Differential expression of aromatase genes as a stress response of different levels of salinity treatments in the Nile Tilapia, *Oreochromis niloticus*

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ABSTRACT

This is the first study on the effects of salinity as a stress on the differential expression of the aromatases (CYP19 a & b) in the Nile tilapia (*Oreochromis niloticus)* tissues (ovary, testis and brain of female). The experiment was conducted to study the effects of five different salinity levels (10‰, 15‰, 20‰, 25‰ and 30‰) as a stress on the expression of aromatase genes. The fish were transferred gradually from lower concentration to higher concentration of salinity for adaptation. The results of the expression of CYP19a in the gonads of *O. niloticus* as a response to the stress of salinity revealed the positive correlation between salinity and the relative CYP19a expression in the ovaries and testes of all treatments with marked higher values in ovaries than testes and this can be ranked in the order: 30‰ > 25‰ > 20‰ > 15‰ > 10‰. With the increase of salinity levels, the relative CYP19a expression in the ovaries varied from 0.8 with the treatment of 10‰ to 7.58 with that of 30‰, while the expression in the testes ranged from 0.65 to 3.48 respectively. Similarly, the direct relationship was observed between the relative expression of CYP19b in the brain of females (0.026 to 0.037) and the increasing levels of salinity.

INTRODUCTION

Cichlids are the most common species and highly economic fishes in most lakes in Egypt. It has many species at Lake Manzala like *Oreochromis niloticus*, *Oreochromis aureus*, *Sarotherodon galilaeus* and *Tilapia zillii*. Although *O. niloticus* is the most popular cultured fresh water species worldwide, it has the least salinity tolerance in contrast of other tilapia species (Kamal and Mair, 2005; El-Saidy and Gaber, 2005; El-Zaeem *et al,* 2011). A previous study by Jaspe and Caipang, (2011) reported that Nile tilapia could tolerate brackish water with salinity up to 25‰.

Aromatases are enzymes that shared in the production of estrogen that works by stimulating the conversion of testosterone (an androgen) to estradiol (an estrogen). Aromatases existed in estrogen-producing cells in the adrenal glands, ovaries, placenta, testicles, adipose (fat) tissue, and brain (Chang *et al.,* 2005). They are essential proteins in the control of steroid balance through sexual differentiation, development, and reproduction (Diotel *et al.,* 2010).

Teleosts express two structurally and functionally P450 aromatase isoforms, named Cyp19a and Cyp19b. They illustrate special regulation mechanisms and tissue

distribution; cyp19a (ovarian aromatase) and cyp19b (brain aromatase), together synthesizing estrogens from androgens (Cheshenko *et al.,* 2008). The gene that adjusted aromatase enzyme and correlated with sex-differentiation, reproduction and behavior regulates sex differentiation in fish. Its activities are exclusive in certain organs in fish that engaged with estradiol synthesis (Callard *et al.*, 2001).

   The present study aims to provide an overview describing the potential of Nile tilapia as a model to determine its response to environmental stress of salinity through studying the effect of different salinity treatments (10‰, 15‰, 20‰, 25‰ and 30‰) on the expression of CYP19a & b in the ovary, testis and brain of *O. niloticus*.

MATERIALS AND METHODS

Experimental fish

   Adult Nile tilapias (*Oreochromis niloticus*) of mixed sex with a mean weight ranging from 50-100 g were collected from a fish farm located at Lake Manzala. For adaptation with the laboratory conditions, the collected fish were acclimatized in rectangular fish holding fiberglass tanks (3m x 1m) for one month before starting the experiment.

Experimental design for salinity as a stress response

   Five groups of fish were subjected to salinity increasing of 3‰ per hour from the reference control (1.5‰) until salinities of 10‰, 15‰, 20‰, 25‰ and 30‰. All the treatments including control were performed in triplicates. For increasing water salinities in experimental groups, underground water (28.8‰) and sea salt were added to adjust the highest concentration of salinity. The fish were transferred gradually from lower concentration to higher concentration of salinity for adaptation. There were ten fish for every 1 cubic meter of water. One-third of the tank water was changed every two days throughout the experimental period in addition to removing uneaten feed, feces or any foreign materials.

   Salinity was measured daily and adjustments were made if required to maintain the experimental conditions. The experiment was carried out at room temperature ($25\pm2^{o}C$) under normal laboratory light conditions for one month as an experimental duration.

