EFFECT OF HYPOTHERMIA ON GENE EXPRESSION OF SOME ENZYMES ACTIVITY IN BRAIN OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Received 6/ 2/ 2012 *Accepted 3/ 3/ 2012*

Abstract

Tilapias are widely recognized as one of the most important fish species for freshwater aquaculture. Temperature is one of the most important factors affecting the metabolism or Nile tilapia (*Oreochromis niloticus*).The present study was conducted to evaluate the effect of low temperature (hypothermia) on some enzymes activity in brain of (*O. niloticus*) such as succinate dehydrogenase, pyrovate and lactate temperature was decreased by permanently used colder. Total number of 50 fish were distributed into 5 groups; each group contain 10 fish, group (1) control group reared at optimal temperature (24-26°C) group (2) fish reared at 15°C for 24 hours. Group (3) fish exposed to 15°C for 30 days. Group (4) fish reared at 17°C for 24 hours. Group (5) fish reared at 17°C for 30 days. The brain tissues were kept in liquid nitrogen tank until analysis.

The results revelated that the brain tissues show a significant decrease in succinat dehydrogenase and pyrovate level in the group subjected to hypothermia and this decrease was clear in group 3. While there a significant increase in lactate level in group 3 subjected to hypothermia and this increase was clear in the group subjected to 15°C for 30 days. . The results showed that the brain tissues of *O. niloticus* show a decrease in the level of Gene expression of Hexokinase enzyme (HK) in group exposed to low temperature at group 3 and 5 when compared to control group. The same results were obtained of Gene expration cytochrome oxidase enzyme (COXII).

Hypothermia has effect on activity and gene expression of metabolic enzymes in brain of Nile tilapia.

Keywords: Nile tilapia, *Oreochromis niloticus*, hypothermia, gene expression, enzymes activity, succinate dehydrogenase,pyrovate and lactate, temperature.

INTRODUCTION

Temperature is one of the most important environmental factors, as it determines the distribution, behaviors and physiological responses of animals. Temperature also affects longevity in insects (Sohal and Allen, 1986), fish (Malek *et al*., 2004), primates and humans (Buffenstein, 2005) Temperature is one of the most important factors affecting the physiology, growth, reproduction and metabolism of tilapia. Temperature is of prime importance in temperate and tropical regions, which are characterized by seasonal fluctuations in water temperature. Tilapias are hemophilic fish and known to tolerate a wide range of water temperatures. Extensive research has been conducted on the effect of water Temperature on tilapia performance. The Temperature range for the normal development reproduction and growth of tilapia is about 20°C to 35°C, depending on fish species, with on optimum range of about 25°C -30°C (Balarin and Haller, 1982 and Chervinsk, 1982).Tilapia can life in optimum temperature 27-28 °C (El-Sayed and Kawanna, 2004).

In contrast to endothermic mammals, ectothcrmic vertebrates such as fish can survive in a wide range of thermal environments, and thus fish cells may utilize different strategies to cope with thermal fluctuations. Many physiological responses, including changes in lipid composition (Hazel, 1979), increases in pump activity and specific Na^+/K^+ -ATPase activity (Sehwarzbaum *et al.,* 1992) and oxygen consumption have been extensively reported when fish are exposed to hypothermia (Raynard and Cossins, 1991).

Tilapia can also tolerate Temperature as low as 7°C -10°C, but only for brief periods (Balarin and Haller, 1982 Chervinskl,1982; Jennings, 1991 and Sifa *et al.,* 2002) longer exposure of tilapia to this low temperature will certainly lead to mass mortality. Tilapia feeding is sharply reduced below 20°**C,** and they stop feeding at about 16°C, while severe mortality occurs at 12°C (Balarin and Haller,1982 and Chervinski,1982).

Hypothermia may have effect on fish especially on growth, reproduction and metabolism , so this study aim to determination the effect of hypathermia on gene expression of some metabolic enzymes and values of some metabolites in brain tissue of Nile tilapia fish.

