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Effect of Stocking Parameters on the Growth of *Cyprinus carpio* (Common Carp) Under Intensive Cage Farming

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SARV acquisition of data, data analysis and interpretation, preparation of manuscript. VSK design of the study, manuscript correction. CJB data analysis and interpretation, manuscript correction. JSSK conception and design of the study, data analysis.

Key words
Common carp, Stocking density, Stocking size, Growth, Survival, Amino acids

ABSTRACT

Cage culture is a globally recognized innovative aquaculture method that offers numerous benefits over traditional fish farming methods. Successful aquaculture management relies on the optimal use of stocking parameters. The present study was conducted to evaluate the influence of stocking parameters such as stocking density and stocking size on the growth of *Cyprinus carpio* under intensive cage farming. In the first set of experiment, *C. carpio* seeds were stocked under three different stocking densities such as 5 nos/m^3 (T1), 10 nos/m^3 (T2) and 15 nos/m^3 (T3). In the second set of experiment, *C. carpio* seeds of three different stocking sizes such as 1" (T4), 2" (T5) and 3" (T6) were stocked in cages at the best performing stocking density arrived at the first experiment (5 nos/m^3). The experiment was carried out for 90 days and the fishes were fed with sinking feed twice a day. Sampling was done fortnightly for the growth parameters, and the water quality parameters were recorded daily. The highest stocking density (T1) and the largest stocking size (T6) showed high growth efficiency, good food consumption metrics, and high concentrations of protein, fat, fiber, ash, and essential amino acids. This study showed that the common carp showed better performance in cages under the stocking density of 5 nos/m^3 with a stocking size of 3" (TL).

INTRODUCTION

Aquaculture is becoming more productive, and cage culture currently contributes significantly in meeting the world's need for fish. Cages are widely used to culture different fish species in various aquaculture environment. In freshwater system, cages are used for rearing fingerlings and fry. High output, tremendous efficiency, running-water culture, and intense farming are typical characteristics of cage culture. The benefits of cage culture could be multiplied by employing fishermen who have been displaced from traditional fisheries so that the economic inputs and fish yield can be increased thereby relieving pressure on traditional fisheries to support their sustainability (Olivares and Brynjolfsson, 2003). The choice of species is crucial for cage culture since not all species are appropriate for all culture systems. Common carp has become one of the widely distributed species around the globe due to its adaptability to a wide range of circumstances. Due to its resilience, quick growth, outstanding meat quality, and omnivorous feeding habits, the species makes a great choice for farming in cage culture. In most culture species, stocking density is crucial factors that influence survival, growth, behaviour, health, water quality, feeding, and productivity (Ferdous *et al*., 2014). According to Sodikin and Joint (1977), the fish culture in cages might be improved by increasing stocking density, using better feeding techniques, choosing species, and controlling the culture cycle to maximize profits. Reduced growth due to stress brought on by higher stocking densities, which raised energy requirements, resulted in decreased growth and decreased food utilization in *Clarias gariepinus* (Hengsawat *et al*., 1997). Sahoo *et al*. (2004) examined the effects of stocking sizes (0.06 g, 0.04 g, and

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0.02 g) on the growth and survival of *Clarius batrachus* fry during fingerling hatchery production and found that the final weight was significantly higher in the larger fish group (0.06 g) than that of the smaller groups (0.02 g). The fundamental variables that determine the meat quality include fish species, catching season, environment, diet, age, sex, and farming procedures (Suarez *et al*., 2014). Hence the present study investigated the effects of different stocking densities and stocking sizes on the growth, survival, meat quality, and cortisol levels of *C. carpio* under intensive cage culture system.

MATERIALS AND METHODS

Experimental fishes

Common carp seeds were procured from Government Fish Seed Farm, Karanthai, Thanjavur, Tamil Nadu, India. The average body weight (ABW) of the fishes were 1.8±0.07 g. Before acclimatizing in FRP tanks, the fish seeds were disinfected with 1% $KMnO_4$. The seeds were then sorted according to their length and weight. Uniform size seeds were used for the experiment. The experiment was carried out at Thanjavur Centre for Sustainable Aquaculture.

Experimental cages

Cages of identical size and design were employed for this study. The experiment included six floating cage units. The dimensions of each cage was 3×3×1.5 m and the total volume of each cage is 13.5 m^3. The cage nets were scrubbed with a brush and treated with $KMnO_4$ to disinfect it. The net was fixed to the cage before commencing the experiment. The mesh size of the inner net was 0.7 mm, and the top end of the outer net was closed in order to protect the fish from bird predators. The cages were deployed in 1 ha open water body at TCeSA. The inlet and outlet of the water body were designed with care to maintain proper water level in the pond.

