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Size dependent variation in cholesterol and fatty acids profile in different tissues of freshwater cyprinid *Ctenopharyngodon idella*

AUTHOR(S): CHAMAN BIBI[1], ALI MUHAMMAD[1*],

Abstract

The present study was carried out to assess variation in cholesterol and fatty acid content in various tissues viz muscle, skin, liver and skull of two different weight groups of farmed herbivore cyprinid *Ctenopharyngodon idella*. The cholesterol content in muscle, skin, liver and skull tissues of small (400 ± 9.50g) and large sized groups of *C. idella* (1005 ± 13.22 g) ranged from 35.93 to 149.06 mg/100 g. Significantly higher (P<0.01) levels of cholesterol were calculated in liver of *C. idella* as compared to muscle. The fatty acid compositions in muscle, skin, liver and skull tissues of small and large sized groups of *C. idella* ranged from 27.34 - 43.43% saturated fatty acids (SFA), 29.20 - 40.78% monounsaturated fatty acids (MUFAs), and 24.04 - 42.02% polyunsaturated fatty acids (PUFAs). There was a wide variation and significant (P<0.05) differences in the fatty acid profiles of the different tissues. The SFA and MUFA contents were significantly higher (P<0.001) in muscle and skin tissues of large sized group of *C. idella* as compared to levels in respective tissues of small sized *C. idella*. However, PUFA levels were higher in different tissues of small sized group of *C. idella*. The Ω-3/Ω-6 ratio in all tissues of *C. idella* ranged from 0.35 - 1.19. Comparison of Ω-3 and Ω-6 fatty acids showed higher levels for small sized group of *C. idella* and as compared to large sized group. The percentage of DHA (22:6n-3) exceeded that of EPA (20:5n-3) in all tissues of small and larger groups. Our present study suggests that *C. idella* have high reserves of essential fatty acids like Ω-3 and Ω-6 and they can be used in cosmetics, pharmaceuticals and biomedical materials.

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About Author

Author(s): CHAMAN BIBI[1], ALI MUHAMMAD[1*]

[1]DEPARTMENT OF ZOOLOGY, ISLAMIA COLLEGE PESHAWAR KPK, PAKISTAN

*Corresponding author: alifishzoology@yahoo.com

Introduction

Malnutrition and hunger remain among the most important problems facing the world's poor population. A major portion of the global population is currently suffering by one or more kinds of nutrient deficiency. Aquaculture plays an essential role to alleviate these problems. Fish has been considered as an excellent food source and perfect diet for human beings for centuries not only due to its excellent taste, high digestibility, but also because of having higher proportions of unsaturated fatty acids, minerals and essential amino acids for the formation of functional and structural proteins (Hassan *et al*., 2010). Regular consumption of fish can promote the defence mechanism for protection against invasion of human pathogens because fish food has antimicrobial peptide.

Polyunsaturated fatty acids, especially, the Ω-3 and Ω-6 PUFAs are most abundant in fish and are considered as useful for human health (Sharma *et al*., 2010). The Ω-3 PUFAs plays a vital role in photoreception (vision), development and function of the nervous system (brain) and the reproductive system and in lowering the risk of cardiovascular diseases (Memon *et al*., 2010a). The Ω-3 PUFAs are known to be antithrombotic (Calder, 2004; Harris, 2004; Givens *et al*. 2006), cardio-protective (Sanderson *et al*., 2002), anti-atherosclerotic and anti-arrythmitic (Givens *et al*. 2006).

Cholesterol is ubiquitous within the body of fish and other animals (Stickney, 2000). Cholesterol is considered to be an important constituent of brain tissue and cholesterol containing phospholipids (Zhang, 2005). Cholesterol is needed by the body in moderate amount. WHO (1982) recommended the upper limit of cholesterol, which is 300 mg per day, while the American Heart Association has recommended 225 mg per day for adult women and 300 mg per day for adult men (Krzynowek and Murphy, 1987).

The chemical composition of fatty acids in tissues of fish varies among species and individuals, depending on their age, size, gender, diet, environmental conditions, season, and method of capture (Luzia *et al*., 2003; Erkan and Ozden, 2007).

Grass carp (*Ctenopharyngodon idella*) an herbivore cyprinid available in the local fish market throughout the year and is very famous amongst the locals for its delicious flesh. Lipid and fatty acid profile of fish muscle has been widely investigated by many authors (Haliloglu *et al*., 2002 & 2004; Hassan *et al*., 2010; Hammed *et al*., 2013), but data on fatty acid and cholesterol profile of other important tissues like liver, skull and skin is rather scarce. These tissues are regarded as useless and often discarded when fish are prepared for consumption. Furthermore, no data is available on cholesterol and fatty acid profile of skin, liver and skull tissues of *C. idella*, which led to the present effort to evaluate the biochemical indices of those important tissues to determine their possible nutritional and therapeutic value.

Materials and Methods

Fresh water cyprinid *C. idella* was obtained from Sherabad carp hatchery, located 15 km away from Peshawar city on Warsak Road near Mathra village.

Fishes were packed in a cool ice box and transported to PCSIR labs. On arrival, the fish specimens were rinsed with tap water, dissected and internal tissues removed. Different tissues viz skin, muscle, liver and the skull were separated, soaked on a filter paper to remove moisture and weighed.

