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Growth Performance and Flesh Fatty Acid Profiles of Nile Tilapia (*Oreochromis niloticus* L.) Fed with Two Leguminous Plant Leaf Meals

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Abstract

The growth performance and proximate general composition along with the fatty acid profile of flesh of 90 days cultured of Nile tilapia fed with leguminous leaf meals were studied. Two almost isonitrogenous (28% Crude Protein) diets were formulated using groundnut (*Arachis hypogea*) leaves (GLM) and arahar (*Cajanas cajan*) leaves (ALM) as the key ingredient. Market available fish feed (MAF), containing 28% CP was supplied to another batch of fish. Fish growth was significantly high ($P < 0.05$) in GLM fed treatment. Crude protein, lipid and ash content showed significantly higher values ($P > 0.05$) in GLM fed treatment over the other. The PUFA content was maximum in GLM fed fish followed by ALM and MAF. Eicosapentenoic acid (EPA) (20:5ω3) was recorded slightly higher in GLM (1.8) fed fish followed by ALM (1.4) and MAF (1.0). The amount of docosahexenoic acid (DHA) (22:6ω3) was found to be highest in GLM (5.8) fed fish and lowest in ALM (4.7) fed fish. Both total n3-PUFA and n3/n6 ratio were high in GLM (14.15 and 1.47) fed fish. The thrombogenic indices (TI) were in the order of GLM (0.59), ALM (0.65) and MAF (0.74) fed fish. Fish PUFA, especially the n3 fatty acids, are affected positively when fed GLM which is good for the quality of the fish produced in regard to the benefits for the health of consumers.

Keywords: Fatty acid profile, leguminous, isonitrogenous, groundnut, arhar and PUFA

1. Introduction

Fish is one of the most vital protein sources for majority of the population in the world. With the increase in global population, there is an urgent need for increasing fish production to meet the ever increasing requirement of protein. For rapid production of fish under cultured condition, feed is considered to be an essential component which constitutes the most expensive operating cost item accounting for over 50% of costs in semi-intensive aquaculture [1] and as high as 70% in intensive aquaculture [2]. Moreover, the feeds are irregular and short in supply, sometimes adulterated, contaminated with pathogen and contain chemicals likely to be harmful to human health. Groundnut (*Arachis hypogea*) and arahar (*Cajanas cajan*), are the two agricultural crop species belong to the legume "bean" family (Fabaceae) grown widely in the tropical countries. The leaves of these plants contain a significant amount (20-23%) of crude protein one of the major feed component, besides considerable amount of crude lipid, carbohydrate etc. claiming as a promising ingredient of fish feed (Table 1). Many studies have been carried out to evaluate the effects of non-conventional ingredients used in diets as FM substitutes on fish fatty acid composition [3-5]. Surprisingly, such an important crop waste potential for fish feed formulation is remained unexplored. Research information on utilization of such alternative fish feed source is scanty.

Keeping the above facts in view the investigation was carried out with the main objective to study the growth performance of *O. niloticus* by using groundnut leaf or arhar leaf as alternative source of protein in fish feed and also to study the qualitative changes in fish flesh as human food.

2. Materials and Methods

Experimental set up

Twenty five Nile tilapia (with male and female ratio 1:1) fingerlings in each group were used in three different treatments. The set was replicated thrice. Altogether 225 fingerlings (average weight 5.5 g and average length 4.5 cm) were used in the experiment. The fish fingerlings were treated with potassium permanganate solution (1 mg L^{-1}) to remove any external parasites and were acclimatized in a big tank for five days. The experiment was conducted for 90 days from 1st June to 29th August in the year 2011 in the tanks of Aquacultural Engineering Section of IIT-Kharagpur, Paschim Medinipur, West Bengal, India. Another batch of fish was cultured fed with feed available in market (MAF). One thousand litre of tap water plus dry inert soil of 40 kg was used for each treatment. The water was exchanged in all the tanks at 7 days interval. A constant depth

of water was maintained adding water at 3 days interval.

