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To evaluate the production performance of carps stocked at various stocking densities

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Abstract
Four different rearing densities T1 (800), T2 (1200), T3 (1600) and T4 (2000) were stocked with ratio of 80% *labeo rohita*, 10% *Catla catla* and 10% *Cirrhinus mrigala* in 12 earthen ponds (in triplicate) of same size in polyculture system to study their effect on growth, survival, condition factor, feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and net production. Each group was fed with same diet; 30% protein @ 3% of entire wet body weight for six months. The growth parameters were inversely affected by stocking density; in T1, maximum weight, length, SGR and PER were observed in all three fish species and gradually decreased in T2, T3 and T4. Condition factor and survival rate showed variation in different groups indicating no effect of their interaction. The best feed conversion ratio was observed in T1 at lower density with insignificant difference. *Labeo rohita* showed maximum weight and length gain (904.25% and 195.4%) followed by *Cirrhinus mrigala* (884.11% and 187.08%) and *Catla catla* (521.84% and 163.87%). Maximum production was observed in T4 in all fish species with maximum number of fish. The overall results presented here indicate that the best growth performance of all fish species was obtained at T1 with maximum production at T4 stocking density.

Keywords: Stocking density, growth, survival, condition factor, FCR, SGR, PER and production.

1. Introduction
Human population is increasing day by day and the situation put more pressure on existing resources for food supply. The problem can be resolved by using fish a quality protein supplement in human diet (Sheikh and Sheikh, 2004) [26]. With the increase in fish demand, fish culture with more intensity to enhance the level of fish production (Hussein, 2012) [17].The productivity of aquatic fish thus increased by more efficiently utilizing ecological resources within the environment. Stocking two or more complimentary fish species can increase the maximum standing crop of a pond by allowing a wide range of available food items and the pond volume to be utilized (Hassan, 2011) [15]. Stocking density is an important parameter in fish culture operations, since it has a direct effect on the growth (Backiel and LeCren 1978) [1]. For the development and rearing techniques of any fish species, stocking density might play a very important role in fish growth. Density-dependent growth is commonly observed in fishes (Walters and Post 1993; Bystro¨m and Garcı´a-Berthou 1999) [30, 4]. Successful aqua culturing requires not only careful selection of species, appropriate feeding and water quality management, but also the extent of density of fish species being stocked (Barua, 1990) [3]. Under natural conditions the growth of fish is dependent on the density to be stocked. Generally, a direct relationship exists between food abundance and growth rate, whereas density of the species to be stocked and its growth rate tend to be inversely related ((Backiel and Le Cren 1967) [2]. Higher stocking density reduces the growth rates during fish culture (Shagunan, 1997). Selection of species plays an important role for any culture practices. For efficient utilization of different strata and zones of a pond, three or more species need to be stocked. Fish culture set up three Indian carps *Catla catla, Labeo rohita, Cirrhinus mrigala* are considered the best combination for growth (Chakrabarti, 1998; Rahman *et al*., 2006) [5, 24]

2. Materials and Methods
2.1 Location
The study was conducted at Al Raheem fish hatchery, Ali Pur Shumali, near Murad Abad, the rural demonstration site (30° 20′ 0″ N, 71° 5′ 0″ E) 18 km away from Muzaffargarh city, Pakistan.

2.2 Experimental design
Trials were conducted in 4 earthen ponds with an area of 0.4 hectares each. A six-month feeding experiment was conducted at hatchery ponds. The experiments were designed, following completely randomized design. All the treatment groups had three replicates.

2.3 Pond preparation
The ponds were cleaned and exposed to sunlight. All the ponds were filled with tube well water up to 1.5 m, and same level was maintained throughout the study period by compensating the daily losses. Prior to stocking of fish, all ponds were fertilized with cattle dung at 90 kg/pond (Jena and Das, 2006; Sahu *et al*., 2007) and poultry manure at 50% of cattle dung, 2.5 kg single super phosphate and 1.25 kg urea/pond to accelerate the production of planktonic life. These doses were repeated after every two weeks during the study period to maintain a constant level of plankton. The inlets of the ponds were properly screened with gauze of fine mesh to avoid the entry of any intruder into or exit of fish from the ponds.

2.4 Experimental fish and stocking density
Indian major carps (*C. catla, C. mrigala* and *L. rohita*) were used as experimental animals. Fish were collected from the AL-Raheem fish hatchery and farm, and were randomly stocked in polyculture. Fishes were stocked with species ratios of 10% *C. catla*, 10% *C. mrigala* and 80% *L. rohita with* various stocking densities given in Table 1. At the time of stocking, the morphometric characteristics of fish i.e. body weight and total lengths were measured, recorded and then fishes were released into their respective ponds. Later on, random sample of 20 fish of each species was taken every one month from all ponds, weighed and measured, and then released into respective pond.

