



## The Effects of Reproduction on Oxidative Stress, vitamins E, A, C and $\beta$ -Caroten in *Capoeta umbla* (Heckel, 1843)

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The aim of this study was to investigate the effect of reproduction on malondialdehyde (MDA), vitamin E (VE), vitamin A (VA), vitamin C (VC) and  $\beta$ -carotene ( $\beta$ C) levels in tissues (liver, muscle, kidney, spleen and gonad) of sexually matured *Capoeta umbla*. For this study was determined three different period; before reproduction period (BR), reproduction period (R) and after reproduction period (AR). The VE, VA, VC,  $\beta$ C and MDA in tissues were made using HPLC. Generally, it was determined that levels of VE, VC, VA and  $\beta$ C in gonad was higher in BR and R periods according to AR period, but these levels in other tissues was lower. In addition, it was observed that the kidney tissue affected least by the oxidative stress occurring during the reproductive period, 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 breeding period.

**Key words:** *Capoeta umbla*, Vitamins, Oxidative stress, Reproduction.

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### 1. Introduction

One of the biggest problems threatening the rapidly increasing world population in the future is food scarcity. Aquatic organisms are a very important key point in solving this problem. Supporting populations of these organisms is one of the most important measures to combat hunger [1,2]. Therefore, investigating the reproductive

cycles of these organisms is very important for their continued existence in the waters.

It is well established that a close connection exists between food intake, energy homeostasis and growth, in terms that feeding provides the energy necessary for supporting

growth processes. In fish, this connection is particularly important for aquaculture, as efforts are continuously being made in order to determine the optimal feeding rate to achieve the optimal fish growth. An inadequate feeding rate (both deficient or in excess) may lead to a decrease in feed efficiency and growth, situation that would considerably increase the cost of fish production [3]. Likewise, food intake and energy stores are tightly related to reproduction, which is largely affected by the amount of energy reserves. Thus, reproductive functions generally take place when enough energy reserves are in the body (achieved by high amounts of food supply), while low food resources inhibit the reproductive system [4].

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 present in the egg. These nutrients are substances that must be present in broodstock feeds during and before oogenesis. The strongest determinants of reproductive performance in these feeds are proteins, fats and antioxidant substances (vitamin E, A, C beta karoten etc.). 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, on the other hand, protects living things, especially from low temperatures and cellular attack of free radicals. Since free radicals contain unshared electrons in their outer orbitals, they are chemical species with very high reactivity [5,6]. 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 [5,7].

In this study; in the tissues (liver, muscle, kidney, spleen, gonad) of *C. umbla*, a species belonging to the cyprinidae family; The efficiency of reproduction was determined on the relationship between MDA, vitamin A, E, C and  $\beta$ -carotene levels. Thus, with the data obtained, both 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 breeding period was emphasized.

## 2. Experimental

*C. umbla* 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 [8]. In the study, the development of fish in 3 different periods was investigated.

A- Before the reproductive period (BR); Samples were taken in February.

B- Reproductive period (R); Samples were taken in May.

C- After reproductive period (AR); 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  $-20^{\circ}\text{C}$  until analysis.

