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Effect of dietary protein level on growth and survival of milkfish *Chanos chanos* fingerlings reared in floating net cages

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Abstract
The present experiment was conducted to study the optimum crude protein level for milkfish, *Chanos chanos* seed reared in floating net cages in closed bay, Bapatla, Andhra Pardesh, India, during May to July, 2016 for a duration of 63 days. Milkfish fry were obtained through natural seed collection. Rectangular floating net cages made of bamboo poles was provided inside with 1×1×2m hapas. Milk fish with mean body weight of 2.81±0.07 g were stocked at the rate of 40 fish/m^3 in each hapa in the rectangular cages. Triplicates were maintained for each treatment. Fish fry were fed with 5 artificial dry diets containing 12.5% (Control - C), 15% (T1), 25% (T2), 35% (T3) or 45% (T4) crude protein. The diets were fed at the rate of 10% of the biomass for a period of 63 days (9 weeks). Water quality parameters like D.O, p^H, salinity, NH^3, NO^{-2}, NO^{-3} and temperature were estimated at weekly intervals and were within the optimum range for culture of milk fish. At the end of 63 days growth experiment, fishes fed on 35% (T3) crude protein feed exhibited highest growth, survival, SGR, ADWG, biomass and best FCR. The subsequent positions were occupied by Treatments T4 (45%), T2 (25%), T1 (15%) and C (control). The present study showed that milkfish seed reared in floating net cages in the closed bay performed best at 35% crude protein. Based on the biological data, it was estimated that the optimal level of protein for Milkfish *Chanos chanos* fingerlings reared in net floating net cages was 35%.

Keywords: Milkfish *Chanos chanos*, growth, survival, crude protein, cage culture

1. Introduction
Aquaculture is one of the fastest growing food production sectors in the world and has been growing steadily over the last three decades and continuing [7]. It is clear that the bulk of fish required to feed the world in the coming decades will come from aquaculture. It is also time to put "nutrition security" at par with "food security". The global aquaculture production has tripled, growing at an average annual rate of 8.8%. Asia accounts for 88% of aquaculture production worldwide [7]. Mariculture is farming of marine organisms in cages, coastal enclosures, or containers with sea water. Presently almost half of all fish consumed by humans are farmed fish exceeding in value and/or in tonnage many capture fisheries worldwide. Cage culture is the most predominant form of mariculture in the Asia-Pacific and the emphasis on the cultured species and the nature, the intensity and scale of the culture operations differ from country to country. Marine aquaculture is a growing industry worldwide [28] due to the increasing demand for marine products by the human population. Milkfish (*Chanos chanos*, Forskal) is an economically important brackish water fish species cultured in Southeast Asia [5]. It is best suited for culture in the tropics because of its fast growth, efficient use of natural foods, herbivorous food habit and propensity to consume a variety of supplemental feeds, resistance to disease, handling and tolerance to a wide range of environmental conditions. It is euryhaline and can thrive in waters of 0 to 150 ppt salinity [5]. It is cultured commercially in waters of widely ranging salinity, i.e., freshwater lakes, brackishwater to hypersaline ponds, and marine cages. The success in cage farming depends on the availability of seeds of appropriate size for stocking. In nurseries the fry can be stocked in higher densities and reared. This would save the space and time in grow-out phase. Nursery rearing is an important phase in the seed production since this transitional phase can be used for acclimatization and weaning to artificial feed and environmental conditions that could be provided in the grow-out systems. Dietary protein is the most important factor affecting growth performance of fish and

feed cost [11] and it is the most expensive component [16]. It is the major macronutrient providing essential and non-essential amino acids for synthesis of protein and energy for maintenance and growth and tissue repair. Several factors influence dietary protein requirement including species, age/size of fish, dietary protein quality, energy level and the protein to energy balance [26] and environmental conditions. For successful aquaculture practices, it is needed to determine the minimum level of protein at which fish can attain maximum growth as well as the operational costs can be saved [16]. Hence, the present study was conducted to assess the protein requirements of milkfish seed reared in floating net cages at natural closed bay.