Extraction of lipids and FAMEs (Fatty Acid Methyl Esters) Preparation

Bligh and Dyer (1959) method was followed for the extraction of lipids from various tissues. For FAMEs analysis, 20- 40 gm of sample (extracted oil) was taken in a test tube, to it was added 1.5 ml of methanolic NaOH and then heat and boiled at 100ºC for 5 minutes. Now the sample was cooled and 2.5 ml BF_3 was added to it and again heated and boiled at 100ºC for 30 minutes 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 minute and then allowed to stand for 2-5 minute to separate the Hexane layer. Now hexane layer was carefully separated with the help of micro pipette in another test tube. Again added 1 ml hexane to the sample containing test tube, shake it vigorously and then wait for a while and extract the hexane layer. 2 ml of hexane and extract FAMEs and allow to filter through 0.45 µm filter paper and then take 1 µl and inject to GC mass and note 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 g fatty acid/100 g oil

Extraction 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 was added 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 layers and the remaining solution was discarded. Now 2 ml of KOH solution (1 M) was added to the test tube containing toluene and gently swirled. The aqueous layer discarded process repeated for one more time. Then added 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. Repeat water washes two times until the toluene layer is crystal clear. Then use sodium sulfate anhydrous to dry. Filter the solution to vial using 0.45 µm syringe filter.

Results

The present study was aimed to analyze the cholesterol contents of various tissues viz muscle, skin, liver and skull of highly delicious freshwater fish specie *C. idella*. Fish were divided into two groups on the basis of body weight i.e. small sized group (400 ± 9.50 g) and large sized group (*C. idella* 1005 ± 13.22 g).

Cholesterol

Cholesterol levels reported in muscle, liver, skin and skull of small and large sized groups of *C. idella* is presented in Table I, Fig I. In *C. idella* (small sized group), cholesterol contents in all analyzed tissues ranged from 24.86 to 149.06 mg/100 g. Liver (149.06 ± 5.00 g/100g) was reported to be the most abundant cholesterol reserve. The cholesterol contents in the liver and the skin were significantly higher ($P < 0.001$, $P < 0.01$ respectively) as compared to muscle. The pattern of cholesterol content was liver > skin > muscle > skull. With the increase in body weight, in *C. idella* (large sized group) cholesterol content of liver (77.2 ± 4.00 mg/100 g) decreased, whereas that of skin (85.7 ± 9.50 mg/100 g) increased as compared to *C. idella* small sized group. The levels in liver, skull and skin were significantly higher ($P<0.01$, $P<0.01$, $P<0.001$ respectively) in large sized *C. idella* when compared with that of muscle. The result showed that cholesterol levels in tissues decreased in order skin > liver > skull > muscle.

The cholesterol content in the skin (85.7 ± 9.50 mg/100 g) and skull (66.6 ± 12.96 mg/100 g) of large sized group of *C. idella* were found significantly higher (P< 0.05 and P<0.01 respectively) as compared to respective tissues in small sized group of *C. idella* (Table I and Fig. I), and hence showed an increase in level with increase in body weight of fish.

Fatty Acids

A total of 35 fatty acids were targeted for analysis on GC mass. The fatty acid contents in various tissues of small and large sized group of *C. idella* are shown in Table II, Fig II. Major classes of fatty acids in muscle, skin and skull of small sized group of *C. idella* were in the order of ∑PUFA> ∑MUFA> ∑SFA. However, in liver the order of major classes of fatty acids was ∑SFA > ∑MUFA> ∑PUFA. The ∑SFA levels in liver (37.45 ± 0.25%) were significantly higher (P<0.01) as compared to muscle. Major classes of fatty acids in the liver and skull of large sized group of *C. idella* were in the order of ∑SFA> ∑PUFA >∑MUFA, in skin ∑MUFA> ∑SFA> ∑PUFA and in muscle ∑SFA> ∑MUFA >∑PUFA. Total SFA contents in various tissues of large sized group of *C. idella* ranged from 32.74 ± 0.81% in skin to 43.43 ± 0.34% in muscle. Palmitic acid (C16:0) was the most abundant SFA in all four tissues of both groups. The SFA contents reported in tissues of large sized group of *C. idella* as compared to small sized group.

In small sized *C. idella*, ∑MUFA contents in all the analyzed tissues ranged from 29.66 ± 0.14% to 34.47 ± 0.66% of the total fatty acids. Oleic acid (C18:1) followed by palmitoleic acid (C16:1c), eladic acid (C18:1n9t) and eicosenoic acid (C20:1) were the major MUFAs in all tissues of both groups. MUFA levels in muscle (32.85 ± 0.25%) of large sized group of *C. idella* were significantly higher (P<0.001) as compared to muscle contents (29.66 ± 0.14%) of the small sized group. A similar trend was also seen in comparison of the skin and skull MUFA contents.

Similarly, in small sized *C. idella*, PUFA levels in muscle (41.59 ± 0.36%) and skin (42.02 ± 0.48%) were statistically not significant (P>0.05) but significantly higher (P<0.05) as compared to liver and skull (Table III). In large sized *C. idella*, PUFA contents ranged from 33.32 ± 0.63% in skull to 24.04 ± 0.14% in muscle. Omega 3 contents were most abundant in skull (17.27 ± 0.41%), which were significantly higher (P<0.05) as compared to liver (12.45 ± 0.10%), which were in turn significantly higher (P<0.05) as compared to muscle. EPA/DHA ratios were statistically comparable (P>0.05) in the skull and liver (Table III).

