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Effect of different protein levels on growth and survival of Nile tilapia (*Oreochromis niloticus*) fry

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Abstract
The present study deals with the "Effect of different protein levels on growth and survival of Nile tilapia (*Oreochromis niloticus*) fry". The experiment was conducted with three replications for each test diet. During experiment of 42 days, the fry of Nile tilapia (*Oreochromis niloticus*) were fed on four different protein (Casein) levels mixed with basal diets containing rice bran, groundnut cake and mineral mixture. The three different feed ingredients have been mixed with conventional Nile tilapia diet at 26%, 28%, 30% and 32% protein levels. The control containing rice bran, groundnut cake and mineral mixture was also run separately. These experimental feeds were given to fishes @ 8% of body weight per day. The growth performance of test fishes was observed at every weekly interval and accordingly the diet of fishes was readjusted. During the experimental period, the water quality parameters were found congenial for fish growth.

Keywords: Protein, *Oreochromis niloticus*, Growth, Survival rate, feed

1. Introduction
Aquaculture of Nile tilapia would require the formulation of efficient food with optimum potency to meet the protein requirements during grow-out period (Kenawy, 1993) [19]. Protein is considered as the main constituent of the fish body thus sufficient dietary supply is needed for optimum growth. Since protein is the most expensive component of the diet, therefore, the amount of protein in the diet should be just enough for fish growth where the excess protein in fish diets may be wasteful and cause diets to be unnecessary expensive (Ahmad *et.al.* 2004) [3]. Thus, reducing feeding costs could be a key factor for successful development of aquaculture. Information on the nutritional requirements of Nile tilapia is available to some extent. Dietary protein requirement has been stated to be between 32 to 50% for juvenile tilapia and for larger tilapia 25 to 30% (El-Saidy and Gaber, 2005 [14]; Ali *et al.,* 2008 [4]; Nguyen *et al*., 2009 [21]; Abdel-Tawab *et al*., 2010) [1].
The protein requirements of tilapia differ with each life stage of the fish (Jauncey and Ross 1982) [18]. According to their study the fry and fingerlings require a diet in high protein, lipids, vitamins and minerals and lower in carbohydrates. Adult fish need more calories from fat and carbohydrates for basal metabolism and a smaller percentage of protein for growth. In view of this, the present study is proposed to investigate the optimum level of protein in tilapia fry diet.

2. Materials and methods
A. Experiment design
The experiment was conducted for 42 days at the Department of Aquaculture, College of Fisheries, Maharana Pratap University of Agriculture and Technology, Udaipur. A total number of 150 (*Oreochromis niloticus*) fry were obtained from fish seed production unit, Directorate of Research, MPUAT, Udaipur. The obtained fish were healthy and free from any infection and were placed in rectangular plastic tanks of 225 liters capacity. After one week of acclimatization period, fish with an average body weight of 0.883g were divided into five groups. Each tank was washed and disinfected before the introduction of fish. Ten fishes were randomly distributed in each tank with three replications. Each plastic tank was filled with Bore-well water. Siphoning was done at every day. Fish were fed two times daily @ 4% in the morning at 9.00 am and 6.00 pm in the evening for 42 days. Five experimental diets were prepared to evaluate the effects of different protein levels 26% (T_1), 28% (T_2), 30% (T_3) and

32% (T_4) of feed protein (Casein) and without casein in control to see impact on growth performance and survival of Nile tilapia (*Oreochromis niloticus*) fry. At the end of every week, the weight of experimental fish was determined using weighing scale. Survival rate was calculated at the end of experiment.

B. Experimental diet

Nile tilapia feed was formulated using groundnut cake, rice bran and mineral mixture and commercially available casein. The treatment tank considered as a control which was fed without casein, while groups T_1, T_2, T_3 and T_4 contained casein. The dry ingredients (consisting of groundnut cake, rice bran, and minerals mixture) of the experimental diets were thoroughly mixed and made in a pellet form by addition of boiled water. The paste was then extruded through a commercial pelletizing machine. The resulting Vermicelli like diet was air dried and stored in air tight containers for further use.

C. Growth parameters

Growth measurement

Following growth parameters were studied using standard methods. (Garg *et al*., 2002) [16].

Weight Gain: Weight gain was determined between the final weight and initial weight of experimental fish.

