

Research Article

A comparative study of fatty acids profile of two Indian major carps (*Gibelion catla*, Hamilton, 1822 and *Cirrhinus mrigala*, Hamilton, 1822) using value added fish feed

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Abstract

Freshwater fishes are not only a major source of protein but they also possess nutritionally valuable lipids in the form of Polyunsaturated fatty acids (PUFAs), which play a crucial role in the normal growth, disease prevention, development, cardiovascular health and reproduction of human. The present study was performed to determine the incorporation rate of fatty acids profile and their composition in two common freshwater carps as *Gibelion catla* and *Cirrhinus mrigala* (*in situ* trial and experimental) in the different experimental time period (0 days, i.e. initial, 90 days and 180 days) by using of value added feed like flaxseed (α -linolenic acids, 51.26% – 54.94%) and soybean oil (α -linolenic acids, 7.95%-9.01%) as omega-3 supplements. To determine the specific growth pattern Length-Weight Relationships (LWRs) are analyzed where it showed positive allometric growth ($b=3.20$ in 90 days, $b=3.11$ in 180 days for *Catla* and $b=3.18$ in 90 days, $b=3.1$ in 180 days for *Mrigala* fish). The Gas Chromatography-Mass Spectrometry (GC/MS) method also confirmed that the percentages of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) increased significantly ($P < 0.05$) in experimental ($0.096a \pm 0.41$, initial; $5.16a \pm 0.27$, 90 days; $6.21b \pm 0.36$, 180 days *Catla* fish species and $0.019a \pm 0.96$ initial; $3.74b \pm 0.37$, 90 days; $3.50a \pm 0.46$ 180 days for *Mrigal* fish species) fishes rather than controls ($4.28a \pm 0.27$, 90 days; $4.36b \pm 0.36$, 180 days for *Catla* species and $2.24b \pm 0.31$ 90 days; $2.50a \pm 0.11$ 180 days for *Mrigal* species). Therefore, it was clearly indicated that formulated diet performed significantly to maintain the positive allometric growth as well as successive enrichment of PUFAs in experimental specimens, which is beneficial for human health as high source of protein and PUFAs as well.

Keywords: Comparative study, *Cirrhinus* sp., Fatty acids profile, *Gibelion* sp., Value added fish feed

INTRODUCTION

Freshwater farming fisheries sustained a positive growth over the years compared to marine fisheries supplying the basic nutritional needs for both developed and developing countries (Subasinghe *et al.*, 2009) to

satisfying livelihood demands for good quality of fish meal contain with reach sources of fat and animal protein. In addition to that, fisheries have supplied fresh fish meal and shellfish to global markets, and there is a collective opportunity for expanding value-added fish and fish by products ((Kaushik *et al.*, 2004). However, a

number of carp species, indigenous to the subcontinent, are available throughout India, which are the major protein sources and contain nutritionally valuable lipids and fatty acids (Mohanty *et al.*, 2019). Further studies now comprise certain aspects on the enhancement of nutritional quality of some freshwater fishes under control and experimental feeding fishes in India. Although fish are considered as a rich animal source of polyunsaturated fatty acids (PUFAs), namely, Eicosapentacenoic acid (EPA; C20:5n3), Docosahexaenoic acid (DHA; C22:6n3), along with that, arachidonic acid (AA) and other PUFAs of the n-6 series, are precursor of biologically important food stuffs, such as epoxides, AA-ethanolamide, considered to be the most beneficial in terms of human health (Galli and Marangoni 1997). Catla (*Gibelion catla*, Hamilton 1822) and Mrigal (*Cirrhinus mrigala*, Hamilton 1822), the most common two freshwater preferred carps among Indian consumers and cultured extensively across the Indian subcontinent (Misra and Samantaray 2004), mostly in culture ponds. The majority of Catla and Mrigal, marketed commercially as the product of fish farming, although a main capture fishery is active on the Ganga River and its tributaries (Dey *et al.*, 2020) also contribute the bulk of fish farm production, with over 2.1 million tonnes (FAO 2018). Yet, the taste and nutritional quality of the fish meat, such as the fatty acid composition, varies with the seasonally and feeding of value added fish feeds (Ackman *et al.*, 2002). The present study was an attempt to a comparative study of the fatty acid profiles between conventional control feeding and feeding by value added food supplements as flaxseeds in experimental cultured carps of the two major commercial carp like *Catla* and *Mrigal* species in India.

