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BIOCHEMICAL AND HISTOPATHOLOGICAL STUDIES ON THE MUSCLES OF THE NILE TILAPIA (*OREOCHROMIS NILOTICUS*) IN EGYPT

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ABSTRACT

Biochemical contents in the muscle of *Oreochromis niloticus* were determined in fish specimens collected from El-Kanater, Benha, Zefta and Talkha stations. Analysis revealed that the water content in muscles was higher in immature than mature fish. The maximum values of protein content in fish flesh were recorded during spring at Benha and Zefta stations. Seasonal variations in the amount of protein, fat, carbohydrate water and ash were observed. The fat in fish muscles was fairly high in winter and early spring in adult specimens, i.e., during the pre-spawning months, then it dropped after the breeding season. A direct relationship between fat and protein was found. The muscle carbohydrate was correlated to feeding and spawning activity. High energy values were found throughout the prespawning period.

Heavy metals concentrations were detected in the muscles of *O.niloticus* and found to follow the order: Fe > Pb > Cu > Zn.

The histopathological study of fish muscles showed marked signs of haemorrhage and hemolysis.

INTRODUCTION

In Egypt several studies have been reported on the biochemical constituents of the Egyptian fishes among them. El-Saby (1934) determined the dietetic value of some Egyptian food fishes and Latif & Fouda (1976) reported the biochemical constituents of most Red sea fishes in Egypt. Similarly, Shakweer *et al.* (1998) mentioned

that. the major biochemical constituents of the muscles of *Mugil cephalus* namely protein, lipid, ash and water contents differed significantly from one fishing area to another.

On the other hand. Saleh *et al.* (1988) studied the heavy metals concentration in whole body. tissues and organs of *Tilapia zillii* in Wady El-Rayan, Egypt. They reported that the heavy metals concentration in fish did not change significantly with the season of collection or with the age of the fish, except for lead. Abdel-Baky *et al.* (1998) studied the heavy metals concentrations in some organs of *Oreochromis aureus* in Lake Manzalah, Egypt. They reported that, the concentrations of the tested metals (Cd., Cu., Zn and Pb). in different organs and tissues of *Oreochromis aureus* varied considerably with regards to seasons and sites.

The effect of a sublethal concentration (6.8 mgL^{-1}) of cadmium chloride on the digestive system of the teleost fish *Heteropneuster fossilis* after exposure for 30 days was examined by Sasstry and Gupta (1978). Lamas *et al.* (1995) cited that the turbot (*Scopthalmis maimusl*) infected by a viral erythrocytic. showed hemorrhages in the head. mouth. fins. exophthalmia and abdominal distension. Degeneration of muscle fibers was accompanied by an intense inflammatory edema.

The present study aimed to investigate the biochemical composition of fish flesh and histopathological changes in muscles of *Oreochromis niloticus* caught from different localities of the Damietta Nile Branch.

MATERIALS AND METHODS

Biochemical analysis:

Fresh samples of *O. niloticus* were collected seasonally during the year 1997 from El-Kanater. Benha. Zefta and Talkha selected stations. Fishes were dissected out. their skin was carefully removed and a flesh sample of about 10 gm was taken from the mid-dorsal region. cut into small pieces and weighed. The muscle samples of each sex were analyzed separately. in order to assess any possible differences in the seasonal cycle of the fish. Moisture content of the samples was determined by drying the flesh in an oven at 100 °C to constant weight. Fat was estimated by extraction of dried muscle samples using ether solvent for about 12h. in a Soxhlet apparatus.

Protein was determined by micro-kjeldal nitrogen estimation method according to El-Aggan (1982).

Heavy metals analysis:

Fish samples were analysed for heavy metals according to Dalziel and Baker (1983), where specimens of muscles were weighed, dried in an oven at 105 °C, for 48 hrs in silica beaker digested by concentrated nitric acid, diluted to 25 ml by distilled water. The results were expressed as Mg/ gm wet weight of the tissue.

Levels of metals in samples were measured by Atomic Absorption spectrophotometry (Hitachi Japan) with detection limit $1 mgL^{-1}$). Results were expressed in part per million (ppm) of the dry weight of the flesh.

Histological examination:

The specimens were carefully dissected; pieces of the muscles were fixed in Bouin fluid. After fixation, the specimens were dehydrated, then muscles were cleared by methyl benzoate. After clearing the process of embedding was performed. After embedding the specimens were supported by paraffin as a block and then transversely cut at a thickness of 4-6 μ.

