

Effect of Different Stocking Rates on Growth, Feed Utilization and Yield of Two Size Group Milkfish, *Chanos Chanos* (Forsskål, 1775) in Inshore Cages at South-East Coast of India

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Received 6 August 2022, Accepted 8 October 2022, Published on 14 December 2022

ABSTRACT

Milkfish (*Chanos chanos*) is one of the most important brackish water finfish species being cultured in south east India. The present study investigated the different stocking densities suitable for better growth, feed utilization of milk fish. Two different size group (351.72 ± 1.05 , 720.80 ± 1.25 g) of fish were stocked at different rates in two different experiments. Milkfish in the cages were fed with 35% formulated diet twice in a day at the rate of 3% body weight for fishes in

experiment I with average body weight of 350g and in experiment II with 2% of body weight for fishes above 500g. In experiment I, the highest percentage of weight gain was observed in 6 fish/ m³ stocking group ($356.7 \pm 2.09\%$) and the lowest weight gain percentage was observed in 12 fish/m³ ($274.4 \pm 6.79\%$). There is significant difference in growth performance, yield, FCR and PER in different stocking groups. In experiment II, the highest final weight was observed in 2 fish/ m³ stocking group (1189.5 ± 10.61 g) and the lowest final weight gain was observed in 8 fish/m³ (1124 ± 14.14 g). The FCR and PER of the different stocking densities varies significantly. Thus, cage culture of milk fish in experiment I with a stocking density of 6 fish / m³ and in experiment II with a stocking of 2 fish/m³ can be considered ideal for better production of the marine fish under Indian context.

Keywords Feed utilization, Milkfish, Marine cage culture, Stocking density, Yield.

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INTRODUCTION

Marine blue food stocks continue to decline worldwide (Hutchings 2000) despite the expansion in the aquaculture sector, causing a significant crisis in the fishing industry. In mariculture, the best grow-out culture of many marine fishes is the use of marine floating cages. For example, they are fattening wild

catch of tuna species in cages for some months (Deveney *et al.* 2005). These cages have the advantage of lower running costs with high returns than land-based facilities of equivalent production capacity (Nowak 2007, Liao *et al.* 2004). Cage farming could be taken up by the fishermen individually or in groups, ensuring high profitability (Aswathy *et al.* 2020).

Some commercially important marine fish species are highly found suitable for cage farming in different parts of the world, including cobia *Rachycentron canadum*, seabass *Lates calcarifer*, snappers *Lutjanus* sp., pompanos *Trachinotus* sp., groupers *Epinephelus* sp. and milkfish *Chanos chanos* (Xavier *et al.* 2016). For many years, milkfish (*Chanos chanos*) has pulled the attention of the farmers and researchers for their better growth performance, efficient use of natural foods and propensity to eat a variety of supplementary feeds, disease resistance, handling and tolerance to a broad range of environmental conditions, it is ideally suited for culture in the tropics (Crear 1980). During 2016 milkfish farming contributed 1188 thousand tons of production, 2% of total finfish produced globally from aquaculture (FAO 2018).

In aquaculture, 'stocking density' must indicate the mass at which fish are initially stocked into a system. However, it is used to refer to the group of fish at any point of time. It is measured as one of the crucial factors that retard growth, feed utilization, and fish yield (Liu and Chang 1992). The full utilization of waterbody for the highest fish production through intensive culture can increase the profitability of the fish culture. The ideal stocking density varies depending on the number of parts and sizes of fish stocked (Chua and Teng 1979). One should avoid accumulating high of slow-growing species for economic viability as they require more seed and feed (Rao *et al.* 2013).

Further, various studies have pointed out an inverted relationship between stocking density and growth rate (Ridha 2006). Again, the relationship between the survival of the fish and stocking density is not found consistent (El-Sayed 2002). The farmers typically practice fish stocking at various densities based on their skill and concepts, using different hand-

books as a guide. Stocking densities of species in cage culture are highly variable and little research has been done to determine the optimum stocking densities for many species (Beveridge *et al.* 2004). Thus, the ideal stocking density of the fish under the different culture techniques and ecological conditions of the Indian subcontinent needs to be addressed coherently. As the need for cage culture in tropical developing countries becomes more apparent due to the fisheries decline in wild stocks and the stocking densities for economic viability, such investigations are urgently needed. This study was therefore designed to address some of these gaps.

