



The Determination of Some Biochemical Parameters in the Tissues of *Luciobarbus esocinus* (Heckel, 1843) in the Reproductive Period

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In the present study was investigated the effect of reproduction on oxidative stress (malondialdehyde (MDA)), vitamin E (VE), vitamin A (VA) vitamin C (VC) and β -carotene (β C) levels in tissues (liver, muscle, kidney, spleen and gonad) of *Luciobarbus esocinus* in three different period. These periods was defined as before reproduction period (A), reproduction period (B) and after reproduction period (C). The VE, VA, VC, β C and MDA levels in tissues were made using HPLC. As a result of these analyzes, it was seen that levels of VE, VC, VA and β C in gonad was higher in A and B periods according to C period, but these levels in other tissues was lower. It was identified according to the results of the analysis that the decrease in the amount of VA in the tissues in general was higher than β C. In addition, it was determined that the kidney tissue affected least by the oxidative stress occurring during the reproductive period. But, significant elevation of MDA levels was observed in other tissues. Furthermore, in this study, MDA levels were found to be higher in females compared to males, especially during the reproduction period.

Key words: *Luciobarbus esocinus*, Vitamins, Oxidative stress, Reproduction.

Submission Date: 02 September 2021

Acceptance Date: 31 October 2021

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1. Introduction

In the developing world conditions, healthy nutrition has a very important place. Decreased food resources in parallel with the increasing world population and climate change brings the concept of food conservation to the agenda. [1]. However, hormones and chemicals used to protect food sources increase the amount of product, reduce the quality of food, and even threaten human health. As a result, people tend to consume high quality foods that develop in the natural environment and contain high nutritional elements such as vitamins, minerals, protein and fatty acids. [2-4]. In this context, fish meat, which is associated with healthy and high quality nutrition, has taken its place among the most preferred food sources in recent years.

Fish, which is an important food for the people of our region as it is for all people, can be caught from the Keban Dam Lake. This Dam Lake is a water source located in the Eastern Anatolia Region and is effective in meeting the fish needs. It was built near Keban Dam Lake, 45 km northwest of Elazig. In our region, it is located between 38° 37' and 39° 20' north latitudes, 38° 15' and 39° 52' east longitudes. [5]. *Luciobarbus esocinus* is a species of Cyprinidae family found in this reservoir. Its meat is very tasty and its economic value is high. Therefore, *L. esocinus* is an important food item for the people living here and a source of income for the fishermen. In order to ensure the continuity of the populations of these fish [6], whose breeding periods are between April and May, cultural studies should be supported under the control of the state, and scientific studies should be carried out in order to carry out conscious for efficient production and breeding. These fish need to be fed with quality feeds in

order to be able to produce and grow efficiently under cultural conditions and to have a high survival rate when stocking them in natural waters in certain periods. Especially the rapid and healthy development of the offspring depends on the essential nutrients available in the egg. These nutrients are substances that must be present in feeds of sexually mature fish during and before oogenesis. The strongest determinants of reproductive performance in these feeds are proteins, fats and antioxidant substances. These substances are effective in cell formation and are effective in the continuation of the fluidity of these membranes by collecting in the subcellular membranes. Membrane fluidity protects living things especially from low temperatures and cellular attack of free radicals. Free radicals are chemical species with very high reactivity because they contain unshared electrons in their outer orbitals. [7,8]. The most important target of the free radical that causes oxidation is fatty acids, since the cell membrane does not contain water. This effect, known as peroxidation of membrane lipids (LPO), disrupts the structure of the membrane and may even cause cell death by increasing its permeability. The resulting peroxidation has a self-sustaining feature and its end product, malondialdehyde (MDA), is very important in biological systems as it is the main indicator of oxidative stress. [7,9]. In order to prevent the formation of reactive oxygen species and their biological effects, many defense mechanisms have been formed in the body. These are known as "antioxidant defense systems" or "antioxidants". Vitamins A, E, C and β -carotene are among the important antioxidant substances that are highly effective in protecting cells against oxidative damage by reacting with free radicals and peroxides. [9,10]. These substances are also among the feed additives that are effective on the reproduction of aquatic organisms. For these reasons, during the breeding period of *L.esocinus*; It is very important to know the oxidative stress affected by the free radical mechanism and the levels of vitamins A, E, C and β -carotene that affect this mechanism.

