BIOCHEMICAL SEGREGATION OF AMINO ACIDS AND FATTY ACIDS OF THREE FRESH WATER TELEOSTS (*Clarias batrachus, Channa punctatus, and Anabas testudineus*)

[1]Sanatan Singh, [2]G. Mishra, [3]P. K. Dixit

[1]Research Scholar, [2]Reader in Zoology, [3]Professor in Zoology
P. G. Dept of Zoology, Berhampur University-760007, Odisha, India

Abstract: Aim of the present piece of work was to biochemically segregate the different amino acids like essential amino acids (EAA), non-essential amino acids (NEAA) in proteins and fatty acids like saturated fatty acids (SFA); mono-unsaturated fatty acids (MUFA) and poly saturated fatty acids (PUFA) of fats in the muscle tissues of three commercially less importance fresh water fishes like *Clarias batrachus*, *Channa punctatus* and *Anabas testudineus*. The parameters under study were estimated in all the three species irrespective of sex, size and maturity. Nine essential amino acids namely Lysine, Leucine, Valine, Isoleucine, Threonine, Phenylalanine, Methionine, Tryptophan and Histidine that are very important for human health were found to be present in all the species examined. Among all the essential amino acids, Leucine is found to be highest in content i.e. 1.29mg, 0.86mg and 1.38mg/100 gm fish muscle among the above three species respectively. However, in NEAA; Glutamic acid content was recorded to be highest in *C.batrachus* and *C.punctatus* with value of 2.41mg and 0.79 mg/100 gm tissue; whereas, Cysteine value of 2.35 mg was recorded in *A.testudineus.* Glutamic acid plays an important role in amino acid metabolism because of its role in transamination reactions and is necessary for the synthesis of key molecules, such as glutathione which are required for removal of highly toxic peroxides and the polyglutamate folate cofactors.

The highest value in SFA of C21:0 was found to be 0.45mg in *C. batrachus,* whereas the C16:0 content was recorded to be 1.07mg and 3.59mg in *C.punctatus* and *A.testudineus* respectively. Similarly, the highest value of MUFA in C18:1n9 has found in *C.batrachus* and *C.punctatus* with a value of 1.54mg and 1.01mg per 100gm tissue respectively. The MUFA of C18:1n9t has highest value found in *A.testudineus* of 1.29 mg. The PUFA like Omega 6 (ω 6) content was recorded highest among above three species with a value of 0.89mg, 0.98mg and 0.69 mg per 100gm of tissues. The total EAA were found to be 7.07mg, 5.19mg, and 7.42mg; whereas the value of NEAA was recorded to be 7.16mg, 4.29mg, and 9.58mg per 100 gm tissue differ between the species. However the total SFA was recorded 1.909mg, 3.25mg, and 4.283mg ; MUFA 2.135mg, 3.650mg, and 1.876mg and the total PUFA content was found to be 2.990mg, 3.402mg, and 1.975mg respectively in the fishes under study. Docosahexaenoic (DHA) and Eicosapentaenoic acid (EPA) were the dominant polyunsaturated fatty acid predominantly found in all the species. The results indicate that all the fish species under experimentation contain high quality of protein and fats with necessary fatty acids and many essential amino acids for normal development and growth of human and can be exploited as alternative fish source like that of commercial important variety. Among all the species under study, *A.testudineus* was found to be the best choice among all the species under study as it contains high nutrient components and the perfect ratio of SFA, MUFA and PUFA followed by *C.batrachus* and *C.punctatus*.

Keywords: amino acids, fatty acids, Clarias *batrachus, Channa punctatus, Anabas testudineus*

Introduction:

Amino acids are useful components in a variety of metabolism. Even though, some roles can be highlighted as a function of an amino acid. It is important to be aware that they are part of complex pathways and biological systems. The function and use of an amino acids and fatty acids has indirect effects that are manifested in myriad metabolisms. Amino acids are essential intermediates in the process of protein synthesis and its degradation products appear in the form of different nitrogenous substances. Amino acid and some nitrogenous compound play an important part during osmotic stress hence increase or decrease in free amino acid content provide valuable information during stress phenomenon at the tissue level. Amino acids are considerably one of the most reliable techniques for detection of changes in protein synthesis in cell and therefore, the protein pattern can be used as a criterion for the differentiation between several organs exposed to some pollutants (Anilkumar *et al.*, 2010). Huynh (2007) observed that fishes are having high poly unsaturated fatty acids that are useful in reducing serum cholesterol. Padmawati and Prema Kumari (2006) reported that changes in biochemical contents of muscles of fish species may also be attributed to alterations due to increased glycogenesis in muscles and accelerated conversion of liver glycogen into muscle glycogen. Chamundeshwari Devi and Vijayaragahwan (2001) observed that changes in biochemical parameters in the fishes are linked to their habitat and nutritive values the available food.

