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Full Length Research Paper

STUDIES ON THE NUTRITIVE VALUE AND FATTY ACID CONTENTS IN FINGERLINGS OF CATLA CATLA FED ON TRADITIONAL DIET AND FREEZE DRIED TUBIFEX

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Abstract

The Indian major carp *Catla catla* is a promising species for aquaculture exploitation for its growth and good market potential. It is commonly preferred, consumed and considered as a low cost fish by all the economic group of people. To gain knowledge on the nutritional stand point of this fish , the nutritive value and fatty acid contents of muscle tissue was analysed based on feeding formulated diet which included freeze dried tubifex instead of fish meal to assess the nutritional value. The feed comprised of groundnut oil cake as control diet, freeze dried tubifex combined with groundnut oil cake as experimental diet. The results show better nutritive values and an increased fatty acid content in fishes fed with freeze dried tubifex combined with groundnut oil cake mixture.

Keywords: Catla catla, Groundnut oil cake, Freeze dried tubifex, Muscle tissue, Nutritive values, Fatty acids.

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INTRODUCTION

Artificial feed plays an important role in semi-intensive fish culture where it is required to maintain a high density of fish, than the natural fertility of the water can support. The role of artificial feed in intensive fish farming cannot be ignored as nutritional requirements of fish depend upon the feed supplied. The quantity and quality of feed consumed have a pronounced effect on growth rate, efficiency of feed conversion and chemical composition of fish (Hassan et al., 1996; Jena et al., 1998). Inexpensive source of high quality animal protein production is essential from farmed species using low cost sustainable farming methods in semi intensive farming systems. The energy yielding nutrients like protein, lipid and carbohydrate are considered as macronutrients are present in high level where as non energy yielding nutrients like vitamins and minerals are micronutrients and are present in small quantities (FAO, 2012; Kumaran et al., 2012).

Polyunsaturated fatty acids are believed to prevent and treat coronary heart disease (CHD), brain development and mental health, hypertension, diabetes, obesity, cancer, thrombosis and lung disease (Ackman, 2002). Among the fatty acids, particular emphasis has been placed on the n-3 (omega-3) and n-6 (omega-6) polyunsaturated fatty acids (PUFA). Omega-3-fatty acids and omega-6-fatty acids are long chain fatty acids, the carbon in the

methyl group is called omega carbon. Omega-3-fatty acid have a double bond, three carbons away from the methyl carbon and omega -6-fatty acids have a double bond, six carbons away from the methyl carbon (Barma and Goswami, 2013). Freshwater animals are not able to synthesize these fatty acids including linoleic, linolenic and oleic acids; hence, one or more fatty acids must be included in their diets (Sargent and Tacon, 1999). In addition, fatty acid composition data are needed by food scientists and nutritionists to aid them in dietary formulation, processing and product development (Ackman, 1989; Stancheva and Merdzhanova, 2011).

It is also been reported that tubifex worms are very popular and cheap source of live food used for feeding larvae of carnivorous and omnivorous fish species (Bucer, 1977). Tubifex make an ideal and suitable diet for ornamental and tropical fishes. Among the natural food organisms red worm (*Tubifex tubifex*) is one of the best candidate owing to its short generation time, occurrence in a vast range of habitats and tolerance to a wide spectrum of environmental variables (Kaster, 1980).

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 *Oreochromis mossambicus* (Pandian and Raghuraman, 1972),

Mystus vittatus (Arunachalam and Reddy, 1981), *Ophiocephalus striatus* (Bouguenec, 1992), *Channa striatus* (Sarowar *et al.*, 2010) and in freshwater prawn *Macrobrachium lanchesteri* (Preetha Panikkar *et al.*, 2010). The nutritional composition of *Tubifex tubifex* was investigated by Ng (2000), Mahmut *et al.* (2003), and Yanar *et al.* (2003).

