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EFFECT OF DIETARY OMEGA-3 FATTY ACID DIETS ON GROWTH AND PHYSICO-CHEMICAL PARAMETERS OF CULTURED WATER IN FINGERLINGS OF LABEO ROHITA

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Abstract: Fish is a rich source of polyunsaturated fatty acids (PUFA's) *viz*. n-6 and n-3 PUFA's, which are beneficial to human health. Rohu considered to be the most important of the "Indian Major Carps" and is the world's 10^{th} highest cultured finfish by production volume. Essential fatty acids such as omega-3 fatty acids (EPA and DHA) play a vital role in fish nutrition. To understand the role of the dietary Omega-3 fatty acids diets in fingerlings of *Labeo rohita*, a 60 days experiment was carried out. 300±20 fingerlings of rohu were randomly distributed in five treatment groups consisting of three replicates of each. Five isonitrogenous (protein 19.60g) and isocaloric (337 k cal/ 100 g) experimental diets *viz.* Control (basal diet), T1 (basal + 1% ω-3 fatty acid), T2 (basal + 3% ω-3 fatty acid), T3 (basal + 5% ω-3 fatty acid) and T4 (basal + 7% ω-3 fatty acid) were prepared with graded levels of omega-3 fatty acids. These were fed to rohu fingerlings (average body weight 1.793 ± 0.022g) twice a day. Sampling of water from all the experimental groups were carried out to estimate water quality parameters *viz* temperature, P^H, dissolved oxygen, free carbon dioxide, total hardness, ammonia, nitrite and alkalinity. These were recorded for every 15 days interval. The water in each tank was changed (80%) daily and replenished with fresh water, for each tank. The results indicated that the fishes which received the feed T1 (127.4±0.1) consisting of 1% of ω-3 fatty acid showed significantly increase of average body weight and specific growth rates (SGR) compared to control and other treatments T2 (120.1±0.3), T3 (112.6±0.3) and T4 (109.0±0.3). The experimental conditions, T1 feed (1% omega-3 fatty acid) had showed better growth amongst the treatments and increase of beyond 1% omega-3 fatty acid had showed poor growth due to of nutrients in general, omega-3 fatty acid in particular. Supplementation of omega-3 fatty acid in the aquaculture diets would also helps maintain the optimum range of physico-chemical parameters of the reared water.

Key words: *Labeo rohita*, Omega-3 fatty acid, Specific Growth rate and Physico-Chemial paramers.

I. INTRODUCTION

Fish is one of the most important components of Asian diets and several reports have exemplified the importance of fish in reducing the risk of cardiovascular diseases. The growth of biomass of fish under intensive culture depends upon various factors notably on feeding regime. One problem facing fish culturist is the need to obtain a balance between a rapid fish growth and optimum use of the supplied feed. In fish farming nutrition is critical because feed represent 40-50 % of production cost [5]. Among cyprinids *Labeo rohita* (rohu) is the most popular fish species cultivated in Indian subcontinent. Rohu is highly delicious and prestigious fish species among other Indian major carps FAO [7]. The use of commercial feed has become inevitable for the success of cyprinid culture under intensive culture conditions particularly rohu alongwith other carps [1]. In general, freshwater fish require either linoleic acid or linolenic acid or both of these fatty acids, while marine fish require a dietary source of highly unsaturated fatty acids (HUFA), mainly eicosapentaenoic acid (EPA, 20;5n-3) and docosahexaenoic acid (DHA), 22;6n-3).

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Because of rapid growth in human population has created unpredictable problem of food shortage in the world. This is more acute in regards to the proteinaceous food in underdeveloped and developing world. Fish and fish products contribute significantly for protein supply in general and white meat in particular. Water is essential for the survival of any form of life. It is universal solvent and one of the precious natural commodities. Water is required for various purposes like drinking, irrigation, fish culture and many other activities. The aquatic organisms are influenced by certain essential hydrological factors such as air and water. The physic – chemical characters of the water is highly influenced by the fish growth and health. Sastry and Malik [22] investigated the seasonal variations in the physic - chemical characteristics of a fresh water fish pond. Water quality influences the growth and survival of fishes.