2.5 Procurement of ingredients, feed formulation and preparation
Feed formulation and composition percentage of ingredients used in trials based on mostly plant origin. All the ingredients were fine ground individually, mixed, steam cooked at 140°C and passed through 5 mm extruder die, dried and crumbled to prepare a mash feed at National Feed Mill, Multan, Pakistan, and were transported to experimental site and carefully stored.

2.6 Feeding protocol
The fish were fed twice a day at 3% body weight by dusting method at 8:30 to 9:00 and 16:30 to 17:00 h (Javed and Sial, 1991). Feeding rate was adjusted after every 1 month with fish growth increments.

2.7 Other growth parameters
Condition factor (K), net weight gain (NWG), percent gain in weight, specific growth rate (SGR%), feed conversion ratio (FCR), protein efficiency ratio (PER), survival (%age), protein utilization (PU), were calculated, according to the following formulae:

Condition factor (K) = W × 100/L3

Net weight gain (NWG) = Average final weight (g) - Average initial weight (g)

Percent weight gain = Final weight (g) - Initial weight (g) / Initial weight (g) × 100

Specific growth rate was estimated by the formula given by Hopkins (1992).

SGR% = ln (Final wet body weight) - ln (Initial wet body weight) / Number of days × 100

Survival (%) = Total number of fish harvested x 100/Total number fish stocked.

2.8 Water quality analysis
Water samples were collected fortnightly from the surface, column and bottom of each pond at five different locations to minimize the possibility of chance error. Water samples were filtered and stored in one liter clean stoppered glass bottles and rushed to the laboratory for analysis. A series of physical and chemical parameters; temperature, dissolved oxygen (DO), pH, Chlorophyll-a, transparency, total alkalinity and total suspended solid (TSS) were determined fortnightly between 9:00 and 10:00 hours. Dissolved oxygen was measured by the Winkler titration method (Stirling 1985), transparency with a Secchi disk, pH with a Jenway 3020 pH meter (Jenway, UK). Total alkalinity was determined by the titrimetric method (Stirling1985). Total suspended solid was determined according to Stirling (1985). Chlorophyll-a was analysed spectrophotometrically after acetone extraction following Boyd (1979).

3. Results
Various parameters of Major Carps (*Labeo rohita, Catla catla and Cirrhinus mrigala*) under different stocking densities have been observed in this experiment. Four rearing densities T1 (800/ acre), T2 (1200/ acre), T3 (1600/ acre), and T4 (2000/ acre) were stocked with ratio of 80% *labeo rohita*, 10% *Catla catla* and 10% *Cirrhinus mrigala* in 12 earthen ponds (in triplicate) of same size in polyculture system as shown in Table 1. Each group of fish was fed with the same type of diet; 30% protein at the rate of 3% of entire wet body weight.

In *Labeo rohita* the group T1 showed maximum growth rate with weight gain as compared to the other groups. The respective weight and length gain values for groups T2, T3 and T4 respectively as shown in Table 2. So, in *Catla catla* the group T1 showed a maximum growth rate with weight gain as compared to the other groups respectively and shown in Table 2 and *Cirrhinus mrigala* the group T1 showed maximum growth rate with weight gain length as compared to the other groups. Here the weight and length gain in T3 and T4 is approximately equal that is shown in Table 2. So the T1 shows the maximum growth rate and weight gain respective to other group in each specie respectively.

In *labeo rohita* and *Catla catla* the group T1 showed a significant increase in weight and length gain compared to the other groups. The respective percentage weight and length gain values for groups T2, T3 and T4. While in *Cirrhinus mrigala* the group T1 showed a significant increase followed by T2, T4 and T3 respectively as given in Table 2.

Labeo rohita, Catla catla the highest SGR was observed in group T1, followed by T2, T3 and T4 while in, *Cirrhinus mrigala* the highest SGR was observed in group T1, followed by T2, T4 and T3 given in Table 2.

Food conversion in *Labeo rohita, Catla catla* was highly efficient in T1, then the respective groups T2, T3 and T4 while in *Cirrhinus mrigala* it was highly efficient in the group T1 and then T4, T2 and T3 respectively as given in Table 2.

Protein conversion ratio in *Labeo rohita, Catla catla* was highest in T1, then the respective groups T2, T3 and T4 while in *Cirrhinus mrigala* it was highest in group T1 and then T4, T2 and T3 respectively as given in Table 2

Percentage Survival rate in *Labeo rohita Catla catla* was highest in T4, then the respective groups T3, T2 and T1 while in *Cirrhinus mrigala, Catla catla* it was highest in group T1 and then T4, T2 and T3 respectively as given in Table 2

Condition factor in *Labeo rohita* the maximum value observed in T3, then respectively in groups T4, T2 and T1 and in in *Catla catla* the maximum value observed in T4, then respectively in groups T1, T2 and T3 while in *Cirrhinus mrigala*, the maximum value observed in T3, then respectively in groups T1, T2 and T4 as given in Table 2

Maximum production was observed in group T4, followed by T3, T2 and T1 in all sampled fishes who are under consideration as given in Table 2.