Fatty acids are one of the defining constituents of lipids and are in large part responsible for the distinctive physical and metabolic properties of the latter where they are of course of vital importance. However, it has become evident that there are a number of more dynamic functions of fatty acids, which are attracting great interest now. Fish and fish products play an important role in human's life. Fish lipids are excellent sources of the essential polyunsaturated fatty acids (PUFAs) in both the omega-3 and omega-6 families. Omega-6 PUFAs are also derived from vegetable oil, whereas long chain omega-3 PUFAs, such as docosahexaenoic acid (DHA) , eicosapentaenoic acid (EPA) derive mainly from fishes. The unsaturated fatty acids analysed in the present study indicate the presence of essential fatty acids linoleic acid (Omega 6) and alpha-linoleic acid (Omega 3) which have many functions for man (food supplement); reduce inflammation throughout the body, keep blood from excessive clotting, maintain the fluidity of the cell membranes, lower the amount of lipids (fats such as cholesterol and triglycerides) circulating in the blood stream, decrease platelet aggregation; inhibit thickening of the arteries by decreasing endothelial cells' production of platelet – derived growth factor, and reduce the production of messenger chemical called cytokines.

Objectives:

- To estimate the amount of amino acids and fatty acids content in the fresh water teleosts
- To separate the types of amino acids like as essential amino acids(EAA) and non-essential amino acids (NEAA)
- To separate the types of fatty acids saturated fat (SFA) , mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA)
- To have a comparative analysis of different amino acids and fatty acids among the three fresh water teleosts.

Materials and methods

The fishes were collected from neighboring fish market in and around Berhampur University without any mechanical injury for biochemical analysis. The sample specimen were packed and stored in refrigerator for laboratory analysis. Each fish sample was gutted, muscle tissue was collected, cleaned, finely minced and homogenized. The present work shows that biochemical composition of amino acids and fatty acids in muscle contents of freshwater fish of *Clarias batrachus*, *Channa punctatus* and *Anabas testudineus*. The fish undergo seasonal changes in growth and energy is reserved for maintenance, somatic growth and reproduction. The fishes are dissected out and then biochemical parameters were estimated from fresh muscles of fishes.

Amino acid analysis

The preparation of the samples was done following the methods of Mason ***et al.*** **(1980).** Half gram sample was weighed into 100cm^3 flat bottomed flask and 1 ml of Nor-leucine standard solution, 5 ml performic acid stand in ice bath in fridge for 16h, 0.84g sodium metabisulphite, 30 ml 6NHCI and anti bumping granules then added. The mixture was hydrolyzed for 24h in polyethylene glycol bath set at 130ºC. It was then allowed to cool and 30 ml of 4 M lithium hydroxide added. The pH was adjusted to 2.1 and the mixture made up to 100 ml final volume. Five ml of the mixture was filtered through 2 µ filter paper and this was run through a Biochrom 20 amino acid analyzer. The data was collected in the form of chromatograms. The analyzer was ion exchange with several buffers at varying pH running through the column. Each sample took about 4 h to run through the system.

Fatty acid analysis

Fats were extracted from the sample and converted to free fatty acids by saponification. The fatty acids were converted to their methyl esters and into heptanes. Internal standards were employed for estimation of actual fatty acids present in the fat. Identification / quantification of fatty acids was achieved by gas chromatography, the former being resolved by elution times (AOAC, 2000).

Statically analysis : Statistical analyses used included correlation coefficient, student t- test and one way analysis of variance (ANOVA). The statistical procedure was adopted from Zar (1998).

RESULTS:

Amino acid composition

There were nineteen different amino acids found in *C.batrachus* ,*C.punctatus* and *A.testudineus*, and total amino acids was recorded as 7.07 mg for *C.batrachus*, 5.19 mg for *C.punctatus* and 7.42 mg for *A.testudineus*. Among all the essential amino acids, Leucine is found to be highest in content i.e. 1.29mg, 0.86mg and 1.38mg/100 gm fish muscle among above three species respectively (Table 1 and figure 1). Leucine is the only dietary amino acid that can stimulate muscle protein synthesis and has important therapeutic role in stress conditions like burn, trauma, and sepsis (De Bandt et al., 2006). Leucine has been found to slow the degradation of muscle tissue by increasing the synthesis of muscle proteins. However, in NEAA; Glutamic acid content was recorded to be highest in *C.batrachus* and *C.punctatus* with value of 2.41mg and 0.79 mg/100 gm tissue whereas, Cysteine value of 2.35 mg was recorded in *A.testudineus*. The total amount of NEAA among the three species were as 9.16 mg, 4.29 mg and 9.58 mg has shown in *C. batrachus*, *C.punctatus* and *A. testudineus* respectively (Table 2 and figure 2). Amino acids were not significantly different among the species. There were no appreciable variations in amino acid composition of fishes of the same species collected from different locations. Arginine plays an important role in cell division, wound healing, ammonia removal, immune function and hormone release. Methionine is used for treating liver disorders, treating depressing, alcoholism, allergies, asthma, radiation and Parkinson's disease (Mischoulon *et al*,.2002) .

