

Influence of Different Sources of Dietary Fats on Fatty Acid Profile of Striped Snakehead (*Channa Striatus*) Fish Carcass

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Abstract Various dietary fats were used to assess their impact on the fatty acid profile of snakehead murrel, saul (*Channa striatus*). The saul grow-out (av. wt. 27.36 ± 0.09 to 32.54 ± 0.41 g) were fed for 12-weeks with seven experimental diets (F1, F2, F3, F4, F5, F6 and a control, F7 of natural foodstuffs, NATFO). F1 (L3HUF) contains 0.5% n-3 fatty acid & 7.5 % saturated oil; F2 (H3HUF) contains 1.0% n-3 fatty acid & 7.0% saturated oil; F3 (MUSOL) contains 8.0% mustard oil; F4 (LINOL) contains 8.0% linseed oil; F5 (MIXOL) contains 4.0% mustard oil and 4.0 % linseed oil; F6 (SATOL) contains 8.0 % saturated oil. The muscle polyunsaturated fatty acids (PUFA) contents varied with different dietary lipids level which impacted on deposition of fatty acid in flesh. The muscle unsaturated fatty acids, including Docosahexaenoic acid (22:6n3, DHA) and Eicosapentanoic Acid {C20:5n-3, EPA}, levels, were comparatively higher in MIXOL diet followed by H3HUF than in the other diets, indicating selective deposition of various fatty acids in each feeding trial. It was concluded that dietary fats in the diet has role in the carcass composition of fatty acid profile in *Channa striatus* and the MIXOL (contains mustard oil and linseed oil: 1:1 w/w) could be safely used for better deposition of beneficial healthy fatty acids like EPA and DHA. The addition of mustard oil and linseed oil in the diet are comparative better if we look towards economizing the cost of the brood-stock feed in comparison to the addition of pure fatty acids in the fish diets.

Keywords Dietary Fats, Fatty Acids, Carcass, *Channa Striatus*, Saul, Grow-Out

1. Introduction

It is reported[1] that fish reared in intensive culture systems require all nutrients in a complete diet. Information on nutritional requirements of major dietary components such as protein and energy is a prerequisite for the formulation of an inexpensive and balanced diet for the fishes. India has huge potential for the production of cheaper plant sources e.g. de-oiled cakes like linseed oil cake etc. rich in essential fatty acid (EFA, omega-3 HUFA) which can be utilized as source of lipid in carnivorous fish nutrition not only for growth purposes but also for gonadal maturation through dietary manipulations. Recycling of these agro-based by-products, like mustard oil cake, linseed oil cake etc. can be used in place of animal origin oils as source of lipid and EFA. Thus, the fatty acid composition of these various ingredients of plant origin have a good source of HUFA which can be utilized for carnivore fishes nutrition. And these can be very well used in place of animal lipid source, and can be studied for the deposition of nutrients in terms of flesh and maturation of the female fishes as well. Sarowar *et al.* [2] have studied the impacts of different diets on growth and survival of *C. striatus* grow-outs. Influence of dietary lipid/protein ratio requirement has been studied in *C. striatus* [3]. Since the fish oil is not only costly but becoming less available, there is an urgent need to assess the dietary potential of various other available sources of fat from both animal and plant sources. In global scenario, the emphasis is being given to dietary replacement of animal fat with less expensive plant fat [5,7]. Replacement of fish oil by vegetable oils has proved in many fishes without impacting on growth performances [4-7]. However, the impacts of various dietary oils on lipid metabolism of fish are still not very

clear, particularly where fish oil provide the only source of highly unsaturated fatty acids, very much essential for catfishes. Variation in dietary oils may lead to imbalances in the essential or non-essential fatty acids, and may be differently affecting tissue profile.

Fish lipids are important because of their high levels of polyunsaturated fatty acids (PUFA), which have been reported to reduce the risk of cardiac diseases [8] and lower plasma triacylglycerol levels [9]. In addition, they can reduce the symptoms of physiological alterations [10,11]. Lipid content and fatty acid composition in fish are known to vary significantly depending on the availability of food items to fish and environmental conditions [12,13]. Previous studies have shown that n-3 PUFA of marine animals vary depending on various biological and environmental factors, such as taxonomy, diet of the animals, water temperature and the latitude at which they were harvested [14].

