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Original Article

EFFECT OF COMMON CARP AND AFRICAN CATFISH OILS ON RATS FED ON HIGH-FAT DIET

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ABSTRACT

Objective: The present study was designed to study the effect of common carp and African catfish oils on the lipid profiles and liver functions in rats fed on high-fat diets, also fatty acids were assessed in both oils by gas-liquid chromatography (GLC).

Methods: A biological experiment on rats was designed to raise fat in the diet and to study the effect of treatment with common carp and African catfish oils for 6 w (by the stomach tube) and then evaluate their impact on blood lipid profile and liver functions (Alanine transaminase [ALT], aspartate transaminase [AST] total protein and albumin).

Results: GLC analysis of fatty acids (FAs) revealed the presence of oleic, palmitic, palmitolic, and linoleic acids more than 70% from total fatty acids in two oils. High-fat diet resulted in a significant increase in plasma lipid profile as well as liver functions. The treatment of rats fed high fat diets with common carp and African catfish oils resulted in a significant decrease in levels of triglycerides (TGs), total cholesterol (TC), low-denisty lipioprotein cholesterol (LDL-c), total cholesterol (TC)/high-denisty lipoprotein cholesterol (HDL-c) and TGs/HDL-c ratios compared with positive control group, while the same treatment with these oils resulted in a significant increase in HDL-c levels compared with positive control group. The intake of common carp and African catfish oils also significantly reduced ALT and AST activities compared with positive control group. Common carp and African catfish oils showed an anti-hyperlipidemic effect in rats feeding on hypercholesterolemic diets.

Conclusion: It could be concluded that these fish oils have a promising role in reducing the harmful effects of high-fat diets.

Keywords: Common carp, African catfish, Oils, High fat diet, Lipid profile

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INTRODUCTION

Excess fats in our diets are a major source of cardiovascular diseases, atherosclerosis, obesity and fatty liver [1-6]. Many previous studies have confirmed that dieting play an important role in lowering blood lipids and reducing the harmful effects of saturated fat and cholesterol [7-10].

Fish consumption has increased in recent years because of the critical increment in the populace. Fish are important sources of high-value protein, essential fatty acids (omega 3 fatty acids), important minerals (selenium and iodine) and vitamins (vitamin D and vitamin B-12) [11]. Commonly, freshwater species show a more prominent ability to desaturate and elongate fatty acids in contrast with their marine partners which are accounted for to be generally wasteful in this process [12-13].

Common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*) have attracted attention as a promising species for fish culture in different countries and also in Egypt due to its rapid growth and easiness of breeding [14-15]. Many researchers [16-18] have studied the fats found in both two fish species and found that they contain a high amount of unsaturated fatty acids, especially oleic acid (omega 9) and linoleic acid (omega 6), as well as not a few amount of omega 3 fatty acids family [α-linolenic, eicosapentaenoic (EPA) and docosahexaenoic acids (DHA)]. In this study, we focused on identify of fatty acid profiles and fat soluble vitamins in common carp and African catfish oils and studying the hypo-lipidemic effect of these oils.

MATERIALS AND METHODS

Sample procedure

The fishes were identified by the exterior shape in the department of Poultry production, Faculty of Agriculture, Menoufia University.

Samples of African catfish (*Clarias garipinus L*), and common carp (*Cyprinus carpio L*) were obtained from a local fish market (Menoufia, Egypt) during the autumn of 2016. They were eviscerated, washed and immediately transported to the laboratory in ice containing boxes. Fresh fish were washed with tap water three times to expel blood, they were then arranged utilizing basic family rehearses, for example, expelling head, spine, skin, tail and balance yielding two filets. The fillets parts of African catfish and common carp were cut into small pieces, allowed to dry in a hot air oven at 65 °C for 96 h, grounded into a powder state using a commercial blender.

Chemicals and reagents

Sodium sulphate, sodium hydroxide, sodium chloride, potassium hydroxide, ascorbic acid, hexane, isopropanol, chloroform, methanol, ethanol and acetonitrile were obtained from El-Gomhoria Company, cairo, Egypt.

Kits of triglycerides (TGs), total cholesterol (TC) and HDL-c, were obtained from Spinreact Co. Girona (Spain) and kits for total protein, albumin and ALT, AST enzymes activity were obtained from Diamond Company, Cairo, Egypt.

