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A PRELIMINARY STUDY TO EVALUATE THE IMPACT OF SUPPLEMENTARY FEEDS ON THE FATTY ACID PROFILE OF TWO SIZE GROUPS OF INDIAN MAJOR CARPS IN SEMI-INTENSIVE CULTURE SYSTEM

Noor Khan[1,*], Naureen Aziz Qureshi[2], Yvan Chouinard[3] and Grant William Vandenberg[3]

[1]Department of Fisheries & Aquaculture, University of Veterinary and Animal Sciences, Lahore, P.O. BOX. 54000, Pakistan; [2]Government College for Women University, Faisalabad, Pakistan; [3]Department des sciences animales, Universite Laval, Quebec, QC, G1V 0A6, Canada.
[*]Corresponding author's e-mail: noorkhanuvas@yahoo.com

Two different size groups (fingerling and grow-out) of Indian major carps (*Catla catla, Cirrhinus mrigala* and *Labeo rohita*) were evaluated for their fatty acid composition in monoculture and polyculture systems with two experimental feeds. All the treatment groups in each trial had one control and three replicates. The studies were conducted for 90 days in earthen ponds (0.03 ha) each and were fertilized with organic and inorganic fertilizers prior to stocking. Diets containing 42% protein (D1) and 35% protein (D2) were applied at 4% to fingerlings (D1) and 3% to grow-out fish (D2). The concentration of palmitic acid (C16:0) (30.5 g $100g^{-1}$), stearic acid (C18:0) (6.1 $g100g^{-1}$), palmitoleic acid (C16:1) (8.1g $100g^{-1}$), oleic acid (C18:1) (25.5 g $100g^{-1}$), and α- linolenic acid (C18:3 n -3) (4.6 g $100g^{-1}$) fatty acids, were found as dominant fatty acids in studied three species. In all three study trials non-significant differences were found between treated and control groups and among species for these fatty acids. The Linoleic acid (C18:2 n-6) (8.7 g 100 g^{-1}), was found significantly higher in treated (D1) than control (D0), while ratio of n-3/ n-6 fatty acids were significantly higher in control (D0) versus treated (D1). It has been concluded that diet (D1) exhibited significant increase in C18:2 n-6 and eicosatrienoic acid (C20:3 n-6) which stimulated significant increase in Σn-6PUFA in all the treated groups compared to controls.
Keywords: Fatty acid profile, Indian major carps, semi-intensive culture, supplementary feed

INTRODUCTION

Fish is one of the richest sources of polyunsaturated fatty acids essential for human diet in addition to, providing an excellent source of protein, vitamins and minerals. A number of studies are available on lipid and fatty acid profile of fish especially on n-3 and n-6 polyunsaturated fatty acids, due to their vital role in human health related disorders (Gustafsson *et al.,* 1992; Jabeen and Chaudhry, 2011). The importance of n-3 highly unsaturated fatty acids (HUFA) have been well recognized due to their anti-arrhythmic, anti-thrombotic, hypolipidemic, anti-inflammatory and vasodilatory properties (Simopoulos, 2002; Simopoulos, 2007). Fish is one of the best sources for n-3 highly unsaturated fatty acids (HUFA) that is not synthesized in human body yet essential in diets (De Deckere *et al.,* 1998; Simopoulos, 2003; 2008; Karalazos, 2007).

The freshwater fish species serves a valuable source of essential fatty acids that contain higher levels of C18 PUFA with a considerable amount of Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and higher proportion of n-6 PUFA with a much lower ratio of n-3/ n-6 than marine fish (Steffens, 1997). Whereas, most of the fatty acids extracted from the carps in 2+ age group are either oleic - linoleic - palmitic or oleic-palmitic-linoleic (Hadjinikolova, 2004).

The variation in fatty acid profile of fish within and between species can be attributed to difference in their natural and artificial diets (Puustinen *et al.,* 1985; Henderson and Tocher, 1987; Ahlgren *et al.,* 1996). The fish being the dietary source of n-3 fatty acids do not synthesize them *de novo* but obtain them from the algal or microalgal plankton in their diets. Phytoplankton, considered as a base of food web, control the lipid quality of fish species directly in herbivorous and indirectly in omnivorous-carnivorous species (Ahlgren *et al.,* 1996).

In case of artificial feed, lipids and proteins are two major organic components of fish and gain considerable importance in artificial or supplementary diets. Lipids are very effective in artificial diet that initiates fat and carbohydrates retention (Zeitler *et al.,* 1984; Viola *et al.,* 1988; Schwarz *et al.,* 1988). Hence, lipids and their constituent fatty acids along with their other metabolic derivatives, such as the eicosanoids, play an important role in growth, health, and reproduction of an organism (Sargent *et al.,* 2002; Tocher, 2003). The nutritive lipid sources of animal and plant origin used in fish diets stimulate their growth, absorption of protein, and the retention of fats and

protein. The fat deposition and increase in flesh has been directly related with size and age of carp (Viola *et al.,* 1988). The level of lipids in carp meat and their modification depends both on the quantity and type of lipids used (Viola *et al.,* 1988). Hence, artificial and supplementary feed, feeding rate and supplementation of deficient diets increase not only growth rate but also increase the fat contents of fish (Shcherbina and Griyayev, 1990; Shemino and Shikata, 1993).

Indian major carps are most popular cuisine fishes in South Asia and are mostly cultured in semi-intensive system with varying degrees of supplementary diets. Information of fatty acid profile of these species is required because of changing food habits and availability of value added products in the country. At present there is only one study showing composition and fatty acid profile in few fresh water fish species *Cyprinus carpio, L. rohita* and *Oreochromis mossambicus* from Indus River, Pakistan (Jabeen and Chaudhry, 2011). It is, therefore, hypothesized that provision of artificial feeding with different protein content to Indian major carps (fingerling and grow-out fish) in semi-intensive system might variably impact fatty acid composition in mono- and poly-culture feeding systems.

