Journal of Materials and Electronic Devices 4 (2022) 27-33

JOURNAL OF MATERIALS AND ELECTRONIC DEVICES
FYTRONIX JMED http://dergi-fytronix.com/

Investigation of oxidative stress and some antioxidant in the tissues of female crayfish (*Pontastacus leptodactylus*) fed with trout diet

[a]Özden Barım-Öz [b]Mine Erişir

[a]Fisheries Faculty, Firat University, Elazig, TURKEY
[b]Department of Biochemistry, Veterinary Faculty of Firat University, Elazig, TURKEY

The effects of trout and control diet on malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and reduced glutathione (GSH) levels in tissues (hepatopancreas, ovarian, muscle and gills) of *Pontastacus leptodactylus* was investigated. The control diet was formulated to contain approximately 37.0% crude protein, 7.6% crude fat. The trout diet was obtained from the feed factory as ready-made. The crude protein content of trout feed was 53.0% and crude fat content was recorded as 15.0%. The experiment was carried out with 3 replicates for each dietary treatment. 15 females were used for each replicate (90 females in total). Statistically significant were detected that the levels of MDA increased in the hepatopancreas, ovarian, muscle and gills tissues of crayfish fed with trout diet according to control diet. In this study, SOD levels were found to be higher in trout diet group compared to control diet group. Furthermore, GSH levels in the trout diet groups were lower in hepatopancreas and ovarian, higher in muscle and gills than control diet group.
This study was planned to give a concrete answer to the question of whether crayfish can be grown with trout diet. At the end of the study, it was concluded that crayfish farming cannot be done with trout diet.

Key words: *Pontastacus leptodactylus, Oxidative stress, Antioxidan, Enzyme.*

Submission Date: 03 November 2022
Acceptance Date: 05 December 2022

**Corresponding author: obarimoz@firat.edu.tr*

1. Introduction

Lipid peroxidation is used as an indicator of oxidative stress in cell and tissues. Lipid peroxides derived from polyunsaturated fatty acids are unstable and can decompose to form a complex series of compounds. These include reactive carbonyl compound, which is the most abundant malondialdehyde (MDA). Therefore, measurement of malondialdehyde is widely used as an indicator of lipid peroxidation [1,2]. Fish and crustacean against this oxidation have built up extensive defence systems, consisting of antioxidant enzymes, endogenous antioxidants, such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), glutathione reductase (GR) and nutritional antioxidants [2].

Aquatic organism are more susceptible to the attack of ROS according to other aerobic organisms, because they have rich source of polyunsaturated fatty acid lipids [2-4]. Studies have shown that the antioxidant defences and oxidative stress in these organism can be affected by several stressors, including intrinsic (age and phylogenetic position, reproduction, feeding habits, etc) or extrinsic (salinity and temperature changes, pathogens, starvation, etc.) factors [5-8]. The induction of antioxidant defence enzymes may provide sensitive early-warning signals of incipient oxidative stress conditions. These enzymes can be induced by various environmental pro-oxidant conditions (such as pollution), endogenous/exogenous factors (such as age, reproductive, diet and temperature variations) [6-10].

Reproductive success is vital for sustaining an aquaculture system. One main factor affecting maturation, fecundity and survival of larvae in aquaculture organism is the fish condition. Crayfish condition is largely affected by their nutrition and environmental conditions [4,7,9-11]. It is very important to investigate the biochemical changes specific to the species in this period in order to create the conditions suitable for the natural environment of the crayfish during the breeding and moulting period [12].

Crayfish are represented in the world by 737 species and subspecies. They are collected in the Decapoda order of the Crustacea (crustaceans) class of the Arthropoda phylum [13]. As a result of the genetic research, it was reported that this species in Turkey is *Pontastacus leptodactylus* [14]. Crayfish can be obtained by catching and aquaculture in the world. According to the aquaculture statistics data, the amount of crayfish, which cannot be farmed in Turkey, obtained by catching was determined to be 1233 tons in 2020 and 1011 tons in 2021[15].