Significantly higher PUFA levels were reported in muscle, skin and skull tissues of small sized group of *C. idella* when compared with corresponding tissues in large sized group. Comparison of the various fatty acid classes and their ratios is depicted in Table III. The Ω-3 contents in muscle tissue of small sized *C. idella* were 21.01 ± 0.04% and found significantly higher (P<0.005) as compared to muscle contents (7.56 ± 0.03%) in large sized group (Fig. III).

Discussion

Fish is one of the chief sources of vitamins (Cahu *et al*., 2004), essential fatty acids, low levels on saturated fatty acids (Stancheva *et al*., 2010) and has significant cholesterol content (Kannel *et al*., 1995). Cholesterol is ubiquitous within the body of the fish (Stickney, 2000).

In *C. idella* small sized group (wt 400 ± 9.50 g) cholesterol contents reported in various tissues were muscle 43.43 ± 9.50, skin 58.76 ± 3.91, liver 149.06 ± 5.00, skull 24.86 ± 4.96 g/100 g of tissue and in *C. idella* large sized group (wt 1005 ± 13.22 g) muscle 35.93 ± 4.12, skin 85.7 ± 9.50, liver 77.2 ± 4.00, skull, 66.6 ± 12.96 g per 100 g of tissue (Table 01). The majority of the values are within the range set by Piironen *et al.* (2002) i.e 49-743 mg/100 g for cholesterol contents in Finland fishes. Great amount of variations has been observed by

different workers in cholesterol content in lipids of carp and it is in the range of 47 to 120 mg/100 g, depending on husbandry system, harvest season, fish breed and age (Vacha and Tvrzicka, 1995; Bieniarz *et al*., 2001; Kopicova and Vavreinova, 2007; Cirkovic *et al.*, 2011). Our reported values varied which are in accordance with Ackman (1992), who analyzed cholesterol levels of sixteen species of fish and 3 species of crustaceans and detected levels ranged from 15 to 151 mg/100g, and also Mathew *et al*. (1999) analyzed 97 samples of Indian fish and shellfish for cholesterol contents and values varied from 22–148 mg/100 g, and 55% of the values were in the range of 45 and 65 mg/100 g. Criner and Feeley (1972) suggested that fish muscle contained cholesterol contents between 50.0 and 90.0 mg/100 g.

Teshima *et al.* (1988) concluded that cholesterol which is supplied to the gonads stems mainly from the muscle stores (edible tissues) and liver. Cholesterol acts as a precursor to produce cortisol hormones, estradiol, testosterone, progesterone (Bastami *et al*., 2012). Donmez (2009) suggested that fish caught during the spawning season have lower cholesterol and fat levels than usual. So, the high values in immature small sized group of *C. idella* and lower values in the muscles and liver of large sized fertile *C. idella* as compared to Memon *et al*. (2011) and Cirkovic *et al.* (2011), can be attributed to thefact that in our study sampling was done in Monsoon, the breeding season for carps.

Higher levels of cholesterol in liver tissues of *C. idella* were reported in our study and Du *et al*. (2006b) suggested that mesenteric and liver fat tissue are both key energy reserves in fish.

Fatty Acid

The Ω-3 and Ω-6 PUFAs are primarily supplied in the diet because they cannot be synthesized by the human body (Calder *et al*., 2009; Hooper *et al*., 2009). Polyunsaturated fatty acids, especially, the Ω-3 and Ω-6 PUFAs are most abundant in fish and are considered as useful for human health (Sharma *et al*., 2010).

The PUFA content was the highest in muscle, skin, skull and liver tissues of small sized group of *C. idella* as compared to SFA and MUFA. Similar results were reported by Li *et al*. (2011) and Hong *et al*. (2014) in cyprinids. SFA levels in muscle of small sized *C. idella* were 28.76% which agrees with levels detected in *C. catla* (29.25%), *C. mrigala* (27.87%) and *L. rohita* (27.35%) (Memon *et al*., 2011). Similarly, 29.03% SFA contents were reported in muscle of *C. idella* (Cirkovic *et al*., 2011), 28.79 % in *C. carpio*, 33.15% in *H. molitrix* and 32.84% in *C. idella* (Cirkovic *et al*., 2012a). In present study, palmitic acid was the most predominant SFA in all tissues of small and large sized groups of *C. idella*. It has been recognized that palmitic acid is the dominant SFA in all tissues in most bony fishes (Ashtom *et al*., 1993). Similarly, palmitic acid has been reported as the most abundant SFA in many species i.e *C. auratus, C. carpio* (Donmez, 2009), *C. catla*, *C. carpio, L. rohita*, *O. niloticus*, *C. mrigala* (Swapna *et al*., 2010), *L. rohita*, *C. catla*, *C. mrigala* (Memon *et al*., 2011), *C. carpio*, rainbow trout, bream and tench (Luczynska *et al*., 2012), *C. idella* and *H. molitrix* (Li *et al*., 2011; Cirkovic *et al*., 2012a; Hong *et al*., 2014), *C. carpio* (Ljubojevic *et al*., 2013). According to Huynh *et al*. (2007), palmitic acid is rich in metabolic energy required for fish growth and formation of roe in females.

The MUFA contents of 29.66% and 32.85% were reported in the muscle tissue of small and large sized *C. idella* respectively, which is in agreement with 31.34% MUFA levels reported in *C. idella* (Endinkeau and Kiew, 1993), 32.90% in *C. carpio* (Jabeen and Chaudhry, 2011), 31.45% in *Carassius gibleo* (Cakmak *et al*., 2012). Kaneniwa *et al*. (2000) reported that MUFA contents in Chinese major carps ranged from 22.8 - 47.8%.

