Indian Journal of Ecology (2021) 48(2): 513-518

Manuscript Number: 3244
NAAS Rating: 5.79

Comparative Study of Amino and Fatty Acids Synthesis in Two Different Groups of Common Carp (*Cyprinus carpio* L.) Cultured in Floated Cages

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Abstract: The purpose of this research was to show the comparative analysis for fatty acids and amino acids in muscle tissues of two groups of common carp (*Cyprinus carpio* L.) H1 (old production line, 1982) and H2 (the new production line,2009)which are important from economical, commercial and nutritional point of Iraqi consumer. These groups were farmed in floating cages at Euphrates River, Babylon province, Iraq lasted for 90 days. The fishes were fed on commercial diet (Alear, Denmark®) with 30% protein and 7% fat. After finishing the experiment, all samples were analyzed. There was a similarity in body components regarding to moisture, protein and ash proportions from both H1 and H2, whereas the total amount of fat content was high in H1(7.21%) and low in H2(4.12%). H2 has ability for body protein synthesis higher than fat deposition as compared with H1.The saturated fatty acids (SFA) in fishes muscle was 34.24 and 27.79% in relation to total fatty acids (TFA) for H1 and H2 respectively. This is in turn reflects on SFA/ unsaturated fatty acids (USFA) which was in high ratio in H2 (2.61%) compared to H1(1.29%). Monounsaturated fatty acids constitute high part of TFA (45.08%) in H2 whereas was 39.23% in H1. The total SFA and MUFA was low in muscle of H2. The SFA/PUFA nearest to 1 in H2 (0.98%). The ω6/ ω3 in H1 and H2 were 0.44-0.48. The muscles of common carp for both groups contain all essential amino acids (EAA) but in different proportions. Although there were individual differences, some of them were significant in the ratios of essential and nonessential amino acids between the two groups of fish. The total ratios of essential and nonessential amino acids did not show significant differences and were similar between the two groups. High levels in EAA especially lysine (Lys), arginine (Arg) and valine (Val) was in new production line (H2). It should take in consideration importance of H2 as one of valuable common carp which was introduced to Iraq in 2009, and is characterized by its high nutritional value due to its contents of USFA and EAA compared to old production line H1.

Keywords: *Cyprinus carpio,* Fatty acids, Amino acids, GC-MS, HPLC

Fishes contribute by 60-70% of protein consumption for many countries (Osman 2001). The amino and fatty acids in protein and fat of muscles and tissues are the key fish meat quality parameters (Saffar et al 2017). Fish protein is belong to high valuable quality and it can exceed the standard chicken egg protein (Krystyna et al 2013). The chemical composition of fish muscle is one of good indicators for physiological and hygienic state (Ali et al 2005). The fresh water fish characterized by high nutritional value and it has been recommended for essential biological protein and amino acids, PUFA and fat soluble vitaminsin addition to major and minor elements (Maqsood and Benjakul 2010). Common carp muscle has low and high protein content compared to trout and fish flat (USDA 2004). Rearing of common carp has much more systemic attention all over the world because of its high productive characteristics and nutritional value (Kocour et al 2005). Based on production changes and nutritive composition of muscles, the efficiency of genetic selection for different lines of common carp are done (Gela et al 2003). There are now two groups or lines of common carp *Cyprinus carpio* L., first group was imported from Indonesia in 1952 (AL- Hamed1971) and was reared in 1982. The second group of common carp was introduced in 2009 to Iraq which was imported from Hungary and it was reared in 2009 in different farming system. There is no available studies regarding to chemical composition and amino and fatty acids contents in common carp. Therefore, the current study was implemented to measure the nutritive value content in fish muscles for each species and to determine the beneficial species in human nutrition.

MATERIAL AND METHODS

Fish samples: After the end of the growth experiment which lasted for a period of 90 days, two groups (2 replicates/ group) of common carp cultured in intensive culture systems in floating cages on the Euphrates River, Iraq.

1st group (H1): common carp fish, the old production line,1982.

2nd group (H2): common carp fish, the new production line-, 2009.

Fish was fed on a commercial floating diet from Danish origin (Alear) with 3% of body weight, with a protein and fat contents of 30 and 7%, respectively. After the completion of the experiment, samples were taken *Cyprinus carpio* from both groups (H1) and H2) with an average weight 557.5 and 670 grams, respectively chemical analysis.

