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Size Dependent Variation in Cholesterol and Fatty Acids Profile of Different Tissues of Carnivore Freshwater Catfish, *Wallago attu*

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ABSTRACT

The purpose of the study was to find out variation in cholesterol and fatty acid content in various tissues *viz*., muscle, skin, liver and skull of two different sized groups of carnivore catfish (*Wallago attu*) *i.e.* small sized group (411.66±10.40 g) and a large sized group (1006.66 ± 8.82 g). Cholesterol contents in different tissues of small and large sized groups of *Wallago attu* showed significant variations ($P<0.05$) and ranged from 40.1 to 183.9 mg/100 g. Higher cholesterol levels were observed in muscle (135.23±12.93 g/100g), skin (102.1±10.76 g/100g) and liver tissues (183.9±13.56 g/100g) of large sized group of *Wallago attu*. Fatty acid analyses were performed on GC-MASS. Fatty acid compositions of different tissues of small and large sized groups of *Wallago attu* consisted of 29.82 to 49.89% saturated fatty acids (SFA), 27.37 to 38% monounsaturated fatty acids (MUFAs) and 17.25 to 39.74% polyunsaturated fatty acids (PUFAs). The SFA and MUFA levels were higher in muscle, skin and skull tissues of small sized group of *Wallago attu*, whereas PUFAs levels were significantly higher ($P<0.001$) in muscle, skin and skull tissues of larger sized group. The Ω-3/Ω-6 ratio in all tissues of *Wallago attu* ranged from 0.60 - 1.79. The percentage of DHA, Docosahexanoic acid (22:6n-3) exceeded that of EPA, Eicosapentanoic acid (20:5n-3) in all tissues of *Wallago attu*. Our present study suggests that *Wallago attu* have high reserves of essential fatty acids like Ω-3 and Ω-6, which provides a strong shield against coronary heart diseases.

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Authors' Contribution
CB and AMY conceived and designed the study. CB executed the experimental work and wrote the article. AK is the corresponding author. All others helped in reviewing the article. AMY supervised the work.

Key words
Fatty acids profile, *Wallago attu*, cholesterol, saturated fatty acids, polyunsaturated fatty acids.

INTRODUCTION

Fish is considered as the best source of the wide variety of minerals, vitamins (Hague, 1992; Edwin *et al.*, 2001) and low energy and high-level protein sources (Weatherley and Gill, 1998), which contain all ten essential amino acids in desirable quantity for human consumption. Fish food has antimicrobial peptides which promote the defense mechanism for protection against invasion of human pathogens (Ravichandran *et al*., 2010). Fish is the principal source of Ω-3 HUFAs, DHA and EPA, which reflects the vital role in different human physiological processes (Cirkovic *et al.*, 2012b).

The influence and contribution of fish to human nutrition have been examined from different aspects and researchers have found that polyunsaturated fatty acids (PUFAs) present in fish lowers the blood pressure and reduce risk of heart diseases (Wang *et al*., 2006). The PUFAs play a significant role in physiological activity of the embryo as it is involved in the formation of retina, brain, muscle etc (Cruzado *et al*., 2011). PUFA is also involved as precursor of functional active molecule prostaglandins and eicosanoids (Jakhar *et al.*, 2012).

Omega-3 PUFAs is useful for the prevention of many diseases and it is a chief component of cell membrane, brain development and visual activity, plays a vital role in prevention of cardiovascular disease, autoimmune disease, in energy production and act as a modulator of inflammation, thrombosis and gene expression (Zibae-Nezahad *et al*., 2010). Omega-3 PUFA has great impact on natural and acquired immunity as well as on the production of inflammatory chemical cytokines (Calder *et al.*, 2002). Omega-3 helps in lowering lipoprotein cholesterol when its synthesis is high (Saify *et al*., 2005). Omega-3 prevents platelets from adhering to one another. It provides prevention from cancer, especially from breast cancer (Gulzar and Zuber, 2000). Fish, meat contains high Ω-3/Ω-6 ratios of PUFAs which is essential for human health (Sharma *et al.*, 2010).

Cholesterol is ubiquitous within the body of fish and other animals (Stickney, 2000). Cholesterol is an important component for eukaryotic cell growth and development (Deng *et al*., 2013). Cholesterol is considered to be an important constituent of brain tissue (Zhang, 2005). Moreover, cholesterol is a precursor of adrenal and reproductive hormones (androgens and estrogens) of vitamin D_3, and of bile acids that facilitate

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dietary lipid digestion and absorption (Stickney, 2000).