Pontastacus leptodactylus is a native freshwater crayfish species in Turkey. It is widely distributed in lakes, pounds and rivers in many parts of Turkey. This species has commercial importance in Turkey and were exported to a number of European countries until 1986. The production of *P. leptodactylus* after 1985 decreased dramatically (from 5000 tonnes annually to 200 tonnes) in most Turkish lakes as a result of the crayfish plague, over-fishing, water pollution, and water withdraws for agricultural irrigation [16]. For these reasons, crayfish especially, broodstocks have to be fed with good quality diet for growth, production and reproduction. So the knowledge of how the biochemical and physiological systems changes during the mating period of *P. leptodactylus* populations are require for this good quality diet.

In this study aimed to determine the oxidative stress (malondialdehyde (MDA)) and some antioxidant (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and reduced glutathione (GSH)) levels in hepatopancreas, gonad, gills muscle, and tissues of female *P. leptodactylus* fed with different two diet (trout and control).

2. Experimental

This study was conducted between September 05 and November 03 at the crayfish reproduction unit of Aquaculture Faculty of Fırat University, Elazığ, Turkey. The crayfish used in the present study was provided from Keban Dam Lake population of *P. leptodactylus*.

Control diet used in this study (Table 1) was modified after Barim [16]. This diet was formulated to contain approximately 37.0% crude protein, 7.6% crude fat, The ingredients for diet were thoroughly mixed, before adding water, in a commercial food mixer, cold-pelleted by forcing through 3-mm holes using a laboratory pellet mill, air-dried at 55 ºC for up to 24 h, and then stored in a refrigerator at 4 ºC until further use.

The trout diet used in the study was obtained from the feed factory as ready-made. This diet contain fish meal, soybean meal, fish oil, corn gluten, wheat flour, vitamin and mineral premix. Crude protein content of trout feed was 53.0% and crude fat content was recorded as 15.0%. This diet included Ca (2.5%), P(1.7%), Na (0,5%), vitamin A (24.000 IU kg^{-1}), vitamin D3 (5000 IU kg^{-1}), vitamin E (500 mg kg^{-1}), vitamin C(400 mg kg^{-1}).

Table 1. Composition and proximate analysis of the control diet [16].

Ingredient	Percent of dry weight
Fish (anchovy) meal	35.78
Soybean meal	38.64
Wheat flour	19.30
Sunflower oil	4.00
Dicalcium phosphate	1.00
Sodium phosphate	0.40
Avilamycine[1]	0.10
Antioxidant [2]	0.10
Vitamin premix [3]	0.50
Mineral premix [4]	0.18

(1) Kavilamycine
(2) Antioxidant (mg/kg dry diet): butylated hydroxytoluene 12.5.
(3) Vitamin premix (IU or mg/kg): vitamin A 2,000,000 IU, vitamin D_3 200,000 IU, vitamin E 20,000 IU, vitamin K 3,000 mg, vitamin B_1 1,000 mg, vitamin B_2 3,000 mg, Niacin 30,000 mg, Calcium D-Pantothenate 10,000 mg, vitamin B_6 2,000 mg, vitamin B_{12} 4 mg, Folic Acid 600 mg, D-Biotin 200 mg, Choline Chloride 100,000 mg and vitamin C 60,000 mg.
(4) Mineral premix (mg/kg dry diet): Mn 80, Fe 35, Zn 50, Cu 5, I 2, Co 0,4, Se 0,15.

The experiment was carried out with 3 replicates for each dietary treatment. 15 females were used for each replicate (90 females in total). Crayfish were housed in tanks (2 x 2 x 0.5 m). Plastic pipes (20 cm in length and 7 cm in diameter) were provided as shelters for the crayfish. *P. leptodactylus* were acclimatised to temperature and flow conditions and starved for one week to standardize their nutritional conditions and to ensure that they were in good health prior to the start of the experiment. After one week, crayfish were weighed and were fed 2 % of their total wet weight daily, divided into three separate feedings [16]. Supplemental water flow was 0,5 l/sec for each tank. During the trial, mean dissolved oxygen was 7,22±0,38 mg/L; mean pH was 7,25±0,08; mean water temperature was 12,16±1,03 ºC.

