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EFFECTS OF BETAINE ON ACCLIMATION OF TILAPIA (*Oreochromis aureus*) TO SEA WATER

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Abstract

This study was performed to determine the effects of betaine on acclimation of tilapia to sea water. Fish was fed with feeds containing different doses of betaine (0% (control dose), 1%, and 2%) during 43 days in fresh water then transferred to SWCRS (Sea Water Fish Culture and Research Station) and acclimated to sea water in seven days gradually. After transfer, fish was fed with the same feeds during 22 days, too. Measurements of Na^+/ K^+/ATPase activity of the gill and histological analysis of osmoregulative tissues (gill and kidney) were carried out.

Na^+/ K^+/ATPase activity values of the gills which were removed on the beginning, 4th, 8th, 15th, and 22nd days of sea water treatment were measured. Whereas its the lowest value was seen on the 4th day after transfer of fish fed with control feed, this enzyme levels in 1% and %2 betaine groups were higher (0.819±0.03 µmol Pi/ mg protein/ hour and 0.963±0.03 µmol Pi/ mg protein/ hour, respectively) than that in control group. Differences among the Na^+/ K^+/ATPase activity of the groups were significant statistically ($P<0.05$). On the following days (8th, 15th,

and 22nd), the activity values of the enzyme were insignificant statistically (P>0.05) among the groups. Histological investigation of osmoregulative tissues (gill and kidney) supported these results.

***Keywords**: Tilapia, betaine, Na^+/ K^+/ATPase activity, gill, kidney, sea water*

1. INTRODUCTION

Tilapias are commercially important culture and euryhaline fresh water fish. They can live in fresh water and saline water (to almost 75- 117 ppt salinity). Their tolerance to high salinity ranges according to species [1, 2]. It is known that, tilapias, especially *O. spilurus* and *O. aureus* can grow better in sea water than in fresh water [3].

Acclimation is one of the most important subjects for sea water culture of euryhaline fish as salmon, trout, and tilapia. Some physiological and histological changes are observed in especially osmoregulative tissues, gill and kidney, of the fishes during acclimation period. Fishes have to be acclimated to new conditions as gradually in order to obtain any or minimum mortality during acclimation period and then for a while, and also healthy and best growth.

Betaine (glycine betaine, trimethylglycine) is an attractant substance and used in order to increase food intake of the fish. Additionally, it has also importance for fish acclimation or adaptation to sea water, because its main physiologic or metabolic functions related to osmoregulation.

Less is known about the effects of betaine on sea water acclimation or adaptation of tilapias. The objective of the present experiment was, therefore, to study whether a dietary betaine supplement could improve the sea water acclimation of the tilapia or not. For this aim, Na^+/ K^+/ATPase activity of the gill and histological structures of the gill and kidney of tilapia (*O. aureus)* fed with feeds containing betaine were investigated in this study.

2. MATERIALS AND METHODS

Experiment was performed in circular tanks (in diameter of 4 m and divided into 4 equal parts with net), in triplicates, and at two environments; Fresh Water Fish Culture and Research Station (FWCRS) and Sea Water Fish Culture and Research Station (SWCRS), of the Fisheries Faculty of Cukurova University, Turkey. Fresh water was from an irrigation channel of the Seyhan Dam Lake. Sea water (35 ppt) was pumped directly from the sea. Water was pumped into the tanks at 10 l/min.

Three different feeds were prepared. For this reason, betaine (from Merck) was added into feed (commercially carp feed containing 28- 30 % crude protein, Pınar, Izmir, Turkey) at the rates of 0% (control), 1%, and 2%. Betaine was dissolved in some water because of its high solubility in the water [4] and then pulverized over the feed. Betaine added feeds were dried by using a ventilator in a shadow place until all water was evaporated.