Feed formulation and preparation

The groundnut leaves and arhar leaves, principal feed ingredients were collected from local agricultural field. Biochemical compositions of groundnut and arhar leaf used for feed for tilapia are shown in Table 1. Diets used for growth trial were prepared in such a manner, the feed formulations remain almost isonitrogenous (25 g 100 g^{-1}) and isoenergetic (4.0 Kcal g^{-1}) in nature. The choice of these nutrient levels, particularly protein, was intended to reflect the practical diets used in India. Diet formulations are presented in Table 2. Mustard oil cake, wheat flour and egg shell dust were common ingredients in the formulated feeds tested. These ingredients were used to compensate lipid, protein and ash deficiency in formulated feed. Wheat flour was used as binder. Each feed was fortified with egg shell dust for calcium supplementation. This was added keeping in mind that the developing fish needs huge quantity of calcium for its bone development. The different ingredients were thoroughly mixed using a food mixer (A200 Hobart Ltd). The proportion of different feed ingredients was determined by using Pearson's square method. The mixture was given the shape of pellets using a Pellet Mill (Model CL2) with a 12 mm die. The resulting pellets were dried in a hot air oven for 48 h at 50 ºC and then packed in polythene bags for future use.

Table 1. Biochemical composition of groundnut and arhar leaf used for feed for tilapia (*O. niloticus*)

Ingredient (%)	Groundnut	Arhar leaf
Dry matter	93.77	93.32
Crude protein	22.25	19.78
Crude lipid	8.89	8.43
Carbohydrate	10.38	9.67
Ash	9.05	9.19
Nitrogen free extract	34.89	37.19
Crude fibre	8.31	9.06
Gross energy (Kcal g^{-1})	3.43	3.34

Table 2 Detailed information of each formulated diet

Name of feed	Ingredients	% of CP in ingredient	% of ingredient in formulated feed	% of crude protein in feed	% of lipid in feed	% of carbohydrate in feed	Calorific value of feed (kcal/g)
GLM	G N Leaves	22.25	40.0	28.16	8.1	10.4	4.0
	MOC	34.65	30.0				
	Wheat flour	9.08	28.0				
	Egg shell dust	1.8	2.0				
ALM	Aarahar Leaves	13.78	39.5	27.95	8.3	10.0	3.9
	MOC	34.65	33.0				
	Wheat flour	9.08	26.0				
	Egg shell dust	1.8	1.5				

Feeding

The feed was given ad libitum in a feeding bag hung from an iron rod in four locations in each tank. Unconsumed feed was removed after 1hour from the beginning of feed administration and dried in a hot air oven at 50 °C and weighed on an electric balance to an accuracy of 0.1 mg.

Growth calculation

Growth and nutrient utilization were determined in terms of feed intake (FI), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and hepatosomatic index (HSI) as follows [6]:

FI (g fish-1 day-1) = Total feed intake per fish/number of days

SGR (% day-1) = 100 × (ln[final body weight]-ln[initial body weight])/no. of Days

FCR = feed intake/live weight gain

PER = live weight gain/crude protein intake

HSI (%) = 100 × (weight of liver/total body weight)

GSI (%) =100 × (weight of gonad/total body weight)

Analysis

Feeds and carcass samples were analyzed following standard procedures [7]: dry matter (DM) after drying in a hot air oven (Gallenkamp, UK) at 105 °C for 24 h; crude protein (CP) by Kjeldahl method (N × 6.25) after acid hydrolysis, crude lipid (CL) after extraction with petroleum ether for 7-8 h by Soxhlet method (40-60 °C boiling range), total ash by igniting at 550 °C for 3 h in muffle furnace (Size 2 Gallenkamp, UK). Organic matter (OM) was calculated by subtracting total ash from DM [8]. Crude fibre was determined using a moisture free

defatted sample which was digested by a weak acid HCl (0.1N) followed by a weak base NaOH (0.1N) using the Fibertec System 2021 (FOSS, Denmark). Nitrogen-free extract was determined by subtracting the sum of crude protein, crude lipid, crude fibre and ash from DM [9]. Gross energy was determined using a Bomb Calorimeter Model-DFU 24. The sample was combusted in a chamber pressurized with pure oxygen and resulting heat measured by increase in the temperature of the water surrounding the bomb.