Formulation and percentage of proximate composition of the diet used for fish feeding during the experimental period

   The diet was offered regularly 4% of fish wet body weight twice a day. The components and the proximate composition are mentioned in Table (1).

Water quality analysis

   Reference water used in this experiment was collected from the reference site close to El-Matariya Research Station for aquatic resources, Dakahlia Governorate, Egypt. Water quality measurements for the reference and underground waters include physicochemical parameters, dissolved polyaromatic hydrocarbons (PAHs) according to Parson *et al.,* (1985) and UNEP (United Nation Environment Program), (1991), extraction of the dissolved heavy metals according to the standard methods of APHA, (1989) and extraction of organochlorine pesticides (OCP) according to UNEP, (1988). The analysis of both sources of water is shown in Table (2).

Table 1: The formulation and proximate composition of the diet used for fish feeding.

Ingredient	Inclusion Level (%)
Wheat Bran	30%
Rice Bran	30%
Fish meal	30%
Yellow corn	6%
Mineral mix	1.5%
Yeast	1%
Vitamin mix	1.5%
Total	100%
Proximate composition	
Crude Protein	25%
Ether extract	12%
Crude fiber	5.5%
Moisture	8.76%
Ash	12.84%

Table 2: Water quality analysis of the reference and underground water

Physicochemical parameters	Reference water	Underground water
Salinity	1.5	28.8
Temperature	22.4	22.4
pH	8.25	7.12
Conductivity	3.68	43.2
PAHs	Reference water	Underground water
Cu	1.33	2.65
Cd	0.23	0.32
Pb	1.32	3.75
Zn	10.69	26.64
PAHs	Reference water	Underground water
∑ **PAHs**	3.46	ND
OCP	Reference water	Underground water
∑ **OCP**	17.58	5.12

Total RNA isolation (AGPC method) with slight modification

Samples of gonads and brain of *O. niloticus* were collected after scarification of fish, immediately stored in liquid nitrogen (-80 °C), then total RNA was isolated from the frozen tissues using Acid Guanidinium Thiocyanate Phenol Chloroform (AGPC) extraction method according to the principles of Chomczynski and Sacchi, 1987 and Chomczynski, 1993 with slight modification.

Cloning of single cDNAs strand then double strands using PCR technique was carried out using SCRIPT RT-PCR two-step Kit (Jena Bioscience) according to the manufacturer protocol.

Primer designing for CYP19 a & b

For designing CYP19a & b specific primers to brain and ovarian aromatases of *Oreochromis niloticus,* cytochrome p450 brain aromatase mRNA; accession number: NM_001279590.1 and ovarian aromatase of *Oreochromis niloticus* cytochrome p450 aromatase mRNA, complete cds; accession number: U72071.1 were retrieved from the Gene Bank and used as the reference sequence for CYP19a & b primers specific to *Oreachromis niloticus*.

To measure the expression level of the target gene in cells, the RNA amount applied in the assay should be normalized to a fixed amount. This can be achieved by performing Quantitative Real Time PCR (QRT-PCR) and amplifying an internal reference template such as a housekeeping gene (Oris and Roberts., 2007). For

designing specific primers of β-actin of *Oreochromis niloticus* , *Oreochromis niloticus* β-actin mRNA; accession number: KJ126772.1 was used. The selected primers were designed and presented in Table (3).

Table 3: Sequences, start, stop and GC% contents of CYP19a (ovarian aromatase), CYP19b (brain aromatase) primers and β-actin (internal control) primers for QRT-PCR.

Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Product length
Ovarian F1	ACGCTGATACTGCTCGTCTG	20	154	173	59.9	55	150
Ovarian R1	GTAGTTGCTGGCTGTGCCTA	20	303	284	60.04	55	
Brain F1	GGAAACAGGAAGGCTACCCA	20	33	52	59.30	55	191
Brain R1	CTGACGCTCTATCAGCCACC	20	223	204	60.25	60	
β-actin F1	CCCAAAGCCAACAGGGAGAA	20	322	341	60.18	55	275
β-actin R1	GTGGTGGTGAAGGAGTAGCC	20	596	577	60.04	55	