The present study, therefore, was conducted to evaluate the beneficial role of dietary omega-3 fatty acid levels on growth and water quality with reference to changes of physic – chemical characteristics of cultured water in Labeo *rohita* fingerlings.

II. MATERIALS AND METHODS

A. Collection Of Rohu Fingerlings

300±20 rohu fingerlings (initial body weight 1.793 ± 0.022g) were collected from recognized a private hatchery. These were brought to the fish nutrition laboratory and acclimatized for 15 days in five synthetic plastic tanks of 100 liter capacity. Each tub was stocked with 20 fingerlings of uniform size. The initial body weight of the rohu fingerlings was recorded prior to stocking in the experimental tubs.

B. Research Design

Complete Randomized Design (CRD) with five different treatment diets each having three replicates was used for the feeding trial. All the test diets were formulated to supply all the essential nutrients required by rohu with different proportions of omega-3 fatty acids. The feeding trial was conducted for 60 days. The fishes were sampled for every 10 days interval for analysis.

C. Experimental Diets

Five isonitrogenous and isocaloric diets were prepared with increasing dietary omeg-3 fatty acid levels 1%, 3%, 5% and 7% respectively. The ingredients such as fish meal, groundnut oil cake, de-oiled rice bran, soya flakes, fish oil, rice bran oil, minerals and vitamins was used for feed preparation. The ingredients were grounded separately in an electric grinder and sieved to remove large particles. The required quantity of feed ingredients was boiled with water in a pressure cooker for 30 min. The boiled mixture was added and mixed well. These mixtures were processed through a hand pelletizer for preparing pellets, which were then dried in room temperature for 2 days. The pellets were analyzed for proximate composition (Table 1) following the standard methods of AOAC [2]. The experimental diets were prepared at M/S Avanthi Feed Private Limited in Kovvuru, E.G, A.P, India.

D. Sampling Of Water

Sampling of water from all the experimental groups were carried out to estimate water quality parameters *viz* temperature, P^H, dissolved oxygen, free carbon dioxide, total hardness, ammonia, nitrite and alkalinity. These were recorded for every 15 days interval. All these parameters were estimated by using standard methods of APHA [3]. The water in each tank was changed (80%) daily and replenished with fresh water, for each tank.

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E. Evaluation Of Growth Parameters, Feed Efficiency And Survivability

Growth parameters were evaluated at every 10days intervals and length and weight were recorded individually for each group of fish. The growth performance of the fishes, in terms of weight gain (%), specific growth rate (SGR) and feed conversion ratio (FCR), were determined by using the following formulae

Weight gain (%) =100 (Final body wt-initial body wt/Initial body wt)
Length gain (in cm) = final length (in cm) – initial length (in cm)
Specific growth rate (SGR) = 100 {final body wt-initial body wt/experimental period (in days)}.
Feed conversion ratio (FCR) = feed consumed (g)/ weight gain (g).
Food conversion efficiency (FCE) = 100(total fish body wt gained/total wt of feed intake).
Fish growth rate = total wt gain of certain fish (g) / no. of fingerlings × Time period (days).
Survival rate= total no. of surviving fish/total no. of fish stocked × 100.

F. Statistical Analysis

The data was analyzed by using one-way analysis of variance

TABLE I Proximate analysis of the experiment diets (g % on dry mater basis)

