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A Comparative Study on Lipid and Protein Composition of *Oreochromis niloticus* Muscles from Five Different Localities in Egypt

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ABSTRACT

This study was aimed to compare the muscle lipid and protein composition of *Oreochromis niloticus* from five different localities in Egypt in order to determine the most nutritionally valuable samples based on the mode of feeding supplied. The fish samples were brought from the River Nile (natural feeding), Ismailia Canal (zooplankton feeding) and three fish aquacultures; Balteem (low protein diet, 18%), Al Abbasa (high protein diet, 30%) and Al Qanater Al Khaireya (zooplankton feeding in concrete tanks). Muscle total protein and total lipid contents were estimated. Fatty acid and amino acid analysis were carried out. Studying muscle protein fractions was undertaken using SDS-PAGE electrophoresis.

Results showed that the highest total proteins content was detected in the River Nile sample, while the lowest was estimated in Al Qanater fish sample. The percentage value of essential amino acids in the fish muscles from Al Abbasa was higher (45.6%) than the other four localities. Total lipids were found to have the highest value in Al Abbasa aquaculture sample whereas the lowest level was detected in the River Nile sample. Analysis of fatty acids and amino acids revealed variable differences between all the examined samples from the five localities. Polyunsaturated fatty acid (PUFA) analysis showed that omega-3 had the highest % value in *O. niloticus* from the River Nile and Al Abbasa localities (30.30 and 30.36 respectively), while omega-6 fatty acids were higher in the fish samples from Balteem and Al Qanater (35.5% and 36.08% respectively) than the other fish samples. Elecrtophoretic data analysis showed that the highest number and percentage values of protein fractions among the five localities were recorded in sample obtained from the River Nile.

It could be concluded that the best nutritional fish muscle quality was related to natural feeding in the River Nile sample and that fed on high protein diet at Al Abbasa aquaculture.

Keywords: Oreochromis niloticus, fatty acids, Amino acids, Electrophoresis, Omega fatty acids, Essential amino acids.

INTRODUCTION

Tilapias are very important in world fisheries, and are the second most important group of food fishes in the world. Nile tilapia, *Oreochromis niloticus* accounted for a harvest of nearly 2.54 million tones in 2009 (FAO, 2011). Although, native to Africa, tilapias are cultured in Asia and the Far East and the Southern United States (Maclean *et al*., 2002).Flesh quality has gained importance among consumers and in the aquaculture industry because it is directly related to human health and nutrition. In order to meet the increase in human fish demand, aquaculture is increasing along the necessity of supplying fish products of high quality and also diversified product (Queméner *et al*., 2002). Generally, an important success factor is that consumers accept farmed fish to be equivalent or superior to the wild fish

(Olsson *et al*., 2003). Quality terms and how they are perceived differ for the fish farmer, processing industry and consumer.

Fish is highly nutritious, tasty and easily digested. Fish contains significant amounts of all essential amino acids, particularly lysine in which cereals are relatively poor. Fish protein can be used therefore to complement the amino acid pattern and improve the overall protein quality of a mixed diet (FAO, 2005).

In human nutrition, fatty acids such as linoleic and linolenic acids - important for preventing skin diseases - are considered essential as they cannot be synthesized by the organism. However, fish oils contain other "essential" polyunsaturated fatty acids which act in the same way as linoleic and arachidonic acids. As members of the linolenic acid family (first double bond in the third position, omega-3, counted from the terminal methyl group), they also have neurological benefits in growing children. Convincing evidence now exists for the significant role fish and fish oils play in decreasing the risk of developing cardiovascular diseases and in improving foetal brain development (Elvevoll and James, 2002). Fish lipids are well known to be rich in long-chain (LC) n-3 polyunsaturated fatty acids (LC n-3 PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Long chain, n-3 PUFA cannot be synthesized by humans and must be obtained from the diet (Alasalvar *et al*.,2002). The importance of fish as a source of polyunsaturated fatty acids (PUFA), particularly n-3 fatty acids in human nutrition is widely recognized (Simopoulos, 2004). These fatty acids (3PUFA) have been associated to numberless benefits to human health. In general, they contribute to the prevention of heart diseases (Lee and Lip, 2003; Leigh-Firbank *et al*., 2002; Krisetherton *et al*., 2002.