The crayfish selected from each replicate were placed on ice in plastic bags and transported to the laboratory. Sample of crayfish taken from each of the two dietary treatments was randomly selected for analysis. The carapace length (mm) were recorded. The tissues of the crayfish for biochemical assays were surgically removed and stored at –80^{0}C.

MDA level assay: The level of MDA as a marker of lipid peroxidation was measured according to the method of Ohkawa, Ohishi and Yagi [17] on the basis of the reaction with thiobarbituric acid (TBA). The formed MDA created a pink complex with TBA and the absorbance was read at 532 nm. The MDA level of tissues was expressed as nmol g^{-1} tissue.

SOD activity assay: The SOD activity was determined according to the Sun, Oberley and Li [18] method. that is based on the principle that xanthine reacts with xanthineoxidase to generate superoxide radicals that react with nitroblue tetrazolium to form a coloured formazan dye. To analyse the SOD activity. 600 µL of the SOD reaction mixture containing 0.1 mM EDTA. 0.1 mM xanthine. 25 µmol L^{-1} of nitroblue tetrazolium and 50 mg of bovine serum albumin was added to 125 µL of the supernatant. Then. 25 µL of 9.9 nM xanthine oxidase solution was added to each tube. The amount of formazan found by measuring the absorbance at 560 nm using a spectrophotometer. The results of SOD activity are provided as U mL^{-1}.

GSH-Px activity assay: The level of GSH-Px was determined using the procedure described by Beutler [19]. which records the disappearance of NADPH through its absorbance at 340 nm. The procedure of analysis performed was based on the oxidation of GSH by GSH-Px coupled with the disappearance of NADPH by glutathione reductase measured at 37°C. The absorbance at 340 nm was placed on record over a period of 5 min. The activity was then calculated from the slope of the lines as µM of NADPH oxidized per minute.

GSH level assay: Glutathione concentration was determined by a kinetic assay using a dithionitrobenzoic acid (DTNB) recycling method described by Ellman [20]. One millilitre of the sample was deproteinated by the addition of a solution containing 0.2 g of Na_2EDTA. 1.67 g of metaphosphoric acid and 30 g of NaCl in distilled water. DTNB and Na_2HPO_4 were added to the supernatants and cleared by centrifugation (10 min. 3000 g/min). The GSH level was measured based on its reaction with DTNB to yield a yellow chromophore. which was measured spectrophotometrically at 412 nm.

Statistical procedures: All results are expressed as mean ± 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

The mean carapace length of females crayfish among the experimental groups (control, trout diet) and within the replicates of each dietary treatments were not significantly different (p>0.05 for each cases) at the beginning of the experiment. The mean carapace length of crayfish was 45.18±0.13 mm for control diet group, 44.88±0.35 mm for trout diet group.

As in all living, many changes occur in metabolic functioning during the oxidative stres in aquatic organisms. The cell and tissues injury caused by oxidative damage that can occur with high levels of free radicals or ROS is one of the consequences of uncontrolled oxidative stres. It is well established that the main ROS generated by cellular metabolism are the superoxide anion (O_2^-), the hydrogen peroxide (H_2O_2), siglet oxygen ($^1O_2$), and hydroxyl radical (HO^*), and these compounds can rapidly react to form other molecules like peroxyl radicals (ROO^*) and alkoxyl radicals (RO^*) [21,22]. Under basal conditions, the advarse effects of oxyradicals in living aerobic organisms are prevented by the antioxidant system. But, the antioxidant and detoxifying systems during high levels of radicals are deficient and not able to neutralise the active intermediates produced by xenobiotics and their metabolites that potential to cause toxin insults to cellular components such as lipids of biological membranes and proteins of enzymes. 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 companents [23-24]. For these reason; a) especially this study; It was done in September, October and November because the crayfish both moulting and develop their ovaries depending on the breeding period, b) thus, the effects of trout and control diets due to changing metabolic activity in *P. leptodactylus* on MDA and antioxidant enzymes were more clearly demonstrated.