AIM OF THE WORK

This study amid to determine the effect of hypothermia on gene expression of some metabolic enzymes such as Hexokinase (HK),and Cytochrome oxidase (Cox II)and determine the levels of lactate ,pyruvate and activity of Succinate dehydrogenase in brane tissueof Nile tilapia fish, (*Oreochromis niloticus*).

MATERIAL AND METHODS

Fish:

Nile tilapia were obtained from local fish hatchery in Abbassa, Abu hammad Skarkia Egypt. Fish were maintained for 1 month in 150 liter tanks under laboratory conditions in normal temperature (24-26°C). Fish were divided into five groups, every group contains 10 fish which into 5 aquarium. The fish fed on diet 25% protein.

Group (1): Fish reared at optimal temperature at 24°C - 26°C (control group) all over the experiment.
Group (2): Fish reared at 15°C for 24 hours.
Group (3): Fish reared at 15°C for 30 days.
Group (4): Fish reared at 17°C for 24 hours.
Group (5): Fish reared at 17°C for 30 days.

Sampling:

Brain tissues were isolated from fish samples of all groups by cutting off the head of fish, set the head on its cut surface with snout facing upward and use the cutter to cut from the nostrils to the upper edge of the eye and remove the front partial pone then remove the brain tissues of fish by forceps. Immediately, the brain tissues isolates were kept in liquid nitrogen tank .

Brain tissues were grinded with a ceramic motor and pestle under approximately 2ml of saline solution under colling and pour the suspension into Homogenization Spin Column.Centrifuged at 12000 x g for min.at room temprature. Samples were kept until analysis at - 168°C

Biochemical Determination:

(1): Determination of brain tissue succinate dehydrogenase according to (Kramer, 1971)

(2): Determination of brain tissue, pyruvate by Enzymatic UV-Test kits according to (George and Peter, 1971).

(3): Determination of brain tissue lactate by enzymatic colorimetric method (LOX | PAP) with lactate oxidase and 4-aminoantipyrine kites according to (Wieser *et al*. 2003).

Molecular determination:

(1) Determination of (Hexokinase and Cytochromeoxidase) Gene Expression: Using a semi-quantitative RT-PCR according to (Meadus, 2003).

(a) Protocol of RNA extraction from tissue; Using Gene JETtm RNA purification Kit (Fermentas).

(b) One step (PCR) Kits: Using beads (Fermentas).

Table (1): The thermal cycler conditions used during PCR.

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycles
Hexokinase	94|5 min	94|1 min	60|1 min	72|1 min	72|10min	35
cytochro-neoxidase (II)	95|2 min	94|30 sec	54|30 sec	72|1 min	72\10min	35
GAPDH	95|5 min	94|30 sec	60|30 sec	72|1 min	727\ min	30

Table (2): Primers used in determination of the expression of the pervious genes.

Gene	Primers	Organ	Annealing Temp.	Expected size
Hexokinase	5'- GCATCTCCGACTTCCTG 3' 5'- GCAGCTTGTACAGGGTG 3'	Brain Tissue	72	447
Cytochrome-oxidase (II)	5'-CGACTAATCATAAAGATATCGGCAC3' 5'-ACTTCAGGGTGACCGAAGAATCAGAA3'	Brain Tissue	72	655
GAPDH	5-CCCGTAGACAAAATGGTGAAGG-3 5-GCCAAAGTTGTCATGGATGACC-3	Brain Tissue	60	215

PCR products were separated on a 1.5% Eithidum bromide treated agarose gel electrophoresis in Tris acetate EDTA buffer with 0.5 mg|ml ethdium bromide. The gel examined by UV transilluminator. The electrophoretic picture was taken by digital camera 12 mega pixels and quantified with image J software.

Statistical analysis of results:

The data obtained in the study, were statistically analyzed according to the methods described by Dixon and Massey (1983) and Snedecor (1980)**.**

RESULTS AND DISCUSSION

The results in tables 3 present values for brain tissue Succinate dehydrogene enzyme activity of Nile tilapia fish exposed to 15°C and 17°c for 24 hours and 30 days. The results show that there in a significant decrease in the activity succinate dehydrogenase enzyme was clear in the groups subjected to hypothermia for 30 days.

