Enhancement of brain booster fatty Acids in brain tissue of *Catla catla*

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Abstract: The growth of aquaculture need to face number of challenges, that challenges are achieved by nutritionally rich and over production using natural antibiotics, herbs and probiotics and live feed culture. The fruit pulp of *A. marmelos* and the leaves of *Spinacia oleracea* used to prepare a low cost protein and fatty acids rich diet for the fish *C. catla.* The present study carried for to have a clean knowledge about the fish fatty acids, SAFA, PUFA and MUFA in the brain tissues of the experimental *C. catla* after treatment with the prepared herbal diets and enriched diets and *L. sporogenes* treatment. The result showed that the increased good cholesterol ratio in the SAFA, PUFA and MUFA fatty acids in all groups. In that there are 17 different types of SAFA; 10 different PUFA and 9 different MUFA in the brain tissues. In control brain tissues, 63% is of SAFA, MUFA 25% and PUFA 12%. The group E has higher SAFA 976.76 mg/100g which are very much higher than the control. PUFA in brain tissues, EPA which is very low 5.72 in control group, has been raised to 98.25 in F group; 67.16 in G group; 70.19 in H group and 256.24 in J group. Among the three MUFA, the ω-9 takes approximately more than 85% of the part in batch – I & 45% of the part in batch - II; whereas ω-7 and ω-5 takes remaining percentage. Therefore formulating fish feed with *Aegle marmelos* and spinach leaves increased the PUFA/SAFA ratio and also would have altered the composition of fatty acids content in the brain tissues of *C. catla* variably.

Key words: Herbals; Probiotics; Brain fatty acids; Gas Chromatography.

1. Introduction

Aquaculture is one of the fast growing food producing sectors of the world and aims to increase productivity continuously per unit space. Many countries engage themselves to harness the potential of aquaculture for the social well being of their people, since it contribute to the food and nutrition security, to generate income and to alleviate from poverty. Generally, there are enzymes called desaturases which can convert SAFA to PUFA by introducing double bonds, in different animals and plants (Vance and Vance, 1985). A fatty acid desaturases enzyme removes two hydrogen atoms from a fatty acid creating double bond. Los *et al.* (1998) have said that fatty acid desaturases appear in all organisms including bacteria, fungus and plants. According to Bourre (2005) the fish like carp have the enzymes required to transform ALA into EPA and DHA. The *A. marmelos* seed have 27.1 (wt%) of linoleic acid (Bhalchadra *et al.,* 2013) and spinach leaves contain 44.5 (wt%) of linoleic acid (Narsing Rao *et al.,* 2015). There are evidence that artemia larvae have C20:4 n6 (ARA) PUFA in considerable (wt%) amount in the control (0.47 wt%) and enriched larvae (98.0 wt%) (Hafezieh *et al.*,2010). Therefore *L. sporogenes* also enhanced the conversion of SAFA and MUFA to PUFA in other groups. A good n-6/n-3 ratio value is an important dietetic parameter because it is the key factor for balanced eicosanoids synthesis in human. In wild and farmed sea bass lipid fraction showed a high proportion of n-3PUFA, specially DHA and

EPA (Mnari Bhouri *et al.*, 2010). Christine Williams (2000) evidenced that long chain omega 3 fatty acids EPA and DHA have beneficial effects on cardio vascular and anti inflammatory properties and their consumption level is insufficient in western diets. The beneficial actions of EPA/DHA on human health was shared possibly by the precursor of omega -3 PUFA, alpha linolenic acid(ALNA) which can be sourced from fish meat. Hence in the present study the fish *Catla catla*, herbals *Aegle marmelos* & *Spinacia oleracea*, probiotic *Lactobacillus sporogenes* and live feed *Artemia salina* is used.

2. Materials and methods

2.1. Selection of fish

Indian major carp *Catla catla* a fresh water teleost belonging to the family cyprinidae was selected for the present investigation. *Catla catla* is a eurythermal animal that grows better in water temperatures between 25–32º C. They are commercially important fish.

2.2. Selection of herbals

The fruit of *Aegle marmelos* possess high nutritional value. The pulp is reported to contain water, sugars, protein, fiber, fat, calcium, phosphorus, potassium, iron, minerals and vitamins (vitamin A, vitamin B, vitamin C, Riboflavin) (Rajan *et al.*, 2011). The different parts of Bael are used for various therapeutic purposes such as for the treatment of asthma, fractures, healing of wounds, swollen joints, high blood pressure, jaundice, diarrhea, and healthy mind (Malviya Rishabha *et al.*, 2012).

Spinacia oleracea has a high nutritional value. They have energy for 23 Kcal and is extremely rich in antioxidants, especially when fresh steamed, or quickly boiled. It is a rich source of Vitamin A (469 mg), Vitamin C (28 mg), Vitamin E (2 mg), Vitamin K (483 µg), Manganese 0.897 mg, folate 194 µg, beta-carotene, iron 2.71 mg, vitamin B2 0.189 mg, calcium 99 mg, potassium 558 mg, vitamin B6 0.195 mg, folic acid, copper, protein 2.9 gm, phosphorus 49 mg, zinc 0.53 mg, niacin 0.724 mg, and selenium.

2.3. Selection of probiotics

The use of probiotics in aquaculture is now widely accepted with an increasing demand for environment friendly aquaculture (Wang and Xu, 2006; Vine *et al*., 2006 Denev *et al*., 2009; Qi *et al*., 2009). According to Delcenserie *et al*. (2009), *L. sporogenes* maintains and promotes the effective functioning of intestines by producing lactic acid. It stimulates both cellular and humoral immune response (Baber *et al*., 2012). *Lactobacillus sporogenes* of trade name Sporlac was procured from local pharmacy. 1gm of sporlac powder contains 150 million spores of *L. sporogenes*.

2.4. Artemia Culture

Artificial sea water (5ppt) was prepared in a glass aquarium. The hatching temperature was maintained at 28 to 30ºC and pH was maintained at 8 – 8.5 by adding sodium carbonate drops. Heavy continuous illumination (40 watt fluorescent bulbs) and aeration were applied. 2grms of cysts were incubated per litre of artificial sea water. After 24hrs harvest newly hatched nauplii were harvested with the help of the cloth net (Sorgeloos *et al.*, 1996).

In the present investigation, the *C. catla* brain tissue fatty acid are clearly studied after the treatment and enrichment of the fish with the herbals *A. marmelos* and *S. oleracea*, probiotic *L. sporogenes* and live feed *Artemia salina*.

2.5. Experimental setup

Experimental fish are divided into two batches (I and II) that include twelve groups such as

Batch – I

Control – Fish fed with control feed

Group A – Fish fed with incorporated *A. marmelos*

Group B – Fish fed with incorporated *S. oleracea*

Group C - Fish treated with *L. sporogenes*

Group D - Fed with *A. marmelos* and treated with *L.s*

Group E – Fed with *S. oleracea* and treated with *L.s*.

Batch – II

Group F- Fish fed with live feed Artemia

Group G – Fish fed with Artemia enriched *A. marmelos*

Group H – Fish fed with Artemia enriched *S. oleracea*

Group I – Fish fed with Artemia enriched *L.s*

Group J – Fish fed with Artemia enriched *A. marmelos* & *L.s*

Group K - – Fish fed with Artemia enriched *S. oleracea* & *L.s*.

2.6. Fatty acid analysis using GC – MS

2.6.1. Lipid extraction

Total lipids were extracted from the brain (1g) tissues according to Blight and Dyer (1959). The brain tissues of each fish were taken after they have been partially thawed. Chloroform/methanol solvent mixture (2:1 v/v) was added of fish tissues sample in the ratio solvent: tissues of 20:1. After phase separation, the chloroform extract were evaporated to dry residue and were quantified by weight.