Preparation and normalization of the QRT-PCR reaction

The QRT-PCR reaction was performed using SYBR green with high ROX, (*enzynomics* kit, Korea). SYBR green participate with cDNA and specific primer of CYP19a or CYP19b of *O. niloticus* and react together in real time PCR according to the method of Bustin, 2004 to get specific gene expression; C_T (TAR) that is normalized to non-targeted β-actin as a reference gene; C_T (REF) within the same sample. To determine ΔC_T, the following equation was applied:

$$\Delta C_T = C_T \text{ (TAR)} - C_T \text{ (REF)}$$

For biological replicates in this experiment, the average of ΔC_T for replicates is exponentially transformed to the ΔC_T Expression in a next equation as the following:

$$\Delta C_T \text{ Expression} = 2^{-\Delta CT}$$

The mean is then normalized to the expression of ΔC_T expression (TAR) from a separate well treated with non-targeting control of reference site to find $\Delta\Delta C_T$. This accounted for any effects associated with the experimental procedure and is expressed as the ratio of the targeted ΔC_T expression to the non-targeted ΔC_T expression.

Percent knockdown was calculated by subtracting the normalized $\Delta\Delta C_T$ Expression from 1 (defined by the level of expression for an untreated sample) and multiplying by 100, with the following equation:

$$\% \text{ KD} = (1 - \Delta\Delta C_T) \times 100$$

RESULTS

Relationship between salinity levels and percentage of survival rate

As shown in Table (4) and Figure (1), the highest survival rate (100%) of *O. niloticus* was recorded with the reference water. On the other hand, the survival rate was inversely related to the increase of water salinity. The lowest value of survival rate (85%) was recorded at the highest salinity treatment (30‰).

Table 4: Percentage of the recorded survival rates

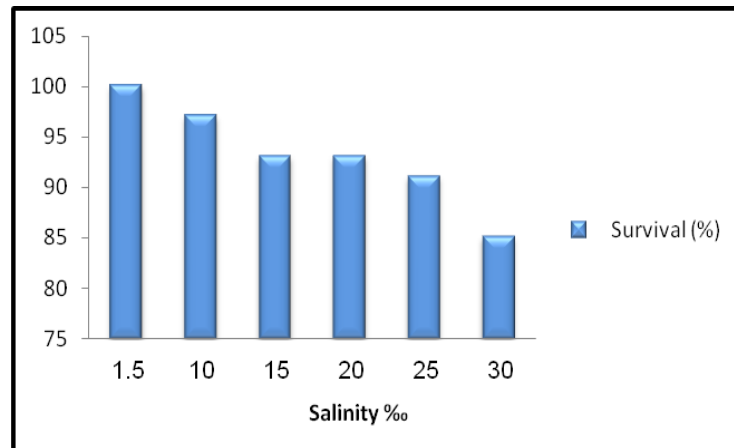| Salinity (‰)	1.5	10	15	20	25	30
Survival rate (%)	100	97	93	93	91	85

Fig. 1: The survival rate of different salinity treatments

Expression of ovarian and brain aromatases of *O. niloticus* of the reference water (1.5‰)

As shown in Table (5), the replicates of C_T values of CYP19a expression in the ovary and testis of *O. niloticus* and the replicates of C_T values of CYP19b expression in brain of female for 1.5‰ concentration of salinity as a control was normalized to the replicates of C_T values of β-actin gene as a reference gene (ΔC_T Ref.). ΔC_T expression (ΔC_T Exp.) is normalized to a corresponding average of ΔC_T of control samples. The obtained results recorded the relative expression ($\Delta\Delta C_T$) of CYP19a for ovary and testis which normally equals to 1. Also, the relative expression of cyp19b in the brain of female is equals to 1.

Table 5: The values of multiple data points are with replicates of treatment 1.5‰ of salinity as reference water.

	Organ	C_T Target	C_T Ref.	ΔC_T	ΔC_T Exp.	ΔC_T Exp. Std. Dev.	$\Delta\Delta C_T$	$\Delta\Delta C_T$ Std. Dev.	% KD
1.5‰	Ovary	38.75	26.30	12.45					
		39.00	26.50	12.50	0.00024	0.0001	1	0.47	-
		38.06	26.86	11.20					
		Average= 12.05							
	Testis	34.88	26.53	8.35	0.003	0.0013	1	0.4	-

Expression of aromatases as a response to environmental stress of salinity

As shown in Tables: (6), (7), (8), (9), and (10), the relative expression of CYP19 a & b were studied by QRT-PCR analysis in female and male of *O. niloticus* with different salinity treatments (10‰, 15‰, 20‰, 25‰, and 30‰) respectively.