The oleic acid was the most abundant MUFA in all analyzed tissues of small sized *C. idella*. Oleic acid content of 19.28 ± 0.16% and 19.04 ± 0.07% in muscle of small and large sized *C. idella* respectively are in accordance with findings in various fish species i.e *C. idella* (Endinkeau and Kiew, 1993), *C. carpio* (Jabeen and Chaudhry, 2011; Luczynska *et al*., 2012; Ljubojevic *et al*., 2013). Oleic acid levels usually reflect the type of diet of the fish and have exogenoous origin (Ackman, 1980; Ackman, 1989).

Variations in fatty acid composition of marine and freshwater fishes may originate from many factors such as species (omnivorous, herbivorous or carnivorous), sexual maturity, sex, size, age, reproductive status of fish, geographical location of the catch, water temperature, salinity, feeding and season (Saito *et al*., 1999; Alasalvar *et al*., 2002). In the present study, PUFA contents were higher as compared to SFA and MUFA in muscle, skin, skull and liver of small sized *C. idella* which are parallel with findings in other fish species ie *S. lucioperca* (Jankowska *et al*., 2003; Guler *et al*., 2007; Ozogul *et al*., 2007), *C. carassius* (Donmez, 2009), *C. giblio* (Camak *et al*., 2012). The *H. molitrix* and *C. idella* fed phytoplankton, zoo-plankton, and macrophytes are rich in Ω-3 PUFAs, especially EPA and DHA (Domaizon *et al*., 2000; Steffens *et al*., 2005).

PUFA contents of 24.04% were seen in muscle tissues of large *C. idella*, which agrees with 24.30% in *C. carpio* (Karacal *et al*., 2011). Hong *et al*. (2014) calculated 21.37 - 41.24% range for PUFA contents in muscle tissue of carps. Linolenic acid (C18:2n6) was the most abundant PUFA in all analyzed tissues of small sized *C. idella*. Cirkovic *et al*. (2012a) also found linolenic acid as the major PUFA in *H. molitrix* and *C. idella*. The shortage of α-linolenic acid (18:3 Ω-3, ALA) is responsible for neurological disorders and poor growth (Cundiff *et al*., 2007).

PUFA contents in small sized *C. idella* ranged from 28.05 ± 0.53% to 42.02 ± 0.48%, whereas in mature and large sized *C. idella* ranged between 24.04 ± 0.14 to 33.32 ± 0.63%. *C. idella* were obtained in their breeding season. Our results are in accordance with Stanek *et al*. (2008) who reported significantly lower levels of PUFA i.e 21.73 ± 3.12% during breeding season in *Perca fluvatilis* and suggested that lower levels of PUFA during breeding season results from decomposition of PUFAs.

Whereas, linoleic acid (C18:2n6) was the major PUFA in muscle, skin, liver and skull tissues in large *C. idella* and levels ranged between 6.45 - 14.99%. Linoleic acid comprises a major portion of feeds fed to all species. Its assimilation into adipose tissue and muscle relative to the quantity in the diet is greater than for other fatty acids (Wood *et al*., 2007). Arachidonic acid was found in significantly higher levels in liver of large sized *C. idella* as compared to small sized group of *C. idella*. Arachidonic acid is essential for fish reproduction and participates in gonadal steroidogenesis (Wade *et al*., 1994).

In large sized *C. idella*, Ω-6 levels were significantly higher in muscle, skin and liver. Similar findings were observed by Jean *et al*. (2004) in *C. carpio.* Freshwater fish are usually characterized by high levels of Ω-6 PUFAs, especially arachidonic acids (20:4 n6) and linoleic (18:2 Ω-6) (Huynh and Kitts, 2009; Ozogul and Ozogul, 2007; Osman *et al*., 2001). Since freshwater fish contain lower proportions of long-chain Ω-3 PUFAs than marine fish (Yildiz *et al*., 2006).

In *C. idella*, Ω-3/Ω-6 PUFAs ratio was significantly higher in all tissues of small sized *C. idella*. And the Ω-3/Ω-6 PUFAs ratio ranged from 0.35-1.19. Bieniarz *et al*. (2001) calculated Ω-3/Ω-6 PUFAs ratio of 0.43-1.46 in *C. carpio*. Memon *et al*. (2010a) calculated Ω-3/Ω-6 in the range of 1.15–3.71 in Indus River fishes. Omega 3 contents were significantly higher in all analyzed tissues in small sized *C. idella*. *C. idella* fed on zooplankton, phytoplankton, and

macrophytes are rich in Ω-3 PUFAs, especially DHA and EPAs (Steffans and Wirth, 2005). In present study, Ω-6 PUFAs contents were higher as compared to Ω-3 PUFAs in various tissues of large *C. idella*. Hong *et al*. (2014) also came up with similar results in carps and Li *et al*. (2011) also confirmed our results with his findings in freshwater fishes of China. In large sized *C. idella*, Ω-3/Ω-6 PUFAs ratio in muscle were 0.49, similarly Karacal *et al*. (2011) showed 0.62 - 0.98 PUFAs ratio in muscle of *C. carpio*. Sharma *et al*. (2010) showed Ω-3/Ω-6 ratio of 0.44 in muscle tissue of *C. idella* and 0.48 in *H. molitrix*. Cirkovic *et al*. (2011) reported 0.45 Ω-3/Ω-6 PUFAs ratio in *C. idella*. An increase in the human dietary Ω-3/Ω-6 fatty acid ratio helps to prevent cardiovascular disease due to strong anti-inflammatory effects of Ω-3 fatty acids (Simopoulos, 2008).