RESULTS

1. Biochemical composition of fish flesh

Variations in the four major biochemical constituents of fish flesh of *Oreochramis niloticus* namely moisture, protein, lipids and ash contents, in addition to the carbohydrates during different seasons are presented in Figure 1.

Seasonal variation in water and lipids content:

For the biochemical analysis specimens inclusion the juvenile specimens, male and female fishes were analysed and the results are presented in Figure 1. Water being the most important constituent, contributing maximum value to the chemical composition in fish tissues and showed high degree of variation.

The amount of fat in the flesh of *O.niloticus* varied from one season to another. The percentage of fat contents in both males and females are presented in Figure 1.

It has to be mentioned that the water content in the flesh of both fish sexes underwent a wide range of variation but was inversely related with the fat content, i.e., as the fat was increased the water content decreased and vice verse.

Seasonal variation in protein content:

Figure (1) shows the protein cycle in muscles of both sexes at different stations. It can be observed that the protein ranged from 73.1 % to 85.6 %, 73.0 % to 87.5 %, 73.5 % to 88.0 % and 73.8 % to 85.2 % for the fish samples caught from El-Kanater, Benha, Zefta and Talkha stations, respectively. The protein content in fish flesh had its lowest percentage (73.0 %) in the adult female sample from Benha during autumn, however the highest percentage (88.0%) was found in fish sample collected from Zefta station (fingerlings) during spring season.

Ash content:

The ash content showed its lowest value (3.7%) in fish flesh sample (fingerlings) from Talkha station during summer season (Figure 1). However, its highest value (10.9%) was found in adult fish flesh sample from El-Kanater station during autumn season.

Carbohydrate content:

Carbohydrate was estimated by calculation (difference). It is obvious from the data obtained that, the values of carbohydrate were relatively higher during winter and spring seasons than during summer and autumn (Figure 1).

2. Heavy metals in fish flesh

The concentration of four selected heavy metals (Iron, Zinc, Copper and Lead) in the flesh of *Oreochromis niloticus* caught during 1997 from the selected different localities at Nile Damietta branch are presented in Table (1). It showed that the highest average value of iron concentration was recorded from fish caught from El-Kanater station (320 µg/g.) during summer but the lowest value was recorded at Zefta station (75 µg/g.) in autumn season. However,

iron concentration showed the highest value along the year round (290, 293, 320 and 240 µg/g) for winter, spring, summer and autumn seasons, respectively.

Zinc concentrations in the fish muscles had been fluctuated from 15.2-45.6 µg/g. A maximum value was observed for fish caught from Benha station during spring though high values were recorded in summer for those caught from all the studied stations. Zn concentrations obtained from all fish examined in autumn were much lower than those obtained during other seasons (25.7, 22.0, 19.5 and 15.2 µg/g) for samples taken from El-Kanater, Benha, Zefta and Talkha stations, respectively.

Concerning Cu concentration in fish, it had reached its maximum value at Talkha station (9.6 µg/g) and falled to its minimum value at Benha station (0.98 µg/g). The results also showed that higher values of Cu was recorded (9.6, 7.4, 4.5 and 3.8 µg/g) during summer, spring, winter and autumn respectively but the lowest values were obtained during winter (1.9, 1.2, 1.3 and 4.5 µg/g) at El-Kanater, Benha, Zefta and Talkha stations, respectively.

The maximum concentration of lead (11.67 µg/g) was recorded in fish caught from El-Kanater station during spring, however, high values of lead (9.40, 5.95, 5.80 and 8.50 µg/g) were recorded during winter at El-Kanater, Benha, Zefta and Talkha stations respectively.

Histopathological changes in the muscles:

The normal skeletal muscles are composed chiefly of segmental myomeres. Each myomere is regarded as apparent muscle and its fibers are parallel to the long axis of the body (Figure 2).

However, the fish caught from the selected stations on the River Nile show that the hypoderm layer between dermis and muscle showed necrosis in connective tissue with hemorrhages and hemocidrine (Figure 3).

The muscle sections showed degeneration and necrosis of muscle fibers and intrafibrilar edema (Figure 4a & 4b) with thickness and dilation in blood vessel (Figures 5a & 5b). Dermal

layer showed degeneration of the collagen bundles that were loose in some regions and collapsed in others (Figure 6).

DISCUSSION

A knowledge of the biochemical composition of fish flesh is of paramount importance to evaluate its nutritive value. Also the biochemical composition specially water and lipids as well as fish protein, contents are important for fish meal or other fishery products.