Chemical analysis: The chemical analyzes were carried out at the Ministry of Science and Technology, Environment and Water Department, Baghdad, Iraq using the (GC-MS) Gas Chromatographic-Mass, (LC-10A Shimadzu) and the fatty acids were estimated according to the methods mentioned by Feng et al (2004).Meat from the dorsal muscles was used for chemical analysis and, the chemical composition of the muscle tissue of the fish was determined by the standard methods AOAC (2000). The water content was estimated, by drying at a temperature of 105 ±2ºC until the weight is stable. The protein content determination was carried out by Kjeldahl apparatus and then multiply the nitrogen content by 6.25. The raw fat is extracted from the muscles by using the organic petroleum ether solvent by using the Soxhlet apparatus. Ash was estimated by combustion for 8 hours in the dry sample by taking 5 grams of the sample and then placed in the Muffle furnace at 550°C (Trbovicet al 2009).

Fatty acid analysis: Total fat was extracted to determine fatty acids from fish muscle tissue by extracting with solvents chloroform / methanol (2/1, v / v) based on method Bligh and Dyer (1959). The resultant oil in this way is called crude oil, and methyl esters of Trimethyl sulfonium hydroxide estimated according to the method of (Spiric et al 2010).

Amino acid analysis: The amino acids of the two groups were estimated from five fish from each group after finishing the field experiment in floating cages, and about 10 grams of meat from the area between the side line and the dorsal fin of each fish was taken and then placed in an electric mixer to mix the meat samples uniformly for each group. Thereafter was placed in tight plastic bottles and preserved at -20°C (Saffaret al 2017) for analysis. The amino acids in fish muscle tissue were identified by High- Performance Liquid Chromatography (HPLC). Amino acids were extracted according to the method Rasmus Dahl – Lassen (2018).

Statistical analysis: The data was statistically analyzed using IBM SPSS statistics 22.

RESULTS AND DISCUSSION

Chemical composition of muscles: There was no significant differences in body components (moisture, protein and ash) of common carp fish of the two groups (H1 and H2). Common carp fish in group (H1) indicated significant increase in the percentage of fat (7.21%) as compared to common carp fish in group H2 (4.12%) after three months of rearing. Ćirkovićet al (2012) also made similar observations. The fat content was compensated by the water content. Özogulet al(2007)observed an inverse relationship between fat and water content. The fat contents were higher in H2 than in H1.

Fatty acids composition: The analysis for crude oil extracted from the muscles of carp fish in the two groups identified group of fatty acids includes SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids) and the relationship between them, and n-3 and n-6 omega acids (Table 2). The saturated fatty acids SFA was significantly high in H1 fish by 34.24%, while this percentage decreased significantly to 27.79% in the new group H2 of total fatty acid content in muscle fat. Ćirković et al (2012) observed that the percentage of SFA was 24.23% in the muscles of carp fish, while SFA (myristic acid, palmatic acid and stearic acid) were prevalent in the muscles of fish of two groups which are considered one of the most fatty acids present in saturated fatty acids of carp fish. Similar trend was observed by in earlier studies (Mahmoudet al 2007, Özogul et al 2007) which indicated that myristic fatty acids, stearic and palmatic are the major acids prevalent in SFA whereas palmitic acid has been the major in other saturated fatty acids in common carp. There was significant decrease in the percentage of these saturated acids in H2. Palmatic acid was major among the saturated acids in muscles of the two groups fish, with a significant decrease in H2 by 16.05% compared with H1 by 20.34%. Mehmet et al (2011) documented that palmatic acid reordered the highest ratio of saturated fatty acids. Analysis of fatty acids showed that the total content of USFA in H2 increased by 72.20% compared to 65.75% inH1. The increase in the percentage of PUFA was observed in fat tissue of the two groups muscles.

PUFA are one of the most beneficial acids for to the human body (Mindaugas et al 2016). PUFA in meat and muscle tissues in common carp ranged from 12.05% (Fajmonovâ et al 2003) to 32.58% (Stanchev et al 2014). The results of the analysis of the muscles of the two groups fish also showed that the content of MUFA in the muscles of the two groups differed significantly in favor of H2 (45.08%) compared to H1 (39.23%). Łuczyńska et al (2012) pointed out that content of MUFA in fish oil in common carp muscle tissue may reach 55%. The concentration of fatty acids, (MUFA and PUFA) in common carp muscles are affected by several environmental and other genetic factors depending on the season and nutrition. Mindaugas et al (2016) also concluded that the highest content of MUFA was 57% in common carp fish. Trbovic (2013) indicated a decrease in the content of saturated fatty acid (SFA) in the muscles of