2.6.2. Preparation of Fatty Acid Methyl Esters (FAME)

The dry residue of the chloroform fraction was methylated by base – catalyzed transmethylation using 2M KOH in methanol and n-hexane (BDS EN ISO 5509 (2000)). After centrifugation (3500rps), the hexane layer was separated and analyzed by GC-MS.

The SIGMA – ALDRICH product Supelco 37 component (FAME) mix used as standard for quantifying the fatty acids profile.

3. Results

The 17 different types of SAFA, 10 different PUFA and 9 different MUFA in the brain tissues were quantified. Tables 1, 2, 3 and 4 showed the composition and amount (mg/100g) of these SAFA, PUFA and MUFA. Figure 1 & 2 showed the percentage of these fatty acids in the brain tissues. In control brain tissues, 63% is of SAFA, MUFA 25% and PUFA 12%. This data varies in the different experimental groups of batch – I and II. Group C

of batch – I has reduced their SAFA to 61%; other groups raised their SAFA after treatment in all groups except group C (61%). The PUFA only increased in batch – I of C group (24%) compared to control. In batch – II all groups show increased level of PUFA. The methyl hexanoate C6:0 and methyl octanoate C8:0 is not available in the batch – I groups, similar results were showed in methyl decanoate (C10:0) in control & group C; methyl undecanoate (C11:0) in group F; methyl tridecanote (C13:0) in group I. The PUFA is highest in group J (28%) and second highest in group H 20%.

Regarding the total amount of SAFA in the control and experimental groups, the control brain tissues have 408.84 mg/100g and C has lower amount 338.19 mg/100g. The group E has higher SAFA 976.76 mg/100g which is very much higher than the control. Among the 17 different SAFA, methyl laurate (C 11:0) is the highest amount of fatty acid in the brain tissues of control. Hence it is clear that the brain of *C. catla* has the meythyl laurate as the major SAFA methyl octanoate (C8:0) and methyl heptadecanote (C17:0) are the two SAFA which are very much lesser from (0.00 to 4.81). Other important SAFA which are higher are methyl stearate (C18:0) 50.95 mg/100g; methyl palmitate (C16:0) 99.65 mg/100g; methyl stearate (C18:0) 50.95 mg/100g and methyl undecanoate (C11:0) 36.66 mg/100g. Other important SAFAs varies between 7.83 to 20.38 mg/100g are methyl tridecanoate (C13:0), methyl myristate (C14:0); methyl pentadecanoate (C15:0); methyl arachidate (C20:0) and methyl heneicosanoate (21:0). The methyl butyrate (C4:0), methyl hexanoate (C6:0), methyl octanoate (C8:0) and methyl decanoate (C10:0) are not available in the control fishes.

After treatment, the experimental groups have changed their amount of SAFA to a significant level. To speak about methyl decanoate (C10:0) it is absent in the control and group C but in other groups the amount were between 370.02 mg/100g to 392.16 mg/100g. Methyl hexanoate is not available in experimental group of batch – I and in the batch – II groups 10.19, 12.53, 14.17, 14.39, 7.00 and 7.61 mg/100g in the groups F, G, H, I, J and K respectively.

From the peak of GC – MS chromatogram of the brain tissues, the identified PUFA are listed in the table 3. Therefore 10 numbers of PUFAs are identified; they are grouped as ω- 3 and ω- 6 fatty acids. Here we can find out the amount of four ω- 3 PUFAs and six ω- 6 PUFAs. The total amount of PUFA is 74.28 mg/100g in control group. The amount is raised in all groups and it is very much higher in J, H and F 324.80 mg/100g, 197.44 mg/100g and 161.44 mg/100g respectively.

The highest amount of ω- 3 is 7.9 mg/100g which is identified as linolenate C18:3 (n-3). Other ω- 3 PUFAs such as DHA; ETE and EPA are important fatty acids present in the brain tissues of *C. catla*. ETE is the second highest ω- 3 PUFA as 7.49 mg/100g in control. Which becomes higher as 8.17 mg/100g in group J; 8.04 mg/100g in group I and 8.00 mg/100g in K methyl linolenate is another ω- 3 fatty acid is reduced to in the batch – I groups but increased amount showed in the batch – II groups compared with control.

The important ω- 6 PUFAs present in the brain tissues are linoleate (C18:2); methyl linolenate (C18:3); cis-11,14-eicosatrienoic acid methyl C20:2 (n-6); cis-8,11,14-eicosatrienoic acid methyl C20:3(n-6); methyl cis-5,8,11,14,17 – eicosapentonic C20:5 (n-3); cis-13,16-docosadienoic acid methyl C22:2 (n-6). The highest amount of ω- 6 is methyl cis-5,8,11,14 –eicosatetraeno C20:4 which is 25.6 mg/100g, in control which is

raised in groups A, B, D & E 39.96, 38.72, 42.76 & 41.27 mg/100g respectively and lowered in all other groups.

Comparing ω- 3 with ω- 6 PUFA in all groups with the control (Figure 3 and 4), ω- 3 is (28%) lower than ω- 6 (72%) fatty acids in control. This percentage varies between the groups. Batch – I groups A, B, C, D, E & F have lowered their ω- 3 PUFA than the control and at the same time increased level showed in the ω- 6. In the batch – II the percentage of ω- 3 was higher than the ω- 6 in all groups compared to the control.

According to the GC – MS chromatogram, there are 9 MUFAs identified in the brain tissues of *C. catla*. They are listed in the table 4 as methyl palmitoleate (C16:1), cis-10- heptadecanoic acid methyl (C17:1), myristoleic acid methyl ester (C14:1), cis – 10- pentadecanoate acids methyl (C15:1), trans-9-elaidic methyl ester (C18:1), cis-9-oleic acid methyl ester (C18:1), methyl eicosanoate (C20:1), methyl erucate (C22:1) and methyl nervonate (C24:1). The total amount of MUFA in control is 162.83 mg/100g. This varies after the experimental period in different groups. They are elevated to 390.13 mg/100g, 385.14 mg/100g, 355.88 mg/100g and 325.73 mg/100g in groups E, B, D & A respectively. Among the nine MUFAs, the cis – 10 pentadecanoate acids methyl C15:1 (n-5) was not available in the control, C, E and F groups but are expressed in all other experimental groups. 86.66 mg/100g of cis-9-oleic acid methyl ester C18:1 (n-9) is present in control group which is the best MUFA in brain tissues of *C. catla*. Second higher amount MUFA is trans – 9 elaidic methyl C18:1 (n-9) which is 43.33 mg/100g. The lowest MUFA is cis – 10 heptadecanoic acid C17:1 (n-7) which is 0.01 mg/100g in control, but other groups the amount showed increased level, higher amount in the group I (9.55 mg/100g).

The percentage figures 5 and 6 explained that the MUFAs are grouped as ω-5, ω-7 and ω-9 MUFAs. Among the three, ω-9 is more than the other two groups ω-9 takes approximately more than 85% of the part in batch – I & 45% of the part in batch - II; whereas ω-7 and ω-5 takes remaining percentage. In control ω-9 is 89%, ω-7 is 3% and ω-5 is 8%. To study about ω-9 alone in all groups, it is reduced in all groups except in groups B, C and E.