Water as an important constituent, contributes highly to the chemical composition in fish tissues and may show high degree of variation (Bruce, 1924).

The obtained data for the analyzed specimens showed that, the mean percentages of water, protein, lipid, ash and carbohydrate are 80.95%, 10.45%, 6.75% and 1.41% respectively. These results are in agreement with those reported by Hammadi *et al.* (1974). Fouda *et al.* (1986) and Wassef & Shehata (1991) who showed that water content seems to be the principal constituent, protein comes next, followed by lipids, ash and carbohydrate.

The higher values of water content during summer and autumn seasons may be related to the spawning period as reported by Abdel Fattah (1979) who gave an explanation for that as to be due to endocrine source.

Lipid content variation was obvious as it is greatly affected by the principal factors such as the stage of sexual development and feeding conditions (Wassef, 1978, Khalil *et al.*, 1986, Wassef & Shehata, 1991). These variations may also declare the effect of the annual reproductive cycle on muscle composition.

Figure (1) shows the protein cycle in muscles of both sexes. Similar to fat, protein values are inversely proportional to water suggesting that depletion in water content is due to the increase in fat and protein levels.

As in most examined fish, with the exception of those caught from Talkha station, protein and ash contents were proved to be more or less stable components. In contrast, lipid content undergoes wide seasonal variations, particularly for adults. These results are in agreement with that reported by Love, (1970) who mentioned that lipid is the most variable component in fish. From the present results

water, lipid and ash contents were found to be higher during autumn and summer than during winter and spring, however, protein content showed a limited variation. It is difficult distinguishing between the effect of many factors which play a part in lipid fluctuation, but the principal ones are the stage of sexual development and feeding conditions (Wassef and Shehata, 1991). These variations may also reveal the effect of the annual reproductive cycle on muscles composition.

The concentrations of heavy metals (iron, zinc, copper and lead) in flesh of *Oreochromis niloticus* seasonally caught from Damietta branch are presented in Table (1). The studied areas are known to receive the wastes of agricultural, industrial and domestic effluents from different sources. The present data for Zn, Cu and Pb studied in fish caught from different stations were found to be less than these reported by WHO (1977) and Hamed (1998).

Abdel-Nasser *et al.* (1996) found that the highest average concentration of Pb and Cu in muscle of *Oreochromis niloticus* caught from Assiut Governorate were 1.78 ppm and 15.93 ppm respectively. The level of Pb was lesser than the permissible limit (2.62 ppm), however Cu level was higher than that limit (10 ppm). Also Abdel Baky *et al.* (1998) found that muscle tissue of *O. aureus* tends to retain lower concentrations of trace metals (Cu 1.5 µg/g, Zn 28.52µg/g and Pb 0.31 µg/g). They also reported that heavy metals concentrations in fish muscle increase with the increase in amounts of drainage and sewage effluents.

The histopathological changes in epidermis, dermis, hypodermis and underlying muscles observed in the present study showed necrosis of epithelial and mucous cells of the epidermis, degeneration, necrosis and edema of muscle fibers. They also revealed congestion and dilation of the dermal blood vessels together with hypodermic inflammatory signs which may extend to the underlying muscle. These results are in accordance with these obtained by Roberts (1972), Easa (1974), Oyuind (1985); Timar and Timar (1986) and Yacoub (1999).

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Table (1): Seasonal variations of some heavy metals accumulation in fish flesh (µg/g dry wt.) of *Oreochromis niloticus* caught from Nile Damietta branch during 1997.

Areas of Sampling	Winter				Spring				Summer				Autumn			
	Fe	Zn	Cu	Pb	Fe	Zn	Cu	Pb	Fe	Zn	Cu	Pb	Fe	Zn	Cu	Pb
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
El-Kanater	290	20.7	1.9	9.4	293	26.85	3.8	11.67	320	32.5	5.6	5.4	240	25.7	2.31	3.9
Benha	86	24.7	1.2	5.95	108	45.6	2.5	6.5	111	38.1	2.9	4.5	98	22	0.98	2.3
Zefta	89	21.2	1.3	5.8	95	403	2.1	4.6	103	31.3	1.9	3.4	75	19.5	1.1	2.4
Talkha	156	17.3	4.5	8.5	270	21.7	7.4	6.4	240	23.5	9.6	7.3	108	15.2	3.8	2.5

