Research Article

Identification of Fatty Acid Profile of Clariid Catfish Species: *Clarias gariepinus* (Burchell, 1822), *Clarias macrocephalus* (Gunther, 1864) and their Hybrids

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Abstract | This study aimed to evaluate the fatty acids profile of the commercial importance fish species. The fish species include the African catfish (*Clarias gariepinus*), Asian catfish (*Clarias macrocephalus*) and hybrid *C. macrocephalus* X *C. gariepinus* (CMxCG). The fatty acid profiles of fishes were performed using a liquid gas chromatographic examination of methyl esters. The overall mean of saturated fatty acid (SFA) composition was significantly higher (p<0.05) in *C. macrocephalus* (48.21 ± 5.11) as compared *C. gariepinus* (32.15 ± 1.23) and hybrid CMxCG (37.70±0.35). Total monounsaturated fatty acid (MUFAs) composition of *C. macrocephalus*, *C. gariepinus*, and hybrid CMxCG was 32.14 ± 5.86, 45.24 ± 3.21, and 30.89 ±0.80, respectively, where palmitoleic (C16:1) and oleic (C18: 1n-9) acids were the dominating MUFAs. The highest levels of Docosahexaenoic acid (DHA) (C22:6n-3) and Eicosapentaenoic acid (EPA) (C22:5n-3) were observed in hybrid CMxCG (2.42 ± 0.40; 2.02 ± 0.09), respectively, as compared to both parent fish. In terms of essential fatty acids such as EPA and DHA, n-6 PUFA, n-3 PUFA, hybrid CMxCG outperformed *C. macrocephalus* and *C. gariepinus*, indicating that it would lead to a better source of diet for humans.

Keywords | African catfish, Asian catfish, Hybrid catfish, Fatty acids profile, DHA

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INTRODUCTION

Fish flesh is widely preferred by the majority of societies all over the world not only due to its nutritional value and excellent taste but also due to its availability and a high digestibility (Louka et al., 2004). The cholesterol level in fish flesh is also low due to its high content of polyunsaturated fatty acid as compared to meat (Harris, 1997; Stansby, 1985). These fatty acids especially omega-3 fatty acids are crucial for maintaining the integrity of members of all living cells and regulate many body processes such as body clotting and inflammation which can guarantee good health and normal development (Connor, 2000) and are thus often recommended in the human daily diet. Regular consumption of fish has also been associated with a broad range of health benefits including aiding in reducing the risk of cardiovascular diseases (CVD), arthritis. and cancer (Mateos et al., 2011).

Thus, fish receives increased attention from time to time as a potential source of both food and income to many people because of its several health benefits (Martha et al., 2014). In recent years, hybrids of *Clarias* catfish (♀*C. macrocephalus* x ♂*C. gariepinus*) has shown heterosis or hybrid vigor where it appeared with the valuable characteristic for culture traits such as the good taste of *C. macrocephalus* and faster growth rate as well as higher resistance to environmental conditions which is inherited from its paternal species, *C. gariepinus* (Na-Nakorn, 1999). The hybrids are also increasingly produced in public and private hatcheries and are commonly consumed in Malaysia. However, relatively little is known about the nutritional value of their flesh particularly for the hybrid species. Therefore, the objective of this study is to evaluate the fatty acids profile of hybrids ♀*C. macrocephalus* x ♂*C. gariepinus* and its parental species.

MATERIALS AND METHODS

Sample collection

Three specimens of *Clarias gariepinus* (adult size with average age: 4-5 months old; BW: 0.14-0.16 kg) used in this study were obtained from a private farm located in Selangor. These species were 50% fed with commercial pellet fish (35% CP) and 50% poultry by-product meal (boiled chicken visceral organs such as kidney, heart, liver, gizzard and intestine) for feeding daily routine. Another three specimens of *Clarias macrocephalus* (adult size with average age: 9-12 months old; BW: 0.10-0.13kg) were collected from a paddy field and were considered as wild species. Meanwhile, three specimens of hybrid CMxCG (adult size with average age: 5-6 months; BW: 0.13-0.15 kg) were obtained from a cultured tank in the freshwater hatchery of Aquatic Animal Health Unit, UPM. The hybrids of CMxCG were previously produced through artificial propagation and maintained in a one-tonne fiberglass tank until adult size. These hybrids were also fed twice daily with 100% commercial pellet containing 35% crude protein (Star Feedmills (M) Sdn. Bhd. Malaysia) throughout the culture period.