Much of the protein in commercial aqua feeds is currently being supplied by fishery resources, and the search for novel and renewable sources of protein is crucial for the continued expansion of the aquaculture industry. The efficiencies of the various alternative animal protein sources as replacements for fish meal have been evaluated in fish diets, e.g., Gambusia meal (Ahmad, 2008), tuna liver meal (Gumus *et al.*, 2009) and sand smelt meal (Gumuş *et al.*, 2010). Realizing the importance of formulated feeding the present work was designed to supplement freeze dried tubifex along with the normal groundnut oil cake diet. In our study freeze dried tubifex is replaced for traditional fish meal. Fish meal is expensive and there is a need to import from other countries. So, this study has been carried out to analyse the effect of different feed ingredients with and without fish meal.

MATERIALS AND METHODS

Collection of animals - 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.

Feed Preparation- Feed ingredients

The diets were prepared by the method of Jayaram and Shetty, 1981 as follows. Rice bran, groundnut oil cake, fish meal, commercially available freeze dried tubifex and vitamin-mineral mixture were procured from local merchants. 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 in preparation of control diet and experimental diet.

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^{9} C. After sun drying, they were stored in air- tight containers and kept in refrigerators for use during feeding trial. The control and experimental diet were formulated as per the composition given in Table A.

Feed composition and Diet formulation

The feed used in this experiment was prepared as given in the table below.

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

| S.No. | Feed Ingredients | Control diet | Experimental diet |
|-------|----------------------|--------------|-------------------|
| 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 |
| 6 | Vitamin- mineral mix | 2.0 | 2.0 |
| | Total | 100 | 100 |

The above feed ingredients used for formulation of feed were a source of various nutrients providing the required dietary and energy requirement for the fish. Groundnut oil cake is source of fat, protein, minerals like magnesium, sulphur and potassium. B-complex vitamins like niacin, pantothenic acid and thiamine, low levels of choline and vitamin E. Rice bran is a source of carbohydrate, protein, fiber, lipid, ash and B-complex vitamins. Another good source of carbohydrate was tapioca flour. Fish meal is a rich source of protein, essential amino acids, ash, B-Complex vitamins biotin, pantothenic acid, niacin, cyanocobalamine, minerals like calcium, phosphorus, iron, copper and zinc. Proximate composition of control and experimental diets analysed are tabulated in Table B.

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

| S.No. | Proximate Composition | Control diet | Experimental diet |
|-------|------------------------|--------------|-------------------|
| 1 | Moisture | 8.61 | 11.5 |
| 2 | Crude Protein | 28.87 | 46.92 |
| 3 | Crude Fat | 10.10 | 16.01 |
| 4 | Ash | 14.68 | 7.79 |
| 5 | Crude Fiber. | 7.2 | 2.42 |
| 6 | Nitrogen Free Extract | 30.54 | 15.36 |
| | (Carbohydrate content) | | |

Based on the composition and the quantity of the particular feed ingredient, proximate content values obtained determined the quality of the particular feed. The energy values is calculated and presented in the tabular column. The effects of feeding this diet for juveniles of *Catla catla* comprising the fingerling stage will be discussed in the foregoing chapters.

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

After an acclimatization period of 15 days and 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 diets were given to control and experimental group of fishes. They 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 body weight and length of the fishes were recorded once in a week. Sampling was done and the quantity of feed given was readjusted, after each sampling, based on the weight recorded.

Collection of tissue samples for nutritive value analysis

At the end of the experiment period of 30 days, the control and experimental diet fed fishes were sacrificed, muscle was dissected out and washed thoroughly in 0.9N saline solution. Nutritive values and fatty acid contents were extracted from the tissues.

Analysis of proximate composition of prepared feed/ nutritive values of muscle (edible flesh) of *Catla catla* fingerlings

Proximate composition of feed for control and experimental diets/ nutritive values of muscle (edible flesh) of *Catla catla* fingerlings was determined using AOAC methods (2003).

Fatty Acid Analysis-Extraction of Lipids

Lipids were extracted by the method of Folch *et al.* (1957) using chloroform: methanol solvent (2.1 V/V).