common carp. The SFA in the muscles of common carp fish in H2 was 27.79% compared to 34.24% in H1. The relationship between PUFA / SFA is used as indicator to assess the quality of fats in fish and should be above 0.45% in fats (Department of Health and Social Security 1994). Woodet al (2008) concluded that the ratio of PUFA / SFA should be higher than 0.4% and this was observed in the muscles of the two fish groups in current study. Therefore, the percentage of SFA / PUFA was close to 1%, especially in the H2 which had a concentration of 0.98% compared to H1with 0.77%.The relationship between the PUFA / SFA ratio is an indicator of the quality of fats in fish. Mayra et al (2017) indicated that low values in the PUFA / SFA ratio are undesirable because they cause increased cholesterol level in blood. Although the relationship between the percentage of PUFA / SFA was high in the fish of the two groups (0.77 and 0.98%) due to the high content of PUFA with a significant superiority in favor of H2, Ivanovo and Hadzhinikolova (2015) observed that feeding common carp fish on complex diets resulted in more appropriate ratio of PUFA / SFA in fat of fish muscle. Simopolous (2013) observed that the percentage of fatty acids (PUFA / SFA) closer to 1% is evident of a balanced distribution of groups of fatty acids in fish meat, and was found in fat muscle of carp fish common in group H1.

Common carp contain the highest levels of ω-3 compared with levels of ω-6 in fatty acids (PUFA) and similar trend was observed by Tocher (2003). The ratio between ω-6 / ω-3 is an important measure in assessing the quality of fats in fish and important in human health. Some studies indicated the that fatty acids (ω-3 and ω-6) in fish muscles prevent heart disease and blood pressure (Kris-Etherton et al 2002). However, excessive quantities of ω-6 have the opposite effect and a balanced relationship between ω-3 and ω-6 in the diet should be maintained. The optimum range for ω-3 / ω-6 ratio for human health ranges from 0.2-0.5 (Steffens et al 2005). The current study indicates that the ratio of ω-6 / ω-3 in fish of two groups was less than 4%, which is recommended by WHO and FAO. The study related to ratio of ω-3 / ω-6 in common carp ranges between 0.8-2.4% (Boukortt et al 2004). Many other observations indicated percentage 0.5 (Ljubojević et al 2013) or 0.2% (Komprda et al 2003). Since the two groups H1 and H2 are omnivorous and fish are on the same commercial diet, so the no variation was observed in PUFA content and in ω-3 and ω-6 ratio between the two groups. The significant increase was observed in the relationship between the ratio (ω-3 / ω-6) and was the highest in the H2 by 2.31% and also the ratio between the USFA / SFA reached a higher percentage in the H2 by 2.61 % compared to H1 group (1.92%). Simopolous (2013) revealed that the balanced distribution of fatty acid groups in muscle tissues of fish is an indication of the high nutritional value, and similar was trend was observed in fat of H2.

Amino acids composition: The total protein ratio of 18.16-19.38 was observed in the tissues of the two groups (Table 3). Polak-Juszczak and Adamczyk (2009) indicated that most of the freshwater fish have an average protein ratio ranging between 16.9-19.5%. The composition of 19 amino acids in the muscles of common carp fish in the two groups was estimated. Dorsal muscle tissue represent the edible part of the muscles, as constitutes 43% of the muscles with a low fat content and makes it easy to extract amino acids (Mayara et

Table 1. Chemical composition of the muscles of two common carps reared in floating cages in the Euphrates river

Parameters (%)	H2	H1
Water content	72.56+1.23[a]	70.07+2.70[a]
Protein content	19.38+0.95[a]	18.16+0.78[a]
Fat content	4.12+0.70[a]	7.21+0.51[b]
Ash content	3.94+0.42[a]	4.57+1.40[a]

The different subscripts in the same row indicate the significant differences (P ≤ 0.05)

Table 2. Fatty acid composition (%) of muscle of two common carps reared in floating cages in the Euphrates river

