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Standardization of stocking density on genetically improved farmed tilapia (GIFT) *Oreochromis niloticus* in pond cage aquaculture system

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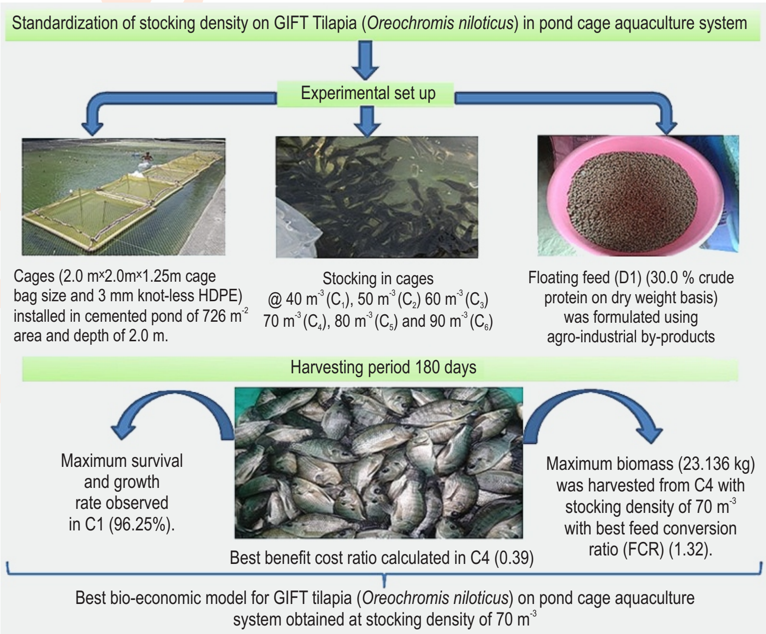
Abstract

Aim: To standardize the stocking density of GIFT strain of Nile tilapia (*Oreochromis niloticus*) to optimize survival, growth, biomass production and maximum economic gain in pond cage culture system.

Methodology: Study was conducted in triplicate for 180 days in fish cages installed in cemented pond of 726 m^{-2} area and depth of 2.0 m. Stocking density of GIFT Tilapia was maintained @ 40 m^{-3} (C1), 50 m^{-3} (C2), 60 m^{-3} (C3), 70 m^{-3} (C4), 80 m^{-3} (C5) and 90 m^{-3} (C6), respectively. Water quality and growth parameters were studied at fortnight basis. Supplementary diet (D_1) (30.0 % crude protein on dry weight basis) was provided @ 2% body weight. Survival rate, average growth rate, biomass production, specific growth rate (SGR), feed conversion ratio (FCR), length-weight relationship, condition factor, economics and benefit cost ratio were evaluated.

Results: All water quality parameters were within the limit suggested for aquaculture. Maximum survival was achieved in C1 (96.25%) and survival rate reduced with increasing stocking density in cages. Decrease in SGR was observed with increasing stocking density. The average growth rate was highest in C1 (112.20 g) and lowest in C6 (61.80 g) whereas the maximum biomass (23.136 kg) was harvested from C4 with stocking density of 70 m^{-3} with best FCR (1.32). Benefit cost ratio was evaluated best in C4 (0.39).



Interpretation: Pond cage aquaculture with improved stocking condition is suitable for GIFT tilapia growth, biomass production and maximum economic gain.

Key words: Food conversion ratio, Growth rate, *Oreochromis niloticus*, Pond cage aquaculture, Supplementary diet

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Introduction

A sustainable and high yield aquaculture system is required for higher fish production per unit area. Inland aquaculture produced 51.3 million tonnes of food fish in 2018 and contributed 62.5 percent of the world's farmed food fish (FAO, 2020). GIFT (genetically improved farmed tilapia) is one of the most popular strains of tilapia (*Oreochromis niloticus*) in freshwater aquaculture (Mohammad, 2006; Santos *et al.,* 2019) and plays a significant role in the development of Nile tilapia culture (Gjedrem, 2012). It is the second most important farmed fish in the world, after carps (FAO, 2018). Utilization of full space through intensive culture for maximum fish production can improve the profitability of fish farm. Intensification by increasing stocking density can be a suitable option to overcome the problem of land shortage (Chakraborty *et al.,* 2010). Cage culture is advantageous for farmers as it offers a chance for maximum utilization of existing water resources. Therefore, the farmers do not have to invest on accumulating water. It has certain advantages over other aquaculture systems that are potentially important in terms of uptake by rural poor and landless people (Bulcock and McAndrew, 2010).

