Cellular and Molecular Biology

Barim-Oz and Sahin *Cell. Mol. Biol.*2016, 62 (14): 1-10
ISSN: 1165-158X
doi: 10.14715/cmb/ 2016.62.14.1

Original Research

The influence of dietary antioxidant on ovarian eggs and levels of vitamin E, C, A, astaxanthin, β-carotene and oxidative stres in tissues of *Astacus leptodactylus* (Eschscholtz) during reproduction

O. Barim-Oz[1*], H. Sahin[2]

[1]Fisheries Faculty, Firat University, Elazig, Turkey
[2]Tunceli University, Fisheries Faculty, Tunceli, Turkey

Abstract: The experiment was conducted to determine the most effective antioxidant (among the vitamin E (VE), vitamin C (VC), vitamin A (VA), astaxanthine (AX), β-carotene (βC)) on the ovarian egg number and size, level of VE, VC, VA, AX, βC and oxidative stress (as malondialdehyde (MDA)) in the hepatopancreas, ovarian, gills and muscle tissue during ovarian development of *Astacus leptodactylus*. One control (C) and five experimental diets (EE, EC, EA, EAX and EβC) were prepared. The EE, EC, EA, EAX and EβC groups were formed by added 150 mg kg^{-1} VE, 200 mg kg^{-1} VC, 240 mg kg^{-1} VA, 200 mg kg^{-1} AX and 200 mg kg^{-1} βC to diet C, respectively. At the end of the experiment found that the dietary antioxidants increased ovarian egg number and size and reduced the level of MDA in the tissues. Ovarian egg number and size were highest in the EE and EAX diet groups in the comparison to control ($p<0.001$). The level of MDA in the tissues was lowest in the EAX diet group in the comparison to control ($p<0.001$). The highest levels of VE, VC, VA, AX and βC were found in the hepatopancreas and ovarian compared with muscle and gills. The highest level of MDA also was determined in the ovarian according to other tissues. In conclusion, the VE and AX in broodstock diets were the most effective antioxidants on the ovarian egg number and size of *A. leptodactylus*.

Key words: *Astacus leptodactylus*, antioxidants, malondialdehyde, vitamins, carotenoids.

Introduction

The reactive oxygen species (ROS) in biology, one types of free radicals, occurs as natural results of aerobic metabolism and it is necessary for the survival of organism. Oxygen radicals exert critical actions such as regulation of some activity in cells, apoptosis, immune system and gene transcription. But, ROS is highly deleterious because of cytotoxic oxidants at pathological levels. To cope with the continuous generation of ROS, there are enzymatic and non-enzymatic antioxidants. In a healthy body, ROS and antioxidants remain in balance. When the balance is disrupted towards an overabundance of ROS, oxidative stress (OS) occurs (1, 2). For example, ROS increase in the several situations such as exercise, pollution, infection and reproductive (3, 4, 5, 6, 7, 8). ROS are very reactive towards all biomolecules (lipids, proteins and nucleotides). The reaction of ROS with polyunsaturated fatty acids (PUFA) generates lipid peroxidation in an auto-oxidation cycle where the product of one cycle is a free radical, which may react with a new PUFA (2). Especially, because aquatic animals are rich source of the n-3 PUFA, it is highly susceptible to attack by oxygen and other organic radicals (9). For these reason, it is inevitable the pursuit of OS indicator such as lipid peroxidation in aquatic animals.

In recent years, in the numerous studies have been reported that OS have an important role on the reproductive parameters of aquatic animals such as oosit maturation, spawning success, eggs and larval quality (1, 3, 4, 10, 11, 12, 13). Some nonenzymatic low molecular weight antioxidant compounds are consumed with increase of OS during reproduction period. However, the antioxidant enzyme systems that are present in the liver and other tissues of fish and crustaceans are not synthesized until late in the embryonic development. For these reason, the body's complex antioxidant system must be supported in the periods of increasing OS. Optimal diet of crustaceans is especially identified as a crucial factor for the sexual maturation because dietary requirements of crustacean are generally higher in sexually maturing adults than in nonreproductive adults and juvenile. In addition, an unbalanced or incomplete diet causes poor reproductive performance or may even stop animals from reproducing. The vitamin E, VC, VA, βC and astaxanthin are some of the major antioxidants used in aquaculture industry in worldwide. But, because most fish and crustaceans are unable to biosynthesize these matters, *de novo*, it must additived in the diet (6, 7, 8).

