Alb and ALT, were affected significantly when stocking density increased to 95 kg m⁻³. Impaired welfare indicators, as well as growth performance, need to be taken into account by husbandry practices to assure that high welfare standards are maintained with increasing profitability.

Key words: African catfish, stocking density, growth performance, hematological indices, biochemical indices.

INTRODUCTION

Raising fish at high stocking density means maximizing usage of the fish production infrastructure, thereby improving farm profitability (Blancheton, 2000). The importance of stocking density on fish growth and welfare has been reported for several species, such as European sea bass (*Dicentrarchus labrax*) (Papoutsoglou et al., 1998), lake sturgeon (*Acipenser fulvescens*) (Fajfer, 1999), Atlantic salmon (*Salmo salar* L.) (Hosfeld et al., 2009), catfish (*Pangasianodon hypophthalmus*) (Slembrouck et al., 2009), piabanha (*Brycon insignis*) (Tolussi et al., 2010), Senegalese sole (*Solea senegalensis*) (Salas-Leiton et al., 2010). It is concluded that increased stocking density can affect fish growth negatively (e.g. trout, Ellis et al., 2002; European seabass, Santos et al., 2010) or positively (e.g. Arctic charr, Jørgensen et al., 1993) depending on species.

There is a contradiction in the effects of high stocking density on growth and welfare of African catfish (*Clarias gariepinus*). Some investigators found that increasing density led to enhanced cannibalism and reduced growth performance (Haylor, 1991; Hossain et al., 1998), some studies showed that increasing density resulted in increased growth performance and decreased aggression
(Kaiser et al., 1995), while it was reported that the welfare of African catfish with increasing density seemed impaired at both the lowest and highest densities (van de Nieuwegiessen et al., 2008). There is a certain threshold density causing stress from which growth of African catfish decreases and welfare was impaired with the increasing stocking density (Hecht and Ulys, 1997; Toko et al., 2007). It is of considerable significance to identify the optimal stocking density to avoid negative impact on the welfare and productivity in fish farm.

The African catfish (Clarias gariepinus) is one of the most important cultured fish in China for its fast growth, high disease resistance and low oxygen tolerance. There are two growth phases in culture. In Phase 1, 30 g juveniles grow to an average size of 80-100g and after which fish are graded and restocked to a market size. Different responses to stocking density can occur during the growth cycle. The purpose of this study was to investigate the effects of different stocking densities on the growth performance of African catfish in Phase 1, as well as some welfare indicators, such as hematological and biochemical responses.

MATERIALS AND METHODS

Fish and experimental design

The study was conducted strictly adhering to the guidelines set by the China Council on Animal Care. Juvenile African catfish weighing 10-12 g were obtained from Deren aquaculture center, Tianjin, China. Prior to the start of the experiment, fish were acclimated to laboratory conditions in 250 L aerated holding tanks (stocking density 5 kg fish m⁻³) for 15d, at optimal temperature of 27±1 °C under a 12 L: 12 D photoperiod. Safe conditions for fish rearing (pH 7.3±0.2, oxygen saturation >50%, NH₄⁺ < 1 mg/L, NO₂⁻ < 1 mg/L, and NO₃⁻ < 1000 mg/L) were maintained with a 100% daily water exchange. Fish were fed at 2.5 % fresh body weight twice daily at 0800 and 1600 hours. One hundred fish were randomly selected and weighed every 3 days, and the daily ration was adjusted accordingly.

After acclimation, fish weighing 30.71±0.89 g were randomly divided into four density-stocked groups each with three replicate tanks (35kg fish m⁻³: n = 134, 135 and 138 fish; 65 kg fish m⁻³: n = 261, 260 and 265 fish; 95 kg fish m⁻³: n = 381, 375 and 355 fish; 125 kg fish m⁻³: n = 495, 467 and 480 fish). Each tank (70 cm x 50 cm x 50 cm) contained 120 L aerated water. To mimic the practical water exchange, water was renewed one and a half tanks every day and water exchange was performed at 1000 and 1800 h. Fish were fed at 2.0 % fresh body weight twice daily at 0800 and 1600 h, with a commercial dry floating pelleted feed for catfish (35 % crude protein [min.], 5% crude fat [min.], 10% crude fiber [max.], 10% ash [max.]; Tianxiang Feed Co. Ltd., Tianjin, China) during both acclimation and experimental period.