MATERIALS AND METHODS

***Experimental site, fish species and study design*:** Current study was conducted at Research and Training Facilities, Department of Fisheries & Aquaculture, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki, Pakistan. The fingerlings were procured from Chenawan, Fish Hatchery, Government of the Punjab Fisheries Department, Pakistan, while the grow-outs used were reared in University ponds. Prior to stocking of fish, ponds were fertilized with cattle dung at 90 kg pond^{-1} (3 ton ha^{-1}) and poultry manure at 45 kg (50% of cattle dung along with 2.5 kg single super phosphate and 1.25 kg urea, one week prior to stocking for the production of planktonic life and followed by fortnightly application of same amount for the entire study period. Three separate trials one on fingerling (20-25g) (Trial I) and other two on grow-out fish (350-400g) in monoculture (Trial II) and polyculture (Trial III) were conducted in earthen ponds with an area of 0.03 ha each for 90 days. The experiments were designed following completely randomized design (CRD). Trial I and II were conducted in 12 earthen ponds with one control and three replicates of each species in monoculture system, while trial III was conducted in four ponds with one control and three replicates in polyculture system. The fish were stocked at 80 in trial I, 70 in trial II and 100/ pond in trial III, respectively. At the time of stocking all the fish were individually weighed (g) and measured (mm). Random sample of 20 fishes were collected from each pond after every 2 weeks through drag net for morphometric measurements and then released back in to their respective ponds, throughout the study period. On termination of each trial five fish was randomly collected from each pond for whole body proximate and fatty acids analysis.

***Procurement of feed ingredients and preparation of supplementary feed*:** The ingredients were purchased from the local market, Bukshi traders, Chowburgi Chwok, Lahore, Pakistan. All the ingredients were fine ground and mixed with mechanical mixer and then passed through extruder at 140°C to prepare a mash feed at National Feed Mill, District Sheikhupura, Pakistan. The crumbles were broken into mash and after drying the feed was sacked and stored properly at the experimental site. Fish were hand fed at 8:00 and 17:00 hours (Javed and Sial, 1991) at 4% body weight to fingerlings in trial I and 3% to grow-out in trials II and III. Amount of feed was readjusted after every 2 weeks sampling of morphometric growth measurements. Detail of ingredient composition, proximate composition and fatty acid profile of artificial feeds are given in Tables 1, 2 and 3. D1 represents 42% crude protein feed used for fingerlings trail I, D2 represents 35% crude protein feed used to fed grow-outs both in trials II and III while D0 natural feed comprised mostly phytoplankton and zooplankton with no artificial feed.

Table 1. Ingredient proportions of formulated diets for *Catla catla, Cirrhinus mrigala* and *Labeo rohita* fingerlings and grow-out fish.

Formulated diets	D1	D2
Components/ composition	Ingredients proportion (%)	Ingredients proportion (%)
Fish meal	20	25
Soya meal	30	32
Canola meal	-	15
Maize gluten	24	-
Wheat bran	5	5
Rice polish	3	5
Maize grains	8	8
Molasses	8	8
Mineral mixtures	1	1
Vitamins	1	1
Total	100	100

***Proximate analysis of feed*:** The proximate analysis of experimental feed, feed ingredients was performed for dry matter, crude protein, crude lipid, ash content, gross energy and crude fiber following (AOAC, 2003), whereas nitrogen free extract (NFE) was calculated according to subtraction. All the samples were dried in a vacuum oven (Model: 524 Precision Scientific, USA), at 105°C for 18 hours to determined the dry matter. Crude protein was determined through Kjeltec Auto analyzer Tecator 1030 (FOSS, Hoganas, Sweden) by digesting the sample at high temperature (415°C) in sulphuric acid (15 ml) in the

presence of potassium sulphate and copper sulphate (catalyst). Crude fat was determined through Soxtec System (Model: HT 1043 Extraction Unit Tecator, Hoganas, Sweden) using diethyl ether as solvent. The ash content was determined by incinerating approximately 1 g of the samples in a muffle furnace (Thermolyne, Dubuque, Iowa, USA) at 550°C overnight. The crude fiber content of each feed and feed ingredients was determined by digesting dry sample with 1.25% H_2SO_4, followed by 1.25% NaOH in Ankom Fiber Analyzer (Ankom 200/220, Model: A200, Macedon, NY, USA). Bomb calorimeter (Parr 6300 Calorimeter, Moline, IL, USA) using benzoic acid as a standard was used to determine gross energy. The ingredients proportion, proximate and fatty acid composition of experimental diets (D1 and D2) are given in Tables 1-3.

Table 2. Proximate composition of experimental diets (D1 and D2) used for fingerlings and grow-out fish under monoculture and polyculture system (all values are on % dry weight basis).

Percentage Composition	Diets	
	D1	D2
Dry matter (%)	92.07	89.55
Crude protein (%)	42.34	35.21
Crude lipid (ether extract) (%)	5.52	4.02
Ash (%)	14.08	16.9
Crude fiber (%)	3.89	7.35
Nitrogen Free extract (%)	26.24	36.52
Gross energy (MJg^{-1})	19.33	17.97

The values for proximate composition represent mean of two determinations.