Table 1: Identity and composition of SAFAs in the brain tissue of *Catla catla* using GC-MS technique

Components	Retention Time	Content in Groups												
Name of Fatty acids		**Amount/ As relative**	**Control**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**
Methyl Butyrate C 4:0	5.42	mg/100g	NA	12.80	17.24	NA	13.12	18.12	4.58	53.11	20.19	24.23	13.36	87.48
		Relative Rt. (%)	NA	0.01	0.01	NA	0.01	0.01	0.03	0.01	0.01	0.12	0.04	0.02
Methyl Hexanoate C 6:0	6.45	mg/100g	NA	NA	NA	NA	NA	NA	10.19	12.53	14.17	14.39	7.00	7.61
		Relative Rt. (%)	NA	NA	NA	NA	NA	NA	0.01	NA	NA	0.03	0.01	0.04
Methyl Octanoate C 8:0	10.30	mg/100g	NA	NA	NA	NA	NA	NA	3.54	3.39	3.67	4.81	3.68	3.58
		Relative Rt. (%)	NA	NA	NA	NA	NA	NA	0.03	0.01	0.01	0.03	0.02	0.02
Methyl Decanoate C 10:0	14.60	mg/100g	NA	372.59	372.95	NA	390.12	392.16	370.02	373.30	371.40	373.50	372.70	362.11
		Relative Rt. (%)	NA	0.01	0.01	NA	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01
Methyl Undecanoate C 11:0	17.35	mg/100g	36.66	36.19	36.48	36.91	38.12	38.28	NA	37.30	37.24	37.37	37.39	36.99
		Relative Rt. (%)	0.01	0.01	0.01	0.01	0.02	0.02	0.01	NA	NA	NA	0.01	0.01
Methyl Laurate C 12:0	20.43	mg/100g	120.27	115.15	116.32	122.51	139.12	141.22	122.71	123.90	117.65	128.16	127.84	82.76
		Relative Rt. (%)	0.05	0.08	0.07	0.07	0.09	0.09	0.38	0.10	0.14	0.36	0.29	0.21
Methyl Tridecanoate C 13:0	23.70	mg/100g	17.95	17.11	17.08	17.94	19.01	19.81	18.00	18.08	18.06	NA	18.07	17.83
		Relative Rt. (%)	0.02	0.01	0.01	0.02	0.02	0.02	0.04	0.01	0.01	0.01	0.06	0.03
Methyl Myristate C 14:0	27.04	mg/100g	20.38	7.89	4.22	22.92	8.12	5.22	22.14	24.39	23.88	27.39	27.42	22.24
		Relative Rt. (%)	0.93	0.60	0.60	0.94	0.99	1.03	0.95	0.71	0.57	1.09	0.92	1.14
Methyl Pentadecanoate C 15:0	30.35	mg/100g	8.56	2.78	1.29	9.17	3.81	2.12	9.67	10.57	11.38	11.89	11.95	10.78
		Relative Rt. (%)	0.60	0.52	0.41	0.62	0.76	0.93	0.73	0.34	0.36	0.64	0.72	0.61

Control – Fish fed with control feed
Group A – Fish fed with *A. marmelos*
Group B – Fish fed with *S. oleracea*
Group C - Fish treated with *L. sporogenes*
Group D – Fish fed with *A. marmelos* and treated with *L.s*
Group E – Fish fed with *S. oleracea* and treated with *L.s*
Group F- Fish fed with live feed Artemia
Group G – Fish fed with Artemia enriched *A. marmelos*
Group H – Fish fed with Artemia enriched *S. oleracea*
Group I – Fish fed with Artemia enriched *L.s*
Group J – Fish fed with Artemia enriched *A. marmelos* & *L.s*
Group K - – Fish fed with Artemia enriched *S. oleracea* & *L.s*

Table 2: Identity and composition of SAFAs in the brain tissue of *Catla catla* using GC-MS technique

Components	Retention Time	Content in Groups												
Name of Fatty acids		Amount/ As relative	Control	A	B	C	D	E	F	G	H	I	J	K
Methyl Palmitate C 16:0	33.60	mg/100g	99.65	179.03	192.65	42.61	192.01	199.62	58.08	29.74	20.05	31.73	31.51	17.73
		Relative Rt. (%)	32.95	27.13	37.53	36.90	19.72	38.36	44.54	34.86	33.03	43.03	38.34	42.50
Methyl Heptadecanoate C 17:0	36.74	mg/100g	5.47	0.32	2.68	4.97	0.92	3.12	7.57	8.38	8.36	9.31	9.33	8.59
		Relative Rt. (%)	1.04	1.45	0.83	1.09	1.68	1.06	2.06	1.36	1.52	1.73	1.99	1.43
Methyl Stearate C 18:0	39.80	mg/100g	50.95	82.58	101.09	20.86	85.12	112.07	22.14	10.45	11.04	18.04	17.90	4.76
		Relative Rt. (%)	32.76	25.31	36.38	32.40	20.00	25.87	34.34	35.49	34.47	33.52	36.82	35.94
Methyl Arachidate C 20:0	45.56	mg/100g	14.16	11.11	10.89	14.50	13.18	13.12	15.78	16.18	16.08	16.57	16.57	16.31
		Relative Rt. (%)	0.85	1.03	0.77	0.98	1.67	1.13	0.53	1.02	0.96	0.63	0.80	0.62
Methyl Heneicosanoate C 21:0	48.30	mg/100g	7.83	7.49	7.45	7.80	9.14	9.01	7.95	7.97	7.96	7.97	7.97	7.96
		Relative Rt. (%)	0.08	0.09	0.05	0.09	0.09	0.05	0.03	0.07	0.06	0.03	0.05	0.04
Methyl Behenate C 22:0	50.98	mg/100g	13.15	9.94	7.54	15.22	10.12	8.15	15.15	15.60	15.34	15.97	15.97	15.74
		Relative Rt. (%)	1.68	0.51	1.21	1.45	1.03	2.32	0.15	0.51	0.40	0.15	0.31	0.30
Methyl Tricosanoate C 23:0	53.47	mg/100g	7.46	6.93	6.52	7.68	7.01	6.91	7.73	7.79	7.74	15.97	7.83	7.81
		Relative Rt. (%)	0.30	0.12	0.18	0.25	0.39	0.34	0.03	0.14	0.12	0.06	0.01	0.12
Methyl Lignocerate C 24:0	55.92	mg/100g	6.35	0.62	6.36	15.10	1.12	7.83	11.83	14.25	13.06	15.62	15.59	14.79
		Relative Rt. (%)	4.45	0.38	4.39	4.33	4.68	4.96	0.11	1.05	0.85	0.18	0.46	0.63
Σ SAFAs	**mg/100g wet brain**		**408.84**	**862.53**	**900.76**	**338.19**	**930.13**	**976.76**	**707.08**	**766.93**	**717.27**	**752.93**	**742.08**	**725.07**

Control – Fish fed with control feed
Group A – Fish fed with *A. marmelos*
Group B – Fish fed with *S. oleracea*
Group C - Fish treated with *L. sporogenes*
Group D – Fish fed with *A. marmelos* and treated with *L.s*
Group E – Fish fed with *S. oleracea* and treated with *L.s*
Group F- Fish fed with live feed Artemia
Group G – Fish fed with Artemia enriched *A. marmelos*
Group H – Fish fed with Artemia enriched *S. oleracea*
Group I – Fish fed with Artemia enriched *L.s*
Group J – Fish fed with Artemia enriched *A. marmelos* & *L.s*
Group K - – Fish fed with Artemia enriched *S. oleracea* & *L.s*

Table 3: Identity and composition of PUFAs in the brain tissue of *Catla catla* using GC-MS technique