MATERIALS AND METHODS

Experimental set up

The present studies were conducted in the closed bay near Suryalanka, Bapthla, Guntur district, Andhra Pradesh, India situating between latitude 15°84'91.38"N and longitude 80°53'32.64"E. Milkfish, *Chanos chanos* seed were collected from wild source at Moolapeta village, UKothapeta Mandal, East Godavari district. Fish seed were brought in aerated, closed bags and released into hapas (2m×2m×1.5m) in brackish water fish pond at FRS, Kakinada and reared for a period of one week. During this period the seed were fed with rice bran. Fish seed were packed in double plastic bags filled with oxygen and 30 ppt saline water in the ratio 3:1 in each bag and the density of fish was 100/bag. Seed was transported in to hapas with in the cage in the experimental site. Before transferring into hapas the seed was slowly acclimatized to water of experimental site for one hour.

Experimental cages for studies

The floating net cages used for experiment Hapas of (1m×1m×2m) sizes, fine meshed polyethylene (PE) net cages (1.25 mm) were fixed in the cages. Outer cage made up of high-density polyethylene (HDPE) was used as protection from predators (Predatory net). The floating net cages were fixed to a bamboo raft. The bamboo raft was used for easy movement, feeding and sampling of the experimental fishes on the cage structure. Sealed and air-filled plastic drums of 200 liter size were used as cage float for buoyancy of cage

structure. Each cage was covered at the top with apieceoflarge mesh size (4.5 cm) net top revent predation by birds and escaping of fish by jumping as reported by Moniruzzaman *et al.* 2015b. The whole structure was tied with an chorsateach corner bynylon rope to make easy movement of floating cages depending on water level and flow. The cages were positioned in a closed bay 500m away from shore with moderate water flow (0.05m second⁻¹). The submerged volume of the cages was invariably 1m³.

Studies on ideal stocking density for milkfish in floating cages

Before the start of the experiment,the transported fish were acclimatized to the sea environment by rearing the minihapa net for one week.Sub-adults with an average initial weight of 351.8±3.42 grams were randomly stocked in the net cages at 6 fish/ m³, 8 fish /m³, 10 fish /m³, 12 fish/m³ as T₁, T₂,T₃, and T₄, respectively, in triplicates (Treatments 4, Replicates 3). Adults with anaverage initial weight of 718.8±3.18g were randomly stocked in the net cages at 2fish/m³, 4 fish/m³,6 fish/m³ and 8 fish/m³ as T₅, T₆, T₇ and T₈ respectively, in triplicates.

Feed preparation and feeding

Formulated floating feed with the 35% crude protein was used for feeding.The feed was prepared with ground nut cake (GNC) at 33.19%, fish meal (FM) at 33.19%,de-oiledrice bran (DOB) at 15.81%,wheat-flour at15.81% and 2% of vitamin and mineral mixture were added to the diets.The diet was estimated for proximate composition (AOAC 1995) is given that Table1. Feed was applied at the ratio 3% of body

Table 1. Proximate analysis of the feed was estimated by the method of AOAC 1995.

Ingredients consumption	Fish meal	Groundnut cake (GNC)	De-oiled rice bran	Wheat flour
Moisture	7.04	8.80	8.20	5.72
Crude protein	55	38.40	12.50	11.30
Crude fiber	3.70	7.30	22.40	0.60
Ether extract	4.03	7.20	3.90	4.02
Total ash	3.46	5.60	15.80	1.55
Acid insoluble ash	5.60	7.60	8.20	4.50

weight for fishes below the 500g and 2% of body weight used for fishes above 500g. Fish were fed twice a day at 8:00 hr and 16:00 hr with daily ration divided into two halves. Feeding was done manually to ensure the ingestion of feed completely by the fish. Fish in each treatment were sampled every 15 days.

Proximate composition Management of cages

The cages were removed from the water at every15 days interval for cleaning and checking the net. Cages were cleaned regularly to remove algae, polychaetes and other organisms. Dead fish were removed from cages immediately and disposed of in a pit,Ancillary work slike the mending of tornnets and realignment/ readjustment of sinkers and anchors were also performed for proper management of cages.

Cage fouling

During the present study, it was observed that the surfaces of cages immersed in water were covered by living organisms and it is called as fouling. Algae, polychaetes, green mussels and other mollusks were the main biofouling organisms on the net of the cage.

Growth performance

The growth parameters of fishes from each net cage were estimated by taking the individual body length and weight at every 15 days.