Vitamin E protects cells from peroxidation of PUFA in membrane phospholipids by inhibiting ROS-induced generation of lipid peroxyl radicals. Vitamin C exhibits a protective effect by stoping the propagation of the peroxidative process, and helps recycle oxidized vitamin E (2, 9). Vitamin A, AX and βC are antioxidants too, but they are generally used as a pigmentation source in aquaculture industry. Moreover, these antioxidants play an important role in crustacea reproduction by acting as biological antioxidants (7, 8).

The narrow-clawed crayfish (*Astacus leptodacty-*

Received June 9, 2016; **Accepted** December 25, 2016; **Published** December 30, 2016

*** Corresponding author:** Ozden Barim-Oz, Fisheries Faculty, Fırat University, 23119, Elazig, Turkey. Email: obarimoz@hotmail.com

lus) is a species of crustacean. It is the native crayfish in Turkey, but there was very little consumption by the people. For this reason, it was exported regularly to Europe (14). There are a few studies on the nutrition for this crayfish (3, 4, 15, 16). However, there is very lack of antioxidants nutritional information on reproduction for the crayfish.

The breeding season in A. *leptodactylus* begins with the decline in water temperature in the autumn, and mating occurs during October and Nowember at 7-12 °C. Thus, the quality and quantity of the egg's vitellin reserves during this stage are crucial for the future offsprings' survial. Especially, because the main characteristic of invertebrate reproduction is the accumulation of reserves made in the oocytes of the maturing female (17), an adequate understanding of the dynamics of nutrient utilization during ovarian development in *A. leptodactylus* will contribute to increasing reproductive success of commercial production of eggs and juveniles

The present study was designed to compare the effects of dietary VE, VC, VA, AX and βC levels on the ovarian egg number and size of *A. leptodactylus* broodstock. Moreover, to test the influence on OS of the diet VE, VC, VA, AX and βC during the ovarian maturation of the crayfish, it was measured the content of MDA in the hepatopancreas, ovarian, gills and muscle. It was also discussed the relationship among the VE, VC, VA, AX and βC levels in these tissues.

Materials and Methods

Crayfish and experimental design

This study was carried out between June 25 and November 15 (143 days) at aquarium laboratory of Fırat University Aquaculture Faculty, Elazığ, Turkey. The crayfish used in this study was provided from Keban Dam Lake population of *A. leptodactylus*.

In this study, one control (C) and five experimental diets (EE, EC, EA, EAX and EβC) were prepared. The EE, EC, EA, EAX and EβC groups were supplemented with 150 mg kg^{-1} VE (4), 200 mg kg^{-1} VC (18), 240 mg kg^{-1} VA (19), 200 mg kg^{-1} AX and 200 mg kg^{-1} βC (20, 6), respectively, and it were set in relation to levels reported by other researchers in a variety of crustacean species. No VE, VC, VA, AX and βC was added to the control diet, except that supplied by the feed ingredients. The control diet (Table 1) was modified after Barim (3) and Harlioğlu and Barim (16). The VE, VC, VA, AX and βC contents of the diets was analysed by High Performance Liquid Chromatography (HPLC) (21, 22, 5). The VE, VC, VA, AX and βC contents of the control were 13.86±1.30 mg kg^{-1}, 17.25±2.50 mg kg^{-1}, 2.00±0.21 mg kg^{-1}, 1.82±0.33 mg kg^{-1}, 20.05±1.90 mg kg^{-1}, respectively. The levels of 138.04±2.31 mg kg^{-1} VE, 185.00±3.22 mg kg^{-1} VC, 227.00±.2.02 mg kg^{-1} VA, 190.00±3.05 mg kg^{-1} AX and 189.00±3.61 mg kg^{-1} βC diet were determined for VE, VC, VA, AX and βC diets, respectively.

The control diet was formulated to contain approximately 38.00% crude protein, on a dry-weight basis and 3.32 kcal g^{-1} gross energy (Table 1). The crude protein content was analysed by Kjeldahl's method; gross energy was calculated based on physiological fuel values of 9 kcal g^{-1} for lipid and 4 kcal g^{-1} for protein and carbohydrate; dry matter after the sample was dried at 105 °C for 6 hours. Ash content was calculated after 24 h at 550 °C in the furnace, and lipid analysed by an ether extraction method (23).

Table 1. Composition of the control diet.