Growth performance

After 60 days experimental period, mean fish weight was calculated using the sum of individual fish weight divided by the fish number in each tank. Growth performance was analyzed in terms of daily weight gain (DWG) and feed conversion ratio (FCR). The following formulae were used:

\[
\text{DWG} (\text{g d}^{-1}) = \frac{\text{final mean fish weight} - \text{initial mean fish weight}}{\text{rearing period (days)}}
\]

\[
\text{FCR} (\text{g g}^{-1}) = \frac{\text{food consumed}}{\text{live weight gain}}
\]

Hematological and biochemical analysis

At day 60, eight randomly selected fish per tank were anesthetized with 200 mg/L MS222 (Tricaine Methanesulfonate). One ml of blood anticoagulated with 3 mg Na₂EDTA was collected from the caudal vein of fish for the hematological assay. Red blood cell (RBC) number, hematocrit (Hct), erythrocyte sedimentation rate (ESR) and hemoglobin (Hb) were determined using hemocytometer, microhematocrit and cyanomethemoglobin methods, respectively (Gui, 2004). Sizes of the RBC (long diameter and short diameter) were measured in the blood smears. A stage micrometer was used to calibrate the microscope prior to the size measurement. The mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) were calculated as follows (Siang et al., 2007):

\[
\text{MCV (µm}^3 \text{cell}^{-1}) = \left( \frac{\text{Packed cell volume as percentage/RBC in millions cell mm}^{-3}}{10} \right)
\]

\[
\text{MCH (pg cell}^{-1}) = \left( \frac{\text{Hb in g 100 mL}^{-1}/\text{RBC in millions cell mm}^{-3}}{1000} \right)
\]

\[
\text{MCHC (100 m}^{-1} \text{Hct}) = \left( \frac{\text{Hb in g 100 mL}^{-1}/\text{packed cell volume as percentage}}{100} \right)
\]

For serum physio-biochemical analysis, another four fish were selected randomly from each tank and anesthetized with 200 mg/L MS222. Blood samples collected from the caudal vein were left at room temperature for 1 h, followed by 4h at 4°C and then serum was harvested by centrifuging at 4000 rpm for 15 min at 4°C. The serum obtained was divided into subsamples and stored at 4°C until the physio-biochemical analysis was done within 24 h. Total protein (TP), albumin (Alb), glucose, triglyceride (TG), and total cholesterol (TC) levels, as well as alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (AP) and lactate dehydrogenase (LDH) activities, were determined using assay kits (Biosino Bio-technology and Science Inc, Beijing, China) (Tietz et al., 1983; Bergmeyer et al., 1986a, b; Ye and Wang, 1997a, 1997b, 1997c, 1997d, 1997e). The ion levels (Na⁺, K⁺, Ca²⁺, Cl⁻) and pH in serum of eight fish per tank were measured using AC-980 Electrolyte Analyzer (Taiyi Medical Apparatus Equipment Co., Ltd., Shanghai, China).

Statistical analysis

The results of data were expressed as mean ± SD and subjected to one-way analysis of variance (SPSS version 10.0) to determine significant differences among groups. Significant differences (P < 0.05) between treatments were determined by least significant difference multiple-range test. Tanks were used as experimental unit for growth performance, hematological and biochemical analysis.

RESULTS

At the end of the experiment, final densities of the four treatment groups reached 95, 189, 252, and 301 kg m⁻³, respectively. As shown in Table 1, final body weight decreased with increasing stocking density, while insignificant difference was observed between 35 and 65 kg m⁻³ treatments (P > 0.05). Decreasing DWG with increasing
Table 1. Effects of four different stocking densities on the growth performance of African catfish (C. gariepinus) during a 60-d trial in 120-L tanks with a 150% daily water exchange.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>35 kg m⁻³</th>
<th>65 kg m⁻³</th>
<th>95 kg m⁻³</th>
<th>125 kg m⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>31.01±0.47</td>
<td>29.77±0.32</td>
<td>30.82±1.17</td>
<td>31.22±0.90</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>100.94±3.42</td>
<td>96.64±3.84</td>
<td>90.70±1.19</td>
<td>82.87±2.71</td>
</tr>
<tr>
<td>DWG (g)</td>
<td>1.16±0.06</td>
<td>1.11±0.07</td>
<td>1.00±0.03</td>
<td>0.86±0.03</td>
</tr>
<tr>
<td>The gross final biomass (kg m⁻³)</td>
<td>37.59±1.35a</td>
<td>71.83±0.97b</td>
<td>94.67±5.10a</td>
<td>106.87±5.72a</td>
</tr>
<tr>
<td>Total feed fed (kg m⁻³)</td>
<td>34.64±2.19a</td>
<td>65.42±3.92a</td>
<td>82.35±4.77ab</td>
<td>90.85±2.23a</td>
</tr>
<tr>
<td>FCR (g g⁻¹)</td>
<td>1.09±0.03</td>
<td>1.10±0.05</td>
<td>1.15±0.03</td>
<td>1.18±0.08</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>3.69±0.76a</td>
<td>3.68±1.13a</td>
<td>5.13±0.36</td>
<td>5.40±0.43b</td>
</tr>
</tbody>
</table>

Values within rows sharing the same letters or no letters are not significantly different (P > 0.05), whereas those with different letters are significantly different (P < 0.05); DWG means daily weight gain and FCR means feed conversion ratio.