Weight increment

The weight was measured with the electrical balance. Weight increment was calculated by subtracting initial body weight from the final body weight.

$$\text{Weight increment} = \text{Final body weight (g)} - \text{Initial body weight (g)}.$$

Specific growth rate

Specific growth rate was calculated by the formula:

$$[(\text{Ln FBW} - \text{Ln IBW})/\text{day}] \times 100$$

Where

Ln = Natural logarithm

FBW = Final body weight
 BW = Initial body weight

Survival rate

Survival of the fishes at the end of each night was noted down and survival rates were calculated as

$$\text{Survival rate (\%)} = \frac{\text{Total number of fish survived}}{\text{Total number of fish stocked}} \times 100$$

Feed conversion ratio (FCR)

Feed conversion ratio was calculated by dividing feed given (dry weight) by body weight gain (wet weight).

$$\text{conversion ratio (FCR)} = \frac{\text{Feed given (dry weight) (g)}}{\text{Body weight gain (wet weight) (g)}}$$

Protein efficiency ratio (PER)

Protein efficiency ratio (PER) is defined as the ratio between the weight gain of fish and the amount of protein fed (De Silva and Anderson 1995).

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Crude protein fed (g)}}$$

Average daily weight gain (ADWG)

$$\text{Average daily weight gain (ADWG)} = \frac{\text{Final fish weight (g)} - \text{Initial fish weight (g)}}{\text{Number of days}}$$

Biomass

Biomass = No. of fish average body weight (g) .

Statistical analysis

The data obtained from the present study were statistically analyzed by using SPSS version 26 (IBM, USA). One way ANOVA and Tuckey's homogeneity of variance test was used to determine significance between the means at 95% probability level. Triplicates were used in each treatment and analysis, the values were expressed as mean SE. The values were considered significant when the *p*-values exceeds 0.05.

Ethics statement

Prior to the experimental design and initiation, the ethical clearance of the Institute Animal Ethical Committee (IAEC) was also obtained. It is under Ministry of Environment and Forests, Government of India. It has been designed to bring out uniformity in the working IAEC so that consistent views are taken while reviewing the proposals entailing use of animals for experimentation.

RESULTS

The primary water quality parameters like temperature, salinity, pH, dissolved oxygen, total alkalinity, ammonia and nitrite did not deviate significantly and remained within optimal ranges for cage culture. The water temperature and salinity were ranged between 27.1-31.6°C and 26-32 ppt respectively. The dissolved oxygen and p^H values were stable around 5.3-6.2

Table 2. The growth and feed utilization of milkfish (350 ± 2.03g) in different stocking densities.

Treatments	T ₁	T ₂	T ₃	T ₄	<i>p</i> value
Initial weight (g)	351.8±3.42	348.2±3.09	352.5±4.53	349.3±4.24	0.676
Final weight (g)	708.5 ^c ±5.52	677.2 ^{bc} ±6.22	654.9 ^{ab} ±9.05	623.7 ^a ±11.03	0.002
WG (g)	356.7 ^d ±2.09	329.0 ^c ±9.32	302.4 ^b ±4.53	274.4 ^a ±6.79	0.001
WG%	101.42 ^b ±0.39	94.51 ^b ±3.52	85.79 ^a ±0.18	78.53 ^a ±0.99	0.001
ADWG (g)	3.96 ^d ±0.02	3.66 ^c ±0.10	3.36 ^b ±0.05	3.05 ^a ±0.08	0.001
SGR	0.78 ^c ±0.002	0.74 ^{bc} ±0.02	0.69 ^{ab} ±0.001	0.64 ^a ±0.01	0.001
FCR	2.52 ^a ±0.01	2.74 ^{ab} ±0.08	2.98 ^{bc} ±0.04	3.28 ^c ±0.08	0.001
PER	1.13 ^d ±0.01	1.04 ^c ±0.03	0.96 ^b ±0.01	0.87 ^a ±0.02	0.001
Yield (g/L)	4.25 ^a ±0.03	5.42 ^b ±0.05	6.55 ^c ±0.09	7.48 ^d ±0.13	< 0.001

WG – Weight gain, WG% - Percentage weight gain, ADWG – Average daily weight gain; SGR – Specific growth rate; FCR – Feed conversion ratio; PER – Protein efficiency ratio. Data expressed in Mean ± SE; Values sharing different superscripts in the same row significantly differs each other (*p*<0.05).