Thus, this study was carried out to determine the proximate, fatty acid and amino acid composition of the common freshwater fish species *Oreochromis niloticus* in order to evaluate and compare the flesh quality (muscles lipid and protein chemical composition) of wild and cultured Nile tilapia, *O. niloticus* collected from River Nile, Ismailia Canal and cultured in Balteem , Al Abbasa and Al- Qanater Al Khairya aquacultures.

MATERIAL AND METHODS

Fish samples

Fish samples of Nile tilapia, *Oreochromis niloticus* were obtained from the River Nile (Cairo Governorate), Ismailia Canal (Ismailia Governorate), Balteem (Kafr Elsheikh Governorate), Al Abbasa (Sharqia Governorate) and Al Qanater Al Khairya (Qalyobia Governorate) aquacultures. Three samples from each locality with equal number of fish were chosen randomly for muscle chemical analysis.

Preparation of fish muscle samples for biochemical analysis

A fixed weight (1.0 g) of the fish dorsal muscle was homogenized in a glass homogenizer for 3 minutes in 5ml saline, then it centrifuged at 3000 r.p.m for 10 minutes. The supernatant was used for determination of total protein and total lipid contents.

Biochemical analysis

Total protein and total lipid contents were determined using commercial colorimetric kits (Diamond Diagnostic, Egypt), according to the methods of Gornal *et al*. (1949) and Zollner and Kirsch (1962) respectively. Fatty acids were analyzed and characterized by the method of Ackman (1991). Amino acids analysis was performed according to the method described by Gam *et al*. (2005).

SDS-polyacrylamide gel electrophoresis

Protein patterns were running gel according to the method of Laemmli (1970).

Statistical analysis

Values were expressed as means ± standard deviation (M±SD). Data were analyzed using t-test (Snedecor, 1962).

RESULTS

Total lipid content

The mean total lipid content of *O. niloticus* ranged from 0.64 to 0.96 mg/100g tissue as shown in Fig. (1).The highest lipid content was obtained on the samples from Al Abbasa, Balteem and Al Qanater aquacultures (0.96, 0.94 and 0.89 mg/100g wet weight respectively), while the lowest was in samples of the River Nile and Ismailia Canal as presented in Fig. (1).

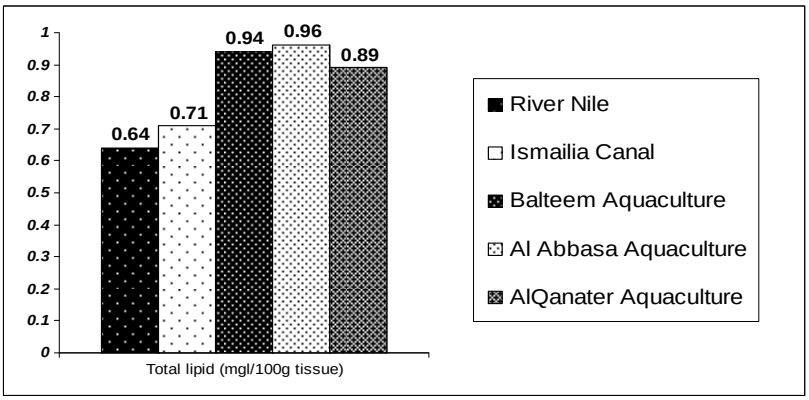

Fig. 1: Total lipid (mg/100g) wet weight of *O. niloticus* muscles from five different localities

Fatty acid content

The fatty acid composition (mg/g tissue) of the freshwater *O. niloticus* is summarized in Table (1). The sequence of the fatty acids is ordered according to their chromatographic retention times.

The fatty acid composition of the studied fish muscles from the five different localities ranged from 27.38 to 32.63 mg/g tissue (in Ismailia canal and the River Nile samples respectively). Omega-3 fatty acid levels in the fish muscles were relatively high in the River Nile and Al Abbasa samples (30.30% and 30.36% respectively) compared to the other groups. Omega-6 also among them those occurring in the highest proportions were Balteem (35.50 %) and Al Qanater (36.08%) aquacultures as shown in Table (2).