In this study; in the tissues of *L.esocinus* (liver, muscle, kidney, spleen, gonad); The efficiency of reproduction was determined on the relationship between MDA, vitamin A, E, C and β -carotene levels. Thus, with the data obtained, the nutritional quality of these fish was determined and the importance of the parameters analyzed in the formation of the feeds to be prepared in order to increase the reproductive performance of the broodstock during the reproduction period was emphasized.

2. Experimental

L. esocinus used in the research was caught between February and August from Keban Dam Lake. By determining the age from the scales of these fish, individuals reaching sexual maturity were preferred [11]. In the study, the development of fish in 3 different periods was investigated.

A- Before the reproductive period: Samples were taken in February.

B- Reproductive period: Samples were taken in May.

C- After reproductive period: Samples were taken in August.

The caught fish were brought to Firat University Faculty in ice and autopsied in the laboratory. Liver, muscle, kidney, spleen and gonads were removed, wrapped in foils, and stored in a deep freezer at -20°C until analysis. All analyses for determination of tissue βC , VA and VE levels were carried out on three aliquots, ranging from 200 to 1000 mg weight. Samples were homogenized in a glass-glass homogenizer in 1 ml of cold acetone. Homogenized samples were transferred into polyethylene tubes and 2 ml ethanol was added to the tubes. After 0.3 ml n-hexane was filled into tubes for vitamins extractions, they were centrifuged. This step was repeated two times. N-hexane in tubes was evaporated using the nitrogen. Then the residues were solved in mobile phase (methanol: acetonitrile: chloroform; 47: 42: 11, v/v) [12]. The fish tissue samples (1,0 g) were homogenized in a glass-glass homogenizer in mixture of 0.5 ml of HClO_4 (0.5 M), 4.5 ml distilled water and 100 μl -500 ppm butylated hydroxytoluene (BHT) for determination of tissues VC and MDA. Then, the samples were centrifuged at 4500 rpm for 5 min and supernatants were injected into HPLC system. Addition of acid was necessary to precipitate proteins and release the MDA bound to the amino groups of proteins and other amino compounds. Acid addition was also needed to maintain the stability of vitamin C [13,14].