Extraction of Lipids

The total lipids were extracted from all the samples, (fish flesh-3, feed-3) following the method of Bligh and Dyer (10) (1959) using methanol-chloroform (2:1, v/v), methanol chloroform-water (2:1:0.8, v/v/v), and then again with the first solvent system viz., methanol-chloroform (2:1, v/v). Sample was ground with the solvent methanol-chloroform (2:1,v/v), filtered through Whatman No. 1 filter paper and residue was extracted with the next solvent system, consisting of methanol-chloroform-water (2:1:0.8, v/v/v). The process was repeated once again with methanol-chloroform (2:1, v/v). Finally, the three extracts were pooled, diluted with three volumes of water (100-200 ml, depending on the volume of pooled extracts) and layer was allowed to separate in a separatory funnel made by Pyrex glass Co.. The chloroform layer at the bottom of the separatory funnel was withdrawn and dried over anhydrous sodium sulphate in glass stoppered conical flasks, by Pyrex. The chloroform solution of lipid was evaporated in a rotary vacuum evaporator by Rotavap under a pressure of 40-50 mm of Mercury, weighed on a micro-balance by Sartorius and redissolved in distilled n-hexane (10-20 ml) and kept at -20 °C for future use. BHT (butylated hydroxy toluene) was added at a level of 100 mg/L to the solvent as antioxidant.

Preparation of Methyl Ester of Fatty acids

Total lipid of various (fish flesh-3, feed-3) samples was dissolved in anhydrous methanol containing concentrated Sulfuric acid (1.0%, v/v) and the mixture was refluxed [11] for 2 hours. Methanol was evaporated to a small volume (1-3 ml) and cooled to 4 °C, in a freezer. Distilled water 10-15 ml was added to the cooled mixture (1-3 ml) in hard glass test tubes by Pyrex and the methyl esters of fatty acids were extracted 3 times with aliquots (5-10 ml) of diethyl ether, vortexed in a Vortex mixer. The ethereal extracts were taken out by Pasteur pipettes, pooled and dried over anhydrous sodium sulphate, (1-2 gm) in conical flasks (25-50 ml capacity) with glass stopper, filtered through Whatman no. 1 filter paper, vacuum dried, redissolved in n-hexane (1-2 ml volume) and kept in a freezer at 4 °C for future use.

Purification of Fatty Acid Methyl Ester (FAME) by Thin Layer Chromatography (TLC)

Fatty acid methyl esters were purified by TLC using a solvent system of n hexane- diethyl ether (90:10, v/v) [12,13]. A standard methyl ester was also run on the same plate in a separate lane, for identification of the methyl ester bands in the samples. The location of methyl ester bands were indicated by placing the TLC plate in an iodine vapour chamber by Pyrex glass co.. The methyl ester bands corresponding to the standard were marked and then scrapped off the plate with a sharp razor blade. Methyl esters were recovered by extracting the silica gel bands containing the methyl ester samples in a mini glass column (10 cm length x 0.8 cm internal diameter, by Pyrex) with chloroform (30-50 ml), the later was evaporated and the methyl esters were kept in n-hexane (1-2 ml) in a freezer at 4 °C till analyzed by Gas Liquid Chromatography (GLC).

Gas Liquid Chromatography (GLC)

GLC of fatty acid methyl esters were done on a Chemito 1000 instrument, equipped with Flame Ionization Detector (FID). Quantifications were done by computer using specific Clarity Lite software.