Lipid and fatty acid profile of fish muscle has been widely investigated by many authors (Haliloglu and Vilmaz, 2002; Haliloglu *et al.*, 2002, 2004; Hassan *et al.*, 2010. The present study was aimed to assess the fatty acids and cholesterol profile muscle, skin, liver and skull tissues of a favorite food fish catfish (*Wallago attu*) to determine their possible nutritional and therapeutic value. *Wallago attu* is available in the local fish market throughout the year and is famous amongst the locals for its delicious flesh.

The main aims and objective of the present study was to show the variation in the fatty acids and cholesterol contents of various tissues *viz*., muscle, skin, liver and skull of a freshwater catfish *W. attu.*

MATERIALS AND METHODS

Fresh water catfish *W. attu* were procured from Tarbela Dam and then were packed in a cool ice box and transported to PCSIR labs for further analysis. On arrival, the fish specimens were rinsed with tap water, dissected and internal tissues were removed. Different tissues *viz*., skin, muscle, liver and the skull were separated, dried on a filter paper to remove moisture and weighed.

Estimation of lipids and fatty acids

Bligh and Dyer (1959) method was followed for the extraction of lipids from various tissues. For FAMEs analysis, 20- 40 g of sample (extracted oil) was taken in a test tube, to it was added 1.5 ml of methanolic NaOH, heat and boiled at 100ºC for 5 min. Now the sample was cooled and 2.5 ml BF_3 was added to it and again heated and boiled at 100ºC for 30 min and then allowed to stand and cool . After cooling off the sample, 5 ml of brine solution and 1 ml hexane was added to it and vigorously shaken for 2-4 min and then allowed to stand for 2-5 minutes to separate the hexane layer. Now hexane layer was carefully separated with the help of micro pipette in another test tube. 1 ml of hexan was added to the sample containing test tube, shake it vigorously and then wait for a while and extract the hexane layer. Two ml of hexane extract FAMEs was taken and filtered through 0.45 µm filter paper and then 1 µl of it was injected to GC mass and noted the respective peaks. The fatty acid profile was determined as FAMEs and the prepared methyl ester was injected to the gas chromatography equipped with flame ionization detector**.** Fatty acid content was calculated, based on the peak area ratio and expressed as fatty acid/100 g oil.

Estimation of cholesterol

Cholesterol was extracted by AOAC (1995) method. A sample 1-2 gm was taken in a test tube, then to it was added 2 ml KOH solution, 8 ml ethanol and allowed to heat at 80ºC for 30 minutes. The sample was allowed to cool and then to it 3 ml toluene and 3 ml of distilled water and then allowed to stand to separate the layers or simply centrifuged it. The upper toluene layer was removed in a separate test tube and then process was repeated for removal of second toluene layer and the remaining solution was discarded. Now 2 ml of KOH solution (1 M) was added to the test tube containing toluene and was gently swirled. The aqueous layer discarded process repeated for one more time. Then added to it 2 ml of KOH (0.5 M) and shaken gently. Layers got separated. Discard aqueous layer and repeat one more time. Now add 3 ml of distilled water to Toluene extract shake gently and draw water layer. Repeated water washes two times until the toluene layer was crystal clear. Then used sodium sulfate anhydrous to dry and filtered the solution to vial by using 0.45 µm syringe filter.

Statistical analysis

For cholesterol, data are expressed in Mean ± SD (n=3) while for fatty acids data are expressed as percentage of total fatty acids as well as mean ± SD (n=3), and ANOVA followed by Tuckey LSD.

RESULTS AND DISCUSSION

The present study was aimed to assess the size dependant variation in the fatty acids and cholesterol contents of various tissues *viz.*, muscle, skin, liver and skull of highly delicious freshwater catfish *W. attu.* Fish were divided into two groups on the basis of body weight *i.e.* small sized group (411.66±10.40 g) and large sized group (1006.66±8.82 g).