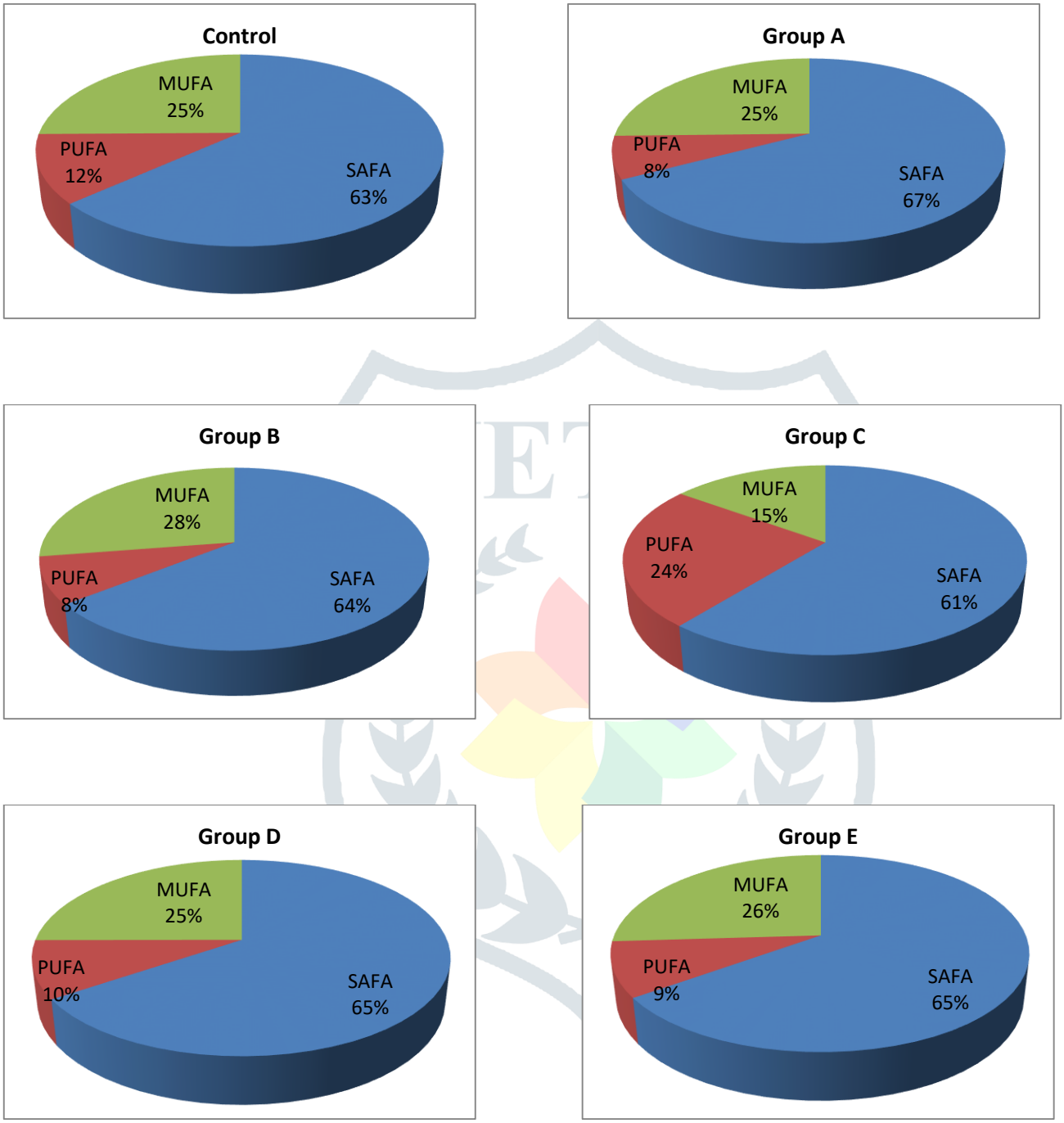
Components	Retention Time	Content in Groups												
Name of Fatty acids		Amount/ As relative	Control	A	B	C	D	E	F	G	H	I	J	K
Methyl Cis-5,8,11,14,17 – Eicosapentonic (EPA) C 20:5 (n-3)	41.73	mg/100g	5.72	NA	0.90	17.62	27.27	3.12	98.25	67.16	70.19	46.59	256.24	18.18
		Relative Rt. (%)	0.01	NA	0.03	0.03	0.05	0.06	0.49	0.05	0.01	0.69	0.55	0.31
Cis-4,7,10,13,16,19-Docosahexane (DHA) C 22:6 (n-3)	45.73	mg/100g	NA	NA	NA	NA	NA	NA	15.26	10.07	77.54	18.37	11.10	20.86
		Relative Rt. (%)	NA	NA	NA	NA	NA	NA	0.03	0.05	0.02	0.03	0.02	0.06
Cis-11,14,17 Ecosatrienoic Acid Methyl (ETE) C 20:3 (n-3)	46.15	mg/100g	7.49	5.94	5.81	7.17	6.82	7.12	7.60	7.91	7.63	8.04	8.17	8.00
		Relative Rt. (%)	0.05	0.07	0.03	0.05	0.09	0.04	0.12	0.18	0.16	0.11	0.15	0.05
Methyl Linolenate C 18:3 (n-3)	40.34	mg/100g	7.90	0.81	0.09	3.99	1.62	0.12	7.67	8.42	8.27	8.59	8.54	8.56
		Relative Rt. (%)	0.17	0.30	0.03	0.19	0.69	0.23	1.10	1.65	0.90	1.09	1.10	0.69
Linolelaidic Acid Methyl Ester C 18:2 (n-6)	39.74	mg/100g	6.43	24.55	29.85	41.59	28.16	30.12	3.03	6.53	5.59	8.58	8.58	7.16
		Relative Rt. (%)	3.17	14.44	1.74	3.17	14.63	4.36	7.61	6.30	9.07	7.92	7.51	5.82
Methyl Linoleate C 18:2 (n-6)	23.40	mg/100g	8.05	7.99	22.18	30.95	9.26	31.26	8.13	8.13	8.11	8.13	8.13	7.04
		Relative Rt. (%)	3.17	14.44	0.01	0.01	14.63	0.01	0.03	6.30	9.07	0.05	0.08	0.08
Cis-11,14-Eicosatrienoic Acid Methyl C 20:2 (n-6)	48.28	mg/100g	2.16	2.75	5.20	3.34	3.12	7.62	0.38	0.63	0.74	1.09	0.50	0.50
		Relative Rt. (%)	0.01	0.02	0.01	0.01	0.03	0.02	0.02	NA	0.01	0.01	0.01	0.01
Cis-8,11,14-Eicosatrienoic Acid methyl (DGLA) C 20:3 (n-6)	45.37	mg/100g	3.60	6.23	5.94	5.22	7.68	7.62	6.00	7.37	6.9	8.37	8.25	7.69
		Relative Rt. (%)	0.40	1.18	0.56	0.49	3.16	1.05	0.38	1.12	1.36	0.70	0.66	0.52
Methyl Cis-5,8,11,14-Eicosatetraeno C 20:4 (n-6)	45.12	mg/100g	25.6	39.96	38.72	17.62	42.76	41.27	7.50	3.68	4.81	6.85	7.67	2.18
		Relative Rt. (%)	0.15	0.25	0.22	0.19	0.32	0.30	0.40	0.43	0.43	0.55	0.48	0.36
Cis-13,16-Docosadienoic Acid Methyl C 22:2 (n-6)	50.99	mg/100g	7.33	7.03	6.77	7.51	8.02	7.06	7.62	7.69	7.66	7.70	7.70	7.70
		Relative Rt. (%)	0.04	0.03	0.04	0.04	0.03	0.04	0.01	0.02	0.02	0.01	0.01	0.01
Σ PUFAs	**mg/100g wet brain**		**74.28**	**95.26**	**115.46**	**135.01**	**134.71**	**135.31**	**161.44**	**127.59**	**197.44**	**122.31**	**324.88**	**87.87**

Table 4: Identity and composition of MUFAs in the brain tissue of *Catla catla* using GC-MS technique