2. Materials and Methods

2.1 Experimental site

The present study was conducted in the closed bay near Suryalanka, Bapatla, Guntur district during May to July, 2016 for a duration of 63 days. It lies between latitude- 15°51' 04.54''N and longitude 80°31'58.87''E. The experimental site is shown in Fig 1.

2.2 Experimental fishes and acclimatization

Milkfish, *Chanos chanos* seed were collected from wild sources in Moolapeta village, U. Kothapeta mandal, East Godavari district (Fig 2). Fish seed were brought in aerated, closed bags and released into hapas (2m×2m×1.5m) in brakishwater 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 water in the ratio of 3:1 in each bag and the density of fish was 100/bag. Seed (Fig 3) was transferred into hapas within the cage in the experimental site. Before transfer into hapas the seed was slowly acclimatized to water of experimental site for one hour. Fish with an average initial weight of 2.81±0.07 g were randomly stocked in the net cages at 40 fish/cage and de-oiled rice bran is used as control feed (C) and four formulated feeds with different protein levels as CP 15% (T1), CP 25% (T2), CP 35% (T3) and CP 45% (T4), respectively were prepared. For each treatment triplicates was maintained.

2.3 Experimental cages

The floating net cages used for experiment was of $20^1 \times 10^1$. (Hapas of 1m × 1m × 2m size, 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 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 litre size were used as cage float for buoyancy of cage structure. Each cage was covered at the top with a piece of large mesh size (4.5 cm) net to prevent escape of fish by jumping and predation of birds as reported by [14]. The top of portion of each experimental hapa was covered with a fine mesh net up to 20 cm depth to prevent floating feeds from escaping the hapa and the whole structure was tied with anchors at each corner by nylon rope to make easy movement of floating cages depending on water level and flow. The cages were positioned in closed bay 500 m away from the shore with moderate water flow (0.05 $m.second^{-1}$). The submerged volume of the cages was invariably 1 m^3. Fish were acclimated with the sea environment by rearing them in hapa net for one week. Experimental setup was shown in Fig 4.

2.4 Feed preparation

Formulated feed with various crude protein levels were used. Feed formulation was done on the basis of Pearson's square method. Experimental diets were prepared with groundnut cake (GNC), fish meal (FM), de oiled rice bran (DOB) and wheat flour. 2% of vitamin and mineral mixture were added to experimental diets (Table 1). De-oiled rice bran was used as control diet (C). Four practical diets were formulated to contain protein levels of 15, 25, 35, and 45% named T1, T2, T3, and T4 respectively (Table 1). All the ingredients like fish meal, groundnut cake, de-oiled rice bran and wheat flour, used in feeds were obtained from local market. Ingredients (Table 2) used in the feed and all the experimental diets (Table 3) were estimated for proximate composition [1]. The experiment lasted for 63 days.

2.5 Feeding

A feed ring (0.5 × 0.5 m) was fixed at middle of each cage. Feed rings are enclosures that float at the water surface. They hold floating feeds and prevent the escape of feed out of cage and thus reduce wastage of feed. Feed was applied at the rate of 10% of body weight. Fish were fed twice a day at 8:00 hr. and at 16:00 hr. with each daily ration divided into two equal halves. Feeding was done manually to ensure ingestion of feed completely by the fish. Total fish in each treatment was sampled to obtain weight (INFRA DIGI, digital weighing machine, and model IN300, Chennai) of fish and the feed amount to be given was adjusted accordingly.

2.6 Water quality parameters

Water quality parameters like temperature (Celsius glass thermometer), salinity (Hand held Refractometer), dissolved oxygen (Titrimetric, Winkler's method APHA, 1995), pH (Digital pH meter), Alkalinity, Ammonia, Nitrite and Nitrate [2] were measured at 8.00 hrs. on weekly basis. Water samples were transported to the laboratory after collection and analysed.

2.7 Management of cages

The cages were removed from water at every 15 days interval to check the net and cleaning purpose. 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 works like mending of torn nets and realignment/readjustment of sinkers and anchors were also performed for proper management of cages.