Analysis of fatty acid methyl esters (FAME)

GLC of FAME was done on a BPX-70 megabore capillary column of 30 mt length and 0.53 mm internal diameter obtained from SGE, Australia. Oven temperature was programmed from 150 °C – 240 °C with a rate of 8 °C/min. Initial and final temperatures were kept isothermal for 1 minute and 20 minutes respectively. Injection port and detector temperatures were 250 °C and 300 °C respectively. Nitrogen gas was used as carrier gas and its flow rate was 6.18ml/min.

Statistical analysis

Data are presented as means ± SD. One-way ANOVA was used to determine the significant effects of different types of feed on growth and growth parameters and also on fatty acid profile of fish flesh.

3. Results

3.1 Growth

Growth was maximum in GLM (88.41 g) fed fish followed by ALM (80.04 g) and MAF (50.24 g) (Figure 1). In the present investigation the amount feed intake (g fish^{-1} day^{-1}) ranged from 1.27–2.14. It was recorded minimum in control treatment (1.27 g) and maximum (Table 3) in ALM (2.14 g). These results show an encouraging response of the fish to the newly formulated feeds. The SGR was obtained maximum in GLM (0.92) followed by ALM (0.83) and MAF (0.50). FCR is an important indicator of feed utilization efficiency, balance of bio-available nutrients, and partitioning dietary nutrients towards growth. It was recorded lowest in GLM (2.26) and highest in ALM (2.58).This indicates that fish can

assimilate and utilize the GLM well than the other feeds. The highest PER value in the present study was recorded from GLM (1.47) fed fish indicating that quality of protein as well as its prosperity of amino acid profile in groundnut leaf is better than the other feed ingredients (Table 3). Hepatosomatic index (HSI) was measured at the end of the experiment to evaluate condition and nutritional status of fish. Gonadosomatic index (GSI) is a tool for measuring the sexual maturity of animals in correlation to ovary and testis development. A significant high value of HSI (1.82) and GSI (1.53) was obtained from GLM fed fish.

The moisture content ranged from 72.77–75.50%. The lowest moisture content was obtained (Table 4) from GLM (72.77%). It indicates that fish fed with GLM contains less amount of moisture in its body, which is desirable. Similar finding also reported from [14].The maximum crude protein (14.74) and lipid (6.56) was obtained from GLM fed fish (Table 4). The ash content of fish under all the treatments ranged between 5.07-5.35%. It is indicative to the fact that the plant sourced feeds contain some such ingredients which increase the ash content of fish [15].

The amount of gross energy (kcal. g^{-1}) significantly differed under different feed formulae for the species studied.

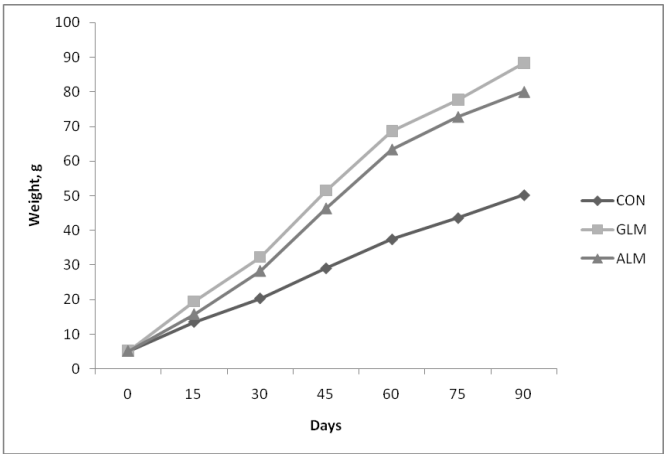

Figure 1. Growth rate of *O. niloticus* fed with GLM and ALM feed

Table 3. Growth performance and nutrient utilization of *O. niloticus* under different feeds