Pond cage aquaculture is a system where different ecological niches can be utilized in a better way for higher biomass production per unit area along with higher economic returns. In pond cage aquaculture, harvesting mechanism is simple and easy. It also helps in conservation of endangered fishes. Mortality due to predation is a big issue which can be controlled by rearing fish in cage net enclosure (Balkhande, 2019). A critical factor for efficient pond cage aquaculture is optimum stocking density. Growth performance of Nile tilapia is density dependent (Alam *et al.,* 2014) and is negatively affected by increase in biomass in cage (Yi and Lin, 2001). This species is considered best suitable for cage aquaculture system owing to hardy and omnivorous nature, acceptability to formulated diets (Arun and Amararatne, 2003) and ability to survive high stocking density (Moniruzzaman *et al.,* 2015). Many researchers have addressed on growth and survival of fish in cage aquaculture system (Carro-Anzalotta and McGinty, 2007; Osofero *et al.,* 2009; Jiwyam, 2011; Kwikiriza *et al.,* 2016) but information on economic analysis is scarce which makes it difficult to make practically suitable model.

Economic analysis is essential along with growth and survival analysis as mismatch of cage cost and other expenditure may ruin the whole motive of cage aquaculture system (Nunoo *et al.,* 2014). Growth performance of tilapia is impressive when reared in cage aquaculture in rivers and other water bodies (Alam *et al.,* 2014). Pond cage aquaculture has tremendous potential for vertical expansion in aquaculture sector as it enhances the production from the same size of pond. Tilapia reared in extensive condition is lower than that of intensive and semi intensive culture conditions. However, on considering the production cost, extensive cage culture of Tilapia can be an alternative among other culture models (Borthakur and Goswami, 2007; Chakraborty and Banerjee, 2009). Culture of GIFT strain of Nile tilapia (*O. niloticus*) is gaining attention in northern states of India owing to its increasing demand in consumer level. The frozen and value added products prepared from this fish are gaining attention due to its taste and low intramuscular spine content. Aquaculture in Punjab is high yielding and profitable despite certain constraints where only two-fifth of farms uses technical efficiency of land. Majority of the farmers in Punjab have less than 2.5 ha land with pond size of 1.4 to 3.9 ha (Singh *et al.,* 2015). Thus a voluminous scope is observed to enhance the aquaculture productivity by adopting pond cage aquaculture technology in future. In view of the above, the aim the study was to standardize the stocking density of GIFT strain of Nile tilapia (*Oreochromis niloticus*) to optimize survival, growth, biomass production and maximum economic return in pond cage culture system.

Materials and Methods

Experimental setup: The experiment was performed at Fish Farm of College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (Punjab), India. The experiment (in triplicate) was conducted in fish cages (2.0 m×2.0 m ×1.25 m cage bag size and 3 mm HDPE knot less net) fabricated by locally available low cost materials installed in cemented pond of 726 m^2 area and 2.0 m of depth. The gap between the cage bottom and pond bottom was maintained at 0.75 m. Certified GIFT Tilapia (average length 2.7$\pm$ 0.2 cm and weight 0.6 + 0.02g) procured from Kolkata were maintained at a density of 40 m^{-3} (a total of 160 individuals) (C_1), 50 m^{-3} (a total of 200 individuals) (C_2), 60 m^{-3} (a total of 240 individuals) (C_3), 70 m^{-3} (a total of 280 individuals) (C_4), 80 m^{-3} (a total of 320 individuals) (C_5) and 90 m^{-3} (a total of 360 individuals) (C_6), respectively. The experiment was conducted for 180 days with additional 15 days for acclimatization of fish before initiation of the experiment from May to October when the ambient temperature of this area varied from 18° C to 45° C.

