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FORMULATED FREEZE DRIED TUBIFEX DIETS ON NUTRITIONAL INDICES IN FINGERLINGS OF *Catla catla* (HAMILTON)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author RS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author KG managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

ABSTRACT

Nutritional quality of fish depends upon the feed supplied. Supplementary feeding is known to increase the carrying capacity of culture system and can enhance the fish production by many folds. Realizing the importance of formulated feeding the present work was designed to supplement freeze dried tubifex along with the normal groundnut oil cake diet, to evaluate the nutritional indices in juveniles of *Catla catla* fingerlings. Experimental diet – I comprised of groundnut cake combined with freeze dried tubifex. Experimental diet – II was commercially available freeze dried Tubifex. Control fishes were fed with normal groundnut cake. Fishes initially weighing about 2.5 to 2.8 gms and length of about 6.3 to 6.4 cms were selected for the present study. The formulated feeding was given for a period of 30 days. A significant increase was observed in the body weight, length and the nutritional indices of the fishes fed on both experimental diets.

Keywords: *Catla catla*; freeze dried tubifex; growth performance; nutritional indices.

1. INTRODUCTION

In the trend of global increase of production in aquaculture, there is a demand for introduction of new components in fish diets and their maximal utilization [1]. The diets are also enriched with feeding stimulants and flavours. Fish feed is formulated to fulfill the requirements of fish in nutrients and energy, since the feed covers 40 to 60% of the total expenses in production [2].

The aquaculture development depends upon the use of high quality and balanced artificial feed [3,4]. Determination of appropriate rations for cultured fish is important to achieve maximum productivity [5,6]. Formulated feeds used in fish farming differ markedly from natural feeds and can be divided into three types viz, wet feed, moist and dry pellets. Evaluation of economically cheap and alternative suitable feed supplement paves way for new fish feed formulation technology [7].

It is reported that tubifex worms are very popular and cheap source of live food used for feeding larvae of carnivorous and omnivorous fish species [8]. Tubifex is a worm which belongs to Phylum - Annelida under the Class-Oligochaeta. It is popularly known as red worm. Tubifex make an ideal and suitable diet for

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ornamental and tropical fishes. Among the natural food organisms red worm (*Tubifex tubifex*) is one of the best owing to its short generation time, occurence in a vast range of habitats and tolerance to a wide spectrum of environmental variables [9]. A perusal of various research investigations using animal materials as source of food reveals that food source of animal origin give more promising results in terms of growth. Tubifex worms have been reported to be nutritionally suitable for rearing the larvae and adult of *Heteropneustes fossilis* [10] , *Ophiocephalus striatus* [11] and *Channa striatus* [12].

The suitability of using formulated feed source will provide cost effective management practices for efficient fish growth with cheaper feed ingredients under culture system in Indian major carp *Catla catla*. The present work was designed to supplement freeze dried tubifex along with the normal groundnut oil cake diet. Freeze dried tubifex will also be given as a separate feed to assess the growth and nutritional indices. In this study freeze dried tubifex is replaced for traditional fish meal in diet formulation.

2. MATERIALS AND METHODS

2.1 Stocking and Maintenance of Fishes

Active and healthy juveniles fishes comprising fingerlings of *Catla catla* weighing about 2.0 to 2.5 gms approximately and 4 - 5 cms in length was procured from Bharat seed fish farm, Budur, Poondi, Tiruvallur District and were brought to the laboratory in polythene bags containing aerated water. The fishes were maintained in circular plastic tubs without any soil base. The tubs were washed thoroughly with water before stocking. The tubs were filled with dechlorinated water. They were randomly assigned to the tubs for laboratory acclimatization for a period of 15 days. They were fed *ad libitum* normal fish feed twice a day in the morning and evening. Left over feed if any was removed by siphoning, 2 hours post-feeding. The water from the fish maintenance tubs was changed thrice in a week and replenished with chlorinated free water. The accumulated faeces from the bottom were siphoned out daily. Continuous oxygen supply was provided through aerator and were maintained at 12 hrs light / dark regime.