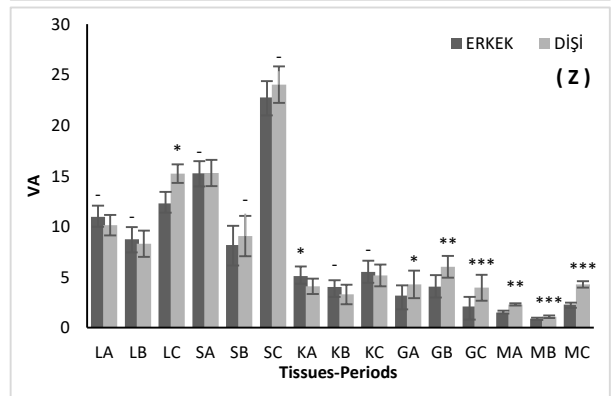
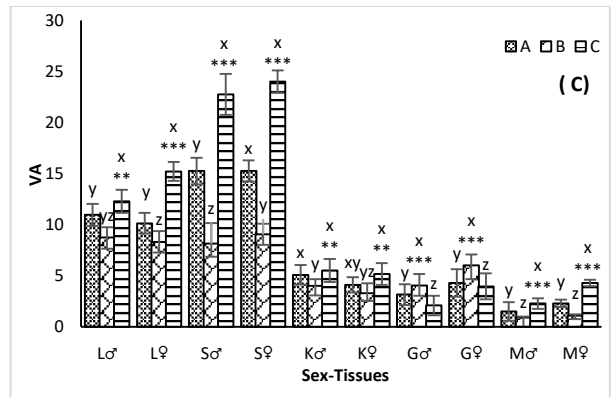
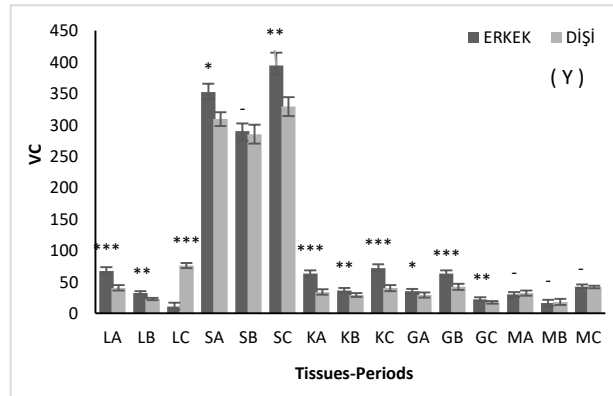
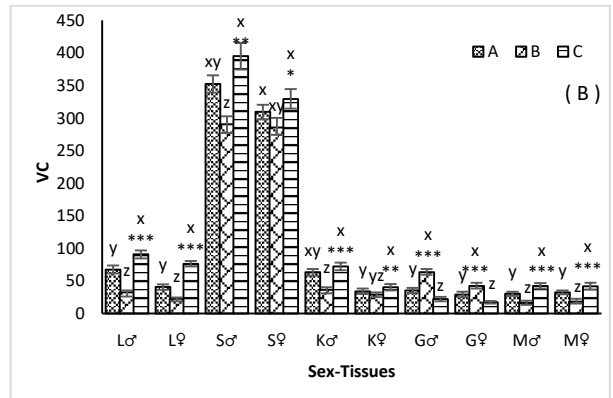
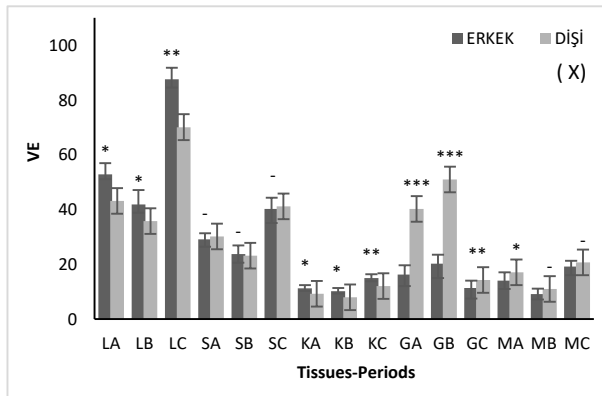
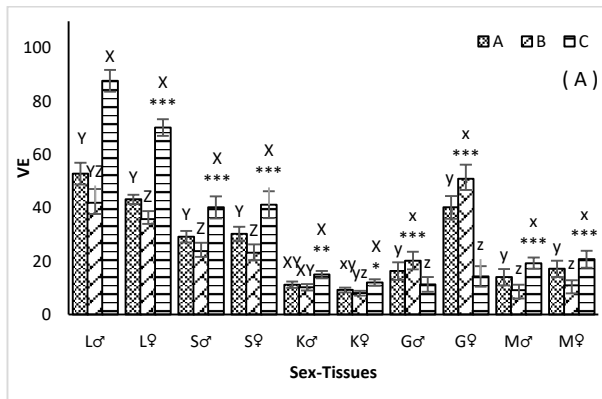
All results are expressed as mean \pm S.E. The data were analyzed with an Independent-Sample T Test and Duncan Test. SPSS 12.0 for Windows was utilized for statistical analysis. The level of significance was set at $p < 0.05$.

3. Results and Discussion

In this study, the data obtained as a result of the analyzes were statistically evaluated and arranged in graphics (Figure 1).

The present study showed the VE level in tissues decreased in the B (liver (male 52.26%, female 49.05%), kidney (male 99.32%, female 99.33%), spleen (male 40.83%, female 43.84%), muscle (male 52.86%, female 47.29%)) and A (liver (male 52.26%, female 49.05%), kidney (male 25.28%, female 23.24%) spleen (male 27.44%, female 26.69%), muscle (male 27.29%, female 17.56%)) periods

compared to the C period (Figure 1A, X). VE is potent antioxidants with lipoperoxyl radical-scavenging activities. Lipid peroxy radicals react approximately 10^5 times faster with α -TOH than with PUFA. Buettner [15] determined that one molecule of α -TOH can protect approximately 1000 molecules of PUFA against oxidation. These findings are corroborated by in vivo studies [16-18]. These decreases in the tissues can be most likely responses to increased ROS generation. In an early study, the importance of vitamin E in reproduction of aquatic animal has been reported [19]. For example, the use of VE in supplemental feeding caused better gonad development and spawning in the *C. carpio* [20] and *C. auratus* [21].