Lipid analysis

Lipid extraction

Lipids from fish tissues were extracted by the hexane-isopropanol method [19]. Samples (5 g) were homogenized in 75 ml hexane: isopropanol (3:2, v/v) using an Wise-Tis homogenizer (Daihan scientific company, Korea). Non-lipids were removed by adding 32.5 ml 6.67% sodium sulphate to each sample. Samples were shaken, centrifuged and evaporated. Dried samples were dissolved in chloroform and stored in-20 °C.

Preparation of fatty acids methyl esters (FAME)

Extracted lipids were methylated following the procedure of [20]. 2 ml 0.01 M NaOH in dry methanol was added to each lipid sample.

The samples were shaken then heated for 10 min on a heating block at 60 ° C. Next 3 ml BF_3 reagent (20% boron trifluoride-methanol complex) was added and the samples were reheated for 10 min. The samples were then cooled and 2 ml 20% NaCl and 2 ml hexane were added. The test tubes were shaken vigorously then centrifuged for 5 min at 2500 rpm at 18 °C. The FAME was transferred to small test tubes and evaporated under nitrogen gas. The dried samples were dissolved in hexane and stored at-20 °C until gas chromatography analyses.

Gas chromatography (GC)

Fatty acids methyl esters were analysed with a gas chromatograph Perkin Elmer Auto System XL (National Research Center, Cairo, Egypt) equipped with flame ionization detector. The samples (1 µl) were injected by autosampler, split mode. The split ratio 1:10 was used. The column temperature was programmed to start at 158 °C for 5 min and then increase at 2 °C/min from 158 ° to 220 ° C and remain at 220 ° C for 10 min. Injector and detector temperatures were 230 ° and 250 ° C, respectively. Fatty acids were identified by comparison with the standard mixture and retention times. Peak areas were integrated. The carrier gas was helium (23 cm/s, flow rate 0.7 ml/min).

High-performance liquid chromatography (HPLC) analysis for retinol, cholecalciferol and α-tocopherol

Retinol, cholecalciferol and α-tocopherol were determined with HPLC using a Shimadzu LC 20 AT, SPD-20 UV Visible detector and SIL 20A autosampler. The HPLC column was a TM 5 µM C18, 25 cm x 4.6 mm. Identification and quantification were done by using external standards. For the analysis of retinol, cholecalciferol and α-tocopherol a modified method by [21] was used. 20 mg lipid was extracted in 2 ml ethanol then 1.2 ml of 20% ascorbic acid solution, 0.6 ml methanol and 1.2 ml of KOH-water (1:1) were added to each tube. After saponification and cooling, retinol, cholecalciferol and α-tocopherol were extracted in 2 times 4 ml of hexane. The hexane-vitamin solution was evaporated under nitrogen gas and diluted with the mobile phase. The mobile phase used consisted of 95% metanol: acetonitrile (1:1) and 5% chloroform with a flow rate of 1.2 ml/min. α-tocopherol and retinol were detected with excitation wavelengths of 290 and 344 nm, respectively, and with emission wavelengths of 327 and 472 nm, respectively. Cholecalciferol was monitored by UV detection at 265 nm.

***In vivo* study**

Animals, diets and blood sampling

The work was carried out at animal house in the Research Institute of Ophthalmology (Giza, Egypt). To study the effect of common carp and African catfish oils on lipid profiles and liver functions of albino rats, thirty-two male albino rats (weighting between 150 and 170 g) were used for the investigation. The rats were obtained from the Research Institute of Ophthalmology (Giza, Egypt).

The rats were fed *ad lipitum* on standard diet and water for two weeks as an adaption period. They were housed individually in stainless steel cages and divided into four groups of eight animals. Negative control group (NC) was fed on standard diet (SD); while the other three groups fed the hypercholesterolemic diet (HCD) was designed as reported by [22] as follow: positive control group (PC) kept without any treatments, common carp oil group (CCO) treated by stomach tube (5g/Kg body weight as daily basis) with common carp oil and African catfish group (ACO) treated by stomach tube (5g/Kg body weight as daily basis) with African catfish oil.

Their food intake was monitored daily and all the rats fasted for 12 h before blood sampling. The blood samples were drawn from eye plexuses after six weeks. The rats were anaesthetized using diethyl ether. The weight gain of the rats was recorded weekly.

