Research Article

Seasonal Variation in the Fatty Acid Composition and Antioxidant Content of Three Air Breathing Fishes of Loktak Lake of Manipur, India

Angom Amala D, Chungkham Sarojnalini*

Department of Life Sciences, Manipur University, Imphal, Manipur, INDIA.

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ABSTRACT

The present study deals with the determination of Fatty acid composition and antioxidant content of three air breathing fishes *Anabas testudineus*, *Clarias batrachus* and *Channa punctata* of Loktak lake of Manipur, India for different seasons. The FAME was prepared from the extracted lipid using boron-triflouride and DPPH radical scavenging method was used to measure antoxidant activity. Fatty acids like SFA, MUFA and PUFA were detected in all the three fishes. SFAs were dominant in all the three fishes. SFAs like stearic acid was dominant in *Anabas testudineus* and *Clarias batrachus* whereas myristic acid was dominant in *Channa punctata* in all the seasons. MUFA like Oleic acid was dominant in *Anabas testudineus* and *Channa punctata* in all the seasons. PUFA like arachidonic acid was dominant in *Clarias batrachus* and *Channa punctata* in all the seasons. Antioxidant was detected in all the three fishes and was recorded higher in winter season in all the three fishes. The quantity of FAs and antioxidant varied in all the three fishes in all the seasons. The results of the present findings could be helpful to the nutritionists, dieticians, researchers, fish farmer, etc for future references.

Keywords: Air breathing fishes, Antioxidant, Fatty acids, Seasonal variation.

Correspondence:
Prof. Chungkham Sarojnalini,
Department of Life Sciences, Manipur University, Imphal-795003, Manipur, INDIA.

Email id: sarojnalini2@gmail.com

INTRODUCTION

Fish is considered as a great food in quality and quantity and a functional food considering principle of reduced risk, enhanced function and basic nutrition, and its validity.[1] Lipid is a macro biomolecule which serves as an energy reserve, helps in hormonal regulation, transmission of nerve impulses, helps in transporting fat-soluble nutrients and act as structural components of cell membranes, etc. Depending on the species, age, size and season, the fat contain of fishes is varied.[2] The calorific value of the fish is contributed by fat which is one of the principal constituent of the fish body and the quantity of fat in the flesh determines the quality and price of the fish.[3]

Observation on several studies have reported that monounsaturated fatty acids (MUFA) have a number of health benefits and there is a relation between an increase intake of monounsaturated fatty acids (MUFA) and a decrease risk of coronary heart disease (CHD), high-MUFA diet reduced LDL cholesterol by 5%,[4] maintain good high-density lipoprotein (HDL) and improves blood vessel function.[5] Polyunsaturated fatty acids (PUFA) are essential fatty acids which cannot be synthesized by the body and depends on food. Omega-3 and Omega-6 are the most common types of PUFA.

Meat and Fishes are prone to oxidation making them off flavor and rancid taste because their flesh content lipids as one of the major component of their body composition. Lipids contain repeating units called Fatty acids which are classified into saturated, monounsaturated and polyunsaturated fatty acids. With the increase in chain length and number of double

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bond, unsatuarated fatty acids increase their reactivity to oxidation.[6] Antioxidant even when present in low concentration can delay or inhibit oxidation.[7]

There are some reports on the high nutrient content in air breathing fishes[8] and some reports on the biochemical analysis and nutrient profiling of the freshwater air breathing fishes.[9,10] There is no report so far on the study of seasonal variation of nutritive values of *Anabas testudineus, Clarias batrachus* and *Channa punctata* of Loktak lake. So, the present study was on the seasonal variation in the nutritional values specifically on fatty acids and antioxidant contents of the three air breathing fishes of Loktak lake of Manipur. Air breathing fishes can survive in land for longer period than other fishes as they contain a special respiratory organ and they are great demand in market of Manipur. There is a popular belief that *Anabas testudineus* has a special nutritive and medicinal quality.[11] *Channa punctata* is recommended as a diet during convalescence[12] and widely consumed in Manipur mostly in smoked form. *Clarias batrachus* in one of the high price fish in the state. Its flesh is soft and red, rich in hemoglobin. It is a believed in Manipur that a curry prepared with *Clarias batrachus* and fermented bamboo shoot is usually given to the night blindness patient to recover from the deficiency disease and also to protect from Cancer.