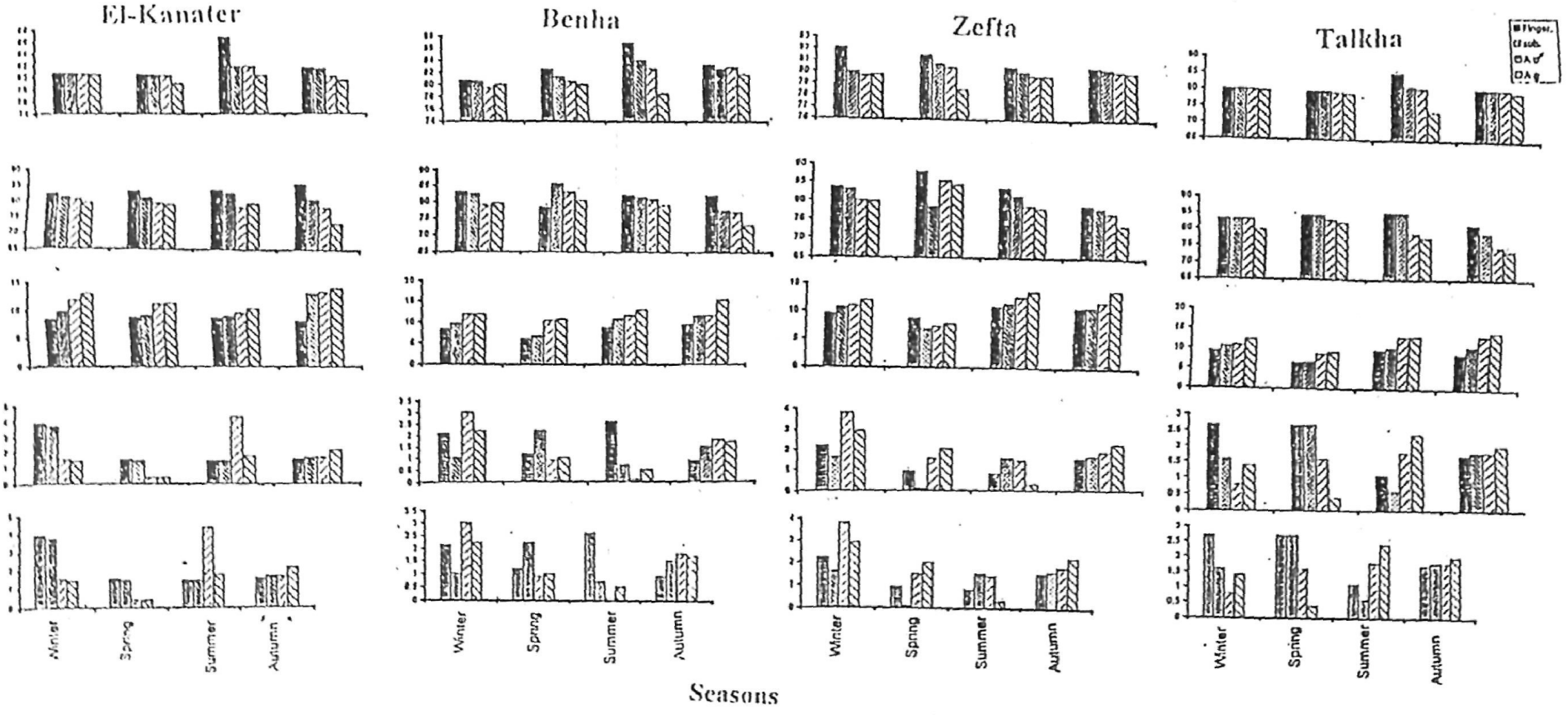


Fig. (1): Seasonal variations in moisture, protein, lipid, ash and carbohydrate contents of *Oreochromis niloticus* caught from the Nile during different Seasons.

Explanation of Figures

Figure (2) : L.S of normal muscle of *Oreochromis niloticus* showing the myomers (M) (Bouin's H& E) x 400.

Figure (3): L.S of muscle of *Oreochromis niloticus* collected from El-Kanater showing: -

1- Necrosis area in hypoderm.
2- Hemorrhage, hemolysis and hemocidrin.
3- Severe degeneration and necrosies in muscle fibers (Bouin's H&E) x 400.