Fish species was *Oreochromis aureus* in this study. One hundred fifty fish (mean 1.10±0.41g initial body weight and 4.32± 0.47cm total length) were stocked into each side of the tank (except one side in each of them, it was left empty) at FWCRS. Fish was

fed with the prepared feeds at FWCRS during 43 days prior to transfer. Fish was weighed and measured (using a 0.01 g sensitive scales and milimetric ruler) at the end of 43 days, then transferred to SWCRS. Fish weight and total length values were as given in Table 2.1. Fish was stocked (ninety fish) into the tanks for each group at SWCRS and acclimated to sea water within seven days. To acclimate the fish, water's salinity was increased gradually during seven days, at the rate of 5 ppt in each day by adding the sea water. Fish was fed there with the feeds given at FWCRS during sea water experiment (22 days), too.

Fish was fed ad libitum and three times a day (in the morning, middle of the day and late afternoon) in both two environments. Water temperature, dissolved oxygen, and salinity (of sea water only) were measured twice a day.

Sampling was carried out on the beginning, 4^{th}, 8^{th}, 15^{th}, and 22^{nd} days of the sea water experiment. Fish weight and length values were as shown in Table 2.1 for each sampling period. Mortality was determined. Gill and kidney tissues of the fish were removed in order to determine the enzyme levels of the gill and histological structures of them.

Groups	Control		Betaine %1		Betaine %2	
Sampling Periods	W	L	W	L	W	L
Beginning	9.87 ± 1.03^{a}	8.53 ± 0.31^{a}	8.83 ± 0.90^{a}	8.17 ± 0.25^{ab}	7.09 ± 0.64^{b}	7.67 ± 0.19^{b}
4^{th} day	10.73 ± 0.84^{a}	8.79 ± 0.25^{a}	8.79 ± 0.78^{a}	8.26 ± 0.23^{ab}	7.63 ± 0.52^{b}	7.87 ± 0.15^{b}
8^{th} day	9.93 ± 0.77^{a}	8.90 ± 0.22^{a}	8.33 ± 0.48^{a}	8.37 ± 0.20^{ab}	7.80 ± 0.58^{b}	8.17 ± 0.23^{b}
15^{th} day	10.65 ± 1.01^{a}	8.96 ± 0.31^{a}	8.92 ± 0.81^{a}	$8.33\pm0.26\pm0.0^{a}$	8.87 ± 0.67^{a}	8.29 ± 0.23^{a}
22^{th} day	11.63 ± 1.51^{a}	9.01 ± 0.36^{a}	10.92 ± 1.25^{a}	$8.79\pm0.31\pm0.0^{a}$	10.61 ± 1.06^{a}	8.66 ± 0.29^{a}

*W (g); L (cm); M (%)

*Different superscripts indicate significant differences between species according to Duncan's Multiple Range Test

Table 2.1 Fish weight (W) and length (L) values in each sampling period

Gill filaments of eight specimens were removed from arches in order to determine the enzyme activity. Filaments (0.2g) were fixed in 1ml SEI buffer (0.3 mol/L reagent grade sucrose (102.7g/L) 0.02mol /L disodium etilendiamin tetraacetat (7.44g/L Na_2 EDTA), and imidazole (0.05mol /L), pH 7.4) [5] and stored freeze at -23ºC until beginning the process. Then they were thawed**,** homogenated with teflone glass homogenizer centrifuged at 1500 rpm [5]. Inorganic phosphate concentration, which was produced as ATP, was determined according to Ames [6]. Na^{+}/ K^{+}/ATPase activity was determined according to Lowry et al. [7].

For histological analysis, after autopsy, the gill and kidney tissues were removed from eight fish and fixed in formaldehyde (40 %), then embedded in paraffin blocks. Paraffin sections were sliced into 5 μm thick and stained with hematoxylin and eosin.