Weight gain (g) = Final weight (g) - Initial weight (g)

$$\% \text{ gain weight in g} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Specific Growth Rate

$$SGR\ (\%) = \frac{(\ln Wt - \ln Wo)}{D} \times 100$$

Where,

Wo = Initial weight of fish (g)

Wt = Final weight of fish (g)

D = Duration of feeding (days)

Feed Conversion Ratio (FCR)

$$FCR\ (g) = \frac{\text{Weight of food given (g)}}{\text{Weight gain of fish (g)}}$$

Gross conversion efficiency (GCE)

$$GCE = \frac{\text{Weight gained (g)}}{\text{Food given (g)}}$$

Survival rate (%)

Survival rate (SR) is the number of fish that survive during the experimental period expressed as a percentage of the stocked fish. It was calculated by subtracting the number of fish dead during the culture period from the fish stocked and then expressing it as percentage (Charo-Karisa *et.al.* 2006) [9]. Survival rate of experiment fish was estimated according to following formula:

$$\text{Survival rate (\%)} = \frac{Nt \times 100}{No}$$

Where,

Nt = Final number of fishes

No = Initial number of fishes

D. Estimation of proximate composition of experimental diets

The experimental diets were analysed for the proximate composition *viz.*, moisture, crude protein, fat, carbohydrates and ash contents as per standard methods of AOAC (1970) [7].

Table 1: Proximate composition of diet

S. No.	Contents	Control	T_1	T_2	T_3	T_4
1.	Moisture (%)	7	6.8	6.2	5.8	6.2
2.	Crude protein (%)	23.59	25.73	27.88	29.60	31.75
3.	Fat (%)	12.33	11.66	11.66	12.66	13.00
4.	Ash (%)	14.4	16.4	16.0	16.6	15.2
5.	Carbohydrate (%)	42.68	39.41	38.26	35.34	33.85

E. Statistical analysis

Mean, percent, frequency, standard deviation, standard error of mean and completely randomized design was used to evaluate the effect of different protein levels on growth and survival of Nile tilapia (*Oreochromis niloticus*) fry.

3. Results

The experimental results of fish growth parameters carried out during the experimental period are presented in (Tables 2 to 6). It is apprehended from the results of the present study that the fish growth parameters were significantly different in all the treatments and control.

Growth parameters

Different doses of Casein-a protein supplementation, revealed significant differences in growth rates among various treatments.

Weight gain

The diet T_4 gave the highest weight gain of (1.139 g), whereas, the lowest (0.676 g) being in control (Tables2). The weight gain of fishes was noticed in the following ranking order T_4 (1.139)> T_3 (0.919)> T_2 (0.855) > T_1 (0.726) > control (0.676).

The total weight gain in percent was highest (127.463%) in T_4 as compared to (75.325%) in control. Whereas, the weight gains in percent T_3, T_2 and T_1 were 102.990%, 100.391% and 82.306% respectively.

Statistical analysis of variance indicated that weight gain was significantly higher in all the treatments as compared to control. Moreover, the test of critical difference (CD) also indicated significant difference CD=0.108 between all the treatments in 0-42 days. Similarly, the per cent weight gain in 42 days of feeding trial has also shown a statistically significant (CD=18.928) increase in weight gain as compared to control.

Percent growth per day in body weight of fry

The growth per day in percent body weight gain was higher 2.110 in $T_4$and lowest 1.416 in control, (Table 3). Whereas, it was 1.836in T_3, 1.794 in T_2 and 1.525 in T_1 respectively.

Statistical analysis of variance indicated that the growth per day in percent body weight gain was significantly higher in all the treatments as compared to control. Moreover, the test of critical difference (CD) also indicated significant difference CD = 0.251 between weight gain in all the treatments in 0-42 days.

Specific growth rate (SGR)

The value of specific growth rate is used to compare growth on a daily basis. The significantly higher growth rate and specific growth rate indicate the effective role of protein in the growth performance. The highest SGR (0.850%) was recorded in treatment T_4after 42 days of experiment which was significantly different from control, T_1, T_2, and T_3. The treatments mean revealed that the best SGR was recorded in T_4 (0.850%), followed by T_3 (0.732), T_2 (0.719), T_1(0.621) and lowest (0.581) in control (Table 4). Thus, the treatment T_4 showed best SGR which was significantly (CD = 0.083) higher as compared to control as well as all other treatments.

Feed given

The amount of feed given to the fishes in 0-42 days, duration of investigation as per requirements. The maximum amount of food was given to the fishes in T_4 (4.116 g) followed by T_3 (3.678 g), T_1 (3.621 g), control (3.581 g) and T_2 (3.500 g) respectively.

The statistical analysis of variance indicates the amount of feed given was significantly higher from T_2 to all other treatments.