Table 2. The hematological parameters of African catfish (C. gariepinus) raised at four different stocking densities during a 60-d trial in 120-L tanks with a 150% daily water exchange.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>35 kg m⁻³</th>
<th>65 kg m⁻³</th>
<th>95 kg m⁻³</th>
<th>125 kg m⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10¹² cell mm⁻³)</td>
<td>2.29±0.15a</td>
<td>2.19±0.04ab</td>
<td>2.08±0.14ab</td>
<td>2.06±0.06ab</td>
</tr>
<tr>
<td>RBCL (µm)</td>
<td>8.27±0.10</td>
<td>8.20±0.10</td>
<td>8.23±0.13</td>
<td>8.18±0.06</td>
</tr>
<tr>
<td>RBCS (µm)</td>
<td>7.75±0.07</td>
<td>7.73±0.08</td>
<td>7.81±0.12</td>
<td>7.75±0.11</td>
</tr>
<tr>
<td>Hb (g dL⁻¹)</td>
<td>7.38±0.24</td>
<td>7.76±0.18</td>
<td>7.27±0.22</td>
<td>7.19±0.62</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>40.77±0.58a</td>
<td>41.77±0.47a</td>
<td>38.85±0.49b</td>
<td>37.4±1.68b</td>
</tr>
<tr>
<td>ESR (mm h⁻¹)</td>
<td>0.72±0.12</td>
<td>0.69±0.20</td>
<td>0.82±0.15</td>
<td>0.87±0.21</td>
</tr>
<tr>
<td>MCV (µm³ cell⁻¹)</td>
<td>181.14±14.85</td>
<td>192.29±5.89</td>
<td>188.41±12.27</td>
<td>183.75±13.02</td>
</tr>
<tr>
<td>MCH (pg cell⁻¹)</td>
<td>32.84±1.94</td>
<td>35.69±1.11</td>
<td>35.28±1.33</td>
<td>35.21±3.54</td>
</tr>
<tr>
<td>MCHC (g 100 mL⁻¹ Hct)</td>
<td>18.15±0.75</td>
<td>18.61±0.55</td>
<td>18.83±0.52</td>
<td>19.23±1.04</td>
</tr>
</tbody>
</table>

Values within rows sharing the same letters or no letters are not significantly different (P > 0.05), whereas those with different letters are significantly different (P < 0.05); RBC means red blood cell number, RBCL means long diameter of RBC, RBCS means short diameter of RBC, Hb means hemoglobin, Hct means hematocrit, ESR means erythrocyte sedimentation rate, MCV means the mean corpuscular volume, MCH means the mean corpusular hemoglobin and MCHC means the mean corpusular hemoglobin concentration.

stocking density was observed and there was no significant difference between 35 and 65 kg m⁻³ treatments (P > 0.05). Feed conversion ratio was not affected by the stocking density (P > 0.05). Mortality was not different between 35 and 65 kg fish m⁻³ treatments, as well as between 95 and 125 kg m⁻³ treatments (P > 0.05).

As shown in Table 2, no significant effects of stocking density on long diameter of RBC (RBCL), short diameter of RBC (RBCS), Hb, ESR, MCV, MCH and MCHC were observed (P > 0.05). RBC count decreased when the stocking density increased, while MCHC values increased with increasing stocking density. Highest values of Hb and Hct were presented at stocking density of 65 kg m⁻³, and lowest values were observed at stocking density of 125 kg m⁻³. There was no significant difference in RBC count and Hct value between 35 and 65 kg fish m⁻³ treatments (P > 0.05).

Figure 1 shows that TP, glucose and TC levels, AST, AP, and LDH activities in serum were not affected by stocking density (P > 0.05). With increasing density, serum TP and Alb levels decreased. A similar trend of decrease in ALT activity was also observed. Highest TG level was presented at stocking density of 95 kg m⁻³, and lowest value was observed at stocking density of 125 kg m⁻³. There was no significant difference in ALT activity, Alb and TG levels between 35 and 65 kg m⁻³ treatments (P > 0.05). Serum ion (Na⁺, K⁺, Ca²⁺, Cl⁻) levels and pH were not affected by stocking density (P > 0.05) (Table 3).