MATERIALS AND METHODS

Experimental design and diets

To complete the present research work, an experimental set-up comprised with 03 (Three) rectangular fibreglass aquarium where 3 control and 3 for experimental, 60 number of two Indian Major Carps (IMC) species like *Gibelion catla* (Hamilton, 1822) and *Cirrhinus mrigala* (Hamilton, 1822) fish species were taken (followed by the proper guideline and permission of the institutional ethical committee) for monoculture in each water tank (with same size and age), each measuring 100 x 60 x 50 cm with a provision for controlling temperature through thermostats, photoperiod for 180 days with automated system and water flow through a digital meter for a continuous flow. Water parameters like pH, salinity, Dissolve Oxygen (DO), BOD are maintained in prescribed range for carp culture throughout the experimental period by using digital water parameter kit (Systronic 375).

The present evaluated the suitability of value added fish feed and to comparative analysis of incorporated fatty

acids (feed developed by different fatty acids enrich ingredients, specifically flaxseed oil) with the comparison of normal diet related to fish feed. To the development of EPA and DHA in the trial/experimental species used flaxseeds as the main ingredients with the normal fish feed diet.

Evaluation of growth parameters

Length measurements were detailed as total length (TL in cm) from the end part of the mouth to the end of the caudal fin measured to the nearest 0.1 cm by using a regular ruler validated against a Vernier calliper. Weight was measured using a digital balance with an accuracy of 0.01. Regression of the log transformed equation: $\log(W) = \log(a) + b \log(L)$, the parameters a and b were calculated with 'a' representing the intercept and 'b' the slope of the relationship. When b is less than 3, fish become slimmer with increasing length, and growth will be negatively allometric. When b is greater than 3.0, fish become heavier, showing positive allometric growth and reflecting optimum conditions for growth. It should be noted that the stomachs of fish were not emptied before weighing. To investigate the LWRs data, ANOVA was used to evaluate the statistical significance of the regression model detected when $P < 0.05$. The coefficient of determination (r^2) is a measure of the quality of a linear regression's prediction (a value close to 1 means a better model) (Chini, *et al.*, 2019).

Extraction of lipids and preparation of fatty acid methyl esters (FAME)

Total lipid was extracted following the Folch *et al.* (1957) method. Evaporation of solvent was done under nitrogen stream and residues are weighed to quantify the amount of lipid extracted. The lipid residue was 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 needed. The FAME's were prepared from the isolated lipids by the AOAC (1990) method.

Gas chromatography

GC/MS analysis of fatty acids profile derived from trial and control cultured sample specimen (*Catla* species and *Mrigal* species) were completed on a Shimadzu QP2010 quadrupole mass spectrometer with ionization 70 eV energy operating in positive electronic where impact set within 100 μ A, coupled with gas chromatography (Shimadzu GC 8060) equipped with a Carbowax (25 m \times 0.25 mm; 0.25- μ m film thickness) column with helium as the carrier gas.

RESULTS AND DISCUSSION

During the research period to the development of fatty acids level in trial fish specimen's muscle tissue, the

Table 1. Feed formulation of control diet and experimental diet (% of dry matter; except moisture). This formulation was followed by feed formulation of ASA 32/6 Of American Soybean Association International Marketing (FAO, 2003). This formulation is specifically dedicated for the IMC culture.

Sl. No.	Dietary components	Inclusion rate of Control Diet (%)	Inclusion rate of Experimental Diet (%)
1.	Soybean meal, 47.5% CP*	54.98	52.00
2.	Wheat flour, 11.7% CP*	30.20	28.20
3.	Corn gluten meal, 60% CP*	6.50	6.50
4.	Rice bran, 15% CP*	5.00	5.00
5.	Crude flax seed oil	NA	4.58
6.	Crude soybean oil	NA	1.40
7.	Soy lecithin	1.00	1.00
8.	Vitamin premix	0.75	0.75
9.	Mineral premix	0.50	0.50
10.	Stay C 35%**	0.04	0.04
11.	Ethoxyquin 100%	0.03	0.03
	Total	100	100

experimental diet mixed with normal feed ingredients as soybean meal, wheat flour, corn gluten meal, rice barn meal with crude flax seed was used. Flax seeds only used in experimental feed formulation as 4.58% in 100 gm of formulated feeds (Table 1). After completion of 90 and 180 days of trial, the current research showed the positive allometric growth in both *G. catla* and *C. mrigala* in LWR pattern. The slope value (b) was calculated as 3.206 and 3.111 for 90 days and 180 days respectively for *G. catla*, as well as 3.183 and 3.104 calculated 90 days and 180 days respectively for *C. mrigala* (Table 2).