Food conversion ratio (FCR)

The results on FCR in different treatment groups have been depicted in (Table 5). The lowest FCR was found with T_4which was significantly different from control, T_1, T_2, and T_3. The mean values of treatments revealed that the best FCR was recorded with T_4 (4.395), followed by T_3 (6.025), T_2 (6.352) and T_1 (7.094) and control (7.580). Thus, treatment T_4 showed best FCR which was significantly (CD=2.732) higher as compared to control as well as all other treatments during 0-42 days of feeding experiment.

Gross conversion efficiency (GCE)

The results on gross conversion efficiency in different treatment groups have been depicted in (Table 6). The best GCE (0.264) was recorded in treatment T_4followed by T_3 (0.230), T_2 (0.224), T_1 (0.191) and lowest in control (0.177).The statistical analysis of variance also indicated significant deference (CD= 0.031) of GCE between treatments and control during 0-42 days of experimental period.

Survival rate

During the experimental period, 100% survival rate of test fishes was observed owing to good water quality maintained in the experimental tanks. The survival rate of each fish group at dietary protein levels or among different sizes of Nile tilapia was unaffected.

4. Discussions

Many authors obtained conflicting results from their studies on the effect of dietary protein levels on the growth of Nile tilapia. The dietary protein requirements of several species of tilapia have been estimated to range between 20% and 56% (EI-Sayed and Teshima, 1991) [15]. De Silva and Perera (1985) [11], Siddiqui *et al*. (1988) [24] and Abdelghany (2000) [2] reported that the optimum dietary protein levels for growth of Nile tilapia fry was 30% crude protein. Hamza and Kenawy (1997) [17] found out that 40% protein was more potent than other levels for Nile tilapia growth. Al-Hafedh (1999) [5] and Al-Hafedh *et al*. (1999) [6] found out that the better growth of Nile tilapia was obtained at high dietary protein levels (40-45%) rather than 25-35%. Khattab *et al*. (2000) [20]. studied the optimum dietary protein level for Nile tilapia collected from Aswan, Abbassa, Manzalah and Maryut. They found that the optimum dietary protein level is 37% for Abbassa strain, 27% for Aswan strain and 32% for Manzalah and Maryut strains.

In general, the growth (net weight gain) in the control and other treatments in the present study was in following order:

T_4 (1.139 g) > T_3 (0.919 g) > T_2 (0.855 g) > T_1 (0.726 g) > control (0.676 g)

The percent weight gain increase in treatments as compared to the control was as follows:

T_4 (127.463%) > T_3 (102.990%) > T_2 (100.391%) > T_1 (82.306%) > control (75.325%)

The mean values of treatments revealed that the best SGR was recorded in T_4 (0.850%), followed by T_3 (0.732%), T_2 (0.719%), T_1 (0.621%) and lowest (0.581%) in control. The results are in conformity with the findings of Bisht and Panday (2013) [8] who found higher SGR as compared to control in *Labeo rohita* fed with *Bacillus subtillis* incorporated diets for 90 days. Sahu *et al.* (2007) [23] and Dhawan and Kaur (2002) [13] also found that the provision of each additional input including fertilization such as cow dung, urea and single super phosphate and supplementary feed did affect fish growth.

The mean values of treatments revealed that the best FCR was recorded with T_4 (4.395), followed by T_3 (6.025), T_2 (6.352), T_1 (7.094) and control (7.580). Ramos *et al*. (2013) [22] conducted an experiment to evaluate the effect of dietary supplementation of multi species (*A. bacillus sp., Pediococas sp., Enterococcus sp., Lactobacillus sp*.) and single species probiotics (*B. pediococcus acidilactici*) on growth performance and gut microbial composition of rainbow trout (*Onchorhynchus mykiss*). The results showed that dietary supplementation changed the gut microbial composition and improved growth in the fish. De Silva and Davy (1992) [12] stated that digestibility of fish plays an important role in lowering the FCR value by efficient utilization of food. The digestibility depends on daily feeding rate, its frequency and the type of food used (Chiu, 1989) [10]. The wastage of food might also lead to poor feed utilization and higher FCR.

In the present study the best GCE (0.264) was recorded in treatment T_4 followed by T_3 (0.230), T_2 (0.224), T_1 (0.191) and lowest in control (0.177). Suzer *et al.* (2008) [25] reported that the influence of commercial probiotic supplementation on the larval stages of Gilthread Sea bream (*Sparus aurata,* L.). Both growth performance and digestive enzyme activities increased in the treatment as compared to control.

On the basis of the results obtained in the present experiment, it can be concluded that the protein supplement Casein has paramount importance in enhancing the growth performance and survival of Nile tilapia. The incorporation of Casein (protein) in fish diet does not show adverse impact on health of Nile tilapia and it is environment friendly.