Components	Retention Time	Content in Groups											
Name of Fatty acids		Amount/ As relative	Control	A	B	C	D	E	F	G	H	I	J	K
Methyl Palmitoleate C 16:1 (n-7)	33.43	mg/100g	4.96	16.48	17.72	1.73	18.17	17.99	3.26	4.15	3.66	10.12	10.22	5.61
		Relative Rt. (%)	0.57	0.35	0.43	0.66	0.58	0.46	0.17	0.24	0.12	0.21	0.32	0.21
Cis-10- Heptadecanoic Acid Methyl C17:1 (n-7)	36.59	mg/100g	0.01	2.26	2.64	0.69	3.16	3.68	7.55	8.31	8.39	9.55	9.54	8.47
		Relative Rt. (%)	0.15	0.08	0.11	0.16	0.16	0.13	0.08	0.14	0.08	0.10	0.19	0.11
Myristoleic Acid Methyl Ester C 14:1 (n-5)	27.14	mg/100g	13.52	13.35	NA	NA	15.18	NA	13.34	13.51	13.54	13.48	NA	13.49
		Relative Rt. (%)	0.01	0.01	NA	NA	0.01	NA	0.02	0.01	0.01	0.01	0.03	0.02
Cis-10-Pentadecanoate Acids Methyl C 15:1 (n-5)	30.48	mg/100g	NA	11.77	11.53	NA	13.12	NA	NA	11.91	11.06	11.84	12.05	11.51
		Relative Rt. (%)	NA	0.02	0.01	NA	0.03	0.01	0.04	0.01	NA	0.02	0.01	0.02
Trans-9-Elaidic Methyl Ester C 18:1(n-9)	39.71	mg/100g	43.33	86.34	108.49	21.74	88.32	110.12	20.93	9.69	10.07	8.77	8.69	5.43
		Relative Rt. (%)	7.91	5.79	6.90	7.60	6.96	7.96	2.67	3.81	3.02	3.32	3.94	3.96
Cis-9-Oleic Acid Methyl Ester C 18:1 (n-9)	39.49	mg/100g	86.66	172.68	216.96	43.47	192.68	227.12	41.85	19.38	20.15	3.33	17.38	10.87
		Relative Rt. (%)	7.91	5.59	6.90	7.60	6.96	7.96	2.67	3.81	3.02	3.32	3.94	3.96
Methyl Eicosanoate C 20:1 (n-9)	45.28	mg/100g	2.10	5.71	8.18	2.51	6.27	9.12	7.08	7.70	7.53	8.39	8.38	7.92
		Relative Rt. (%)	0.22	0.26	0.18	0.27	0.36	0.21	0.05	0.13	0.14	0.07	0.04	0.05
Methyl Erucate C 22:1 (n-9)	50.70	mg/100g	6.46	4.27	4.38	6.54	5.86	5.92	7.26	7.51	7.43	7.58	7.61	7.59
		Relative Rt. (%)	0.04	0.05	0.03	0.05	0.05	0.03	0.03	0.01	0.01	0.01	0.01	0.01
Methyl Nervonate C 24:1 (n-9)	55.76	mg/100g	5.79	12.87	15.24	6.83	13.12	16.18	3.06	4.98	4.58	7.82	5.51	6.19
		Relative Rt. (%)	0.31	0.01	0.32	0.32	0.09	0.40	0.10	0.06	0.05	0.16	0.08	0.11
Σ MUFAs	**mg/100g wet brain**		**162.83**	**325.73**	**385.14**	**83.51**	**355.88**	**390.13**	**104.33**	**84.14**	**86.41**	**80.88**	**79.38**	**77.08**

Control – Fish fed with control feed
Group A – Fish fed with *A. marmelos*
Group B – Fish fed with *S. oleracea*
Group C - Fish treated with *L. sporogenes*
Group D – Fish fed with *A. marmelos* and treated with *L.s*
Group E – Fish fed with *S. oleracea* and treated with *L.s*
Group F- Fish fed with live feed Artemia
Group G – Fish fed with Artemia enriched *A. marmelos*
Group H – Fish fed with Artemia enriched *S. oleracea*
Group I – Fish fed with Artemia enriched *L.s*
Group J – Fish fed with Artemia enriched *A. marmelos* & *L.s*
Group K - – Fish fed with Artemia enriched *S. oleracea* & *L.s*

Fig. 1 Percentage (%) of fatty acids in the brain tissues of batch – I groups - *Catla catla*



