EFFECTS OF DIFFERENT STOCKING DENSITIES ON GROWTH PERFORMANCE, STRESS RESISTANCE, ANTIOXIDANT CAPACITY AND IMMUNITY OF GRASS CARP

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Introduction

Grass carp is a globally important freshwater aquaculture species valued for its rapid growth and adaptability. In intensive systems, high stocking density is used to maximize yield but inevitably causes chronic crowding stress that disrupts homeostasis and welfare. Excessive density intensifies competition, reduces feed intake and growth, and elevates cortisol levels, which trigger metabolic and oxidative disturbances. Antioxidant defenses (e.g., catalase, glutathione) are suppressed, malondialdehyde accumulates indicating lipid peroxidation, and immune-related genes are downregulated, compromising disease resistance. Despite existing knowledge, the integrated effects of density on growth, stress, antioxidant capacity, and immunity in grass carp remain poorly understood. This study examined three densities in pond cages, assessing growth, serum biochemistry, antioxidant status, and innate immunity to identify an optimal balance between productivity and health. Results showed that high density impaired growth, induced stress, and disrupted immunity, whereas moderate density optimally supported growth and physiological health, offering a sustainable strategy for grass carp aquaculture.

Materials and Methods

This experiment was conducted in pond cages measuring 2 m in length, 1.5 m in width and 1 m in height, located in Liulihe, Fangshan District, Beijing. A total of 420 healthy grass carp of uniform size with an initial body weight of 81.76 g were randomly assigned to three groups with three replicates (0.57, 1.13, and 2.27 kg/m^3) . Growth performance were measured after 75 days of cultivation. Reagent kits were used to detect serum biochemical indicators, kidney immune enzyme activity, and liver antioxidant indicators in each group. The expression of spleen immune-related genes was detected using real-time quantitative PCR. Data are expressed as mean ± standard deviation. Oneway ANOVA and Tukey–Kramer's post hoc tests were conducted.

Results and Discussion

Impact of Densities on Growth Trait

As demonstrated in Table 1, the MSD group exhibited significantly higher final body, and the FCR was significantly lower than that of the other two, indicating that the MSD fish group has a better utilization efficiency of feed and the best growth performance. In addition, the dissolved oxygen in the water decreased numerically with the increase in stocking density, indicating that high density leads to water quality deterioration.

Table 1. Effects of different stocking density on growth performance, morphometric parameters and water quality indicators of *Ctenopharyngodon idella*.

Parameters	LSD	MSD	HSD
Initial body weight/g	89.82 ± 5.63	88.61 ± 4.41	86.76 ± 3.04
Final body weight/g	187.33 ± 10.46 ^{b}	237.78 ± 10.67 ^{a}	204.18 ± 9.03 ^{b}
WGR/%	125.0 ± 10.73 ^{c}	178.12 ± 8.38 ^{a}	139.84 ± 7.86 ^{b}
SGR/(%/d)	1.08 ± 0.06 ^{c}	1.40 ± 0.04 ^{a}	1.16 ± 0.03 ^{b}
FCR	1.87 ± 0.13 ^{a}	1.24 ± 0.41 ^{c}	1.35 ± 0.43 ^{b}
CF	1.99 ± 0.12 ^{b}	2.08 ± 0.13 ^{a}	2.11 ± 0.14 ^{b}
dissolved oxygen/mg·L^{-1}	5.67 ± 0.19	5.50 ± 0.20	5.00 ± 0.28
ammonia nitrogen/mg·L^{-1}	0.09 ± 0.03	0.10 ± 0.04	0.11 ± 0.03
nitrite/mg·L^{-1}	0.15 ± 0.04 ^{b}	0.24 ± 0.08 ^{a}	0.30 ± 0.07 ^{a}
pH	7.45 ± 0.14	7.25 ± 0.12	7.52 ± 0.16

Note: Different lowercase superscripts within the same row represent statistical significance ($p < 0.05$). LSD, low stocking density; MSD, medium stocking density; HSD, high stocking density; WGR, weight gain rate; SGR, specific growth rate; FCR, feed conversion ratio; CF, condition factor. Values are expressed as the mean ± standard deviation (SD). Differences among multiple groups were assessed using one-way ANOVA, followed by Tukey–Kramer's post hoc tests to determine specific group differences.

Impact of Densities on Serum Biochemical Indicators

Increasing stocking density progressively elevated serum cortisol and glucose, while decreasing cholesterol and triglycerides. Liver and kidney indicators (ALT, AST, CRE, BUN) rose with density. In kidney, immune enzyme activities (IgM, LZM, AMP, AKP, ACP) declined as density increased. High density significantly worsened all parameters compared to medium and low groups; medium density showed intermediate effects, with low density consistently maintaining the best physiological and immune status.