Cholesterol

In *W. attu* (small sized group), muscle was reported to be the primary storage tissue for cholesterol with highest contents (108.83±5.00 mg/100 g). Cholesterol content of muscle was significantly higher from that of skin (86.56±1.56 mg/100 g) and skull (47.36±3.62 mg/100 g) ($P < 0.01$ and $P < 0.001$ respectively). The order of cholesterol level in different tissues was muscle > liver > skin > skull (Table I, Fig. 1). In *W. attu* (large sized group) liver appeared to be the tissue with highest cholesterol content (183.9±13.56 mg/100 g). The tissues were arranged on the basis of cholesterol content in descending order as liver > muscle > skin > skull (Table I). Cholesterol contents in liver were significantly higher ($P < 0.001$) as compared to muscle (135.23±12.93 mg/100 g). Content in muscles, skin and liver increased with the

increase in body weight of fish (Fig. 2). Comparison of small and large sized groups of *W. attu* revealed that cholesterol contents in muscle (135.23±12.93 mg/100 g) and liver (183.9±13.56 mg/100 g) were significantly higher in large sized group ($P<0.05$, $P<0.001$ respectively) (Table I), as well as in small size fish muscle is the main reservoir for cholesterol and in large size fish, liver is main organ.

Table I.- Cholesterol contents (mg/100 g) in different tissues of *W.attu* depending upon body weight.

Mean body wt. (g) of fish	Cholesterol levels			
	Muscle	Skin	Liver	Skull
411.66± 10.40	108.83± 5.00	86.56± 1.56^{b}	98.1± $8.70^{(ns)}$	47.36± 3.62^{c}
1006.66± 8.82	135.23± 12.93*	$102.1\pm 10.76^{(ns),a}$	$183.9\pm 13.56^{***,b}$	$40.1\pm 3.76^{***,c}$

Data are expressed as Mean ± SD (n = 3).
*,0.05, **,0.01, ***,0.001 value vs corresponding tissue in small sized *W. attu*.
[a],0.05, [b],0.01, [c],0.001 value vs muscle content of cholesterol in the same sized

Cirkovic *et al.* (2012b) found cholesterol levels of 33 mg/100 g in catfish (*Silurus glanis*). Memon *et al.* (2010a) determined 125 mg/100g levels in *W. attu* (1500 g body wt of fish) from the Indus River. Cholesterol level in liver ranged from 98.1-183.9 mg/100 g which suggests that the liver is the main storage tissue for cholesterol in many fish species. According to Kinsella, (1987) cholesterol level varied among menhaden, silver salmon and cod liver oil cholesterol concentrations were reported at 521, 485 and 570 mg/100 g of oil, Mullet (2,400 mg/100 g oil) and Ocean perch (2,600 mg/100 g oil) respectively.

Fatty acids

The fatty acid contents in various tissues of small sized group of *W. attu* are shown in Table II. A total of 35 fatty acids were targeted for analysis. Fatty acid contents in muscle, skin, liver and skull of a small and large sized group of *W. attu* is represented in Table II. SFA levels in all tissues ranged from 29.82±0.14% to 49.89 ± 0.02%. In small sized group of *W.attu*, the order of major classes of fatty acids in all tissues was ∑SFA> ∑MUFA> ∑PUFA (Fig. 1). The SFA levels ranged from 40.13±0.05% in skin to 49.89±0.02% in the skull. In large sized group of *W. attu*, the liver, SFA contents of 43.15±0.73% were found to be significantly higher ($P<0.05$) as compared to muscle (34.55±0.10%) which was in turn significantly higher when compared with that of skull (31.53±0.31%). Palmitic acid (C18:0) followed by stearic acid (C18:0) was the most abundant SFA in all tissues. SFA contents in muscle, skin, liver and skull of small sized group of *W. attu* were found significantly higher as compared to the larger group.

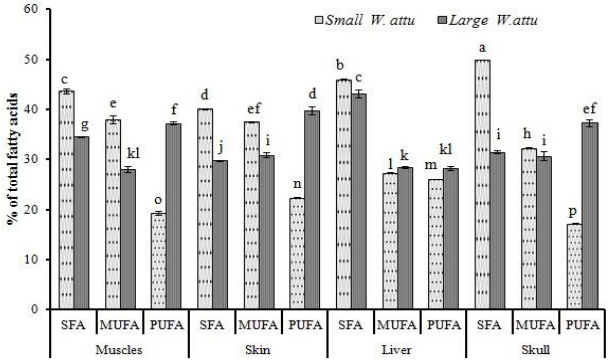

Fig. 1. Major classes of fatty acids in different tissues of small and large sized groups of *W. attu.* Values expressed as Mean ± SE (n=3). Values not sharing a common superscript letter are significantly different ($P<0.05$). ANOVA followed by Tuckey LSD.

