Research Article

Diverse Protein Levels and their Impact on Survival and Growth Proficiency of Monosex Tilapia, *Oreochromis niloticus* in Captivity

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Abstract | This study was conducted to evaluate the impact of dietary protein concentration on survival and growth efficiency of monosex tilapia, *Oreochromis niloticus* which were maintained in aquarium during the period of April-June 2020. Three diets were prepared by using locally available ingredients (wheat and rice bran, powder of mustard oil cakes, whole wheat flour etc.) to have varying levels of crude protein, specifically 35%, 40%, and 45%. The aeration provided to the aquaria was continuous for 24 hours, and feeding occurred twice daily. The findings of this study indicate that T-II demonstrated the highest performance in terms of growth metrics, followed by T-III. Conversely, a significant drop in performance was observed in T-I ($p < 0.05$). The highest temperature, measured at T-III, was recorded as 32 °C during the month of June. Conversely, the lowest temperature, observed at T-I, was recorded as 25.5 °C in the month of April. The month of June exhibited a higher dissolved oxygen (DO) concentration in T-II, measuring at 5.6 mg/l. Conversely, the month of April recorded a minimum DO concentration in T-III group, measuring at 3.6 mg/l. The study determined that the feed with a protein content of 40% was suitable for promoting the growth efficiency of *Oreochromis niloticus* when grown in glass aquaria.

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Introduction

Tilapia has now become the prioritized farmed fish after carps (FAO, 2014; Cao *et al*., 2015; Saikia and Das, 2015; Fitzsimmons, 2016). Its farming is being expanded world widely in both developed and developing countries. Being cultured in tough environmental conditions and breed easily

in captivity are the features making them ideal for culturing. Dietary protein is considered as the main nutrient for good growth; hence adequate nutritional amount is required for optimal growth of any living body. The quantity of energy in the diet of fish holds considerable importance, as an excessive amount of useable energy can potentially limit the intake of protein and other essential elements, leading to hindered growth (Abbas and Siddiqui, 2013). Therefore, it is crucial to upsize appropriate feed stuffs that will be practiced moreover as additional régime in artificial condition and in whole feed in cisterns. Escalation of tilapia culture is a virtuous solution for increasing fish production. Therefore, it is essential to manufacture appropriate feeds either additional supplemental and ample diet in different conditions and cisterns. Various researches have been conducted on tilapia such (El-Saidy and Gaber, 2005; Abdel-Tawwab *et al*., 2010; Ng and Romano, 2013; Saikia and Das, 2015; Cao *et al*., 2015; Saikia and Das, 2015; Nguyen, 2016; Yue *et al*., 2016; Fitzsimmons, 2016). Shiau and Lin (2006) conducted experiments and discovered that amino acids are needed since the fish cannot synthesize them in sufficient numbers and hence must be provided by the meal for muscle production, enzymatic processes, and energy supply to the fish, which helps them grow. Agbo *et al*. (2011), on the other hand, observed that 50% crude protein was sufficient to sustain the growth of brooder fish fed fishmeal-added cottonseed-based diets. Excessive amounts of dietary protein that are metabolized for energy should, when suitable, be substituted by cheaper alternative sources of energy (Li *et al*., 2023). Hence, this experiment initiates to find outcome of various protein ratios in diets on the growth of mono-sex Nile Tilapia. As obtained protein ratio in the diet for this fish species might be significantly in preparation of a nutritious feed for successful intensive culture.

Materials and Methods

Fingerlings availability

A healthy group of experimental fish with uniform size (0.5 g) was obtained from a mono sex seed production hatchery. The fish were acclimatized in 10 aquaria size (W-18, L-6 and H-8 inches) and were maintained and designed as different treatments namely T-I, T-II and T-III with three replications. The experimental aquaria were supplied aeration through blower. The growth and water quality parameters were noted monthly during the experimental period of three months.