**DISCUSSION**

In China, African catfish is reared in concrete ponds with daily water exchange, as well as in flowing water aquaculture systems. Some investigations have been carried out to study how stocking density affects the growth and welfare of African catfish reared in recirculation systems (van de Nieuwegiessen et al., 2008, 2009), while information on the effects of stocking density on the African catfish reared in concrete ponds with daily water exchange is scarce. Usually stress response in fish...
is evaluated by measuring levels of biochemical, hematological parameters in the blood, as well as growth and feeding parameters (Wagner et al., 1997; Braun et al., 2010). In this study, all the examined parameters gave little indication of impaired fish welfare between 35 kg m⁻³ and 65 kg m⁻³ treatments.

In optimizing controlled fish production, a number of parameters directly related to the stocking density, including water quality, fish nutrition and the size-physiological stage of the fish, the type of production system, and the type and size of the rearing tanks, must be considered (Malison and Held, 1992; Tidwell and Webster, 1993; Bagley et al., 1994; Papoutsoglou et al., 1998). High stocking density can affect fish performance and welfare through crowding stress, and/or through water quality deterioration in recirculating systems. As compared to water recirculation, daily water exchange was insufficient in the concrete pond. Under practical conditions, high stocking density often coincides with reduced water quality, so decreased growth was attributed to the combined effects of fish crowding and water quality deterioration (Santos et al., 2010).

**Table 3.** The levels of K⁺, Na⁺, Cl⁻, Ca²⁺ and pH in serum of African catfish (*C. gariepinus*) raised at four different stocking densities during a 60-d trial in 120-L tanks with a 150% daily water exchange.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>35 kg m⁻³</th>
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<th>125 kg m⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺ (mmol L⁻¹)</td>
<td>2.46±0.06</td>
<td>2.49±0.05</td>
<td>2.43±0.15</td>
<td>2.46±0.09</td>
</tr>
<tr>
<td>Na⁺ (mmol L⁻¹)</td>
<td>139.56±3.95</td>
<td>141.90±4.69</td>
<td>140.88±3.32</td>
<td>141.27±3.99</td>
</tr>
<tr>
<td>Cl⁻ (mmol L⁻¹)</td>
<td>144.99±8.17</td>
<td>143.48±8.13</td>
<td>143.51±7.84</td>
<td>141.68±9.60</td>
</tr>
<tr>
<td>Ca²⁺ (mmol L⁻¹)</td>
<td>1.33±0.02</td>
<td>1.36±0.05</td>
<td>1.36±0.04</td>
<td>1.35±0.04</td>
</tr>
<tr>
<td>pH</td>
<td>7.50±0.01</td>
<td>7.50±0.02</td>
<td>7.52±0.01</td>
<td>7.50±0.02</td>
</tr>
</tbody>
</table>

Values within rows sharing no letters are not significantly different (P > 0.05). Values within rows sharing no letters or no letters are not significantly different (P < 0.05).
study, fish were fed restrictively, because young catfish can experience a feed-related problem known as “Ruptured Intestine Syndrome” (RIS) or “Open Belly Syndrome” when feeding high feed loads (Hariati et al., 1994). The effects of stocking density on African catfish were not uniform throughout the growth cycle. The African catfish is the second most important cultured fish in the Netherlands and known for its ability to be cultured at densities ranging between 100 to 300 kg m⁻³ at the end of phase 1 in intensive recirculation systems while being reared at high densities up to 500 kg m⁻³ at the end of phase 2 (van de Nieuwegiessen et al., 2009). African catfish is more sensitive to chronic stress induced by high density in the former phase as compared to the later phase of grow-out. It is important to note that the actual group sizes and tank sizes under practical culture conditions are considerably higher than those used in the laboratory conditions. Practical density of African catfish is 55 to 60 kg m⁻³ in some farms in Tianjin, China. e. g. 200,000 fish weighing 30 g were reared in concrete pond containing water (area 60 m²; height 1.6 to 1.7 m), with a 150 % water exchange daily. The data about how increasing group sizes and tank sizes affect growth performance of African catfish is lacking while keeping stocking density fixed, and further study in this field is required to provide more information for the commercial culture.

The increases in skin lesions of African catfish in higher density treatments (95 and 125 kg m⁻³) were observed as compared with fish in treatments with lower density (35 and 65 kg m⁻³). Some examined welfare parameters, such as Hct, Alb and ALT, were affected significantly when stocking density increased to 95 kg m⁻³. These differences in welfare indicators, as well as growth performance, need to be taken into account by husbandry practices when high stocking density was adopted to improve farm profitability.

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