Table 1: Fatty Acid composition (mg/g tissue) of *O. niloticus muscles* from five different localities in Egypt.

Fatty acid	Fish localities				
	River Nile	Ismailia Canal	Balteem Aquaculture	Al Abbasa Aquaculture	Al Qanater Aquaculture
C14:0 Myristic acid	0.521	0.650	0.578	0.669	0.551
C15:0 Pentadecanoic acid	0.290	0.235	0.209	0.318	0.307
C16:0 Palmitic acid	3.452	3.527	4.077	3.628	2.658
C16:1n-7 Palmitoleic acid	2.459	1.991	1.770	2.048	2.601
C17:0 Heptadecanoic acid	1.026	0.831	0.738	0.845	0.790
C18:0 Stearic acid	1.368	1.108	0.985	1.139	1.447
C18:1n-9 Oleic acid	1.628	1.318	1.172	1.356	1.722
C18:١n-7 Vaccenic acid	0.959	1.197	1.384	1.232	1.015
C18:2n-6 Linoleic acid (omega6)	1.511	1.590	1.837	1.324	1.843
C18:3 n-6 Linolenic acid (omega6)	0.438	0.461	0.410	0.384	0.464
C18:3n-3 Linolenic acid (omega3)	0.595	0.626	0.724	0.691	0.458
C20:0 Arachidic acid	ND	ND	ND	ND	ND
C20:1 Gadoleic acid	ND	ND	ND	ND	ND
C20: 2n-6 Eicosadienoic acid (omega6)	0.142	0.219	0.253	0.208	0.150
C20 :3n-6 Eicosatrienoic acid (omega6)	0.554	0.408	0.363	0.555	0.586
C20:4n-6 Arachidonic acid (omega6)	1.437	1.512	1.748	1.442	1.521
C20 :3n-3 Eicosatrienoic acid (omega3)	ND	ND	ND	ND	ND
C20 :4n-3 Eicsoatetraenoic acid (omega3)	0.758	0.510	0.590	0.694	0.583
C20 :5n-3 Eicosapentaenoic acid (omega3)	1.763	1.187	1.055	1.615	1.357
C22: 0 Behenic acid	0.238	0.257	0.348	0.403	0.290
C22: 4n-6 Docosatetraenoic acid (omega6)	1.059	1.014	0.901	0.706	1.121
C22 :5n-6 Clupanodonic acid (omega6)	5.656	3.808	4.403	5.181	5.984
C22 :5n-3 Clupanodonic acid (omega3)	5.850	3.939	3.502	5.359	6.189
C22 :6n-3 Docosahexaenoic acid (omega3)	0.921	0.995	0.884	0.985	0.709
Total	32.63	27.38	27.93	30.78	32.35

Table 2: Omega fatty Acid percentage values of *O. niloticus muscles* from five different localities in Egypt.

Omega fatty acids	Percentage values				
	River Nile	Ismailia Canal	Balteem Aquaculture	Al Abbasa Aquaculture	Al Qanater Aquaculture
Omega 3	30.30	26.50	24.18	30.36	28.74
Omega 6	33.09	32.91	35.50	31.84	36.08

Total protein content

The total protein content of *O. niloticus* ranged from 12.26 to 16.77 g/100g wet weight (Fig., 2). No significant difference was noted ($p > 0.05$) between the five fish samples. The highest protein content was recorded on the River Nile fish muscles.