The results obtained in our study reveal that the changes in antioxidant defence and MDA levels of crayfish in the trout diet and control diet groups were generally higher in the hepatopancreas compared with muscle, gills and ovarian (Table 2). These findings are in agreement with a previous observation that was made by Paital and Chainy [25] who found that the oxidative stress physiology markers (SOD, GSH-Px, GR) were higher in hepatopancreas in comparison to gills and abdominal muscle of *S. serrata* in all seasons. Similarly, Verlecar et al. [26] determined that digestive gland is specific tissues in seasonal variation of ROS level such as H_2O_2 and lipid proxidation of *P. viridis*. It is known that crustacea hepatopancreas, the main digestive gland, contains fat-soluble vitamins, regulates the metabolism of the body and exhibits high oxygen consumption [27,28]. Thus, the generation of O_2^{-*} and H_2O_2 can be comparatively more in this organ than other organs.

Table 2. Comparison of the mean concentration of the tested malondialdehyde (MDA (nmol g-1 tissue)), superoxide dismutase (SOD (U ml-1)), glutathione peroxidase ((GSH-Px (U g-1)), reduced glutathione ((GSH) (µmol mL^{-1})) in hepatopancreas (H), ovarian (O), muscle (M) and gills (G) tissues (T) of crayfish (*P. leptodactylus*)) in the trout diet group (TD) and the control diet group (CD), P_{CD}: Comparison of the control diet group among the tissues (a,b,c,d), P_{TD}: Comparison of the trout diet group among the tissues (x,y,z,t). Significance between groups was shown as asterisk (*p < .05, **p < .01, ***p < .001).

D	T	Parameters			
		MDA	SOD	GSH-PX	GSH
CD	H	1.55±0.12^{a}	1,76±0.22^{a}	262.28±17.28^{a}	5.83±0.59^{a}
TD	H	2.89±0.43^{x}	4.28±0.52^{x}	612.21±22.15^{x}	3.22±0.31^{y}
	P	***	***	***	***
CD	O	1.31±0.16^{b}	1.65±0.16^{a}	102.47±9.56^{c}	6.09±0.36^{a}
TD	O	2.18±0.19^{y}	3.56±0.30^{y}	311.84±28.77^{y}	4.36±1.06^{x}
	P	***	***	***	***
CD	M	0.96±0.09^{c}	1.18±0.13^{b}	167.66±17.11^{b}	2.16±0.34^{b}
TD	M	1.48±0.11^{z}	2.25±0.28^{z}	305.97±19.26^{y}	3.42±0.61^{y}
	P	***	***	***	***
CD	G	0,81±0,11^{d}	0,90±0.09^{bc}	115.14±7.34^{c}	2.25±0.24^{b}
TD	G	0,97±0,07^{t}	1.42±0.12^{t}	224.28±18.36^{z}	3.59±0.58^{y}
	P	**	***	***	***
	P_{CD}	***	***	***	***
	P_{TD}	***	***	***	***

In this study, gastroliths were observed in crayfish. It was determined by Barim-Oz et al. [12] that male and female crayfish molt in the August, September and October months and the MDA levels were higher in hepatopancreas according to other tissues. Moulting influences all aspects of crustacean biology (cellular metabolism, physiology and behaviour). Metabolism is elevated because organic reserves such as mineral deposits, glucose, α-chitin-protein, gastrolith matrix protein, glycoprotein and ecdysteroids in tissues (especially hepatopancreas) and hemolymph are conversion and release [29,30] Aiken and Waddy [29] reported that tissue metabolism can elevate oxygen consumption by as much as 1900% during premoult. Additionally, Yudkovski et al. [31] determined that during late premoult stage occured up-regulation of genes and three additional gene changes effecting oxidative stress in gastrolith disc. This increase in MDA levels could be related to direct damage to biological molecules and tissues of excessive free radical generation due to an increase of the physiological activity caused primarily by the varying metabolic activity during moulting because those described above.