Water temperature, dissolved oxygen, and salinity (of sea water only) were measured twice a day. In fresh water tanks, water temperatures were maintained between 23.5±0.3 °C (mean minimum temperature) and 30.8±0.2 °C (mean maximum temperature). Dissolved oxygen ranged between means of 7.00±0.49 mg/l and 7.72±0.63 mg/l. The temperature of sea water tanks changed between means of 24.1±0.3 °C and 28.6±0.2 °C; dissolved oxygen was between 7.34± 0.55 and 7. 56± 0.97. Salinity value was between 34.98±0.7 and 35±0.4 ppt.

Statistical analyses for Na^+/ K^+/ATPase activity values of the groups were carried out with Duncan's Multiple Range Tests at SPSS 8.0 package programme for windows [8].

3. RESULTS

3. 1. MORTALITY

Mortality was not observed until 4th sampling period (15th day), but observed at the later periods (Table 3.1).

Sampling Periods	Mortality (%)		
	Control	Betain %1	Betain %2
Beginning	0	0	0
4th day	0	0	0
8th day	0	0	0
15th day	6.7±0.0 [a]	6.7±0.0 [a]	5.6±0.0 [a]
22th day	13.3±0.0 [a]	14.4±0.0 [a]	10.1±0.0 [a]

Table 3.1 Mortality values of the groups in the sampling periods

3. 2. Na^+/ K^+/atpase Activity

Na^+/ K^+/ATPase activity values of the groups were similar in the beginning and changed between minimum 0.477± 0.03 μmol Pi/ mg protein/ hour and maximum 0. 489±0.10 μmol Pi/ mg protein/ hour (P>0.05). After transferring to sea water, Na^+/ K^+/ATPase activity values of the groups increased in each sampling period, as seen in Table 3.2 and Chart 3.2. After fish was transferred to sea water, the lowest Na^+/ K^+/ATPase activity level (0.702±0.03 μmol Pi/ mg protein/ hour) of the fish was found in the control group on the 4th day (in 20ppt salinity). Its values were higher on the 4th day than those on the beginning. These values increased to 0.819±0.03 μmol Pi/ mg protein/ hour and 0.963±0.03 μmol Pi/ mg protein/ hour in the 1 % and 2 % betaine application groups, respectively (P<0.05). On the last sampling day (22nd), enzyme activity level reached the highest value in each three groups (2.443±0.02 μmol Pi/ mg protein/ hour, 2.608± 0.10 μmol Pi/ mg protein/ hour, and 3.325±0.03 μmol Pi/ mg protein/ hour, respectively). The last group's (betaine 2 %) enzyme level was significant statistically from that of the first group in this sampling day (P<0.05).

	Na^+/ K^+/ATPase Activity (µmol Pi/ mg protein/ hour)				
Sampling Periods / Groups	Beginning	4th day	8th day	15th day	22th day
Control	0.478±0.06[a*]	0.702±0.03 [a]	0.867±0.16 [a]	2.212±0.05 [a]	2.443±0.02 [a]
Betaine 1%	0.477±0.03 [a]	0.819±0.03 [b]	1.384±0.15 [a]	2.245±0.09 [a]	2.608±0.10 [ab]
Betaine 2%	0.489±0.06 [a]	0.963±0.03 [c]	1.411±0.15 [a]	2.497±0.23 [a]	3.325±0.03 [b]

[*]Different superscripts indicate significant differences between species according to Duncan's Multiple Range Test

Table 3.2 Na^+/ K^+/ATPase Activity Levels (µmol Pi/ mg proteine/ hour) of the Fish Fed Feeds Containing Different Dose of Betaine in Sampling Periods