Control – Fish fed with control feed

Group A – Fish fed with *A. marmelos*

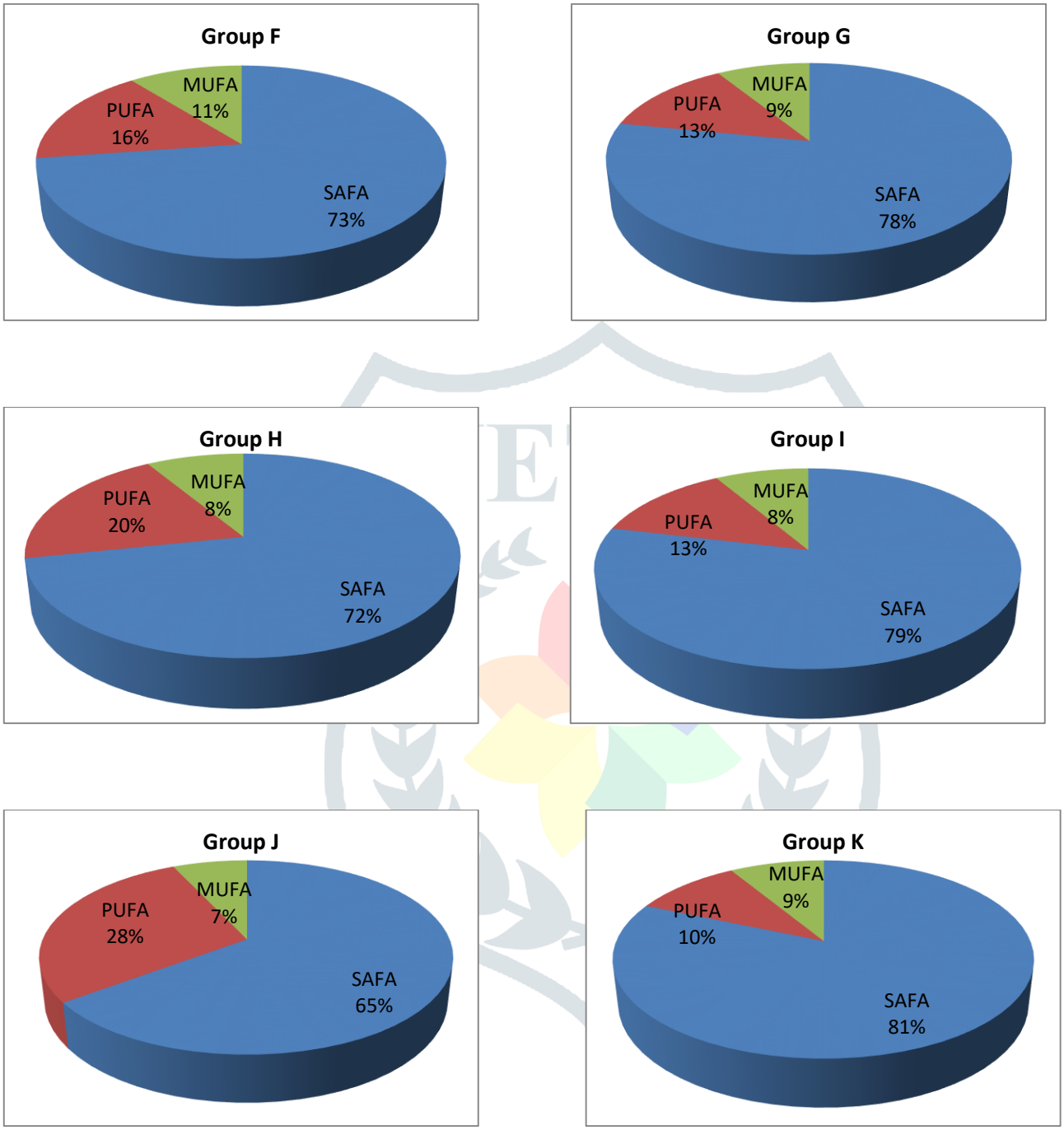
Group B – Fish fed with *S. oleracea*

Group C - Fish treated with *L. sporogenes*

Group D – Fish fed with *A. marmelos* and treated with *L.s*

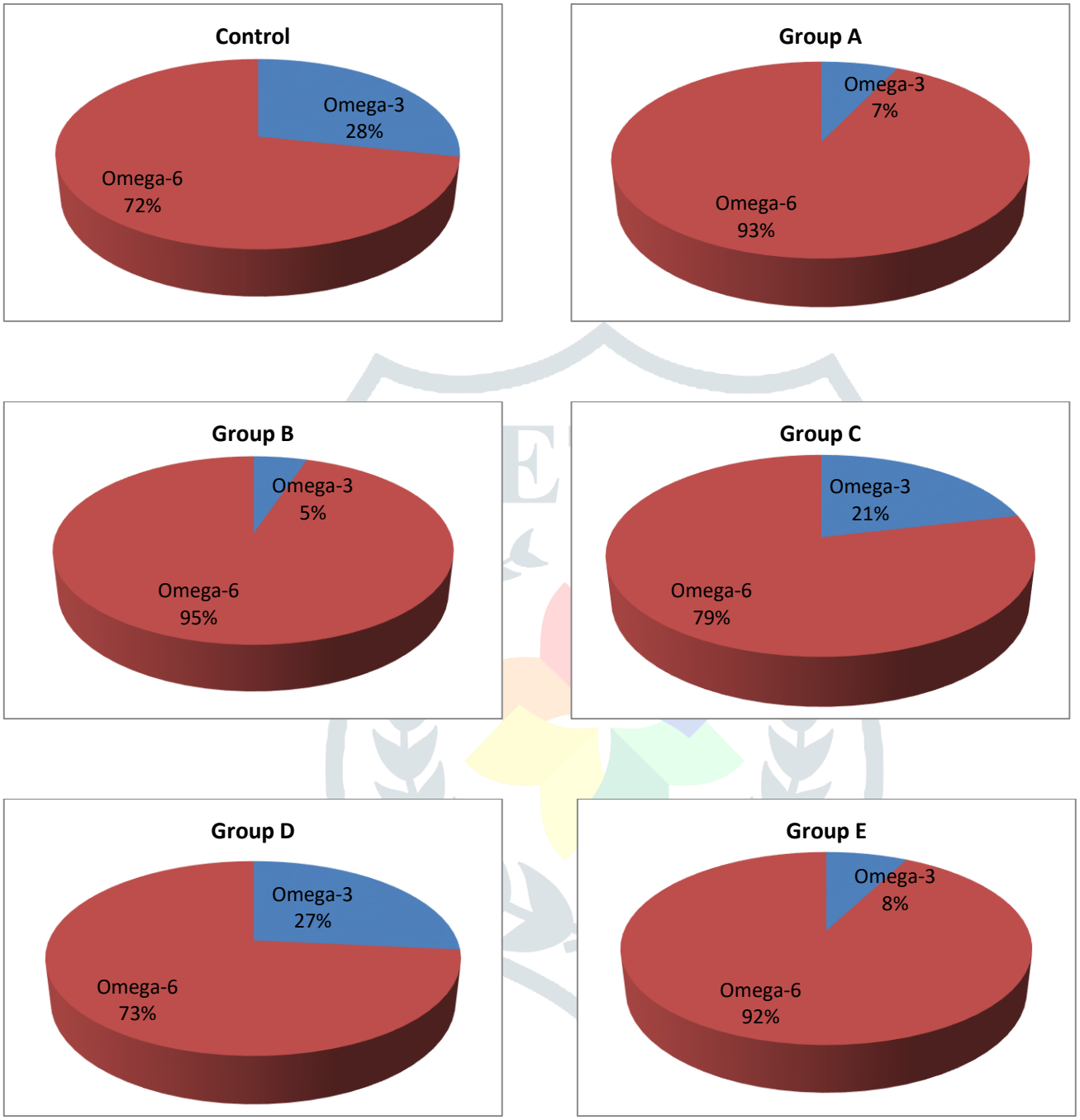
Group E – Fish fed with *S. oleracea* and treated with *L.s*

Fig. 2 Percentage (%) of fatty acids in the brain tissues of batch – II groups - *Catla catla*



Group F- Fish fed with live feed Artemia
Group G – Fish fed with Artemia enriched *A. marmelos*
Group H – Fish fed with Artemia enriched *S. oleracea*
Group I – Fish fed with Artemia enriched *L.s*
Group J – Fish fed with Artemia enriched *A. marmelos* & *L.s*
Group K - – Fish fed with Artemia enriched *S. oleracea* & *L.s*

Fig. 3 Percentage (%) of PUFAs in the brain tissues of batch – I groups - *Catla catla*