Table 1: Composition of different Essential amino acids in fish's muscles (mgg^{-1}).

	ESSENTIAL AMINO ACIDS (EAA)		
Parameters	***Clarias batrachus***	***Channa punctatus***	***Anabas testudineus***
Arginine	0.71	0.87	0.36
Histidine	0.65	0.27	0.73
Lysine	0.69	0.84	0.51
Threonine	0.83	0.53	0.94
Methionine	0.43	0.36	0.29
Leucine	**1.29**	**0.86**	**1.38**
Isoleucine	0.77	0.48	0.91
Valine	1.08	0.51	1.27
Phenyle alanine	0.62	0.47	1.03
Total	**7.07**	**5.19**	**7.42**

Fig 1 : Essential amino acids content in muscle's of fishes .

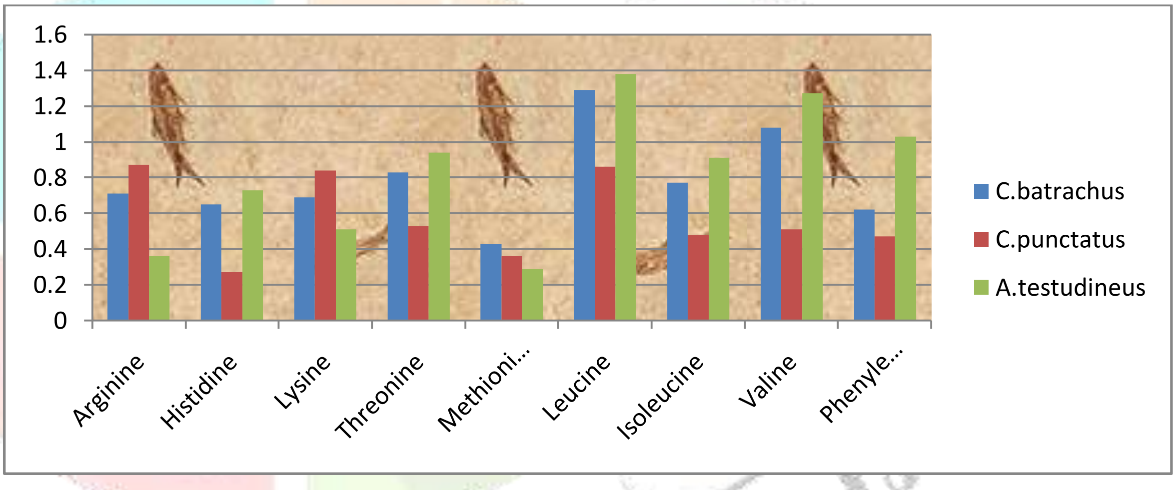

Table 2: Composition of different Non-essential amino acids in fish's muscles (mgg^{-1}).

	NON-ESSENTIAL AMINO ACIDS (NEAA)		
Parameters	***Clarias batrachus***	***Channa punctatus***	***Anabas testudineus***
Tyrosine	0.11	0.41	0.17
Alanine	1.19	0.58	1.33
Proline	0.21	0.37	0.27
Serine	0.85	0.46	0.87
Glycine	2.32	0.56	**2.51**
Aspartic acid	1.85	**0.87**	1.88
Glutamic acid	**2.41**	0.79	2.35
Cysteine	0.04	0.12	0.03
Tryptophan	0.18	0.13	0.17
Glutamine	ND	ND	ND
Total	**9.16**	**4.29**	**9.58**