Experimental design

In the present study, two sets of experiments were carried out in duplicate. In the first set of experiment, seeds were stocked in cages at three different stocking densities such as 5/m^3, 10/m^3, and 15/m^3. Based on the stocking density, the number of seeds stocked in T1, T2 and T3 were 68, 135 and 203 numbers, respectively. The stocking density that resulted in the best growth and survival rate was chosen for the second experiment. In the second experiment, fishes with three different stocking sizes such as 1”, 2” and 3” were used for stocking. The size of the seeds in T4, T5 and T6 were 1.5±0.0 g (1 inch), 1.8±0.07 g (2 inch), and 2.2±0.0 g (3 inch), respectively. Based on the results of first experiment, a total of 68 fishes were stocked in each treatment groups. The duration of each study was 90 days.

Feeding, sampling and harvesting

Fish were fed at 10% body weight (BW) during the first month of fish rearing; after which it was reduced to 5% BW of fish throughout the culture period. The fishes were fed with commercial sinking feed twice a day at 10 am and 4 pm. The fishes were sampled every fortnight and the growth parameters were noted. Accordingly, the feeding rate was adjusted. During sampling, the bio-growth parameters and feed utilization parameters were documented. Visual inspection was done to evaluate the health of the fish. Fish were given a prophylactic 10-minute soak in 5 mg/l concentration of $KMnO_4$ solution after each handling. At the end of the experiment, the fish were harvested from the cages by lowering the cage net. The total fish biomass was counted and weighed in each treatment.

Physico-chemical parameters of water

Water quality parameters such as temperature, pH, and dissolved oxygen were recorded daily throughout the experiment, whereas total alkalinity, total hardness, chemical oxygen demand, total ammonia nitrogen, nitrite nitrogen, and nitrate were tested once a week. The parameters were estimated following standard procedures (APHA, 1998).

Meat quality analysis

According to the AOAC protocol (AOAC, 1990), the proximate composition of the fish meat such as the protein, fat, ash and moisture was determined. Following the methodology outlined by Osibona *et al*. (2009), the aminoacids were examined. An amino acid analyzer LC3000 (Eppendorf-Bio-tronik; Germany) was used to analyze the samples.

Cortisol assays

The cortisol radioimmunoassay measurement was done following Zhang *et al*. (2020). In a chilled centrifuge (4°C), tissue samples were homogenized in cold PBS and centrifuged at 3000 rpm for 20 min. The supernatants were collected and the cortisol content was estimated using Sigma ELISA kits.

Statistical analysis

All the observations were recorded, tabulated and processed. Data were presented as mean standard deviation and analysed using one-way ANOVA. The significant difference between the treatments was determined by

Duncan's multiple range test (DMRT) using SPSS (version 20.0). The level of significance employed was 0.05.

RESULTS AND DISCUSSION

Growth performance of C. carpio

Oreochromis niloticus produced best growth performance at the lowest stocking density (50 nos/cage) (Osofero *et al*., 2009). Ahmed *et al*. (2002) found that the common carp produced the highest net weight gain, net weight gain percentage, daily weight gain, and daily weight gain percentage at the lowest stocking density (25 nos/m^2). Similar findings were reported by Allison *et al*. (1976), Hastings (1973), and Marek (1975), showing that more significant growth occurred at lower stocking numbers. In the present study also, net weight gain, net weight gain percentage, daily weight gain, daily weight gain percentage and specific growth rate were higher in lowest stocking density (T1) (Table I).

Rahman *et al*. (2006) found that increasing stocking density led to higher yield. In the present study, the highest net biomass production and biomass production/m^3 (Table II) were found in highest stocking density (T3). Lembi *et al*. (1968), Kilambi *et al*. (1977) and Hasan *et al*. (2010) suggested that intense rivalry while feeding in crowded cages may be the cause of the individual growth drop with stocking density. In the present study, the lowest FCR and the highest FCE, PER and survival rate were found in lowest stocking density (T1). Similarly, Kohinoor and Rahman (2014) reported that the lowest stocking density (80 nos/m^3) of common carp cultured in cages had the highest survival rate.

Table I. Bio-growth parameters of *C. carpio* cultured in cages under different stocking parameters.