***Preparation and purification of fatty acid methyl esters of fish feed and ingredients*:** The fatty acid profile of fish feeds and ingredients were determined as described by Sukhija and Palmquist (1988). Lipids were extracted by toluene and methanolic HCL. Fish samples were purified for fatty acid analysis was done by following Folch *et al.* (1957) as modified by Dryer (1970). The composition analysis of fatty acid methyl esters (FAMEs) was done with gas chromatography (Model: HP 5890 Series II, Hewlett Packard, Palo Alto, CA Mississauga, Ontario, Canada) equipped with a 100 m CP Sil 88 capillary column (i.d., 0.25 µm, film thickness, 0.20 µm, Chrompack, Middleburg, Netherlands) and a flame ionization detector. The column temperature was 80°C for 1 minute at the time of sample injection, then ramped at 2°C min^{-1} to 215°C and maintained for 30 minutes. Inlet temperatures was 220°C and detector temperatures was 230°C. Split ratio was 100:1. The flow rate for H_2 carrier gas was 1 mL/min. Most of the fatty acid peaks were indentified and quantified using either a quantitative mixture or pure methyl ester standards using a software programme Chem station and data processor Chem station.

Table 3. Fatty acid profile of the experimental diets (D1 and D2) (values are g/ 100 g of fatty acids).

Fatty acid profile	Diets	
	D1	D2
C12:0	0.13	0.29
C14:0	2.16	3.17
C16:0	22.14	24.84
C18:0	6.27	9.25
C20:0	0.70	0.70
C22:0	0.54	0.41
ΣSFA[a]	31.96	38.65
C14:1c9	0.06	0.08
C16:1c9	1.55	2.47
C18:1c9	23.12	26.59
C18:1c11	1.50	2.48
C24:1c15	0.31	0.12
ΣMUFA[b]	26.55	31.74
C18:1 t 6-8	0.26	0.17
C18:1 t 9	0.08	0.59
C18:1 t 10	0.07	0.26
C18:1 t 11	0.19	0.56
C18:1 t 12	0.11	0.23
ΣMUTFA[c]	0.72	1.81
C18:3 n-3	1.93	2.02
C20:3 n-3	0.10	0.25
C22:5 n-3	0.93	0.79
C22:5 n-3	0.22	0.21
C22:6 n-3	1.56	1.08
Σ n-3PUFA[d]	4.74	4.35
C18:2 n-6	32.57	19.01
C18:3 n-6	0.06	0.03
C20:2 n-6	0.10	0.13
C20:3 n-6	0.04	0.06
20:4 n-6	0.34	0.32
C22:4 n-6	0.10	0.07
C22:5 n-6	0.14	0.11
Σ n-6PUFA[e]	33.34	19.74
ΣPUFA[f]	38.08	24.09
n-3/ n-6[g]	0.14	0.22

The values for fatty acid profile represent mean of two determinations; [a]ΣSFA = sum of saturated fatty acids; [b]ΣMUFA= sum of monounsaturated fatty acids; [c]ΣMUTFA= sum of monounsaturated trans fatty acids; [d]Σ n-3PUFA= sum of n-3polyunsaturated fatty acids; [e]Σ n-6PUFA= sum of n-6 polyunsaturated fatty acids; [f]ΣPUFA= sum of polyunsaturated fatty acids; [g] n-3 / n-6= ratio of n-3 / n-6fatty acids.

***Statistical analysis*:** The data was analyzed by using general linear model (GLM) procedure and the mixed procedure of SAS (2009) software version 9.2 (TSIM0), SAS Institute, Cary, NC, USA. Pair wise comparisons were made using Tukey adjustment. The normality and homogeneity of variance were verified and transformation was used if necessary. The results were accepted at the probability

value of 0.05 or less for significant difference. The results in the tables are presented as mean with pooled mean standard error (PSEM) throughout the text.

RESULTS

***Trial I fingerling monoculture*:** The fatty acid profile of post-trial fingerlings of *C. catla*, *C. mrigala* and *L. rohita* for experimental group (D1) and control (D0) are presented in Table 4. The experimental group was fed on artificially prepared diet while the control group received natural food from pond. Twenty four fatty acids were identified in all selected fish species in both the treatment groups. The concentration of C14:0 in the control group (D0) was observed significantly different ($P \leq 0.05$) between experimental and control group, while species showed non-significant differences. The concentration of dominating C16:0 and C18:0 acids indicated non-significant differences among species and diets.

The results revealed a significant difference ($P \leq 0.05$) among species for C22:0 and C24:0, while a non-significant difference ($P > 0.05$) was observed for C20:0 among species and between diets. In case of C22:0 all the three species showed significant differences ($P \leq 0.05$), whereas for C24:0 *C. mrigala* exhibited significant higher concentration ($P \leq 0.05$) than *L. rohita* and *C. catla* (Table 4). Non-significant ($P \geq 0.05$) differences were observed for sum of all the saturated fatty acids (ΣSFA) for all three fish species in D0

Table 4. Fatty acids composition of three fish species fingerlings fed 42% protein diet (D1) with control (D0) in monoculture system post treatment(values are g/ 100 g of fatty acids).

