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Optimizing Stocking Density for Rearing Ornamental Koi Carp in Pond Cage Aquaculture System

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Abstract: The experimental study (90 days) was carried out to optimize the stocking density of ornamental koi carp in pond cage aquaculture system in integration with Indian major carps (IMCs). Koi carp fingerlings were stocked in 15 (5 treatments in triplicate) bamboo net cages (1.0 m^3) fixed in earthen pond (380 m^2) at stocking densities (fish m^{-3}) of 20, 40, 60, 80 and 100, whereas, IMCs (catla, rohu and mrigal) were stocked at standard stocking density of 4000 fingerlings acre^{-1} (380 fish) in ratio of 3:4:3 in earthen pond. Koi carp were fed with commercial feed @ 5% of fish body weight (BW) twice daily and IMCs were fed with formulated pelleted feed @ 1% of BW once daily throughout the experiment. The physico-chemical parameters did not revealed any negative impact of stocking density on water quality. Significantly higher fish survival, growth as well as enhanced feed efficiency in terms of FCR was observed in low stocking density treatments (20 and 40 fish m^{-3}). Haematological parameters w.r.t. Hb and Ht increased with maximum value in treatment with 100 fish m^{-3}, whereas TLC decreased with minimum value in 60 fish m^{-3}, and TEC did not revealed any significantly change. Among biochemical parameters, significant variations were observed for blood serum glucose, total protein and total immunoglobulins, whereas, there were no significant variations in albumin, globulins and A/G ratio. Increase in serum concentration of cortisol, decrease in triiodothyronine and thyroid stimulatinmg hormone and no change in tetraiodothyronine were observed with increasing stocking density. Survival of all the three major carps remained above 90% with impressive growth and FCR. It can be concluded from the present study, that ornamental koi carp can be successfully integrated with carps in pond cage system up to 40 fish m^{-3} for higher economic gain from the available water resource.

Keywords: Cage culture, Stocking density, Koi carp, Crowding stress, Physiology

In Punjab, carp farming remained most viable option in view of availability of raw material in the form of quality seed and environmental conditions. However, recently, in addition to carp culture, farmers are showing interest in adopting diverse culture systems and species for higher productivity and profitability. In this context, ornamental fish culture is one of the easily adoptable activity as a diversified component along with carp farming due to various reasons including small investment, ease of adoption as an ancillary activity and easy involvement of women. In comparison with food fish culture carried out in large sized earthen ponds, ornamental fish culture can be taken up in small concrete tanks, earthen ponds or even cages/hapas installed in earthen ponds. A management strategy based on the concept of integrating intensive and semi-intensive culture practices in cages and ponds simultaneously (cage-cum-pond-integrated system) has been suggested as an endeavour to increase per unit fish production (Mokoro et al 2014). Moreover, compared to nutrient utilization efficiency of about 30% in most intensive culture systems, the nutrient utilization efficiency could reach more than 50% in integrated pond-cum-cage culture system, resulting in release of much less nutrient to the surrounding environment (Asaduzzaman et al 2006). Further, the integrated pond-cum-cage system provide an option to small scale farmers to utilize limited resources in terms of land and water to generate additional income from high value ornamental fish cultured in cage integration with carps cultured in pond, so as to improve their livelihood. Stocking density is the most important factor determining the productivity of a culture system which affects survival, size and growth of fish (Swain et al 2018). Among various stresses which affects the fish while rearing under restricted environment like cages, is the inappropriate stocking density, as it represent a potential source of chronic stress, which may have adverse effects on physiological, behavioural and health status. It is a crucial factor in determining productivity and profitability of aquaculture systems (North et al 2006, Braun et al 2010). Among various freshwater ornamental fishes, 'Koi carp', *Cyprinus carpio* (family – Cyprinidae), ornamental mutation of common carp is a native from Asia, especially China and Japan. The term “Koi” refers to many strains of ornamental carp that have been genetically selected over many generations (Feldlite and Milstein 2000). In view of the importance of culturing ornamental fish especially koi carp in cages, there is need to standardize the stocking density for rearing koi carp fry to marketable size for getting maximum production without disturbing physiology of fish.