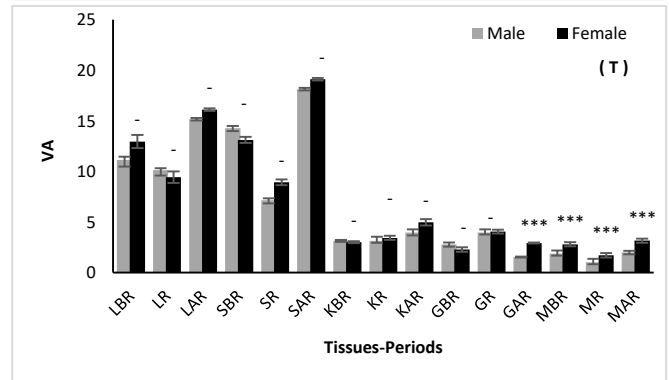
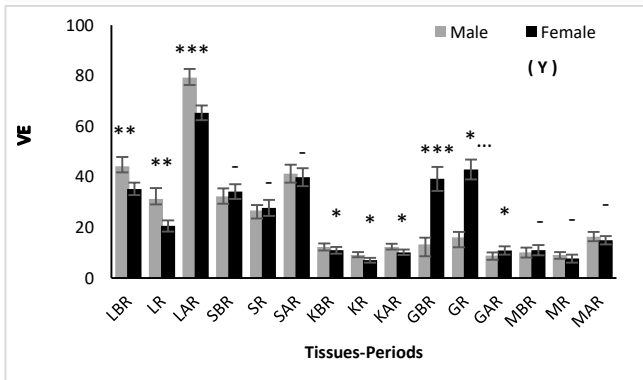
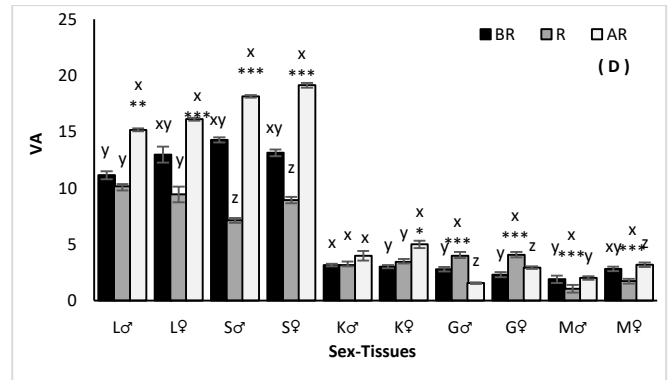
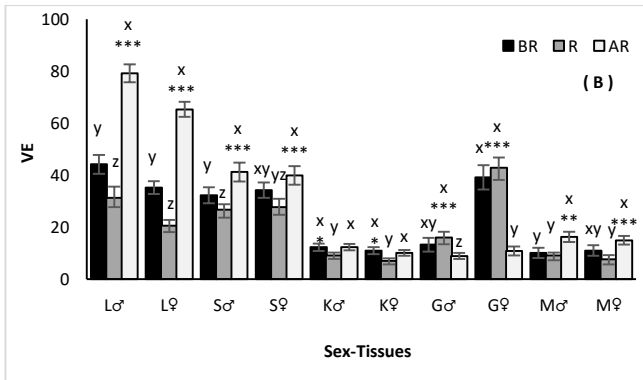
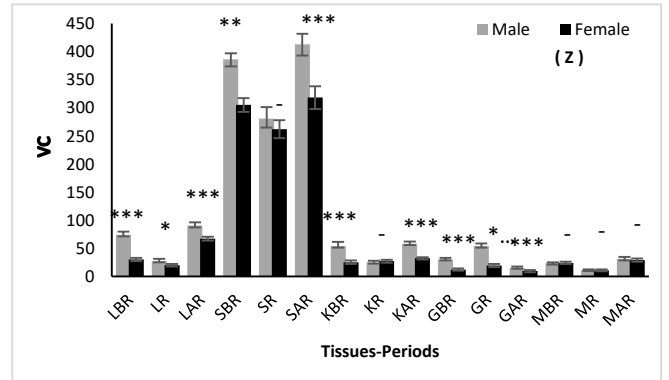
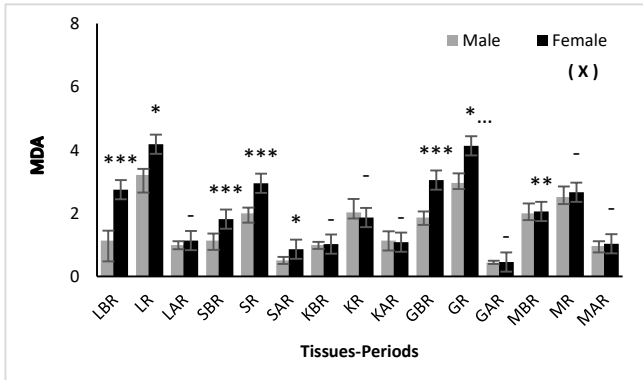
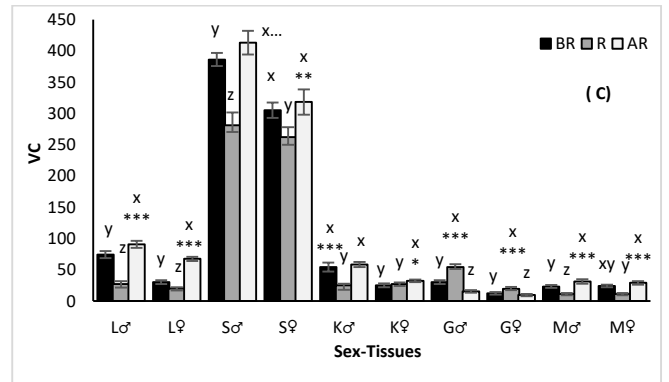
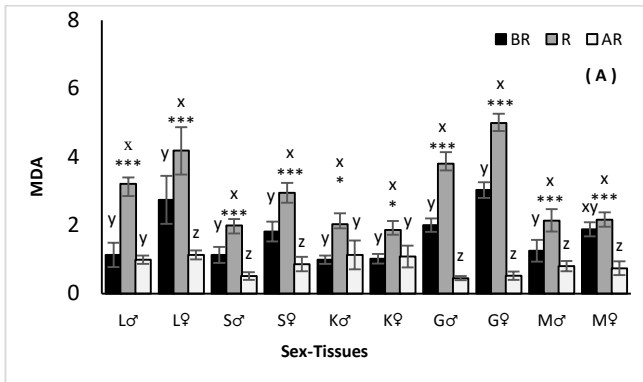
All analyses for determination of tissue  $\beta\text{C}$ , VA and VE levels were carried out on three aliquots, ranging from 200 to 1000 mg weight. Samples were homogenized in a glass-glass homogenizer in 1 ml of cold acetone. Homogenized samples were transferred into polyethylene tubes and 2 ml ethanol 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