	Species and Diets										
	Catla catla		*Cirrhinus mrigala*		*Labeo rohita*				ANOVA *P values*		
Fatty acids	**D0**	**D1**	**D0**	**D1**	**D0**	**D1**	**SEMD0**	**SEMD1**	**species**	**diet**	**sp*diet**
C14:0	4.33^{a}	3.20^{b}	4.73^{a}	3.17^{b}	3.25^{a}	2.95^{b}	0.32	0.18	0.21	0.03	0.42
C16:0	32.44	30.78	40.59	37.19	31.33	30.75	2.77	1.60	0.16	0.58	0.94
C18:0	8.67	7.37	6.85	6.04	5.40	5.79	0.76	0.44	0.15	0.54	0.73
C20:0	0.61	0.46	0.62	0.42	0.38	0.39	0.05	0.03	0.13	0.09	0.36
C22:0	0.40^{a}	0.31^{a}	0.29^{b}	0.16^{b}	0.18^{b}	0.17^{b}	0.03	0.02	0.01	0.07	0.37
C24:0	0.11^{a}	0.10^{a}	0.20^{b}	0.15^{b}	0.13^{ab}	0.15^{ab}	0.01	0.01	0.02	0.46	0.19
ΣSFA	46.55	42.22	53.29	47.13	40.67	40.20	3.78	2.18	0.26	0.43	0.87
C16:1c-9	8.42	6.72	6.99	5.83	8.84	7.10	0.77	0.44	0.39	0.13	0.96
C18:1c-9	18.47	21.99	17.96	24.42	24.66	25.25	1.82	1.05	0.23	0.14	0.56
C18:1c-11	4.39^{a}	3.48^{a}	3.10^{b}	2.59^{b}	3.00^{ab}	3.10^{ab}	0.24	0.14	0.04	0.16	0.38
C20:1c-11	1.38^{a}	1.52^{a}	1.06^{b}	1.11^{b}	1.23^{ab}	1.10^{ab}	0.08	0.04	0.03	0.83	0.47
C22:1c-13	0.26	0.19	0.19	0.11	0.08	0.13	0.06	0.03	0.37	0.64	0.66
ΣMUFA	32.91	33.90	29.30	34.07	37.81	36.68	2.30	1.32	0.29	0.58	0.67
C18:3 n-3	6.87	5.06	5.99	4.51	5.84	6.13	0.71	0.41	0.72	0.27	0.56
C20:3 n-3	0.68	0.54	0.39	0.43	0.38	0.52	0.13	0.08	0.55	0.94	0.75
C22:5 n-3	0.94	1.06	1.14	0.80	1.59	1.31	0.23	0.14	0.34	0.56	0.75
C22:5 n-3	0.79	0.79	0.71	0.52	0.97	0.88	0.14	0.08	0.36	0.57	0.89
C22:6 n-3	1.19^{a}	1.49^{a}	1.53^{ab}	1.51^{ab}	2.25^{b}	2.23^{b}	0.20	0.11	0.04	0.71	0.80
Σ n-3PUFA	10.46	8.94	9.76	7.76	11.03	11.07	1.10	0.62	0.38	0.398	0.79
C18:2 n-6	6.49^{a}	11.27^{b}	4.87^{a}	8.28^{b}	6.73^{a}	8.53^{b}	0.87	0.50	0.25	0.02	0.52
C18:3 n-6	0.33	0.37	0.26	0.40	0.46	0.41	0.04	0.02	0.22	0.33	0.31
C20:2 n-6	0.34	0.54	0.26	0.30	0.29	0.35	0.05	0.03	0.11	0.11	0.45
C20:3 n-6	0.58^{a}	0.75^{b}	0.34^{c}	0.50^{d}	0.51^{ac}	0.51^{bd}	0.04	0.02	0.01	0.04	0.24
20:4 n-6	1.33	1.02	1.14	0.80	1.30	1.21	0.20	0.12	0.62	0.34	0.89
C22:2 n-6	0.60	0.63	0.35	0.32	0.58	0.45	0.09	0.05	0.16	0.69	0.84
C22:4 n-6	0.19	0.14	0.16	0.17	0.19	0.20	0.01	0.01	0.32	0.58	0.27
C22:5 n-6	0.22^{b}	0.22^{b}	0.27^{b}	0.26^{b}	0.42^{a}	0.38^{a}	0.03	0.02	0.01	0.59	0.91
Σ n-6PUFA	10.07^{a}	14.94^{b}	7.65^{a}	11.03^{b}	10.49^{a}	12.05^{b}	1.00	0.60	0.18	0.03	0.56
ΣPUFA	22.59	23.19	16.56	19.08	21.52	23.12	1.90	1.10	0.19	0.49	0.94
n-3/ n-6	1.04^{a}	0.60^{b}	1.28^{a}	0.72^{b}	1.05^{a}	0.92^{b}	0.09	0.05	0.35	0.01	0.30

D0= Control; D1= Diet 1; SEMD0= Pooled standard error of a mean of D0; SEMD1= Pooled standard error of a mean of D1;Means are of three replicates of D1 and one of D0 and the values in each represents means of two determinations; Figures with different superscripts are significantly different ($P \leq 0.05$).

Table 5. Fatty acids composition of three fish species grow-out post-treatment fed 35% protein diet (D2) and control (D0) in monoculture system (values are g/ 100 g of fatty acids).