Fatty acid	Percent of total fatty acids	
	H2	H1
Myristic (C14:0)	5.68 + 0.41[a]	5.10 + 0.70[a]
palmitic (C16:0)	16.05 + 0.62[b]	20.34 + 0.59[a]
Stearic (C18:0)	6.07 + 0.44[b]	8.81 + 0.30[a]
Palmitoleic (C16:1)	11.02 + 0.92[a]	9.54 + 0.71[b]
Oleic (C18:1)	34.07 + 1.20[a]	29.69 + 1.69[b]
Linoleic (C18:2)	18.79 + 0.83[a]	17.91 + 0.71[a]
Linolenic (C18:3)	8.34 + 0.90[a]	8.62 + 0.40[a]
Σ SFA	27.79 + 1.01[a]	34.24 + 1.43[b]
Σ MUFA	45.08 + 1.23[a]	39.23 + 2.39[b]
Σ PUFA	27.12 + 1.73[a]	26.53 + 1.10[a]
Σ USFA	72.21+1.01[a]	65.76+1.43[b]
ω3	8.34 + 0.90[a]	8.62 + 0.40[a]
ω6	18.79 + 0.83[a]	17.91 + 0.71[a]
ω3/ω6	0.44+0.03[a]	0.48+0.01[a]
ω6/ω3	2.26+0.14[a]	2.08+0.04[a]
Σ PUFA/Σ SFA	0.98+0.09[a]	0.77+0.02[b]
Σ USFA / Σ SFA	2.60+0.13[a]	1.92+0.12[b]

The different subscripts in the same column indicate the significant differences (P ≤ 0.05). SFA saturated fatty acids, MUFA monounsaturated fatty acids, USFA unsaturated fatty acids, PUFA polyunsaturated fatty acids from the ω3 (ω3PUFA) and ω6(ω6 PUFA)

al 2017). The nutritional value in a food product, including fish protein, it must be evaluated through its content of amino acids, especially the essential ones. Human beings are unable to synthase essential amino acids in the body which included histidine (His), isoleucine (Ile), lysine (Lue), phenylalanine (Phe), lysine (Lys), methionine (Met), tryptophan (Try) and valine (Val) (Peckenpaugh 2011). Amino acids composition in fish muscle protein are similar to that in humans. The current study indicates that muscles of the two types or groups of common carp are currently used in different culture systems and contain all the essential amino acids in different proportions. The analysis indicated differences in the levels of the presence of those acids.

In the current muscles of common carp for both studied groups contain all essential amino acids (EAA) but in different proportions. Although there were individual differences, some of them were significant in the ratios of essential and nonessential amino acids, the total ratios of essential and nonessential amino acids did not show significant differences and were similar between the two groups of studied fish. However, Krystynaet al (2013) also indicated that there were no significant differences in essential amino acids of carp fish. The muscles of the common carp fish in H2 can be considered a source of a protein of good nutritional value because essential amino acids constitutes 39% of total amino acids compared to H1. Fish muscles are considered to be of high and good nutritional value (Jensen et al 2013), or perhaps the difference in the ratios of essential amino acids in the muscle protein of the two groups might explain the degradation of the genetic traits of H1 (old production line) as a result of inland inbreeding for more than thirty-six years in different culture systems. Hana et al (2009) observed that genetic characteristics of the common carp groups differed in the composition of total essential amino acids and in different proportions between different lines in common carp fish. Perhaps these differences in EAA are linked to changes in the genetic makeup. AL-Jubouri (2012) and Isa and Al-Azzawi (2019) also stated that carp fish in the new production line have better genetic traits than the old production line fish. In the current study it was observed that there are differences between the ratios of essential amino acids between the muscle content of the fishes of the two groups showed a significant superiority in the ratio of Val by 4.37% Val is one of the acids that determines the nutritional value of the common carp muscle protein (Krystyna et al 2013). Val presence is an important essential amino acid in fish muscle protein. Gertig and Przyslawski (2006) indicated that any deficiency or decrease in percentage of Val can cause movement disorders, weight loss and loss of appetite. The ideal content of Val in fish muscles has a positive effect on the normal functioning of stem cells, especially in people with cirrhosis (Kakazu et al 2007). The higher acid content Lys was found in the muscles of H2 (8.4%) with significant superiority over H1. The presence of high-level of Lys in carp muscle tissue protein is a good indication of the high nutritional value of carp fish in the new production line group, and this is confirmed by Krystyna et al (2013) pointed out that the presence of Lys in a muscle tissue of common carp fish is a positive indicator of the nutritional value in human nutrition. In case of any deficiency in the Lys it must compensate in the body from other food sources, because any deficiency in Lys causes muscular dystrophy because it participates in the representation of calcium in the bone (Peckenpaugh 2011). In current study higher presence of methionine was in the muscles of H1 compared to H2 in which the ratio of methionine decreased. This is a good indication that the muscles of that group's fish are the best nutritional value. In