2.8 Cage fouling

During the present study, it was observed that the fish and crustacean population around the cages increased. Algae, polychaetes, green mussels and other molluscs were the main bio fouling organisms on the net of the cage.

2.9 Fish samplings

Individual fish samples were randomly taken (Fig 5) weekly for enumeration of various growth parameters as below:

Weight increment = Final body weight (g) – Initial body weight (g).

Specific growth rate (SGR) = $[(L_n \text{ FBW} - L_n \text{ IBW}) / \text{day}] \times 100$, Where: L_n = Natural logarithm, FBW = final body weight, IBW = initial body weight.

Survival Rate (%) = Total number of fish survived / Total number of fish stocked×100

Feed Conversion Ratio (FCR) = Feed given (dry weight) (g) / Body weight gain (wet weight) (g).

Protein Efficiency Ratio (PER) = Weight gain (g) / Crude protein fed (g)
Average Daily Weight Gain (ADWG) = Final fish weight (g) – Initial fish weight (g) / Number of days.
Biomass = No. of fish × average body weight (g)

2.10 Statistical analysis
The data obtained on Growth, Weight Gain, Survival and Feed Conversion Ratio was treated statistically by applying two way ANOVA classifications according to [22]. The results were presented as mean ± standard error (SE).

3. Results
The details of the water quality parameters recorded during the study period in the floating cages are given in Table 4. The temperature, salinity, pH, D.O, Total alkalinity, Ammonia, Nitrite and Nitrate recorded in the cages were in the range 27.2 to 30.9^{0}C, 26 to 32 ppt, 7.7 to 8.3, 4.7 to 6.2 mg/l, 139 to 162 mg/l, 0.01 to 0.25 mg/l, 0.01 to 0.03 mg/l and 2.15 to 3.25 mg/l respectively. In the present study salinity of water was slowly decreased. This may be due to rains in that particular period and riverine inflow into the bay. Growth response and feed utilization of milkfish *Chanos chanos* after 63 days of feeding on experimental diets containing different levels of protein were presented in Table 5. During the experimental period, increments of growth at weekly intervals are shown in Fig 6. On the final day the highest average weight was observed in the T3. The Treatment T3 (35% CP) is found to be significantly superior when compare to other Treatments. The subsequent positions were occupied by Treatments T4 (45%), T2 (25%), T1 (15%) and C (control). However, the weight increment in the fishes also observed as significant and pair wise comparison was done by computing RBD. The Treatments T3 (35%) is found to be significantly superior when compare to other Treatments. The subsequent positions were occupied by Treatments T4 (45%), T2 (25%), T1 (15%) and C (control). Observations on the survival during the first week (7th day) revealed that 100% survival rate is maintained in all treatments except in T4 (97.5%). At the end of the experiment the highest average survival was observed in T3 and lowest survival was recorded in T4 (Fig 7). The treatments T1, T2 and C stood second, third and fourth respectively. Treatment T4 was found to be significantly lower FCR when compared to the other Treatments. Treatment T3, T2, T1 and C- Control occupied second, third, fourth and fifth positions. The Protein Efficiency Ratio (PER) values were ranged between 0.38±0.08 and 3.12 ±0.09 during the experimental period. Treatment C- Control was found to be significantly superior when compared to the other Treatments. Treatment T1, T2, T3 and T4 occupied second, third, fourth and fifth positions. The specific growth rate (SGR) was calculated for the total experimental period (i.e. 63 days). The maximum SGR was recorded in the T3 (1.73±0.02) and the minimum SGR was observed in the C- Control (1.10±0.04). The other treatments SGR is T4 (1.70±0.03), T2 (1.52±0.03) and T1 (1.31±0.02). The maximum Average daily Weight Gain (ADWG) was recorded in the T3 (0.419±0.02 g) and the minimum ADWG was observed in the C-Control (0.201±0.04 g). The other treatments T1, T2 and T4 recorded ADWG of 0.215±0.03, 0.339±0.04 and 0.383±0.06 respectively. The maximum biomass was recorded in the T3 (1022±0.25) and the minimum biomass was observed in the C- Control (496.6±0.12). The other treatments T1, T2 and T4 recorded net yield of 558.6±0.11, 824.5±0.20 and 832.3±0.18 respectively.
Biological preferences of milkfish fed with different crude protein level were presented in Table 3.