	Species and Diets										
	Catla catla		*Cirrhinus mrigala*		*Labeo rohita*				ANOVA *P values*		
Fatty acids	**D0**	**D2**	**D0**	**D2**	**D0**	**D2**	**SEM D0**	**SEM D2**	**species**	**diet**	**sp*diet**
C14:0	2.83	2.94	2.74	2.88	2.96	2.46	0.30	0.20	0.93	0.83	0.76
C16:0	28.54	28.38	28.16	29.12	29.59	29.73	0.50	0.30	0.25	0.60	0.72
C18:0	7.17^{a}	7.20^{a}	4.39^{b}	4.36^{b}	5.16^{b}	6.34^{b}	0.30	0.20	0.00	0.32	0.38
C20:0	0.44	0.48	0.33	0.36	0.36	0.39	0.04	0.02	0.18	0.49	0.99
C22:0	0.27	0.27	0.15	0.12	0.17	0.17	0.04	0.02	0.14	0.79	0.96
C24:0	0.16^{a}	0.10^{b}	0.14^{a}	0.12^{b}	0.14^{a}	0.09^{b}	0.02	0.01	0.76	0.04	0.67
ΣSFA	39.41	39.36	35.90	36.95	38.36	39.17	0.84	0.49	0.10	0.56	0.89
C16:1c-9	6.77	6.78	6.95	7.97	9.02	6.61	0.88	0.51	0.71	0.66	0.42
C18:1c-9	23.86	27.24	29.00	30.38	27.01	26.09	2.00	1.20	0.39	0.61	0.77
C18:1c-11	4.17^{a}	3.48^{a}	3.34^{ab}	3.22^{ab}	3.37^{b}	3.14^{b}	0.13	0.08	0.04	0.06	0.33
C20:1c-11	1.61^{a}	1.65^{a}	1.47^{ab}	1.49^{ab}	1.20^{b}	1.14^{b}	0.09	0.05	0.03	1.00	0.92
C22:1c-13	0.11	0.14	0.08	0.08	0.06	0.05	0.03	0.02	0.40	0.89	0.92
ΣMUFA	36.52	39.29	40.84	43.14	40.65	37.03	1.61	0.93	0.25	0.80	0.36
C18:3 n-3	5.48	4.07	3.16	4.19	5.20	5.59	0.68	0.40	0.27	0.99	0.47
C20:3n-3	0.47	0.39	0.25	0.27	0.31	0.37	0.09	0.05	0.43	0.99	0.83
C20:5 n-3	1.31	1.07	0.98	0.99	1.62	1.60	0.28	0.16	0.36	0.81	0.95
C22:5 n-3	1.12	0.82	0.62	0.63	0.93	1.02	0.17	0.10	0.34	0.73	0.72
C22:6 n-3	1.90	1.63	2.30	1.71	2.11	2.62	0.29	0.17	0.39	0.74	0.43
Σ n-3PUFA	10.28	7.97	7.31	7.79	10.16	11.20	0.24	0.72	0.28	0.86	0.62
C18:2 n-6	10.05	9.96	11.35	9.05	7.56	8.77	0.73	0.42	0.18	0.66	0.30
C18:3 n-6	0.24^{b}	0.27^{b}	0.60^{a}	0.32^{b}	0.35^{ab}	0.46^{ab}	0.03	0.02	0.01	0.24	0.01
C20:2 n-6	0.44^{a}	0.46^{a}	0.52^{a}	0.35^{a}	0.28^{b}	0.34^{b}	0.02	0.01	0.01	0.35	0.03
C20:3 n-6	0.65^{b}	0.61^{b}	0.96^{a}	0.55^{bc}	0.42^{c}	0.55^{bc}	0.02	0.01	0.00	0.00	0.00
20:4 n-6	1.43	1.12	1.48	1.03	1.30	1.43	0.15	0.09	0.86	0.26	0.40
C22:2 n-6	0.47	0.53	0.28	0.34	0.40	0.46	0.11	0.07	0.52	0.67	0.99
C22:4 n-6	0.22^{ab}	0.17^{b}	0.36^{a}	0.22^{ab}	0.15^{b}	0.19^{b}	0.02	0.01	0.00	0.03	0.02
C22:5 n-6	0.29	0.26	0.40	0.26	0.35	0.41	0.04	0.03	0.30	0.47	0.36
Σ n-6PUFA	13.79	13.38	15.95	12.12	10.82	12.60	0.56	0.32	0.06	0.25	0.03
ΣPUFA	20.29	22.61	19.02	21.33	24.40	22.67	1.31	0.76	0.27	0.55	0.50
n-3/ n-6	0.75	0.60	0.46	0.65	0.94	0.90	0.11	0.06	0.14	1.00	0.58

D0= Control; D2= Diet 2; SEMD0= Pooled standard error of a mean of D0; SEMD2= Pooled standard error of a mean of D2. Means are of three replicates of D2 and one of D0 and the values in each represent means of two determinations. Figures with different superscripts are significantly different ($P \leq 0.05$).

and D1.

The monounsaturated fatty acids C18:1n-11 and C20:1n-11 showed significant differences ($P \leq 0.05$) among species. Comparison of means of *C. mrigala* showed significantly ($P \leq 0.05$) less concentration of C18:1n-11 as compared to *C. catla* and *L. rohita,* whereas in case of C20:1n-11 same trend was observed among species. The diets effect in all the monounsaturated fatty acids remained non-significantly different ($P \geq 0.05$). The sum of all the monounsaturated fatty acids (ΣMUFA) concentration in three fish species indicated non-significant differences among species and between diets.

Among n-3fatty acids, the C18:3n-3 was observed in largest concentration followed by C22:6n-3, C20:5n-3, C22:5 n-3 and C20:3n-3, in *C. catla*, *C. mrigala* and *L. rohita* under control (D0) and experimental unit (D1), respectively. The statistical analysis revealed non-significant differences ($P \geq 0.05$) for all the Σn-3PUFA, except for C22:6 n-3 which was found significantly different ($P \leq 0.05$). The comparison of means for C22:6 n-3 indicated that *L. rohita* possess significantly ($P \leq 0.05$) higher concentration than *C. catla,* while *L. rohita* and *C. mrigala* exhibited non-significant difference. The concentration of Σn-3PUFA indicated non-significant differences among species and between diets.

The results of n-6fatty acids revealed that C18:2 n-6 was found significantly different ($P \leq 0.05$) among diets (D0 and D1), whereas species showed non-significant difference regarding this acid. C20:3 n-6 was also found significantly different ($P \leq 0.05$) both among species and diets. C22:5 n-6 was observed significantly different ($P \leq 0.05$) among species, while diets effect remained non-significant. The concentration of Σn-6PUFA revealed a significant difference ($P \leq 0.05$) among diets, where experimental diet (D1) showed higher concentration than control (D0). Non-significant difference was observed among three fish species for Σn-6PUFA.