MATERIAL AND METHODS

Preparation and maintenance of experimental pond: The experiment was carried out in bamboo net cages (1.0 m^3) installed in lime disinfected earthen pond (380 m^2). 15 cages were installed in the form of battery of 5 cages each in one row (3 rows x 5 cages=15 cages). Tube well water was used for filling, maintaining and exchanging the water in the pond. After one week of liming and initial filling of water, fertilizer slurry was added in the pond for natural food (plankton) production for fish. 1/4th of water from experimental tanks was exchanged with fresh water once every fortnight. Cages was cleaned thoroughly once in a week to get rid of algae. The water quality parameters of all the cages and earthen pond were analysed before stocking the fish.

Acclimatization and stocking of experimental fishes: Equal sized active and healthy ornamental Koi carp (length – 5.50cm; weight–3.01g) was acclimatized under well oxygenated conditions for 15 days under controlled conditions. During acclimatization, fish were fed @ 5% of their body weight twice a day with commercial feed. Acclimatized fish were stocked in 15 cages (5 treatments in triplicate) @ 20, 40, 60, 80 and 100 fish m^{-3} designated as SD1, SD2, SD3, SD4 and SD5 respectively in June 2021 by following complete randomized design. Indian major carps (IMCs) i.e. catla (length – 4.13 cm &. weight – 0.96 g), rohu (length – 10.35 cm and. weight – 13.47 g) and mrigal (length – 12.06 cm and weight – 17.07 g) were stocked in ratio of @ fish m^{-3} 3:4:3 in earthen pond, in which cages were installed.

Fish feeding: Experimental koi were fed with commercial feed @ 5% of fish body weight (BW) twice daily (10:00 and 16:00 h) and IMCs were fed with formulated pelleted feed (rice bran and mustard cake - 49% each, vitamin mixture – 1.5% and salt 0.5%) @ 1% of BW once daily (10:00) throughout the experiment (June-August 2021). Amount of feed for Koi carp was adjusted after each sampling according to increase in fish weight.

Water quality parameters: Water quality parameters in terms of temperature, pH, dissolved oxygen (DO), total alkalinity (TA), total hardness (TH), ammonical - nitrogen (NH_3-N), nitrite - nitrogen (NO_2^--N) and nitrate - nitrogen (NO_3^--N) (NH_3) were analysed at fortnightly intervals following by the method of APHA (2012).

Survival of fish: Survival (%) of fish in each treatment was recorded by comparing the live fish recovered at the end of the experiment with that of total fish stocked according to following formula

$$\text{Fish survival (\%)} = \frac{\text{No. of fish recovered after completion of experiment}}{\text{No. of fish stocked at initiation of experiment}} \times 100$$

Growth of fish: Fish sampling was done at monthly intervals for koi carp and at the completion of experiment for IMCs to record fish growth in terms of total body length and body weight. Total body length gain (TBLG) and %TBLG, net weight gain (NWG) and %NWG, specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (K) of fish for each treatment were calculated. (Halver 1976).

$$\text{SGR (\% weight gain day}^{-1}\text{)} = \frac{\text{ln final BW (g)} - \text{ln initial BW (g)}}{\text{Culture days}} \times 100$$

Where, ln = natural logarithm

Feed conversion ratio (FCR) = Feed given (g)/weight gain (g)

Condition factor (K-value) = Body weight (g)/ body Length $(cm)^3 \times 100$

Haematological parameters: Haematological parameters were analysed at the completion of experiment for total erythrocytes count (TEC), total leukocytes count (TLC), haemoglobin (Hb), haematocrit (Ht) by following the methods of Sahli (1962) and micro-capillary (Mukherjee 1988).

Biochemical parameters: Biochemical parameters in terms of blood glucose (Trinder 1969), total proteins (Gornall et al 1949), globulin, albumin and total immunoglobulin were estimated and albumin/globulin ratio was calculated in blood serum after completion of the experiment with Erba Diagnostic Mannheim GmbH kits

Hormonal profile: Hormonal profile were analysed at monthly intervals for Cortisol, triiodothyronine (T_3), tetraiodothyronine (T_4) and thyroid-stimulating hormone (TSH) with the help of ELISA kit.

Statistical analysis: Statistical analysis of the data was performed with a Statistical Package for the Social Science (SPSS v.16.0) with Duncan's Multiple Range Test to determine significant differences among the treatments (Duncan 1955).