Particulars	**MAF**	**GLM**	**ALM**
Initial weight (g)	5.10±0.02^{a}	5.10±0.03^{a}	5.10±0.02^{a}
Final weight (g)	50.24±0.16^{a}	88.41±0.15^{c}	80.04±0.14^{b}
Feed intake (g $fish^{-1}$ day^{-1})	1.27±0.12^{a}	2.09±0.18^{bc}	2.14±0.21^{c}
Specific growth rate (% day^{-1})	0.50±0.01^{a}	0.92±0.01^{c}	0.83±0.01^{b}
Feed conversion ratio	2.55±0.05^{b}	2.26±0.03^{a}	2.58±0.04^{b}
Protein efficiency ratio	1.31±0.06^{a}	1.47±0.06^{b}	1.29±0.06^{a}
Hepatosomatic index	1.60±0.04^{a}	1.82±0.02^{c}	1.71±0.03^{b}
Gonadosomatic index	1.25±0.05^{a}	1.53±0.05^{c}	1.39±0.08^{b}

Values are mean±SD, n=3
Values in the row superscripted by different alphabets are significantly different from each other (P<0.05, Duncan's new multiple range test).
Separate analysis was done for each row.

Table 4. Effect of feed formulae on proximate composition of whole body of *O. niloticus* at harvest time (%fresh weight basis, mean±SD)

Particulars	**MAF**	**GLM**	**ALM**
Moisture	75.50±1.21^{b}	72.77±1.23^{a}	75.20±1.19^{b}
Crude protein	13.36±0.20^{a}	14.74±0.19^{b}	13.39±0.17^{a}
Crude lipid	4.60±0.05^{a}	6.56±0.08^{c}	5.53±0.06^{b}
Ash	5.10±0.06^{a}	5.07±0.06^{a}	5.35±0.07^{b}

3.2 FA profile of three formulated feeds

The FA profile of total lipids of 3 types of feeds (MAF, GLM and ALM) is represented in Table 5. The amount of MUFA was found to be maximum amount in the FA classes followed by DUFA, SFA and PUFA. It was more or less similar in all supplied fish feeds (MAF, GLM and ALM). The amount of SFA was recorded maximum in ALM (13.6) and minimum in GLM (11.8). Among SFAs, palmitic acid (16:0) was dominant occupying more than 50% of the total SFA (Table 5). MUFA ranged from 59.6 – 60.5. Oleic (18:1ω9) was one of the dominant MUFA in these feeds. The amount of DUFA was maximum in GLM (21.5) and minimum in MAF (20.6). PUFA was found to be maximum in MAF (8.8) and minimum in ALM (5.4). Total amount of ω3 FAs was maximum in MAF (7.6) and minimum in ALM (5.3). The n3/n6 ratio was maximum in MAF (0.35) and minimum (Table 5) in ALM (0.25).

Table 5. Fatty acid profile of tested feeds and flesh of *O. niloticus* fed with feeds (% w/w of each component in total fatty acids)