Bio-growth parameters	Stocking density			Stocking size		
	T1 (5/m^3) (n = 68)	T2 (10/m^3) (n = 135)	T3 (15/m^3) (n = 203)	T4 (1 inch) (n = 68)	T5 (2 inch) (n = 68)	T6 (3 inch) (n = 68)
Initial MBW (g)	1.7±0.07^{a}	1.8±0.07^{a}	1.8±0.14^{a}	1.5±0.0^{c}	1.8±0.07^{b}	2.2±0.0^{a}
Final MBW (g)	96.3±0.1^{a}	85.2±0.1^{b}	72.3±0.1^{c}	73.1±0.0^{c}	96.3±0.1^{b}	130.7±0.07^{a}
Total WG (g)	94.5±0.07^{a}	83.3±0.07^{b}	70.50±0.0^{c}	71.6±0.0^{c}	94.4±0.2^{b}	128.5±0.07^{a}
Weight gain (%)	5407.1±214.4^{a}	4508.6±168.5^{ab}	3928.7±308.7^{b}	4773.3±0.0^{b}	5109.3±206.7^{b}	5843.1±3.2^{a}
ADWG(g)	1.05±0.0^{a}	0.9±0.0^{b}	0.7±0.0^{c}	0.7±0.0^{c}	1.04±0.0^{b}	1.42±0.0^{a}
DWG (%)	60.05±2.4^{a}	49.7±1.9^{ab}	43.4±3.4^{b}	52.6±0.0^{b}	56.5±2.5^{b}	64.5±0.0^{a}
SGR (%)	4.4±0.04^{a}	4.2±0.04^{ab}	4.1±0.08^{b}	4.31±0.0^{b}	4.39±0.04^{b}	4.5±0.0^{a}

The number of seeds stocked in T1, T2 and T3 were 68, 135 and 203. The size of the seeds in T1, T2 and T3 were 1.5g (1 inch), 1.8g (2 inch) and 2.2g (3 inch). Data expressed as Mean ± Standard deviation. Values with different superscripts within the column differ significantly (P<0.05). MBW, mean body weight; WG, weight gain; DWG, daily weight gain; ADWG, average daily weight gain; SGR, specific growth rate.

Table II. Feed utilization parameters of *C carpio* cultured in cages under different stocking parameters.

Feed utilization parameters	Stocking density			Stocking size		
	T1 (5/m^3) (n = 68)	T2 (10/m^3) (n = 135)	T3 (15/m^3) (n = 203)	T4 (1 inch) (n = 68)	T5 (2 inch) (n = 68)	T6 (3 inch) (n = 68)
IBM (kg)	0.12±0.0^{c}	0.25±0.01^{b}	0.37±0.03^{a}	0.10±0.0^{c}	0.12±0.0^{b}	0.14±0.0^{a}
FBM (kg)	6.31±0.06^{c}	10.69±0.04^{b}	13.27±0.03^{a}	4.5±0.0^{c}	6.3±0.05^{b}	8.6±0.08^{a}
FCR	1.4±0.01^{c}	1.6±0.01^{b}	1.8±0.01^{a}	1.52±0.01^{a}	1.43±0.0^{b}	1.34±0.01^{c}
FCE (%)	70.8±0.6^{a}	63.6±0.2^{b}	56.2±0.1^{c}	67.1±0.7^{c}	70.8±0.6^{b}	75.6±0.7^{a}
PER	2.02±0.01^{a}	1.8±0.01^{b}	1.6±0.0^{c}	1.9±0.0^{c}	2.02±0.01^{b}	2.16±0.02^{a}
NBM (Kg)	6.19±0.06^{c}	10.44±0.05^{b}	12.9±0.05^{a}	4.4±0.05^{c}	6.1±0.05^{b}	8.5±0.08^{a}
Production rate/m^3 (kg)	0.45±0.01^{c}	0.77±0.0^{b}	0.96±0.0^{a}	0.33±0.00^{c}	0.45±0.0^{b}	0.63±0.0^{a}
Survival (%)	96.2±1.1^{a}	92.6±0.5^{b}	90.3±0.3^{b}	91.8±1.06^{b}	96.2±1.09^{a}	97.5±0.7^{a}

Data expressed as Mean ± Standard deviation. Values with different superscripts within the column differ significantly (P<0.05). IBM, initial biomass; FBM, final biomass; NBM, net biomass; FCR, food conversion ratio; FCE, food efficiency ratio; PER, protein efficiency ratio. For details of treatment group, see Table I.