The present study was taken up to evaluate the utilization impact of dietary lipids on the carcass fatty acid profile by the striped murrel, *C. striatus*. The aim of the present study was to assess the impact of different dietary fats in the edible tissue composition of muscle and FA profile enrichment with essential fatty acid of this commercially important fish.

2. Materials and Methods

2.1. Feed Preparation and Feeding

After the acclimation period of 7 days Six type of feeds were formulated having similar feed ingredients in same quantities excepting different source of fat namely low level of highly unsaturated fatty acid (L3HUF, F1); high level of highly unsaturated fatty acid (H3HUF, F2); mustard oil (MUSOL, F3); linseed oil (LINOL, F4); mixed oil (MIXOL, F5); saturated fat (SATOL, F6) and a control (NATFO, F7) comprising of natural foodstuffs (Table 1). Six diets (L3HUF, F1; H3HUF, F2; MUSOL, F3; LINOL, F4; MIXOL, F5; SATOL, F6) and a control (NATFO, F7) with natural food. F1, contains 0.5% n-3 fatty acid and 7.5% saturated oil; F2, contains 1.0% n-3 fatty acid and 7.0% saturated oil; F3, contains 8.0% mustard oil; F4, contains 8.0% linseed oil; F5, contains 4.0% mustard oil and 4% linseed oil; F6, contains 8% saturated oils. (Table 1).

In order to evaluate the effect of different oil sources on the carcass fatty acid profile of *C. striatus*, the experiment was conducted in indoor condition in 14 (7 types of feed, 2 replicates) round plastic pools of 300 litre capacity, each filled-up with 100 litre tube well water. Each having two replications, stocked with 20 grow-out having an initial average weight 27.36 ± 0.09 g to 32.54 ± 0.41 g were plotted in each of the plastic pool after proper acclimatization. The tanks were provided aeration from a portable aerator round the clock. During the experiment, the fishes were fed twice a day at 10:00 and 17:00 hours *ad libitum* per day. Rearing pools were cleaned every second day and about half of the water was replaced with fresh bore-well water to reduce the nitrogenous waste accumulated as debris and fecal matters.

Table 1. Ingredients composition (w/w) of feeds

Feed Ingredients	F-1 (L3HUF)	F-2 (H3HUF)	F-3 (MUSOL)	F-4 (LINOL)	F-5 (MIXOL)	F-6 (SATOL)	F-7 (NATFO)
Soybean meal	41.0	41.0	41.0	41.0	41.0	41.0	-
Starch Soluble	25.0	25.0	25.0	25.0	25.0	25.0	-
Casein	20.0	20.0	20.0	20.0	20.0	20.0	-
Carboxy Methyl Cellulose	2.0	2.0	2.0	2.0	2.0	2.0	-
Papain	0.5	0.5	0.5	0.5	0.5	0.5	-
Vitamin & Mineral Mix.	3.5	3.5	3.5	3.5	3.5	3.5	-
Omega – 3 HUFA	0.5	1.0	-	-	-	-	-
Saturated Oil	7.5	7.0	-	-	-	8.0	-
Mustard Oil	-	-	8.0	-	4.0	-	-
Linseed Oil	-	-	-	8.0	4.0	-	-
Live Fish/ NATFO	-	-	-	-	-	-	100.0

L3HUF = Low Omega – 3 HUFA; H3HUF = High Omega – 3 HUFA; MUSOL = Mustard Oil; LINOL = Linseed Oil; MIXOL = Mixed Oil (Mustard Oil : Linseed Oil :: 1 : 1 w/w); SATOL = Saturated Oil; NATFO = Natural Food

2.2. Lipid Extraction

Total lipid was extracted from muscle following Folch[15]. Muscle tissue (5 g) was homogenized in 10 volume of methanol (w/v) followed by 20 volume of chloroform (w/v) in a homogenizer (ART Micra, Germany). The homogenate was filtered (using a funnel with a folded defatted filter paper) to recover the liquid phase and the filter residue was re-homogenized with a second volume of chloroform-methanol. The filtrate was washed with 0.2 volume (4 ml for 20 ml) of 0.9% NaCl solution and phases were vigorously mixed. The mixture was poured into a separating funnel and allowed to separate. The lower chloroform phase containing lipids was collected and evaporated under vacuum in a rotary evaporator to bring down the volume to 2-3 ml. Further evaporation of chloroform was done under nitrogen stream and residue was weighed to quantify the amount of lipid extracted. The lipid residue was re-dissolved in chloroform/ methanol (2:1, v/v) and then stored in a 25 ml conical flask with glass stopper under nitrogen at -20 °C until used.