ND –Not detected

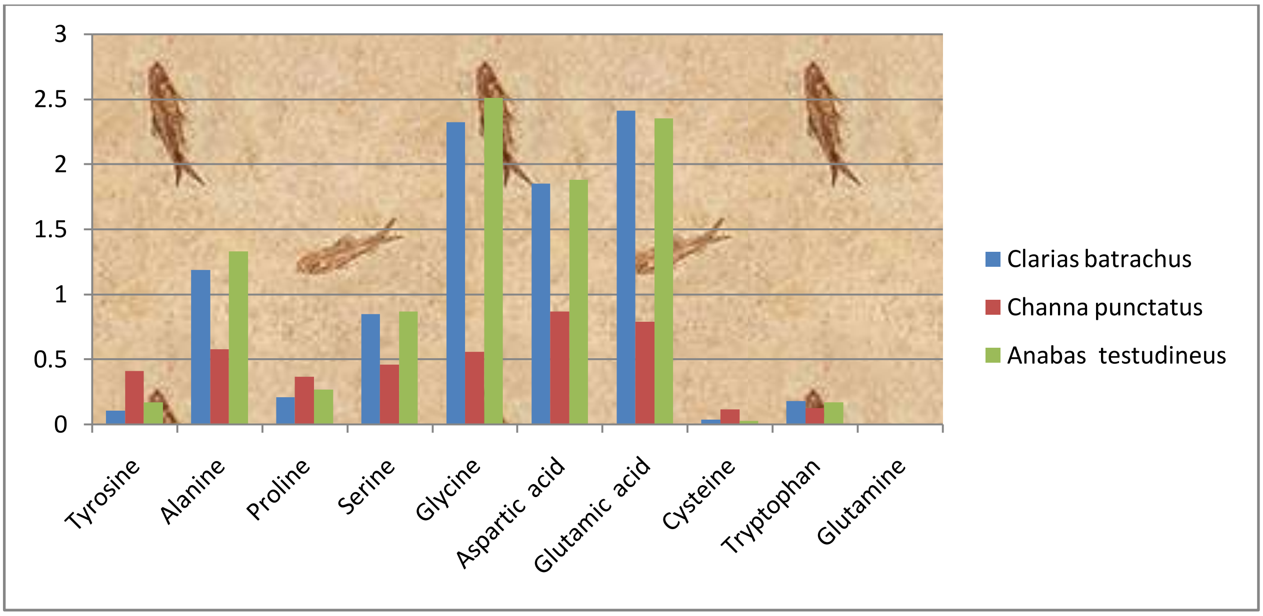

Fig 2: Non-essential amino acids content in muscle's of fishes .

Fatty acid composition of the different species

The compositions of the different fatty acids of the three species are shown in table and fig 3, 4 and 5. The total SFA content among the above three species were found to be 1.909 mg, 3.25mg and 4.283 mg respectively. *C. batrachus* has maximum amount of steric acid C18:0 (0.40mg) and least content is Caprylic acid (C10:0) i.e. 0.004mg. However, Palmitic acid (C16:0) content has recorded 1.07 mg and 3.59 mg among the *C. punctatus* and *A. testudineus* respectively (Table 3 and figure 3). Out of seven monounsaturated fatty acids, Oleic acid (C18:1) content was highest i.e. 1.54 mg / 100gm of muscle tissue of *C.batrachus* and 1.01mg and 0.19 mg present in *C. punctatus* and *A. testudineus* respectively. C18:1n9t content showing highest value of 1.29 mg /100 gm of muscle tissue of *A.testudineus*. The total amount of MUFA were of 2.135mg, 3.65 mg and 1.876 mg among the above respective fishes (Table 4 and figure 4). The PUFA content like Omega 6 (ω 6) is estimated to be 0.89mg, 0.98mg and 0.69mg among the three freshwater teleost *C. batrachus*, *C. punctatus and A. testudineus* respectively. However, the total amount of PUFA has recorded to be 2.99mg , 3.402 mg and 1.975 mg in the muscle tissues of fishes under study (Table 5 and figure 5).

Table 3: Composition of different SFA in fish's muscle

	Saturated fatty acids (SFA)		
No. of carbon atoms	***Clarias batrachus***	***Channa punctatus***	***Anabas testudineus***
C4 :0/Butyric acid	0.005	0.07	ND
C 10:0 / Caprylic acid	0.004	0.24	ND
C11:0 / Undecanoic acid	0.05	0.13	ND
C12:0 / Lauric acid	0.14	0.25	0.06
C13:0 / Tridecanoic acid	0.04	0.06	0.004
C14:0 /Myristic acid	ND	0.09	0.11
C15:0 / Pentadecanoic acid	0.13	0.34	0.05
C16:0/ Palmitic acid	0.36	**1.07**	**3.59**
C17:0/ Heptadecanoic acid	0.29	0.31	0.009
C18:0 / Stearic acid	0.40	0.29	0.41
C20:0 / Arachidic acid	0.04	0.08	0.05
C21:0/Heneicosanoic Acid	**0.45**	0.32	ND
TOTAL	**1.909**	**3.25**	4.283