2.2 Feed Preparation

The diets were prepared by the following method of Jayaram and Shetty [13]. Rice bran, groundnut oil cake, fish meal, commercially available freeze dried tubifex and vitamin-mineral mixture were procured from local market. Weighed amounts were mixed thoroughly using a common blender and mixer. The powered ingredients were mixed with vitamin-mineral premix. Tapioca flour was used as binder for preparation of control diet and experimental diet-I.

Finely ground ingredients were mixed thoroughly with 200 ml water and tapioca flour to make a thick smooth dough. Freeze dried tubifex cubes were powdered, weighed accurately and added to cooled dough and mixed thoroughly to ensure uniform dispersal before pelletization. The dough was then transferred to an aluminum container and steam cooked in pressure cooker for 15 minutes. The dough pellets (2 mm diameter size) were prepared by a hand pelletizer and were air dried in an hot air oven at 40˚C. After sun drying, they were stored in air- tight containers and kept in refrigerators for use during feeding trial. The control diet and the experimental diet -I were formulated as per the composition given in Table 1.

2.3 Feed Composition and Diet Formulation

The feed used in this experiment was prepared as given in the Table 1.

Table 1. Composition of formulated feed (in percentage) for control and experimental group of fishes (Ingredients refer to 100 gm of feed prepared)

S. no	Feed ingredients	Control diet	Experimental diet- I	Experimental diet- II
1	Groundnut oil cake	35	30	-
2	Rice bran	20	10	-
3	Tapioca flour	13	8	-
4	Fish meal	30	-	-
5	Freeze dried tubifex	-	50	100
6	Vitamin- mineral mix	2.0	2.0	-
	Total	**100**	**100**	**100**

2.4 Experimental Design

Group-I (Control): *Catla catla* fingerlings which was given normal feed groundnut oil cake for a period of 30 days (initially 3% for one week, followed by 5% of the body weight twice daily)

Group-II (Experimental diet-I): *Catla catla* fingerlings which was fed on combined mixture of groundnut oil cake and freeze dried tubifex for a period of 30 days (initially 3% for one week, followed by 5% of the body weight twice daily)

Group-III (Experimental diet–II): *Catla catla* fingerlings which was fed on freeze dried tubifex alone for a period of 30 days (initially 3% for one week, followed by 5% of the body weight twice daily).

The fishes were acclimatized for a period of 15 days. At the beginning of the experiment the fishes weighed 2.5 to 2.8 gms and 6.3 - 6.4 cms in length approximately. About 10 juvenile fishes were stocked in each tub. Fishes were handled with a clean hand net. Aeration was continuously provided from air compressors through air stones. Weighed formulated and commercial diets were given to control and experimental group of fishes. Diets were fed twice daily at 09:00 and 15:00 hours. Feeding period was two hours. After the feeding time, the unconsumed food remaining in the tub was collected by siphoning out with a tube, causing least disturbance to the fish. About 75% of the water in the tubs was changed daily.

On the subsequent day before feeding, faecal matter accumulated in the tubs was siphoned out. The unconsumed feed and faecal matter were oven dried and weighed to calculate the feeding rate. All weighing were made by using digital balance to an accuracy of 1 mg. The amount of food given, the amount food unconsumed were recorded daily before and after feeding. The body weight and length of the fishes were recorded once in a week. Sampling was done and the quantity of feed given was re-adjusted, after each sampling, based on the weight recorded. Nutritional indices was calculated based on standard formula [14].

2.5 Statistical Analysis

The data collected on the different parameters of the experimental study were subjected to statistical analysis [15] by SPSS software 16.0 version. Data was expressed as Mean ± SD. Paired sample 't 'test was applied for body weight and length analysis. Nutritional indices were compared by one way analysis of variance (ANOVA) followed by Tukey multiple range tests. Statistical significance was tested at 1% and 5% levels.

3. RESULTS AND DISCUSSION

3.1 Analysis of Proximate Composition of Prepared Feed

Proximate composition (Table 2) of feed for control and experimental diets was determined by AOAC methods [16].