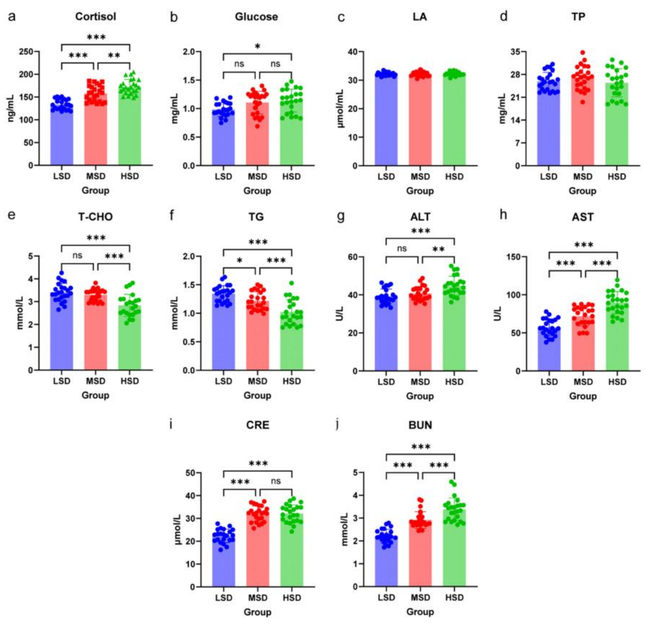


Figure 1. The biochemical index of grass carp was affected by different stocking densities. (a) Cortisol; (b) Glucose; (c) Lactate, LA; (d) Total protein, TP; (e) Total cholesterol, T-CHO, (f) Triglyceride, TG; (g) Alanine aminotransferase, ALT; (h) Aspartate aminotransferase, AST; (i) Creatinine, CRE; (j) Blood urea nitrogen, BUN.

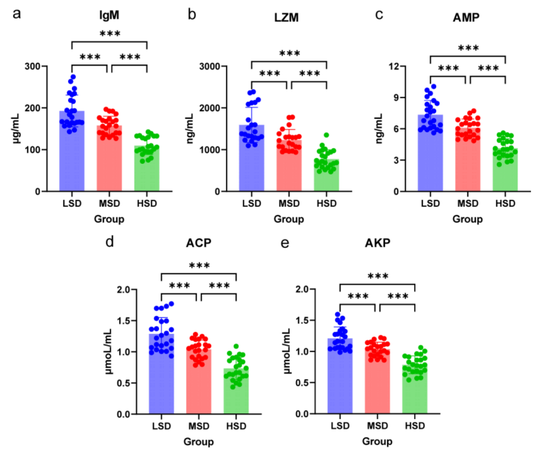


Figure 2. Effects of different stocking densities on immune-related enzyme activities. (a) Immunoglobulin M, IgM; (b) Lysozyme, LZM; (c) Antimicrobial peptides, AMP; (d) Acid phosphatase, ACP; (e) Alkaline phosphatase, AKP.

Impact of Densities on the antioxidant capacity and immune related genes

Liver T-AOC initially rose slightly then declined significantly at high density, with CAT and GSH activities dropping and MDA increasing compared to lower densities. Kidney immune enzymes (IgM, LZM, AMP, AKP, ACP) decreased with rising density, all significantly lower in HSD and MSD versus LSD. Splenic pro-inflammatory cytokines (IL-1β, IL-6, TNF-α), along with IL-10 and IL-8, were upregulated, while IgM gene expression was downregulated under high density, indicating impaired hepatic antioxidant capacity and suppressed systemic immunity.

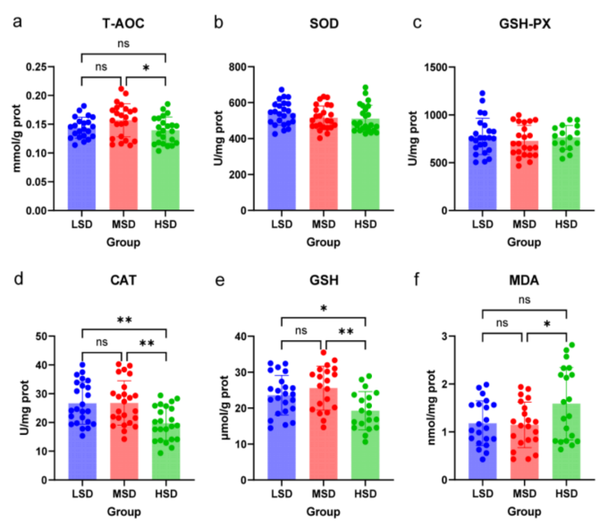


Figure 3. Effects of different stocking densities on the antioxidant capacity of grass carp. (a) Total antioxidant capacity, T-AOC; (b) Superoxide dismutase, SOD; (c) Glutathione peroxidase, GSH-Px; (d) Catalase, CAT; (e) Glutathione, GSH; (f) Malondialdehyde, MDA.

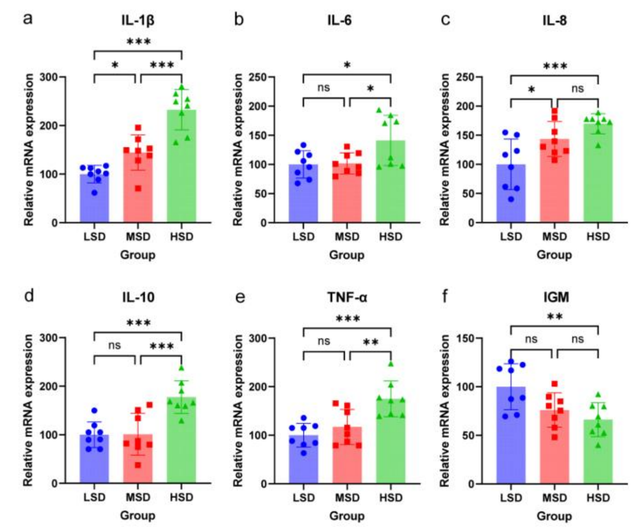


Figure 4. Effects of different stocking densities on the expression of immune-related genes. (a) Interleukin 1β, IL-1β; (b) interleukin 6, IL-6; (c) interleukin 8, IL-8; (d) interleukin 10, IL-10; (e) tumor necrosis factor alpha, TNF-α; (f) immunoglobulin M, IgM.

Conclusions

The present study systematically demonstrates that stocking density exerts a significant regulatory effect on the growth performance, serum biochemical profiles, immune competence, antioxidant capacity, and tissue morphology of grass carp.