Blood samples were collected in tubes containing ethylene diaminetetraacetic acid (EDTA) and centrifuged at 3600 rpm at 5 °C for 15 min, and the plasma was transferred to new tubes and stored at 4 °C.

Table 1: Compositions of standard diet (SD) and hypercholesterolemic diet (HCD)

Ingredients (%)	SD	HCD
Starch	47	41.25
Sucrose	23	17.5
Casein	12.5	12.5
Corn oil	10	-
Salts mixture	3.5	3.5
Vitamins mixture	1	1
Fiber	3	3
Coconut oil	-	20
Cholesterol	-	1
Cholic acid	-	0.25

Plasma biochemistry

Plasma levels of triglycerides (TGs) were analyzed according to [23]. The total cholesterol (TC) was analyzed according to [24]. HDL-c was determined and LDL-c was calculated according to [25]. Total cholesterol/HDL-c and TGs/HDL-c ratios were calculated. ALT and AST activities were measured according to the method described by [26]. Total protein was determined according to [27]. Albumin was determined according to [28].

Statistical analysis

The results of the animal experiments were expressed as the mean±SD and they were analyzed statistically using the one-way analysis of variance (ANOVA) followed by Duncan's test.

RESULTS AND DISCUSSION

Fatty acid profile of common carp and African catfish oils:

Fish lipid content and fatty acids composition varies significantly depending on many factors such as fish species, season, locality, and life phase [29-31].

The fatty acid composition of common carp and African catfish oils is presented in table 2. These data denoted that, in the common carp oil seventeen fatty acids were identified, whereas eighteen fatty acids were identified in the African catfish oil. The most dominant fatty acids in both fish oils were oleic (C18:1 n-9), palmitic (C16:0) and linoleic (C18:2 n-6) and they presented the following distribution oleic > palmitic > linoleic in common carp oil; while the distribution in African catfish oil were palmitic > oleic > linoleic. These results are in agreement with [32-34] for catfish oil and [35-36] for common carp oil.

Linoleic acid (C18:2 n-6) was the primary n-6 fatty acid, 18.65 and 14.66% for common carp and African catfish oils, respectively. Omega 3 fatty acids (n-3 fatty acids) identified in common carp oil were α-linolenic acid (1.68%), EPA (2.44%), DHA (2.35%) and eicosatrienoic acid (0.85%) which are in agreement with [37-38]; while in African catfish oil were DHA (2.99%), EPA (2.34%), α-linolenic acid (1.78%) and eicosatrienoic acid (0.69%) which are in line with data observed for catfish oil in earlier investigations [33-34].

There are two important reasons for variation in EPA and DHA contents in fish oils the first is: fish diet and second is: fish are wild or fish farms [39].

The presence of EPA, DHA and arachidonic acid in two fish oils in current study can be explained in light of the concept of [40-41] who suggested that freshwater fishes have a high capacity for bioconversion of α-linolenic acid (C18:3 n-3) and linoleic acid (C18:2 n-6) into higher unsaturated fatty acid EPA, DHA and arachidonic acid, respectively.

Table 2: Levels of fatty acids (FAs) (%) in common carp (CC) and African catfish (AC) oils

FAs		CC oil	AC oil
C14:0	Myristic acid	1.11±0.021	3.41±0.16
C16:0	Palmitic acid	19.25±0.11	23.75±0.12
C18:0	Stearic acid	6.33±0.11	4.98±0.1
C20:0	Arachidic acid	0.44±0.05	1.21±0.095
C22:0	Docosanoic acid	0.25±0.04	0.77±0.056
C: 24:0	Lignoceric acid	ND	0.88±0.055
Σ Saturated fatty acids		27.39±0.33	34.99±0.58
C16:1 (n-9)	Palmitolic acid	6.23±0.95	15.63±0.025
C18:1 (n-9)	Oleic acid	33.56±0.49	19.87±0.08
C20:1 (n-9)	Gadoleic acid	2.83±0.15	0.57±0.05
C22:1 (n-9)	Erucic acid	0.47±0.06	0.38±0.04
Σ Monounsaturated fatty acids		43.09±0.38	36.45±0.15
C18:2 (n-6)	Linoleic acid	18.65±0.092	14.66±0.1
C18:3 (n-6)	γ-Linolenic acid	0.37±0.051	0.87±0.03
C20:3 (n-6)	Dihomoγ-linolenic acid	1.07±0.05	1.11±0.025
C20:4 (n-6)	Arachidonic acid	1.13±0.55	1.66±0.09
Σ n-6 fatty acids		21.22±0.047	18.31±0.25
C18:3 (n-3)	α-linolenic acid	1.68±0.095	1.78±0.045
C20:3 (n-3)	Eicosatrienoic acid	0.85±0.04	0.69±0.035
C20:5 (n-3)	Eicosapentaenoic acid (EPA)	2.44±0.044	2.34±0.03
C22:6 (n-3)	Docosahexaenoic acid (DHA)	2.35±0.12	2.99±0.14
Σ n-3 fatty acids		7.32±0.29	7.79±0.24