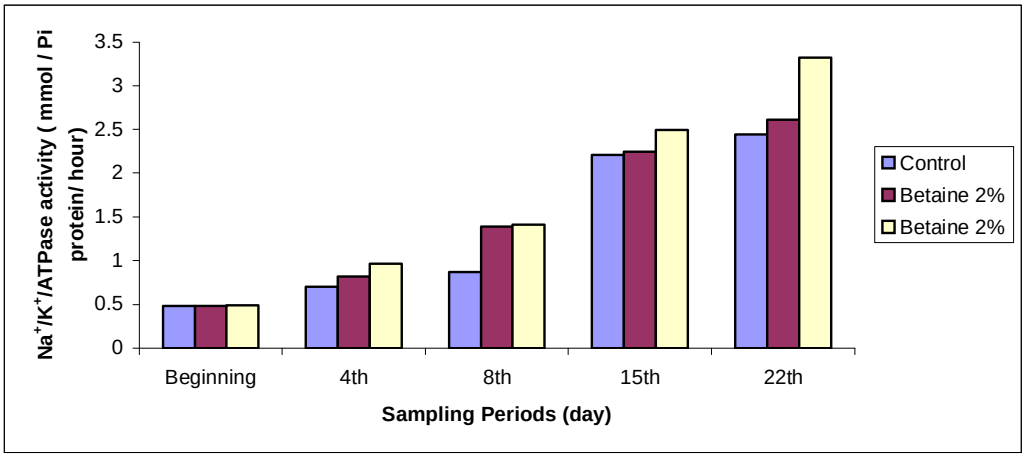
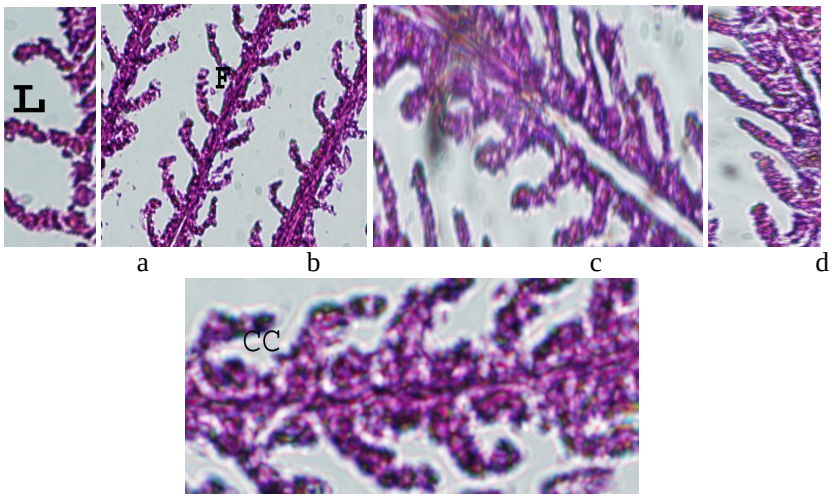


Chart 3 2 Na^+/ K^+/ATPase Activity (µmol Pi/ mg protein/ hour) of the Fish Fed Feeds Containing Different Dose of Betaine in Sampling Periods

3. 3. THE HISTOLOGY OF GILL AND KIDNEY TISSUES

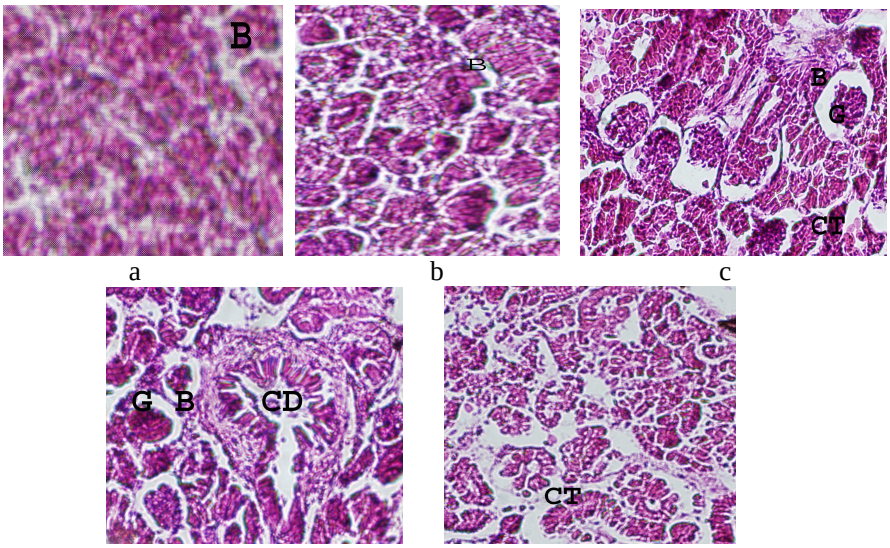
The gill and kidney tissues of the fish were shown in Chart 3.3.1 and 3.3.2 Series in the figures were given as range of the control group.