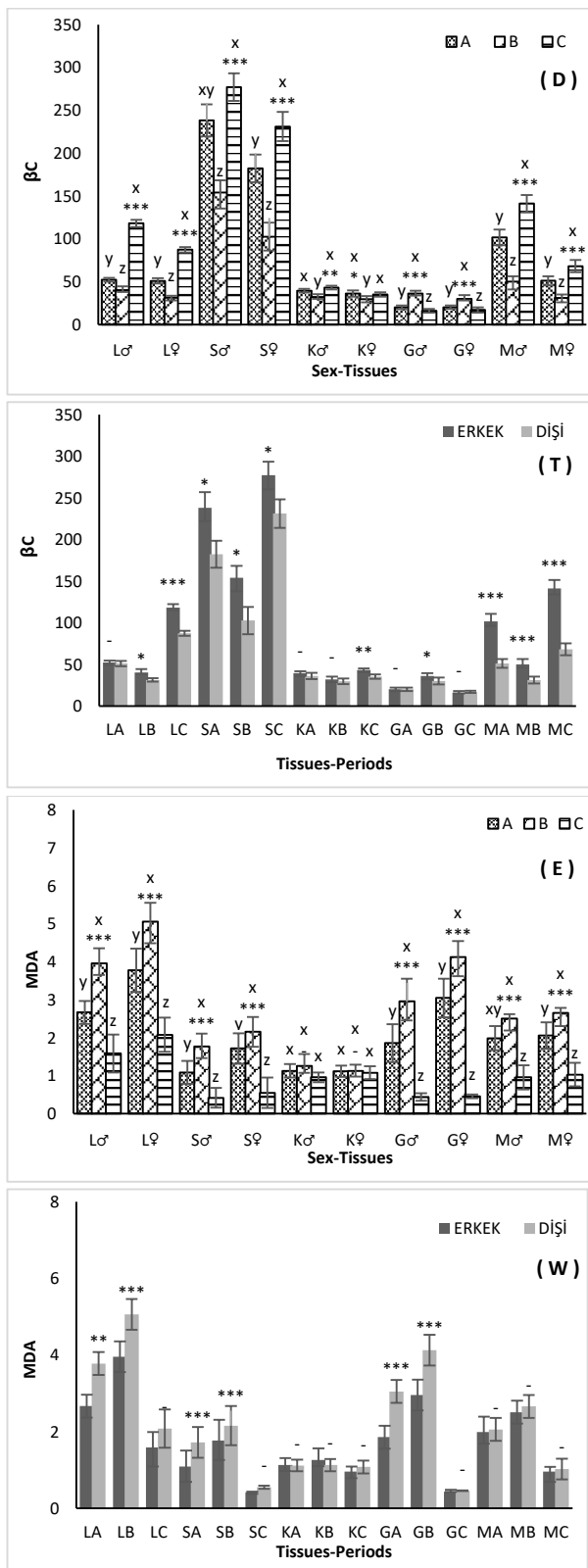


Figure 1. A, B, C, D, E: Comparison of the mean concentration of the tested vitamin E ($\mu\text{g/g}$) (A), vitamin C ($\mu\text{g/g}$) (B), vitamin A ($\mu\text{g/g}$) (C), beta carotene ($\mu\text{g/g}$) (D) and MDA ($\mu\text{g/g}$) (E), in tissues (L σ : Liver-male, L ϕ : Liver-female, S σ : Spleen-male, S ϕ : Spleen-female, K σ : Kidney-male, K ϕ : Kidney-female, G σ : Gonad-male, G ϕ : Gonad-female, M σ : Muscle-male, M ϕ : Muscle-female) of same-sex *L. esocinus* in three different periods (A: Before

reproduction, B: Reproduction, C: After reproduction). Significance between groups was shown as asterisk (* $p < .05$, ** $p < .01$, *** $p < .001$). Different letters on the bar show statistical significance among different groups (x, y, z), one-way ANOVA, Duncan's test,

X, Y, Z, T, W: Comparison of the mean concentration of the tested vitamin E ($\mu\text{g/g}$) (X), vitamin C ($\mu\text{g/g}$) (Y), vitamin A ($\mu\text{g/g}$) (Z), beta carotene ($\mu\text{g/g}$) (T) and MDA ($\mu\text{g/g}$) (W), in tissues of different-sex *L. esocinus* in three different periods (LA: Liver before reproduction, LB: Liver reproduction, LC: Liver after reproduction, SA: Spleen-before reproduction, SB: Spleen-reproduction, SC: Spleen-after reproduction, KA: Kidney-before reproduction, KB: Kidney-reproduction, KC: Kidney-after reproduction, GA: Gonad-before reproduction, GB: Gonad-reproduction, GC: Gonad-after reproduction, MA: Muscle-before reproduction, MB: Muscle-reproduction, MC: Muscle-after reproduction). Significance between groups was shown as asterisk (* $p < .05$, ** $p < .01$, *** $p < .001$), Independent-sample T test.