Ingredients	Percent of dry weight
Fish (anchovy) meal	35.78
Soybean meal	38.64
Wheat flour	19.40
Sunflower oil	4.00
Dicalcium phosphate	1.00
Sodium phosphate	0.40
Antioxidant [1]	0.10
Vitamin E, A, C-free vitamin premix [2]	0.50
Mineral premix [3]	0.18

(1) Antioxidant (mg/kg dry diet): butylated hydroxytoluene 12.5.
(2) Vitamin premix (IU or mg/kg): vitamin D_3 200,000 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.
(3) Mineral premix (mg/kg dry diet): Mn 80, Fe 35, Zn 50, Cu 5, I 2, Co 0,4, Se 0,15.

Fish (anchovy) meal, soybean meal, wheat flour, sunflower oil, antioxidant, dicalcium phosphate, sodium phosphate, vitamin-mineral premix, dietary VE (50% dl-α-tocopheryl acetate), VC (33% L ascorbic acid monophosphate), VA (1000000 IU per gram retinyl acetate), AX (8% astaxanthin, Carophyll Pink,) and βC (10% β-Carotene) was donated by a food producer firm (DSM Nutritional Products). The ingredients for each 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 55ºC for up to 24 h, and then stored in a deep freeze at -4ºC until further use (4, 24, 25).

The experiment was carried out with 3 replicates for each dietary treatment. Ten females and three males were used for each replicate (180 females and 54 males in total). Crayfish were housed in 18 glass aquariums (25 x 25 x 110 cm). Plastic pipes (15 cm in length and 7 cm in diameter) were provided as shelters for the crayfish. Adequate aeration was provided for each aquarium by a simple air pump. The carapace length (mm) and weight (g) were recorded for each crayfish. Crayfish were fed 2 % of their total wet weight daily, divided into three separate feedings (26).

During the trial, dissolved oxygen, pH and water temperature were measured daily. Ammonia, iron, copper, alkalinity, calcium and water flow were measured twice a week. Mean dissolved oxygen was 7.40±0.80 mg L^{-1}; mean ammonia, iron and copper content were less than 0.001 mg L^{-1} (for each parameter); mean calcium was 64.20±1.96 mg L^{-1}; mean alkalinity was 276.20±7.34 mg $CaCO_3$ L^{-1}; mean pH was 7.21±0.42 (27); and mean water temperature was 13.05±2.6 C.

At the end of the study, the size of ovarian eggs was measured by use of a light microscope and graticule. For biochemical assays, the hepatopancreas, muscle, gonad and gills in the crayfish were removed and were stored at -80 °C until used.

Sample preparation and biochemical assays

Determination of tissues vitamin C and MDA levels

An aliquot portion of (1,0 g) crayfish tissue samples 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 100µl-500 ppm butylated hydroxytoluene (BHT) (21, 5). These 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. The mobile phase contained 30 mM KH_2PO_4-Methanol (82.5+ 17.5, v/v %, pH 3.6). The flow rate of this phase was 1.2 mL min^{-1}. Chromatograms were monitored at 250 nm and injection volume was 20 µL. A Wakosil II 5C18 RS 5µm (150 x 4.6 mm SS, SGE, AUS) column was used at room temperature (5).

Determination of tissue vitamin A, E and β-carotene levels

The analyses 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 hepatopancreas and muscle 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, and they were centrifuged. This step was repeated two times (5).

N-hexane in tubes was evaporated with the nitrogen. Then the residues were solved in mobile phase (methanol: acetonitrile: chloroform; 47: 42: 11, v/v). Chromatograms were monitored at 326, 296 and 450 nm (vitamin A, E and β-carotene, respectively). Injection volume was set 50 µL. Techsphere ODS-2 packed column (5 mm particle, 250x4.6 ID) was used and flow rate was 1.0 ml min^{-1}(22).

Statistical procedures

The results in this study are expressed as mean ± S.E. The data were analyzed with an One-Way Anova Test. SPSS 21 for Windows was utilized for statistical analysis. The level of significance was set at $p < 0.05$.

Results

The carapace length of crayfish among the experimental groups (C, EE, EC, EA, EAX, and EβC) 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 carapace length of crayfish was 48.66±1.09 mm for C, 48.84±1.19 mm for EE, 48.73±1.08 mm for EC, 48.92±0.92 mm for EA, 48.90±0,86 mm for EAX, 48.64±1.00 mm for EβC, respectively.