Table 1: Stocking ratios of fish species in treatment ponds

Fish species	T1	T2	T3	T4
Labeo rohita	640	960	1280	1600
Catla catla	80	120	160	200
Cirrhinus mrigala	80	120	160	200
Total	800	1200	1600	2000

Table 2: Growth parameters (Mean ± SD) of Major Carps under different stocking densities.

Parameters	*Labeo rohita*				*Catla catla*				*Cirrhinus mrigala*			
Parameters	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
Rearing Period (days)	180	180	180	180	180	180	180	180	180	180	180	180
Mean Initial Weight (g)	169.84±26.58	169.84 ±26.58	169.84 ±26.58	169.84 ±26.58	271.87 ±34.85	271.87 ±34.85	271.87 ±34.85	271.87 ±34.85	96.5 ±13.51	96.5 ±13.51	96.5 ±13.51	96.5 ±13.51
Mean final weight (g)	1535.79 ±67.58	1429.42 ±151.14	1219.69 ±195.57	1032.76 ±99.13	1418.74 ±47.55	1206 ±37.56	1157.57 ±38.43	1004.96 ±23.95	853.17 ±28.2	743.41 ±33.59	732.08 ±25.78	732.17 ±21.6
Weight gain (g)	1365.95	1259.58	1049.85	862.92	1146.87	934.13	885.7	733.09	756.67	646.91	635.58	635.67
(%) Weight gain	904.25	841.62	718.14	608.08	521.84	443.59	425.78	369.64	884.11	770.37	758.63	758.72
Mean Initial Length (cm)	24.64 ±1.63	24.64 ±1.63	24.64 ±1.63	24.64 ±1.63	26.49 ±1.0	26.49 ±1.0	26.49 ±1.0	26.49 ±1.0	21.76 ±1.15	21.76 ±1.15	21.76 ±1.15	21.76 ±1.15
Mean final Length (cm)	48.15 ±1.02	46.65 ±1.37	43.62 ±2.42	41.6 ±1.24	43.41 ±0.26	41.98 ±0.15	41.52 ±0.52	39.1 ±0.21	40.71 ±0.2	39.04 ±0.28	38.65 ±0.37	38.8 ±0.14
Length gain (cm)	23.51	22.01	18.98	16.96	16.92	15.49	15.03	12.61	18.95	17.28	16.89	17.04
(%) Length gain	195.4	189.32	177.03	168.83	163.87	158.47	156.74	147.6	187.08	179.41	177.62	178.31
Condition factor	1.38 ±0.23	1.40 ±0.19	1.46 ±0.06	1.43 ±0.34	1.64 ±0.002	1.63 ±0.03	1.62 ±0.02	1.68 ±0.02	1.26 ±0.02	1.25 ±0.03	1.27 ±0.01	1.25 ±0.02
SGR	0.587	0.568	0.525	0.481	0.44	0.4	0.38	0.35	0.581	0.544	0.540	0.540
FCR	3.15	3.32	3.47	3.83	3.71	4.26	4.43	5.24	3.76	4.15	4.19	4.05
PER	1.056	1.004	0.962	0.869	0.9	0.78	0.75	0.63	0.87	0.8	0.79	0.82
Survival (%)	94.69	95.0	97.14	98.03	95	90.83	88.75	91.5	96.25	92.5	88.75	94
Production Kg/m^2/180 Days	0.0013	0.0018	0.002	0.0021	0.00014	0.00017	0.00021	0.00022	0.00009	0.00011	0.00015	0.00019

4. Discussion

Obtaining higher yield in terms of growth in fish accompanied with optimum qualitative characteristic of meat is main target of contemporary aquaculture processes. Fish growth is attributed as an increase in overall length and weight of fish under feeding regimen. Fish growth and feed utilization were significantly retarded herein with increasing the rearing density irrespective of protein levels. It has been demonstrated that rearing fish at high density may reduce their growth due to factors such as social interaction and the deterioration of water quality, which can affect the feed utilization by fish (Ellis *et al.*, 2002) [9]. Fish density can affect the efficiency of feed utilization; as the number of fish stocked in a pond increases, the amount of feed available to each fish decreases (Chang, 1988) [6].