Vitamin C or L-ascorbic acid is considered to be the most powerful water-soluble antioxidant in extracellular fluids. Especially, this vitamin can protect biomembranes against peroxidative damage by trapping peroxyradicals in the aqueous phase before they can initiate LPO [22, 23]. As a result of the analysis, that the VC level in tissues decreased in the B (liver (male 70.07%), kidney (male 49.89%, female 27.42%), spleen (male 26.48%, female 13.36%), muscle (male 61.58%, female 56.75%)) and A (liver (male 18.32%), kidney (male 12.51%, female 15.43%) spleen (male 10.79%, female 6.04%), muscle (male 28.52%, female 23.19%)) periods compared to the C period (Figure 1B, Y). Very interesting, the level of VC in the liver of male fish increased significantly in periods A (532.83%) and B (202.53%) compared to period C. Vitamin C can also act to protect membranes against peroxidation by enhancing the activity of tocopherol, chain-breaking antioxidant.

Vitamin A (retinol) is one of the fat soluble vitamins, works as a good antioxidative agent as it neutralizes the free radicals [24, 25]. Li *et al.* [24] determined that VA status and its metabolism in the fasting and refeeding cycle contribute to the regulation of the expression of genes in hepatic glucose and lipid metabolism in rats. In the current study that the VA and βC levels in tissues decreased in the B (liver (male 28.74%, 65.85%, female 45.46%, 64.02%), kidney (male 27.04%, 25.57%, female 99.36%, 15.78%), spleen (male 64.14%, 44.39%, female 62.32%, 55.59%), muscle (male 60.52%, 64.43%, female 74.53%, 54.32%)) and A (liver (male 10.74%, 55.70%, female 33.44%, 41.52%), kidney (male 20.09%, 0.96%, female 39.75%), spleen (male 32.95%, 14.07%, female 36.38%, 21.09%),

muscle (male 34.21%, 27.95%, female 46.26%, 25.03%)) periods compared to the C period, respectively (Figure 1C,D, Z,T). In many studies was reported that the carotenenes are converted to VA as it is essential for the epithelial cells differentiation [25, 26]. The antioxidant activity of carotenoids when reacting with the O_2^* anion through oxidation of this radical to oxygen arises in the capacity to prevent the formation of peroxides (H_2O_2/O_2^{2-}) and other reactive oxygen species such as HO^* derived from peroxides through Fenton-type reactions [27, 28]. It was determined that the decrease in the amount of VA in the tissues in general was higher than βC . For VA this may reflect conversion from provitamin A carotenoids [29]. It was reported that VA requirement for *P. chinensis* increases during the spawning season, because they may shift VA from their tissues to their eggs [30].

In this study was determined that the increases in the gonad VE, VC, VA and βC levels occurred at the A (VE (male 44.45%, female 181.89%), VC (male 59.34%, female 68.63%), VA (male 51.19%, female 8.33%), βC (male 24.55%, female 18.21%)) and B (VE (male 79.05%, female 252.21%), VC (male 185.36%, female 144.27%), VA (male 93.77% female 52.02%), βC (male 123.81%, female 76.06%)) periods according to C period. In some aquatic species was reported that the remobilisation of nutritional stores from existing tissues is required during the latter stages of gonadal development [29]. Rodriguez-Gonzalez et al. [31] found continuous transfer of nutrients from the hepatopancreas to the gonad for activating and sustaining gametogenesis of female crayfish (*C. destructor*). Lie et al. [32] found that α -tocopherol is transported from muscle to liver by high density lipoprotein, and then from liver to ovary by low density lipoprotein in *S. salar* during vitellogenesis, and determined that the massive transfer of α -tocopherol were seen from the filet to the ovary during the final part of vitellogenesis. Previous studies found that the carotenoids generally is transfer from muscle to ovary during spawning period [29, 33]. Sagi et al. [34] reported that carotenoids are accumulated in the oocyte to suffice the embryonic need of *C. quadricarinatus*. The possible reason for this was reviewed by Liñán-Cabello et al. [33] that oogenesis in aquatic organism is characterized by deposition of yolk in the oocyte. The vitelline in the yolk, a source of nutrition for the developing embryo, is a lipoglyco-carotenoprotein. For this reason, these organism eggs accumulate carotenoids in high levels for these functions during vitellogenesis. In our study, VE, VC, VA and βC levels decreased in liver, kidney, spleen and muscle tissue, while increased in gonad tissue in B and A periods compared to C period. Reason for that, during ovarian maturation, *L. esocinus* may be transport the VE, VC, VA, and βC from other tissues to ovaries. In this study, in general, VE and VC level in the liver, VA level in spleen,