Analysis of water quality parameters: Physico–chemical parameters of water samples including water temperature, pH, dissolved oxygen, total alkalinity, hardness, ammonia- nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N) and phosphate-phosphorous (PO_4-P) were measured at weekly interval from each corner of pond following the standard methods of APHA (2017).

Feed formulation and utilization: Floating feed (30.0 % crude protein on dry weight basis) was formulated using agro- industrial by-products like rice bran (30%), de-oiled ground nut cake (30%), de-oiled soybean cake (25%), fish meal (13 %) and vitamin – mineral mixture (2%) in extruder machine installed in College of Fisheries and dried in oven to maintain the final moisture level below 10 %. Initially floating feed of 1 mm size were fed @ 5% of body weight for initial 15 days and gradually reduced to 2% with 2 mm feed (two meals of 1.0 % body weight at 9.00 am and 5.00 pm, respectively) for rest of the culture period. To regularize feeding, 30 fish each from all the replications were sampled from each treatment. Proximate composition of feed ingredients and formulated diets were determined as per the standard methods of AOAC (2005).

Growth analysis: Random sampling of 30 fish from each of the replicate of each treatment were measured for total length (TL) and total weight (TW) at fortnightly interval basis to analyze the growth of fish. Survival rate, feed conversion ratio (FCR), specific growth rate (SGR) and length-weight (log-transformed) relationships were determined by linear regression analysis and scatter diagrams of length and weight. The length-weight relationship was evaluated as per Le Cren (1951). Fulton's condition factor (K) was calculated according to Htun Hun (1978) equation.

Economics and Benefit cost ratio: All the fixed cost, variable cost including seed cost, feed cost, medicine and miscellaneous expenditure (electricity, repairing, netting, labour cost); cost of locally fabricated nylon net was calculated to evaluate the total expenditure. Profit and benefit analysis was calculated based on the procedure described by Datta *et al.* (2014) and Janssen *et al.* (2018).

Statistical analyses: The analysis of co-variance was performed to determine variation in 'b' values following the method of Snedecor and Cochran (1967). The statistical significance of Isometric exponent (b) was examined as a function: ts = (b-3)/Sb (Sokal and Rohlf, 2009) having 'ts' as t- test value; 'b' as slope and Sb as standard error of 'b'. Statistical software SPSS v16 and XLSTAT were used for statistical analysis.

Results and Discussion

All the mean values of water quality parameters *viz.* temperature (15-35°C), pH (7.0- 9.5), dissolved oxygen (> 5.0 ppm), total alkalinity (50-200 mg l^{-1}), hardness (< 300 mg l^{-1}), NH_3-N (< 0.1 mg l^{-1}), NO_3-N (0.02-2.0 mg l^{-1}), and PO_4-P (0.03-2.0) were within the acceptable range as suggested by Bhatnagar and Devi (2013) for general aquaculture in pond. The extreme variations were recorded in ambient and water temperatures although the values remained under the acceptable limits. The survival of fish under such fluctuations determines that GIFT Tilapia is hardy and has wide range of tolerance for temperature (Nivelle *et al.,* 2019). The maximum survival rate was observed in C1 (96.25%) and minimum in C6 (89.44%) in the present study. Survival rate decreased with increase in stocking density in cages. Similar trend was also observed by Moniruzzaman *et al.* (2015) where survival rate of more than 90% was achieved in Nile Tilapia reared

Table 1: Stocking and production of fish in different treatments (initial average length 2.7+ 0.2 cm and wt. 0.6 + 0.02g)