EPA and DHA levels in muscle of large *C. idella* were 0.24% and 1.10% respectively. Hong *et al*. (2014) showed EPA and DHA levels of 0.35% and 1.92% in *C. idella*. Whereas levels in other investigated carps were variable and ranged from 0.35-6.94% EPA and 1.92-7.40% DHA.

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).

Conclusion

Our present study concludes that liver, skin and skull tissues of *W. attu* have high levels of Ω3 and Ω6 PUFAs and other important fatty acids. So, the present study recommends that instead of discarding those tissues, they can be used in various industries to produce various materials like cosmetics, pharmaceuticals and biomedical materials.

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

Fish Specie	Mean Body wt (grams)	Cholesterol levels			
		Muscle	Skin	Liver	Skull
C. idella (Small sized group)	400 ± 9.50	43.43 ± 1.41	58.76 ± 3.91^{b}	149.06 ± 5.00^{c}	24.86 ± 4.96^{a}
C. idella (Large sized group)	1005 ±13.22	35.93 ± 4.12*	85.7 ± 9.50*^{c}	77.2±4.00***^{b}	66.6±12.96**^{b}

Data are expressed as Mean ± SD (n = 6).
*=0.05, **=0.01, ***=0.001 value vs corresponding tissue in small sized *C. idella*.
^{a}=0.05, ^{b}=0.01, ^{c}=0.001 value vs muscle content of cholesterol in the same sized specie.
Values having **ns** as superscript are statistically non significant

Values having **ns** as superscript are statistically non significant

Table II. Comparison of the fatty acid content (% of total fatty acids) in different tissues of small and large sized group of *C. idella*