liver, kidney and spleen, and βC level in muscle and liver of fish in the A and B periods compared to the C period showed a great decrease. The largest percentage of these reductions were in the liver. It is known that liver contains fat-soluble vitamins, regulates the metabolism of the body and exhibits high oxygen consumption. For these reason, liver represents the status of antioxidants and OS in aquatic organism [35, 36]. For that matter De Vlaming et al. [37] reported that vitellogenin is synthesized in the liver under the control of estradiol produced by the follicle cells in the ovaries.

As in all living, many changes occur in metabolic functioning during the reproductive period in aquatic organisms. Oxidative stress, especially expressed as lipid peroxidation, is one of the prominent parameters in this period [29, 38,]. Lipid peroxidation which is the result of interactions of lipid radicals and/or formation of nonradicals species by ROO^* is used to be a valuable indicator of the oxidative damage of cellular components [39, 40]. Because of this reason, in the present study was investigated levels of MDA, as a secondary lipid peroxidation product. As a result of the analysis, that the MDA level in tissues increased in the B (liver (male 149.05%, female 143.26%), kidney (male 31.25%, 4.62%), spleen (male 321.42%, female 292.72%), muscle (male 161.45%, female 158.25%), gonad (male 572.72%, female 797.82%)) and A (liver (male 67.924%, female 81.73%), spleen (male 159.52%, female 212.72%), kidney (male 17.70%, 3.70%), muscle (male 107.29%, female 100.00%), gonad (male 322.72%, female 563.04%)) periods compared to the C period (Figure 1E, W). The results of our study illustrated that the MDA level in gonad, liver and spleen according to kidney and muscle of female *L. esocinus* increased significantly in B period compared to other periods. Immunocytochemical identifications showed that vitellogenin is synthesized in hepatopancreas of crustacean in liver of fish under the control of estradiol produced by the follicle cells in the ovaries [29, 33, 41, 42]. These data indicate that high MDA in liver may be due to the increase of free radicals during the transfer of energy from the liver to the gonads during gamet formation. The marked reduction of MDA in liver after B period may also be associated with the decrease in liver lipid content because of higher energetic investment of fish for gametogenesis.

In many studies have been shown that OS plays a role in multiple physiological processes from oocyte maturation to fertilization and embryo development. Especially, in the initial stages of oogenesis increases the number of cell in mitochondria, and with metabolism and incomplete reduction of oxygen during cell respiration, more O_2^* is unavoidably synthesized from the liberation of electrons [41]. The aquatic organism were determined that OS

observed in reproduction period were approximately double those found in the rest of the year, and their gonads have a higher protein synthesis, lipid, acylglycerides and carbohydrate mobilization [43, 44]. Similarly, Barim Oz and Yılmaz [45] determined that level of MDA increased approximately 100 % in the hepatopancreas and ovarian tissues of *A. leptodactylus* during reproduction. It was reported that highly unsaturated fatty acids are particularly susceptible to attack by reactive oxygen radicals, and uncontrolled damage to membrane fatty acids and the accumulation of their oxidized breakdown products can have deleterious consequences for cell function [39, 40]. The main cause for increase of MDA level in gonad may be the inability of the antioxidant mechanism as the results of excessive production of O_2^* -generation because of the accumulation of lipids and protein [45, 46]. Moreover, interestingly, the MDA level in gonad was highest in female compared with male. In immunocytochemical identifications of Lee and Chang [47] was found that the amount of the incorporated vitellogenin was high in the vitellogenin stage (stage III-V) according to the early stage (stage I-II) of ovarian development in *M. rosenbergi*. For this reason, the rise in MDA can be associated to peroxidation increasing because maximal gamet formation with high in precursor protein of egg yolk.

4. Conclusions

During the reproduction period of *L. esocinus*, MDA level increased in liver, spleen, kidney, gonad and muscle and reached the highest level especially in the gonads. However, VE, VC, VA and β C levels decreased in liver, spleen, kidney and muscle, while these levels increased in gonads. For this reason, antioxidant substances should be added to the diets of these fish during the reproduction period in production studies to be carried out under culture conditions.

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