Although, to estimate the incorporation rate of fatty acids profile in two carp's species showed that deposition was remarkably increased day by day regular feeding of formulated feed staff rather than the normal feeds. The results also clearly indicated that mono saturated fatty acids (MUFA) increased in experimental *Catla* fish species' muscle 9.65% to 35.77% for the initial stage to first 90days culture period and 35.77% to 38.93% for the next 180days of culture. Whether, in *Mrigala* fish species muscle showed MUFA developed 9.48% in the initial stage to 32.25% for 90days culture and 38.93% in final stage at 180days cultured period. However, feeding with conventional fish feed did not show such development of fatty acids profile in control culture of *Catla* and *Mrigal* fish species muscle. Accordingly, PUFAs like Linoleic Acid (C18:2n6), α - Linolenic Acid (C18:3n3), Eicosa-pentacenoic acid (EPA; C20:5n3), Docosahexaenoic acid (DHA; C22:6n3) and others were also well developed in experimental species rather than the control cultured species (Table 3 and 4).

The present investigation carried out the comparison of incorporation rates of fatty acids profile by conventional feeding and formulated feeding during the cul-

ture of 180 days experiments. Although, in carp culture, variations in the fatty acid composition might be related to changes in the nutritional habits of the fish (Guler *et al.*, 2008) and fat content was influenced by species, season, geographical regions, age and maturity (Osman *et al.*, 2001) (Dalbir *et al.* 2015). In the present study, variations in MUFA and PUFA content in the two carps might be attributed to the reason of their feeding habits as conventional and formulated (Roques, *et al.*, 2020). Our analyses of the proximate composition of cultured with conventional feed and value added fish feed revealed that value added feed cultured fish contained significantly higher lipid levels and a lower moisture content than its control feeding. It was found that Flaxseed (*Linum usitatissimum*) was one of the most important oilseed crops for industrial as well for food and feed purposes because of its exceptionally high content of α -linolenic acid (ALA, 18:3n-3) and lignans and considered traditional source of dietary omega-3 fatty acids while flaxseed oil could be used as a vegetarian source of dietary omega-3 fatty acids (Singh *et al.*, 2011). Thus, current research dealt with flax seeds as a value added supplements to enhance the fatty acids level in cultured fish muscle. However, without value added control feeding cultured fishes, there was no found such development of poly unsaturated fatty acids in fish muscle tissue. In addition to that, the EPA and DHA incorporation level was significantly increased with 4.28% to 5.16% in *Catla* fish species and 2.24% to 3.74% in *Mrigal* during 90days of cultured periods by the value added feeding. The results also explained that the development rate of fatty acids was higher in 90days of culture than next 180days (Fig. 1a and 1b). However, the most imperative finding of the present study was the distinct variance between controlled and experi-

Table 2. Length-Weight relation of total cultured species of fishes.

Scientific name	Culture period	No. of sample (n)	Length (L) (cm)		Weight (W) (gm)		Length Weight Relationship (LWR)		
			Max	Min	Max	Min	b	a	r ²
<i>Gibelion catla</i> (Hamilton, 1822)	90	40	28.1	24.4	436.80	280.23	3.206	0.0095	0.945
	180	30	33.8	31.3	649.94	537.01	3.111	0.0114	0.93
<i>Cirrhinus mrigala</i> (Hamilton, 1822)	90	40	31.9	28.0	388.76	253.44	3.183	0.0060	0.954
	180	30	37.8	35.2	599.01	500.05	3.104	0.0076	0.936

Table 3. Fatty acids deposition rate of *Catla* and *Mrigala* fish during 90 days culture period.