Extraction of total lipids

The total fatty acids were extracted from muscle tissues based on the method described by Folch et al. (1956) with minor modification by Rajion (1985). About 0.5 g of fish muscle tissue samples were placed in glass extraction tubes containing 5 mL of chloroform: Methanol 2:1 (v/v) for homogenizing process using an Ultra-Turrax T5 FU homogenizer (IKA Analysentechnik GmBH, Germany). The tubes were shaken vigorously using vortex for 5 minutes. 5 mL of normal saline solution was added to each extraction tubes. After that, the mixture was centrifuged for one minute at 30 x 100 rpm and allowed to rest for four hours. The aqueous layer on top of the tubes was discarded with a Pasteur pipette, and the organic layer at the bottom of the tubes was collected and transferred into new 10 ml stoppered ground-glass extraction tubes, where it was evaporated at a temperature of 70°C by rotary evaporation (Heidolph GmBH, Germany). The complete lipid extract was then diluted with five mL fresh chloroform-methanol 2:1 (v/v) and immediately transferred to a capped methylation tube.

Preparation of fatty acid methyl esters (FAME)

Transmethylation with 14 percent methanolic boron trifluoride (BF2) was used to make fatty acid methyl esters, according to AOAC Methods (2007). Prior to transmethylation, heneicosanoic acid (21:0) (Sigma Chemical Co., St. Louis, Missouri, USA) was added to each sample for the evaluation of the individual fatty acid concentrations inside the samples. A steady and gentle flow of pure nitrogen gas was applied to the sample extract on the heating block (70°C). After that, the samples were taken off the heating block and allowed to cool to 30-40 °C (20 minutes) before adding 2 mL of potassium hydroxide solution (KOH, 0.66N). These tubes were vortexed for 30 seconds before being put in a heating block at 90 °C for 10 minutes and then allowed to cool.

Gas liquid chromatography

A 7890N gas chromatograph (Agilent Technologies, Santa Clara, CA) with an autosampler fitted with an SP-2330 fused silica capillary column, 30m X 0.25mm ID (0.20 m film thickness) was used to measure fatty acid methyl esters (Supelco, Inc., Bellefonte, PA, USA). An auto sampler injected one microlitre of sample into the chromatograph, which was fitted with a split/splitless injector and a FID detector. At a rate of 40 ml/min, high purity nitrogen was used as the carrier gas. The flame ionisation detector in the gas-liquid chromatography was made of high purity hydrogen (Dominick Hunter, Parker Hannifin ltd, UK) and compressed air (Malaysian Oxygen Bhd., Malaysia). The injector temperature was set to 250 °C, and the flame ionisation detector temperature was set to 300 °C. To allow optimum separation, the column temperature programme started at 100°C for 2 minutes, then the temperature was increased to 170°C at 10°C/min for 2 minutes, and lastly increased to 220°C at 7.5°C/min for 2 minutes, and then further held for another 20 minutes. Fatty acid detection was achieved by comparing relative FAME peak retention times of samples to Sigma standards (St. Louis, MO, USA). The variations in FA composition were determined using gravimetric measurements and a normalised percentage (percent) of total FA. A personal computer integrator was used to assess and calibrate peak areas (Hewlett-Packard, Avondale, PA). A programmed PC under Microsoft Excel 2000 (Microsoft Corp., Redmond, USA) was used to obtain automatic expression of the peak areas as absolute and percentage quantities of a detected fatty acid. The

Table 1: Fatty acid profiles of *Clarias macrocephalus*, *Clarias gariepinus*, and hybrids CM x CG.