The sum of all the polyunsaturated fatty acid (ΣPUFA) illustrated non-significant differences among species and diets (D0 and D1). In conclusion the experimental diet (D1) significantly increased the concentration of C18:2n-6 which increased the concentration of C20:3 n-6 in the treated fish species and ultimately resulted in significantly higher concentration of Σn-6PUFA in the experimental group. The ratio of n-3 / n-6 was observed significantly different in treated and control group, where significantly higher concentration was observed in (D1) compared to (D0). However, the ratio among species was found non-significantly different.

***Trial II grow-out monoculture*:** Fatty acid profile of grow-out *C. catla*, *C. mrigala* and *L. rohita* for experimental group (D2) and control (D0) are presented in Table 5. A total of twenty four fatty acids were identified under both the treatments. The concentration of C14:0 and the dominating C16:0 revealed non-significant differences ($P > 0.05$) among species and between diets. C18:0 revealed significant difference ($P \leq 0.05$) among species, where *C. catla* was found significantly different from *C. mrigala* and *L. rohita*. The treatments effect was noticed non-significantly different. Lower levels were again observed for C20:0, C22:0 and C24:0 during this trial for all the species. The results showed non-significant differences ($P \geq 0.05$) among species for C20:0 and C22:0, whereas C24:0 showed significant ($P \leq 0.05$) difference among diets. The concentration of ΣSFA for three fish species under both diets (D0 and D2) indicated non-significant differences ($P \geq 0.05$) among species and between diets.

C18:1 n-9 occurred in higher concentration followed by C16:1 n-9, C18:1 n-11, C20:1 n-11and C22:1 n-13, in all the three fish species under both diets. Significant differences ($P \leq 0.05$) among these acids were shown only in C18:1 n-11 and C20:1 n-11 among species. The treatments effect in these monounsaturated fatty acids remained non-significant ($P \geq 0.05$). The concentration of ΣMUFA revealed non-significant differences ($P \geq 0.05$) among species and diets (Table 5).

Among n-3fatty acids, the C18:3 n-3 was found in higher concentration in *C. catla, C. mrigala* and *L. rohita* under control (D0) and experimental unit (D2), respectively (Table 5). The results revealed non-significant differences ($P \geq 0.05$) for all the Σn-3PUFA. The concentration of Σn-3PUFA in all the three species and treatment groups showed non-significantly different ($P \geq 0.05$) among species and diets.

The results of different n-6 fatty acids in all the three fish species and dietary groups (D0 and D2) revealed that C18:2 n-6 was found in higher concentration (Table 5). Statistically C18:3 n-6, and C20:2 n-6 were found significantly different ($P \leq 0.05$) among species and in the interaction effect of (species x diets). The interaction effect showed that *C. mrigala* in control (D0) showed significantly higher ($P \leq 0.05$) concentration than treated (D2) as well as from other species under both the diets. The C20:3 n-6, and C22:4 n-6, were also found significantly different ($P \leq 0.05$) among species, diets and in interaction (species x diets). The concentration of Σω-6PUFA revealed non-significant difference ($P \geq 0.05$) between diets and among species.

The sum of all the polyunsaturated fatty acid (ΣPUFA) showed non-significant differences among species and diets. The ratio of n-3 / n-6 was observed non-significantly different among species and between diets.

***Trial III grow-out polyculture*:** The results of fatty acid profile of grow-out fish in polyculture system of the same species both for experimental group (D2) and control (D0) are presented in Table 6. Again total of twenty four fatty acids were identified under both the dietary groups in three fish species. The concentration of C14:0 and C16:0 were found non-significantly different among species and between diets. Significant ($P \leq 0.05$) difference was observed for C18:0 among species where *C. catla* and *L. rohita* were significantly different ($P \leq 0.05$) from *C. mrigala*. The diets effect was observed non-significant. The C20:0, C22:0 and C24:0 were again observed in lower concentration in all the species as per above two trials. The C22:0 and C24:0 were observed non-significantly different ($P \geq 0.05$) among species and diets, whereas C20:0 was observed significantly different ($P \leq 0.05$) among diets and experimental diet (D2) showed higher concentration of this acid than control (D0) while species were found non-significantly different. The concentration of ΣSFA for three fish species under both the diets (D0 and D2) indicated non-significantly different ($P \geq 0.05$) concentration among species and diets.

Among monounsaturated fatty acids, C18:1 n-9 occurred in higher concentration followed by C16:1 n-9, C18:1 n-11, C20:1 n-11and C22:1 n-13, in all the three fish species under both the dietary groups. Significant differences among these acids were observed only in C16:1 n-9 among species where *C. mrigala* and *L. rohita* were found significantly different ($P \leq 0.05$) from *C. catla.* The C18:1 n-11 was also found significantly different ($P \leq 0.05$) between diets (D0 and D2) where D2 was found significantly higher ($P \leq 0.05$) than D0. Other acids of this group did not show significant

Table 6. Fatty acids composition of three fish species grow-out post-treatment fed 35% protein diet (D2) and control (D0) in polyculture system(values are g /100 g of fatty acids).