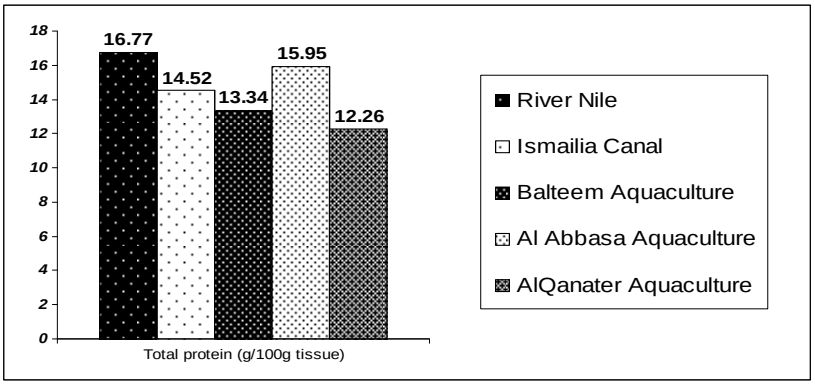

Fig. 2: Total protein (g/100g) wet weight of *O. niloticus* muscles from five different localities.

Amino acid content

Seventeen different amino acids were obtained in the freshwater fish *O. niloticus*. The total amino acid content was presented in Table (2) as µmol/g protein.

Eight of the nine essential amino acids that are very important for the human body are separated in this fish species. These essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine. Tryptophan could not be detected by this technique. Total amino acid concentration had the highest level in the River Nile sample (91.646 µmol/g protein), while the essential amino acid percentage value was 43.99% (Table 3). The major essential amino acids values (45.60 %) was recorded in the fish muscles obtained from Al Abbasa aquaculture compared to the other samples while the total amino acid content of this sample was 79.487 µmol/g protein (Table, 3).

Table 3: Amino Acid composition (umol/g protein) of *O. niloticus muscles* from five different localities in Egypt

Amino acid	Fish localities				
	River Nile	Ismailia Canal	Balteem Aquaculture	Al Abbasa Aquaculture	Al Qanater Aquaculture
Alanine	3.612	4.009	4.450	4.495	4.541
Arginine	8.339	7.338	6.458	5.001	3.873
Aspartic acid	8.229	8.640	7.604	5.888	4.560
Glutamic acid	13.422	12.205	12.751	12.113	14.778
Glycine	4.221	4.007	4.010	3.809	3.619
Histidine*	2.841	2.010	2.699	2.564	2.436
Isoleucine*	2.294	2.108	2.179	2.070	1.967
Leucine*	8.055	8.221	7.286	8.088	7.316
Lysine*	8.611	7.022	8.180	7.771	7.383
Methionine*	3.147	3.328	2.868	2.615	2.383
Phenylalanine*	5.044	5.116	4.598	4.191	3.820
Proline	3.416	3.113	3.245	3.082	2.928
Serine	5.055	5.282	4.608	4.200	3.828
Threonine*	5.216	5.112	4.955	4.707	4.472
Tyrosine	4.221	4.015	4.010	3.809	3.619
Valine*	5.108	5.213	4.656	4.244	3.868
Cystine	0.815	0.756	0.806	0.840	0.805
Tryptophan	ND	ND	ND	ND	ND
Total amino acids content	91.646	87.495	85.363	79.487	76.196
% value of Essential amino acids	43.99	43.58	43.84	45.60	44.16

ND : Not detected *: Essential Amino acid

SDS-polyacrylamide gel electrophoresis

Protein patterns determined by SDS-polyacrylamide gel electrophoresis revealed 21 protein bands in the muscle tissue of the River Nile sample and Al Abbasa (19 band), which is the highest band number among the tested samples from the different localities; while the least band number (14) was in Balteem sample. The band numbers and percentages of all samples are summarized in Table (4). Fig. (3) shows the electrophoregram and densitometric profile of protein fractions of the studied fish muscles of *O. niloticus*. Analysis of electrophoretic data in Fig. (3) revealed variable changes specially in the high (bands 1- 5) and low (bands 15 - 21) molecular weight regions in all the four localities compared to the that of the River Nile. It was also noticed that bands 17- 21 disappeared in Ismailia Canal and Balteem and Al Qanater fish muscle samples.

Table 4: Number and percentages of *O. niloticus* muscle protein fractions separated by SDS-PAGE from different localities.