Fatty Acids	Initial		90 days			
	<i>G. catla</i>	<i>C. mrigala</i>	<i>G. catla</i>		<i>C. mrigala</i>	
			Control	Experiment	Control	Experiment
Myristic acid (C _{14:0})	3.49 ^a ±0.23	4.60 ^a ±0.42	7.48 ^b ±0.20	6.13 ^b ±0.11	5.69 ^b ±0.32	4.71 ^b ±0.23
Pentadecanoic acid (C _{15:0})	ND	3.72 ^a ±0.09	2.25 ^b ±0.09	2.15 ^b ±0.06	2.10 ^a ±0.13	2.11 ^a ±0.03
Palmitic acid (C _{16:0})	30.18 ^c ±0.51	12.84 ^b ±0.69	ND	1.61±0.03	31.28 ^c ±0.61	33.28 ^c ±0.52
Heptadecanoic acid (C _{17:0})	ND	ND	3.80±0.12	2.35±0.02	1.89±0.04	1.09±0.05
Stearic acid (C _{18:0})	12.41 ^b ±0.18	ND	17.02 ^c ±0.18	15.02 ^c ±0.07	ND	0.03±0.13
Arachidic acid (C _{20:0})	ND	0.12 ^a ±0.01	ND	0.03±0.01	0.45 ^a ±0.13	0.46 ^a ±0.07
Heneicosanoic acid (C _{21:0})	1.55 ^a ±0.19	4.73 ^b ±0.28	8.31 ^b ±0.07	8.01 ^b ±0.005	3.21 ^a ±0.03	3.62 ^a ±0.12
Tricosanoic acid (C _{23:0})	ND	ND	ND	ND	1.16 ^c ±0.01	1.06 ^c ±0.03
Others	1.62±0.31	1.02±0.17	2.73±0.40	2.61±0.33	1.49±0.07	1.89±0.01
Σ SFA	49.18 ^d ±1.56	27.31 ^a ±1.65	41.56 ^c ±0.04	39.17±0.02	48.93 ^b ±0.34	52.95 ^b ±0.06
Pentadecenoic acid (C _{15:1})	ND	ND	ND	0.07 ^a ±0.02	ND	0.07±0.19
Palmitoleic acid (C _{16:1n7})	1.48 ^a ±0.17	2.48 ^a ±0.17	0.18 ^a ±0.02	1.21 ^a ±0.07	23.89 ^b ±0.25	34.09 ^b ±0.05
Oleic Acid (C _{18:1n9})	6.15 ^b ±0.73	4.13 ^c ±0.56	0.68 ^a ±0.05	0.38 ^a ±0.02	5.42 ^b ±0.99	6.76 ^b ±0.09
Eicosenoic acid (C _{20:1n9})	ND	2.74 ^b ±0.55	28.24 ^a ±0.40	30.04 ^a ±0.85	3.49b±0.49	3.98b±0.39
Erucic acid (C _{22:1n9})	ND	1.89 ^b ±0.15	ND	0.003±0.01	3.60 ^c ±0.14	4.05±0.12
Others	0.90±0.08	0.73±0.06	3.17±0.18	3.07±0.15	0.48±0.10	0.28±0.17
Σ MUFA	09.65 ^b ±1.01	09.48 ^d ±1.21	32.25 ^a ±0.45	35.77 ^a ±0.45	37.87 ^c ±0.21	38.93 ^c ±0.15
Linoleic Acid (C _{18:2n6})	0.34±0.17	0.974 ^c ±1.27	8.99 ^b ±0.64	10.09 ^b ±0.61	ND	0.84±0.07
α- Linolenic Acid (C _{18:3n3})	7.02 ^a ±0.11	4.34±0.17	5.53 ^b ±0.94	6.13 ^b ±0.85	3.47±0.57	4.17±0.73
Arachidonic acid (C _{20:4n6})	ND	0.28 ^a ±0.13	ND	ND	0.31 ^a ±0.01	1.25 ^a ±0.05
Eicosatrienoic acid (C _{20:3n6})	ND	ND	ND	ND	ND	0.13±0.03
Eicosapentaenoic acid (C _{20:5n3})	0.008 ^a ±0.11	0.019 ^a ±0.96	3.78 ^a ±0.24	4.08 ^a ±0.41	1.92 ^a ±0.07	2.02 ^a ±0.11
Docosahexaenoic acid (C _{22:6n3})	0.078 ^a ±0.30	ND	0.50 ^a ±0.03	0.98 ^a ±0.32	0.32±0.3	1.72±0.05
Others	0.97±0.43	1.23±0.61	2.86±0.35	2.86±0.51	1.80±0.14	1.18±0.19
Σ PUFA	09.23 ^a ±0.60	06.74 ^b ±2.89	19.36 ^b ±0.75	23.54 ^b ±0.75	10.21 ^a ±0.55	11.91 ^a ±0.47
Σ ω-3 PUFA	10.01 ^a ±0.49	5.16 ^a ±1.05	13.89 ^b ±0.87	14.09 ^b ±0.63	7.41 ^a ±0.74	8.41 ^a ±0.33
Σ ω-6 PUFA	1.24 ^a ±0.47	19.62 ^b ±2.08	13.74 ^d ±1.34	14.06 ^d ±0.57	2.41 ^a ±0.34	2.84 ^a ±0.41
ω-3: ω-6 PUFA	9.24±3.11	0.26 ^a ±0.03	1.0±0.02	1.56±0.02	3.10 ^b ±0.13	3.49 ^b ±0.03
EPA+DHA	0.096 ^a ±0.41	0.019 ^a ±0.96	4.28 ^a ±0.27	5.16 ^a ±0.27	2.24 ^b ±0.31	3.74 ^b ±0.37