Treatments	C_1	C_2	C_3	C_4	C_5	C_6
Stocking density (number m^{-3})	40	50	60	70	80	90
Total number of fish stocked in one cage (4 m^{-3})	160	200	240	280	320	360
Survival %	$96.25^{a}\pm0.17$	$96.00^{a}\pm0.21$	$93.33^{ab}\pm0.07$	$92.85^{ab}\pm0.34$	$90.00^{b}\pm0.56$	$89.44^{b}\pm0.44$
Final average wt. gain (g)	$112.20^{a}\pm0.32$	$105.0^{ab}\pm0.85$	$99.10^{b}\pm0.98$	$89.10^{c}\pm1.25$	$74.23^{d}\pm0.60$	$61.80^{e}\pm0.88$
Biomass Produced (kg m^{-3})	$4.319^{a}\pm0.05$	$5.040^{c}\pm0.02$	$5.549^{ab}\pm0.06$	$5.784^{a}\pm0.04$	$5.344^{b}\pm0.02$	$4972^{cd}\pm0.01$
Total Biomass produced in one cage (4 m^{-3}) (kg)	$17.278^{a}\pm0.12$	$20.160^{a}\pm0.16$	$22.196^{a}\pm0.05$	$23.136^{a}\pm0.08$	$21.376^{a}\pm0.02$	$19.888^{a}\pm0.03$

*The values (mean ± S.E.) with different alphabetical superscripts (a, b, c…) differ significantly within the treatments (in a row) ($p\leq 0.05$)

Table 2: Growth parameters of fish in different treatments

Treatments	C_1	C_2	C_3	C_4	C_5	C_6
Feed Conversion Ratio (FCR)	$1.620^{c}\pm0.012$	$1.550^{cd}\pm0.026$	$1.440^{d}\pm0.040$	$1.320^{e}\pm0.036$	$1.680^{ab}\pm0.021$	$1.70^{a}\pm0.042$
Specific growth rate (%/day)	$2.91^{a}\pm0.013$	$2.32^{ab}\pm0.015$	$2.02^{bc}\pm0.038$	$1.78^{c}\pm0.091$	$1.55^{de}\pm0.064$	$1.33^{e}\pm0.055$
Log W = log a+b log L	Log0.049+2.607 log L	Log0.043+2.685 log L	Log0.070+2.433 log L	Log0.055+2.507 log L	Log 0.059+2.521 log L	Log 0.051+ 2.605 log L
Correlation Coefficient ®	0.983	0.981	0.995	0.985	0.989	0.957
Coefficient of determination 'r^2'	0.966	0.963	0.99	0.971	0.979	0.916
Condition factor 'K'	1.92	2.14	2.42	2.39	2.20	2.03

*The values (mean ± S.E.) with different alphabetical superscripts (a, b, c…) differ significantly within the treatments (in a row) ($p\leq0.05$)

Table 3: Economics of fish in different treatments (cost in Rs) (calculation is based on single cage of area 4m^2)

Particulars	C_1	C_2	C_3	C_4	C_5	C_6
Fixed expenditure						
Cost of indigenous cage made of PVC pipes and Nylon net (2mX2m×1.25 m)(durability 8 yrs)	2500	2500	2500	2500	2500	2500
Recurring expenditure						
Seed Cost @ Rs 1/piece	160	200	240	280	320	360
Total feed cost @ Rs 27/kg	755.38	843.70	862.91	824.56	969.61	912.85
Lime/Medicine (Rs)	7.50	7.50	7.50	7.50	7.50	7.50
Miscellaneous expenditure (electricity, repairing, netting, labour cost etc.)	20.00	20.00	20.00	20.00	20.00	20.00
Cost of locally fabricated Nylon net (2mX2m ×1.25 m) = Rs 400 (durability 2 yrs)	200.0	200.0	200.0	200.0	200.0	200.0
Total recurring expenditure	1142.88	1271.2	1330.41	1332.06	1517.11	1500.35
Total income						
Total income (Biomass produced per cage X Rs 80/- per kg sale of fish)	1382.24	1612.8	1775.68	1850.88	1710.08	1591.04
Net profit (Total income - Total recurring expenditure)	239.36	341.6	445.27	518.82	192.97	90.69
Benefit Cost ratio (based on recurring expenditure and net profit)	0.21	0.27	0.33	0.39	0.13	0.06

in cages maintaining stocking density of 50, 75,100 and 125 fish m^{-3} in Bangladesh. Constant decrease in survival rate with increasing stocking density was also observed by Chakraborty *et al.* (2010) in Nile Tilapia, reared in cages. Thus, Tilapia can sustain the crowding environment in fish cages.