Table III. Proximate composition (%) and cortisol level (µg/dL) of *C. carpio* cultured in cages under different stocking parameters.

Proximate composition and cortisol level	Stocking density			Stocking size		
	T1 ($5/m^3$) (n = 68)	T2 ($10/m^3$) (n = 135)	T3 ($15/m^3$) (n = 203)	T4 (1 inch) (n = 68)	T5 (2 inch) (n = 68)	T6 (3 inch) (n = 68)
Crude protein (%)	16.2±0.1^{a}	14±0.2^{b}	12.4±0.2^{c}	14±0.2^{c}	16.2±0.1^{b}	17±0.1^{a}
Crude Fat (%)	3±0.1^{a}	2.6±0.2^{a}	2.5±0.1^{a}	2.6±0.2^{b}	3±0.1^{b}	3.7±0.1^{a}
Crude Fiber (%)	2±0.1^{a}	1.8±0.2^{a}	1.6±0.1^{a}	1.8±0.2^{a}	2±0.1^{a}	2.4±0.2^{a}
Ash (%)	0.8±0.1^{a}	0.6±0.1^{a}	0.5±0.1^{a}	0.6±0.1^{a}	0.8±0.1^{a}	0.9±0.1^{a}
Moisture (%)	78±0.2^{c}	81±0.1^{b}	83±0.1^{a}	81±0.1^{a}	78±0.2^{b}	76±0.1^{c}
Cortisol level (µg/dL)	1.27±0.01^{c}	1.55±0.01^{b}	1.95±0.01^{a}	1.15±0.01^{c}	1.27±0.01^{b}	1.43±0.01^{a}

Data expressed as Mean ± Standard deviation. Values with different superscripts within the column differ significantly (P<0.05). For details of treatment group, see Table I.

Table IV. Essential amino acid profile (g/100g of crude protein) of *C. carpio* cultured in cages under different stocking parameters.

Essential amino acid profile	Stocking density			Stocking size		
	T1 ($5/m^3$) (n = 68)	T2 ($10/m^3$) (n = 135)	T3 ($15/m^3$) (n = 203)	T4 (1 inch) (n = 68)	T5 (2 inch) (n = 68)	T6 (3 inch) (n = 68)
Tryptophan (g)	3.56±0.02^{a}	3.28±0.01^{b}	3.02±0.01^{c}	3.28±0.01^{c}	3.56±0.02^{b}	3.74±0.02^{a}
Threonine (g)	3.48±0.01^{a}	3.18±0.01^{b}	2.78±0.01^{c}	3.18±0.01^{c}	3.48±0.01^{b}	3.66±0.02^{a}
Valine (g)	3.34±0.02^{a}	3.08±0.01^{b}	2.62±0.01^{c}	3.08±0.01^{c}	3.34±0.02^{b}	3.53±0.02^{a}
Phenylalanine (g)	2.78±0.01^{a}	2.52±0.01^{b}	2.22±0.01^{c}	2.52±0.01^{c}	2.78±0.01^{b}	2.92±0.01^{a}
Leucine (g)	2.44±0.01^{a}	2.22±0.01^{b}	2.02±0.01^{c}	2.22±0.01^{c}	2.44±0.01^{b}	2.56±0.01^{a}
Histidine (g)	2.18±0.01^{a}	2.06±0.02^{b}	1.84±0.02^{c}	2.06±0.02^{c}	2.18±0.01^{b}	2.38±0.01^{a}
Isoleucine (g)	2.12±0.01^{a}	2.02±0.01^{b}	1.82±0.01^{c}	2.02±0.01^{c}	2.12±0.01^{b}	2.32±0.01^{a}

Data expressed as Mean ± Standard deviation. Values with different superscripts within the column differ significantly (P<0.05). For details of treatment group, see Table I.

Proximate composition of C. carpio

Due to the negative impacts of greater stocking density on body composition, *Ictalurus punctatus* (Refaey *et al*., 2018) and *Clarias gariepinus* (Toko *et al*., 2007) both had reduced protein contents. In line with the aforementioned results, the present investigation (Table III) found that the group with the lowest stocking density (T1) had the highest concentrations of protein, fat, fiber, and ash. Similarly, Toko *et al*. (2007) found that *H. longifilis* and *C. gariepinus* had the highest lipid contents in lower stocking densities. However, *Oncorhynchus mykiss* exhibited the maximum protein content at intermediate stocking densities (Cagiltay *et al*., 2015).