Fatty acids	*C. macrocephalus* (n=6)	*C. gariepinus* (n=6)	Hybrid CM x CG (n=6)
Lauric acid (C12:0)	2.32 ± 0.49[a]	0.95 ± 0.09[b]	2.94 ± 0.57[a]
Myristic acid (C14:0)	0.94 ± 0.32[a]	0.28 ± 0.26[b]	0.81 ± 0.30[ab]
Palmitic acid (C16:0)	30.74 ± 5.61	20.62 ± 0.51	25.58 ± 0.68
Stearic acid (C18:0)	14.20 ± 2.42[a]	6.31 ± 0.49[b]	8.37 ± 0.34[b]
Σ Saturated fatty acid (SFA)	48.21±5.11[a]	32.15±1.23[b]	37.70±0.35[b]
Palmitoleic acid (C16:1)	5.06 ± 0.80	4.41 ± 1.31	4.22 ± 0.57
Cis-9-Oleic acid (C18: 1n-9)	27.08 ± 5.10[a]	40.82 ± 2.02[b]	26.67 ± 1.15[a]
Σ Monounsaturated fatty acid (MUFA)	32.14 ± 5.86[a]	45.24 ± 3.21[b]	30.89 ±0.80[a]
Cis-9,12-Linoleic acid (C18:2n-6)	10.30 ± 0.34[a]	19.10 ± 1.05[b]	22.22 ± 0.73[c]
α-Linoleinic acid (18:3n-3)	3.06 ± 0.63[a]	1.32 ± 0.74[b]	3.25 ± 0.65[a]
Arachidonic acid (C20:4n-6)	1.91 ± 0.81[a]	0.83 ± 0.29[b]	1.17 ± 0.08[ab]
cis-5,8,11,14,17- Eicosapentaenoic acid (C20:5n-3) EPA	1.67 ± 0.42[ab]	1.22 ± 0.35[a]	2.02 ± 0.09[b]
cis-5,8,11,14,17- Eicosapentaenoic acid (C22:5n-3) EPA	0.68 ± 0.48[a]	0.04 ± 0.03[b]	0.33 ± 0.06[ab]
4,7,10,13,16,19-Docosahexaenoic acid (C22:6n-3) DHA	2.03 ± 1.12[a]	0.10 ± 0.02[b]	2.42 ± 0.40[b]
n-6 PUFA	12.21 ±0.77[a]	19.93 ± 1.29[b]	23.39 ± 0.68[c]
n-3 PUFA	7.45 ± 0.65[a]	2.68 ± 0.98[b]	8.01 ± 0.76[a]
n-6/n-3	1.64 ± 0.06[a]	7.97± 2.18[b]	2.94 ±0.32[c]
n-3/n-6	0.61 ± 0.02[a]	0.13 ± 0.04[b]	0.34 ± 0.04[c]
Σ Polyunsaturated fatty acid (PUFA)	19.65 ± 1.39[a]	22.61 ± 2.11[a]	31.41 ± 0.94[b]

Notes: [a, b, c, ab] Mean values ± standard deviation (SD) within the same row with different superscripts are significantly different ($p<0.05$).

volume of fatty acid was shown by the relative proportions (normalised percentages of the total fatty acids) (Alfaia et al., 2006), while the actual amount of fatty acids in tissues, which is linked to dietary intake was showed by the gravimetric concentration.

Statistical analysis

SPSS 17.0 was used to perform statistical analysis on the results. Meanwhile, an HP-3393A Integrator (Hewlett-Packard, Avondale, PA) was used to achieve peak areas for fatty acids. The peak areas were represented as the absolute amount of detected fatty acids using a Microsoft Corp., Redmond, USA). The significant difference ($p<0.05$) for each parameter evaluated in this analysis was calculated using Post-Hoc Tukey's tests.