Dietary Ingredients (g 100g^{-1})	**C**	**T1**	**T2**	**T3**	**T4**
Gelatin	6.51	6.51	6.51	6.51	6.51
Cellulose	6.5	6.5	6.5	6.5	6.5
Starch	20.00	20.00	20.00	20.00	20.00
Dextrin	10.00	10.00	10.00	10.00	10.00
Vitamin + Mineral mix	1.0	1.0	1.0	1.0	1.0
Protein	19.60	19.60	19.60	19.60	19.60
Fat	**7.02**	**6.02**	**4.02**	**2.02**	**0.02**
EPA and DHA	**--**	**1.0**	**3.0**	**5.0**	**7 .0**
Moisture	10.02	10.02	10.02	10.02	10.02
Fiber	9.66	9.66	9.66	9.66	9.66
Ash	8.33	8.33	8.33	8.33	8.33
Sand Silica	1.36	1.36	1.36	1.36	1.36
Total	100	100	100	100	100

Composition of vitamin mineral mix (EMIX PLUS) (quantity 2.5/kg). Vitamin A, 55, 00,000 IU; Vitamin D3, 11,00,000IU; Vitamin B2, 2000mg; Vitamin E, 750mg; Vitamin K, 1000mg; Vitamin B6, 1000 mg; Vitamin B12, 6µg; Calcium pantothenate, 2500mg; nicotinamide, 10g; chloride, 150g; Mn 27,000 mg; I 1000MG; Fe 7500 mg; Zn 5000 mg; Cu 2000 mg; Co 450 mg; Ca 500 g; P 300 G; L-lysine 10 g; DL-methionine 10 g; Selenium 50 ppm.

<u>Control;</u> (Basal Diet), <u>T1;</u> (Basal + 1% omega-3 fatty acid), <u>T2;</u> (Basal + 3% omega-3 fatty acid), <u>T3;</u> (Basal + 5% omega-3 fatty acid), <u>T4;</u> (Basal + 7% omega-3 fatty acid)

III. RESULTS

A. Growth Performance

The final body weight, weight gain and specific growth rates (SGR) of fishes were significantly higher in 1% omega-3 fatty acid diets (T1) than with 3%, 5% and 7% omega-3 fatty acid diets (Table 2).

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TABLE II Effect of dietary Omega-3 fatty acid levels on growth performance.

Growth parameters	C	T1	T2	T3	T4
Initial weight	35.7 ± 0.75	36.13 ± 0.50	35.96 ± 0.32	35.60 ± 0.87	35.60 ± 0.36
Initial Length(cm)	4.5 ± 0.847	5.09 ± 0.842	4.9 ± 0.788	4.96 ± 0.847	5.24 ± 0.708
Final weight	95.53 ± 0.8	112.60 ± 1.4	108.07 ± 2.3	103.2 ± 4.2	101.01 ± 1.2
Final Length(cm)	13.42 ± 1.11	13.48 ± 1.14	13.24 ± 1.27	13.21 ± 1.26	13.48 ± 1.14
Body Weight Gain %	167.59 ± 16.08	211.64 ± 58.09	200.47 ± 105.33	189.86 ± 96.70	183.72 ± 91.78
Length Increase(cm)	8.51 ± 0.31	8.39 ± 0.30	8.30 ± 0.48	8.24 ± 0.42	8.24 ± 0.44
Specific Growth Rate	99.72 ± 0.061	127.45 ± 0.18	120.17 ± 0.32	112.66± 0.33	109.02 0.32

B. Feed Efficiency

The food conversion efficiency (FCE) is highly influenced by the omega-3 fatty acid levels in the diets. The food consumption and wet weight production played an important role in the increase or decrease of food conversion efficiencies. In the present experiment the diet with 1% omega-3 fatty acid level (T1 group) showed the minimum (1.781 ± 0.13) food conversion ratio (FCR), whereas maximum (2.05 ± 0.43) FCR is recorded in control group (Table 3). The change in FCR was also due to the level of ω-3 fatty acid diets. The highest FCE is observed in T1 (103.52) and lowest FCE is observed in control (88.72) group. No significant differences in feed intake were observed among the experimental groups, but feed consumption decreased with increasing omega-3 fatty acid diets.

Table II Effect Of Dietary Omega-3 Fatty Acid Levels On Feed Efficiency And Survivability.