Feed ingredients availability

The test diets were prepared by the locally obtainable raw materials including mustard oil cake (MOC), wheat and rice bran powder and wheat flour was assembled from the market of Hyderabad and Thatta. The experimental diets were formulated after grinding, sieving and after mixing were manually put in the pellet machine for the pellets (1mm). Diet composition feed is presented in (Table 1). All the diets were assigned in triplicates and were hand fed at 20-15 per body weight 4 times daily.

Table 1: *Feed ingredient (%) for the rearing of Oreochromis niloticus.*

Ingredient	Protein/ kg (%)	T-I (35%)	T-II (40%)	T-III (45%)
MOC	30.33%	250	250	300
Fish meal	59.61%	400	500	600
Rice bran	10.26%	150	70	30
Wheat bran	12.92%	140	120	30
Wheat flour	12.92%	50	50	30
Salt	-	5	5	5
Vitamin	-	5	5	5
		1000 g	1000 g	1000 g

Sampling

The sampling was done once a month. Fish body weight and length were measured carefully. The aquaria were syphoned to prevent the growth of resident ammonia.

Water quality

During the culture phase, water temperature (°C), pH, dissolved oxygen (DO) (mg/L), total dissolved salts (TDS), and salinity were all measured and maintained. Temperature was measured using a glass Celsius thermometer, salinity with a refractometer, total dissolved salts with a TDS meter, and pH and dissolved oxygen (DO) with digital pH meter and DO meter, respectively.

Data analysis

One-way analysis of variance (ANOVA) was used to investigate how protein levels affected the results. Then, Duncan Multiple Range Test (DMRT) was applied with a 5% threshold of significance (Zar, 2001).

Results and Discussion

Cost of experimental feeds

Price of the ingredients is presented in Table 2. Feed

T-I had the price of Rs. 24.9/kg, T-II Rs. 26.9/kg and T-III had a total cost of 28.25/kg.

Table 2: *Costs of the experimental feeds (Rs./kg).*

Ingredient	T-I (35%)		T-II (40%)		T-III (45%)	
	Quantity	Cost	Quantity	Cost	Quantity	Cost
MOC	250	7.50	250	7.50	300	9.00
Fish meal	400	12.0	500	15.0	600	18.0
Rice bran	150	1.80	70	0.90	30	0.25
Wheat bran	140	1.60	120	1.50	30	0.25
Wheat flour	50	1.50	50	1.50	30	0.25
Salt+Vitamin	10	0.50	10	0.50	10	0.50
		24.9		26.9		28.25

Proximate analysis

Proximate analysis of experimental diets is presented in Table 3. Highest NFE appeared in the diets of T-II followed by T-III, though T-I possessed lowest NFE content.

Table 3: *Proximate compositions of the diets for the rearing of Oreochromis niloticusjuveniles.*

	Moisture	Crude protein	Crude lipid	Ash	Crude fiber	NFE
T-I	15.2± 0.19[a]	35± 0.19a	10.5± 0.19[a]	14.2± 0.19[a]	13.1± 0.19[a]	12.0± 0.19[b]
T-II	10.0± 0.19[a]	40± 0.19[b]	10± 0.19[a]	10.8± 0.19[b]	10.0± 0.19[b]	19.20± 0.19[a]
T-III	9.1± 0.19[a]	45± 0.19c	11± 0.19[a]	11± 0.19[b]	11.0± 0.19[b]	13± 0.19[b]

Nitrogen Free Extract calculated as: 100-% (Moisture + Protein +Lipid +Ash + Crude Fiber).

Growth performance

Fish body initial and final mean weight, WG (% g), SGR, survival rate, FCR, and yield is given in Table 4. *O. niloticus* having an initial body weight of 0.5 g attained a weight of 40.5 g, 52.2g, and 49.3g for T-I, T-II, and T-III. The maximum gain in weight was recorded in T-II (51.5 g) and the lowest was observed in T-I (39.5g). The survival rate was unaffected in T-I and T-II when compared to the T-III. Specific growth rate (SGR) was higher in T-II - 0.023 (%/day) and lower in T-I, 0.021 (%/day). Feed conversion ratio (FCR) values were 0.9 g, 1.4g and 3.0g for T-I, T-II and T-III, respectively. The fish production values were 0.09 kg/m2, 0.12 kg/m2 and 0.11 kg/m2 for T-I, T-II and T-III throughout the study period.