SFA contents of 43.67% in muscle tissues in the present study agrees with 40.17% value in *W. attu* from Indus River (Memon *et al.*, 2010a) and 45.22% SFA in muscles of Giant catfish (*Pangasianodon gigas*) (Chaijan *et al.*, 2010). Our findings are in agreement with Luczynska *et al.* (2008) and Ljubojevic *et al.* (2013) where higher SFA contents were reported in predatory fish as compared to non predatory. Similarly Ho and Paul (2009) calculated significantly higher SFA content in Tra catfish (*Pangasius hypophthalmus*) as compared to *Lates calcarifer* and *Salmo solar*. SFA levels decreased with increase in size of fish, which may be because of change in feeding habits of fish. Young ones of *W. attu* are insectivores, while adults are strong carnivores and feed on shrimp, mollusks and fishes (Hossain *et al.*, 2008).

MUFA levels stretched from 27.37±0.10% to 38.00±0.88% in all analyzed tissues. In small sized *W. attu*, MUFA contents in muscle (38.00 ± 0.88%) and skin (37.53±0.08%) were statistically not significant but significantly higher ($P<0.05$) as compared to skull. Whereas large sized *W. attu*, MUFA contents ranged from 28.12±0.64% in muscle to 30.95 ± 0.56% in skin.

MUFAs constitute a main energetic source for development and growth in marine and freshwater fish, especially during the larval stage when fish need energy for tissue ogenesis, metamorphosis, fast growth, and basal metabolism (Abi-Ayad *et al.*, 2004). Our results are

Table II.- Comparison of the fatty acid contents (% of total fatty acids) in different tissues of small and large sized group of *W. attu*