Components	MAF	GLM	ALM	MAF	GLM fed fish	ALM fed fish
Saturated						
14:0	0.3	0.2	0.2	5.2	5.4	4.9
15:0	0.1	0.0	0.3	1.0	0.9	1.5
16:0	7.4	7.6	7.5	31.2	31.3	31.3
17:0	1.1	0.4	1.4	0.9	0.8	1.9
18:0	2.6	2.2	2.4	8.5	7.7	8.1
20:0	0.2	0.3	0.2	0.4	0.1	0.5
22:0	0.8	0.7	1.2	4.1	4.0	5.1
24:0	0.3	0.4	0.4	0.8	0.5	0.8
∑SFA	**12.8**	**11.8**	**13.6**	**52.10**	**50.95**	**54.1**
Monoene						
14:1	0.2	0.0	0.4	0.9	0.7	0.7
15:1	0.1	0.0	0.0	0.4	0.2	0.3
16:1	1.2	0.7	1.0	7.7	7.1	8.4
17:1	0.3	0.0	0.0	0.5	0.2	0.3
18:1ω9	20.4	23.2	23.2	13.8	13.6	12.4
20:1ω9	7.2	7.4	7.6	1.5	1.1	1.3
22:1ω11	27.5	28.7	27.5	1.7	0.8	0.6
24:1	0.0	0.5	0.6	1.8	1.4	1.5
∑MUFA	**56.9**	**60.5**	**60.3**	**28.3**	**25.1**	**25.5**
Diene						
16:2	1.1	0.0	0.0	0.76	0.1	0.2
18:2ω6	20.4	21.5	20.8	6.1	6.4	6.0
20:2				0.0	0.03	0.00
∑DUFA	**21.5**	**21.5**	**20.8**	**6.86**	**6.53**	**6.2**
Polyene						
18:3ω6	0.4	0.0	0.0	0.3	0.5	0.4
18:3ω3	5.9	5.7	5.2	3.0	3.3	2.6
20:3ω6	0.5	0.3	0.1	1.1	1.5	1.1
20:3ω3	0.5	0.2	0.1	0.04	0.05	0.03
20:4ω6	0.1	0.0	0.0	1.0	1.0	0.9
20:5ω3	0.3	0.0	0.0	1.0	1.8	1.4
21:5ω3	0.2	0.0	0.0	0.1	0.3	0.1
22:5ω6	0.2	0.0	0.0	0.2	0.3	0.2
22:5ω3	0.3	0.0	0.0	1.3	2.9	2.3
22:6ω3	0.4	0.0	0.0	5.1	5.8	5.2
∑PUFA	**8.8**	**6.2**	**5.4**	**13.14**	**17.45**	**14.23**
Total -ω3	**7.6**	**5.9**	**5.3**	**10.54**	**14.15**	**11.63**
Total -ω6	**21.6**	**21.8**	**20.9**	**8.7**	**9.6**	**8.2**
n3/n6	**0.35**	**0.27**	**0.25**	**1.21**	**1.47**	**1.41**
TI				**0.73**	**0.59**	**0.65**

3.3 FA profile of *O. niloticus* fed with different feeds

The FA profile of total lipids of fishes (*O. niloticus*) fed with 3 different feeds (MAF, GLM and ALM) are represented in Table 5. SFA was maximum in amount than the other classes of FAs. SFA was maximum in ALM (54.10%) fed fish and minimum in GLM (50.95%) fed fish. Oleic (18:1ω9) was dominant MUFA which was maximum in MAF (13.8) followed by GLM (13.6) and ALM (12.4). Linoleic acid (18:2ω6), the most predominant DUFA was recorded highest in GLM (6.4) fed fish than others (Table 5). EPA (20:5ω3) was recorded highest in GLM (1.8) fed fish followed by ALM (1.4) and MAF (1.0). The amount DHA (22:6ω3) was found to be highest in GLM (5.8) fed fish and lowest in MAF (5.1) fed fish. Both total ω3 and ω6 FAs were high in GLM (14.15 and 9.6) fed fish (Table 5). Whereas, n3/n6 ratio was highest in GLM (1.47) and lowest in MAF (1.21) feed fed fish respectively. The thrombogenic indices (TI) were in the order of GLM (0.59), ALM (0.65) and MAF (0.74).

Discussion

Growth of animal is a complex process influenced by its genotype, hormonal status, nutrition and the environment under which it grows [16]. In the present study it is observed that the fish fed with different diets had different effects on various growth parameters like body weight, SGR, PER and FCR. This might have happened possibly because of differences in acceptability and palatability of feeds and the environmental condition of the tank. Although the genetic potential for growth may differ among fish, nutritional and hormonal factors are significant contributors to the expression of that genetic potential for growth and efficiency of nutrient utilization [16].

Ackman [17] stated that only 14 fatty acids are really needed to describe the fatty acids of fish. However, Ackman et al. [18] listed 64 fatty acids from 5 fresh water fishes of West Bengal, India. The fish under discussion recorded 28 fatty acids of the total lipid (TL) and the result is more or less similar to those reported from other tropical and certain temperate zone fresh water fishes. According to Ackman et al. [18], dominant fatty acids in lipids of all the fishes were myristic (14:0), palmitic (16:0), stearic (18:0), palmitoleic (16:1ω7), oleic (18:1ω9), linoleic (18:2ω6), linolenic (18:3ω3), arachidonic (20:4ω6), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids. The present results corroborate with the above findings. The total SFA of our experimental fish was nearly double than the amount reported from [18].