2.3. Preparation of Fatty Acid Methyl Esters (FAME)

The method as per AOAC[16] was followed to esterify the lipid. FAME was prepared from the isolated lipids by heating with the methanolic NaOH and then with BF₃ methanol for esterification. An aliquot of 5 ml n-heptane was added to recover the methyl esters in organic phase. The mixture was washed with saturated NaCl solution and two phases were separated using a separating funnel. The upper n-heptane phase was collected and stored in 10 ml all glass vials until further analysis.

2.4. Gas Chromatography – Mass Spectrometry (GC-MS)

Fatty acid methyl esters were separated using a Shimadzu QP2010 quadruple Gas Chromatography Mass Spectrometer (GCMS) equipped with a Carbowax (30 m x 0.25 mm ID; 0.25- μ m film thickness) capillary column (Cromlab S.A.). Helium was used as the carrier gas. Injector and detector temperatures were set at 250 °C. Injection was performed in split mode (1:15). The column temperature was programmed initially at 50 °C for 2 min and then increased at a rate of 10 °C per min to a final temperature of 230 °C. FAME was separated at constant pressure (23.1 kpa) and peaks were identified by comparing standard mass spectra with the relative abundances of *m/z* ranging from 40 to 550. The values of fatty acids are presented in peak area percentage of total identified fatty acids.

3. Results and Discussion

The data on total lipid content (%) recorded in the muscle tissue of *Channa striatus* significant differences ($p < 0.05$) were observed in different feedings (F1-F7). The total lipid content in muscle was found to be in the range of 7.2 \pm 0.4 to 9.5 \pm 0.4%. According to the one suggested by Kleimenov[17], fishes belongs to the group of low fat fishes having an average lipid content of 2-8%. The lipid contents in the carcass of fishes as well as prey animals have been reported to be very high [18]. As the fish consume prey with moderate lipid content, the additional lipid would be deposited in the body of fishes due to higher lipid levels in the feed.

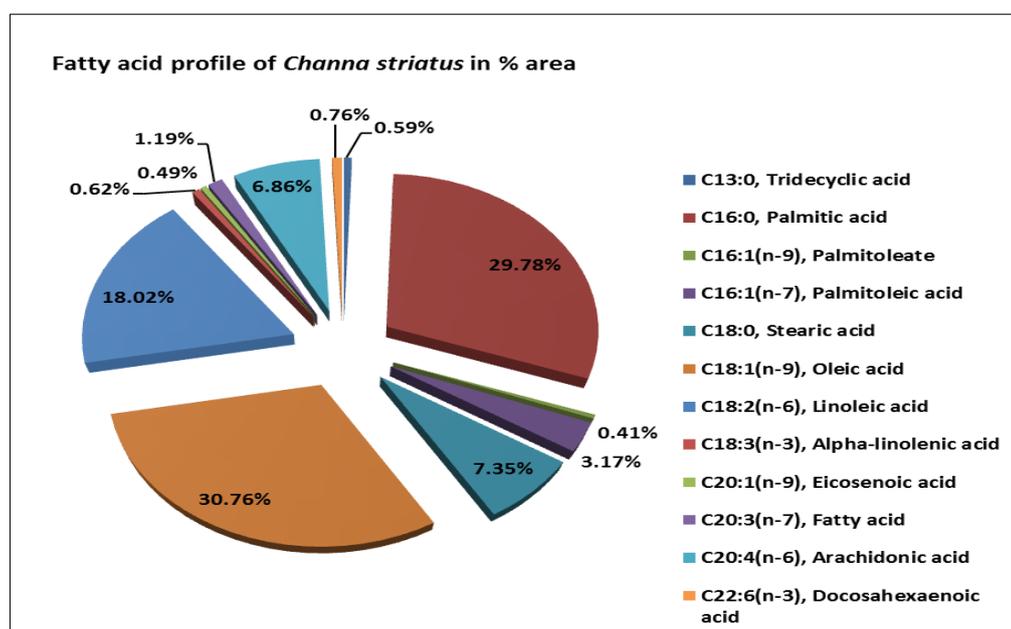


Figure 1. Fatty acid profile of *Channa striatus* fed with F₁(L3HUF)