MATERIALS AND METHODS

Study Site and Sample Collection

The live fish samples were collected from the Loktak lake with the help of local fisherman. The samples were collected during the pre-monsoon (March-May), monsoon (May – August), post-monsoon (September – November) and winter (December – February) seasons of 2017 and 2018. The live fishes were brought to the Fishery Laboratory, Life Sciences Department (Zoology), Manipur University. Six numbers of *Anabas testudineus*, *Clarias batrachus* and *Channa punctata* weight of about 70-80g, 125-155g and 80-90g and standard length of about 16-18cm, 24.5-27cm and 16-18.5cm respectively were used for different seasons. The fishes were washed thoroughly with running tap water, beheaded, eviscerated and the edible muscle parts were taken for various analyses.

Fatty Acid Analysis

Fat Extraction

The lipid content in the muscle was extracted following the modified method of Singh *et al.*[13] 2:1 ratio of chloroform and methanol was used to extract the total lipid content from the tissue in a single phase of solvent.

Fatty acid extraction

2g oil was taken into a round bottom flask and 10 ml alcoholic KOH was added to it. Refluxed for 20 min and cooled to room temperature. The Non-saponifiable matter was extracted with 10 ml hexane or petroleum spirit. Then the fatty acid portion was repeatedly washed with water and passed through anhydrous Sodium Sulphate and evaporated to dryness.

Fatty acid methyl esters preparation

Fatty acid methyl esters (FAME) were prepared from the extracted lipid using boron-triflouride methanol following the method of Metcalfe *et al.*[14] The fatty acid methyl esters was injected into the Gas Chromatograph with high resolution Mass Spectrometer (GC-HRMS: AccuTOF model, GC-Agilent 7890, FID detector, head space injector, combipal autosampler and HRMS-Jeol USA; Library Software-NIST MS Search 2.0.) for analysis of fatty acids.

Antioxidant Analysis

The antioxidant activity of the sample was measured by modified method of Thaipong *et al.*[15] to measure the DPPH radical scavenging activity. In this assay, 100µl (appropriately diluted) of each sample was reacted with 1900µl of 0.1mM DPPH solution prepared in methanol in a test tube and mixed uniformly. The tubes were shaken vigorously and incubated for 1 hr at 25°C in dark. The reduction of stable DPPH free radical (*i.e.* decolourization of purple colour) was read at 517nm against methanol as blank using UV-VIS Spectrophotometer (Prove 300, Merck, USA). A standard curve of ascorbic acid was run and antioxidant activity is expressed as Ascorbic acid equivalent (AAE).

Statistical Analysis

Data were analyzed using SPSS package (version 16.0) and Microsoft Excel (2007). The data were presented as mean ± standard deviation.

RESULTS

The Fatty acid profile of the three air breathing fishes *Anabas testudineus, Clarias batrachus* and *Channa punctata* were analyzed and the results were given in percentage of total area of fatty acids and the result are shown in Table 1, 2 and 3 respectively:

In *Anabas testudineus*, fifteen fatty acids (from C13:0 to C20:4) were identified including seven saturated fatty acids (SFA), five monounsaturated fatty acids (MUFA) and three polyunsaturated fatty acids (PUFA). Saturated fatty acids (SFA) such as pentadecylic acid (C15:0), palmitic acid (C16:0) and stearic acid

Table 1: Fatty acids composition (% of total area) of *Anabas testudineus* for four different seasons.