The expression of the target gene (C_T Target) was normalized to the expression of the reference gene; β-actin (C_T Reference) to obtain ΔC_T Expression. The normalization to the control was as the ratio of the targeted ΔC_T Expression to ΔC_T Expression of the control to find $\Delta\Delta C_T$.

Table 6: The values of multiple data points are with the replicates of 10‰ treatment of salinity and the reference water.

	Organ	C_T Target	C_T Ref.	ΔC_T	ΔC_T Exp.	ΔC_T Exp. Std. Dev.	$\Delta\Delta C_T$	$\Delta\Delta C_T$ Std. Dev.	% KD
10‰	Ovary	36.40	23.30	13.10					
		36.00	24.00	12.00	0.0002	7.7E-05	0.8	0.26	74.90
		36.70	24.74	11.96					
		Average = 12.35							
	Testis	33.91	24.10	9.81					
		32.70	24.35	8.35	0.002	7.7E-05	0.65	0.03	34.60
		33.20	24.38	8.82					
		Average = 8.99							
	Brain of female	32.91	26.12	6.80					
		33.70	26.30	7.39	0.008	0.0016	0.026	0.006	97.30
		33.20	26.33	6.88					
		Average = 7.02							

Table 7: The values of multiple data points are with replicates of 15‰ treatment of salinity and the reference water.

	Organ	C_T Target	C_T Ref.	ΔC_T	ΔC_T Exp.	ΔC_T Exp. Std. Dev.	$\Delta\Delta C_T$	$\Delta\Delta C_T$ Std. Dev.	% KD
5‰	Ovary	25.60	13.30	12.30					
		24.90	14.10	10.80	0.0004	0.0002	1.65	0.66	-65.25
		25.30	14.50	10.80					
		Average = 11.30							
	Testis	37.50	29.80	7.70					
		36.53	29.20	7.33	0.005	0.0014	1.56	0.47	-55.6
		37.20	29.00	8.20					
		Average = 7.74							
	Brain of female	33.50	26.40	7.10					
		33.12	26.00	6.12	0.008	0.012	0.028	0.004	97.12
		33.70	26.11	7.59					
		Average = 6.90							

Table 8: The values of multiple data points are with the replicates of 20‰ treatment of salinity and the reference water.

	Organ	C_T **Target**	C_T **Ref.**	ΔC_T	ΔC_T **Exp.**	ΔC_T **Exp. Std. Dev.**	$\Delta\Delta C_T$	$\Delta\Delta C_T$ **Std. Dev.**	**% KD**
20‰	Ovary	32.60	22.01	10.59	0.0005		2.19	0.09	-118.6
		32.50	22.00	10.50		2.6E-05			
		33.00	22.40	11.60					
		Average = 10.90							
	Testis	37.50	30.00	7.50	0.006	0.0026	1.98	0.86	-98.7
		36.53	29.76	6.77					
		37.20	29.30	7.90					
		Average = 7.56							
	Brain of female	32.12	25.30	7.10	0.009	0.006	0.029	0.019	97.11
		31.16	25.01	6.12					
		33.04	24.73	7.59					
		Average = 6.94							

Table 9: The values of multiple data points are with the replicates of 25‰ treatment of salinity and the reference water.

	Organ	C_T Target	C_T Ref.	ΔC_T	ΔC_T Exp.	ΔC_T Exp. Std. Dev.	$\Delta\Delta C_T$	$\Delta\Delta C_T$ Std. Dev.	% KD
25‰	Ovary	32.15	22.20	9.59					
		32.50	22.80	9.70	0.0014	0.0003	5.20	1.00	-939.6
		32.69	23.40	9.29					
		Average = 9.65							
	Testis	35.75	28.24	7.51					
		35.08	28.21	6.87	0.007	0.002	2.40	0.56	-143
		34.92	28.00	6.92					
		Average = 7.10							
	Brain of female	31.37	24.21	9.16					
		31.53	24.51	6.12	0.087	0.003	0.03	0.01	97
		32.00	25.65	7.59					
		Average = 6.84							

Table 10**:** The values of multiple data points are with the replicates of 30‰ treatment of salinity and the reference water.