Fatty acids		Muscle		Skin		Liver		Skull	
		Small *W. attu*	**Large** *W. attu*	**Small** *W. attu*	**Large** *W. attu*	**Small** *W. attu*	**Large** *W. attu*	**Small** *W. attu*	**Large** *W. attu*
Hexanoic acid	C6:0	0.18 ± 0.01^{b}	Nd	0.03^{c}	Nd	0.38 ± 0.06^{a}	Nd	0.17 ± 0.03^{b}	Nd
Caprylic acid	C8:0	0.04 ± 0.01^{cd}	0.03 ± 0.01^{cd}	0.01^{d}	0.09 ± 0.03^{cd}	0.54 ± 0.03^{a}	0.11 ± 0.07^{bc}	0.06 ± 0.01^{cd}	0.19 ± 0.12^{b}
Capric acid	C10:0	0.07^{cd}	0.04 ± 0.02^{de}	0.01^{e}	0.11 ± 0.02^{bc}	0.47 ± 0.02^{a}	0.09 ± 0.04^{c}	0.14 ± 0.01^{b}	0.09 ± 0.05^{c}
Undecanoic acid	C11:0	0.01^{c}	0.05 ± 0.03^{bc}	0.01^{c}	0.01^{c}	0.02^{c}	0.07 ± 0.04^{b}	0.02 ± 0.01^{c}	0.23 ± 0.04^{a}
Lauricacid	C12:0	0.30 ± 0.01^{cd}	0.26 ± 0.04^{cd}	0.20 ± 0.01^{d}	0.71 ± 0.03^{b}	2.87 ± 0.09^{a}	0.64 ± 0.14^{b}	0.37 ± 0.03^{c}	0.61 ± 0.08^{b}
Tridecanoic acid	C13:0	0.12 ± 0.01^{bc}	0.08 ± 0.02^{bc}	0.14 ± 0.02^{b}	0.03 ± 0.01^{c}	0.04 ± 0.01^{bc}	0.10 ± 0.05^{bc}	0.06 ± 0.01^{bc}	0.55 ± 0.13^{a}
Myristic acid	C14:0	4.53 ± 0.08^{b}	3.58 ± 0.03^{b}	6.10 ± 0.01^{a}	2.17 ± 0.08^{cd}	3.43 ± 0.05^{bc}	1.17 ± 0.20^{d}	3.45 ± 0.04^{bc}	2.16 ± 0.07^{cd}
Pentadecanoic acid	C15:0	1.83 ± 0.04^{b}	2.27 ± 0.02^{a}	2.19 ± 0.01^{a}	0.70 ± 0.08^{d}	1.43 ± 0.03^{c}	1.80 ± 0.04^{b}	1.68 ± 0.05^{b}	0.50 ± 0.13^{e}
Palmitic acid	C16:0	27.31 ± 0.12^{b}	19.88 ± 0.06^{h}	24.99 ± 0.01^{d}	20.70 ± 0.12^{g}	21.43 ± 0.14^{f}	25.76 ± 0.18^{c}	27.90 ± 0.14^{a}	22.14 ± 0.22^{e}
Margaric acid	C17:0	1.62 ± 0.06^{c}	1.67 ± 0.03^{c}	1.81 ± 0.02^{bc}	0.66 ± 0.09^{d}	1.93 ± 0.04^{ab}	1.63 ± 0.11^{c}	2.03 ± 0.02^{a}	0.80 ± 0.08^{d}
Stearic acid	C18:0	6.66 ± 0.19^{d}	5.48 ± 0.03^{e}	3.91^{f}	4.07 ± 0.05^{f}	12.33 ± 0.14^{a}	10.51 ± 0.16^{c}	11.02 ± 0.05^{b}	3.81 ± 0.15^{f}
Arachidic acid	C20:0	0.37 ± 0.01^{abc}	0.48 ± 0.02^{a}	0.39 ± 0.01^{ab}	0.26 ± 0.02^{c}	0.27 ± 0.02^{bc}	0.37 ± 0.03^{abc}	0.48 ± 0.04^{a}	0.28 ± 0.09^{bc}
Heneicosanoic acid	C21:0	0.05^{b}	0.18 ± 0.03^{a}	0.07 ± 0.01^{b}	0.03 ± 0.01^{b}	0.19 ± 0.01^{a}	0.16 ± 0.05^{a}	0.17 ± 0.03^{a}	Nd
Behenic acid	C22:0	0.27^{bcd}	0.36 ± 0.02^{bc}	0.16 ± 0.01^{d}	0.20 ± 0.03^{d}	0.25 ± 0.02^{cd}	0.36 ± 0.04^{b}	0.81 ± 0.02^{a}	0.17 ± 0.07^{d}
Tricosanoic acid	C23:0	0.07^{b}	Nd	0.01^{c}	Nd	0.15 ± 0.01^{a}	Nd	0.17 ± 0.03^{a}	Nd
Lignoceric acid	C24:0	0.23 ± 0.51^{c}	0.18 ± 0.04^{cd}	0.09 ± 0.01^{d}	0.08 ± 0.01^{d}	0.36 ± 0.01^{b}	0.40 ± 0.08^{b}	1.36 ± 0.02^{a}	Nd
∑ SFA		43.67 ± 0.51^{c}	34.55 ± 0.10^{e}	40.13 ± 0.05^{d}	29.82 ± 0.14^{g}	46.03 ± 0.03^{b}	43.15 ± 0.73^{c}	49.89 ± 0.02^{a}	31.53 ± 0.31^{f}
Myristolic acid	C14:1	3.41 ± 0.20^{a}	2.24 ± 0.03^{b}	0.2 ± 0.02^{e}	0.02 ± 0.01^{e}	Nd	1.27 ± 0.13^{c}	2.22 ± 0.08^{b}	0.78 ± 0.06^{d}
Pentadecenoic acid	C15:1	1.85 ± 0.20^{b}	0.19 ± 0.04^{f}	2.62 ± 0.02^{a}	0.45 ± 0.04^{e}	1.25 ± 0.02^{c}	1.02 ± 0.09^{d}	1.32 ± 0.03^{c}	0.59 ± 0.11^{e}