Our results mentioned above are in close agreement with the values reported by other worker as Campbell and Davies (1978) reported that the aerobic enzymes activities are found to be lower at the cold temperature in blennies (Blemmios phalis) acclimated to 10°C compared to 20°C; the activities of aerobic enzymes reman mach lower at cold then warm temperature Johnston and Dunn (1987). Yasuyuki *et al.* (1985) reported that in carpmeat, the enzyme a activities at optional temperature (30°C) because 8.5 fold higher there at 25 degree of temperature.

The data obtained in tables 4 revealed that the pyruvate value in brain tissue of Nile tilapia fish exposed to 15°C and 17°C for 24 hours are decreased; while the pyruvate content in brain tissue of Nile tilapia fish exposed to 15°C and 17°C for 30 days decreased significantly.

Our findings mentioned above are similar to many findings of other researcher; pyruvate decreased in both muscle and liver of some fishes at low temperature (Zakhartsev *et al.*, 2004); levels of pyruvate decreased in rainbow trout (oncorhynchus mykiss) when fish exposure to low temperature from till 8°c (George and Peter, 1971).)

Our investigation in table 5 showed that the lactate values in bran tissue of Nile tilapia fish exposed to 15°C and 17°C for 24 hours are increased, while the lactate levels in brain tissue of Nile tilapia fish exposed to 15°C and 17°C for 30 days were increased significantly.

Our results obtained are agreed with finding of Trygve and Bengt (2003) who said that exposure Atlantic salmon (*Salmo Salar*) to low temperature leads to increase in lactate level; lactate level were determined in rainbow trout (*Salmo gairdneri*) acclimated to 8°c 12°C and 16°C; the 8°C acclimated fish had the highest lactate level **.** At 20°C gold fish survive anaerobic conditions for only a few hours while at 4°C survival in extended to several days, during the course of low temperature anaerobiosis these was a rise in blood glucose and lactate, a decline in liver glycogen concentration and an increase in liver water content. It is concluded that liver glycogen is a necessary energy source during cold an aerobiosis (Walker and Johansen, 1977).

Findings of other authors are not in agreement with our result, on in (Rutilus rutilus L.) fish acclimated to 4°C , lactate accumulation after activity were only half those in fish to 12 and 20°c (Wieser *et al.*, 2003), also changes in blood lactate and glucose concentration was monitored in common carp (*Cyprinus carpio*) exposed to decreasing temperatures (from 24°C to 4°C), blood lactate decreased in the cold treated common carp in both rapid and slow change experiments (Guan, 2010).

Crockett and Sidell (1990) found that low lactate level when exposure polar fish such as (Antarctic notothenioids) to low temperature.

As illustrated in table 5 appeared that elevation of temperature increased the lactate level of fish exposed for 24 hours; slight differences in elevation was noticed between both group exposed for 30 days and this findings are in agreement with conclusions of may authors mentioned before.

In response to differences in environmental temperature, however, significant changes in the levels of activity of fructose biphosphate aldolase, pyruvate kinase. Succinate dehydrogenase, cytochrome oxidase and cytochrome oxidase were observed in one or more tissue. One group of enzymes increased in the cold while the other group decreased, indicating that major metabolic reorganizations were occurring, the patterns of change among tissues were distinctly different, therefore changes observed in one tissue can not readily be generalized to other tissue (James *et al.,* 2005).

Table (3): Showed succinate dehydrogenase activity (m/u/ml) in Nile tilapia (*O. niloticus*) reared at different levels of temperatures for different periods.