mental cultured fish species in terms of fatty acids level in the muscle tissue: experimental fishes had higher levels of PUFAs comparatively than control feeding. Ackman et al. (2002) stated that incorporation of polar lipid levels reached 10.6% in Indian major carps feed-

ing with value added natural food, while Sharma et al. (2010) reported 8.51% in *Mrigal* and 14% in *Catla* carp species. Although a general trend towards improvement of standard living worldwide, human beings were attract-

Table 4. Fatty acids deposition rate of *Catla* and *Mrigala* fish during 180 days culture period.

Fatty Acids	180 days			
	<i>G. catla</i>		<i>C. mrigala</i>	
	Control	Experiment	Control	Experiment
Myristic acid (C _{14:0})	ND	ND	ND	0.51 ^b ±0.08
Pentadecanoic acid (C _{15:0})	0.01 ^a ±0.02	0.31 ^a ±0.01	6.44 ^b ±0.10	7.19 ^b ±0.005
Palmitic acid (C _{16:0})	0.04 ^a ±0.02	0.09 ^a ±0.06	5.18 ^a ±0.07	7.08 ^a ±0.32
Heptadecanoic acid (C _{17:0})	ND	0.09±0.05	0.75 ^b ±0.03	0.95 ^b ±0.07
Stearic acid (C _{18:0})	14.16 ^b ±0.50	15.06 ^b ±0.16	3.37±0.38	4.07±0.81
Arachidic acid (C _{20:0})	ND	0.99 ^a ±0.05	5.70 ^c ±0.03	7.35 ^c ±0.11
Heneicosanoic acid (C _{21:0})	0.99 ^a ±0.05	1.02 ^a ±0.01	ND	0.41 ^b ±0.17
Tricosanoic acid (C _{23:0})	ND	ND	0.60 ^b ±0.08	0.54 ^b ±0.14
Others	5.31±0.40	4.91±0.70	1.25±0.02	2.05±0.12
Σ SFA	20.84 ^a ±0.43	20.84 ^a ±0.59	28.35 ^a ±0.75	30.15 ^a ±0.48
Pentadecenoic acid (C _{15:1})	ND	0.32 ^a ±0.03	ND	0.95 ^c ±0.10
Palmitoleic acid (C _{16:1n7})	2.62 ^b ±0.21	3.82 ^b ±0.11	24.75 ^c ±0.20	35.95 ^c ±0.10
Oleic Acid (C _{18:1n9})	38.84 ^c ±0.15	39.54 ^c ±0.58	7.08 ^a ±0.84	8.37 ^a ±0.04
Eicosenoic acid (C _{20:1n9})	ND	ND	0.07 ^a ±0.04	1.03 ^a ±0.01
Erucic acid (C _{22:1n9})	ND	0.007±0.03	0.009 ^a ±0.005	0.007 ^a ±0.002
Others	1.04±0.14	1.47±0.04	2.07±0.64	3.01±0.07
Σ MUFA	46.01 ^c ±0.41	48.22 ^c ±0.52	26.01 ^b ±1.00	27.21 ^b ±1.00
Linoleic Acid (C _{18:2n6})	5.52 ^b ±0.12	6.02 ^b ±0.02	09.55±0.04	09.55±0.04
α- Linolenic Acid (C _{18:3n3})	10.84 ^c ±0.06	13.14 ^c ±0.07	2.34±0.67	2.34±0.67
Arachidonic acid (C _{20:4n6})	ND	ND	15.08 ^b ±0.51	15.08 ^b ±0.51
Eicosatrienoic acid (C _{20:3n6})	ND	1.02±0.04	ND	1.13 ^a ±0.01
Eicosapentaenoic acid (C _{20:5n3})	3.14 ^b ±0.18	4.14 ^b ±0.11	1.18 ^a ±0.10	1.98 ^a ±0.16
Docosahexaenoic acid (C _{22:6n3})	1.02±0.14	2.07±0.15	1.32±0.25	2.52±0.31
Others	2.54±0.44	2.59±0.14	6.01±0.62	5.91±0.71
Σ PUFA	31.74 ^b ±0.07	32.98 ^b ±0.31	35.15 ^c ±0.75	38.41 ^c ±1.06
Σ ω-3 PUFA	15.09 ^c ±0.08	20.09 ^c ±0.44	5.02 ^a ±0.87	7.02 ^a ±0.09
Σ ω-6 PUFA	4.18 ^c ±0.46	7.11 ^c ±0.51	28.14±1.11	30.14±1.13
ω-3: ω-6 PUFA	1.12±0.07	2.17±0.32	0.20 ^a ±0.02	0.40 ^a ±0.05
EPA+DHA	4.16 ^b ±0.36	6.21 ^b ±0.36	2.50 ^a ±0.11	3.50 ^a ±0.46