Conversion of lipid into corresponding fatty acid methyl esters (FAMES)

The dried chloroform layer with lipid is evaporated to dryness in a rotary evaporator. It is dissolved in 2.0 ml of freshly prepared mixture of acetyl chloride and methanol in ratio of 1:20 (v/v). The mixture is placed in teflon capped pyrex tube and the reaction is continued at 100° C for one hour under atmosphere of nitrogen in darkness. After cooling to $30-40^{\circ}$ C, 1.0 ml of extracting solvent (hexane) is added and then vortexed for about 20 seconds. Purification of solution is achieved by washing with 1.0 ml of distilled water causing the formation of two immiscible phases, which are then allowed to separate. The upper extracted solvent phase is recovered dried over anhydrous sodium sulphite and analysed by gas chromatography (GC).

Statistical Analysis

Data was expressed as Mean \pm SD. The data collected on the different parameters of the experimental study were subjected to statistical analysis (Snedecor and Cochran, 1989). Software package SPSS 16.0 version was used to carry out the statistical analysis.

RESULTS

Analysis of proximate composition of formulated control and experimental diet

The various ingredients in the experimental diet increased percentage of crude proteins, fat, and moisture when compared to control diet

| Nutritive values | Control diet (Fed on groundnut oil cake) | Experimental diet (Fed on groundnut oil cake combined with freeze dried tubifex) | P - value |
|-----------------------|--|--|-----------|
| Nitrogen free extract | 15.34 ± 1.19 | 11.45 ± 0.91 | < 0.001** |
| (Carbohydrates) | | | |
| Crude Protein | 19.22 ± 0.08 | 28.60 ± 0.07 | < 0.001** |
| Crude Fat | 4.23 ± 0.31 | 9.46 ± 3.62 | < 0.001** |
| Ash | 2.27 ± 0.01 | 3.09 ± 0.01 | < 0.05* |
| Moisture | 35.23 ± 1.38 | 33.10 ± 0.39 | NS |
| Fibre | 7.20 ± 1.2 | 3.35 ± 1.7 | < 0.001* |

Table 1. Impact of formulated diet on the nutritive value in muscle tissue of Catla catla fingerlings

Values are expressed as %

Values are Mean \pm SD (n=5) observations

** denotes significance at 1% level ; * denotes significance at 1% level; NS - Non significant

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| Fatty acid content | Control diet (Fed on groundnut oil cake) | Experimental diet (Fed on groundnut cake oil combined with freeze dried tubifex) | |
|-------------------------------------|--|--|--|
| Saturated fatty acid | | | |
| (SFA) | 24.5 | 40.4 | |
| Palmitic acid (16:0) | | | |
| Stearic acid (18:0) | 105.5 | 210.4 | |
| Mono-unsaturated | | | |
| fatty acid (MUFA) | 304.5 | 315.6 | |
| Oleic acid (18:1 n-6) | | | |
| Poly-unsaturated | | | |
| fatty acid (PUFA) | 100.0 | 211.4 | |
| Linoleic acid (18:2 n- | 129.8 | | |
| 6) | | | |
| Alpha- linolenic acid (18:3 n-3) | 90.5 | 112.5 | |

Values are expressed as mg /100 gm tissue





| | Fatty acid | Carbon Number | Retention Time | Area | Area% |
|----|----------------------|---------------|----------------|-----------|--------|
| 1 | Myristic acid | C 14 | 26.5 | 3452.6 | 6.88 |
| 2 | Pentadecanoic | C 15 | 28.1 | 2364.7 | 4.794 |
| 3 | Palmitic acid | C 16 | 30.7 | 3125.3 | 6.01 |
| 4 | Moroctic acid | C 17 | 30.3 | 2159.746 | 4.08 |
| 5 | Stearic acid | C 18 | 33.7 | 1059.531 | 2.03 |
| 6 | Oleic acid | C 18 :1 | 35.2 | 8145.561 | 14.269 |
| 7 | Linoleic acid | C 18 :2 | 37.8 | 7649.265 | 14.72 |
| 8 | Alpha linolenic acid | C 18 :3 | 39.9 | 23745.642 | 44.32 |
| 9 | Moroctic acid | C 18 :4 | 44.9 | 426.345 | 0.89 |
| 10 | Burucic acid | C 22 :1 | 45.4 | 123.9 | 0.237 |
| 11 | Arachidonic scid | C 22 ; 2 | 46.1 | 89.264 | 1.77 |
| | | | | 52344.921 | 100 |