Fatty Acids		Muscle		Skin		Liver		Skull	
		Small *C. idella*	**Large** *C. idella*	**Small** *C. idella*	**Large** *C. idella*	**Small** *C. idella*	**Large** *C. idella*	**Small** *C. idella*	**Large** *C. idella*
Caprylic acid	C8:0	0.06±0.01^{b}	0.04±0.01^{b}	0.18±0.01^{ab}	0.01^{b}	Nd	0.01^{b}	0.29±0.01^{a}	0.02^{b}
Capric acid	C10:0	0.07±0.01^{a}	0.08±0.03^{a}	0.08±0.01^{a}	0.01^{b}	0.03^{b}	0.02^{b}	0.09±0.01^{a}	0.02^{b}
Undecanoic acid	C11:0	0.01^{b}	0.02±0.01^{ab}	0.01^{b}	Nd	0.02^{ab}	0.02±0.01^{ab}	0.05^{a}	0.02^{ab}
Lauric acid	C12:0	0.62±0.01^{b}	0.34±0.06^{cd}	0.54±0.04^{bc}	0.17±0.02^{d}	0.62±0.02^{b}	0.18±0.02^{d}	0.85±0.02^{a}	0.30±0.05^{d}
Tridecanoic acid	C13:0	0.02^{ab}	0.03±0.01^{ab}	0.04^{ab}	0.01^{b}	0.04^{ab}	0.06±0.01^{a}	0.06^{a}	0.02^{ab}
Myristic acid	C14:0	2.19±0.02^{c}	2.72±0.06^{b}	2.23±0.01^{c}	3.68±0.14^{a}	3.81±0.23^{a}	3.87±0.24^{a}	3.71±0.52^{a}	2.47±0.15^{bc}
Pentadecanoic acid	C15:0	0.62±0.01^{b}	0.47±0.01^{bc}	0.59±0.05^{b}	0.43±0.04^{bcd}	0.06^{e}	2.43±0.07^{a}	0.25±0.01^{de}	0.31±0.03^{cd}
Palmitic acid	C16:0	20.33±0.34^{d}	32.85±0.21^{a}	19.16±0.20^{e}	23.75±0.75^{c}	26.53±0.41^{b}	23.06±0.37^{c}	20.04±0.28^{de}	25.80±1.36^{b}
Margaric acid	C17:0	0.66±0.02^{b}	0.49±0.03^{cd}	0.58±0.10^{bc}	0.34±0.03^{ef}	0.11^{f}	2.50±0.03^{a}	0.67±0.01^{b}	0.41±0.14^{de}
Stearic acid	C18:0	3.66±0.21^{d}	5.88±0.03^{b}	3.58±0.29^{d}	4±0.13^{d}	6.05±0.55^{b}	8.27±0.08^{a}	3.67±0.05^{d}	5.07±0.21^{c}
Arachidic acid	C20:0	0.24±0.01^{bc}	0.29±0.01^{b}	0.19±0.05^{c}	0.19±0.10^{c}	0.03^{d}	0.40±0.01^{a}	0.26±0.04^{bc}	0.23±0.06^{bc}
Heneicosanoic acid	C21:0	0.05±0.01^{b}	0.05^{b}	0.05±0.01^{b}	0.03^{c}	0.01^{d}	0.09±0.01^{a}	0.03^{c}	0.03^{c}
Behenic acid	C22:0	0.10±0.01^{bc}	0.14±0.02^{b}	0.09±0.02^{c}	0.09±0.04^{c}	0.04^{d}	0.32±0.01^{a}	0.12±0.01^{bc}	0.10^{bc}
Tricosanoic acid	C23:0	0.04±0.01^{bc}	0.05±0.01^{b}	0.02^{de}	0.03±0.01^{cd}	0.01^{e}	0.10^{a}	0.02^{de}	0.02^{de}
Lignoceric acid	C24:0	0.09±0.01^{cd}	0.10±0.01^{bc}	0.08^{cd}	0.09±0.02^{cd}	0.11^{b}	0.48±0.02^{a}	0.07^{d}	0.08±0.01^{cd}
∑ SFA		28.76±0.38^{g}	43.43±0.34^{a}	27.43±0.48^{h}	32.74±0.81^{e}	37.47±0.25^{c}	41.33±0.46^{b}	30.16±0.11^{f}	34.83±1.26^{d}
Myristolic acid	C14:1	0.02^{cd}	0.12±0.02^{b}	0.02^{cd}	0.11±0.01^{b}	0.05^{cd}	0.18±0.01a	0.02^{cd}	0.07±0.01^{bc}
Pentadecenoic acid	C15:1	0.02±0.01^{e}	0.48±0.03^{a}	Nd	0.21±0.01^{c}	0.04^{e}	0.19±0.01^{d}	0.40±0.02^{b}	0.47±0.01^{a}
Palmitoleic acid	C16:1	6.56±0.05^{f}	7.99±0.05^{d}	6.62±0.01^{f}	10.79±0.03^{a}	9.55±0.45^{c}	10.02±0.05^{b}	6.61±0.09^{f}	7.36±0.57^{e}
Heptadecenioc acid	C17:1	0.05±0.01^{d}	0.28±0.04^{ab}	0.05^{d}	0.34±0.06^{a}	0.07^{d}	0.21±0.02^{bc}	0.06^{d}	0.17±0.01^{c}
Oleic acid	C18:1c	19.28±0.16^{b}	19.04±0.07^{b}	19.95±0.27^{b}	23.04±0.36^{a}	19.89±1.20^{b}	12±0.69^{c}	19.53±0.28^{b}	19.90±1.26^{b}
Elaidic acid	C18:1n9t	2.78±0.03^{d}	3.56±0.16^{c}	2.87±0.22^{d}	4.29±0.52^{b}	4.14±0.15^{b}	5.68±0.25^{a}	2.91±0.04^{d}	2.71±0.23^{d}
Eicosenoic acid	C20:1c	0.91±0.02^{cd}	1.19±0.02^{b}	1.04^{bc}	1.88±0.24^{a}	0.73±0.13^{d}	0.71±0.01^{d}	1.19±0.31^{b}	1.06±0.05^{bc}
Errucic acid	C22:1n9	0.04±0.01^{d}	0.18±0.02^{b}	Nd	0.11±0.01^{c}	Nd	0.22±0.02^{a}	Nd	0.10±0.01^{c}
∑ MUFA		29.66±0.14^{f}	32.82±0.25^{c}	30.55±0.19^{e}	40.78±0.35^{a}	34.47±0.66^{b}	29.20±0.47^{f}	30.73±0.41^{e}	31.85±0.67^{d}
Linoleic acid	C18:2n6	15.95±0.41^{bc}	12.34±0.14^{d}	16.10±0.07^{a}	14.99±0.14^{c}	9.72±0.12^{e}	6.45±0.11^{f}	15.45±0.41^{bc}	12.61±0.47^{d}
Octadecadienoic acid	C18:2t	0.09±0.01^{bc}	0.07±0.01b^{cd}	0.02^{de}	0.09±0.01^{bc}	0.02^{de}	0.16±01^{a}	0.10^{b}	0.04^{cde}
Linolenic acid	C18:3n3	17.23±0.03^{a}	5.76±0.03^{e}	17.41±0.19^{a}	4.45±0.50^{f}	9.34±0.22^{d}	3.81±0.09^{g}	16.38±0.12^{b}	13.04±0.36^{c}
g-linolenic acid	C18:3n6	0.25±0.01^{b}	0.20^{b}	0.25±0.01^{b}	0.18±0.01^{b}	0.03±0.01^{c}	0.39^{a}	0.37±0.12^{a}	0.18±0.01^{b}
Eicosadienoic acid	C20:2c	0.98±0.12^{cd}	0.90±0.01^{d}	1.03±0.03^{c}	1.17±0.10^{b}	0.11±0.01^{f}	1.49±0.04^{a}	1.07±0.13^{bc}	0.76±0.05^{e}
Eicosatrienoic acid	C20:3n3	1.31±0.02^{ab}	0.46±0.01^{ef}	1.39±0.01^{a}	0.38±0.07^{f}	0.89±0.05^{d}	0.47±0.03^{e}	1.28±0.03^{b}	1±0.07^{c}
Eicosatrienoic acid	C20:3n6	1.73±0.02^{a}	1.52±0.04^{b}	1.76±0.01^{a}	1.77±0.16^{a}	0.19±0.01^{d}	1.31±0.02^{c}	1.69±0.07^{a}	1.48±0.02^{b}
Arachidonic acid	C20:4n6	1.58±0.02^{c}	1.36±0.01^{d}	1.44±0.05^{cd}	1.53±0.13^{c}	2.82±0.09^{b}	6.37±0.17^{a}	1.34±0.02^{d}	0.91±0.11^{e}
Eicosapentanoicacid (EPA)	C20:5n3c	0.68±0.06^{d}	0.24±0.03^{e}	0.63±0.02^{d}	0.20±0.02^{e}	0.91±0.05^{b}	1.78±0.03^{a}	0.63±0.02^{d}	0.76±0.05^{c}
Docosahexanoicacid (DHA)	C22:6n3c	1.78±0.01^{e}	1.10±0.04^{g}	1.98±0.22^{d}	1.51±0.03^{f}	4.02±0.15^{b}	6.39±0.16^{a}	1.54±0.06^{f}	2.46±0.05^{c}
Locosadienoic acid	C22:2c	Nd	Nd	Nd	0.11±0.01^{b}	Nd	0.37±0.03^{a}	Nd	Nd