In this study, the data obtained as a result of the analyzes were statistically evaluated and it was determined that MDA level was higher in gonads compared to gills and muscle. The changes in biochemical levels occur as aresult of maturation stage, so this was considered as an additional variable because of its effect on the mobilization and accumulation of reserves in several tissues. Accumulation of biochemical components, especially lipids, in the maturing ovary has been reported in fish and crustaceans [2,4,12]. For example, Palacious et al. [32] found that the level of total lipid, acylglycerides, cholesterol and total protein in mature ovaries of *Penaeus vannamei* increased, and acylglycerides represent the bulk of lipids in the hepatopancreas, whereas in the ovary, this lipid class represents a lower fraction because phospholipids are reported to be predominant. Wilhelm Filho et al. [4] determined that in *Perna perna*, the TBA-reactive substance content observed in reproductive period were approximately double than those found in the rest of the year. Their gonads have a higher lipid and carbohydrate mobilization and protein synthesis. Bell et al. [33] and Cavalli et al. [34] demonstrated that highly unsaturated fatty acids, which are vital components of cellular membranes, are particularly susceptible to attack by reactive oxygen radicals. Uncontrolled damage to membrane fatty acids and the accumulation of their oxidized breakdown products can have deleterious consequences for cell and organ function and may increase the requirement for antioxidants. According to these studies, the increase in MDA levels may be linked to the accumulation of lipids in tissues and the increased metabolic activity in the ovarian tissues.

The results of the study illustrated that the levels of MDA increased in the hepatopancreas (86.45%), ovarian (61.48%), muscle (54.16%) and gills (19.75%) tissues of trout diet group crayfish according to control diet group crayfish. Many studies have been carried out on the nutritional needs of crayfish. As a result of this study, it was determined that the high fat content in the diets negatively affected the growth of crayfish. For example, Ackefors et al. [35] *Astacus astacus* crayfish in different proportions protein (22,31.40%), carbohydrates (9.2-25.8), fat (5.5-16) and P/E (19-153 mg/day) kcal) have investigated the evaluation of 12 diets. In this study, crayfish with an initial mean body weight of 146±5 mg were fed at a water temperature of 18.3-19.6°C for 394 days. At the end of this period, it was found that crayfish fed with feed containing 40% protein showed approximately 3% live weight gain every day, the optimum P/E ratio was 114-123 mg/kcal, and high fat content (13-16%) caused slower growth and survival rates. Jussila [36] *C. tenuimanus* (11.5±0.3 g) crayfish with diet containing 30% protein, 10% fat, *A. astacus* (21.2±0.5) with food containing 48% protein, 22% fat g) and *Pasifastacus leniusculus* (31.5±0.9 g) crayfish were fed at 22-24 °C water temperature with 6-10% of their body weight weekly for 125-182 days, and their growth performance and HSI were examined. At the end of the research, they determined that high fat content caused slowing in growth. Fotedar et al. [37] crayfish (*C. tenuimanus*, mean starting weight 1.99±0.09 g) with three different feeds containing 25.57% crude protein, 0.28%, 6.28% and 12.28% fat fed for 108 day. At the end of this period, the mean live weight of the crayfish at the end of the trial was 6.34±0.72 g, 5.03±0.55 g, 3.54±5 g for feeds containing 0.28%, 6.28% and 12.28% fat, respectively. They determined SBO as 1.07±0.06, 0.82±0.07, 0.61±0.09 and the high amount of fat added to the crayfish feeds would cause a slowdown in growth. In addition, Hajra et al. [38] fed shrimp (*P. monodon*) with an average weight of 0.510 g with feeds containing different ratios of protein (45.10-46.90%) and fat (25.90-33.0%). As a result of the study, the average weight of the crayfish fed with diet containing 46.30% crude protein and 10.60% fat, which was determined to be the best diet, was determined as 1.3376 g. In the light of these studies, high fat content (%24) in trout feed according to control diet (15.0%) may cause high MDA levels. Furthermore, as a result of feeding crayfish with diets containing high fat content, they cannot utilize this fat with metabolic activities. Thus, the LPO level and oxidative stress may increase with radical as the generation of O_2^{-*}.