Fatty acids	Pre-monsoon	Monsoon	Post-monsoon	Winter
C13:0	ND	1.80±0.00	ND	ND
C14:0	ND	1.77±0.00	1.31±0.00	ND
C15:0	1.40±0.00	5.86±0.01	2.07±0.00	1.43±0.00
C16:0	8.58±0.00	15.54±0.63	12.28±0.01	11.22±0.97
C17:0	24.56±0.00	21.57±0.03	ND	26.48±0.00
C18:0	25.68±0.00	22.47±0.00	23.79±0.00	21.87±0.00
C27:0	ND	ND	18.80±0.00	ND
ΣSFA	60.22±0.00	69.01±0.67	58.25±0.01	61.00±0.97
C11:1	ND	2.29±0.00	ND	ND
C16:1	1.24±0.00	1.19±0.00	0.97±0.00	1.11±0.00
C16:1 n7	1.39±0.00	0.50±0.00	0.45±0.00	0.89±0.00
C18:1 n9	3.51±0.06	7.92±0.77	8.32±0.57	11.27±2.00
C22:1 n9	1.31±0.00	ND	ND	ND
ΣMUFA	7.45±0.06	11.9±0.77	9.74±0.57	13.27±2.00
C18:2 n6	0.25±0.00	ND	ND	0.21±0.00
C20:3 n6	6.24±0.00	3.72±0.00	3.86±0.00	5.93±2.58
C20:4 n6	2.74±0.00	4.48±0.00	1.06±0.00	2.33±0.00
ΣPUFA	9.23±0.00	8.20±0.00	4.92±0.00	8.47±2.58

ND- Not Detected
Values are mean of three replicates and expressed in Mean (±SD).

Table 2: Fatty acids composition (% of total area) of *Clarias batrachus* for four different seasons.

Fatty acids	Pre-monsoon	Monsoon	Post-monsoon	Winter
C13:0	ND	ND	ND	3.25±0.85
C14:0	28.98±0.00	1.99±0.00	3.32±0.00	ND
C15:0	2.56±0.00	4.87±0.67	7.69±1.41	11.55±0.00
C16:0	6.89±0.00	4.21±0.00	3.27±0.00	7.73±0.89
C17:0	3.28±0.00	25.93±0.71	26.65±0.00	19.81±0.00
C18:0	23.74±0.00	29.01±0.00	24.61±0.00	15.35±0.00
C27:0	ND	3.98±0.00	ND	ND
ΣSFA	65.45±0.00	69.99±1.38	65.54±1.41	57.69±1.74
C16:1	0.99±0.00	2.03±0.47	1.05±0.00	1.19±0.00
C18:1	ND	ND	ND	2.29±0.00
C18:1 n9	0.22±0.00	3.02±0.00	2.19±0.00	ND
C18:1 n12	ND	ND	ND	0.98±0.00
ΣMUFA	1.21±0.00	5.05±0.47	3.24±0.00	4.46±0.00
C18:2n6	2.12±0.00	2.54±0.00	3.13±0.98	2.16±0.00
C18:3 n3	ND	4.11±0.00	0.83±0.00	ND
C20:3 n6	2.28±0.00	ND	0.79±0.00	6.90±0.00
C20:4 n6	1.59±0.00	8.35±0.00	1.55±0.00	4.27±0.00
ΣPUFA	5.99±0.00	15.00±0.00	6.30±0.98	13.33±0.00

ND- Not Detected
Values are mean of three replicates and expressed in Mean (±SD).

(C18:0); monounsaturated fatty acids (MUFA) such as 7z-Hexadecenoic acid (C16:1), palmitoleic acid (C16:1) and oleic acid (C18:1) and polyunsaturated fatty acids such as DGLA (C20:3) and arachidonic acid (C20:4) were detected in all the seasons (Table 1).

In *Clarias batrachus*, fifteen fatty acids (from C13:0 to C20:4) were identified including seven saturated fatty acids (SFA), four monounsaturated fatty acids (MUFA) and four polyunsaturated fatty acids (PUFA). SFA such as pentadecylic acid (C15:0), palmitic acid (C16:0), margaric acid (C17:0) and stearic acid (C18:0); MUFA such as 7z-Hexadecenoic acid (C16:1) and PUFA such as linoleic acid (C18:2) and arachidonic acid (C20:4) were detected in all the seasons (Table 2).