Band number	Band % of muscle protein fractions				
	River Nile	Ismailia Canal	Balteem Aquaculture	Al Abbasa Aquaculture	Al Qanater Aquaculture
1	5.72	8.49	6.30	3.07	5.57
2	1.77	5.25	5.36	2.30	3.10
3	3.17	6.35	9.67	3.21	10.52
4	2.31	16.32	15.69	3.67	10.82
5	3.45	5.00	8.39	7.76	12.14
6	5.26	1.55	3.71	14.88	1.64
7	1.03	3.51	0.75	7.40	4.51
8	2.43	3.15	5.47	2.26	2.82
9	14.79	3.95	1.43	3.60	1.61
10	2.86	1.94	1.84	1.70	2.17
11	0.96	1.74	19.39	1.17	3.43
12	1.19	19.38	2.69	1.78	1.97
13	4.15	7.50	6.73	2.24	20.77
14	4.35	5.61	12.58	2.25	5.56
15	1.94	10.27	_	1.64	4.54
16	1.19	_	_	19.34	8.82
17	4.27	_	_	6.20	_
18	11.65	_	_	5.20	_
19	14.58	_	_	10.35	_
20	4.27	_	_	_	_
21	8.68	_	_	_	_

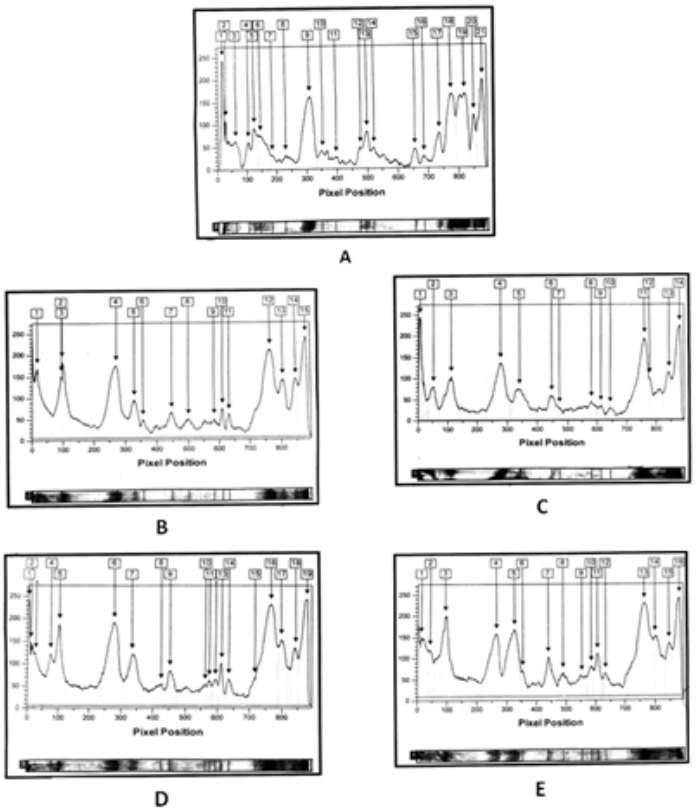

Fig. 3 : Elecrtophoregram and densitometric profile of O. niloticus muscle proteins separated by SDS-PAGE.

DISCUSSION

Fish is a vital component of food in most developing countries especially in Egypt. The production and consumption of freshwater fish, has increased during recent years. Therefore effort is needed to improve the output performances and quality of the freshwater fish. *Oreochromis niloticus*, commonly known as the Nile tilapia, is a popular food fish that has been farmed in ponds for thousands of years. Lipids are an important component in human diets, both as energy and fatty acids (FA) sources (Sargent *et al*., 1989). Fish, like other animals, have the ability to accumulate lipids in their body. Fat content is influenced by species, geographical region, age, and diet (Tacon, 1990).

The physiological status of farmed fish is an integral part for evaluating their health status. However, physiological alterations might be used as indicators for unsuitable environmental conditions (Cnaani *et al*. 2004 and Řehulka *et al*. 2004).