Treatment	15°C/ 24h.		15°C/ 30 day.		17°C/ 24h.		17°C/ 30 day.	
	Control	Grop 2	Control	Grop 3	Control	Grop 4	Control	Grop 5
Minimum	7.94	3.06	7.67	4.23	7.44	4.76	7.76	4.88
Maximum	14.53	11.18	14.34	10.71	14.53	11.23	11.34	11.54
Mean	11.64	8.26	10.84	5.66	11.64	4.18	10.84	6.733
±SE	0.68	0.57	0.86	0.57	0.68	0.83	0.68	0.38
***T. test**	2.572		3.667		3.742		2.874	

* P<0.01

Table (4): Showed Pyruvate content (mg/dl) in Nile tilapia (*O. niloticus*) reared at different levels of temperatures for different periods.

Treatment	15°C/ 24h.		15°C/ 30 day.		17°C/ 24h.		17°C/ 30 day.	
	Control	Grop2	Control	Grop3	Control	Grop4	Control	Grop5
Minimum	73.00	2.58	2.81	1.71	3.00	3.17	2.81	2.35
Maximum	8.71	7.24	8.32	4.32	8.71	7.31	8.32	5.17
Mean	5.34	4.11	5.53	2.76	5.34	4.68	5.53	3.31
±SE	0.96	0.43	0.48	0.63	0.96	0.85	0.98	0.68
***T. test**	2.53		3.21		2.74		2.36	

*P<0.01

Table (5): Showed lactate content (mg/dl) in Nile tilapia *(O. niloticus*) reared at different levels for temperatures for different periods.

Treatment	15°C/ 24h.		15°C/ 30 day.		17°C/ 24h.		17°C/ 30 day.	
	Control	Grop 2	Control	Grop 3	Control	Grop 4	Control	Grop 5
Minimum	11.0	10.0	11.6	11.3	11.0	10.3	11.6	11.7
Maximum	12.5	12.0	13.8	14.2	12.5	13.4	13.8	14.4
Mean	10.5	10.75	11.60	12.25	10.50	11.25	11.6	13.0
±**SE**	0.30	0.70	0.30	0.28	0.30	0.73	0.30	0.46
***T. test**	1.17		1.38		0.98		2.98	

*P<0.01

In the study, the brain tissue showed a decrease in level of gene expression of Hexokinase gene in both group (3) and (5) exposed to hypothermia for long duration comparing with control group (Table 6 and Fig. 1) o n fending support the opinion of Dawn *et al*., (1991) who stated that there in a decrease in the activities of Hexokinase in tissue removed from cold – acclimated fish , also our result was in agreement with foundation of Johnston (1977) that said , low temperature acclimation, the activities of hexokinase was approximately 2-3 time lower in carp (*Cyprinus carpio*) than rainbow trout (*Salmo gairdneri*) white muscles.

When exposure fish to low temperature in our study, we found a significant decrease in level of Cox II gene expression in both group (3) and (5) that exposed to hypothermia for 30 days comparing with control group (Table 7) and (Fig. 2); these results are in agree with others findings and disagrees with others. At hypothermia, Antarctic eelpout (*Pachycara bracephalum*) failed to reflect such a compensatory increase in the cox II activities, whereas cold acclimated eelpout from the north sea show lower enzyme activities then expected on the basis of mitochondrial m RNA level (Hardewig *et al*. 1999); transfer of green

sunfish (*Lepomis cyanellus*) from 25°c to 5°c resulted in a rapid decrease of approximately 40% in rates of synthesis of skeletal muscle cytochrome c and a concomitant decrease in the degradation rate constant for this molecule of approximately 60% (Sidell, 1977).

Cold acclimation of the eurythermic carp is acrapanied by a partial compensation of the acute effect of decreasing temperature on the activity of cytochrome oxidase in red muscle mitochandria (Ekkehart, 2003).

Leigh (2011) measured metabolic enzyme activity in terrapin muscle tissue to assess thermal dependence and the role of temperature in seasonal metabolic down regulation in this species. Activity of cytochrome c oxidase was assayed at 10**,** 20**,** 30 and 40°c for tissue collected during summer and winter; he found that the activity of cytochrome oxidase was significantly lower in winter collected tissue compared with summer collected tissues. Results indicate that temperature affects contribute to seasonal metabolic down regulation and dormancy in terrapins.