In many cultivated fish species, growth and feed utilization are inversely related to rearing density, and this is mainly attributed to social interactions such as competition for food and/or space that can negatively affect fish growth (Irwin *et al*. 1999) [18]. Similar results were obtained by Huang and Chiu (1997) [16], El-Sayed (2002) [10], who found that the increase of stocking density inversely affected the growth of fish. Feed efficiency was correlated with stocking density. Wang *et al*. (2000) [31] have also reported a reduced rate of gross growth efficiency with increased density. In contrast, Matthews (2000) [21] reported better survival in ponds with increasing stocking density.

The condition factor and survival rates were not affected significantly by the rearing density, suggesting that there was no competition for space. Moreover, the good survival rate of fish at high densities indicates the amenability of fish to the intensive culture practice. Various authors have reported no effect of stocking density on the survival in aquaria or ponds (Tidwell *et al*. 1994; Wang *et al*. 2000) [29, 31]. Huang and Chiu (1997) found that condition factor and survival had not been significantly affected by rearing density. Stocking densities had no effect on the survival or relative condition of the fish in this study. However, a decrease in growth rate and feed efficiency and an increased coefficient of variation in fish weights were related to increase stocking density. McComish (1971) [23] also showed that an increase in stocking density caused a decrease in growth among fish maintained in indoor tanks. Matthews (2000) [22] found a reduction in the growth rate in ponds with increasing stocking densities. Reductions in growth which occurred at high density appeared to be due to poor water quality because dissolved oxygen concentration at dawn (total hours when DO was less than one) and NH3 levels all differed significantly among treatments. The present experiment showed growth depreciation of fish maintained in ponds as stocking densities increased.

The dietary protein level for all stocking densities was remained constant (30%) which is a standard protein level. From previous reports, insufficient dietary protein levels resulted in poor growth performance in many fish species (Yang *et al*., 2002; Giri *et al*., 2003; Kim and Lee, 2005) [34, 11, 19] due to insufficiency of amino acids supplied to maintain the body composition (Halver and Hardy, 2002) [13, 14]. Excess dietary protein level, however, did not improve the growth of the fish because the excess amino acid is metabolized by oxidative deamination and used to generate energy (Cho *et al*., 1985; Shiau and Huang, 1989; Vergara *et al*., 1996; Kim and Lee, 2009) [7, 27, 29, 20]. Using excess protein levels in the diet therefore cannot enhance fish growth, and therefore show negative effects on the fish production. Excess protein levels in the feed increased the amino acid catabolism in the fish body, and this resulted in higher ammonia excretion and accumulation of nitrogen waste in the culture system (Yang *et al*., 2002; Webb Jr. and Gatlin III, 2003) [34, 32]. Moreover, increasing excess protein in a practical diet results in higher feed costs, which is the major variable cost in the aquaculture production system (Goddard, 1996) [12]. Fish density can affect the efficiency of feed utilization; as the number of fish stocked in a pond increases, the amount of feed available to each fish decreases (Chang 1988) [6].

Feed intake and FCR herein were significantly affected by protein levels and rearing density, but not their interaction. In our studies FCR increases as the number of stocking densities increases; minimum FCR observed in T1 in all fish species and gradually increases which indicates reduced feed utilization at high stocking densities. The reduced feed utilization at high rearing density had been attributed to changes in metabolism. This effect is based on the assumption that chronic stress, due to the high density, increases the fish's overall energy demand, which is then unavailable for growth (Wendelaar Bonga, 1997) [33]. Likewise, high levels of locomotory activity have been shown to cause elevated metabolic rates as measured in rainbow trout (Cooke et al. 2000) [8]; however, the activity level of fish increased with increasing density, resulting in energy demands. Better feed utilization was observed in *Labeo rohita* as it shows minimum FCR (3.15) as compared to *Catla catla* (3.71) and *Cirrhinus mrigala* (3.76) in T1. FCR value increases as stocking density increases which is inversely proportional to feed utilization.

SGR (specific growth rate), PER (protein efficiency ratio), percentage weight gain, percentage length gain shows maximum values in T1 in all fish species and gradually decreases as the stocking densities increases. Though, in our experiment growth, % age growth, SGR and PER increases in T1 and gradually decreases in T2, T3, and T4 but production shows inverse results. The maximum production rate was observed in T4 followed by T3, T2 and T1. In T1, minimum production was observed as there was less number of fish stocked. Increased fish yields in conventional, static ponds or reservoirs was accomplished by a combination of management procedures, the most important among them being the use of supplementary feed, polyculture and auxiliary aeration during the night (Sarig,1989) [25].

5. Conclusion

It is concluded from the above experiment that at low stocking densities the fish growth, SGR, PER, % age weight and length gain showed maximum values with low FCR but net production is minimum. As the stocking densities increases the inverse results were obtained. So for a profitable culturing it is recommended to stock maximum number of fish in a pond to get maximum weight. Though, T4 produces maximum mass but with less average size of the fish which may not be acceptable to the consumer.

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