Values are mean±standard deviation (SD) of three samples.

Fat-soluble vitamins composition of common carp and African catfish oils

The amounts of retinol, cholecalciferol, and α-tocopherol contents are presented in table 3 in µg/100 g; the results were expressed as mean and standard deviations.

Common carp oil contained a large amount (1576 µg/g) of α-tocopherol, while low levels of retinol (33.63 µg/g) and cholecalciferol (17.49 µg/g) were measured. In African catfish oil distribution of fat-soluble vitamins were α-tocopherol (1240 µg/g) followed by retinol (17.97 µg/g) and cholecalciferol (8.5 µg/g). This means that both two fish oils are good sources of α-tocopherols which may play important role in protecting unsaturated fatty acids from peroxidation.

These data denoted that the three fat-soluble vitamins (retinol, cholecalciferol, and α-tocopherol) were found in lower amounts in African catfish oil compared with common carp oil, these results run with published by [34, 42, 43]; who confirmed a similar relationship between the amounts of those vitamins in the two types of fish.

In comparison with the current study [44] found fewer amounts for the same vitamins (retinol, cholecalciferol, and α-tocopherol). The difference in results may be attributed to the difference in the type of feeding, which in turn depends on the livelihood of the fish in a natural way or on fish farms. Wild fish feeds on benthos and plankton, which is a good source of fat-soluble vitamins, while fish farms foods are rich in carbohydrates [45].

Table 3: Total content of fat soluble vitamins in common carp (CC) and African catfish (AC) oils

Vitamins	Total content (µg/g)	
	CC oil	AC oil
Retinol	33.63±1.77	17.97±1.25
Cholecalciferol	17.49±0.78	8.5±0.9
α-Tocopherol	1576±95.01	1240±66.64

Values are mean±standard deviation (SD) of three samples.

Impact of common carp and African catfish oils supplementation on the plasma lipid profile

The control group (CC) gave the lowest levels in all lipid estimates tested. Groups treated with common carp (CCO) and African catfish (ACO) oils showed a significant reduction in levels of TGs, TC, LDL-c, TC/HDL-c and TGs/HDL-c ratios compared with positive control group (PC). HDL-c produced a different response, PC group had the lowest level followed by ACO and then CCO groups (table 4).

Many researchers [22, 46-50] have observed the elevation in the ratios of TC/HDL-c and TGs/HDL-c in experimental animals fed high fat and high cholesterol diets and cholesterol, which are believed to be closely related to atherosclerosis. In current study (table 4), it can be noticed that the PC group showed significantly higher levels of blood lipids compared with the NC group. On the other hand, CCO and ACO groups indicated bring down levels of lipid profile parameters which are in harmony with many researchers have addressed the relationship between the use of diets rich in oleic and linoleic acids and reduce the levels of lipid profile parameters [7, 51-53]; common carp oil and African catfish oil are characterized by high content of oleic (33.56 and 19.87%, respectively) and linoleic (18.65 and 14.66%, respectively).