Low temperature leads to low citrate synthase / cytochrome oxidase ratio that reflect the energy surplus available (Ibarz *et al*., 2010); Juvenile lake white fish (*Coregonus clupeaformis*), a species while is extensively distributed in northern latitudes and which experiences a fairly wide range of temperature acclimated at 5 and 18°c , cold acclimation during six months do not increase the levels of cytochrome oxidase and citrate synthase (Blier and Guderley, 1988).

Acclimated goldfish for 2-4 weeks to either 10°c or 30°c and found that activities of cytochrome c oxidase in gill tissue were higher at all temperature between 10℃ - 40℃ in the cold acclimated.

Flounder (*Platichthys flesus L*.) were acclimated in sea water for 1-2 month to either 5°C or 23°C; activities of marker enzymes for

mitochondrial metabolism, cytochrome oxidase and hexokinase are 1.5-2.8 times higher in muscles of cold - acclimated compared to worm acclimated flounders. Increases in the activities of these enzymes with cold acclimation may serve to offset the effects of low temperature on aerobic ATP supply. Glycolylic enzyme activities as lactate dehydrogenase, however, are similar at both acclimation temperature (Johnston and Wokoma, 1986).

Table (6): Analysis of PCR product of Hexokinase gene in brain tissue of Nile tilapia fish.

Group	Area	Mean	Stadev	Min	Max	XM	YM	Medium	%area
G1	130	158.038	1.116	155	161	110.987	392.502	158	100
G2	130	1570146	10.107	153	160	156.991	391.5	157	100
G3	130	145.731	1.385	142	160	200.001	390.499	157	100
G4	130	157.754	2.359	149	169	149.987	389.497	146	100
G5	130	151.23	1.146	143	158	231.874	318.348	150	100

The brain tissue showed a decrease in level of gene expression of (HK) gene in both group (3) and (5) that exposed to hypothermia for long time comparing with control group.

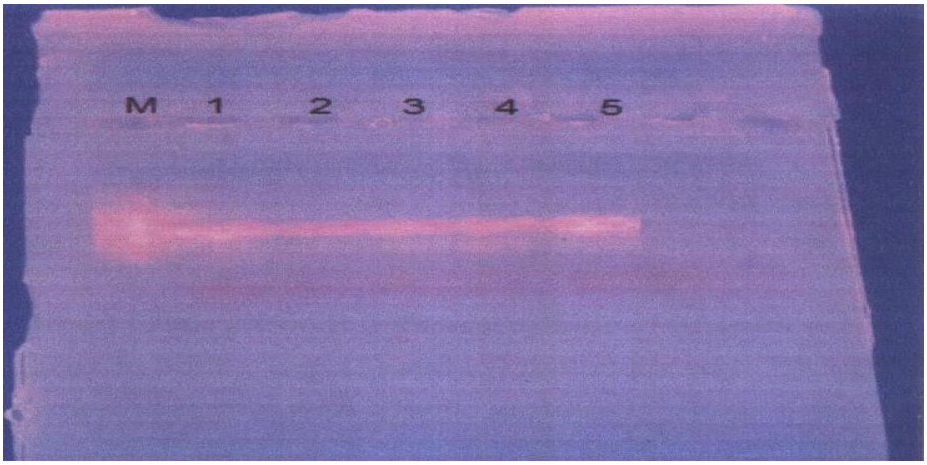

Figure (1): The electrophotography of m RNA of Hexokinase gene in brain tissue of Nile tilapia fish.

Where:

M: Marker. (1) Group (1) (2) Group (2)
(3) Group (3) (4) Group (4) (5) Group (5)

Table (7): Analysis of PCR product of Cox II gene in brain tissue of Nile tilapia fish.