Table 4: *Growth parameters of Oreochromisniloticus throughout the study period.*

Parameters	T-I	T-II	T-III
Mean initial weight (g)	0.5±0.19	0.5±0.19	0.5±0.19
Mean final weight (g)	40.5±3.86	52.2±7.84	49.3±3.95
Weight gain (g)	39.5±1.23[b]	51.5±2.63[a]	48.5±1.95[a]
[1]SGR (%/day)	0.021	0.023	0.022
Survival	98%	98%	92%
[2]FCR	0.9±3.86[a]	1.4±3.86[a]	3.0±3.86[b]
Production/kg/m^2	0.09±3.86	0.12±3.86	0.11±3.86

[1]SGR= Specific Growth Rate; [2]FCR= Feed Conversion Rate.

Water quality parameters

The temperature varied significantly (p 0.05) over the course of the research period (Table 5). T-III experienced the highest temperature (32 C) in June, while T-I experienced the lowest temperature (25.5) in April (Table 5). Dissolved oxygen was maintained by supplying the aeration. T-II showed higher DO (5.65 mg/l) in the month of June, while T-III showed the lowest (3.63 mg/) in the month of April. Salinity level remained constant throughout the study period. Total dissolved solids (TDS) appeared to be lowest in the treatment 45% in the month of April.

Although *O. niloticus* growth was suppressed when fed 45% protein in the current study, it is possible that feeding fish with 45% protein in the diet is not cost-effective. Therefore, it could be reasonable to add 40% more protein to the diet of *O. niloticus*. At a higher protein ratio (45%), growth and conversion efficiency significantly decreased, demonstrating that a diet with

Table 5: *Monthly changes in water quality parameters throughout the study.*

Parameter	T-I (35% protein)			T-II (40% protein)			T-III (45% protein)		
	April 2016	May 2016	June 2016	April 2016	May 2016	June 2016	April 2016	May 2016	June 2016
T (°C)	25.5±0.5	28.5±0.3	29.5±0.5	26.5±0.2	29.5±0.4	31.0±01.0	28.5±0.5	30.5±0.3	32.5±0.5
DO (mg/L)	4.16±0.062	4.83±1.24	3.8±0.4	4.2±0.49	4.75±0.75	5.65±0.45	3.63±0.98	3.8±1.5	4.25±0.65
Salinity (ppt)	0.25±0.05	0.25±0.05	0.25±0.05	0.25±0.05	0.25±0.05	0.25±0.05	0.25±0.05	0.25±0.05	0.25±0.05
TDS	314±23	250±7.5	305±5.5	300±10	305±6.5	230±50	226±16	300.5±6.5	287±7.5
pH	8.1±0.4	8.22±0.17	8.18±0.18	8±0.3	8.1±00.1	8.05±0.05	6.8±0.3	8.0±0.5	8.75±0.25