Table 1: Feed formulation of the diets used for study (Ingredients g/100g):

Diets Parameters	De-oiled Rice bran (Control)	CP (15%) T1	CP (25%) T2	CP (35%) T3	CP (45%) T4
Fish meal	-	4.0	18.82	33.19	47.0
GNC	-	4.0	18.82	33.19	47.0
De oiled rice bran	100	45.0	30.18	15.81	2.0
Wheat flour	-	45.0	30.18	15.81	2.0
Vitamin and mineral mix	-	2.0	2.0	2.0	2.0
Total	100	100	100	100	100

Control = Diet with 12.5% crude protein
T1 = Diet with 15% crude protein
T2 = Diet with 25% crude protein
T3 = Diet with 35% crude protein
T4 = Diet with 45% crude protein

Table 2: Proximate composition of the feed ingredients (% on dry matter basis).

Ingredients Composition	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 fibre	3.70	7.30	22.40	00.60
Ether extract	4.03	7.20	3.90	4.02
Total ash	3.46	5.60	15.80	01.55
Acid insoluble ash	5.60	7.60	8.20	4.50

Table 3: Proximate composition of the diets (g/100g dry diet) used for study

Feeds Composition	Control (C)	T1 (15%)	T2 (25%)	T3 (35%)	T4 (45%)
Dry matter	89.90	92.81	91.91	93.11	91.81
Moisture	8.20	07.19	08.09	06.89	08.19
Crude protein	12.50	14.94	24.99	35.00	44.89
Crude Fat	8.11	06.14	06.37	06.32	06.13
Crude fibre	22.40	05.43	13.59	11.00	09.00
Ash	15.80	02.54	02.57	02.57	02.47
NFE	32.99	63.76	44.39	38.22	70.78

Table 4: Water quality parameters in all floating net cages with milkfish (*Chanos chanos*) fed with different % of dietary protein levels.

Diets Parameters	Temperature (°C)	Salinity (ppt)	p^H	D.O (mg/l)	Total Alkalinity (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)
Initial	29.5	28	8.3	6.2	139	0.01	0.01	2.15
7	27.5	30	8.1	5.8	145	0.02	0.02	3.25
14	30.1	28	8	6.1	150	0.03	0.01	3.20
21	30.5	32	7.7	5.5	148	0.25	0.03	2.45
28	29.8	29	7.8	5.6	158	0.05	0.02	2.28
35	27.2	26	7.8	4.8	160	0.01	0.03	3.10
42	30.9	27	7.9	5.1	145	0.02	0.01	2.30
49	29.3	28	8.1	4.7	150	0.01	0.02	2.35
56	27.9	27	8.1	5.2	162	0.25	0.03	2.85
63	28.7	29	7.9	5.1	160	0.25	0.02	3.00

Table 5: Growth response and feed utilization of milkfish *Chanos chanos*, feeding on experimental diets containing different levels of protein

Diets Parameters	Control (C)	T1 (15%)	T2 (25%)	T3 (35%)	T4 (45%)
Initial weight(g)	2.81±0.08	2.86±0.08	2.85±0.04	2.80±0.07	2.72±0.04
Final weight(g)	15.52±0.10	16.43±0.09	24.25±0.09	29.20±0.11	26.85±0.13
Weight gain (g)	12.71 ± 0.11	13.57 ± 0.15	21.40 ± 0.10	26.40 ± 0.16	24.13 ± 0.18
Survival (%)	80	85	85	87.5	77.5
SGR (%)	1.10 ±0.04	1.31 ±0.02	1.52 ±0.03	1.73 ±0.02	1.70 ±0.03
FCR	3.66±0.14	3.61±0.10	2.95±0.13	2.76±0.11	3.02±0.13
PER	2.39±0.09	1.93±0.06	1.56±0.10	1.27±0.08	0.94±0.05
ADWG(g)	0.201 ±0.05	0.215 ±0.03	0.339 ±0.04	0.419 ±0.02	0.383 ±0.06
Biomass(g)	496.6 ±0.12	558.6 ±0.11	824.5 ±0.20	1022 ±0.25	832.3 ±0.18