Group	Area	Mean	Stadev	Min	Max	XM	YM	Medium	%area
G1	175	121.966	3.905	116	138	442.703	266.502	124	100
G2	175	118.72	2.465	114	123	383.357	265.507	121	100
G3	175	107.017	1.101	99	119	324.495	266.494	111	100
G4	175	120.177	11.568	117	124	269.514	269.543	122	100
G5	175	110.132	4.103	104	118	214.87	227.131	131.117	100

The brain tissue showed significant decrease in level of gene expression of (Cox II) gene in both group (3) and (5) the exposed to hypothermia for long time comparing with control group.

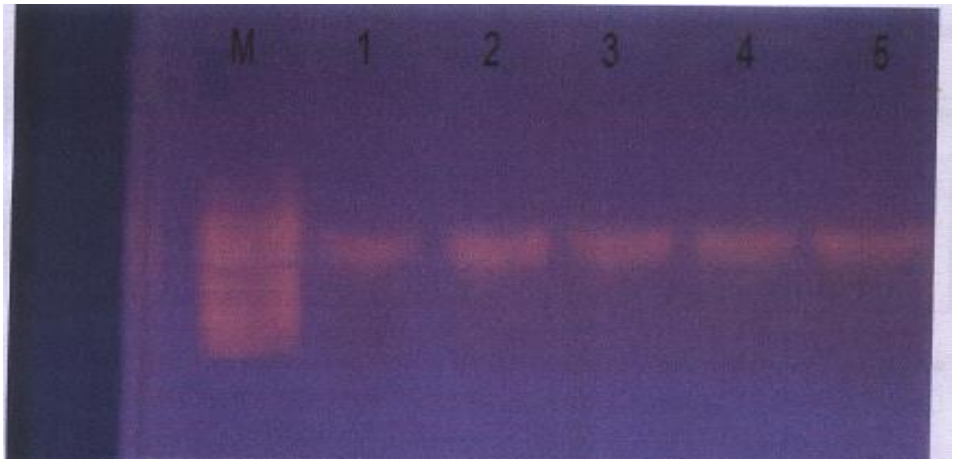

Figure (2): The electrophotography of m RNA of GK gene of (Cytochrome oxidase) in brain tissue of Nile tilapia fish.

Where:

M: Marker.	(1) Group (1)	(2) Group (2)
(3) Group (3)	(4) Group (4)	(5) Group (5)

Table (8): Analysis of PCR product of GAPDH gene in brain tissue of Nile tilapia fish.

Group	Area	Mean	Stadev	Min	Max	XM	YM	Medium	%area
G1	250	267.5	3.146	166	186	140.43	318.004	174	100
G2	250	165.304	2.968	159	171	188.033	320.025	166	100
G3	250	162.592	2.562	155	167	241.497	320.001	159	100
G4	250	163.648	2.668	164	181	289.497	320.002	173	100
G5	250	164.67	3.138	163	184	291.497	321.002	175	100

The internal control gene showed almost stable pattern of expression in brain tissue of Nile tilapia.