Table 4: Plasma lipid profile parameters of rats fed different experimental diets

Groups	NC	PC	CCO	ACO
Parameters				
TGs (mg/dl)	126.25±3.8 a	314.75±5.7 d	241.5±4.1 b	273.62±5 c
TC (mg/dl)	106.62±3.9 a	248.87±6.1 d	192.12±4.2 b	215±4.8 c
HDL-c (mg/dl)	31.87±3.6 a	54.62±3.5 b	74.75±4.2 d	63.12±3.3 c
LDL-c (mg/dl)	49.5±3.6 a	131.3±7.3 d	69.07±5 b	97.15±5.8 c
TC/HDL-c	3.38±0.35 b	4.57±0.29 c	2.58±0.14 a	3.41±0.18 b
TGs/HDL-c	4±0.44 b	5.78±0.39 c	3.24±0.19 a	4.34±0.23 b

Values represent means±SD obtained from 8 rats, Means in the same row followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.01$)

Also, the reduced effect of plasma lipid profile levels can be correlated with common carp and African catfish oils with a significant content of fatty acids EPA, DHA (omega-3 family), it is possible to confirm the previous recommendations of [54-56] who emphasized that omega-3 fatty acids play an important role in reducing the risk of atherosclerosis caused by elevated blood lipids. The data in the table (4) showed that CCO group has better reduction in TGs, TC, LDL-c, TC/HDL-c and TGs/HDL-c ratios than ACO group, which may be due to the high content of oleic, linoleic acids in common carp oil compared with African catfish oil.

The hypolipidemic effect may be attributed to the presence of high amounts of α-tocopherol (table 3) in both fish oils, as noted by a number of researchers in previous studies [7, 57].

There are many explanations that may explain how fish oils improve lipid profile such as changes in LDL production and lipoprotein composition [58]. Omega 3 fatty acids (EPA and DHA) prevent triglycerides synthesis [59]. Moreover, polyunsaturated fatty acids activate the enzymes involved in β-Oxidation [60].

Impact of common carp and African catfish oils supplementation on liver functions

The obtained results in table (5) clarified that PC group showed the highest levels of AST and ALT activities (39.5 and 25.35 U/l respectively) compared other groups.

Such findings coincide with that obtained by [6, 7, 61, 62], who found that when experimental animals feed on high-fat diets, this leads to a significant increase in both AST and ALT activities compared with animals feed on normal diets. While those results don't agree with [22]; which may be explained by different feeding period on high-fat meals, where in the current study six weeks, while in the study referred to one month only.

Increases in plasma AST and ALT activities are usually indicative of possible liver damage or fatty liver. Although the fatty liver (FL) has not been classified as a disease, but many recent studies have shown the relationship of FL with many health problems such as atherosclerosis, kidney disease, colon cancer and osteoporosis [63-65].

Treatment with common carp and African catfish oils induced a significant decrease in AST and ALT enzymes activities compared with positive control group. These results are consistent with many previous studies [7, 10] that have indicated the vital role played by diets containing unsaturated fatty acids in improving the levels of liver enzymes. Common carp and African catfish oils have high contents of unsaturated fatty acids (oleic and linoleic acids), in addition to their good amounts of omega 3 fatty acids. Therefore, both CCO and ACOgroups showed enhanced levels of AST and ALT in plasma, which might be because of the enhancing impact of these oils in lipid metabolism.

Table 5: Plasma liver function parameters of rats fed different experimental diets

Groups	NC	PC	CCO	ACO
Parameters				
AST (U/l)	24.87±1.03 a	39.5±1.6 c	31.7±1.09 b	32.07±1.1 b
ALT (U/l)	16.54±1.08 a	25.35±1.22 c	20.19±1.07 b	21±1.58 b
Total protein (g/dl)	3.36±0.19 a	3.37±0.25 a	3.4±0.29 a	3.34±0.2 a
Albumin (g/dl)	5.66±0.16 a	5.61±0.19 a	5.59±0.27 a	5.58±0.15 a

Values represent means±SD obtained from 8 rats, Means in the same row followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.01$)

CONCLUSION

Common carp and African catfish oils significantly improve the negative effects of high-fat diets on plasma lipid profile levels as well as liver enzymes and these effects are mainly mediated by high amounts of unsaturated fatty acids and omega 3 fatty acids. Future studies need to assess the level of contamination of these species of fish in the local environment to recommend their use as an alternative to cold water fish characterized by high mercury content.

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AUTHORS CONTRIBUTIONS

Medhat M. Abozid developed the design of the experiment, carried out the biological experiment and wrote the manuscript. Hossam Zein shared in the design and implementation of experiments. Amer Abd El-Halem carried out blood analysis.

COMPETING INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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