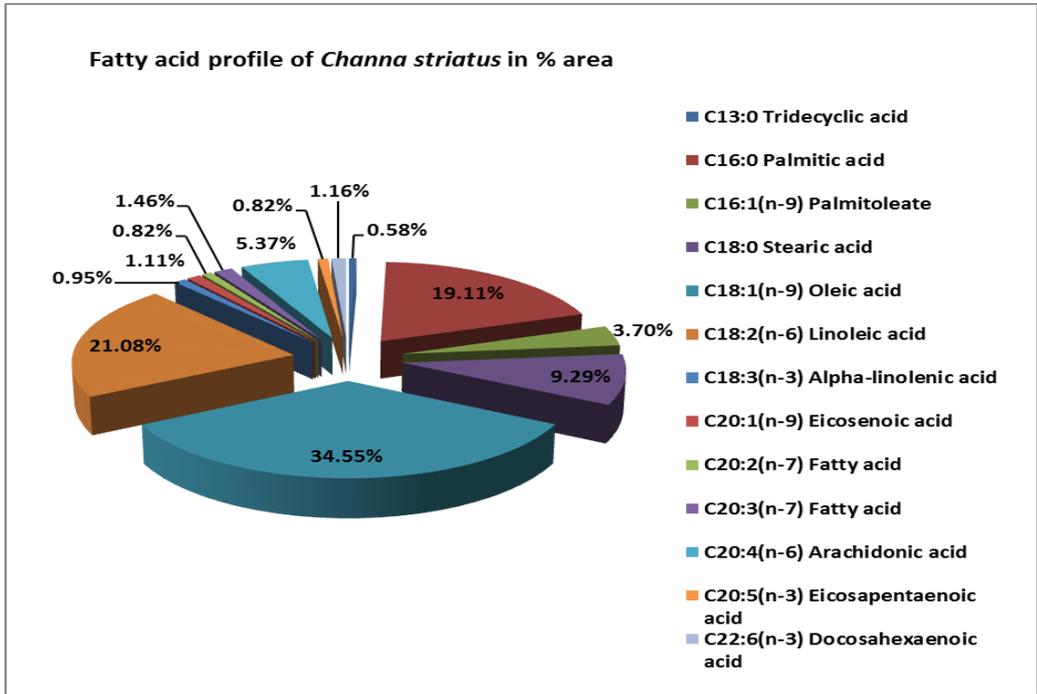


Figure 2. Fatty acid profile of *Channa striatus* fed with F₂(H3HUF)

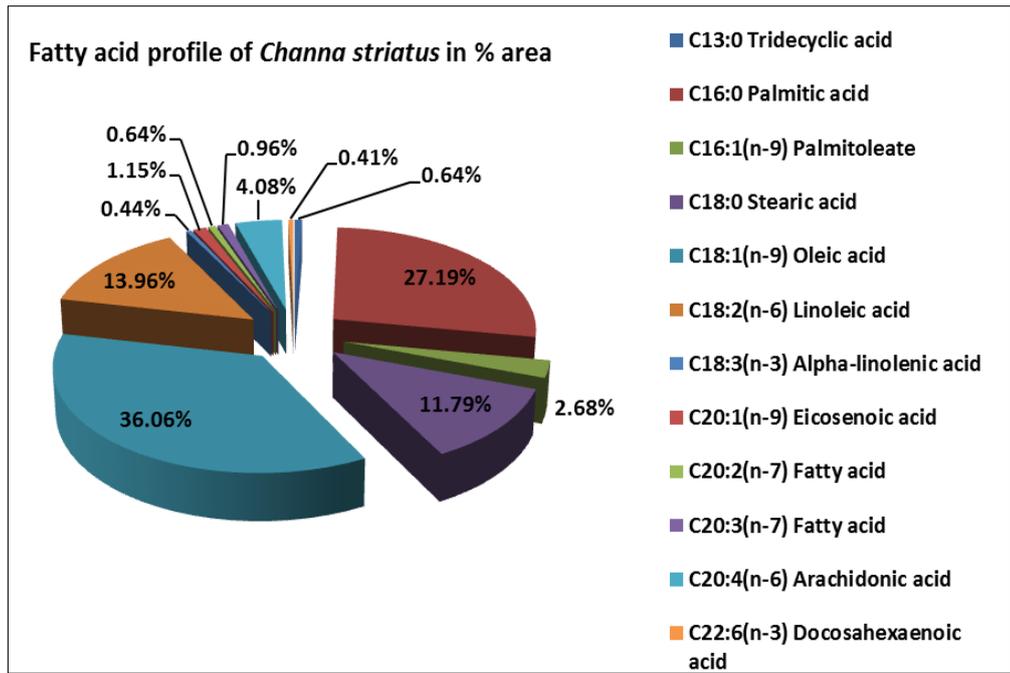


Figure 3. Fatty acid profile of *Channa striatus* fed with F₃(MUSOL)