The maximum average weight gain was recorded in C_1 (112.20 g) followed by C_2 (105.00 g),C_3 (99.10 g), C_4 (89.10 g), C_5 (74.23 g) and C_6 (61.80 g), however, the maximum biomass produced in cage of 4m^3 area was observed in C_4 (23.136 kg) followed by C_3 (22.196 kg), C_5 (21.376 kg), C_2 (20.160 kg), C_6 (19.888) and C_1 (17.278 kg), respectively (C_4 recorded 3.88 %, 14.76%, 4.23%, 8.23% and 16.33 % more biomass than C_1, C_2, C_3, C_5 and C_6, respectively) (Table 1). The stocking density of 70 m^{-3} (C_4) was found best in the pond cage aquaculture of Gift Tilapia in present study. The maximum carrying capacity of unit volume of water reached highest at stocking density of 70 m^{-3} after that at stocking density of 80 m^{-3} onwards biomass production per unit area decreased (Table 1). Mengistu *et al.* (2020) observed that fish kept in higher stocking density showed slow growth rate. Individual growth and population density are closely linked, and the growth of large sized fish is dramatically affected by stocking density (Coulibaly *et al.,* 2007). Rearing tilapia in cages contributes to the higher biomass production and increase a profitability (Sakr *et al.,* 2015).

Various studies have shown that the stocking density plays a critical role to standardize the maximum biomass production model for cage aquaculture (Moniruzzaman *et al.,* 2015; Datta *et al.,* 2017 and Mengistu *et al.,* 2020). In the present study, similar feeding rate was followed for all the treatments, therefore variation in growth in treatments was largely related to stocking density. Significant variations in FCR were also observed among different treatments. The lowest feed conversion ratio was observed in C_4 (1.320) as compared to C_1 (1.620), C_2 (1.550), C_3 (1.440), C_5 (1.680) and C_6 (1.700) indicating higher stocking density having positive relation with FCR upto stocking density of 70 m^{-3}, followed by increased FCR with increasing stocking density (Table 2). Better FCR was recorded in the present study as compared to Moniruzzaman *et al.* (2015) who reported FCR in the range of 1.81 to 2.05 with stocking density of 50 fish m^{-3} to 125 fish m^{-3} in *O. niloticus*. SGR provides information about growth rate in relation to time period which is important for culture fisheries. SGR was inversely proportional to stocking density, this might be due to more space and less competition for feeds in low density as compared to higher stocking density (Datta *et al.,* 2017).

The maximum SGR observed in C_1 (2.91), followed by C_2 (2.32), C_3 (2.02), C_4 (1.78), C_5 (1.55) and C_6 (1.33) revealed that the SGR gradually decreased with increasing the stocking density of *Oreochromis niloticus* in cages. The results corroborates with the findings of Moniruzzaman *et al.* (2015) and Datta *et al.* (2017) that SGR generally decreases on increasing the stocking density. Ferdous *et al.* (2014) also studied the influence of stocking density on growth and survival performances in monosex tilapia (*Oreochromis niloticus*) fry reared in pond in Bangladesh. The finding suggest that fish reared at highest stocking density revealed lowest growth and survival. Length – weight relationship of fishes stocked in different cages, values of regression co-efficient 'b' and logarithmic relationship between length and weight with regression equation is given in Table 2 and Fig. 1. Growth is designated as negative allometric when length increase is more than weight (b<3) and positive allometric when b>3. When total length was regressed with body weight, final 'b' varied between 2.433 to 2.685 in the present study. The slope value was