Figure (4a & 4b): L.S of muscle of *oreochromis niloticus* collected from talkha showing:-

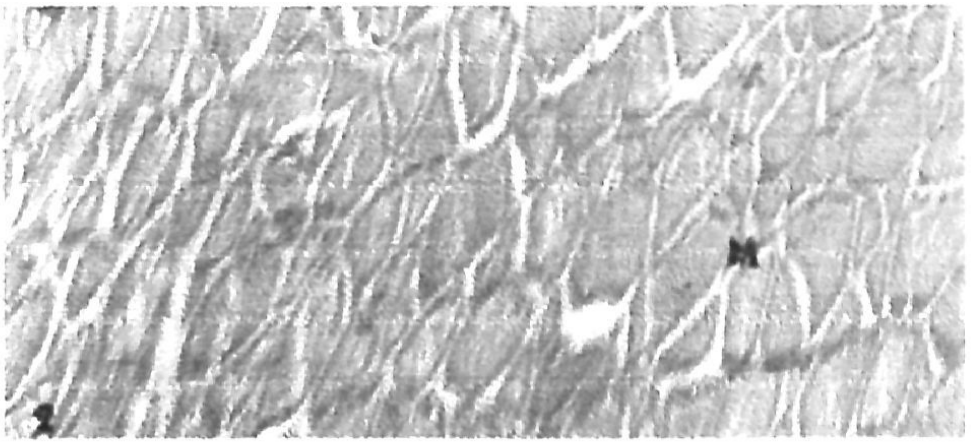
4- Severe destruction in muscle fibers.
5- Intrafibrillar edema. (Bouin's H & E) x 400.

Figure (5a & 5b): L.S of muscle of *Oreochromis niloticus* collected from Benha showing:-

6- Epidermal cells are missing.
7- Thickness and dilation in blood vessel.
8- Hemolysis and hemocidrin around blood vessel.
9- Severe destruction in muscle fibers (Bouin's H & E)x400

Figure (6): L.S of muscle of *Oreochromis niloticus* collected from Zefta showing:-

10- Necrosis in hypodermis layer.
11- Severe degeneration in muscle fibers.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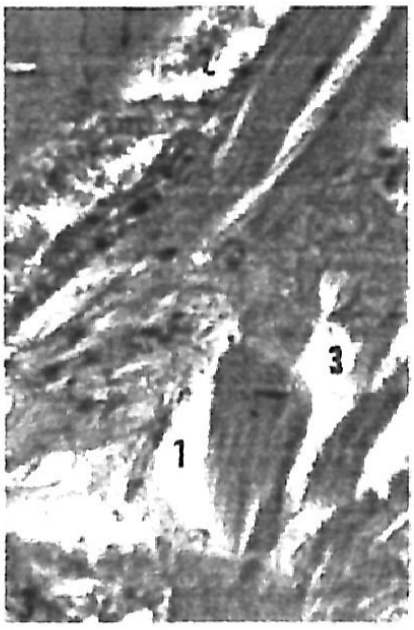
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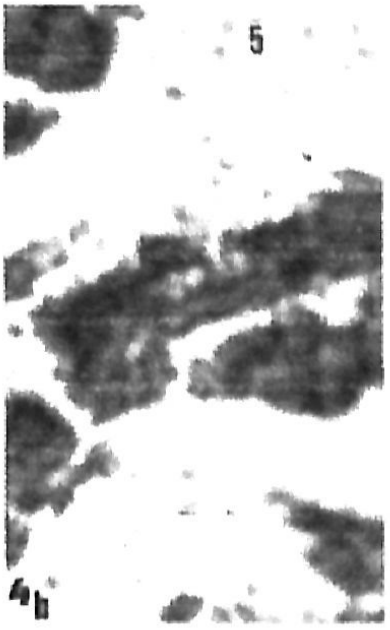
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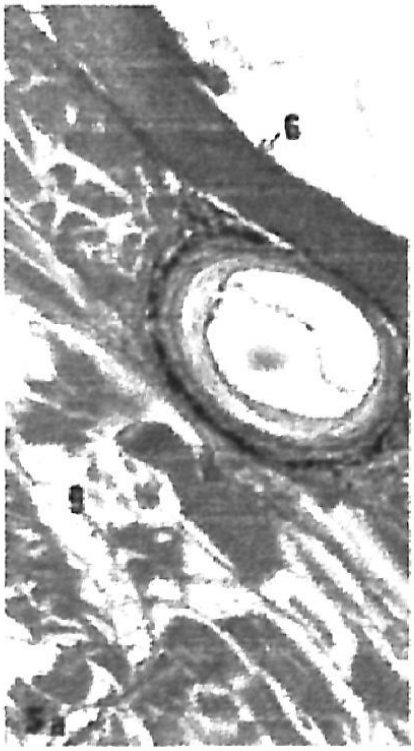
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