ND – Not detected

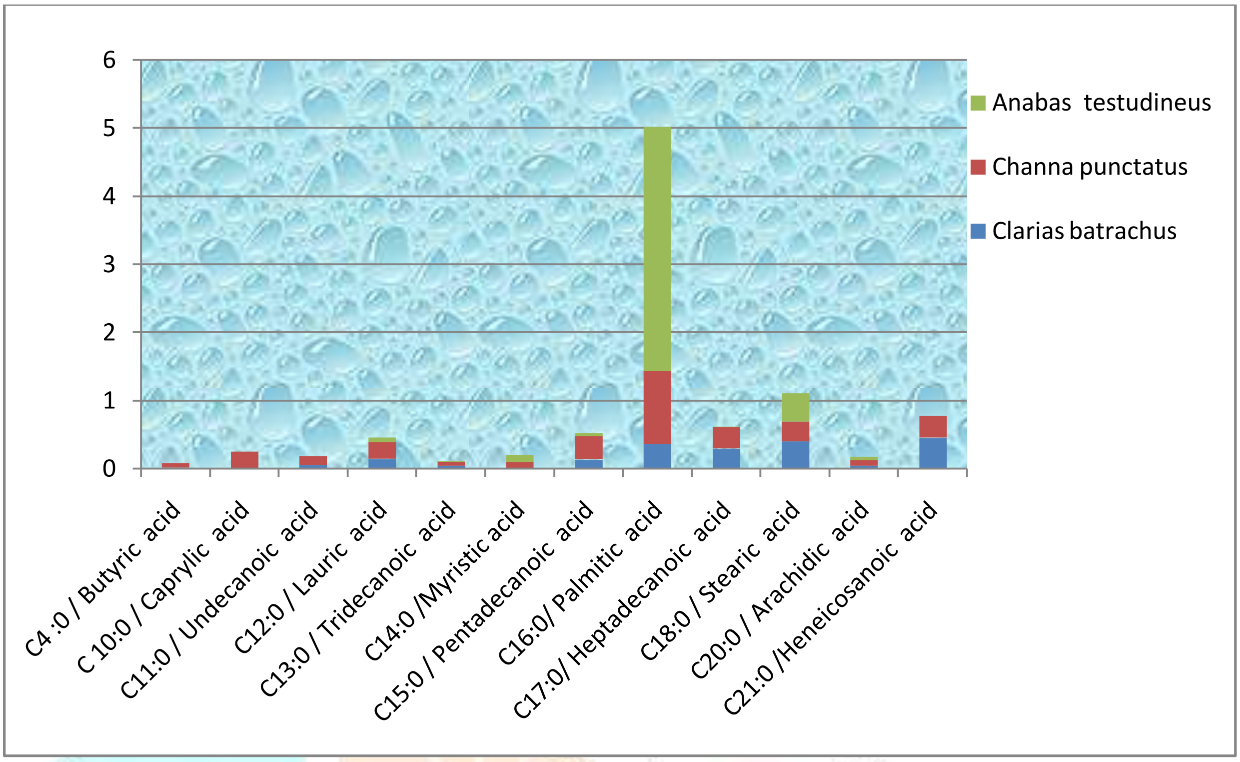

Fig 3: Saturated fatty acid (SFA) content in Fish's muscle .

Table 4: Composition of different MUFA in fish's muscle .

	Mono unsaturated fatty acid (MUFA)		
No. of carbon atoms	***Clarias batrachus***	***Channa punctatus***	***Anabas testudineus***
C 14:1/ Myristoleic acid	0.005	0.08	0.006
C 15:1/Pentadecanoic Acid	0.16	ND	0.08
C 16:1/ Palmitoleic Acid	0.39	0.92	0.20
C 17:1/ Heptadecanoic Acid	0.04	0.09	0.07
C 18:1n9/ Oleic Acid	**1.54**	1.01	0.19
C 18:1n9t / Elaidic Acid	ND	**0.96**	**1.29**
C 20 :1n9c / Eicisanoic Acid	ND	0.59	0.04
TOTAL	**2.135**	**3.65**	**1.876**