RESULTS AND DISCUSSION

Water quality- The water quality parameters viz. temperature, pH, DO, TA,TH, NO_2-N and NO_3-N ranged between 28.33 - 35.30˚C, 7.38-8.23, 7.6-16.93 mg l^{-1}, 228.67 – 282.00 $CaCO_3$ mg l^{-1}, 226.00 – 282.00 $CaCO_3$ mg l^{-1}, 0.01 - 0.04 mg l^{-1}, 0.18 - 0.49 mg l^{-1} in all treatments and earthen pond, whereas mean NH_3 remained nil. The differences were insignificant for all the parameters among all treatments except pH. Further, the values for all the water quality parameters were well within the desirable range required for normal growth and physiological activities of fish (Boyd and Tucker 1998), which revealed that stocking densities did not affected the water quality.

Survival and growth of fish - Survival (%) of fish ranged from 87.33 to 96.67 with significantly higher fish survival in SD1 (96.67) and SD2 (94.17) as compared to other treatments (SD3-SD5), which were insignificantly different from each other. At the end of experimental period, TBLG and TBLG (%) was significantly higher (2.47 and 45.44 cm) in SD2, but with no significant difference from SD1. The differences were insignificant for both the parameters among rest of the treatments. In accordance to length of fish, weight parameters (NWG and SGR) were also significantly higher in SD1 and SD2 as compared to other treatments. The remaining parameters i.e. % NWG and condition factor (K) did not differ significantly. Feed efficiency in terms of FCR was also significantly reduced in SD1 and SD2 as compared to all other treatments (Table 1). The best growth performance of fish in terms of weight gain and specific growth rate at lower stocking density of 20 and 40 fish m^{-3}, got affected with further increase in stocking density. The enhanced fish growth at lowest stocking densities may be explained as accessibility of comfortable space to fish for growth. Most of the previous studies also revealed that lower stocking densities had positive effect on fish in terms of weight gain, growth and survival of species like *Cyprinus carpio* Lin. (Ahmed et al 2002), goldfish (Stone et al 2003, Jahedi et al 2012), *Anabas testudineus* (Uddin et al 2016), *Oreochromis niloticus* (Gibtan et al 2008). In present study, fish growth decreased significantly with significantly increased feed conversion ratio with increase in stocking densities. Yang et al (2020), also reported decreased growth performance and increased FCR with the increase in stocking density.

Haematological parameter: At highest stocking densities, haematological parameters w.r.t. Hb and Ht of koi carp increased and maximum in SD5 (100 m^{-3}), whereas TLC decreased, with minimum in SD3, whereas TEC did not revealed any significantly change (Fig. 1). Most of the

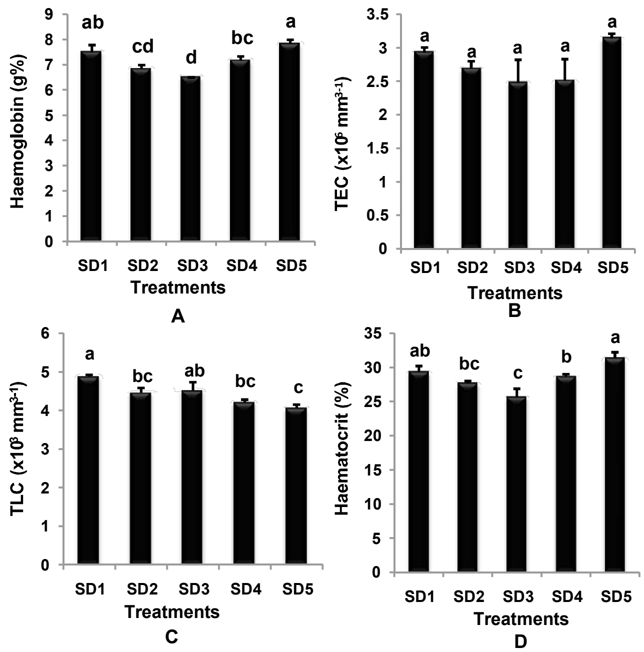

Fig. 1. Comparative A) Hb (g %) B); TEC ($x10^{6}$ mm^{3-1}); C) TLC ($x10^{3}$ mm^{3-1}) and D) Haematocrit (%) content in blood of Koi carp in different treatments after completion of the experiment