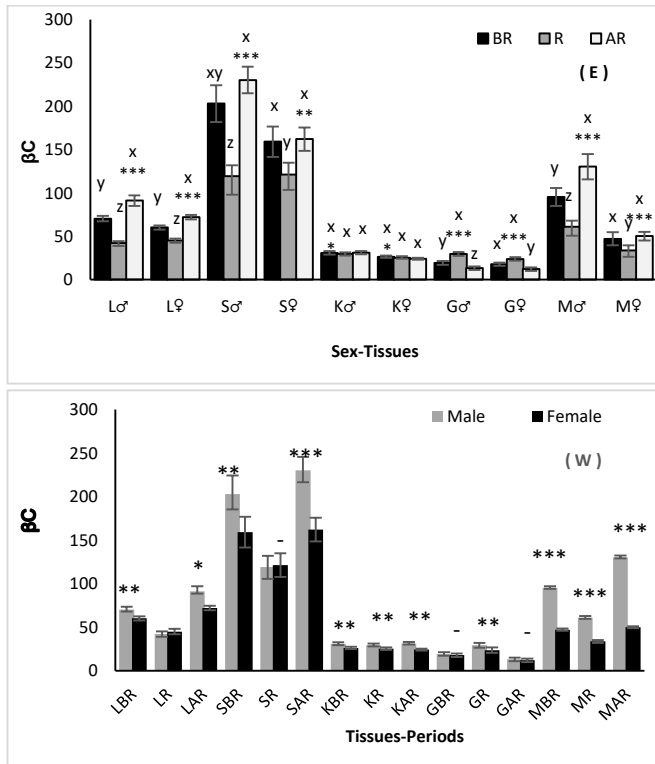
tubes was evaporated using the nitrogen. Then the residues were solved in mobile phase (methanol: acetonitrile: chloroform; 47: 42: 11, v/v) [9]. The fish tissue samples (1,0 g) were homogenized in a glass-glass homogenizer in mixture of 0.5 ml of  $\text{HClO}_4$  (0.5 M), 4.5 ml distilled water and  $100\mu\text{l}$ -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 [10,11].