Graph II. Chromatogram showing the peaks for fatty acid content in muscle tissue of *Catla catla* fingerlings fed with control diet (Groundnut oil cake) for 30 days

| No. | Fatty acid | Carbon Number | Retention Time | Area | Area% |
|-----|----------------------|---------------|----------------|---------|-------|
| 1 | Palmitic acid | C 16 | 30.6 | 155.6 | 4.72 |
| 2 | Stearic acid | C 18 | 33.5 | 458.760 | 13.95 |
| 3 | Oleic acid | C 18; 1 | 35.5 | 658.890 | 20.1 |
| 4 | Linoleic acid | C 18: 2 | 37.2 | 901.400 | 27.42 |
| 5 | Alpha linolenic acid | C 18 :3 | 40.2 | 998.500 | 30.48 |
| 6 | Moroctic acid | C 18:4 | 44.6 | 106.560 | 3.33 |



Graph III. Chromatogram showing the peaks for fatty acid contents in muscle tissue of *Catla catla* fingerlings fed with experimental diet (Groundnut oil cake with freeze dried tubifex combined) for 30 days

| No. | Fatty acid | Carbon Number | Retention Time | Area |
|-----|-----------------------|---------------|----------------|----------|
| 1 | Palmitic acid | C 16 | 30.6 | 220.3 |
| 2 | Stearic acid | C 18 | 33.7 | 145.500 |
| 3 | Oleic acid | C 18 :1 | 35.7 | 673.500 |
| 4 | Linoleic acid | C 18 :2 | 37.4 | 189.400 |
| 5 | Alpha- linolenic acid | C 18 :3 | 40.4 | 1477.500 |
| 6 | Moroctic acid | C 18 :4 | 44.6 | |
| 7 | Behenic acid | C 22:0 | 46.2 | 120.9 |

A decrease in the percentage of Nitrogen free extract (carbohydrate), fiber and ash contents in experimental diet was recorded when compared to control diet (Table - A and B).

Analysis of nutritive value in muscle tissue of *Catla catla* fingerlings fed on formulated diet

Protein, fat and ash in the muscle tissue were significantly (p< 0.001) increased in experimental diet fed fishes, when compared to control diet fed animals. Carbohydrates and fiber content (p< 0.001) declined in experimental diet fed fishes and significant change was not noticed in moisture content in both groups (Table 1, Fig 1).

Analysis of fatty acid content in muscle tissue of *Catla catla* fingerlings fed on formulated diet

Analysis of fatty acid content in muscle tissue of *Catla catla* fingerlings fed on formulated diet in muscle showed a marked increase in saturated fatty acids palmitic acid and stearic acid, monounsaturated fatty acid oleic acid and polyunsaturated fatty acid linoleic acid (omega- 6) and alpha-linolenic acid (omega- 3) in experimental diet fed groups when compared to fishes fed on control diet. The chromatogram peaks of the control and experimental samples were compared with the standard chromatogram peaks (Table 2; Fig 2; Graphs I – III). Among the polyunsaturated fatty acids no peaks were observed for gamma - linolenic acid and arachidonic acid in the control and experimental samples.

DISCUSSION

Intensive fish culture of in recent years is the result of higher demand and good market prices offered for this fish (Brugere and Ridler, 2004). However, limited fry production due to poor survival has made it necessary to improve hatchery techiques and upgrade nutritional qualities of diets for fry and fingerlings. The nutritional requirements of several species have been determined in previous studies. Various ingredients such as lumbricid worms (Tacon *et al.*, 1983), krill, silkworm pupae powder (Akiyama *et al.*, 1984) have been incorporated into fish feeds to act as feed attractants or feeding stimulants. The suitability of frozen and freeze dried zooplankton (Grabner *et al.*, 1981) and worms such as earthworms, tubificid worms, mealworm larvae, blood worm, silkworm pupae as potential food for fish larvae has also been assessed (Ng, 2000).