SOD catalyses the transformation of O_2^{-*} to H_2O_2 and water [39]. For this reason, SOD and GSH-Px enzymes, used as a biomarker of ROS production, are the first line of defence against oxidative stress. In the study by Barim-Oz et al. [12] was determined that the SOD activity in hepatopancreas was higher in moulting and breeding periods according to other months. Increased SOD level during gonadal development and breeding period has also been determined in *P. perna* by Wilhelm Filho et al. [4], in *P. viridis* by Verlecar et al. [26] and in *S. glanis* by Bayir et al. [27]. High level of H_2O_2, change cell physiology through the production of OH^* by Fenton reaction, in these periods were also observed (Verlecar *et al*. 2008).The increase of SOD may be associated to neutralise the overproduction of O_2^- anions and H_2O_2 due to peroxidation. The present study illustrates that SOD activity was higher in hepatopancreas (%143.18), ovarian (%115.75), muscle (%90.67) and gills (%57.77) tissues of crayfish in trout diet group according to control diet group. It may indicates an increasing need to destroy O_2^- in tissues during metabolic activity [40].

GSH-Px is mainly involved in the removal of organic peroxides. Hence, GSH-Px is considered to play an very important role in protecting membranes from damage due to LPO [22,24,41]. The present study illustrates that GSH-Px activity in crayfish was higher according to other tissues in hepatopancreas of both diet groups. This observation is in good agremeent with an earlier report [4,22,26,42] which the production of O_2^{-*} radicals increase during metabolic activities. The increased GSH-Px activity in the hepatopancreas protected the organ from the formation of lipid peroxides by reducing H_2O_2 levels, which in turn attenuated OH^{-*} generation. It was reported that H_2O_2 is neutralized by two different enzymes present in the cellular system, they are GSH-P_x and CAT. Each differs in its affinity for H_2O_2, and intracellular H_2O_2 concentration is one of the factors in deciding which of these enzymes will be functional since each has a different Km value [43]. Furthermore, GSH-P_x is responsible for the neutralization of both inorganic and organic hydroperoxides. As paralel of our study, Nahrgang et al. [44] also determined that in *M. edulis*, during gonadal development and spawning season, the level of GSH-Px was higher those found in the rest of the year. Moreover, Barim-Oz and Yılmaz [42]. was also determined that GSH-Px level in gonad of *A. leptodactylus* increased during moulting period. Our results also indicate that the percentages of increase in the GSH-Px level was 133.41%, 204.32%, 82.49% and 94.78%, respectively for hepatopancreas, ovarian, muscle and gills at the trout diet group compared with control diet group. As mentioned earlier, high level of GSH-Px might not be sufficient to reduce OS in reproduction and moulting period as evidenced by high level of MDA.

GSH, the non-enzymatic antioxidants, is a primary reductant and is the most abundant thiol-containing substance of low molecular weight in the cells. In this way, it serves multiple functions in protecting tissues from oxidative damage and keeping the intracellular environment in the reduced state. In addition, this enzyme reduces hydrogen- and organic-peroxides *via* a reaction catalyzed by GSH-Px; it serves as a scavenger of OH^* and $^1O_2$ [22,24]. Our study found that the GSH activity of crayfish in control diet group was lower in muscle and gills tissues according to hepatopancreas and ovarian tissues, but the GSH activity of crayfish in trout diet group was lower in hepatopancreas, muscle and gills tissues according to ovarian tissues. This idea was corroborated by the observations of Wilhelh Filho et al. [4], who described that the concentration of the GSH in *P. perna* decreased during reproduction period. The decrease in this enzyme activities were likely responses to an increased utilization of GSH. Furthermore, severe oxidative stress may suppress GSH levels due to the impairment of moulting and reproduction mechanisms in *A. leptodactylus* [1,41,42]. We found that the GSH activity in the hepatopancreas and ovarian tissues (44.76%, 28.40% respectively) were lower at the trout diet group compared with the control diet group, but the GSH activity in the muscle and gills tissues (58.33%, 59.55% respectively) were higher at the trout diet group compared with the control diet group. Earlier findings also suggest that the presence of high GSH level is associated with the attenuation of oxidative stres. The decrease in the GSH level may be caused by the utilisation of nonenzymatic antioxidants to control the enormous of free radicals produced in the metabolism against high fat level in trout diet [22].