Palmitoleic acid	C16:1	11.88 ± 0.07^{b}	8.42 ± 0.04^{c}	16.03 ± 0.04^{a}	6.56 ± 0.05^{d}	3.93 ± 0.09^{f}	6.04 ± 0.17^{de}	8.27 ± 0.07^{c}	5.78 ± 0.16^{e}
Heptadecenioc acid	C17:1	1.17 ± 0.02^{ab}	0.13 ± 0.02^{c}	0.13 ± 0.02^{c}	0.43 ± 0.03^{c}	0.97 ± 0.01^{b}	1.40 ± 0.33^{a}	1.02 ± 0.02^{ab}	0.38 ± 0.15^{c}
Oleic acid	C18:1c	12.28 ± 0.12^{d}	9.87 ± 0.07^{f}	13.25 ± 0.06^{c}	20.31 ± 0.28^{a}	13.30 ± 0.02^{c}	10.56 ± 0.22^{e}	12.66 ± 0.05^{d}	19.03 ± 0.10^{b}
Elaidic acid	C18:1n9t	5.53 ± 0.05^{b}	5.24 ± 0.04^{bc}	5.03 ± 0.01^{c}	2.15 ± 0.09^{e}	6.28 ± 0.01^{a}	5.56 ± 0.08^{b}	6.14 ± 0.03^{a}	3.37 ± 0.26^{d}
Eicosenoic acid	C20:1c11	0.94 ± 0.03^{c}	1.74 ± 0.34^{ab}	0.11 ± 0.01^{d}	0.97 ± 0.04^{c}	1.89 ± 0.03^{a}	1.44 ± 0.07^{b}	0.73 ± 0.02^{c}	0.80 ± 0.11^{c}
Errucic acid	C22:1n9	0.77 ± 0.04^{a}	0.10 ± 0.03^{bc}	0.15 ± 0.01^{b}	0.03 ± 0.01^{c}	0.11 ± 0.03^{bc}	0.17 ± 0.03^{b}	0.50 ± 0.02^{a}	Nd
Nervonic acid	C24:1	0.18 ± 0.01^{a}	Nd	Nd	Nd	Nd	Nd	Nd	Nd
∑ MUFA		38 ± 0.88^{a}	28.12 ± 0.64^{d}	37.53 ± 0.08^{a}	30.95 ± 0.56^{c}	27.37 ± 0.10^{d}	28.53 ± 1.21^{d}	32.32 ± 0.06^{b}	30.73 ± 0.95^{c}
Linoleic acid	C18:2n6	2.91 ± 0.03^{f}	9.87 ± 0.09^{b}	3.74 ± 0.05^{e}	9.93 ± 0.05^{b}	4.50 ± 0.05^{d}	6.14 ± 0.05^{c}	2.23 ± 0.04^{g}	14.58 ± 0.50^{a}
Octadecadienoic acid	C18:2t	3.17 ± 0.08^{a}	0.11 ± 0.01^{c}	0.07 ± 0.01^{c}	0.14 ± 0.02^{c}	0.12 ± 0.01^{c}	0.39 ± 0.04^{b}	0.09 ± 0.01^{c}	0.20 ± 0.09^{c}
Linolenic acid	C18:3n3	4.82 ± 0.07^{d}	9.00 ± 0.02^{c}	9.01^{c}	18.28 ± 0.14^{a}	1.88 ± 0.05^{f}	3.31 ± 0.14^{e}	2.40 ± 0.07^{f}	15.40 ± 0.40^{b}
g-linolenic acid	C18:3n6	0.32 ± 0.03^{e}	6.54 ± 0.08^{a}	0.65 ± 0.01^{cd}	2.55 ± 0.06^{b}	0.09 ± 0.02^{f}	0.79 ± 0.04^{c}	0.15 ± 0.02^{f}	0.61 ± 0.08^{d}
Eicosadienoic acid	C20:2c	0.35 ± 0.01^{e}	1.70 ± 0.02^{b}	0.37 ± 0.01^{e}	1.06 ± 0.03^{c}	1.67 ± 0.05^{b}	2.00 ± 0.06^{a}	0.37 ± 0.01^{e}	0.89 ± 0.08^{d}
Eicosatrienoic acid	C20:3n3	0.29 ± 0.02^{d}	1.60 ± 0.03^{ab}	0.48 ± 0.02^{d}	1.44 ± 0.05^{b}	1.15 ± 0.04^{c}	1.77 ± 0.21^{a}	0.28 ± 0.01^{d}	1.06 ± 0.09^{c}
Eicosatrienoic acid	C20:3n6	0.84 ± 0.05^{g}	$1.55\pm0.03d^{e}$	1.23 ± 0.02^{ef}	2.02 ± 0.28^{c}	2.87 ± 0.04^{a}	2.45 ± 0.08^{b}	1.06 ± 0.03^{fg}	1.67 ± 0.13^{cd}
Arachidonic acid	C20:4n6	2.58 ± 0.04^{d}	2.59 ± 0.12^{d}	2.24 ± 0.02^{e}	1.66 ± 0.07^{g}	7.73 ± 0.05^{a}	5.86 ± 0.08^{b}	5.15 ± 0.02^{c}	1.28 ± 0.08^{f}
Eicosapentanoicacid(EPA)	C20:5n3c	1.37 ± 0.07^{b}	0.88 ± 0.02^{c}	2.60 ± 0.02^{a}	0.70 ± 0.03^{d}	0.39 ± 0.09^{e}	0.37 ± 0.04^{e}	0.97 ± 0.02^{c}	0.66 ± 0.09^{d}
Docosahexanoicacid(DHA)	C22:6n3c	2.66 ± 0.12^{e}	3.46 ± 0.05^{d}	1.96 ± 0.01^{f}	1.95 ± 0.08^{f}	5.68^{a}	5.21 ± 0.11^{b}	4.55 ± 0.05^{c}	1.39 ± 0.15^{g}
∑ PUFA		19.33 ± 0.41^{f}	37.30 ± 0.27^{b}	22.33 ± 0.10^{e}	39.74 ± 0.81^{a}	26.07 ± 0.07^{d}	28.31 ± 0.39^{c}	17.25 ± 0.07^{g}	37.34 ± 0.63^{b}