	Organ	C_T Target	C_T Ref.	ΔC_T	ΔC_T Exp.	ΔC_T Exp. Std. Dev.	$\Delta\Delta C_T$	$\Delta\Delta C_T$ Std. Dev.	% KD
30‰	Ovary	30.68	21.00	9.68					
		30.63	21.53	9.28	0.0018	0.001	7.58	3.24	-657
		30.04	21.69	9.35					
		Average = 9.10							
	Testis	33.50	26.04	7.46					
		32.10	26.01	6.09	0.01	0.005	3.48	1.63	-247.6
		32.73	26.53	6.20					
		Average = 6.58							
	Brain of female	35.43	28.90	6.53					
		35.11	29.00	6.11	0. 01	0.003	0.037	0.01	96.23
		35.70	28.80	6.90					
		Average = 6.51							

As shown in Fig. (2), the present study of the relative expression of CYP19a in the ovary of *O. niloticus* with different treatments of salinity reported that the highest relative expression of CYP19a in the ovary was 7.58 with the treatment of 30‰ of salinity, followed by 5.2 with the treatment of 25‰, 2.19 with the treatment of 20‰ and 1.65 with the treatment of 15‰.
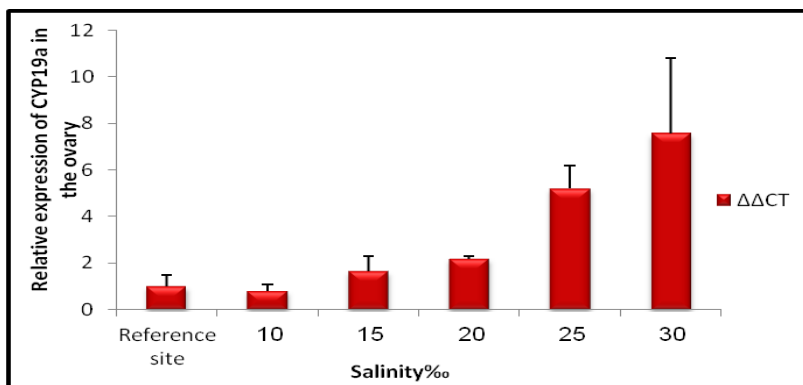

Fig. 2: The relative expression of aromatase gene (CYP19a) analyzed by QRT-PCR in the ovary of *O. niloticus* with different salinity treatments.

Finally, the lowest relative expression of CYP19a in the ovary (0.8) was recorded with the treatment of 10‰ that is the only treatment which recorded mRNA knockdown by 74.9%.

Generally, the direct relationship was clearly observed between salinity and the relative gene expression of CYP19a in the ovary.

The relative expression of CYP19a in the testis of *O. niloticus* under the stress of salinity with different treatments is shown in Fig. (3). The treatment of 30‰ recorded the highest relative expression of CYP19a in the testis (3.48), then 2.4 with 25‰, followed by 1.98 with 20‰, while 15‰ showed 1.56. The lowest relative expression of CYP19a in the testis (0.65) was recorded with the treatment of 10‰ of salinity. The treatment of 10‰ is the only treatment which recorded mRNA knockdown by 34.6%.

Generally, the relative expression of CYP19a in the testis is directly proportional to the increment of salinity.
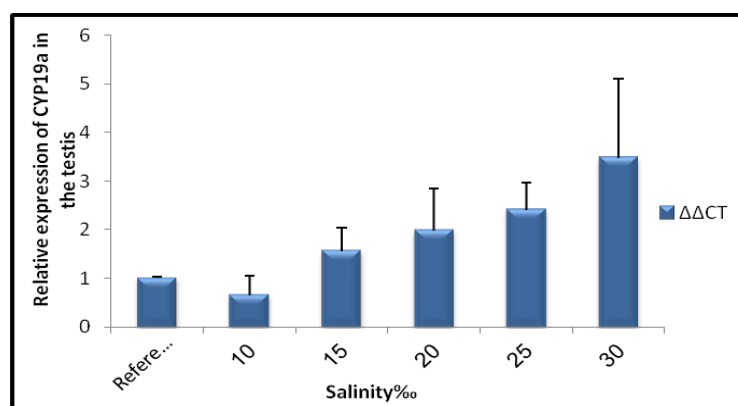

Fig. 3: The relative expression of aromatase gene (CYP19a) analyzed by QRT-PCR in the testis of *O. niloticus* with different salinity treatments.