RESULTS AND DISCUSSION

In this study, fatty acid profiles of *Clarias macrocephalus*, *Clarias gariepinus* and hybrids CMxCG was shown in the Table 1. the value of saturated fatty acid (SFA) was found to be the highest in *C. macrocephalus* (48.21 ± 5.11%), whereas PUFA was dominant in the hybrid CMxCG (31.41 ± 0.94%). In contrast, the percentage of SFA was the lowest in *C. gariepinus* (32.15 ± 1.23%) and PUFA was the lowest in *C. macrocephalus* (19.65 ± 1.39%). Meanwhile, the MUFA accounted highest value in *C. gariepinus* (45.24 ± 3.21%) followed by *C. macrocephalus* (32.14± 5.86%) and lowest in hybrid CMxCG (30.89± 0.80%). The high percentage value of SFA in *C. macrocephalus* is considerably higher than the study done by (Tao et al., 2012) on the same fish species with the reported value of 26.24 ± 0.12%. In different studies, total SFA contents range between 42.63-46.5% for Tra catfish (*P. hypopthalmus*) which was almost similar to the mean value of SFA recorded in this study (Men et al., 2005; Ho and Paul, 2009). Furthermore, SFA content was found to be high in all of Saudi Arabia's most significant fish species, ranging from 34.19 ± 1.70% in golden threadfin bream (*Nemipterus japonicus*) to 54.67 ± 3.61% in grey mullet (*Liza ramada*) (Shady et al., 2016). The different values of the total SFA amount recorded in all fish species in this current work can be attributed to the fact that the fatty acid composition of the fish can be affected by many parameters such as biological variations, environmental conditions, diet, and seasonal changes (Tao et al., 2012).

Palmitic acid (C16:0) was found to be the most abundant SFA in muscle among all the fish species examined, accounting for 30.74 ± 5.61 %, 20.62 ± 0.51%, and 25.58 ± 0.68 % of total saturated fatty acids in the lipids for *C. macrocephalus*, *C. gariepinus*, and hybrid CMxCG,

respectively. Palmitic acid was present in the highest proportion in the SFA population of both marine and freshwater animals, as found in raw and hot-smoked sturgeon (*Huso huso*, L. 1758) (Kaya et al., 2008), hybrid sturgeon (*Acipenser naccarii* x *Acipenser baerii*) (Vaccaro et al., 2008), hybrid trout (*Salvelinus fontinalis* x *Salmo trutta labrax*) (Sahin et al., 2011) and freshwater rainbow trout (*Oncorhynchus mykiss*) (Haliloglu et al., 2004). According to (Mohanty et al., 2016), palmitic acid was considered fundamental to most of the metabolic processes in fish and other aquatic animals.

Another major type of saturated fatty acid that were found to be present at the second-highest level in this study is stearic acid (C18:0). For instance, stearic acid values ranged between 6.31± 0.49% to 14.20 ± 2.42% in all fish samples. Similar results were observed in a previous study performed by (Ibhadon et al., 2015) on juvenile pond catfish (PCF_{ju}). The authors concluded that a high amount of stearic acid PCF_{ju} reflects a resultant low amount of oleic acid (C18:1n-9) which was commonly identified as the major monounsaturated fatty acid (MUFA), since stearic acid is a precursor of oleic acid (George, 1995). On the other hand, it is evident from this present study that *C. macrocephalus*, *C. gariepinus* and hybrid CMxCG have a high amount of SFA than MUFA. The proportion of SFA and MUFA in total fatty acids is in agreement with the reports of several authors in earlier research (Ozogul and Ozogul, 2007; Wangcharoen et al., 2015; Paul et al., 2016). Furthermore, the findings revealed that lauric acid (C12:0) and myristic acid (C14:0) were in the third and fourth-order, of SFA, respectively. Myristic acid can be found in dietary fats, plant oils, and marine animals (Ibhadon et al., 2015).