Table 1. Growth parameters of Koi carp in different treatments

Parameters	Treatments				
	SD1	SD2	SD3	SD4	SD5
			Length parameters		
ITBL (cm)	5.38^{a}	5.44^{a}	5.48^{a}	5.51^{a}	5.70^{a}
FTBL (cm)	8.14^{a}	7.97^{a}	6.99^{b}	6.99^{b}	6.94^{b}
TLG (cm)	2.37^{a}	2.47^{a}	1.53^{b}	1.43^{b}	1.50^{b}
TLG (%)	42.90^{a}	45.44^{a}	28.68^{b}	26.90^{b}	29.10^{b}
			Weight parameters		
IBW (g)	3.20^{a}	3.07^{a}	2.93^{a}	2.93^{a}	2.93^{a}
FBW (g)	11.60^{a}	11.27^{a}	8.47^{b}	7.40^{c}	6.53^{d}
NWG (g)	8.40^{a}	8.20^{a}	5.53^{b}	4.47^{c}	3.60^{d}
NWG (%)	264.43^{a}	253.43^{a}	188.57^{b}	152.47^{c}	122.73^{d}
SGR (%)	1.43^{a}	1.40^{a}	1.18^{b}	1.03^{c}	0.89^{d}
Condition Factor (K)	2.15^{a}	2.24^{a}	2.48^{a}	2.18^{a}	1.95^{a}
FCR	2.00^{b}	1.99^{b}	2.55^{a}	2.61^{a}	2.50^{a}

* SD = Stocking Density SD1 = 20 fish m^{-3}, SD2= 40 fish m^{-3}, SD3 = 60 fish m^{-3}, SD4= 80 fish m^{-3}, SD5 = 100 fish m^{-3}
Values with same superscript (a, b,…….d) in row do not differ significantly ($p \leq 0.05$)
ITBL – Initial total body length; FTBL – Final total body length; TLG – Total length gain; NWG – Net weight gain; SGR – Specific Growth Rate; FCR – Feed conversion ratio

previous studies also revealed variations in physiological responses (Bacchetta et al 2020, Khairnar et al 2020). Insignificant change in TEC and decreases in TLC at highest stocking densities coincides with the results of Yarahmadi et al (2015), in terms of decreased WBC at highest stocking densities. It may be due to sub chronic overcrowding stress and increased activity of pituitary internal axis caused by increased secretion of cortisol level (Tort 2011)

Biochemical Parameters: The blood serum glucose, total protein and total immunoglobulins of koi carp was significantly higher in SD5, SD3 and SD1 and SD2 respectively, whereas, the differences were insignificant for albumin, globulins and A/G ratio (Table 2). Increase in serum glucose level at highest stocking densities coincides with the findings of earlier researcher in different fish species like Asian seabass (Sadhu et al 2014); tilapia (Aly et al 2009); golden pompano (Yang et al 2020). The increase in serum glucose level is an indication of strategy to cope up with high stocking density stress. Decrease in total protein content at highest stocking densities, supported with the findings of Yang et al (2020), whereas the results of other parameters coincide with the investigation of Yarahmadi (2015), where

Table 2. Biochemical parameters in blood serum of Koi carp in different treatments after completion of the experiment

Biochemical parameters (g dl^{-1})	Treatments				
	SD1	SD2	SD3	SD4	SD5
Glucose	97.43^{b}	92.27^{b}	90.97^{b}	94.16^{b}	108.60^{a}
Total Protein	2.87^{ab}	2.79^{b}	2.90^{a}	2.84^{ab}	2.04^{c}
Albumins	1.34^{a}	1.38^{a}	1.40^{a}	1.30^{a}	1.34^{a}
Globulins	1.53^{a}	1.45^{a}	1.51^{a}	1.57^{a}	0.86^{a}
A/G Ratio	0.88^{a}	1.21^{a}	1.04^{a}	0.93^{a}	1.62^{a}
Total Ig	1.42^{a}	1.42^{a}	1.15^{ab}	1.23^{ab}	1.01^{b}

Values with same superscript (a, b,…….d) in row do not differ significantly (p≤0.05)
A/G ratio = Albumin/Globulins ratio; Ig - Immunoglobulins

Table 3. Variations in hormonal profile of blood serum of Koi carp in different treatments at monthly intervals