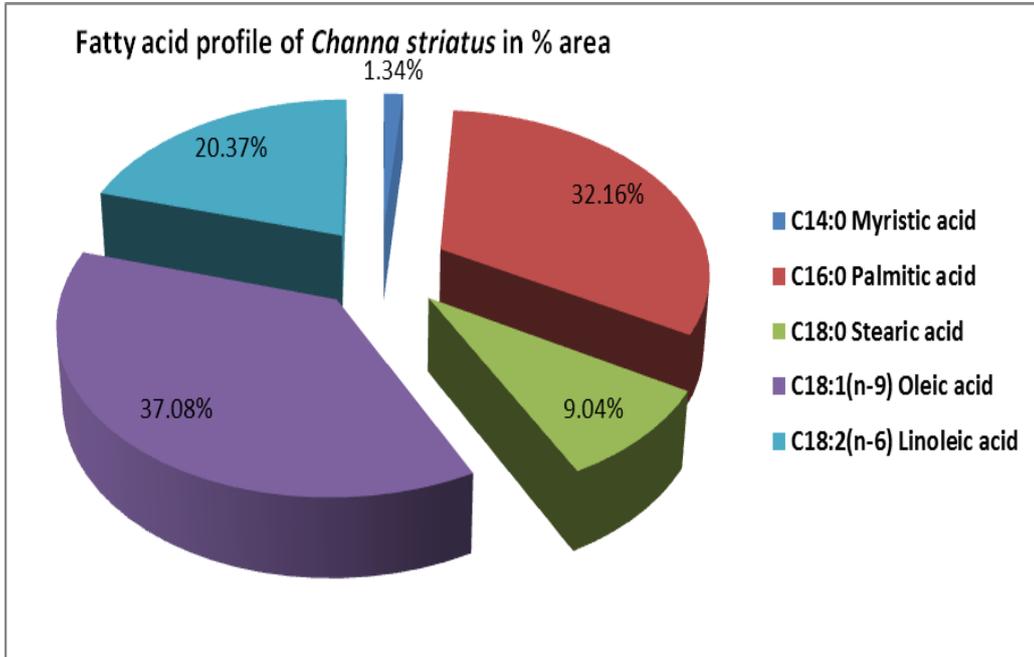


Figure 4. Fatty acid profile of *Channa striatus* fed with F₄(LINOL)