4. Conclusion

This study was planned to give a concrete answer to the question of whether crayfish (*P. leptodactylus*) can be grown with trout diet. For this, the crayfish were fed with trout diet obtained from the factory as ready-made and a control diet prepared for the needs of the crayfish. The results of the study illustrated that the levels of MDA increased at a high level in the hepatopancreas, ovarian and muscle tissues of trout diet group crayfish according to control diet group crayfish. In addition, antioxidant levels increased. For these reason, it was concluded that crayfish farming cannot be done with trout diet.

References

[1] O. Barim, Journal of Animal and Veterinary Advances, 8(6) (2009) 1190-1197.

[2] G. W. Winston, R. T. D. Giulio, Aquatic Toxicology, 19 (1991)137-161.

[3] Ö. Barım Öz and S. Köprücü, J. Mater. Electron. Device. 6 (2021) 1–6.

[4] W. Filho, T. Tribess, C. Gáspari, F.D. Claudio, M.N. Torres, A.R.M. Magalhães, Aquaculture, 203 (2001) 149-158.

[5] R.G. Ahmed, Medical Journal of Islamic World Academy of Sciences, 15(2) (2005) 55-63.

[6] A. Muhlia-Almazan, A. Sanchez-Paz, F. Garcia Carreno, A.B. Peregrino-Uriarte, G. YepizPlascencia, Comparative Biochemistry and Physiology B, 142 (2005) 209-216.

[7] M. Furne, M. Garcia-Galloge, M.C. Hidalgo, M.C. Morales, A. Domezain, J. Domezain, A. Sanz, Aquaculture Nutrition, 15 (2009) 587-595.

[8] S. Barata, H. Şahin, S. Köprücü, Nevşehir Bilim ve Teknoloji Dergisi Cilt 6(2) (2017) 476-482.

[9] Ö. Barım Öz, M. Karatepe, Sakarya Üniversitesi Fen Bilimleri Dergisi, 21 (6) (2017**)** 1537–1543.

[10] Ö. Barım Öz, H. Şahin, S. Köprücü, Türk Doğa ve Fen Dergisi, 6(2) (2017) 57–61.

[11] O. Barim, M. Karatepe, Ecotoxicology and Environmental Safety, 73 (2010) 138-142.

[12] O. Barim-Oz, S. Yilmaz, H. Sahin, F. Benzer, Fresionus Environmental Bulletion, 26(4) (2017) 3038-3047.

[13] S. Berber, M. Palaz, Su Ürünleri Sempozyumu, Sinop, (2000) 1-11.

[14] S. Akhan, Y. Bektas, S. Berber, G. Kalaycı, Genetica, 142 (2014) 381–395.

[15]https://www.tarimorman.gov.tr/BSGM/Belgeler/Icerikler/Su%20%C3%9Cr%C3%BCnleri%20Veri%20ve%20D%C3%B6k%C3%BCmanlar%C4%B1/Su-Urunleri-%C4%B0statistikleri-temmuz-2021-1.pdf Erişim Tarihi: 20.09.2022.

[16] Ö. Barım, Graduate School of Natural and Applied Sciences. Department of Aquaculture, PhD Thesis, (2005) 73 s.

[17] H. Ohkawa, N. Ohishi, K. Yagi, Anal Biochem., 95 (1979) 351-358.