The levels of VE, VC, VA, AX and βC in the tissues of the crayfish fed with the control and experimental diet

The percentage of VE in hepatopancreas, ovarian, gills and muscle were found higher in the groups EE (300.00%, 2400%, 1033.33% and 566%), EC (107.14%, 700.00%, 500.00% and 333.33%), EA (200.00%, 600.00%, 600.00% and 233.33%), EAX (142.86%, 333.33%, 800.00% and 266,67) and EβC (192.86%, 600.00%, 133.33% and 233.33%) compared with control group, respectively (for each of the diets p<0.001) (Fig. 1A). However, in the peresent study was determined that VE level was statistically higher in the ovarian of the EE (40.54% of 1.85 µg g^{-1} total VE) group, in the hepatopancreas of the C (60.87% of 0.23 µg g^{-1} total VE), EC (34.52% of 0.84 µg g^{-1} total VE), EA (44.68% of 0.94 µg g^{-1} total VE), EAX (40.00% of 0.85 µg g^{-1} total VE) and EβC (51.90% of 0.79 µg g^{-1} total VE) groups according to other tissues (for each of the diets p<0.001) (Fig. 2A).

In this study, VC level in the hepatopancreas was lower for the EA diet group (11.52%), higher for the EE (140.46%), EC (267.20%), EAX (143.35%) and EβC (128.97%) diet groups according to C group (p<0.001). The percentage of VC level in ovarian were found higher in the groups EE (301.07%), EC (575.36%), EA(286.45%), EAX (183.27%) and EβC (270.22) compared with control group (for each, p<0.001) (Fig. 1B). This level was higher in the gills (EE;42.06%, EC; 51.02%, EA; 44.41% and EAX; 36.89) and muscle (EE; 45.89, EC; 111.96 and EA; 47.98) of crayfish in the some experimental diet groups compared with control group, respectively (for each, p<0.001). In addition, in this study was showed that VC level was statistically higher in the ovarian of the EA (44.59% of 225.88 µg g^{-1} total VC) and the EC (40.28% of 436.89 µg g^{-1} total VC) group, in the hepatopancreas of the C (32.23% of 127.99 µg g^{-1} total VC) and EAX (40.10% of 250.33 µg g^{-1} total VC) groups according to other tissues (for each of the diets p<0.001) (Fig. 2B). This level fed with EE and EβC diets was statistically higher than other tissues in both hepatopancreas (34.08%, of 291.03 µg g^{-1} total VC, 36.34%, of 259.09 µg g^{-1} total VC respectively) and ovarian (35.91%, of 291.03 µg g^{-1} total VC, 37.12%, of 259.09 µg g^{-1} total VC respectively) (for each of the diets p<0.001).

The VA level in hepatopancreas of crayfish fed with the diets EA was significantly higher (776.92%) than those of crayfish fed control diet (Fig. 1C).. It was found the level of the VA in ovarian of crayfish fed the diets EA and EAX were higher than the control (54.55%, 45.45% respectively), but this level in EC groups were lower (54.54%). The VA level in gills of crayfish in EC, EA, EAX and EβC group was higher than control (50.00%, 300.00%, 225.00%, 225.00% respectively). This level in the muscle of crayfish in EE, EA and EAX group were higher according to C group (75.00%, 125.00%, 75.00% respectively). Moreover, in the peresent study was determined that VE level was statistically higher in the hepatopancreas, ovarian and gills of the EβC (30.23%, 30.23%, 30.23% of 0.43 µg g^{-1} total VA, respectively) in the ovarian of the EAX (33.33% of 0.48 µg g^{-1} total VA) group, in the hepatopancreas of the C (40.63% of 0.32 µg g^{-1} total VA), EE (35.48% of 0.31 µg g^{-1} total VA), EC (33.33% of 0.21 µg g^{-1} total VA) and EA (73.08% of 0.85 µg g^{-1} total VE) groups according to other tissues (for each of the diets p<0.001) (Fig. 2C).

The percentage of AX in ovarian, gills and muscle were found higher in the groups EE (211.43%, 666.67% and 366.67%), EC (131.43%, 133.33% and 311.11%), EA (196.57%, 633.33% and 188.89%), EAX (937.14%, 333.33% and 333.33%) and EβC (268.57%, 1333.33%