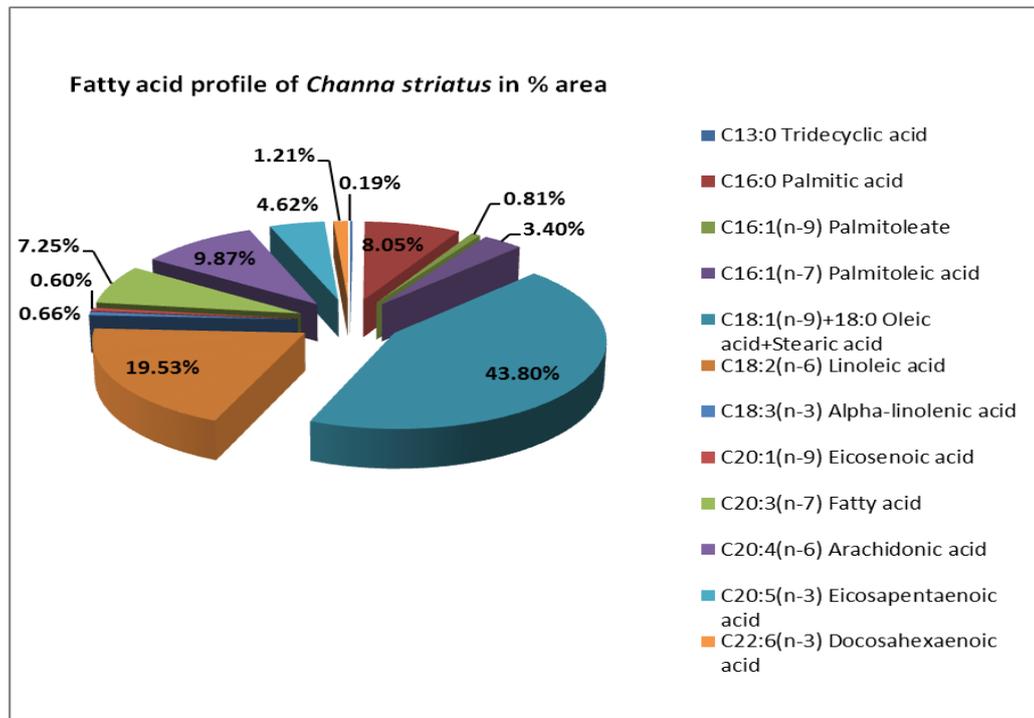


Figure 5. Fatty acid profile of *Channa striatus* fed with F₅(MIXOL)

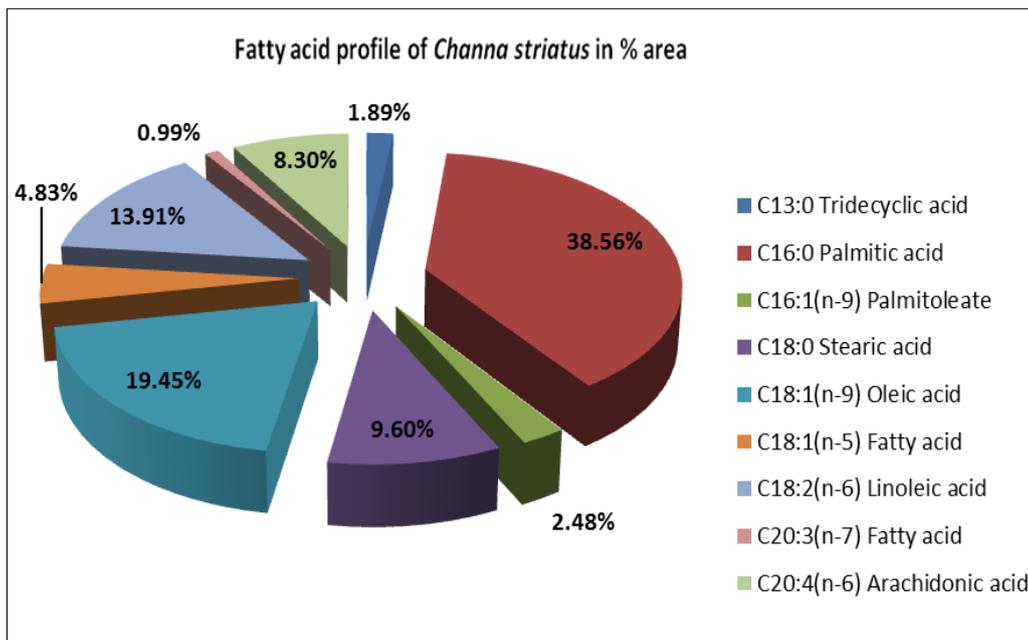


Figure 6. Fatty acid profile of *Channa striatus* fed with F₆(SATOL)