ND – Not detected

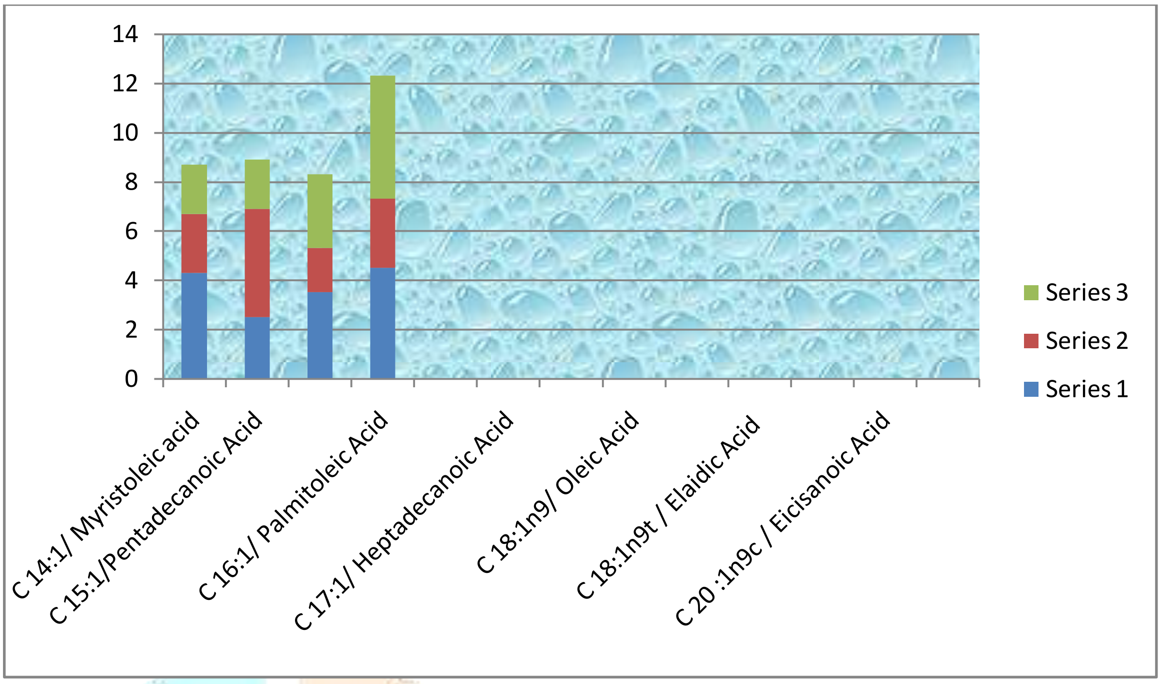

Fig 4: Mono unsaturated fatty acid (MUFA) content in Fish's muscle .

The biochemical content of fish provides information on physiological and nutritive values of fishes but also helps in better management practices in inland fisheries and prevention of fish capture in breeding season to maintain the diversity of fishes .

Table 5: Composition of different PUFA in fish's muscle .

	Poly unsaturated fatty acids (PUFA)		
No. of carbon atoms	***Clarias batrachus***	***Channa punctatus***	***Anabas testudineus***
C 18:2n6c	0.77	0.88	0.58
C 18:2n6t	ND	0.002	0.006
C 18:3n3	0.11	0.23	0.15
C 18 :3n6	0.12	0.48	0.009
C 20 :2n6	0.05	0.06	0.05
C 20 :3n3	0.06	ND	ND
C 20 :3n6	0.03	0.09	0.11
C 20 :5n3	0.19	0.23	ND
C 22 :6n3	0.15	0.41	0.13
ω 3(Alpha linolenic acid)	0.37	0.04	0.25
ω6(Linolenic acid)	**0.89**	**0.98**	**0.69**
EPA + DHA	0.25	ND	ND
TOTAL	**2.99**	**3.402**	**1.975**

ND – Not detected

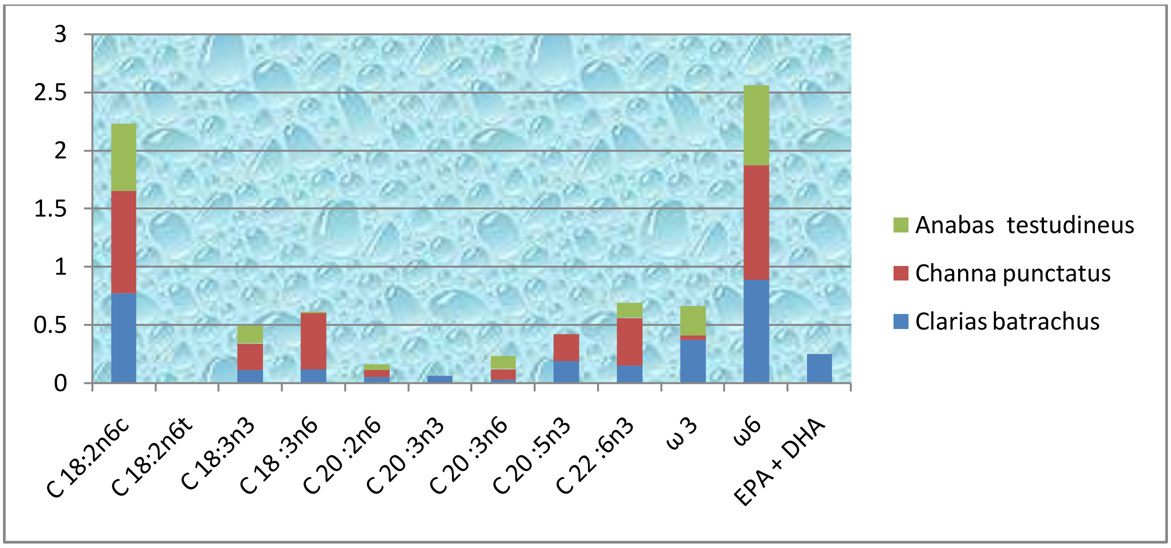

Fig 5: Poly unsaturated fatty acid (PUFA) content in Fish's muscle.