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

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 [5,6]. Lipid peroxidation which is the result of interactions of lipid radicals and/or formation of nonradicals species by  $\text{ROO}^*$  is used to be a valuable indicator of the oxidative damage of cellular components [12,13]. 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 R (liver (male 224.24%, female 269.91%), kidney (male 79.64%, 72.22%), spleen (male 290.19%, female 243.02%), muscle (male 166.25%, female 191.89%), gonad (male 744.44%, female 859.61%)) and BR (liver (male 14.14%, female 142.47%), spleen (male 121.56%, female 110.46%), muscle (male 56.25%, female 154.05%), gonad (male 344.44%, female 482.69%)) periods compared to the AR period (Figure 1A, X). The results of our study illustrated that the MDA level in gonad, liver and spleen according to kidney and muscle of female *C. umbla* increased significantly in R 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 [6,14-16]. 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 R period may also be associated with the decrease in liver lipid content because of higher energetic investment of fish for gametogenesis.





**Fig. 1. A, B, C, D, E:** Comparison of the mean concentration of the tested MDA ( $\mu\text{g/g}$ ) (A), vitamin E ( $\mu\text{g/g}$ ) (B), vitamin C ( $\mu\text{g/g}$ ) (C), vitamin A ( $\mu\text{g/g}$ ) (D), beta carotene ( $\mu\text{g/g}$ ) (E) in tissues (L♂: Liver-male, L♀: Liver-female, S♂: Spleen-male, S♀: Spleen-female, K♂: Kidney-male, K♀: Kidney-female, G♂: Gonad-male, G♀: Gonad-female, M♂: Muscle-male, M♀: Muscle-female) of same-sex *C. umbla* in three different periods (BR: Before reproduction, R: Reproduction, AR: 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 MDA ( $\mu\text{g/g}$ ) (X), vitamin E ( $\mu\text{g/g}$ ) (Y), vitamin C ( $\mu\text{g/g}$ ) (Z), vitamin A ( $\mu\text{g/g}$ ) (T), beta carotene ( $\mu\text{g/g}$ ) (W) in tissues of different-sex *C. umbla* in three different periods (LBR: Liver before reproduction, LR: Liver reproduction, LAR: Liver after reproduction, SBR: Spleen-before reproduction, SR: Spleen-reproduction, SAR: Spleen-after reproduction, KBR: Kidney-before reproduction, KR: Kidney-reproduction, KAR: Kidney-after reproduction, GBR: Gonad-before reproduction, GR: Gonad-reproduction, GAR: Gonad-after reproduction, MBR: Muscle-before reproduction, MR: Muscle-reproduction, MAR: Muscle-after reproduction). Significance between groups was shown as asterisk (\* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ ), Independent-sample T test.

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  $\text{O}_2^{\cdot-}$  is unavoidably synthesized from the liberation of electrons [15]. 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 [17,18]. Similarly, Barim Oz and Yılmaz [19] 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 [12,13]. 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  $\text{O}_2^{\cdot-}$  generation because of the accumulation of lipids and protein [19,20]. Moreover, interestingly, the MDA level in gonad was highest in female compared with male. In immunocytochemical identifications of Lee and Chang [21] 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.

The present study showed the VE level in tissues decreased in the R (liver (male 60.48%, female 68.50%), kidney (male 25.57%, female 30.53%), spleen (male 35.16%, female 30.47%), muscle (male 43.71%, female 48.75%)) and BR (liver (male 44.23%, female 46.04%), kidney (male 0.32%, female 7.90%) spleen (male 21.70%, female 14.27%), muscle (male 37.74%, female 26.49%)) periods compared to the AR period (Figure 1B, Y). VE is potent antioxidants with lipoperoxyl radical-scavenging activities. Lipid peroxyl radicals react approximately  $10^5$  times faster with  $\alpha$ -TOH than with PUFA. Buettner [22] determined that one molecule of  $\alpha$ -TOH can protect approximately 1000 molecules of PUFA against oxidation. These finding are corroborated by in vivo studies [23-25]. These decreases in the tissues can be most likely responses to increased ROS generation. In an early studies, the importance of vitamin E in reproduction of aquatic animal has been reported [26]. For example, the use of VE in supplemental feeding caused better gonad development and spawning in the *C. carpio* [27] and *C. auratus* [28].