Proximate composition of formulated control and experimental feed

The ingredients improved the nutrient contents of the experimental diet. Proportions of groundnut oil cake, rice bran, tapioca flour in the control diet show increase in ash, crude fiber and nitrogen free extract. Proportional changes in the traditional ingredients and supplementation with freeze dried tubifex powder instead of fish meal in the experimental diet resulted in decline. In above mentioned constituents, but showed an increase in protein and fat contents.



Values are Mean \pm SD (N = 5) Values expressed as %



Fig.1. Impact of formulated diet on the nutritive value in muscle tissue of Catla catla fingerlings

Fig. 2. Impact of formulated diet on muscle tissue fatty acid contents in

fingerlings of Catla catla

Nutritive value in muscle tissue of *Catla catla* fingerlings fed on formulated diet

The type of feed ingested and their nutritional quality is known to be one of the main factor affecting fish muscle composition (Meyers, 1999; NRC, 2011; Alltech, 2013). Superior performance of fish with the experimental diet reflects on the ability of *Catla catla* to utilize the supplementary ingredients effectively. Ingredients of the experimental diet have shown to improve the nutrient contents. Carps are known to utilize high levels of carbohydrates (Stone, 1993; Wilson, 1994; Manjappa *et al.*, 2009). The carbohydrates as spared proteins for growth as revealed from the results of this study. This could have been due to the changes in the proportions of ground nut oil cake, rice bran and tapioca flour in our formulated experimental diet. Further, carbohydrates improve the pelleting quality and nutrient value of the diet (Lovell, 1989).

Fiber content of the diet affects feed digestibility and food retention in the gut thereby influencing absorption of nutrients. Nutritive value of edible flesh revealed that experimental diet

affected protein and fat, both being lowest in control and highest in experimental diet fed groups. However, there was no difference in moisture level in control and experimental diet fed groups. This is indicative of protein accretion and true growth involving an increase in the structural tissue such as muscle and various organs (Fafioye *et al.*, 2005). It has been reported that fish growth would be better when fed with higher protein containing feed especially animal protein (Rai and Bista, 2001)

Dietary lipid levels is known to influence muscle lipid positively (Guler *et al.*, 2008). A significant increase in fat level in fish receiving the experimental diet indicates enhanced lipid production which can be related to the fat and NFE levels of the diet. The level of protein, fat and ash change according to the nutrition and size of the fish. Influence of nutrients on body composition has also been reported in major carp rohu (Umer *et al.*, 2011) and also on other major carps (Khan *et al.*, 2012).

Fatty acid content in muscle tissue of *Catla catla* fingerlings fed on formulated diets

In the present study, a few analysed saturated fatty acid palmitic acid and stearic acid, mono-unsaturated oleic acid and polyunsaturated fatty acid linoleic acid (omega- 6) and alphalinolenic acid (omega- 3) showed a significant increase in the fishes which were fed on experimental diets, comprising a mixture of groundnut oil cake and tubifex.

Freshwater carp may be as nutritionally valuable as marine fishes (Ozugal and Ozugal, 2007; Manivannan and Saravanan, 2012). However, quantity of these acids varies largely in dependence on the fish species (herbivorous, omnivorous or carnivorous), if they are wild fish or farm-raised, on the age of fish and on origin of diets mainly natural food, cereal, plant, vegetable, animal based or plankton supplement (Ackman, 2002; Gumus and Erodgan, 2010). These fatty acids are important factors that affect the nutritional quality of fish muscle (Donmez *et al.*, 2009; Barma and Goswami, 2013).

Omega-3 and 6 fatty acid in the fish species taken for the study also serve as a valuable source of essential fatty acids (Andrade *et al.*, 1995; Saravanan *et al.*, 2013). Higher total fatty acids and omega fatty acids contents is reported in *Tubifex tubifex* (Mahmut *et al.*, 2003; Yanar *et al.*, 2003).

The study attributes that edible flesh of freshwater Indian major carp *Catla catla* is desirable in human diet because of the good source of PUFA containing essential fatty acids which could enhance the nutritional quality of the consumer. Further research should be conducted on usage of tubifex freeze dried forms to determine acceptable fatty acid composition for large pond fish.

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