Table 2: Weekly average weight gain of Nile tilapia fry fed with different levels of Protein (Casein) mixed diet

S. No.	Treatments	Initial weight (g)	Fish weight gain (g)						Total net weight gain (g)	Percent weight gain
			I	II	III	IV	V	VI		
1.	Control	0.897	0.030	0.056	0.089	0.098	0.175	0.227	0.676	75.325
2.	T_1	0.882	0.042	0.062	0.124	0.054	0.236	0.207	0.726	82.306
3.	T_2	0.852	0.050	0.046	0.122	0.079	0.183	0.376	0.855	100.391
4.	T_3	0.892	0.062	0.083	0.083	0.067	0.193	0.431	0.919	102.990
5.	T_4	0.893	0.104	0.064	0.161	0.231	0.270	0.309	1.139	127.463
	SEm±	0.032	0.015	0.012	0.021	0.023	0.033	0.042	0.034	6.007
	CD at 0.05%	0.100	0.047	0.038	0.067	0.071	0.103	0.133	0.108	18.928

Table 3: Weekly growth per day in percent body weight of Nile tilapia fry fed with different levels of Protein (Casein) mixed diet

S. No.	Treatments	Growth per day in percent body weight of fry						
		I	II	III	IV	V	VI	Average
1.	Control	0.472	0.868	1.298	1.310	2.131	2.414	1.416
2.	T_1	0.681	0.964	1.797	0.695	2.901	2.112	1.525
3.	T_2	0.833	0.724	1.840	1.051	2.281	4.035	1.794
4.	T_3	0.993	1.238	1.144	0.851	2.328	4.463	1.836
5.	T_4	1.663	0.912	2.168	2.700	2.658	2.559	2.110
	SEm±	0.286	0.200	0.307	0.317	0.417	0.487	0.080
	CD at 0.05%	0.901	0.631	0.967	1.000	1.314	1.535	0.251

Table 4: Weekly specific growth rate (SGR) of Nile tilapia fry fed with different levelsof Protein (Casein) mixed diet

S. No.	Treatments	Specific growth rate (SGR) of fryin percent						
		I	II	III	IV	V	IV	Average SGR
1.	Control	0.20	0.37	0.54	0.54	0.86	0.97	0.581
2.	T_1	0.29	0.41	0.73	0.29	1.15	0.86	0.621
3.	T_2	0.35	0.31	0.75	0.44	0.92	1.54	0.719
4.	T_3	0.42	0.52	0.48	0.36	0.94	1.69	0.732
5.	T_4	0.68	0.38	0.88	1.07	1.06	1.02	0.850
	SEm±	0.109	0.080	0.120	0.124	0.156	0.173	0.026
	CD at 0.05%	0.343	0.251	0.378	0.390	0.491	0.544	0.083

Table 5: Weekly food conversion ratio (FCR) of Nile tilapia fry fed with different levels of Protein (Casein) mixed diet

S. No.	Treatments	Food conversion ratio (FCR) of fry						
		I	II	III	IV	V	IV	Average (FCR)
1.	Control	16.932	9.212	6.162	6.107	3.753	3.314	7.580
2.	T_1	11.756	8.298	4.453	11.511	2.758	3.788	7.094
3.	T_2	9.606	11.057	4.348	7.612	3.507	1.983	6.352
4.	T_3	8.057	6.463	6.994	9.405	3.436	1.793	6.025
5.	T_4	4.810	8.772	3.690	2.962	3.010	3.127	4.395
	SEm±	0.889	1.384	1.382	4.246	0.938	1.037	0.867
	CD at 0.05%	2.802	4.363	4.354	13.379	2.955	3.268	2.732

Table 6: Weekly gross conversion efficiency (GCE) of Nile tilapia fry fed with different levels of Protein (Casein) mixed diet

S. No.	Treatments	Gross conversion efficiency (GCE)of fry						
		I	II	III	IV	V	VI	Average (GCE)
1.	Control	0.059	0.109	0.162	0.164	0.266	0.302	0.177
2.	T_1	0.085	0.121	0.225	0.087	0.363	0.264	0.191
3.	T_2	0.104	0.090	0.230	0.131	0.285	0.504	0.224
4.	T_3	0.124	0.155	0.143	0.106	0.291	0.558	0.230
5.	T_4	0.208	0.114	0.271	0.338	0.332	0.320	0.264
	SEm±	0.036	0.025	0.038	0.040	0.052	0.061	0.010
	CD at 0.0 5%	0.113	0.079	0.121	0.125	0.164	0.192	0.031

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