in accordance with findings in catfish *Pangasius hypothalamus* (Muhammad and Mohammad, 2012) and Giant catfish (*Pangasianodon gigas*) (Chaijan *et al.*, 2010). Similarly, SFA and MUFA contents in muscle of large sized *W. attu* were 34.55% and 37.90%, respectively, which are in accordance with those calculated in muscle of two catfishes *Clarias gariepinus* and *Arius argyropleuron* (Suloma *et al.*, 2008).

PUFA levels ranged from 17.25±0.07 to 39.74±0.81% in all tissues. In small sized *W. attu*, PUFA content in liver (26.07±0.07%) was significantly higher ($P<0.01$) as compared to muscle (19.33±0.41%). In large sized *W. attu*, PUFA content in the skin were significantly higher ($P<0.05$) as compared to muscle. However, no significant difference ($P>0.05$) was observed between PUFA levels in muscle and skull. PUFA content in muscle, skin, liver and the skull were found significantly higher in large sized group of *W. attu* as compared to small sized group. The SFA, MUFA and PUFA contents in various tissues of all studied groups in present research are parallel with the ranges of SFA, MUFA and PUFA found in muscle tissues of cyprinids and catfishes from River Indus (Memon *et al.*, 2010a). Significantly higher PUFA contents were seen in muscle (37.30%), skin (39.74%) and skull (37.34%) in large sized carnivorous catfish *W. attu*, which is in accordance with the findings of Bulut (2010) and Kucska *et al.* (2006) in carnivorous pike.

In small sized *W. attu*, the Ω-3 contents ranged from 8.20±0.14% in skull to 14.05±0.04% in skin, whereas in large sized *W. attu*, the Ω-3 contents in the skin (22.37±0.31%) were found to be significantly higher ($P<0.001$) as compared to muscle (14.94±0.12%). Linolenic acid was the predominant Ω-3 PUFA and arachidonic acid appeared to be the most abundant Ω-6 PUFA in all tissues. In small *W. attu*, significantly higher ($P<0.05$) Ω-3/Ω-6 ratio of 1.79 was seen in skin as compared to muscle (1.37) (Table III). In large sized *W. attu*, the SFA/MUFA, SFA/PUFA, MUFA/PUFA ratios were significantly higher in liver as compared to muscle, skin and skull (Table III).

The Ω-3 content of 14.94 ± 0.12% in muscle tissue of large sized group was found significantly higher ($P<0.001$) than muscle content of 9.15 ± 0.28% in small sized group. Similarly Ω-3 and Ω-6 contents in the skin and skull also followed a similar trend with significantly higher levels in large sized group (Fig. 2).

The Ω-3 and Ω-6 PUFAs are primarily supplied in the diet because they cannot be synthesized by the human body (Calder and Yagoob, 2009; Hooper *et al.*, 2009). In our study, Ω-3/Ω-6 ratio in muscle tissue of small sized *W. attu* was 1.37, which is slightly lower as compared to

Table III.- Comparison of the Ω-3, Ω-6 PUFAs and ratios of various fatty acid classes in different tissues of small and large sized group of *W. attu*