Essential amino acid profile of C. carpio

There was a reduction in the amount of several amino acids in *Brachymystax lenok* cultured under high stocking density (Liu *et al*., 2019). Alanine, valine, and glutamate are among the amino acids that have decreased in the liver, which suggests that amino acids may be used by the lenok as an energy source to deal with high stocking density. Corresponding to the previous findings, the present study recorded that *C. carpio* grown at high stocking density (T3) had lower levels of essential amino acids while lower stocking density (T1) had higher level of essential amino acids. As shown in Table IV, the examined samples included seven essential amino acids such as tryptophan, threonine, valine, phenylalanine, leucine, histidine, and isoleucine that are vital to human health.

Cortisol level of C. carpio

According to Vijayan and Leatherland (1988), and Wedemeyer (1997), high stocking density has been identified as an aquaculture-related chronic stressor that causes a chronic elevation of plasma cortisol, the primary corticosteroid in fish (Wendelaar, 1997; Mommsen *et al*., 1999). Bolasina *et al*. (2006) found that *Paralichthys olivaceus* populations with the highest stocking densities had the highest cortisol levels. In the present study also, cortisol level was higher in highest stocking density (T3) (Table III).

Growth performance of C. carpio

The size of fish used for stocking determines the economic sustainability of the various production systems (Sahoo *et al*., 2004). In the present study, larger stocking size (T6) showed the best growth performance. Arce and Lopez (1981) assert that larger fingerlings live longer, grow faster, and mature more quickly. In the present study, net weight gain, net weight gain percentage, daily weight gain, daily weight gain percentage and specific growth rate were higher in largest stocking size (T6) (Table I).

Suresh and Lin (1992) found that as the density increased, the net yield also increased. In the present study, highest net biomass production and biomass production/m^3 were higher in largest stocking size (T6) (Table II). Sweilum *et al*. (2005) asserted that small fish had greater FCR values than large fish did. In the present study, the lowest FCR and the highest FCE and PER were noticed in largest stocking size (T6). According to Sahoo *et al*. (2004), the survival rate was also considerably lower when the stocking size was smallest. In the present study, survival rate was higher in larger stocking size (T6).

Proximate composition of C. carpio

The present study found increased concentrations of protein, fat, fiber, ash and decreased moisture in largest stocking size (T6) (Table III). This concurs with the findings of Breck (2014), who stated that larger fish contained less water per unit of protein. Similarly, Naeem and Ishtiaq (2011) found positive correlation between the body weight (wet and dry weight), protein and organic contents of *Mystus bleekeri*. In *Aristichthys nobilis*, moisture content decreased with body weight, whereas protein and fat content increased as body weight increased (Naeem and Salam, 2010).

Essential amino acid profile of C. carpio

The edible portion of the farmed *C. carpio* has higher quantities of amino acids than the wild fish (Sarma *et al*., 2013; Mohanty *et al*., 2014). Ahmed (2022) reported that farmed common carp contained a high concentration of tryptophan followed by threonine, valine, phenylalanine, leucine, histidine, and isoleucine in decreasing order. This is consistent with the findings of the present study that the farmed common carp under different sizes contained a high amount of tryptophan followed by threonine, valine, phenylalanine, leucine, histidine, and isoleucine in decreasing levels. As a result (Table IV), larger stocking sizes (T6) had higher essential amino acid levels and smaller stocking sizes (T4) had lower essential amino acids.

Cortisol level of C. carpio

Barnett and Pankhurst (1998) documented the stress response to a variety of aquaculture procedures in greenback flounder, *Rhombosolea tapirina*. Waring *et al*. (1992) described the stress reaction of flatfish, *Platichthys flesus* to handling and net confinement. These two investigations demonstrated that stresses like crowding, confinement, and handling can raise plasma cortisol levels. When groups are larger, dominants have slightly greater cortisol levels than subordinates (Bessa *et al*., 2021). In the present study, cortisol level was higher in largest stocking size (T6) (Table III).

From the present study it can be concluded that highest stocking density (5 nos/m^3) and larger stocking size (3” TL) resulted in higher growth, better survival, superior meat quality, and lower cortisol levels in *C. carpio.*

DECLARATIONS

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IRB approval

The research committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tuticorin, India has approved the work.

Ethical statement

The experiment was conducted following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division), Govt. of India on care and use of animals in scientific research. The fish were raised and handled in accordance with the guidelines set forth by the animal ethics committee, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam.

Data availability statement

The article contains the data that support the study’s conclusions.

Statement of conflict of interest

The authors have declared no conflict of interest.

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