[18] Y. Sun, Y. W.L. Oberley, Y. Lı, Clin Chem, 34 (1988) 97-500.

[19] E. Beutler, O. Duron, B.M. Kelly, Journal of Laboratory and Clinical Medicine, 61 (1975) 882- 888.

[20] G.L. Ellman, Arch Biochem Biophys, 82 (1) (1959) 70-7.

[21] F.F. Benzie,. J. Nutr. 398 2000), 53-61.

[22] A. Ayala, M.F. Munoz, S. Argüelles, Medicine and Cellular Longevity (2014) 31 pp,

[23] A. Valavanidis, T. Vlahogianni, M. Dassenakis, M. Scoullos, Ecotoxicology and Environmental Safety 64, (2006) 178-189.

[24] F. Regoli, M.E. Giuliani, Marine Enviromental Research 93 (2014) 106-117.

[25] B. Paital, G.B.N. Chainy, Ecotoxicology and Environmental Safety 87 (2013) 33-41.

[26] X.N. Verlecar, K.B. Jena, G.B.N. Coastal and Shelf Science, 76 (2008) 745-752.

[27] A. Bayir, A.N. Sirkecioglu, H.I. Haliloglu, E. Aksakal, M. Gunes, N.M. Aras, Fresenius Environmental Bulletin, 20(1) (2011) 3-11.

[28] S.S. Borković, S.Z. Pavlović, T.B. Kovačević, A.Š. Štajn, V.M. Petrović, Z.S. Saičić, Comprative Biochemistry and Physiology Part C, 147 (2008) 122-128.

[29] D. E. Aiken, , S.L. Waddy, Reviews in Aquatic Sciences 6(3-4) (1992)335-381.

[30] J. Ghanawi, I.P. Saoud, Aquaculture, (2012) 358-359 183-195.

[31] Y. Yudkovski, L. Glazer, A. Shechter, R. Reinhardt, V. Califa-Caspi, A. Sagi, M. Tom, Comperative Biochemistry and Physiology Part D: Genomics and Proteomics, 5(2) (2010) 171-177.

[32] E. Palacious, A.M. Ibarra, I.S. Racotta, Aquaculture, 185, (2000) 353-371.

[33] J.G. Bell, , J. McEvoy, D.R. Tocher, J.R. Sargent, The Journal of Nutrition (Nutrient Interaction and Toxicity)**,** (2000) 1800-1808.

[34] R.O. Cavalli, F.M.M. Batista, P. Lavens, P. Sorgeloos, H.J. Nelis, A.P.D. Leenheer, Aquaculture, 227 (2003) 131-146.
[35] H. Ackefors, J.D. Castell, L.D. Boston, P. Raty, M. Svensson, Aquaculture, 104 (1992) 341-356.

[36] J. Jussila, Universty of Kuopio, Austraalia, (1997) 140 p.

[37] R.K. Fotedar, L.H. Evans, B. Knott, Freshwater Crayfish, 11 (1997) 417-427.
[38] A. Hajra, A. Ghosh, S.K. Mandal, Aqucalture, 71 (1988) 71-79.
[39] R. Oost, J. Beyer, N.P.E. Vermeulen, Environ. Toxicol. Pharmacol., 13(2) (2003)57-149.
[40] A.M. Pisoschi, A. Pop, Europan Journal of Medicinal Chemistry, 97 (2015) 55-74.
[41] O. Barim, F. Benzer, M. Erisir, M. Dorucu, Fresenius Environmental Bulletin, 6, (2009) 948-954.
[42] O. Barim-Oz, S. Yilmaz, E-Journal of New World Sciences Academy 4(3), (2009) 39-46.
[43] E. Beytut, Ö. Barim, N.N. Kamiloglu, Journal of Animal and Veterinary Advances 8(6), (2009) 1177-1184.
[44] J. Nahrang, L. Camus, F. Broms, J.S. Christianses, H. Hop, Marine Pollution Bulletin, 60 (2010) 1336-1345.