Based on the composition and the quantity of the particular feed ingredient, proximate content values obtained determined the quality of the particular feed which reflects on the growth and nutritional indices of fishes

3.2 Body Weight and Length Analysis in Fingerlings of *Catla catla* Fed on Different Freeze Dried Tubifex Formulated Diets

A significant increase was obtained in the body weight and length of the fishes fed on experimental diet-I and II when compared to the initial body weight and length. An average body weight gain of 3.51 gm / fish and average length gain of 1.78 cm / fish was observed in experimental diet- I fed fishes. In experimental diet-II fed fishes average body weight gain of 2.87 gm / fish and average length of 1.38 cm / fish was observed. The observations on body weight and length were statistically significant ($p<0.05$) in both the experimental diet fed groups according to paired sample 't' test (Tables 3 & 4).

Table 2. Proximate composition (in percentage) of control and experimental diets

S. no	Proximate composition	Control diet	Experimental diet- I	Experimental diet -II
1	Moisture	8.61	11.5	10
2	Crude Protein	28.87	46.92	55
3	Crude Fat	10.10	16.01	14
4	Ash	14.68	7.79	10
5	Crude Fiber	7.2	2.42	2
6	Nitrogen Free Extract	30.54	15.36	9
7	Gross Energy (KJ/g)	16.15	20.11	20.13

Table 3. Impact of freeze dried tubifex formulated diets on body weight in fingerlings of *Catla catla*

Experimental groups (Feeding Regime)	Body weight (gm)		Paired sample 't' test			
	Initial	Final	Paired sample test difference	t-value	df	2-tailed significance
Control diet (Fed on groundnut oil cake)	2.86±0.72	4.73±0.90	1.87±1.30	3.213	4	0.032*
Experimental diet- I (Fed on groundnut oil cake combined with freeze dried tubifex)	2.59±0.46	6.10±0.66	3.52±1.05	7.507	4	0.002*
Experimental diet- II (Fed with freeze dried tubifex alone)	2.72±0.50	5.59±0.39	2.87±0.68	9.486	4	0.001*

*Values are Mean ± SD (n=5) observations ; Statistical significance tested at 5% level : *P < 0.05 (Compared between initial and final weight for each group)*

Table 4. Impact of freeze dried tubifex formulated diets on length in fingerlings of *Catla catla*

Experimental groups (Feeding regime)	Length (cm)		Paired sample 't' test			
	Initial	Final	Paired sample test difference	t-value	df	2-tailed significance
Control diet (Fed on groundnut oil cake)	6.32 ± 0.54	6.82 ± 0.79	0.50 ± 0.76	1.456	4	0.219
Experimental diet- I (Fed on groundnut oil cake combined with freeze dried tubifex)	6.48 ± 0.16	8.26 ± 0.39	1.78 ± 0.30	13.122	4	0.000*
Experimental diet- II (Fed with freeze dried tubifex alone)	6.42 ± 0.29	7.80 ± 0.72	1.39 ± 0.52	5.863	4	0.004*

*Values are Mean ± SD (n=5) observations; Statistical significance tested at 5% level: *P < 0.05 (Compared between initial and final length for each group)*

The pattern of growth performance was remarkable in experimental diet-I and II, suggesting that the diet was acceptable. The difference in weight gain in experimental fishes of both the groups may be attributed to variation in the feed used and their ingredients. The results are corrborating with other similar findings on various feeding studies on *Catla catla* and growth in fingerlings of *Labeo rohita* fed on rice bran and commercial feed [17,18]. Protein is the most expensive dietary macronutrient which directly effects fish weight gain [19,20]. *Ompak pabo* hatchlings fed on earthworms in captive conditions also showed similar results on survival and growth [21].

3.3 Nutritional Indices in Fingerlings of *Catla catla* Fed on Freeze Dried Tubifex Formulated Diets

Feed conversion ratio, feed conversion efficiency and feeding rate was significantly ($p<0.001$) altered in both the experimental groups when compared to fishes fed with control diet. A significant decline ($p<0.05$) was seen in feed conversion ratio of experimental diet-II fed fishes when compared to experimental diet-I fed fishes, whereas other feeding parameters show significant increment ($p <0.05$) when the experimental groups of different diets were compared. Food absorbed, absorption rate, absorption efficiency, gross and net conversion efficiencies was significantly ($p<0.001$) increased in the experimental diet fed groups when compared to that of the control diet fed fishes The values also show a marked difference which is significant ($p<0.05$) when both experimental diet fed groups are compared (Table 5).