In *Channa punctata*, eighteen fatty acids (from C10:0 to C20:4) were identified including nine saturated fatty acids (SFA), five monounsaturated fatty acids (MUFA) and four polyunsaturated fatty acids (PUFA). SFA such as myristic acid (C14:0), pentadecylic acid (C15:0), palmitic acid (C16:0) and stearic acid (C18:0); MUFA such as oleic acid (C18:1) and PUFA such as arachidonic acid (C20:4) were detected in all the seasons (Table 3).

Antioxidant content of the three air breathing fishes of the Loktak lake were analyzed and the results were expressed in Ascorbic Acid Equivalent (AAE) and are shown in Table 4.

Table 3: Fatty acids composition (% of total area) of *Channa punctata* for four different seasons.

Fatty acids	Pre-monsoon	Monsoon	Post-monsoon	Winter
C10:0	ND	ND	32.02±0.00	ND
C14:0	25.44±0.00	1.58±0.00	2.26±0.00	32.05±0.00
C15:0	1.69±0.00	2.49±0.00	3.13±0.00	1.50±0.00
C16:0	6.17±0.00	6.72±0.00	16.67±0.00	8.21±0.00
C17:0	0.97±0.00	1.48±0.75	ND	0.84±0.00
C18:0	17.77±0.00	3.24±0.00	2.78±0.00	21.26±0.00
C20:0	1.82±0.00	ND	ND	ND
C22:0	ND	24.55±0.00	ND	1.77±0.00
C27:0	ND	2.44±0.00	ND	ND
ΣSFA	53.86±0.00	42.50±0.00	56.86±0.00	65.63±0.00
C11:1	ND	1.27±0.00	ND	ND
C15:1	ND	ND	1.18±0.00	ND
C16:1	2.35±0.00	2.09±0.00	ND	1.09±0.00
C18:1 n7	1.42±0.00	ND	16.36±0.00	0.78±0.00
C18:1 n9	2.36±0.00	17.91±0.00	2.33±0.00	2.62±0.00
ΣMUFA	6.13±0.00	21.27±0.00	19.87±0.00	4.49±0.00
C18:2 n6	1.47±0.00	2.28±0.00	ND	1.06±0.00
C18:2	ND	ND	1.98±0.00	ND
C20:3 n6	ND	ND	ND	1.68±0.00
C20:4 n6	10.66±0.00	12.28±0.00	1.14±0.00	9.56±0.00
ΣPUFA	12.13±0.00	14.56±0.00	3.12±0.00	12.3±0.00

ND- Not Detected
Values are mean of three replicates and expressed in Mean (±SD).

Table 4: Antioxidant content (µg/100g) of the three air breathing fishes of Loktak lake for four different seasons.

Species	Pre-monsoon	Monsoon	Post-monsoon	Winter
Anabas testudineus	860.05±0.00	750.00±0.00	790.15±0.00	862.18±0.00
Clarias batrachus	830.00±0.00	812.00±0.00	810.20±0.00	840.44±0.00
Channa punctata	880.05±0.00	860.60±0.00	890.00±0.00	910.51±0.00

The antioxidant content ranges from 750.00±0.00 to 862.18±0.00 µg/100g, in *Anabas testudineus*; 810.20±0.00 to 840.44±0.00 µg/100g, in *Clarias batrachus* and 860.60±0.00 to 910.51±0.00 µg/100g, in *Channa punctata*.

DISCUSSION

In the present investigation, fatty acids profile of the three air breathing fishes showed that the SFAs were dominant followed by MUFAs and PUFAs which is not in agreement with the study report of Sarma *et al*.[16] in Rainbow trout but in agreement with the study report in *Mugil cephalus* by Kumaran *et al*.[17] and Ali *et al*.[18] The dominant SFA in all the season was stearic acid (C18:0) in *Anabas testudineus* and *Clarias batrachus* whereas myristic acid was the most dominant SFA in *Channa punctata* which is similar with the findings of Mohanty *et al*.[19] in Hilsa. However, Paul *et al*.[10] reported that the FAs content of *Anabas testudineus* was in the order of MUFA<PUFA<SFA. Age related decline in the function of white blood cells'is related with lack of saturated fatty acid in the body.[20]