ing more and more health conscious, preferring to ingest nutritious food added with some health benefits. Fish enrichment in PUFAs had numerous health benefits, as PUFAs played a vital role in mitigating anti-atherosclerotic, cardioprotective, antithrombotic, and anti-arrhythmic disease, along with the improvement of normal eye and brain development in the human body (Jakhar *et al.*, 2012). However, based on our current study results, the variation in the fatty acid profiles of value added feeding culture *catla* and *mrigala* fish species might be attributed primarily to the dietary intake of the fish rather than controlled feeding species.

Conclusion

The present investigation showed that dietary feed supplements regulated and enhanced the incorporation rate of PUFAs in the two common IMCs (*Catla* and *Mrigala*). Using flaxseeds as a value added fish feed diet confirms better growth performance and efficiency of feed utilization. Thus, the nutritional value of cultured species can be improved by integrating desired fatty acids, such as linoleic acid, linolenic acid, EPA, and DHA, into the value added feed. A well-designed comparative present research work of lipid and fatty acids

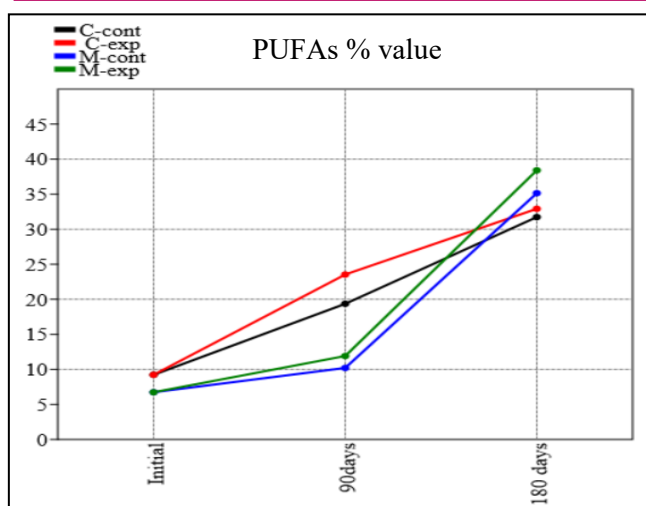


Fig.1a. Comparative analysis of PUFAs in Catla and Mrigala fish muscle content during control and experimental culture period.

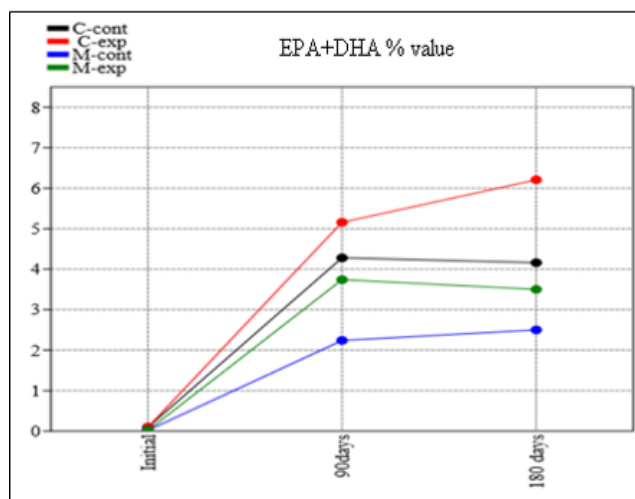


Fig.1b. Comparative analysis of EPA and DHA in Catla and Mrigala fish muscle content during control and experimental culture period.

of the two carp species of fish needs to be carried out in the future diet formulation.

Conflict of interest

The authors declare that they have no conflict of interest.

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