Table 3. The growth and feed utilization of milkfish (720.5± 1.44g) in different stocking densities.

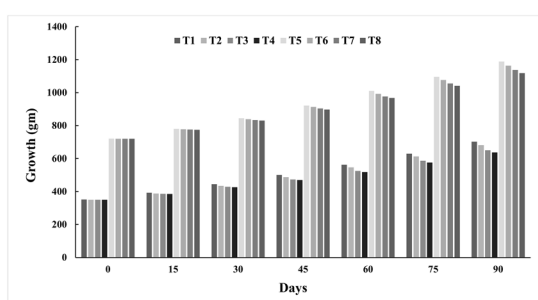
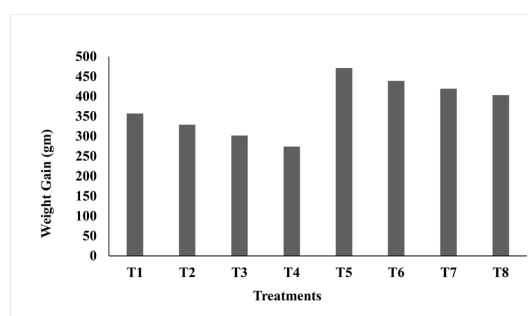
Treatments	T ₅	T ₆	T ₇	T ₈	p value
Initial weight (g)	718.8 ± 3.18	720.3 ± 4.24	722.3 ± 2.71	720.7 ± 2.56	0.765
Final weight (g)	1189.5 ^b ± 10.61	1159 ^{ab} ± 8.49	1141.5 ^{ab} ± 13.44	1124 ^a ± 14.14	0.021
WG (g)	470.8 ^b ± 7.42	438.8 ^{ab} ± 4.24	419.3 ^{ab} ± 10.72	403.3 ^a ± 11.58	0.007
WG%	65.49 ^c ± 0.74	60.92 ^{bc} ± 0.23	58.05 ^{ab} ± 1.27	55.96 ^a ± 1.41	0.003
ADWG (g)	5.23 ^b ± 0.08	4.88 ^{ab} ± 0.05	4.66 ^{ab} ± 0.12	4.48 ^a ± 0.13	0.007
SGR	0.56 ^c ± 0.005	0.53 ^{bc} ± 0.002	0.51 ^a ± 0.009	0.49 ^a ± 0.01	0.001
FCR	2.75 ^a ± 0.04	2.95 ^{ab} ± 0.03	3.09 ^b ± 0.08	3.21 ^b ± 0.09	0.008
PER	1.041 ^b ± 0.02	0.97 ^{ab} ± 0.01	0.92 ^a ± 0.02	0.89 ^a ± 0.03	0.006
Yield (g/L)	7.14 ^a ± 0.06	9.27 ^b ± 0.07	11.41 ^c ± 0.13	13.49 ^d ± 0.17	< 0.001

WG – Weight gain, WG% - Percentage weight gain, ADWG – Average daily weight gain, SGR – Specific growth rate, FCR – Feed conversion ratio, PER – Protein efficiency ratio. Data expressed in Mean ±SE, Values sharing different superscripts in the same row significantly differs each other (p<0.05).

mg/l and 7.8-8.3 respectively. The average alkalinity values in the cages were measured as 150.23 ± 6.83. The ammonia and nitrite values ranged between 0.02-0.11 ppm and 0.01-0.05 ppm, respectively, during the experiment.

The growth performance (initial weight, final weight, weight gain, percentage weight gain, average daily weight gain, specific growth rate and yield) and feed utilization (feed conversion ratio, protein efficiency ratio) of milkfish from the experiment I were presented in Table 2. The growth trend of different treatment group fishes in experiment I have been shown in Fig. 1. The initial mean weight of fishes in each experimental group (350±2.03g) did not significantly differ among them (p<0.05). At the end of the experimental period, the T₁ treatment group showed significantly highest mean final weight (708.5±5.52g) followed by T₂ (677.2±6.22g), T₃ (654.9±9.05g) and T₄ (623.7±11.03g). The final

weight of all experimental groups has significantly differed among each other. The mean individual weight gain (Fig. 1), average daily weight gain and specific growth rate of the fishes followed a similar trend with the final weight of different experimental groups. The highest percentage of weight gain was observed in T₁ (356.7±2.09%) followed by T₂ (329.0±9.32%); however, the differences were not significant (Mandal *et al.* 2018). The lowest weight gain percentage was observed in T₄ (274.4±6.79%) followed by T₃ (302.4±4.53%) and they were insignificant to each other but significantly lower than T₁ and T₂. The T₁ has posed significantly lowest feed conversion ratio (2.52±0.01) followed by T₂ (2.74±0.08), T₃ (2.98±0.04) and T₄ (3.28±0.08) and all treatments were differed significantly from each other (Fig. 2). The protein efficiency ratio of different treatment groups showed a relatively inverse trend to feed conversion, with the highest (1.13±0.01) and