Table 3. Amino acid composition (%) of muscle of two common carps reared in floating cages in the Euphrates river

No.	Amino acid (%)	Mean± SD	
	Amino acids	H2	H1
1	Tryptophan(Try)	2.67±0.62 ^{a}	3.66±3.39 ^{a}
2	Histidine (His)	1.28±1.16 ^{a}	2.27±1.19 ^{a}
3	Isoleucine (Ile)	5.74±0.84 ^{a}	4.93±2.75 ^{a}
4	Leucine (Leu)	3.75±0.65 ^{a}	2.64±1.13 ^{a}
5	Lysine (Lys)	8.73±1.01 ^{a}	3.46±1.85 ^{b}
6	Methionine (Met)	2.92±1.07 ^{b}	4.49±1.50 ^{a}
7	Phenylalanine (Phe)	3.54±0.29 ^{a}	2.56±2.19 ^{a}
8	Threonine (Thr)	2.60±1.18 ^{a}	5.48±2.89 ^{a}
9	Arginine (Arg)	1.22±0.03 ^{a}	0.96±0.03 ^{b}
10	Valine (Val)	4.37±0.05 ^{a}	3.14±0.64 ^{b}
Essential amino acids (sum EAA)		39.00±3.14 ^{a}	36.89±6.19 ^{a}
11	Alanine (Ala)	7.72±0.99 ^{a}	3.22±2.17 ^{b}
12	Tyrosine (Tyr)	3.36±1.68 ^{a}	4.77±2.04 ^{a}
13	Aspartic acid (Asp)	6.84±0.53 ^{a}	5.17±1.27 ^{a}
14	Glutamic acid (Glu)	3.43±1.62 ^{b}	8.01±1.19 ^{a}
15	Glycine (Gly)	15.83±0.54 ^{a}	14.82±1.14 ^{a}
16	Butyric acid (But)	2.75±0.62 ^{a}	2.03±1.79 ^{a}
17	Proline (Pro)	8.39±0.55 ^{a}	9.54±3.07 ^{a}
18	Serine (Ser)	6.39±1.56 ^{a}	3.79±4.35 ^{a}
19	Taurine (Tau)	8.38±0.2 ^{b}	15.51±3.45 ^{a}
Non-essential amino acids (sum NEAA)		60.99±3.14 ^{a}	63.10±6.19 ^{a}
	($_{sum}$ EAA / $_{sum}$ NEAA)	0.63 ±1.02 ^{a}	0.58 ±1.25 ^{a}
	($_{sum}$ NEAA / $_{sum}$ EAA)	1.56 ±1.42 ^{a}	1.71 ± 1.06 ^{a}

Means with the different small subscripts within a row were significantly different at ($P < 0.05$)

the organs, Homocysteine in turn leads to high blood pressure and heart disease (Hashimoto et al 2007). On the other hand, a deficiency of the amino acid methionine in the protein intake can affect the liver and weaken the body's immune system (Gertig and Przyslawski 2006). The results of the amino acid analysis show the presence of all non-essential amino acids in the protein of the muscles of the two fish groups, but with different levels of occurrence. The significant difference in the alanine and taurine acids and glutamic acid was observed. The dominant amino acid (Gly) in H2 was 15.83%, while Taurine (Tau) was the dominant in H1 (15.51%). Hana (2009) indicated that there was only significant difference in the ratio of the amino acid (Gly) between the different lines of common carp and that Gly constituted the smallest ratio among other amino acids. There was no significant difference was recorded in the ratios of other non-essential amino acids (Pro, Tyr, Ser, and Gly). Maximum growth and feed conversion efficiency can be achieved by manipulating the formation of nutritional amino acids. However, it is not only the levels of dietary protein and amino acid profiles, but also body fat levels that affect levels of formation of amino acids in tissues (Yamamoto et al2000). H2 showed the higher amino acids and the lowest of non-essential amino acids as compared to H1.

CONCLUSION

The common carp muscles in H2 had high protein with medium content of fat whereas was high fat and medium protein in muscles of H1. Moreover, H2 is considered as a new production line and used as source of unsaturated fatty acids essential amino acids (lysine, arginine and valine), omega 3, omega 6, MUFS, medium content of PUFA. Therefore, can be part in nutritional diet to avoid the high consumption of saturated fatty acids, solidarity the health state and preserving body from diseases.

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Received 20 October, 2020; Accepted 07 March, 2021