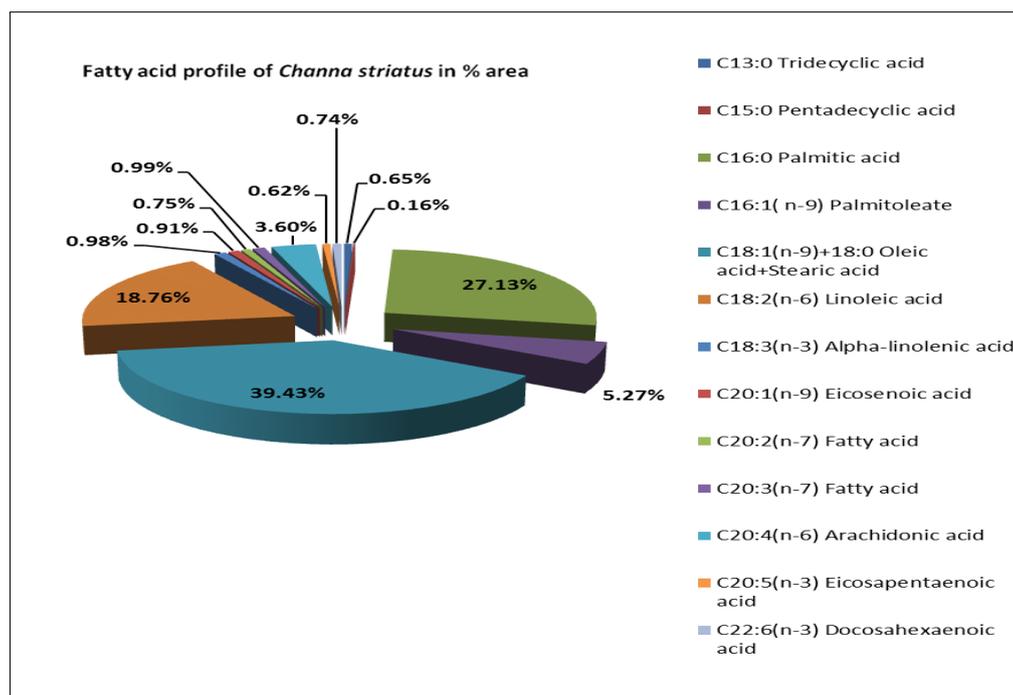


Figure 7. Fatty acid profile of *Channa striatus* fed with F₇ (NATFO)

3.1. Fatty Acid Composition

A total fatty acids were identified in muscle tissue of *Channa striatus* fed with different diets are presented in Fig1-7 and results indicate that the carcass profile changes as per the dietary sources of the fats fed to the fishes.

In the earlier studies on fatty acids profiles of a marine fish, 15 fatty acids were identified by Gopakumar [19] and 19 were reported by Joydeep et al.[20]. In muscle tissue, the most important and common essential fatty acids like Arachidonic acid (AA, 20:4n-6) was the principal n-6 PUFA at a level of 3.6-9.87% of total fatty acids. Eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) were the major n-3 PUFA identified, accounting 0.82-4.62 and 0.74-1.21% respectively (Table 2). The detailed fatty acid composition of fish tissue are depicted in Table-3.

Table 2. Important fatty acid profile of fish muscle

Fatty Acid	Emperical formula	F1 (L3HUF)	F2 (H3HUF)	F3 (MUSOL)	F4 (LINOL)	F5 (MIXOL)	F6 (SATOL)	F7 (NATFO)
Linoleic acid	C18:2(n-6)	18.02	21.08	13.96	20.37	19.53	13.91	18.76
Alpha-linolenic acid	C18:3(n-3)	0.62	0.95	0.44	-	0.66	-	0.98
Eicosenoic acid	C20:1(n-9)	0.49	1.11	1.15	-	0.60	-	0.91
Arachidonic acid	C20:4(n-6)	6.86	5.37	4.08	-	9.87	8.30	3.60
Eicosapentaenoic acid	C20:5(n-3)	-	0.82	-	-	4.62	-	0.62
Docosahexaenoic acid	C22:6(n-3)	0.76	1.16	0.41	-	1.21	-	0.74