**Fig. 1.** Growth trend of different treatment groups in experiment I & II.**Fig. 2.** Mean weight gain (g) of different treatments in experiment I & II.

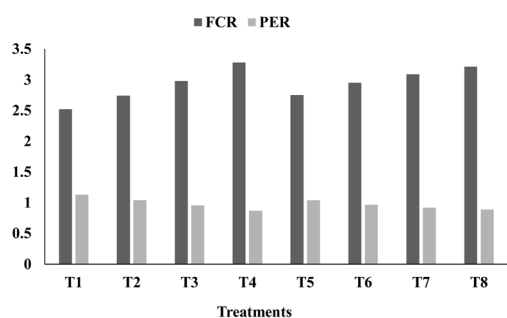


Fig. 3. Feed conversion ratio and protein efficiency ratio of different treatments in experiment I & II.

lowest (0.87 ± 0.02) was observed in T_1 and T_4 respectively (Figure 2). The highest yield was obtained in T_4 (7.48 ± 0.13 g/L) followed by T_3 (6.55 ± 0.09 g/L), T_2 (5.42 ± 0.05 g/L) and T_1 (4.25 ± 0.03 g/L) and the values have significantly differed among each other (Fig. 3).

The growth performances and feed utilization of milkfish resulted from experiment II were presented in Table 3. The growth trend of different treatment group fishes has been shown in Fig. 3. The initial mean weight of fishes of all treatment groups (720.5 ± 1.44 g) in experiment II was not significantly different among each other ($p < 0.05$). The T_5 treatment group resulted in the significantly highest final weight (1189.5 ± 10.61 g) followed by T_6 (1159 ± 8.49 g) and T_7 (1141.5 ± 13.44 g). The T_6 and T_7 were significantly lower than T_5 , but they are insignificant to each other. The T_8 treatment group resulted in significantly lowest final body weight (1124 ± 14.14 g) of all treatment groups. The mean weight gain (Fig. 3), percentage

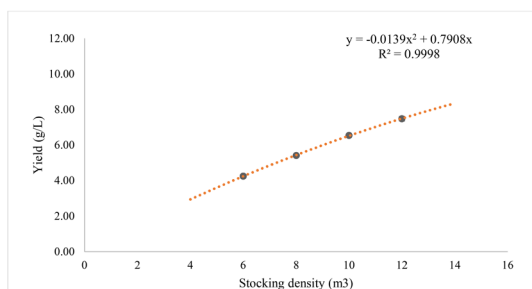


Fig. 5. Mean yield (g/L) of different stocking density (m^3) in experiment I.

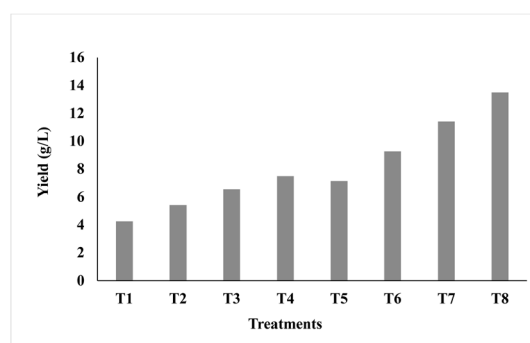


Fig. 4. Mean yield (g/L) of different treatments in experiment I & II.

weight gain, average daily weight gain and specific growth rates were followed a similar trend with the final body weight of different treatment groups. The T_5 treatment group showed a significantly reduced feed conversion ratio (2.75 ± 0.04) followed by T_6 (2.95 ± 0.03), T_7 (3.09 ± 0.08) and T_8 (3.21 ± 0.09) and the values were significantly differing among each other (Fig. 3). The protein efficiency ratio followed a relatively inverse trend with feed conversion ratio with the highest in T_5 (1.041 ± 0.02) and the lowest in T_8 (0.89 ± 0.03) respectively (Fig. 4). In the experiment II, the highest yield was obtained in T_8 (13.49 ± 0.17 g/L) followed by T_7 (11.41 ± 0.13 g/L), T_6 (9.27 ± 0.07 g/L) and T_5 (4.25 ± 0.03 g/L) and the values are significantly differed among each other (Fig. 4).