Growth parameters	C	T1	T2	T3	T4
Feed Conversion Ratio	2.05 ± 0.43	1.78 ± 0.13	1.85 ± 0.06	1.92 ± 0.07	1.94 ± 0.09
Food Conversion Efficiency	88.72	103.52	99.08	94.4	93.44
Fish growth rate	0.0490	0.0630	0.0610	0.0563	0.0545
Survivability	100	100	100	100	100
Protein Efficiency Ratio	2.49	2.87	2.76	2.65	2.62

C. Analysis Of Physico-Chemical Parameters Of Water

The effect of incorporation of increased levels of dietary omega-3 fatty acid in the test diets, broadcasted in the water medium as a supplementary feed to the experimental fishes, indicated zero effect on water quality. The water physic-chemical parameters were within the acceptable range for carps, with no drastic variation between treatments. The results pertained to the effect of omega-3 fatty acid diets on water quality was presented in Table.4.

Table IV Effect of omega - 3 fatty acid test diets on water quality parameters

Sl. No.	Parameter	Control Diet (C)	Test Diets			
			T_1	T_2	T_3	T_4
1.	Temperature(°C)	28±0.81	28±0.47	28±1.25	28±1.72	28±1.29
2.	p^H	7±0	7±0	7±0.4	7±0.4	7±0.4
3.	Dissolved oxygen (ppm)	7.7±0.95	7.3±0.41	7.5±0.55	7.8±0.52	8.1±0.8
4.	Total hardness (mg/l)	119.2±0.2	119.7±0.8	118.7±0.7	119±0.9	119±1.1

Mean ± Standard deviation; n=4

IV. DISCUSSION

In the present study the fingerlings of *Labeo rohita* fed with 1% omega-3 fatty acid diet showed the best growth performance, while the 7% omega-3 fatty acid diets had shown lowest growth performance (Fig 1). The increase of omega-3 fatty acid beyond 1% had not shown influence on the growth performance. The excess of omega-3 fatty acid in the fish diets would become waste due to its insignificant role on growth. High protein digestibility and lipid utilization are possible factors in the good growth performance for fingerlings of rohu with the 1% omega-3 fatty acid diet. Protein digestibility and lipid utilization might be reduced when the 7% omega-3 fatty acid diet was fed. The results reported in the present experiment agree that *Labeo rohita* fed diets supplemented with omega-3 fatty acid, especially (EPA/DHA), exhibited good performance and influencing the survivability of rohu fingerlings.

Dietary supplement of omega-3 fatty acid containing high level of EPA and DHA improved weight gain, feed efficiency and food conversion ratio of rohu fingerlings. The results showed that rohu fingerlings with the diet containing 1% and 3% omega-3 fatty acid achieved the best feed efficiency and food conversion ratio. It revealed that dietary EPA and DHA are important for the normal growth of rohu as previously reported for marine fish [20]. The optimum dietary n-3 fatty acid level of 1% for fingerlings of rohu (determined in this study) was higher than those (9-10 g kg^{-1}) for fingerling gilthead seabream [11] [10], hybrid striped bass [19] and rock fish [14]. The feed utilization of rohu tended to decrease when dietary n-3 HUFA level become excessive. Similar phenomenon was observed in other studies of [16] [8] [13]. The negative effects of excessive n-3 HUFA might be due to the disturbance of membrane polar lipid caused by the excessive accumulation of EPA and DHA in tissue. However, in other studies for rockfish [14] and starry flounder [15], negative effects of excessive n-3 HUFA on growth and feed utilization were not observed. The exact reason of different response among fish fed with excessive n-3 HUFA is not clear, but it may be due to differences in fish species, interaction with other nutrients, etc. The decrease in feed utilization can also be attributed to the feed palatability and taste. But these inferences can be further referred with substantial evidence.