The FCR values were lower in the fishes fed on experimental diets in the present study compared to that of fishes fed on control diet. Result indicate tubifex freeze dried worms incorporated in the groundnut oil cake feed was much acceptable, than diet containing proteins of plant or vegetable origin, but the potential for including this combination in the feed of fish needs more evaluation. A low FCR is a good indication of a high quality feed. As the amount of protein in the diet increases the FCR gets smaller

Table 5. Impact of freeze dried tubifex formulated diets on feeding parameters in fingerlings of *Catla catla*

Feeding parameters	Control diet (Fed on groundnut oil cake)	Experimental diet- I (Fed on groundnut oil cake combined with freeze dried tubifex)	Experimental diet- II (Fed with freeze dried tubifex alone)	F- value	P- value
Feed conversion ratio (gm)	$0.720^{c} \pm 0.02$	$0.348^{a} \pm 0.14$	$0.442^{b} \pm 0.0.04$	3.234	< 0.001**
Feed conversion efficiency (%)	$139.10^{a} \pm 0.87$	$286.51^{c} \pm 1.73$	$227.88^{b} \pm 1.39$	1.448	< 0.001**
Feeding rate (gm/day)	$0.008^{a} \pm 0.0007$	$0.011^{b} \pm 0.0008$	$0.015^{c} \pm 0.005$	131.933	< 0.001**
Food absorbed (mg /day)	$0.53^{a} \pm 0.01$	$0.78^{b} \pm 0.03$	$0.95^{c} \pm 0.07$	507.630	< 0.001**
Absorption rate (mg /gm /day)	$0.006^{a} \pm 0.0008$	$0.0118^{b} \pm 0.001$	$0.016^{c} \pm 0.001$	66.94	< 0.001**
Absorption efficiency (%)	$74.05^{a} \pm 0.45$	$87.53^{b} \pm 0.56$	$89.59^{c} \pm 0.47$	1.433	< 0.001**
Gross conversion efficiency (%)	$514.48^{a} \pm 3.02$	$957.83^{b} \pm 1.13$	$647.03^{c} \pm 0.83$	6.971	< 0.001**
Net conversion efficiency (%)	$7.82^{a} \pm 0.07$	$14.54^{c} \pm 0.18$	$10.84^{b} \pm 0.56$	474.492	< 0.001**

*Values are Mean ± SD (n=5) observations; ** denotes significance at 1% level*
Different alphabets between groups denotes significance at 5% level using Tukey multiple range test (P < 0.05) ; Means carrying atleast one common superscript between groups do not differ significantly

[22]. The quantity and quality of feed consumed have a pronounced effect on growth rate, efficiency of feed conversion. Higher protein content present in the experimental diet-II indicate its growth promoting potential and feed conversion efficiency (FCE). The present findings are in accordance with studies carried out in *Cirrhinus mrigala* [23] and *Labeo rohita* [24]

In addition to growth characteristics, food utilization parameters were also considered equally important indices for assessing the suitability of formulated feed preparation [25]. The food utilization efficiency is mainly dependent on the quality of the diet [26,27]. Results obtained in this study can be regarded as an index of food utilization. The present study indicates food absorption, absorption rate and efficiency, gross and net conversion ratio are remarkably higher in juvenile fishes fed on experimental diet-I and II, when compared with the fishes that were fed on a normal basal diet. Higher nutritional values for tubifex is also reported [28].