Fig 1: Experimental site at suryalanka in Guntur district.

Fig 3: Milkfish, *Chanos chanos* seed.

Fig 2: Wild seed (*Chanos chanos*) collection.

Fig 4: Experimental setup (Floating net cages).

Fig 5: Milkfish *Chanos chanos* length measurement.

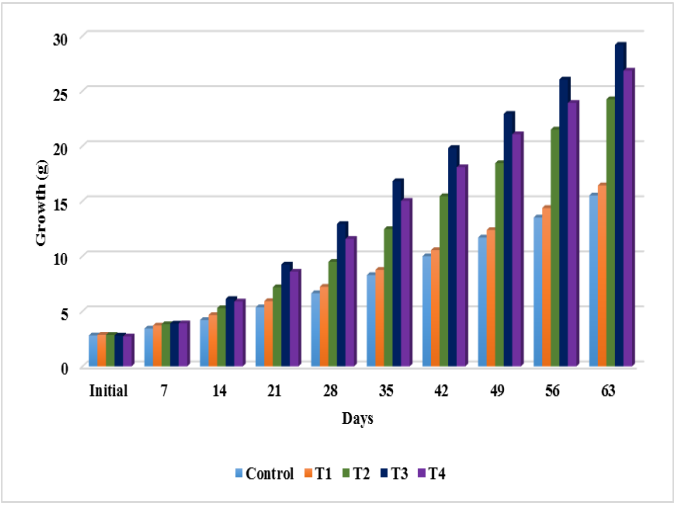

Fig 6: Growth performance (g) of milkfish (Chanos chanos) fed with different % of crude protein (CP) experimental diets

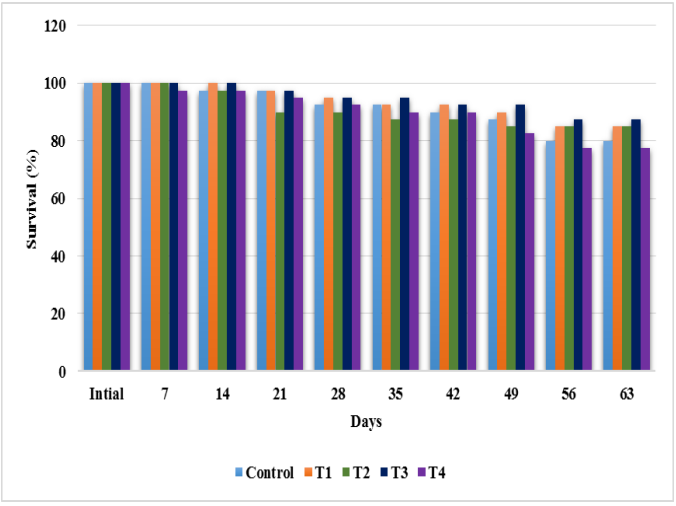

Fig 7: Survival rate of milkfish (Chanos chanos) fed with different % of crude protein (CP) experimental diets