Ackman et al. [18] stated that n3/n6 ratio should range 1–2 for fresh water fish. The n3/n6 ratio of our experimental fish was within the same range.

Mnari et al. [19] observed significantly high n3 PUFA levels in cultured sea bream in the muscle and liver; the same findings were reported by De Silva et al. [20] in Murray cod. In the present study n3 in experimental fish show much higher level fed with low level of n3 in supplied fish. However, a reverse result is recorded in case of n6 content. This has resulted increase of n3/n6 ratio even in experimental fish and the values are approximately to that of control fish. This is due to the inherent mechanism of fish physiology to maintain internal homeostasis.

Fish in general contain more n3 than n6-PUFA although fresh water fishes have higher level of n6 FAs than marine species as can be seen in the present study. Fishes have a higher dietary requirement for n3 PUFA and dietary EFA requirement of marine fish for n3-PUFA may be higher than that of fresh water fish as average value of n3/n6 ratios, as worked out Cowey [21], ranged from 1–4 for fresh water and 5–14 for marine fish respectively.

Valfré et al. [22] also approves such values for freshwater fishes. The difference between fresh water and marine fish may be due to two reasons. The difference in the FAs contents in the diet may be one of the reasons and the specific requirements related to physiological adaptation to the environment the other [23]. However, the n3/n6 ratio of three freshwater fishes studied in Pakistan show much lower value (<1), where the value in *O. mossambicus* was 0.23 [24].

In the present study, the n3/n6 ratio of fed fishes is always much higher than the feed provided to them. The results indicate that the fish adjust their own n3/n6 ratio for their own physiological adaptation. The most interesting aspect of the present experiment is that the fishes provided with high level of n6 fatty acids however, the fishes were able to covert n6 fatty acids to n3 fatty acids efficiently to a very low n3/n6 ratio (<1) to higher value of n3/n6 (>1). The mechanism of this conversion could not be explained at present. However, it proves that these fishes have the ability for such a conversion for the maintenance of physiological homeostasis. The reason behind is that in formulated feeds, 20:1ω11 fatty acid is the major MUFA (28.5% and 28.7% respectively) which has been reduced to less than 2 in fed fishes. The value of linoleic acid (18:2ω6) of these feeds was more than 20 which has been found to be much lower (nearly 6) in the fed fishes. The mechanisms behind this conversion depend on the efficiency of the experimental fish by adopting desaturation and chain elongation process. Sharma et al. [25] stated that the fatty acid profile of fish may be attributed primarily to the dietary intake of the fish, but the present experimental results are not in conformity with the above proposition.

Fish oil and fish meal lipids and their constituent fatty acids have high digestibility and energy value.

Fish lipids contain high levels of n3 PUFA which may be essential for worm blooded animals. The substitution of SFA from MUFA in experimental fishes might play a major role in reducing TC levels and CAD in human who take such fish as diet. Fish is more beneficial than fish oil but for CAD patients prescribed amount of n3 PUFA (EPA and DHA) is required as the best insurance against sudden death.

Low fat and easy digestibility of the two batches of experimentally fed fishes under investigation together with its EFA resource and other attributes discussed above can be recommended as a better diet on par with the recognized fish diet.

Conclusion

The feed prepared from groundnut leaves enhance growth and thereby yield of *Oreochromis niloticus*. It improves quality of fish by accumulating more n-3 PUFA in the flesh of the fish as well as increasing the n3/n6 ratio which is beneficial for human health. The cost of GLM feed is very nominal since the key ingredient is available as one of the widely grown agricultural crop waste. Moreover, the feed can be formulated at local level leading to employment generation in rural areas.

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