40% protein (T-II) met the requirement and was the most effective for maximizing growth and achieving outstanding conversion efficiency. Researches with various species have been found that are in agreement with our current study (Jauncey, 1982; Cho *et al*., 1985; Khan and Jafri, 1990; Vergara *et al*., 1996; Bai *et al*., 1999; Ng *et al*., 2001; Kim *et al*., 2002; Kim and Lee, 2005; Wang *et al*., 2006). The fact that the fish stop consuming the protein once the ideal level has been reached may be the cause of the growth reduction at protein levels higher than 40%. A surplus of protein in the diet may reduce performance because catabolism requires more energy than protein synthesis (Siddiqui and Khan, 2009). Due to a lack of non-protein energy required to deaminate and eliminate extra absorbed amino acids, weight gain may be reduced at greater or excessive protein levels (Cho *et al*., 1985; Jauncey, 1982; Kim *et al*., 2002; Vergara *et al*., 1996). Various researchers have noted varying protein requirements for tilapia species, such as Balarin (1982), who came to the conclusion that tilapia fry needed 35–50% protein. He also noted that fish weighing 1–5g needed 30–40% protein, and fish weighing 5–25g needed 25–35% protein. According to Hamza and Kenawy (1997), in the instance of Nile tilapia, a feed with a 40% protein content performed better than other levels of other feedstuffs. Hafedh (1999) reported that fish fed with 40 – 45% exhibits significantly better feed conversation, survival, and growth in contrasts to feed with 25 – 35%. These results may be because of fish pertains to live with optimum protein level may be insufficient for such species. The results of a few researchers are in agreement with present work. Other researchers found alike results on the studies based on various feed trials with different protein concentrations for the rearing of Nile tilapia. The protein ratios of several species of tilapia have been estimated to range between 20% and 56% (De Silva and Perera, 1985; Siddiqui *et al*., 1988; El-Sayed and Teshima, 1991; Hamza and Kenawy, 1997) observed maximum growth of fish fed with 40% protein then other stuffs, (Hafedh, 1999; Khattab *et al*., 2000); observed maximum protein requirement ranged between 38-40% which is in range with present study. Few authors from different countries reported little bit variation in results like El-Saidy and Gaber (2005) reported 25% protein diet at 2% body weight is suggested for Nile tilapia cultured in concrete cisterns. The decreasing trend in our study in terms of growth has also been reported in *Catla catla* (Dars *et al*., 2010), *Anguilla japonic* (Nose and Arai, 1972), *Pleuronectes platessa* (Cowey, 1972) and *Ctenopharyngodon idella* (Dabrowski, 1977) identical to those described for this investigation, with the increasing level of protein above the optimum. On the other hand, researchers like Narejo *et al*. (2002) studied the growth of the snake eel Pisodonophis boro and discovered that fish fed with 35% protein produced noticeably higher growth than fish fed with 40% and 45% protein. For higher growth, survival, and output when raising *Mastacembelus armatus* in cemented cisterns, Narejo *et al*. (2003), reported 35% gross protein. According to Narejo *et al*. (2015), the pellet feed with 38% (gross protein) was shown to be the ideal protein requirement for *Labeorohita* intensive cultivation. All of these observations lend weight to the conclusions of the current investigation. The observed water quality parameters were determined to be within acceptable ranges during the study period, as reported by (Narejo *et al*., 2002, 2003, 2015).

Conclusions and Recommendations

It was concluded that the 40% protein feed and stocking density 20 fish/L was found to be the best for the culture of Nile Tilapia *Oreochromis niloticus* in aquaria for the intensive culture under control conditions. Fish growth is influenced by the amount of protein in the diet and survival rate by the stocking density. Hence feeding optimum protein with suitable stocking density will increase the production. Environmental conditions and stocking density should be optimum since they affect feed intake and efficiency. These parameters are very important since they determine the general survival of the fish.

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Novelty Statement

Fish growth is influenced by the amount of protein in the diet and survival rate by the stocking density. Hence feeding optimum protein with suitable stocking density will increase the production.

Author's Contribution

Naeem Tariq Narejo: The experiment was designed and conceptualized by.
Muhammad Hanif Chandio: Contributed to the collection of data.
Faheem Saddar: Gathered samples of young fish for the study.
Bushra Ainy Dars and Majida Parveen Narejo: Feed preparation
Hafeez ur Rehman Narejo and Athar Mustafa Laghari: Data analysis
Shafiq ur Rahman Shaikh: Contributed to the chemical analysis
Ghulam Abbas and Shahnaz Rashid: Helped in format setting and provided appropriate materials.

Conflict of interest
The authors have declared no conflict of interest.

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