The chloride cell was not seen in gill filaments and lamellae on the beginning day. Large chloride cells were seen on the basement of the gill lamellae and the filaments in the fish in sea water application (Chart 3.3.1). Chloride cells were seen on 3rd sampling period (on the 8th day) in control group but on the 2nd sampling period (on the 4th day) in both two betaine groups (Chart 3.3.1c).



a b c d e

Chart 3.3.1 The Gill Tissue of O. aureus in control group (HE, X40) (a, b: beginning; c: 4th day; d: 8th day; e: 15th and 22nd days) L: Lamella, F: Flament, CC: Chloride Cell.



a b c d e

Chart 3.3.2. Kidney Tissue of O. aureus (HE, X40) in control group (a: Beginning period, b; 4th day; c: 8th day; d: 15th day; e: 22nd day) B: Bowman's capsule, G: Glomerulus, CD: Collecting Duct, C: Collecting Tubule

Collecting tubule, collecting duct, Bowman's capsule and glomerulus in the kidney were seen as a compact structure on the beginning in the control group. These parts were recognized clearly in the following sampling days. The glomerulus size reduced in the kidney. Widening was seen on collecting tubule and collecting duct (Chart

3.3.2 d and e). View of (Chart 3.3.2c) was observed in both betaine groups as from 4th day.

4. DISCUSSION

When cultured in sea water, tilapias have to adapt to large chances in salinity in the surrounding environment. Tilapias can accommodate to new salinity conditions in for a while. The time changes between 4 or 8 days according to species [9]. Because of taking salt to their body from the environment by drinking water continuously, fish has to excrete the more salt. In addition, fish also losses water from over body continuously. So glomerular structure of the kidney lessens. In culture conditions, desired thing is its ending in a short time to obtain any or minimum mortality and in later time, better daily growth rate.

Therefore, effects of betaine on tilapia transferred to sea water were observed by investigating Na^+/ K^+/ ATPase activity of gill, gill and kidney tissues histologies during 3 weeks in this study.

Betaine is one of the most important osmotic effector. It has ability to protect cells against dramatic changes in osmotic pressure in fish. It has been found that within marine vertebrates, an increase in salinity and temperature stimulates mitocondrial betaine synthesis naturally; results in being that betaine is accumulated at high levels and prevents abnormal water loss from the cells. This has importance when transporting euryhaline fish or other animals from fresh water into sea water [10].

Present and quantity of betaine in marine and fresh water animals such as Atlantic salmon (*Salmo salar*), brook charr (*Salvelinus fontinalis*), Artic charr (*S. alpinus*), rainbow trout (*Oncorhynchus mykiss*), some crustaceas, and molluscs during transfer to sea water has been more increased by feeding the animals with betaine added feed before transfer [10, 11, 12, 13, 14, 15]. Polat and Beklevik [4] emphasized that feeding of salmon smolts with betaine supplemented diets for a 5- 8 week prior to sea water transfer resulted in improved fish adaptation and increased growth performance.

This allows accumulation of a sufficient muscle betaine reserve so as to reduce the consequences of osmotic stress [16], to improve the maintenance of ionic and osmotic homeostasis during sea water adaptation in teleost fish [17].