DISSCUSION

Essential amino acids supplements were reported to increase the bone strength, trabecular architecture and cortical thickness (Yin et al., 2005). The dominance of Glutamic acid as a major amino acid reported in this study is similar to previous reports on amino acid composition of channel catfish (Wilson and Poe, 1985). The same amino acids were present in the three fish species analyzed and the fact that there was no significant difference in their concentration may be because it was only the tissues that were analysed in this study. Studies have also shown that concentration and differential distribution of the amino acids depends on the nature of the tissues analyzed (Sadiku and Oladimeji,1989). The amino acid were not significantly different between the species and thus, eating any of these species could provide virtually the same type of amino acids in the diet. This amino acid was found to be more in the collagen that in the muscle (Nelson and Cox, 2005). This may be that freshwater species have more collagen than the marine species. In the present study, the analysis indicated the presence of 9 unsaturated fatty acids including eicosapentanoic acid (EPA) and 4 saturated fatty acids which are being increasingly used to treat and prevent a wide variety of lifestyle related diseases and to improve the quality of life. Our results were similar to the findings recorded by Bimal *et al.*, 2014, Singh *et al.*, 2012 and Tantarpale *et al.*,2011.

The present investigation also reveals the amount of amino acids (EAA, NEAA) and fatty acids (SFA, MUFA, PUFA) content of the muscle tissue among the *C.batrachus* , *C.punctatus* and *A. testudineus*. The fatty acids content varied among the species under study. Previous studies also noted significant difference among the fatty acids profiles (SFA, MUFA, PUFA) of other freshwater and marine fish species (Diraman and Dibeklioglu, 2009). The low concentrations of lipid in the muscle of this species could be due to poor storage mechanism and the use of fat reserves during spawning activities. Although slight mean monthly variations were observed for the dry and rainy season, protein levels were not statistically different in the fishes. The high tissue protein content may result from the equally high protein content of their diets (fish items, crustaceans, molluscs, algae and diatoms). Fatty acid profiles showed that palmitic acid (C16:0) was the predominant saturated fatty acid in the *C.gariepinus*. Ackman (1988) observed that palmitic acid (C16:0) was a key metabolite in fish which level was not influenced by diet. Palmitic acid level (70 % of saturated fatty methyl esters-FAME) observation in this study was higher than that reported for the Atlantic herring. Oleic acid (C18:1), the major monounsaturated fatty acid in this species, was considered to be of exogenous origin and usually a reflection of the type of fish diet (Ackman, 1980). Dutta *et al.*, 1985 reported the lipid, fat and protein content in various organs affected by the species, sex, age, water temperature, degree of pollution, nutritional condition, seasonal variation and its origin too. The above result have similar finding reported by several scientist (Osibona *et al.*, 2006, Edesola Olayinka, 2011). Fatty acid composition of aquatic animals is influenced by intrinsic variables, such as species, sex, age and size; as well as extrinsic factors, such as diet, salinity, temperature, geographical regions, and the general rearing conditions (Sener *et al.*, 2005). Fatty acids in fishes are derived from two main sources, namely, biosynthesis and diet (Kamler *et al.*, 2001).

CONCLUSION :

In the present study, response of the amino acids and fatty acids content was significantly observed. Among amino acid, Leucine, Glycine, Aspartic acid and Glutamic acids content were highly observed in three fishes. Among the SFA, palmitic acids and stearic acids content were found higher in these fishes. Oleic acid and Elaidic acid were significantly higher than other fatty acid among the MUFA. Linolenic acid content was comparatively dominant than other acids among the PUFA. Study results support the established hypothesis of use of above products to assess the nutritional status and starving condition of an organism. *Anabas testudineus* is an ideal dietetic food and its consumption would help prevent nutritional deficiencies and can be commercially exploited.

ACKNOWLEGEMENT

The author is grateful to Head, Post Graduate Department of Zoology, Berhampur University for providing necessary laboratory facilities and valuable support in preparing this manuscript.

REFERENCE

Ackman, R.G. (1980). Fish lipids, part1. In: J. J. Connell, ed. Advances in Fish Sciences and Technology. Pp. 86-103. Fishing News Books Ltd. Farnham, Surrey.

Ackman, R.G. (1988). Concerns for utilization of marine lipids and oils. Food Technology 42: 151-155.

Anilkumar V, Janaiah C and Venkateshwarlu P. (2010). Impact of thiamethoxan on proteases, aminases and glutamated dehydrogenase in some tissue of fresh water fish, Channa punctatus (Bloch). An. Int. J. Life. Sci. 5(1): 135-137.