On the other hand, the relative expression of CYP19b in the brain of the females with different salinity treatments is shown in Fig. (4). The present study of the relative expression of CYP19b in the brain of female recorded its highest value (0.037) with the treatment of 30‰ of salinity, while the treatment of 25‰ recorded 0.03, then 0.029 with the treatment of 20‰, followed by 0.028 with 15‰. Finally, the lowest relative expression of CYP19b in the brain of female (0.026) was recorded with 10‰.
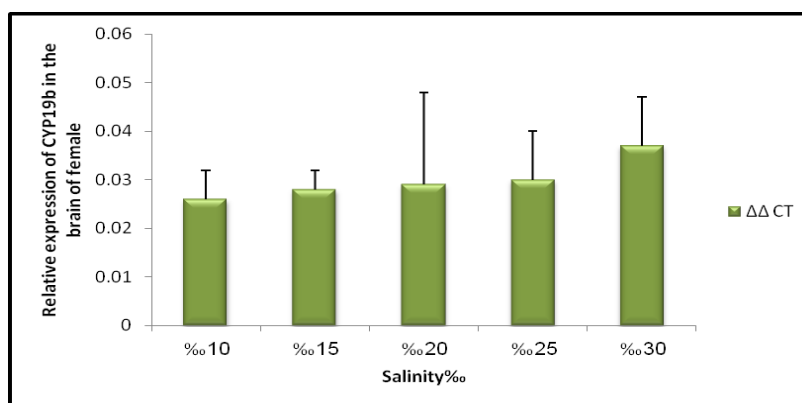

Fig. 4: The relative expression of aromatase gene (CYP19b) analyzed by QRT-PCR in the brain of the female of *O. niloticus* with different salinity treatments.

The relative expression of CYP19b in brain of females was accompanied with mRNA knockdown percentage which was inversely proportional to salinity increment. The recorded values were 97.3%, 97.12%, 97.11%, 97% and 96.3% with 10‰, 15‰, 20‰, 25‰ and 30‰ respectively.

In conclusion, the positive relationship was obviously observed between the relative expression of CYP19b in the brain of females and salinity.

DISCUSSION

Regarding the survival rates, what is well known among *Tilapia* spp. is that Nile tilapia is the least tolerant to high salinity levels. Previous studies indicated that the maximum capacity of Nile tilapia to survive in 25‰ (Jaspe and Caipang, 2011). However, in this study, we exceeded the limits of this range to be 30‰ and this affects the survival rate to become 85% in this treatment. Previous studies observed that the survival rate of fish is significantly varied with different salinity levels (Kang'ombe and Joseph, 2008). Watanabe *et al.*, (2007) observed that growth and survival in fish are not affected at different salinity levels when the temperature exceeds 27°C but salinity has a pronounced effect at temperatures below 25°C. Contradictory to our and previous studies, Iqbal *et al.*, (2012) observed that the higher salinity (40‰) levels have a pronounced effect on fish growth which might be due to improved osmoregulation.

Nowadays, gene expression is a biomarker to different biological responses studies that allow early detection of toxic effects occurring due to pollution charges (Contardo-Jara and Wiegand, 2008). In this study, the results of the expression of CYP19a in the gonads of *O. niloticus* as a response to the stress of salinity revealed the positive relation between salinity and the relative CYP19a expression in the ovary and testis of all treatments with marked higher values in ovary than testis. This finding is agreed with the previous studies that generally reported that CYP19 transcripts are expressed in the gonads of females higher than those recorded for the male in teleost fishes, such as zebrafish (Tchoudakova and Callard, 1998), goldfish (Kishida and Callard, 2001), and tilapia (Kwon *et al.*, 2001). Similarly, the direct relationship was observed between the relative expression of CYP19b in the brain of female and salinity. This may be an indication that different levels of salinity have a direct effect on the expression of CYP19 genes.