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 [29,30]. As a result of the analysis, that the VC level in tissues decreased in the R (liver (male 70.07%, female 70.52%), kidney (male 56.69%, female 15.94%), spleen (male 31.98%, female 17.62%), muscle (male 63.90%, female 62.17%)) and BR (liver (male 18.32%, female 55.12%), kidney (male 6.87%, female 21.87%) spleen (male 6.53%, female 4.09%), muscle (male 25.62%, female 16.45%)) periods compared to the AR period (Figure 1C, Z). 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 [31,32]. Li *et al.* [31] 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  $\beta$ C levels in tissues decreased in the R (liver (male -, 53.73%, female 41.46%, 37.54%), kidney (male 20.90%, 4.97%, female 31.12%, -), spleen (male 60.63%, 48.20%, female 55.22%, 25.20%), muscle (male 48.50%, 53.04%, female 45.88%, 33.26%)) and BR (liver (male 28.65%, 22.79%, female 19.55%, 16.58), kidney (male 20.09%, 0.96%, female 39.75%), spleen (male 21.27%, female 31.38%, 1.86%), muscle (male 6.0%, 26.85%, female 11.39%, 6.04%)) periods compared to the AR period, respectively (Figure 1DE, TW). In many studies was reported that the carotenes are converted to VA as it is essential for the epithelial cells differentiation [32,33]. The antioxidant activity of carotenoids when reacting with the  $O_2^{\cdot -}$  anion through oxidation of this radical to oxygen arises in the capacity to prevent the formation of peroxides ( $H_2O_2/O_2^{\cdot -}$ ) and other reactive oxygen species such as  $HO^{\cdot}$  derived from peroxides through Fenton-type reactions [34,35]. It was determined that  $\beta$ C level increased in the kidney of female in the BR and R periods, and in the spleen of male in the BR period. In addition, in this study, the decrease in the amount of VA in the tissues in general was higher than  $\beta$ C. For VA this may reflect conversion from provitamin A carotenoids [6]. 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 [36].

In this study was determined that the increases in the gonad VE, VC, VA and  $\beta$ C levels occurred at the BR (VE (male 49.26%, female 261.92%), VC (male 97.51%, female 29.08%), VA (male 80.51%),  $\beta$ C (male 45.61%, female 47.52%)) and R (VE (male 80.83%, female 296.11%), VC (male 254.77%, female 105.52%), VA (male 157.79%

female 38.69%),  $\beta$ C (male 122.58%, female 95.29%)) periods according to AR 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 [6]. Rodriguez-Gonzalez *et al.* [37] found continuous transfer of nutrients from the hepatopancreas to the gonad for activating and sustaining gametogenesis of female crayfish (*C. destructor*). Lie *et al.* [38] found that  $\alpha$ -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  $\alpha$ -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 [6,14]. Sagi *et al.* [39] 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.* [14] 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 lipo-glyco-carotenoprotein. For this reason, these organisms eggs accumulate carotenoids in high levels for these functions during vitellogenesis. In our study, VE, VC, VA and  $\beta$ C levels decreased in liver, kidney, spleen and muscle tissue, while increased in gonad tissue in R and BR periods compared to AR period. Reason for that, during ovarian maturation, *C. umbla* may be transport the VE, VC, VA, and  $\beta$ C 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  $\beta$ C level in muscle and liver of fish in the BR and R periods compared to the AR 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 [40,41]. For that matter De Vlaming *et al.* [42] reported that vitellogenin is synthesized in the liver under the control of estradiol produced by the follicle cells in the ovaries.

#### 4. Conclusions

As a result, in reproduction period of *C. umbla* decreased the level of VE, VC, VA and  $\beta$ C in liver, spleen, kidney and muscle, but these levels increased in gonad. In general, the MDA level increased in all tissues, reaching the highest level in gonads in particular. For this reason, VE, VC, VA, and  $\beta$ C should be added to the diets of these fish in the reproduction period in production studies to be carried out under culture conditions.

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