The predominant MUFA in *Anabas testudineus* and *Channa punctata* in all the seasons was oleic acid (C18: 1n9) whereas 7z-Hexadecenoic acid (C16:1) was dominant in *Clarias batrachus*. Other workers also reported oleic acid as the most abundant MUFA in their study and stated that, oxidative desaturation of stearic acids results in the formation of oleic acids therefore increased levels of stearic acids could be associated with the composition of oleic acid.[21,22] Oleic acid helps the body in different ways such as maintaining blood sugar balance, act as immune system booster, reduce arthrosclerosis, etc..[21] The value of oleic acid fluctuates in all the seasons in *Anabas testudineus* and *Clarias batrachus* whereas found in highest concentration in monsoon in *Channa punctata*. Vaccenic acid was detected only in *Channa punctata* with highest concentration in post-monsoon season.

And the most abundant PUFA in all the seasons was dihomo-g-linolenic acid (DGLA) (C20:3) in *Anabas testudineus* whereas arachidonic acid (C20:4) was dominant in *Clarias batrachus* and *Channa punctata*. On a review study by Wang *et al*.[23] two oxidative metabolites of DGLA, have been found effective on some diseases such as suppression of chronic inflammation, lowering of blood pressure, inhibition of smooth muscle cell proliferation associated with atherosclerotic plaque development, Cessation of growth and differentiation of tumor cells. Arachidonic acid has been recommended by the Food and Agricultural Organisation (FAO)/ World Health Organisation (WHO) to be added in the infant formula due to its useful role in the development of the central nervous system and retina.[24] Significant variation was observed in the Fatty acids values between the fishes as well as within the fishes. Seasonal variations, fish size, age, area of availability, breeding period, maturity, migration and nutrient availabilty might be the factors which influenced the differences in the values of fatty acid in different fish species.[25,26] Linoleic acid and arachidonic acid are the main type of n-6 PUFA contain in freshwater fishes.[27] Linoleic acid are converted to a substance in the body that regulates inflammation and blood pressure as well as heart, gastrointestinal and kidney functions, also, in human, synthesis of Alpha-linolenic acid (ALA) takes place with the excessive intake of linoleic acid which is also beneficial as Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA).[21] Therefore, inclusion of these three air breathing fishes in the human diet might be useful in wound healing process. Henderson[28] preferentially utilized SFA and MUFA for energy production in the mitochondria. Guner *et al*.[29] stated that nutritional value of fish could be determined with reference to the amount of crude fat and fatty acids.

The antioxidant content for *Anabas testudineus,Clarias batrachus* and *Channa punctata* was recorded higher in winter season. Antioxidant activity was also found in *Esomus danricus* by Sarojnalini and Sarjubala;[30] in fishes of different feeding habit by Sarower *et al*.[31] Antioxidant enzymes level depends on age, nutrition and spawning period of the fish species.[32] The antioxidants plays a very important role in protecting the organism from protein oxidation, breaking of DNA strand and the induction of lipid peroxidation.[33]

CONCLUSION

The study shows these three air breathing fishes obtained from Loktak lake are a good source of healthy nutrition. The fatty acid composition and antioxidant content varied between the different species and among the same species in all the seasons. SFA was found dominant in all the three fishes and high antioxidant content was recorded in winter season in all the fishes. The variation in the nutrient composition might be due to factors such as seasonal changes, availability of foods, breeding seasons, sex and maturity of the fishes. The present findings could be helpful to the nutritionists, dieticians, researchers, fish farmers, etc for future references.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FAs: Fatty acids: **MUFA:** Monounsaturated fatty acid; **PUFA:** Polyunsaturated fatty acid; **SFA:** Saturated fatty acid; **FAME:** Fatty acid methyl esters; **DPPH:** 2,2-diphenyl-1-picryl-hydrazyl-hydrate; **CHD:** Coronary heart disease; **LDL:** Low density lipoprotein; **HDL:** High density lipoprotein; **NIST:** National Institute of Standards and Technology.

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