Of the three fish species analysed, hybrid CMxCG (31.41±0.94%) had the highest percentage of PUFA, while the percentage amount of PUFA for *C. macrocephalus* and *C. gariepinus* were 19.65±1.39% and 22.61±2.11%, respectively. Cis-9, 12-Linoleic acid (C18:-2n-6) was the major PUFA in all studied fish species with the highest value recorded in hybrid CMxCG (22.22±0.73%). The highest percentage of linoleic acid in hybrid CMxCG is a good sign in this case of crossbreeding because linoleic acid was particularly beneficial for metabolism as well as for human the immune system. Other than that, docosahexaenoic acid (C22:6n-3) was another notable fatty acid in the PUFA fraction of all analysed fish. The high PUFA content of catfish in the present experiment is in agreement with the similar work performed on other freshwater fish (Kenari et al., 2009; Swapna et al., 2010; Paul et al., 2016). There are many studies reported on the beneficial effects of PUFA in various chronic diseases such as cardiovascular disease, high blood pressure, diabetes, autoimmune disorder, cancer and inflammatory ailments (Simopoulos, 2002). Due to this advantage in curing illnesses, PUFA was considered as essential in the human diet to improve health conditions.

Emphasis on the contents of n-3 fatty acids, especially docosahexaenoic acid (22:6n-3, DHA) and eicosapentaenoic acid (20:5n-3, EPA), crude lipids of the hybrids CMxCG (2.42±0.40%; 2.02±0.09%) could be considered as the best as compared to *C. gariepinus* (0.10 ± 0.02%; 1.22±0.35%) and *C. macrocephalus* (2.03±1.12%; 1.67±0.42%). Through the results observed in this study, it showed that changes in the compositions of fish or other animal lipids could be manipulated through crossbreeding and this is in agreement with the study in other commercialized freshwater catfish (Wangcharoen *et al.*, 2015). All fish species in this study were a good source for EPA and DHA, but the content was generally low as compared to fishes from marine counterparts such as *Tenualosa toli* (Terubok), *Rastrelliger kanagurta* (Kembung) and *Stolephorus baganensis* (Bilis) which contain a high amount of omega-3 fatty acids in their tissue muscle due to consumption of oceanic plankton in their daily diet (Steffens, 1997; Airina and Jamaludin, 2012). Other than size, age, climate, and season the fish feed was the major factor that influenced the type and amount of fatty acids in the fish muscles in previous studies (Ackman, 1989; Satio *et al.*, 1999; Shady *et al.*, 2016). Apart from that, the highest PUFA/SFA ratio was found to be 0.83 for hybrid CMxCG, 0.70 for *C. gariepinus*, and 0.50 for *C. ma*crocepahalus, with the lowest value coming from *C. macrocepahalus* (0.41). The UK Department of Health recommends that the PUFA/SFA ratio be greater than 0.4, which was lower than the values found in all fish species in this study.

The ratio of n6/n3 obtained in the current study also varied considerably and showed a significant differences between all fish species with the highest value obtained from *C. gariepinus* (7.97±2.18%) exceeded the maximum value recommended by HMSO (1994). Values higher than recommended value may cause cardiovascular disease which is harmful to health (Moreira *et al.*, 2001). As for hybrid CMxCG and *C. macrocephalus*, the ratio of n6/n3 was lower which were 2.94±0.32% and 1.64±0.06%, respectively, indicating the beneficial side of this species and their hybrid. It was noteworthy that the hybrid CMxCG muscle in this present study had a comparable fatty acid profile, which was not seen in previous reports on this species.

CONCLUSIONS AND RECOMMENDATIONS

In comparison to *C. macrocephalus* and *C. gariepinus*, hybrid CMxCG had higher nutritional values in terms of essential fatty acids like EPA, DHA, n-6 PUFA and n-3 PUFA. As a result of this research, it is concluded that aquaculture

of a combination of *C. macrocephalus* and *C. gariepinus* is beneficial to the future aquaculture industry and it will lead to a better source of diet for humans.

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NOVELTY STATEMENT

Indeed, at present, little work has been carried out on the nutritional value of the flesh of hybrid CMxCG and is still in its infancy. Therefore, it is sensible to dedicate a research work on fatty acid profiles of hybrids CMxCG and its parental species for better utilisation of global aquaculture demand.

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AUTHOR'S CONTRIBUTION

All authors provided critical feedback and contributed equally in the interpreatation of data, manuscript writing, and approved the final manuscript.

Conflict of interest

Authors declare that there in no conflict of interest.

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