4. Discussion

In the present study fishes fed on 35% CP (T3) resulted in high final body weight, weight gain and SGR. The present study is an agreement with the studies of [15]. They reported that diet containing 32% protein gives best results in milkfish fingerlings (12-18 g). They reported slightly less CP. This may be due to larger size of fish in their study. 30 to 40% dietary protein is required by milkfish fingerlings weighing 0.5 to 0.8 g [18]. Another study conducted by [20] on wild milkfish fry in net enclosures in ponds, reported that 40% protein feed showed maximum growth. Milkfish fingerlings showed the best growth in terms of both weight gain and increase in body length when reared on a purified diet containing 35% casein and 15% gelatin as protein sources [26]. Supplemental diet containing 34.3% protein showed better growth performance in milkfish than the natural food (chicken manure) [23]. Fish should be fed supplementary diets containing appropriate (40%) protein levels to get a high-yield in milkfish culture [8]. In the present study fishes fed on supplemental feed with 35% CP exhibited significantly higher growth than the fishes fed on DOB (11% CP-Control) or 15, 25% CP. However, there was no significant in growth of fish fed on diet with 45% CP compared to fish fed on with 35% CP. Fish fed diet beyond 35% Crude protein could not produce additional growth. There is some evidence that at very high feeding levels, protein deposition tends to level off (plateau) [4]. Feeding the milkfish on formulated diets resulted in significantly higher mean fish weight gain than that of fish fed on nonformulated diets like coconut cake and maize bran [12]. In the present study also non formulated feed (DOB - Control) showed lower growth than the formulated diets (T1, T2, T3 or T4). Milkfish showed better growth when fed the diet with 30% protein originated from fishmeal than those given diets of lower fishmeal content. They concluded that the diets for milkfish fry should contain not less than 15% of the protein from fishmeal to support good growth [19]. In the present study T3 (35% CP) feed contain 18% fishmeal. According to [19] the weight gain of milkfish fry were directly related to fishmeal content of the diet. In the present study also milkfish showed better weight gain in Treatments T3 and T4 which are having more than 30% fishmeal in their diet. This may be reason for good growth. Crude protein requirement varies with species and size. According to [17] a diet with 35% protein was found to be best for rabbitfish, *Sigunus guttatus* fry. According to [21] optimum dietary protein level is about 32% for juvenile *Zacco barbata*. Optimal level of protein for *O. niloticus* weighing between 1.0 g and 5.7 g was 35% [6]. Tilapia fingerling and advanced juvenile showed optimum growth performance with the 35%-CP diet [13]. Current study is in agreement with the observations made by [4, 6, 8, 17, 20, 21, 26]. In the present study result was higher than the observations of [25]. They reported that the most cost-effective protein range in supplemental feeding for milkfish is between 22% to 27% protein for milkfish size range of 15 g. Likewise according to [3] milkfish grown to marketable size are fed with commercial pellets containing 23–27% protein. Culture of milkfish (*Chanos chanos*) in brackishwater ponds, profits can be earned by using 24% protein diet [24]. According to [9] compounded diets containing 23%–27% crude protein have been used successfully for deep-water pond culture of milkfish in Taiwan. In the present study results was lesser than the observations of [10]. He reported that diet containing 40 percent protein was optimum for the growth of milkfish fry stocked at 500 per m^2 in concrete tank. In the present study natural food available also might have contributed for the growth of milkfish. In the present study significantly higher efficiency of feed conversion observed in fish fed diets 35 and 45% CP. Similar results were reported by [19] in milkfish. In this study, salinity and temperature evidently influenced fish growth, since dissolved oxygen and other water quality parameters, NH_3, NO_2, pH, alkalinity were within tolerable ranges.

The feed was well accepted by milkfish from 1st day itself. Fishes were approaching the feed within 5 min. Fish fed the diets containing CP 35% and 45% (T3 and T4) exhibited a schooling behavior during feeding. No such behavior was observed in fish given diets containing 12.5% (D.O.B), 15% or 25% CP (Control, T1 and T2). The feeds given in all treatments were consumed within 20 min after feeding but fish fed the diets containing 35 and 45% protein (T3 and T4) consumed the feed within a shorter period of time. It may be concluded that for *Chanos chanos* juveniles a dietary protein level of 35% CP is the optimum for maximum growth and good feed conversion ratio in cages grown in closed bay. In addition, the natural food contribution under cage culture conditions should also be considered. Further, the study provides important evidence that milkfish fed with 35% CP performed well in net cages at that particular environment.

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