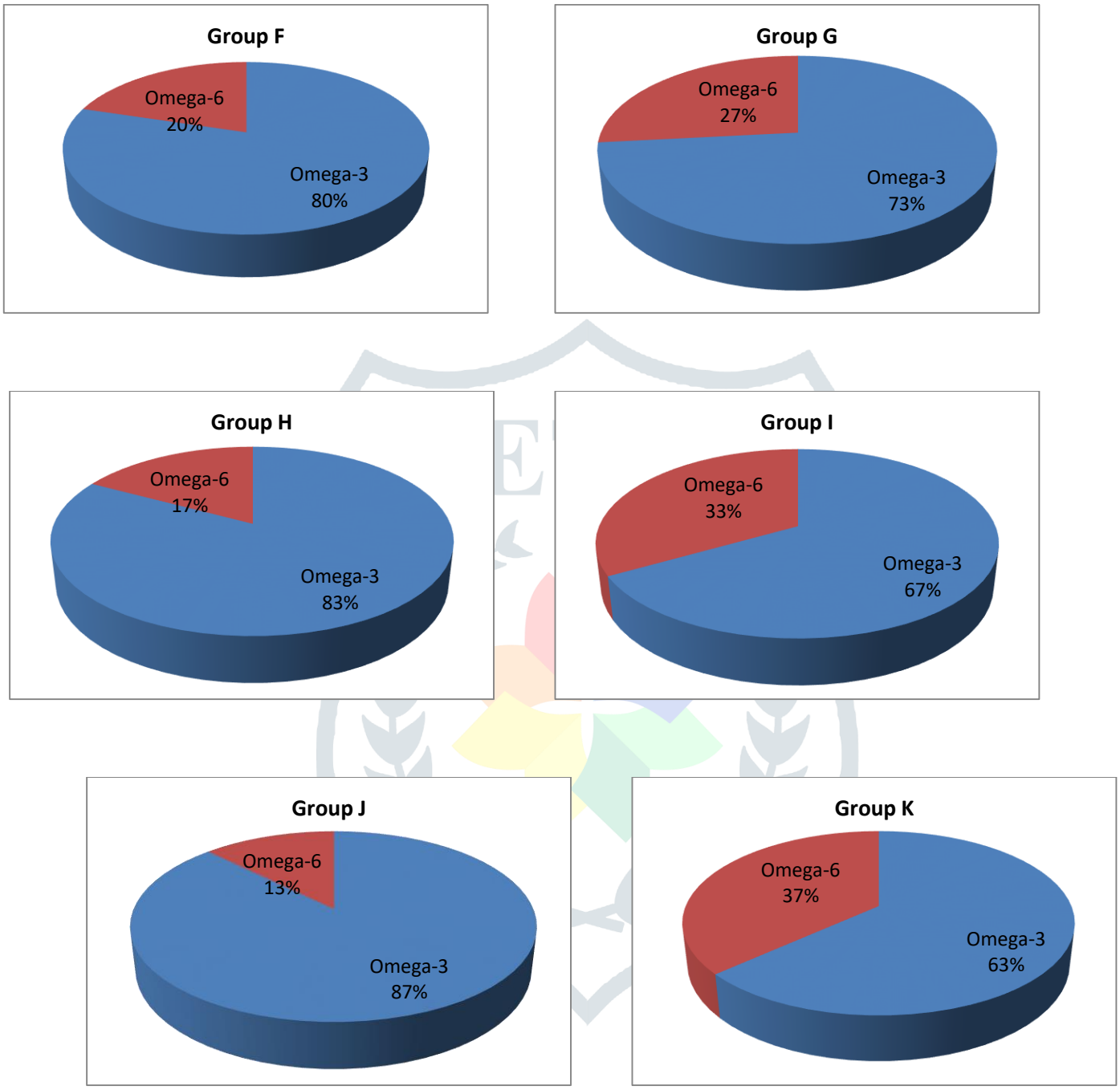


Control – Fish fed with control feed
– Fish fed with *S. oleracea*
Fish fed with *A. marmelos* and treated with *L.s*

Group A – Fish fed with *A. marmelos*
Group C - Fish treated with *L. sporogenes*
Group E – Fish fed with *S. oleracea* and treated with *L.s*

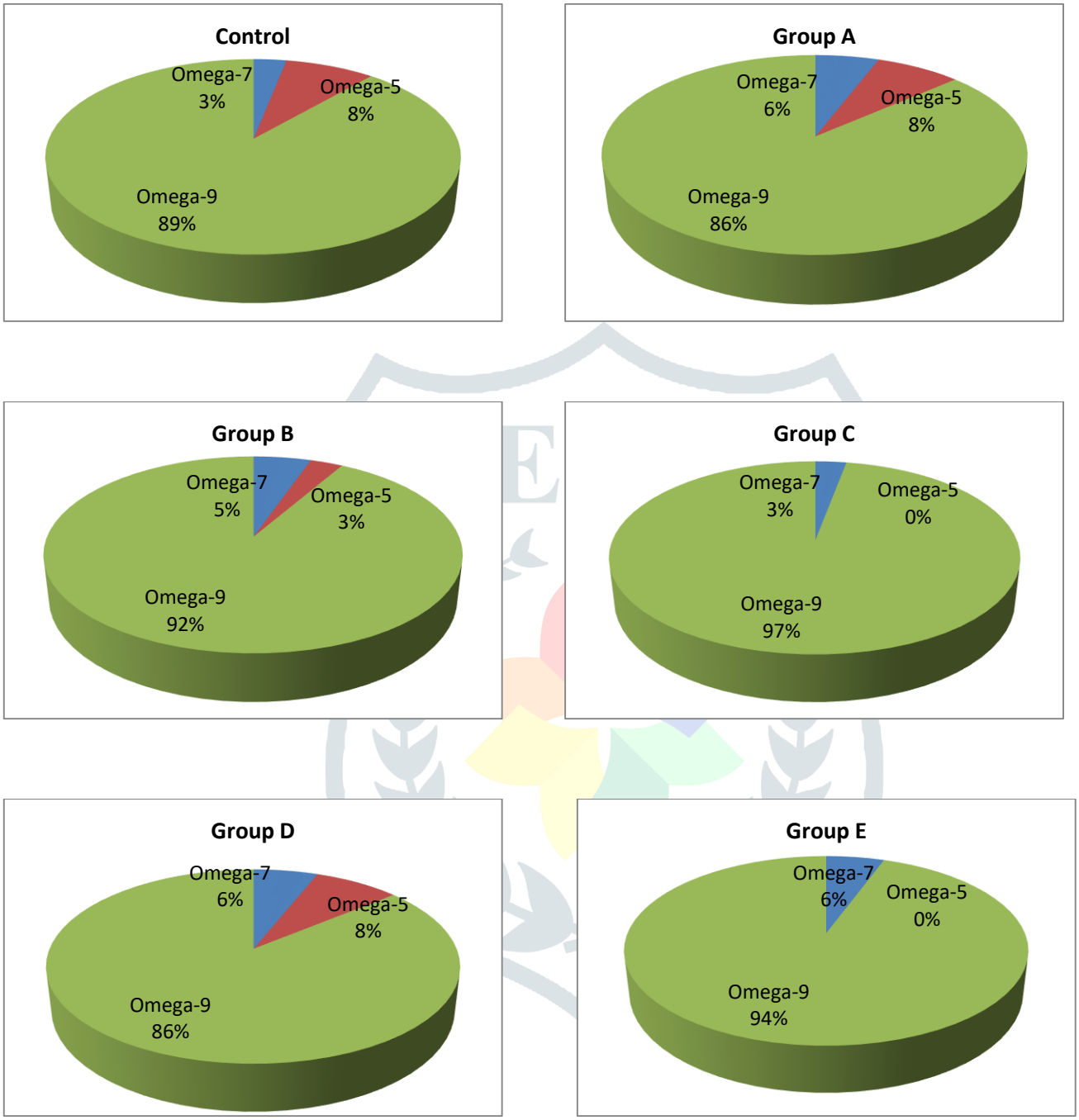
Gr
Group I

Fig. 4 Percentage (%) of PUFAs in the brain tissues of batch – II groups - *Catla catla*



Group F- Fish fed with live feed Artemia
Group G – Fish fed with Artemia enriched *A. marmelos*
Group H – Fish fed with Artemia enriched *S. oleracea*
Group I – Fish fed with Artemia enriched *L.s*
Group J – Fish fed with Artemia enriched *A. marmelos* & *L.s*
Group K - – Fish fed with Artemia enriched *S. oleracea* & *L.s*

Fig. 5 Percentage of MUFAs in the brain tissues of batch – I groups - *Catla catla*