	Species and Diets										
	Catla catla		*Cirrhinus mrigala*		*Labeo rohita*				ANOVA *P values*		
Fatty acids	**D0**	**D2**	**D0**	**D2**	**D0**	**D2**	**SEM D0**	**SEM D2**	**species**	**diet**	**sp*diet**
C14:0	2.22	2.62	2.74	3.22	2.88	3.08	0.25	0.15	0.11	0.35	0.83
C16:0	27.36	27.39	29.73	28.63	28.61	31.53	0.90	0.52	0.46	0.61	0.62
C18:0	6.24^{a}	7.41^{a}	4.41^{b}	4.28^{b}	5.21^{a}	6.90^{a}	0.31	0.18	0.02	0.13	0.29
C20:0	0.37^{a}	0.51^{b}	0.31^{a}	0.38^{b}	0.35^{a}	0.45^{b}	0.02	0.01	0.50	0.05	0.89
C22:0	0.16	0.30	0.10	0.18	0.13	0.19	0.03	0.02	0.44	0.09	0.85
C24:0	0.07	0.08	0.12	0.10	0.09	0.09	0.04	0.02	0.14	1.00	0.74
ΣSFA	36.43	38.31	37.41	36.79	37.27	42.24	1.44	0.83	0.42	0.34	0.46
C16:1c-9	6.84^{a}	8.28^{a}	11.38^{b}	12.44^{b}	10.00^{ab}	9.16^{ab}	0.51	0.30	0.04	0.45	0.57
C18:1c-9	31.39	24.16	31.48	25.01	27.04	22.77	1.97	1.14	0.47	0.12	0.85
C18:1c-11	3.81^{a}	4.89^{b}	3.42^{a}	4.00^{b}	3.56^{a}	3.85^{b}	0.08	0.05	0.47	0.02	0.78
C20:1c-11	1.59	1.23	1.30	1.04	1.04	1.04	0.09	0.05	0.50	0.20	0.83
C22:1c-13	0.09	0.10	0.04	0.07	0.05	0.07	0.01	0.01	0.26	0.32	0.94
ΣMUFA	43.72	38.66	47.62	42.55	41.70	36.89	2.49	1.44	0.18	0.23	0.99
C18:3 n-3	4.07	3.83	2.46	2.47	3.73	3.56	0.42	0.24	0.12	0.81	0.97
C20:3 n-3	0.27	0.29	0.10	0.16	0.18	0.20	0.05	0.03	0.12	0.63	0.92
C20:5 n-3	0.92^{a}	1.83^{b}	1.43^{a}	2.54^{b}	2.07^{a}	1.16^{b}	0.06	0.04	0.61	0.04	0.28
C22:5 n-3	0.60^{a}	1.06^{b}	0.72^{a}	1.14^{b}	0.84^{a}	0.67^{b}	0.01	0.01	0.82	0.01	0.50
C22:6 n-3	1.27	1.84	1.26	2.15	1.73	1.75	0.30	0.17	0.87	0.29	0.53
Σ n-3PUFA	7.13	8.84	5.98	8.46	8.55	7.32	0.64	0.37	0.87	0.31	0.54
C18:2 n-6	9.92^{a}	10.54^{a}	6.26^{b}	7.88^{b}	9.31^{a}	10.14^{a}	0.68	0.39	0.02	0.32	0.76
C18:3 n-6	0.26	0.37	0.24	0.37	0.40	0.47	0.09	0.05	0.69	0.44	0.97
C20:2 n-6	0.40	0.42	0.27	0.36	0.41	0.40	0.05	0.03	0.15	0.66	0.54
C20:3 n-6	0.59	0.55	0.39	0.51	0.46	0.50	0.06	0.04	0.15	0.62	0.43
20:4 n-6	0.86	1.37	1.16	2.03	1.07	1.18	0.15	0.09	0.53	0.11	0.72
C22:2 n-6	0.32	0.42	0.19	0.26	0.31	0.28	0.06	0.04	0.34	0.60	0.74
C22:4 n-6	0.15	0.21	0.23	0.35	0.19	0.23	0.02	0.01	0.36	0.09	0.84
C22:5 n-6	0.23	0.32	0.26	0.44	0.32	0.36	0.05	0.03	0.76	0.21	0.80
Σ n-6PUFA	12.73	14.19	8.99	12.20	12.48	13.55	0.90	0.52	0.22	0.21	0.75
ΣPUFA	19.85	23.03	14.97	20.66	21.03	20.87	1.53	0.89	0.46	0.24	0.62
n-3/ n-6	0.56	0.62	0.66	0.69	0.69	0.54	0.01	0.01	0.64	0.23	0.49

D0= Control; D2= Diet 2; SEMD0= Pooled standard error of a mean of D0; SEMD2= Pooled standard error of a mean of D2;Means are of three replicates of D2 and one of D0 and the values in each represent means of two determinations; Figures with different superscripts are significantly different ($P \leq 0.05$).

differences. The ΣMUFA concentration in three fish species under both the treatment (D0 and D2) revealed non-significant differences among species and diets.

The n-3fatty acids showed similar trend as in trial I and II. The statistical results illustrated that C22:5 n-3 and 22:5 n-3 were found significantly different ($P \leq 0$ .05) among diets (D0 and D2), while non-significant differences were observed among species (Table 6).

The results of different n-6 fatty acids in all selected fish species and treatment groups (D0 and D2) indicated that C18:2n-6 found in higher concentration in the present trial (Table 6). The statistical analysis of these acids revealed that C18:2 n-6 was found significantly higher in *L. rohita* and *C. catla* than *C. mrigala*.