High levels of n-3 PUFA have been reported to depress the growth of tilapia [9]. It has been suggested that T. zillii requires approximately 1% n-6 fatty acid in the diet which can be met with linoleic acid (18:2 n-6) or arachidonic acid (20:4 n-6); [12] [25]. Final body weights of experimental fishes were negatively affected by increasing the EPA/DHA ratios in the diets. This might be due to the poor growth for the fingerlings fed diets containing high EPA/DHA ratios, especially more than 7% (T4 group).

Earlier Studies on *Cyprinus carpio* had showed that it requires 18:2n6 and 18:3n3 with best weight gain and feed conversion in fish receiving a diet with both 1% of 18:2n6 and 1% of 18: 3n3 [26] [23]. On the other hand, omnivorous fish such as the carp can utilize effectively both carbohydrate and lipids as dietary energy sources. Addition of lipid at levels of 5-15% to diets resulted in no improvement in growth, feed conversion, or the value of NPU (Net Protein Utilization), when the dietary protein level remained around 32% in craps [24].

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In Indian carp culture, a mixture of rice bran and groundnut oil cake (1:1) is generally practiced [18]. However, studies pertaining to nutrition in freshwater aquaculture in the recent years have led to the development of new feed formulations for Indian carp [17] [4] [21].Dietary lipid source can influence the composition of fatty acids in muscle of hybrid tilapia [6]. Consequently, the use of vegetable oils that contain very high levels of linolenic acid and lower levels of EPA and DHA will decrease the concentrations of beneficial n-3 HUFA in fish fillets destined for the human consumer. Either EPA or DHA is known to provide positive health benefits such as decreasing cardiovascular diseases, cancer, and many others. Further studies on HUFA synthesis, gene level observations are essential to utilize the beneficial aspects of omega-3 fatty acids.

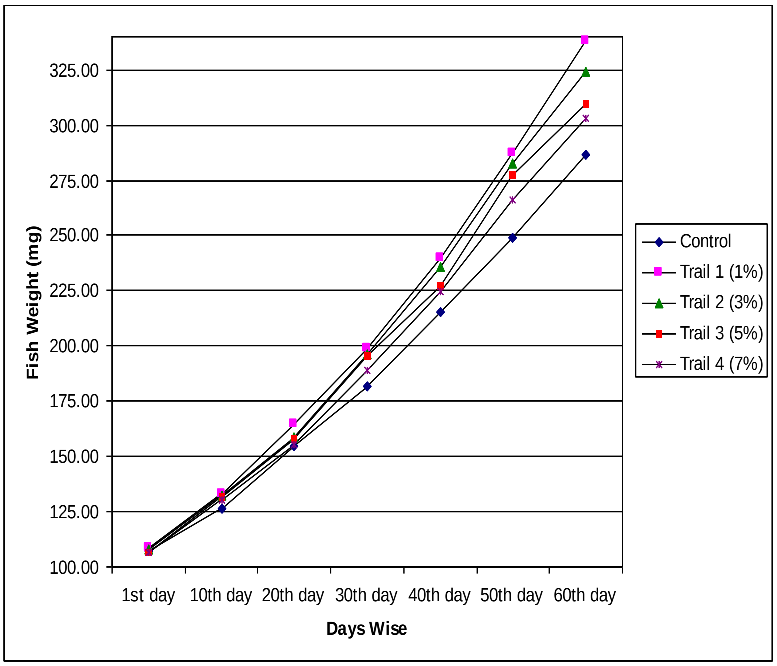

Fig 1. The Effect of dietary Omega-3 fatty acid levels on growth performance fingerlings of *L. rohita*

IV. CONCLUSION

These findings have practical importance in maximizing the growth and survival of fingerlings by feed managers during fingerlings rearing. The present study demonstrated that the optimal Omega-3 fatty acid requirement of *Labeo rohita* fingerlings for best growth performance was 1% dry diet. Supplementation of omega-3 fatty acid in the aquaculture diets would also helps maintain the optimum range of physico-chemical parameters of the reared water. As regards the gross production of fish for consumption, the present finding gave encouraging results which can support fish farmers and can improve the economics of the fish farming sector significantly.

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