However, the results of fishes fed on experimental diet-II showed absorption rate and efficiency parameters on higher side which can be suggested that animal source ingredients in the diets are easily and efficiently digested and absorbed. The differences in the values obtained on various feeding parameters and nutrient related bioenergetics occurs due to variation of voluntary feed intake, digestion, absorption, transport and metabolism [29,30]. The increase in absorption activities and its conversion efficiency could be due an increase in activity of digestive enzymes which could have increased rate of assimilation [31]. Similar results of tubifex worm utilization under different feeding regimes have been reported in *Macrobrachium lanchesteri* [32].

4. CONCLUSION

It is evident from the present study, that the increased nutritional indices is due to better acceptability and utility of freeze dried tubifex worms by *Catla catla* fingerlings. The study reveals that commercial feed (freeze dried tubifex) available in local market are good to enhance growth and nutritional indices when combined along with groundnut oilcake or when given as direct feed with a lower production costs. Further research is required to establish the credibility of the formulated diet with nutrient composition of edible tissue.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO. State of the World Fisheries and Aquaculture. Contribution to Food Security and Nutrition for All. Rome. 2016;200.
2. Aarseth KA, Sorensen M, Storebakken T. Effects of red yeast inclusions in diets for salmonids and extrusion temperature on pellet tensile strength. Journal of Animal Feed, Science and Technology. 2006;126:75–91.
3. Shaheen T, Ayub M, Hayat S, Abidi SZA, Tahir S. Impact of different levels on the survival and growth of *Labeo rohita* at constant level of protein. Pakistan Journal of Fisheries. 2000;1(1):95-102.
4. Srivastava PP, Jena JK, Chowdhary S, Sharma P, Raizadas, Dayal R. Performances of Catla (*Catla catla*) fingerling reared on locally available feed ingredients. Online Journal of Animal Feed Research. 2013;2(4):153-158.
5. Khan MA, Abidi SF. Optimum ration level for better growth conversion efficiencies and body composition of fingerling of *Heteropneustes fossilis* (Bloch). Aquaculture International. 2010;18:175-188
6. Roopma Gandotra, Dalbir Singh Parihar, Meenakshi Koul, Vaini Gupta, Ritu Kumari. Effect of varying dietary protein levels on growth, feed conversion efficiency (FCE) and feed conversion ratio (FCR) of *Catla catla* (Ham) fry. Journal of International Academic Multidisciplinary Research. 2014;2(1):2320-2325.
7. Hadjinikolova I. The influence of nutritive lipid sources on the growth and chemical and fatty acid composition of carp (*Cyprinus carpio*). Archives of Polish Fisheries. 2005;12:111-119.
8. Bucer J., Food and feeding, culturing tubifex worms. Tropical fish hobbyist., 1977; 25(7): 99-102.
9. Kaster JL. The reproductive biology of *Tubifex tubifex*. Muller (Annelida: Tubificidae). American Midland Natural. 1980;104:364-366.
10. Reddy SR, Katre S. Growth rate and conversion efficiency of the air-breathing catfish *Heteropneutes fossilis* in relation to ration size. Aquaculture. 1979;18:35-40.
11. Bouguenec V. Oligochaetes (Tubificidae and Enchytraeidae) as food in fish rearing: a review and preliminary tests. Aquaculture. 1992;102: 201-217.
12. Sarowar MN, Jewel MZH, Sayeed MA, Mollah MFA. Impacts of different diets on growth and survival of *Channa striatus* fry. International Journal of Biological Research. 2010;1(3):08-12.
13. Jayaram MG, Shetty HPC. Formulation processing and water stability of two pelleted fish feeds. Aquaculture. 1981;23(1-4):355-359.
14. Castell JD, Tiews K. Report of the EIFAC, IUNS and IECS. Working Group on the standardization methodology in fish nutrition research. Hamburg, Federal Republic of Germany. European Inland Fisheries Advisory Commission. Technical paper 36; 1980.
15. Snedecor GW, Cochran WG. Statistical methods. 8th edn. The Lowa State College Press. 1989;135.
16. AOAC (Association of Official Analytical Chemists). Methods of analysis of the AOAC International, 17th edition, Washington DC USA; 2003.
17. Abdul Kadhar M, Saiyad Musthafa M, Chitrarasu P, Arunkumar MS, Jawahar Ali A. Growth response of two Indian major carps *Catla catla* and *Cirrhinus mrigala* fingerlings fed diet containing different nutritional supplements. International Journal of Applied Biology and Pharmaceutical Technology. 2012; 3(3):280-286.
18. Abid M, Ahmed MS. Growth response of *Labeo rohita* fingerlings fed with different feeding regimes under intensive rearing. Journal of Animal and Plant Sciences. 2009a; 19(1):45-49.
19. Ahmed MS, Shatiq K, Kiani MS. Growth performance of major carp, *Labeo rohita* fingerlings on commercial feeds. Journal of Animal and Plant Sciences. 2012;22(1):93-96.
20. Ng WK, Soon SC, Hashim R. The dietary requirement of bagrid catfish, *Mystus nemurus* (Cuvier & Valenciennes), determined using semipurified diets of varying protein level. Aquaculture Nutrition. 2001;7:45-51.
21. Sarma D, Das J, Datta J. Effect of certain feeds on the growth and survival of *Ompok pabo* (Hamilton – Buchanan) hatchlings in captive condition. International Journal of Scientific Research Publication. 2012;2(1):1-5.
22. Ebtehal EL-Sayed, Hussein M. Effect of seaweed supplemented diets on Nile Tilapia, *Oreochromis niloticus* performance. International Journal of Fish Aquatic Studies. 2017; 5(2):205-210.
23. Ashraf M, Ayub M, Rauf A. Effect of different feed ingredients and low temperature on diet acceptability, growth and survival of Mrigal, *Cirrhinus mrigala* fingerlings. Pakistan Journal of Zoology. 2008;40(2):83-90.
24. Abid M, Ahmed MS. Efficacy of feeding frequency on growth and survival of *Labeo rohita* (Ham.) fingerlings under intensive