values are in %

Table 3. Fatty Acid Composition of *Channa striatus* fish carcass

Sr.NO.	Fatty Acid	F1 (L3HUF)	F2 (H3HUF)	F3 (MUSOL)	F4 (LINOL)	F5 (MIXOL)	F6 (SATOL)	F7 (NATFO)
Percentage								
1	C13:0, Tridecyclic acid	0.59	0.58	0.64	-	0.19	1.89	0.65
2	C14:0, Myristic Acid	-	-	-	1.34	-	-	-
3	C15:0, Pentadecyclic Acid	-	-	-	-	-	-	0.16
4	C16:0, Palmitic Acid	29.78	19.11	27.19	32.16	8.05	38.56	27.13
5	C16:1 (n-9), Palmitoleate	0.41	3.70	2.68	-	0.81	2.48	5.27
6	C16:1 (n-7), Palmitoleic Acid	3.17	-	-	-	3.40	-	-
7	C18:0, Stearic Acid	7.35	9.29	11.79	9.04	-	9.60	-
8	C18:1 (n-5), Fatty Acid	-	-	-	-	-	4.83	-
9	C18:1 (n-9), Oleic Acid	30.76	34.55	36.06	37.08	-	19.45	-
10	C18:1 (n-9), Oleic Acid + C18:0, Stearic Acid	-	-	-	-	43.80	-	39.43
11	C18:2 (n-6), Linoleic Acid	18.02	21.08	13.96	20.37	19.53	13.91	18.76
12	C18:3 (n-3), Alpha-linoleic Acid	0.62	0.95	0.44	-	0.66	-	0.98
13	C20:1 (n-9), Eicosenoic Acid	0.49	1.11	1.15	-	0.60	-	0.91
14	C20:2 (n-7), Fatty Acid	-	0.82	0.64	-	-	-	0.75
15	C20:3 (n-7), Fatty Acid	1.19	1.46	0.96	-	7.25	0.99	0.99
16	C20:4 (n-6), Arachidonic Acid	6.86	5.37	4.08	-	9.87	8.30	3.60
17	C20:5 (n-3), Eicosapentaenoic Acid	-	0.82	-	-	4.62	-	0.62
18	C22:6 (n-3), Docosahexaenoic Acid	0.76	1.16	0.41	-	1.21	-	0.74

The fatty acid composition of fish fillet in general is established and well known to be modified by diet [21], food efficiency [22] and non-food factors including climatic temperature [23]. It has been documented that the PUFA content in fish and shellfish varies inversely with ambient temperature, while the SAFA content varies positively with ambient temperature [14]. Climatic temperature variations is known to affect the fatty acid profile of the fillet of fish so that the unsaturation increases with lowering the temperature [24]. Though the total lipid content in the muscle tissue recorded was low, the concentration of EPA and DHA which are considered as human health beneficial fatty acids [20], observed towards high side. The fatty acid (FA) profile of *C. striatus* muscle was influenced by the FA composition of the feed, reported for many species [25-29]. The decline in PUFA in the muscle with different fats level at the FA levels are recorded in the present study. This observation concurs with what has been reported in the published materials, that dietary fat provide essential FA for the normal growth and development of body [30], and assure the established fact that the metabolic activities of carnivorous fish is set to a high level of intake protein [31]. It also indicates that *C. striatus* has the ability to differentiate between intake fatty acid, and using them for specific deposition and/or catabolize for energy release, similar to the results with other fish species reported by many researchers [27,28,32]. Bell et al. [33] had earlier reported that there is accumulation of MUFA and SFA being preferential substrates for beta-oxidation in salmonids. It is recorded that even overall, feed FA proximate commonly impact that of the carcass, alterations in the FA composition of carcass in this species followed alteration in the dietary FA composition and also changed among carcass lipid classes and between carcass, as similarly demonstrated by Trushenski et al. [34,35] for a lean fillet fish sp. This deduce that *C. striatus* conforms to the metabolic pattern in freshwater fish as ability of bioconversion of dietary fats to C20 and C22 HUFA as demonstrated by many researchers [36-38].

Evaluation of chicken fat and its mixture with cold-pressed linseed oil as supplemental dietary lipid for sablefish (*Anoplopoma fimbria*) assessed by Friesen *et al.* [40]. The linseed oil, has long been assessed as an alternative to fish oils in aquaculture feed. But only in the last decade it has been used for some marine species including: sablefish [39-41]; gilthead sea bream [6,42], European sea bass [6,43] and turbot [7,44,45]. Results from these studies indicate that dietary oil can be partially substituted with linseed oil, however, at higher substitution levels, reductions in growth may happen due to the deficiencies in n-3 HUFAs [6,42,43,45]. The same researcher group of present study, worked with same fish supplemented the MIXOL and it was recorded that the growth performance is improved in comparison to other groups [46]. The effects of different dietary fats on the fatty acid profile of *C. striatus* carcass of have been investigated

in the present study. In all the treatments it is indicated that all types of oils tested in the study were impacting beneficial effects to striped murrel, *Channa striatus*, in terms of fatty acid profile, at their levels of maximum supplementation of 8%. Hence they may be used in combinations which did create significant changes in carcass fatty acid profile in 12 week growth studies.

The sources of dietary fats positively affected the deposition of fatty acids (EPA and DHA) in this fish. This piece of research work may not be able to give an economical feed, as it is an indicative in the findings that various dietary fats alter the flesh composition, however, a cheaper natural lipid source may economize the cost of the brood-stock feed and for this more research is required in future.

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