The present results showed that the total lipid content was highest in fish muscle from Al Abbasa compared to the River Nile sample which had the lower muscle total lipid content. Serum lipids increased significantly due to the increase in protein level, and it may be because the muscle is a pivotal compartment directly linked to amino acid turnover. This involves protein synthesis or breakdown of those molecules as energetic substrates (Gallagher, 1999). It is generally recognized that PUFA composition may vary among species of fish but little attention has been paid to the PUFA composition of different species when selecting fish for diets. All fish were considered of similar nutritional value and selection was made primarily on the basis of availability, freshness, flavour and similar factors (Turker *et al*. 2003). Among the fatty acids, particular emphasis has been placed on the omega-3 and omega-6 polyunsaturated fatty acids (PUFA). Polyunsaturated omega-3 fatty acid, eicosapentaenoic acid (EPA, C-20:5) and docosahexaenoic acid (DHA, C-22:6), are of interest because they reduce the risk of cardiovascular diseases (Leaf and Weber, 1988and Kang and Leaf, 1996). Since these fatty acids composition may vary among fish species, it is necessary to determine both the lipid content and the PUFA distribution. This importance in fatty acid profile of fish stems from their importance in human health, mainly in reducing the occurrence of heart diseases, strokes and various inflammatory injuries, and improving neurological function in newborn infants (Kefatos *et al* 1997 and Maina *et al*. 2003). In that area, findings have confirmed that body fatty acid profile to a large extent mirrors the dietary fatty acid composition (Maina *et al*. 2003 and Williams *et al*.2003). Hence, differences could appear between tank and pond rearing systems, due to the natural food present in the latter, when feeding fish with same artificial diets.

Biochemical analysis of the fish muscle of *Oreochromis niloticus* in the present study showed no significant change in the protein content among the five studied samples. These results confirm the results obtained by El –Kasheif *et al* (2011) who found no significant change in the serum total protein in response to change of fish diet in *O. niloticus*. On the other hand, Kheir (1997) found that total protein content increased with increasing supplemented oil and the highest protein content in muscles of *Oreochromis aureus* was recorded with 6% lipid. Also, Serum and muscle proteins tended to increase with increased dietary protein level in Nile tilapia (Abdel-Tawwab *et al*. 2010 and Abdelhamid *et al*., 2014).

Amino acids are also important in healing processes and the composition of amino acids in fish is similar to that in man, people can acquire essential amino acids in abundance and proper balance by eating fish. The essential amino acids cannot be

manufactured in human bodies, but can be obtained from food. The present study indicated that the samples of fresh water *O. niloticus* muscles obtained from the five studied localities had all the essential amino acids. Deficiency in the essential amino acids may hinder healing recovery process (Zenebe *et al*. 1998). Leucine promotes the healing of bones, skin and muscle tissue. Isoleucine is necessary for hemoglobin formation, stabilizing and regulating blood sugar and energy. Glycine, which is one of the major components of human skin collagen, together with other essential amino acids such as alanine form a polypeptide that will promote re-growth and tissue healing (Kang'ombe *et al*., 2006). Other reports of similar nature provided valuable information on selecting fish and fish oils for nutritional purposes (Saha and Dewan, 1979 and Bahurmiz and Ng, 2007) .

Electrophoresis is an analytical method in which substances are separated from one another based upon their differing rates of migration in a strong electric field. Muscle protein consists mainly of actin and myosin, but numerous other proteins also make up muscle tissue. Variations in an organism's proteins may reflect physiological adaptations to an ecological niche and environment. Polyacrylamide SDS gel electrophoresis was used in the present study to separate muscle protein fractions. Numerous studies had special concern in analyzing the fish muscle proteins electrophoretically. In Nile Tilapia *O. niloticus* (Tongnuanchan *et al*., 2011 and Monter-rey-Quintero and Sobral, 2000). Also, Ferreira *et al*., (2013) studied the muscle proteins of the White mouth Croaker, *Micropogonias furnieri*. Furthermore, the present results showed that muscle proteins of *O. niloticus* separated by SDS-PAGE revealed that the River Nile fish muscle was separated to 21 electrophoretic bands that was the largest number compared to the other fish samples, while the least band number (14) was in Balteem sample. Protein fractions were characterized to high, medium and low- molecular band proteins. Variable changes were also recorded between the different fish samples.