∑ PUFA		41.59±0.36[a]	24.04±0.14[g]	42.02±0.48[a]	26.48±0.48[f]	28.05±0.53[e]	29.46±0.08[d]	39.85±0.28[b]	33.32±0.63[c]

Nd: Not Detected, Saturated Fatty acids SFA; Monounsaturated fatty Acid, MUFA; Polyunsaturated Fatty Acid PUFA. Values are expressed as percentage of total fatty acids. Mean ± SD (n=6). Data within the same row not sharing a common superscript letter are significantly different (P<0.05). ANOVA followed by Tuckey LSD.

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 *C. idella*

	Muscle		Skin		Liver		Skull	
	Small *C. idella*	**Large *C. idella***	**Small *C. idella***	**Large *C. idella***	**Small *C. idella***	**Large *C. idella***	**Small *C. idella***	**Large *C. idella***
Total Ω-3 PUFAs	21.01±0.04^{a}	7.56±0.03^{f}	21.04±0.40^{a}	6.55±0.50^{g}	15.16±0.44^{d}	12.45±0.10^{e}	19.82±0.24^{b}	17.27±0.41^{c}
Total Ω-6 PUFAs	19.51±0.38^{a}	15.42±0.10^{c}	19.56±0.08^{a}	18.48±0.39^{b}	12.76±0.12^{e}	14.52±0.12^{d}	18.85±0.21^{b}	15.17±0.66^{c}
Ω-3/Ω-6	1.08±0.02^{c}	0.49^{e}	1.09±0.02^{bc}	0.35±0.03^{f}	1.19±0.03^{a}	0.86±0.01^{d}	1.05^{c}	1.14±0.06^{b}
EPA/DHA	0.38±0.03^{a}	0.22±0.03^{c}	0.32±0.05^{b}	0.13±0.02^{d}	0.23±0.01^{c}	0.28±0.01^{b}	0.41±0.02^{a}	0.31±0.03^{b}
SFA/MUFA	0.97±0.01^{d}	1.32±0.01^{b}	0.90±0.02^{e}	0.80±0.03^{f}	1.09±0.03^{c}	1.41±0.04^{a}	0.98±0.01^{d}	1.09±0.06^{c}
SFA/PUFA	0.69±0.01^{g}	1.81^{a}	0.65±0.02^{g}	1.24±0.05^{d}	1.34±0.02^{c}	1.40±0.02^{b}	0.76^{f}	1.05±0.06^{e}
MUFA/PUFA	0.71±0.01^{f}	1.37±0.01^{b}	0.73±0.01^{f}	1.54±0.02^{a}	1.23±0.05^{c}	0.99±0.02^{d}	0.77±0.01^{e}	0.96±0.01^{d}

Nd: Not Detected, Saturated Fatty acids SFA; Monounsaturated fatty Acid, MUFA; Polyunsaturated Fatty Acid PUFA. Data are expressed as percentage of total fatty acids. Mean ± SD (n=6). Values within the same row not sharing a common superscript letter are significantly different ($P<0.05$). ANOVA followed by Tuckey LSD.

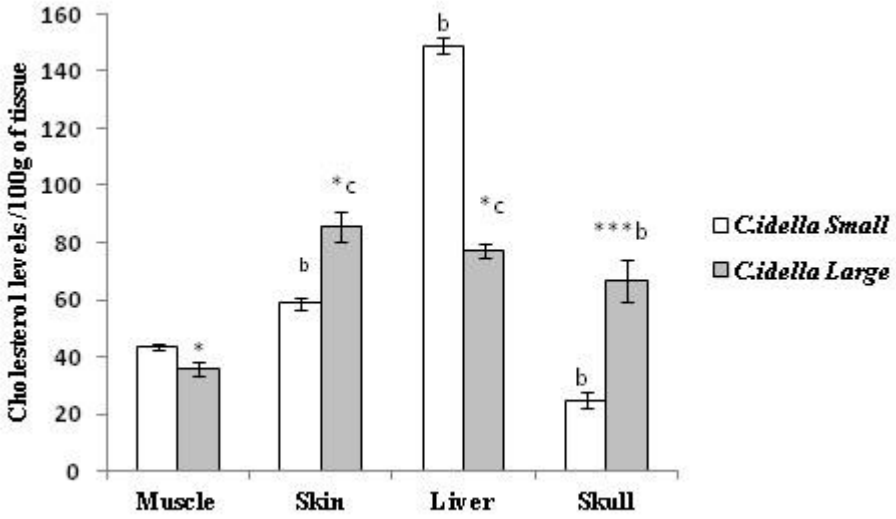


Figure I. Showing the cholesterol content (mg/100 g) in different tissues of *C.idella* (small sized vs large sized group). Each bar represent the values as Mean ± SE. (n=6). (ANOVA followed by Tukey test).
*=0.05, **=0.01, ***=0.001 value vs corresponding tissue in small sized *C. idella*.
[a]=0.05, [b]=0.01, [c]=0.001 value vs muscle content of cholesterol in the same sized specie.