rearing. Journal of Animal and Plant Sciences. 2009b;19(2):111-113.
25. Abdul Kadhar M, Saiyad Musthafa M, Chitrarasu P, Arunkumar MS, Jawahar Ali A. Growth response of two Indian major carps *Catla catla* and *Cirrhinus mrigala* fingerlings fed diet containing different nutritional supplements. International Journal of Applied Biology and Pharmaceutical Technology. 2012; 3(3):280-286.
26. Khan MA, Ahmed I, Abidi SF. Effect of ration size on growth, conversion efficiency and body composition of fingerlings of mrigala, *Cirrhinus mrigala* (Hamilton). Aquaculture Nutrition. 2004;10:47-49
27. Khan N, Ashraf M, Qureshi NA, Sarker PK, Vandenberg GW, Rasool F. Effect of similar feeding regime on growth and body composition and Indian major carps (*Catla catla, Cirrhinus mrigala* and *Labeo rohita*) under mono and polyculture. African Journal of Biotechnology. 2012;11(44):10280-10290.
28. Evangelista AD, Fortes NR, Santiago CB. Comparison of some live organisms and artificial diet as feed for Asian catfish *Clarias macrocephalus* (Gunther) larvae. Journal of Applied Ichthyology. 2005;21:437-443.
29. Manivannan S, Saravanan TS. Impact of formulated protein diets on growth of the Indian major carp, *Labeo rohita* (Hamilton). Fish Aquaculture Journal. 2012; 20:57.
30. Otochoumou K, Celestin MB, Olivier AE, Yao LA, Sebastian LN, Jacques KD. Effects of increasing dietary proteins levels on growth, feed utilization and body composition of *Heterobranchus longifilis* (Valenciennes, 1840) fingerlings. African Journal of Biotechnology. 2012;11(2):524-529.
31. Poongodi R, Saravana Bhavan. Growth promoting potential of garlic, ginger, turmeric and fenugreek on the freshwater prawn *Macrobrachium rosenbergi*. International Journal of Pharmaceutical and Biological Sciences. 2012;3(4):914-926.
32. Preetha Panikker, Ayyar SP, Katre Shekuntala. Utilization of tubifex worms by *Macrobrachium lanchesteri* (de Man) under different feeding regimes. Indian Journal of Fisheries. 2010;57(4):81-84.