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ARABIC SUMMARY

دراسة التعبيرالجيني لجينات الأروماتيز كاستجابة لمعاملات مختلفة من الملوحة في البلطي النيلي ،
Oreochromis niloticus

علي حسين أبو المعاطي[1] ، محمد عبد الحليم حجازى القاضى[2] ، هالة عبد السميع عبد الحميد منصور[3]
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أجريت هذه الدراسة بمحطة بحوث الثرة المائية بمدينة المطرية بمحافظة الدقهلية ـ جمهورية مصر العربية ، واستهدف هذا البحث دراسة تأثيرات بعض الضغوط البيئية مثل الملوحة على تعبير جينات الأروماتيز (CYP19a & b) فى البلطي النيلي *O. niloticus* من خلال دراسة التعبير الجيني النسبى للأروماتيز (CYP19a & b) في أنسجة البلطي النيلي (المبيض ، الخصية ، ومخ الإناث). ولتحقيق هذا الهدف ، كان من الأهمية بمكان عدم إهمال تأثير العوامل البيئة المحيطة .لذلك فقد قمنا بدراسة الخصائص الفيزيائية والكيميائية للبيئة المحيطة بجانب الهيدروكربونات متعددة العطرية (PAHs)ومبيدات الآفات العضوية (OCP).

تم تجميع عينات البلطي النيلي البالغ *O. niloticus* من جنس مختلط بمتوسط وزن يتراوح من 50 إلى 100 جم من مزرعة أسماك تقع في بحيرة المنزلة .وللتكيف مع ظروف المعمل المائى ،فقد تم تأقلم الأسماك المجمعة في أحواض مستطيلة مصنعة من الألياف الزجاجية لمدة شهر واحد قبل بدء التجربة.

تعرضت خمس مجموعات من الأسماك لملوحة متزايدة بواقع 3% في الساعة بداية من المياه الموجودة فى المنطقة المرجعية (1.5‰) حتى إستقرار الملوحة لتصل إلى 10‰و 15‰ و 20‰ و 25‰ و 30‰ وتم إجراء جميع المعاملات بما في ذلك مياه المنطقة المرجعية في ثلاثة أحواض مكررة.

ولزيادة ملوحة المياه في المعاملات ، تم إضافة المياه الجوفية (28.8‰) وملح البحر لتعديل أعلى تركيز للملوحة .وتم نقل الأسماك تدريجيا من تركيز أقل إلى تركيز أعلى من الملوحة عن طريق الأقلمة.تم وضع عشرة أسماك لكل 1 متر مكعب من الماء. تم تغيير ثلث مياه الأحواض كل يومين بصورة منتظمة خلال فترة التجربة بالإضافة إلى إزالة الأعلاف غير المأكولة والفضلات أو أي مواد غريبة . تم قياس الملوحة يوميا وتم إجراء تعديلات إذا لزم الأمر للحفاظ على ظروف التجربة. أجريت التجربة في درجة حرارة الغرفة. وتم تقديم العليقة الغذائية بصورة منتظمة بواقع 4% من وزن الجسم مرتين يوميا.

تم اجراء تجارب موحدة على العينات المأخوذة كالآتى :
تم ذبح الأسماك فى التجربتين وتجميع الغدد التناسلية والمخ ، وتخزينها على الفور في النيتروجين السائل. وتم عزل الحمض النووي الريبي (total RNA) من الأنسجة المجمدة باستخدام طريقة AGPC وحساب تركيزه باستخدام الاسبكتروفوتوميتر. تم قياس تركيز total RNA لاستخدامه فى عمل cDNA ثم اعداد بادئات للجينات المطلوب معرفة تعبيرها الجيني وايضا تجهيز بادئات ل β-actin كجين مرجعى ثم تحديد التعبير الجيني باستخدام QRT-PCR.

في هذه الدراسة ، أظهرت نتائج التعبير الجيني عن CYP19a في المناسل كاستجابة للضغط البيئى للملوحة العلاقة الإيجابية بين الملوحة والتعبير النسبي لجين CYP19a في المبيض والخصية لجميع المعاملات مع قيم أعلى وملحوظة في المبيض عنها فى الخصية ويتفق هذا الاستنتاج مع الدراسات السابقة التي أفادت عموما أن CYP19 يتم التعبير عنه في الغدد التناسلية للإناث أعلى من تلك المسجلة للذكور في الأسماك مثل zebrafish و goldfish و tilapia. وبالمثل فقد لوحظت العلاقة المباشرة الإيجابية بين التعبير النسبي ل CYP19b في مخ الإناث وزيادة نسبة الملوحة.