Control – Fish fed with control feed

Group A – Fish fed with *A. marmelos*

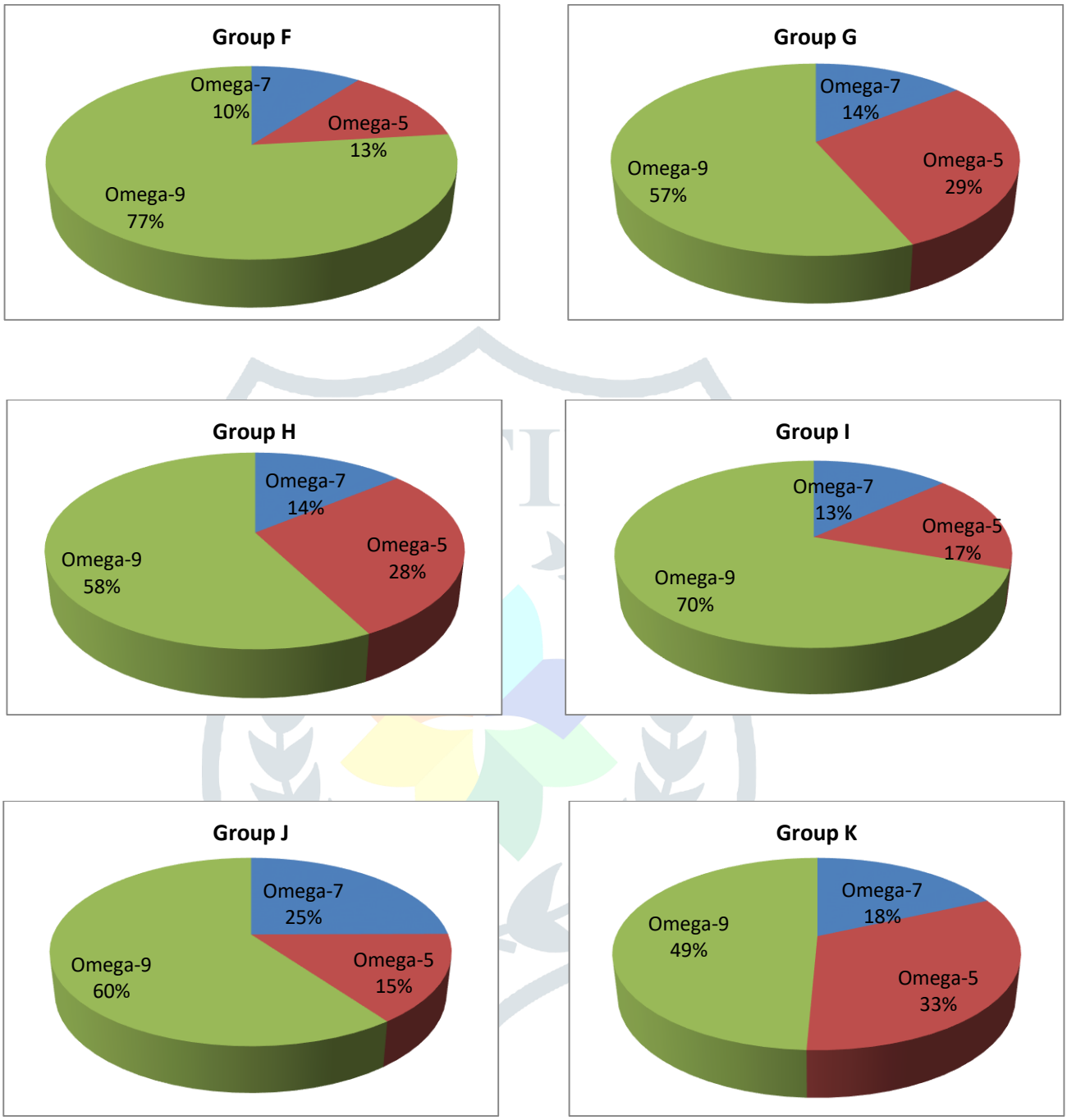
Group B – Fish fed with *S. oleracea*

Group C - Fish treated with *L. sporogenes*

Group D – Fish fed with *A. marmelos* and treated with *L.s*

Group E – Fish fed with *S. oleracea* and treated with *L.s*

Fig. 6 Percentage of MUFAs in the brain tissues of batch –II groups - *Catla catla*



Group F- Fish fed with live feed Artemia
Group G – Fish fed with Artemia enriched *A. marmelos*
Group H – Fish fed with Artemia enriched *S. oleracea*
Group I – Fish fed with Artemia enriched *L.s*
Group J – Fish fed with Artemia enriched *A. marmelos* & *L.s*
Group K - – Fish fed with Artemia enriched *S. oleracea* & *L.s*

4. Discussion

These are seventeen different SAFA analyzed in brain tissues; among which the methyl laurate is the highest content *C. catla.* It is the fatty acids which increase total serum good cholesterol, it is non – toxic. After the treatment, the experimental groups of batch – II have methyl hexanoate and methyl decanoate. This may be attributed to the esterification of fatty acids. Methyl palmitate which is increased over the control in batch – I group has reduced in batch – II. This may be attributed to the artemia enriched *A. marmelos*, spinach and *L. sporogenes*. It is an advantage because excess of palmitic acid increases the risk of developing cardiovascular diseases (WHO, 2003).

Regarding PUFA in brain tissues, EPA which is very low 5.72 in control group, has been raised to 98.25 in F group; 67.16 in G group; 70.19 in H group and 256.24 in J group. This is attributed only to the feed provided to these groups; J group fish were provided with artemia enriched *A. marmelos* and treated with *L. sporogenes.* Hence artemia have influenced the fish to convert the fatty acid to ω - 3 fatty acids. Likewise the F, G and H also have increased their content with the help of artemia. Similarly DHA which is not available in control and batch – I fishes, is now present in batch II fishes there is 77.54 in group H; 18.37 in I group; 20.86 in K; 15.26 in F; 11.1 in J and 10.07 in G groups. The study of Hafezieh, *et al.* (2010) has proved the effects of different artemia enrichment containing variable amount of DHA and EPA on the growth and survival of larval Persian sturgeon *Acipenser persicus*.

Leger *et al.* (1986) and Navarro; 1992 have said that the fatty acid content of artemia nauplii is of major nutritional value for fish and crustacean larvae. Among the two main categories of artemia, Watanabe *et al.* (1978) have classified marine type artemia have a high content of EPA. Moreover the EPA and DHA content play a role in growth and neural tissue development of cultured organisms (Bell *et al.*, 1995 and Harel *et al.*, 2002). Therefore in the present investigation enrichment of artemia with *A. marmelos* and treated with *L. sporogenes* have been formulated and it has increased the amount of DHA and EPA in the brain tissues of *C. catla.* While analyzing the lipid profile of spinach, Narsing Rao *et al.* (2015) found high saturated fatty acids (23.9%), PUFA (68%) and MUFA (8.1%). This may be attributed to the increase of PUFA from 12% in control group to 20% in H group which were fed with artemia enriched *S. oleracea*. The presence of high amount of PUFA compared to SAFA make spinach leaf oil suitable for nutritional applications (Narsing Rao *et al.*, 2015). Therefore formulating fish feed with spinach leaves increased the PUFA/SAFA ratio in the tissues of *C. catla*.

Among MUFA, the control brain tissues are lacking cis – 10 – pentadecanoate acid methyl C15:1 (n-5) ω - 5 MUFA which were present in experimental A, B, D, G, H, I, J & K groups. Similarly ω - 9 fatty acids cis – 9 – oleic acid methyl ester C 18:1 (n- 9) is considerably increased over the control tissues in group B, D & E, which were fed with *S. oleracea* and *A. marmelos* and treated with *L. sporogenes*. Hence it is attributed to the presence of oleic acid in the spinach leaves. Moreover *L. sporogenes* could have also influenced the increase of this MUFA in the brain tissues of *C. catla*.

L. sporogenes maintains the effective functioning of the intestine of *C. catla* and helps to promote healthy intestinal functioning by producing lactic acid (Delcenserie *et al.,* 2009). It maintains a healthy balance of microflora in the gut and support the growth of beneficial microflora in the gut. It prevents diarrhea, inflammatory bowel disease and ulcers. When *C. catla* were treated with *L. sporogenes*, their gut ability would have enhanced and digestion of fish would have properly been done to enhance the absorption of fatty acids present in the spinach and *A. marmelos*. Since probiotic consumption is said to put a beneficial effect on immune response, the results obtained in the present study indicate that *L. sporogenes* is a potent probiotic to protect the fish health mechanisms. According to Lara-Flores (2003) the uses of probiotic *Streptococcus* strain increase the content of crude protein and crude lipid in the Nile tilapia *O. niloticus*. Hence in the present investigation also, the increase of lipid content in the brain tissues of *C. catla* may be attributed to the treatment of these groups fish with *L. sporogenes*.

5. Conclusion

The study is clear that the diet prepared for the fish groups would have altered the composition of fatty acids content in the brain tissues of *C. catla* variably. The result showed that the increased good cholesterol ratio in the SAFA, PUFA and MUFA fatty acids in all groups. The brain booster fatty acids EPA and DHA content and the ratio of the brain tissues have been increased in the case of the groups of fish which were fed with artemia enriched *A. marmelos* and S. *oleracea* diets.

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