CONCLUSION

The present study indicated some differences and similarities in the biochemical composition of the muscles of *Oreochromi niloticus* obtained from five different localities belonging to five Governorates in Egypt. Fish samples were collected from the River Nile (Cairo G.), Ismailia Canal (Ismailia G.), Balteem (kafr El-Sheikh G.), Al-Abbasa (Al Sharqya G.) and Al-Qanater Al-Khairya (Al-Qalubyia G.) Fish muscle lipid and protein contents were estimated to evaluate the change in the fish muscle composition according to the mode of feeding available. Fish from the River Nile had natural feeding. Ismailia Canal fish samples were fed with zooplanktons. Balteem aquaculture provided low-proten diet (18%), Al-Abbasa aquaculture provided relatively high protein Diet (30%) and in Al-Qanater Al-Khairya aquaculture, fish were reared in concrete tanks and fed zooplanktons. The biochemical variations of the fish samples were to determine the nutritional value of each sample. The current results, based on the biochemical analysis performed, indicated that the fish samples obtained from natural habitat had better flesh qualities than that collected from aquacultures.

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ARABIC SUMMRY

دراسة مقارنة على محتوى الدهون والبروتينات في عضلات سمكة البلطي النيلي من مناطق مختلفة في مصر

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تهدف هذه الدراسة إلى مقارنة محتوى الدهون والبروتينات في سمكة البلطي النيلي "*أوريوكروميس نيلوتيكاس*" في خمس مناطق مختلفة تنتمي إلى خمس محافظات في مصر، وذلك بهدف تقدير القيمة الغذائية لكل منها وتحديد أفضلها بناء على التغذية التي تعتمد عليها. وقد تم الحصول على عينات الأسماك من نهر النيل (محافظة القاهرة، تغذية طبيعية)، ومن ترعة الإسماعيلية (محافظة الاسماعيلية، هائمات حيوانية)، ومن مزارع سمكية في كل من بلطيم (محافظة كفر الشيخ، عليقة ذات بروتين منخفض – ١٨%)، والعباسة (محافظة الشرقية، عليقة ذات بروتين مرتفع – ٣٠%) والقناطر الخيرية (محافظة القليوبية، هوائم حيوانية في أحواض أسمنتية). تم قياس البروتينات والدهون الكلية في عضلات السمك، وكذلك تحليل الأحماض الدهنية والأحماض الأمينية، كما تم فصل بروتينات عضلات السمكة باستخدام طريقة الفصل الكهربي على هلام " إس دي إس عديد الأكريلامايد".

وقد أظهرت النتائج أن أعلى نسبة للبروتين الكلي كانت في عينات نهر النيل بينما كانت أقلها في عينة المزرعة السمكية بالقناطر الخيرية. أما النسبة المئوية الأعلى (٤٥،٦ %) للأحماض الأمينية فقد سجلت في عينة مزرعة العباسة مقارنة بالأربع مناطق الأخرى. وقد وجد أن أعلى نسبة للدهون الكلية في عضلات سمكة البلطي كانت في عينات مزرعة العباسة بينما كانت أقل نسبة للدهون الكلية في عينة نهر النيل. أظهرت تحاليل الأحماض الدهنية والأحماض الأمينية اختلافات متباينة بين عينات المناطق الخمسة، وقد بيّن تحليل الأحماض الدهنية غير المشبعة أن أعلى نسبة لأحماض أوميجا ٣ كانت في عينتي نهر النيل ومزرعة العباسة (٣٠،٣٠% & ٣٠،٣٦ % على التوالي)، بينما كانت أعلى نسبة لأحماض أوميجا ٦ في عينات مزرعتي بلطيم والقناطر الخيرية (٣٥،٥ %& ٣٥،٠٨ % على التوالي) مقارنة بعينات باقي المناطق. أوضحت نتائج الفصل الكهربي لبروتينات عضلات سمكة البلطي النيلي من المناطق الخمسة أن أكثرعدد من الشرائط البروتينية ونسبها المئوية تم فصلها من عينة نهر النيل.

ويستخلص مما سبق أن القيمة الغذائية ونوعية عضلات السمكة ترتبط بنوعية الغذاء الطبيعي في عينة نهر النيل والتغذية على عليقة ذات بروتين عالي في مزرعة العباسة.