In spite of these explanations, it could be observed that dietary betaine resulted in a 5- fold increase in muscular betaine content, but had no significant effect on either growth or the degree of ion/ osmotic imbalance following transfer to sea water in Atlantic salmon parr [13].

Na^+/ K^+/ ATPase is a key enzyme in the NaCl excretion mechanisms of the teleost gill in sea water [17]. Although betaine alone has no significant effect on gill Na^+/ K^+/ ATPase activity for Atlantic salmon parr [13] it generally seems to accelerate active ion extrusion in gills via elevation of Na^+/ K^+/ ATPase activity. The gill Na^+/ K^+/ ATPase activity of the betaine-fed fish was significantly elevated during the sea water acclimation period and stayed slightly higher than that of the control fish during sea water stage [10, 20]. The results in this study are harmonious with that of Virtanen et al. [10]. In the present study, enzyme levels of all groups increased until end of the experiment. It can show that biochemical accommodation of *O. aureus* to sea water does not finish in few days but continues longer time. Enzyme levels were higher in both betaine applications in

each sampling period than in the control. In control group, it increased as depending on the following time naturally. This is like the result suggested by Virtanen et al. [10].

It must be considered in this point that fish was taken entirely to sea water in the course of seven days. After one day (on 8^{th} day) from completely transfer to sea water, gill enzyme levels were found unaffected from betaine ($P>0.05$). Similar event were valid for histological structures, too.

An increase in chloride cell number was observed with increasing of salt concentration in *O. niloticus* and *O. aureus* [2]. The small glomerular structure in Bowman's capsule was determined in *O. niloticus* cultured in sea water [19]. The results belonging to kidney in this study is same that of the previous study.

On the 4^{th} day only, there were histological differences in the gill and kidney tissues of fish in three groups. On the 8^{th} day and in the later periods, structures of the gill and kidney tissues of the groups did not change with betaine application.

S. fontinalis were fed experimental diets containing NaCl (10 %), betaine (6 %) or a combination of both, one month prior to sea water transfer. Osmoregulatory performance (Na^+/ K^+/ ATPase, Ionic composition) was different among the experimental groups. NaCl initially generated significantly higher gill Na^+/ K^+/ ATPase compared to betaine, but both supplements were effective osmoprotective agents (mortality was reduced) [15]. Chronic mortality was observed in all triploid groups in spite of adequate Na^+/ K^+/ ATPase activity levels [15].

It was found in the present study that there was no mortality until 15^{th} day. Then it was observed an increase in the mortality of the groups. Betaine in both doses did not affect the mortality.

In conclusion, betaine treatment in these dosages prior (almost 45 days) to transfer o sea water cannot supply any physiological or histological benefit on the subject of acclimation of *O. aureus*.

BIBLIOGRAPHY

[1] Suresh, A., A., Lin, C. K., 1992. Tilapia Culture in Saline Waters: A review Aquaculture, 106: 201- 226;

[2] Avella, M., Berhaut, J., Bornancin, M., 1993. Salinity Tolerance of Two Tropical Fishes, *Oreochromis aureus* and *Oreochromis niloticus*. I. Biochemical and Morphological Changes in the Gill Epithelium. Journal of Fish Biology, 42: 243- 254;

[3] Altun, T., Sarihan, E., Kayrin, L., Doran, F., 2004. Effects of Fresh and Sea Water on Growth, Estradiol-17β and Ovarian Development in Tilapia.The Israeli Journal of Aquaculture - Bamidgeh 56 (2): 102-112;

[4] Polat A., Beklevik, G. 1999. The Importance of Betaine and Some Attractive Substances as Fish Feed Additives. In Brufau J. (ed.), Tacon A. (ed.). Feed Manufacturing in the Mediterranean Region: Recent Advances in Research and Technology. Zaragoza: CIHEAM-IAMZ. p. 217-220;