DISCUSSION

Studying the fatty acid composition of any food product is utmost important due to composition, nutritional quality and oxidative stability of lipids. Among lipid classes the polyunsaturated fatty acids (PUFA) is considered to be one of the most important. Indian major carps (*C. catla, C. mrigala* and *L. rohita*) are popular and commercially important food fishes of South Asia. Very little information regarding fatty acid profile of these species is available in

the literature. It is generally considered that carps derive their lipid requirement from natural feed available in the pond as planktonic and other biotic communities rich in such compounds. The present work is the first attempt to study the fatty acid composition of Indian major carps with the application of supplementary feed in semi-intensive system. The most dominating fatty acids in all the trials and fish species during current study were C16:0, C18:0, C16:1, C18:1, C18: 3 n-3, C22:6 n-3, C18: 2 n-6 and C20:4 n-6. The results are in line with the findings of Jabeen and Chaudhry (2011) from Pakistan; Aggelousis and Lazos (1991) from Greece and Andrade *et al.* (1995) from Brazil who reported that dominant fatty acids in fresh water fish species are C16:0, C16:1, C18:1, C20:5 n-3, and C22:6 n-3 C16:0, C16:1, C18:1, C20:5 ω -3, and C22:6 ω-3. These dominating fatty acids are responsible for curing various diseases and disorders such as arachidonic acid (C20:4 n-6) act as a precursor for prostaglandin and thrombox-ane biosynthesis (Pompeia *et al.,* 2002) also help in blood clotting and wound healing (Rahman *et al.,* 1995). Some other studies also revealed that freshwater fishes are characterized by containing high levels of C16:0, C16:1 n-9 and C18:1 n-9 and n-6PUFA (Ahlgren *et al.,* 1994; Zenebe *et al.,* 1998; Kozlova and Khotimchhenko, 2000; Maazouzi *et al.,* 2010). Among monounsaturated fatty acids Oleic acid (C18:1) was found most dominating acid than others. Jabeen and Chaudhry (2011) also reported that Oleic acid was found as dominating monounsaturated fatty acid in freshwater species with 53%, 55.5% and 71.3%, in *L. rohita*, *O. mossambicus and C. carpio,* , respectively and is hypotensive role (reducing blood pressure) effects.

Viola *et al.* (1988) and Hadjinikolova (2004) illustrated that lipid level in carp meat and its modification depends on the type and quantity in the diet, whereas the increase in dietary lipid does not increase in the general lipid contents in carp. Henderson and Tochar (1987) reported that freshwater fish contain higher contents of n-6PUFA than in marine fishes which may be due to their prey comprising both terrestrial and aquatic insects along with benthic communities that are rich in such PUFA. The ratio of n-3 / n-6 in the present study was recorded as 0.6-1.28 in post-fingerlings stage, 0.46-0.94 in monoculture grow-out and 0.54-0.69 in grow-out polyculture which are concordance with the findings of Henderson and Tocher (1987), Ahlgren *et al.* (1994) and Abd. Rahaman *et al.* (1995) who reported that n-3 / n-6 ratio in freshwater fish species ranged from 0.5 and 3.8. Maazouzi *et al.* (2010) reported that in *L. gibbosus* the ratio of n-3 / n-6 did not exceed 3.1 and they tend to decrease with fish size.

Lipid content of fish body generally increased with the increase in fish size and increase in C18:1n-9 and MUFA with increase in fish size could be due to increased storage of lipid contents (Heermann *et al.,* 2009). In case of low food availability the retention or synthesis of these fatty acids in fish body may give energy stores for months (Abi-Ayad *et al.,* 2004).

During present investigation it was observed that supplementary diets had no significant effect on the total concentration of saturated, mono-unsaturated, and poly-unsaturated fatty acids of *C. catla, C. mrigala* and *L. rohita*. Jankowska *et al.* (2004) reported that in catfish, feed type had no significant impact on the combined share of saturated, unsaturated, MUFA or PUFA acids. The proportion of these acids in the fish fed natural or artificial feed was found similar, while share of the majority of the fatty acids within each group differed. Higher concentration of saturated, mono-unsaturated and n-3 fatty acids in three fish species under all the three trials was observed higher except n-6 PUFA and sum of polyunsaturated fatty acids versus their contents in the experimental feeds (D1 and D2). The higher concentration of saturated, monounsaturated fatty acids and presence of docosahexaenoic acids (DHA) and Eicosapentaenoic acid (EPA) with other PUFAs in all the three fish species exhibited their good nutritional profile and medicinal importance such as alleviating muscular pain, inflammation, preventing coronary artery disease in human (Leaf and Webber, 1988) as well as playing a vital role as anti-arrhythmic, anti-thrombotic, hypolipidemic, anti-inflamatory, autoimmune disorder and vasodilatory properties, (Hooper *et al.,* 2004; Simopoulos, 2002). The increased concentration of saturated, mono-unsaturated and n-3 fatty acids in this study might be due to their higher amount in the natural fish food. The fatty acid profile of phyto and zooplankton reported by Domaizon *et al.* (2000) and Guo *et al.* (2008) demonstrated a close relationship to the fatty acid profile of three fish species observed in this study. It may be explained by the fact that fish during present study consumed more fat from natural feed compared to experimental diets. Jankowska *et al.* (2004) reported that meat of catfish reared under artificial feed contained low level of 20:4 n-6, differed from catfish fed with natural food. In another study by Jankowska *et al.* (2003) for pikeperch, *Sander lucioperca* L. found that meat of wild fish contained more 20:4 n-6 than cultivated under artificial feed. Reason might be due to higher levels of this acid in natural food and fact that C18:2n-6 fatty acid was not transformed into its long chain derivative. The elongation and desaturation of n-6 fatty acids is effective when fish diet contain fewer amounts of n-3PUFA or due to competition between n-3 and n-6 fatty acids for the desaturation enzyme process (Henderson, 1996).

***Conclusion*:** It is concluded from the present investigation that artificial diets (D1 and D2) did not played any significant role on fatty acid profile except in trial I where significant increase has been recorded in C18:2n-6 and 20:3 n-6. The above mentioned increase stimulated significant increase in Σω-6PUFA in all the treated groups when

compared to controls. Therefore, the preliminary data of fatty acid profile of the Indian major carps presented here, which may be limited in scope, but important to establish baseline values for future studies.

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