AOAC (2000), *Official Method of Analysis*, Association of Official Analytical Chemists, Gaithersburg, Md, USA, 17th edition, 2000.

Bimal Mohanty, Arabinda Mohanty (2014). Amino Acid Compositions of 27 Food Fishes and Their Importance in Clinical Nutrition. Hindawi Publishing Corporation . J. of Amino Acids.Vol. 2014:1-7 .

Chamundeshwari Devi and Vijayaragahwan S. (2001) . Biochemical composition of carcass, muscle and liver of Labeo rohita fed on soybean and Glycine based diets with lysine and Methionine. J. Aqua. Bio. 16 (2):81-83.

Dutta H, Das AB, Farkas T (1985). Role of environmental temperature in seasonal changes of fatty acid composition of hepatic lipid in air breathing Indian Teleost, *Channa punctatus* (Bloch). Comp. Biochem. Physiol. 81(B):341-347.

Diraman H., Dibeklioglu H. (2009) : Chemometric characterization and classification of selected freshwater and marine fishes from Turkey based on their fatty acid profiles – J. Am. Oil Chem. Soc. 86: 235-246.

D .Mischoulon and M.Fava .(2002) :" Role of S-adenosyle –L –Methionine in the treatment of depression : a review of the evidence," American journal of clinical Nutrition. Vol 76 , no 5, 2002 .

Edesola Olayinka Osibona (2011). Comparative study of proximate, amino and fatty acids of some economically important fish species in Lagos, Nigeria. African J. of Food Sci. Vol. 5(10): pp. 581-588.

Huynh M.D., Kitts, D.D., Hu C. and Trites A.W. (2007) . Comparions of fatty acids profiles on spawning and non-spawning Pacific herring, Chupea harengus pallasi. Comparative Biochemistry and Physiology., 146: 504-511.

J.-P. De Bandt and L. Cynober, (2006) :"Therapeutic use of branched chain amino acids in burn, trauma, and sepsis," *Journal of Nutrition*, vol. 185, no. 1, pp. 308S–313S.

Kamler, E., Krasicka, B. and Rakusa-Suszczewski, S. (2001). Comparison of Lipid content and fatty acid composition in muscle and liver of two notothenoid fishes from Admiralty Bay (Antartica), and eco-physiological perspective. *Polar Biology*, 24: 735-743

Mason VS, Beck-Anderson S, Rudemo M (1990). Hydrolysate preparation for amino acids determination in feed constituents. Teirnahrg U Lattermittlekde, 43: 146-164.

Nelson DL, Cox MM (2005). Lehninger's Principles of Biochemistry, 4th edition, W.H. Freeman and Company, New York.

Osibona, A.O., Kusemiju, K. and Akande, G.R. (2009). Proximate composition and fatty acids profile of the African Catfish , Clarias gariepinus. acta SATECH Vol. 3(1):85-89.

Padmawati G. and Prema Kumari R. (2006) . Impact of starvation on tissue lipid content in climbing perch, Anabas testudineus. J. Aqua. Biol. 21(2): 147-150

Sadiku SOE, Oladimeji AA (1991). Amino acid composition of some freshwater fish obtain from Zaria dam, Nigeria. Biosci. Res. Comm. , 1(2): 81-86.

Sener, E., Yildiz, M. and Savas, E. (2005). Effects of dietary lipids on growth and fatty acid composition in Russian sturgeon (*Acipenser gueldenstaedtii*) juveniles. *Turkish Journal of Veterinary and Animal Science,* 29: 1101-1107

Singh C.P , Ram R.N ,Mohd.Danish and Anup kumar (2012) . Biochemical modulation in male specimens of channa punctatus (Bloch) under different habitats and season.African journ.of biotech. Vol 11(97) ,16365-16376 .

Tantarpale V.T , Tantarpale S.A , and K.M.Kulkarni 2011 : Impact of changed temperature variations on free amino acids in muscle of fresh water fish *Channa Striatus* . Science research reporter 1(3): 143 -145

Wilson RP, Poe WE (1985). Relationship of whole body and egg amino acid requirement patterns in channel catfish (*Ictalurus punctatus*). Comp. Biochem. Phys. 80B: 385-388.

Yin J. Tezuka Y. Subehan Liying S. Ueda J. Matsushige K. Kadota S (2005). A combination of soft-shell turtle powder and essential oil of a unicellular chorophyte prevents bone loss and decreased bone strength in ovariectomized rats. Biol. Pharm. Bull., 28(2): 275-279.

Zar JH (1998). Biostatistical Analysis. 4th edition . Prentice-Hall International Inc. London. p. 652.