[5] Caberoy and Quınıtıo, 2000. Changes in Na^+/ K^+/ ATPase Activity and Gill Chlorid cell Morphology in the Grouper *Epinephelus coioides* Larvae and Juveniles in Response to Salinity and Temperature. Fish Physiology and Biochemistry, 23: 83-92;
[6] Ames, B. N., 1966. Assay of Inorganic Phosphate Methods in Enzymology. Vol. 8. New York, Academic Pres. 115p;
[7] Lowry, O. H., Roserrouh, N., J., Farr, A. L., Rondel, R. J., 1951, Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. 193: 265- 275;
[8] SPSS,1998. Computer Program, MS for Windows, Version 8.0. USA: SPSS Inc.
[9] Perschbacher, P. W.,1992, A Review of Sea Water Acclimation Procedures for Commercially Important Eurohaline Tilapias, Asian- Fish Sci., 5(2): 241- 248;
[10] Virtanen E., Junnila, M., Soivio, A.1989, Effect on Containing Betaine / Amino Acid Additive on the Osmotic Adaptation of Young Atlantic salmon, *Salmo salar* L. Aquaculture, 83: 109- 122.
[11] Gilles, R., 1979, Intracellular Organic Osmotic Effectors. Mechanisms of Osmoregulation of Animals. R. Gilles, ed., John Wilevy and Sons, New York, NY. Pages 111- 154;
[12] Staurnes, M., and Eliassen, R., 1986. Smoltifisering og SjØvannstoleranse II (Smoltification and Seawater Tolerance II) Norsk Fiskeoppdrett, 9: 52-53;
[13] Duston, J., 1993. Effects of Dietary Betaine and Sodium Chloride on Seawater Adaptation in Atlantic Salmon Parr (*Salmo salar* L), Comparative Biochemistry and Physiology Part A: *Physiology*: Volume 105, Issue 4: 673-677;
[14] De Vooys, C. G. N., Geenevasen, J. A. J., 2002, Biosynthesis and Role in Osmoregulation of Glycin Betaine in the Mediteranean Mussel *Mytilus galloprovinsialis* LMK, Comparative Biochemistry and Physiology Part B, 132: 209- 414;
[15] Le François, N. R., and Lammarre, S. G., 2003. Can We Reduced Mortality of O^+ Triploid Brook Charr (*Salvelinus fontinalis*) Following Sea Water Transfer, Aquaculture Canada Victora Conference Center, Victoria, BC Poster Session, Abstract.
[16] Clarke, W. C., Virtanen, E., Blackburn, J., Higgs, D. A., 1994. Effects of Diatery Betaine/ Amino Acid Additive on Growth and Seawater Adaptation in Yearling Chinook Salmon, Aquaculture, 121: 137- 145;
[17] Amlund, H., and Berntssen, M. H. G., 2004. Arsenobetaine in Atlantic Salmon (*Salmo salar* L.): Influence of Sea Water Adaptation, Comparative Biochemistry and Physiology, Part C 138: 507- 514;
[18] Payan, P., Girard, J. P., Mayer-Gostan, N.,1984. Branchial Ion Movements in Teleost: The Roles of Respiratory and Chloride Cells. In: Fish Physiology: Gills. Ion and Water Transfer, Vol. XB (ed. by W. S. Hoar and D. J. Randall), Academic Press, New York, pp: 39-63;
[19] Sarıhan, E., Altun, T., Şahan (Azızoğlu), A., Cengızler, I., Genç, E., 1997. Gill Na^+/ K^+/ATPase Activity, Histological Structures of Gill and Kidneys and Some Hematological Parameters of *O. niloticus* Grown in Fresh and Seawater. Proceedings Vol.1. Edited by B. Papathanassiou 4th Balkan Conference on Operational Research, 20-23 October, Thessaloniki, Greece;
[20] Castro, H., Battaglıa, J., Vırtanen, E., 1998. Effects of Finnstim on Growth and Sea Water Adaptation of Coho Salmon. Aquaculture, 168: 423- 429.