	Muscle		Skin		Liver		Skull	
	Small *W. attu*	Large *W. attu*	Small *W. attu*	Large *W. attu*	Small *W. attu*	Large *W. attu*	Small *W. attu*	Large*W. attu*
Total Ω-3 PUFAs	9.15±0.28^{f}	14.94±0.12^{c}	14.05±0.04^{d}	22.37±0.31^{a}	9.10±0.11^{f}	10.66±0.19^{e}	8.20±0.14^{g}	18.50±0.19^{b}
Total Ω-6 PUFAs	6.65±0.12^{g}	20.56±0.16^{a}	7.65±0.07^{f}	16.16±0.59^{c}	15.19±0.09^{d}	15.25±0.19^{d}	8.59±0.06^{e}	18.15±0.61^{b}
Ω-3/Ω-6	1.37±0.03^{b}	0.73^{e}	1.79±0.03^{a}	1.38±0.02^{b}	0.60±0.01^{g}	0.70±0.01^{f}	0.95±0.10^{d}	1.02±0.03^{c}
EPA/DHA	0.52±0.01^{b}	0.25^{d}	1.32±0.01^{a}	0.36^{c}	0.07^{e}	0.07±0.01^{e}	0.21±0.04^{d}	0.48±0.08^{b}
SFA/MUFA	1.15±0.01^{cd}	1.23±0.01^{c}	1.07^{de}	0.96±0.01^{e}	1.66±0.01^{a}	1.51±0.05^{b}	1.52±0.08^{b}	1.03^{e}
SFA/PUFA	2.26±0.03^{b}	0.93±0.01^{e}	1.80±0.01^{c}	0.75±0.02^{g}	1.77^{c}	1.52±0.05^{d}	2.89±0.13^{a}	0.84±0.02^{f}
MUFA/PUFA	1.97^{a}	0.75±0.01^{g}	1.68±0.01^{c}	0.78±0.02^{fg}	1.06±0.01^{d}	1.01±0.01^{e}	1.91±0.22^{b}	0.81±0.02^{f}

Nd, not detected, SFA, saturated fatty acids; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Data are expressed as percentage of total fatty acids. Mean ± SD (n=3). Values within the same row not sharing a common superscript letter are significantly different ($P<0.05$). ANOVA followed by Tuckey LSD.

1.50 ratio calculated in muscle of *W. attu* from River Indus (Memon *et al.,* 2010a). In small sized catfish *W. attu,* Miroslav *et al.* (2011) calculated Ω-3/Ω-6 value of 1.29 in muscle of welsh catfish *Silurus glanis.* Cirkovic *et al.* (2012a) showed slightly lower ratio of 0.45 in catfish *Siluris glanis.* Whereas Osibona *et al.* (2009) reported Ω-3/Ω-6 ratio of 0.39 in African catfish *C. gariepinus.*

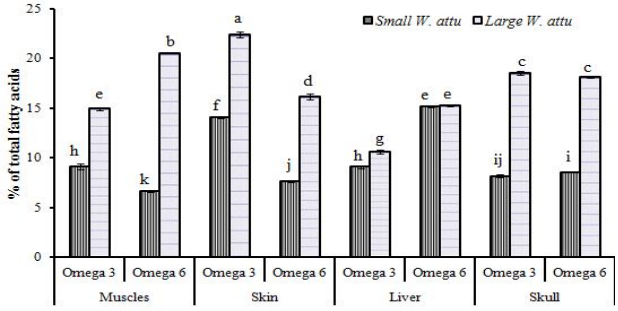

Fig. 2. Ω-3 and Ω-6 PUFAs in different tissues of small and large sized group of *W. attu.* Values expressed as Mean ± SE (n=3). Values not sharing a common superscript letter are significantly different (P<0.05). ANOVA followed by Tuckey LSD.

EPA levels in muscle, skin, liver and skull ranged from 0.39 to2.60%, and DHA ranged from 1.96% to 5.68% in small sized group of *W. attu*. Ho and Paul (2009) found EPA and DHA levels of 0.76% and 10% in Trash catfish (*Pangasius hypophthalmus*). The levels of DHA and EPA are generally higher in seawater fish than in their freshwater counterparts (Haard, 1992), because marine fish species obtain these Ω-3 fatty acid from oceanic plankton (Steffens, 1997), or are fed fishmeal comprising of these fatty acid (Henderson, 1996). Gruger, (1967) after analyzing the fatty acids composition of 95 marine fish species, reported the EPA contents varied from 2.4 to 22% whereas DHA contents varied from 1.3 to 37.5%. Ward and Singh (2005) concluded that DHA is a major component of the eye retina, the brain, heart muscles and also plays a fundamental role in the eye and the brain development.

CONCLUSION

The present study was the first of its nature to evaluate the cholesterol and fatty acid contents in muscle, skin, liver and skull of delicious freshwater fish species, a carnivore catfish *W attu*. Our findings revealed significant differences in cholesterol and fatty acid contents amongst various tissues. Our present study concludes that both fish species have high reserves of essential fatty acids like Ω-3 and Ω-6 PUFAs, which have beneficial effects on health. Moreover, it is evident from our findings that liver, skin and skull of both species have high levels of Ω-3 and Ω-6 PUFAs and other important fatty acids. So, the present study recommends that instead of discarding those tissues, they can be used for the extraction of Ω-3 and Ω-6 PUFAs and can also be used for the production of various materials like cosmetics, pharmaceuticals and biomedical materials.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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