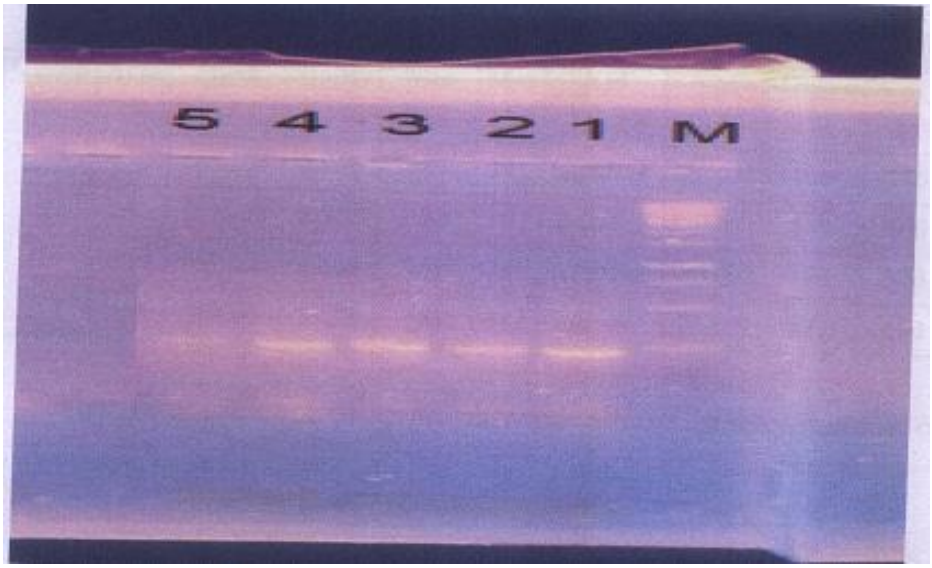

Figure (3): The electrophotography of Glyceraldhyde 3 phosphate dehydrogenase m RNA (GAPDH) expression in brain tissue of Nile tilapia fish.

Where:

M: Marker. (1) Group (1) (2) Group (2)
(3) Group (3) (4) Group (4) (5) Group (5)

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تأثير انخفاض درجة الحرارة على التعبير الجينى لنشاط بعض الإنزيمات الموجودة بالمخ لأسماك البلطي النيلي

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الملخـــص العربـــى

تعتبر أسماك البلطي من أهم الأسماك التي تصلح للتربية في المزارع السمكية ودرجة الحرارة من أهم العوامل المؤثرة على العمليات الحيوية لأسماك البلطي النيلي. استهدفت هذه الدراسة تأثير انخفاض درجة الحرارة على نشاط بعض الإنزيمات الموجودة بالمخ لأسماك البلطي النيلي مثل إنزيم السكسينك ديهيدروجنيز ومستوى البروفات واللاكتات وقد تم خفض درجة الحرارة عن طريق كولدير واستخدم في هذا البحث ٥٠ سمكة بلطي نيلي وقسمت هذه الأسماك إلى ٥ مجموعات وتحتوي كل مجموعة على ١٠ اسماك، المجموعة الأولى وهي مجموعة الكنترول (٢٤-٢٦°م)، المجموعة الثانية وهي مجموعة معاملة الأسماك تحت تأثير ١٥°م لمدة ٢٤ ساعة، المجموعة الثالثة وهي مجموعة معاملة الأسماك تحت تأثير ١٥°م لمدة ٣٠يوم، المجموعة الرابعة وهي مجموعة معاملة الأسماك لدرجة حرارة ١٧°م لمدة ٢٤ ساعة، المجموعة الخامسة وهي مجموعة تأثير درجة حرارة ١٧°م على الأسماك لمدة ٣٠يوم، وقد أظهرت النتائج انخفاض معنوي في مستوى إنزيم سكسينك ديهيدروجنيز ولبيروفات في مخ أسماك البلطي النيلي في جميع المجموعة بالمقارنة بمجموعة الكنترول، بينما كان هناك ارتفاع معنوي في مستوى اللاكتات لجميع المعاملات بالمقارنة بمجموعة الكنترول٠.

مما يدل على أن انخفاض درجة الحرارة يؤثر على انزيم السكسينك ديهيدروجنيز والبروفيك، وأظهرت النتائج أيضا انخفاض ملحوظ في مستوى التعبير الجيني لإنزيم الهكسوكينيز مما يدل على أن انخفاض درجة الحرارة تؤثر على التعبير الجيني لإنزيم الهكسوكينيز. كما أنه لوحظ انخفاض في التعبير الجيني لإنزيم السيتوكروم أكسيديز في مخ سمكة البلطي النيلي في المجموعة الثالثة والخامسة مما يدل على أن انخفاض درجة الحرارة تؤثر على التعبير الجيني لإنزيم السيتوكروم أكسيديز.

من هذه الدراسة نجد أن انخفاض درجة الحرارة يؤثر تأثيراً ملحوظاً على التعبير الجيني لبعض إنزيمات التمثيل الغذائي لأسماك البلطي النيلي.