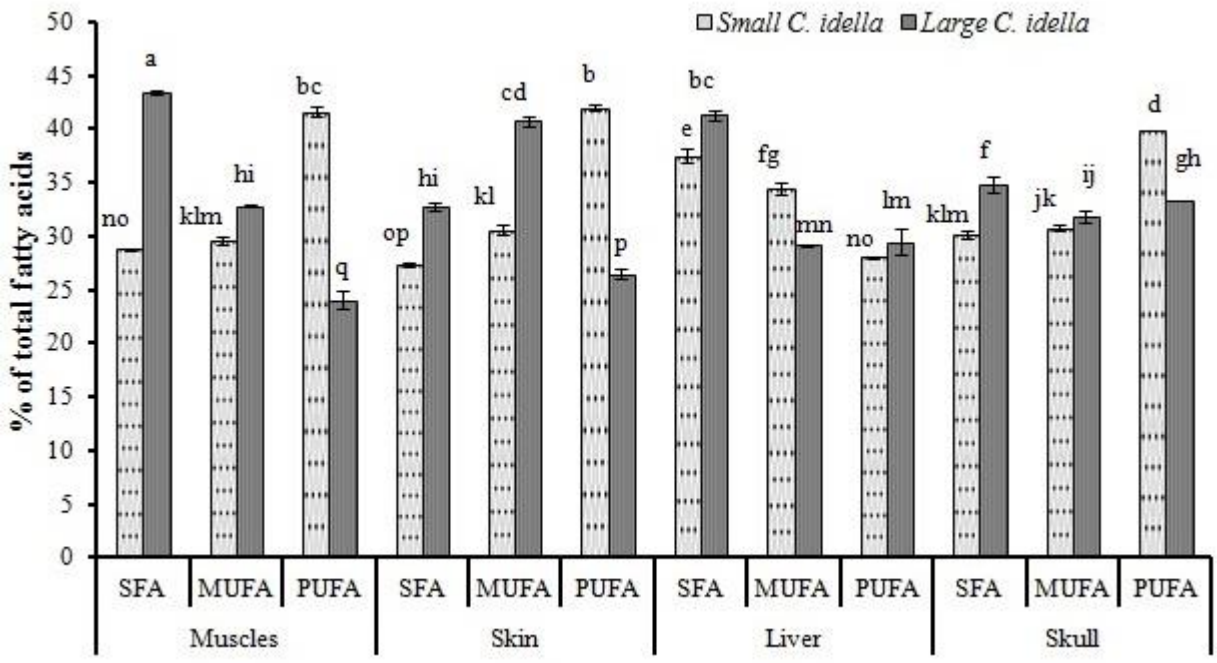
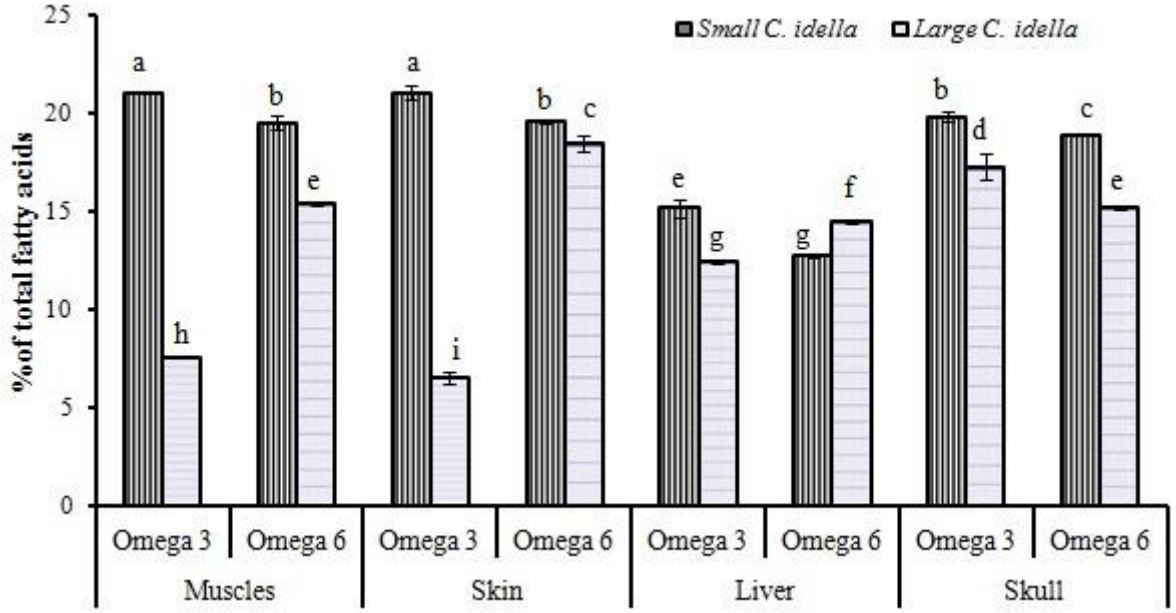


Figure II. Comparison of major classes of fatty acids in different tissues of small and large sized groups of *C. idella.* Values expressed as Mean ± SE (n=6). Values not sharing a common superscript letter are significantly different (P<0.05). ANOVA followed by Tuckey LSD.



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