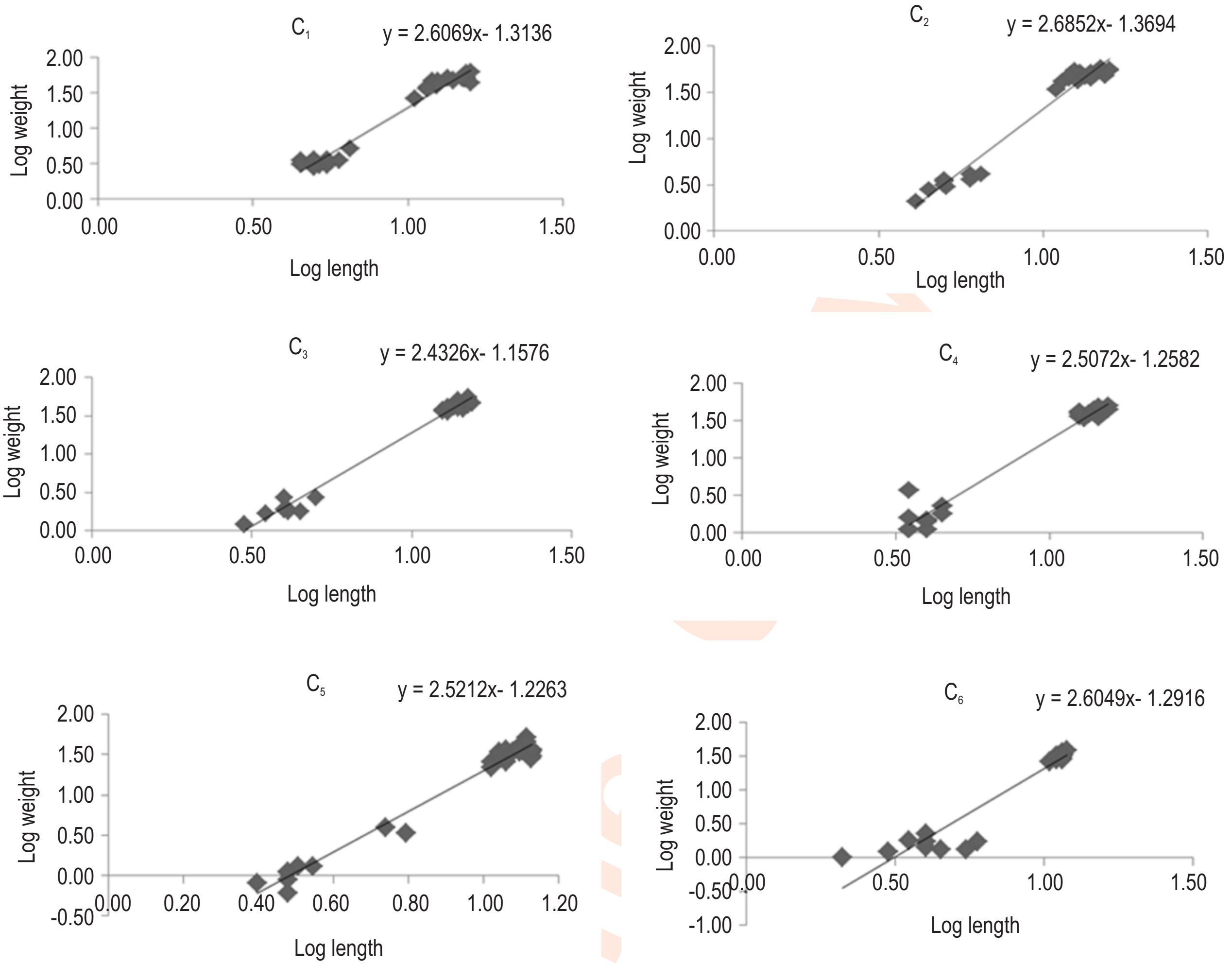

Fig. 1: Linearized length-weight relationship of fish in different treatments.

recorded significantly lower in all treatments (C_1 to C_6) than critical isometric value, indicating negative allometric growth; thus species become slender due to increase in length. The results of the present study is in conformity with the study of Nehemia *et al.* (2012) who reported that fish normally does not retain same shape or body outline throughout their lifespan and specific gravity of tissue in fish may not remain constant. Stocking density and environmental factors may lead to variation in slope (Yusof *et al.*, 2011; Deka and Gohain, 2015; Mortuza and Al-Misned, 2015).