DISCUSSION

Stocking density is one of the prime factors that

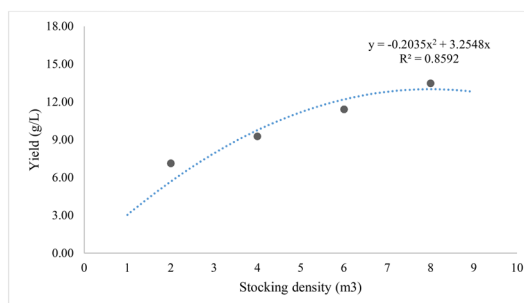


Fig. 6. Mean yield (g/L) of different stocking density (m^3) in experiment II.

could potentially affect the survival and production performance of aquatic organisms. Thus, using an appropriate density can increase the profitability of farming systems by maximizing the utilization of water and the other resources in the rearing system (Fairchild and Howell 2001). The present study has been conducted to determine the optimal stocking density of milkfish at different size groups for inshore cage culture. During both experiments, basal water quality parameters including temperature, dissolved oxygen, salinity, pH, alkalinity, ammonia and nitrite were within the ideal range for milkfish culture to support optimal growth and feed utilization (Sumagaysay-Chavoso and San Diego-McGlone 2003). In both experiments the growth performances of milkfish (FBW, WG, WG%, ADWG, SGR) were reduced significantly with the increment of stocking density. The corresponding feed utilization variables (FCR and PER) also showed a reducing trend with the increasing stocking densities in both experiments. Typically, the growth and feed utilization of fishes reduces when the biomass density increases in the culture systems. The higher densities elevate the chronic stress related to crowdedness in the culture systems and redirect the metabolic energy into stress amelioration instead of tissue synthesis (Andrade *et al.* 2015, Costas *et al.* 2008). In intense culture systems, the competition for space and food also increases among fishes (Qi *et al.* 2016). Usually, higher stocking densities result in a social hierarchy among fishes where dominant individuals have more access to food and space than other fishes (Wedemeyer 1996). Similar kind of results has been found in striped catfish (Chowdhury *et al.* 2020), tambaqui (Merola and de Souza 1988), African catfish (Coulibaly *et al.* 2007), Korean rockfish (Hwang *et al.* 2014), Nile tilapia (Gibtan *et al.* 2008) and olive barb (Upadhyay *et al.* 2022). There was no mortality observed in both experiments during the culture period.

Optimizing stocking densities for each species with a specific culture phase must be determined to ensure effective management and maximize production and profitability. In both experiments, controversial to growth response, the yield was increased significantly in every treatment group with the increase in the stocking density (Akyurt and Gokcek 2007). The yield (g/L) was almost doubled from the

lowest stocking density to the highest in both experimental conditions. Thus, it is evident that the yield is most likely to be increasing in further increment of stocking density of milkfish. Therefore, a simple regression analysis was carried out for stocking density (Nos/m³)– yield (g/L) responses of milkfish for both experiments in order to elucidate the probable yield response in varying stocking densities of milkfish at two different size groups. The yield response (g/L) of experiment I (Fig. 5) showed an increasing trend for the further increment of stocking densities for a larger extent and the results were controversial to the optimal stocking biomass of milkfish in cages reported by (Nowak 2007). Whereas in the experiment II the yield response (g/L) of milkfish were almost satiated in the stocking density of T₈ treatment (8 Nos/m³). From the available data, the further increment in stocking densities above 8 nos/ m³ will probably result into reduced yield responses (Fig. 6).

From the results of the present study, it can be concluded that milk fish sub-adults and adults can be stocked at 6 fish/m³ and 2 fish/m³ respectively for the higher growth rates and lower FCR. However, the highest production in cage culture depends on the volume and depth of the cages than those used in this study. Further, there is need for research by using the bigger size cages which can confirm the present study and also need to find the economic perspective for small scale farmers.

ACKNOWLEDGMENT

The authors are thankful for Dean, College of Fisheries Science, Muthukur, Andhra Pradesh, India for providing necessary facilities.

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