Time of observation (days)	Treatments				
	SD1	SD2	SD3	SD4	SD5
T3 (ng ml^{-1})					
0	3.9^{a1}	3.9^{a1}	3.9^{a1}	3.8^{a1}	3.9^{a,1}
30	3.7^{a2}	3.5^{bc2}	3.3^{d2}	3.6^{ab2}	3.4^{cd,2}
60	3.6^{a2}	3.5^{a2}	3.3^{b2}	3.5^{a2,3}	3.2^{b,3}
90	3.5^{a2}	3.5^{ab2}	3.2^{bc2}	3.3^{ab3}	3.2^{c,3}
T4 (ng ml^{-1})					
0	3.3^{a1}	3.4^{a1}	3.5^{a1}	3.3^{a1}	3.3^{a1}
30	3.3^{a1}	3.3^{a1}	3.4^{a1}	3.3^{a1}	3.3^{a1}
60	3.3^{a1}	3.3^{a1}	3.4^{a1}	3.4^{a1}	3.2^{a1}
90	3.2^{a1}	3.1^{a1}	3.4^{a1}	3.4^{a1}	3.2^{a1}
TSH (ng ml^{-1})					
0	3.88^{a1}	3.91^{a1}	3.88^{a1}	3.90^{a1}	3.92^{a1}
30	3.53^{b2}	3.59^{ab2}	3.79^{a1}	3.53^{b2}	2.26^{c2}
60	2.61^{b3}	2.14^{a3}	2.13^{a2}	2.83^{a3}	2.40^{c2}
90	0.58^{a4}	0.35^{bc,4}	0.50^{ab3}	0.44^{ab4}	0.17^{c3}
Cortisol (ng ml^{-1})					
0	3.43^{a2}	3.51^{a12}	3.36^{a3}	3.49^{a2}	3.37^{a3}
30	3.43^{b2}	3.45^{b2}	3.61^{a2}	3.69^{a12}	3.76^{a2}
60	3.44^{c12}	3.51^{c12}	3.76^{b1}	3.78^{b1}	3.93^{a1}
90	3.52^{d1}	3.62^{c1}	3.83^{b1}	3.84^{b1}	3.99^{a1}

Values with same superscript (a, b,…….d) in row and column (1, 2……4) do not differ significantly (p≤0.05) no significant changes on globulin and albumin with increasing stocking density. The biochemical parameters of the fishes are affected by several factors such as species, environmental, response of condition, dietary, age, maturation and nutrition. The variation in biochemical parameters is indicative of crowding stress along with poor nutritional status of fish at high stocking density (Wu et al 2018). Significantly high total immunoglobulins in SD1 & SD2 and minimum in highest stocking density (SD5) can be the result of crowding stress leading to decreased plasma lysozyme activity in fish (Yarahmadi et al 2015, Costa et al 2017). Lysozyme is a major index of the immune system that involves a lysis process of Gram-positive bacterial cell walls. These results coincides with the findings of Long et al (2019) in terms of significant decline in serum immunoglobulin M (IgM), lysozyme, alkaline phosphatase, and acid phosphatase activities at highest stocking density.

Hormonal profile: The present study in terms of decreased concentation of T3 and TSH and insignificant change in T4 (Table 3) with increasing stocking density supported with the results of Kpundeh et al (2013) and Li et al (2012) respectively. Reduced T3 & TSH levels in the present study with increase in stocking density must be associated with physiological stress. At monthy intervals, with increase in stocking density there is a significant increase in the level of cortisol (Table 3) corresponds well with the findings of earlier researcher in different fish species like Asian seabass (Sadhu et al 2014), Fancy carp (Yin et al 1995); Golden pompano (Yang et al 2020),and Jundia (Barcellos et al 2004).

Survival and growth of Indian Major Carps (IMCs): Survival of all the IMCs remained above 90% with maximum growth in terms of % NWG and SGR in catla (2787 and 6.3). Further, FCR of 0.072 also revealed that the available feed (IMC fed @ 1% BW + unfed feed of Koi carp, if any) was also efficiently utilized by the carps.

CONCLUSIONS

The koi carp can be reared at stocking density of 40 m^{-3} in cages in integration with carps in earthen pond with no negative impact on water quality, without compromising survival and growth of fish and without any major effect on other physiological parameters.

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