In different studies, slope value (b) of *O. niloticus* was observed as 2.313 by Marx *et al.* (2014) from Barur Reservoir, Krishnakri district of Tamil Nadu whereas Silva *et al.* (2015) recorded slope value (b) of 3.060 in *O. niloticus* in cage farming in Brazil. Thus, it can be stated that the slope value (b) of *O. niloticus* may vary widely depending on culture practices, size of stocking, stoking density, feed provided and environmental conditions. Information on condition factor (K) provides vital information about management of fish culture system as it provides information about specific conditions of developing organism (Araneda *et al.*, 2008). It reflects physiology and biology of fish caused by interaction among factors like feeding conditions and physiological factors. This also indicates general fish condition via changes in food reserves in body. Besides all aspects, K value is an excellent alternative to expensive *in vitro* methods of proximate analyses of tissues (Datta *et al.*, 2013). The range of condition factor 'K' recorded in different treatments in the present study was 1.92 - 2.42. Condition factor of greater than one showed the well being of fishes fed with experimental diet, suggesting that formulated diet was preferred by GIFT Tilapia and digested well and they were well adjusted in higher socking density in different treatments. K value greater than 1.0 indicate that the culture environment and conditions were suitable, thus

GIFT Tilapia is a suitable candidate species for cage culture also. Proper fit of model for growth was explained by co-efficient of determination (r^2) values. In the present study, the lowest value of r^2 of GIFT Tilapia was 0.916 (91.6 % variability) in C_6 and the highest was 0.990 (99.0% variability) in C_3 (Table 2) indicating more than 91.0% variability by the model and good fitness. Economic evaluation of any culture model is important before recommending in field for mass scale adoption. Cage aquaculture can provide more income than the traditional aquaculture from same pond area which can help in generating additional income sources for small and marginal farmers (Rao *et al.,* 2013; Nunoo *et al.,* 2014). In the present study economics of a single cage unit of 4 m^3 area were calculated. The cage frame made up of PVC material fabricated at College of Fisheries, GADVASU with a self-life of 8 years costing Rs 2500.00. Total variable cost including seed cost, feed cost, medicine, miscellaneous expenditure (electricity, repairing, netting, labour cost) and cost of locally fabricated nylon net varied from Rs 1142.88 to Rs 1500.35 in different treatments.

Total income (biomass produced per cage X Rs 80/- per kg sale of fish) and net profit generated calculated were maximum in C_4 and minimum in C_1. Benefit cost ratio (based on recurring expenditure and net profit) was calculated highest in C_4 (0.39) and it was lowest in C_6 (0.06) (Table 3). The cost of production should be lower than the total income for significant benefit from cage aquaculture (Datta *et al.,* 2014). Based on the economics and benefit cost analysis, it can be stated that GIFT Tilapia is a suitable Species for pond cage aquaculture and can produce maximum return at stocking density of 70 m^{-3}. Cage aquaculture in a deeper pond can be a better option for farmers with small land holdings without putting additional pressure in existing water resources. It could be the best possible way to utilize different ecological niches in pond ecosystem. Based on the results of economics, survival and growth rate, it can be stated that GIFT Tilapia is suitable specie for pond cage aquaculture.

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Add-on Information

Authors' contribution: S. N. Datta: Planning and designed the study, conducting the experiment, data analysis, manuscript writing; **S. Kumar:** Conducting the experiment, manuscript writing.

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