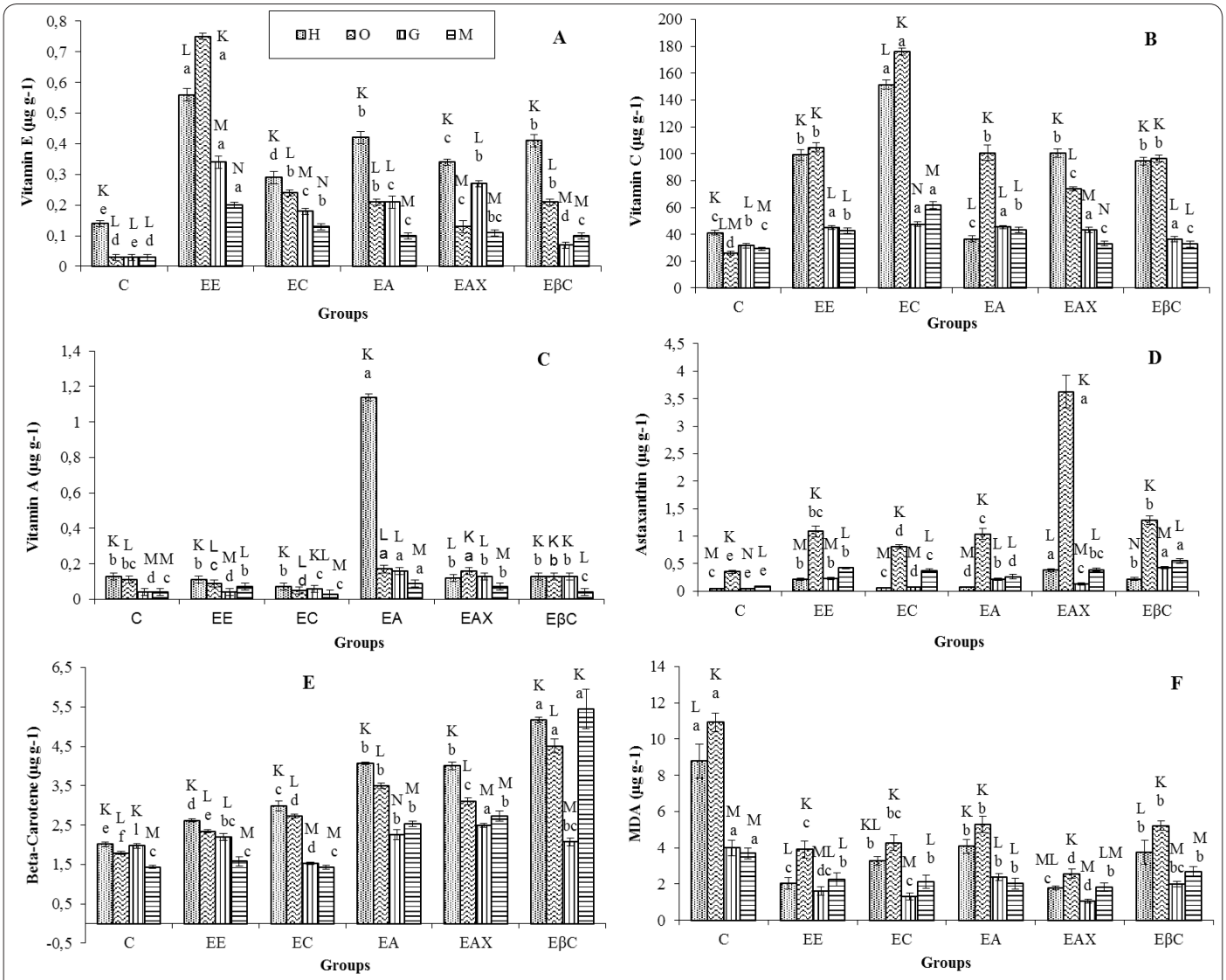

Figure 1. The comparison of the mean levels of the tested Vitamin E (A), vitamin C (B), vitamin A (C), astaxanthine (D), βeta-carotene (E) and malondialdehyde (F) according to tissues of the C and experimental (EE, EC, EA, EAX, EβC) groups (Note: a,b, c, d, e and f show the comparison in the tissues of the C and experimental groups; hepatopancreas, ovarian, gills and muscle were $p<0.001$ for each, but, only it was $p<0.01$ for MDA in muscle. K, L, M and N show the comparison of the tissues in the group; EE, EC, EA, EAX and EβC were $p<0.001$ for each group).

and 511.11%) compared with control group, respectively (for each of the diets $p<0.001$) (Fig. 1D). This level in the hepatopancreas was determined higher in the groups EE (320.00%), EC (20.00%), EAX (660.00%) and EβC (340%), lower in the EA group (80.00%) compared to control ($p<0.001$). This level was higher in the ovarian of the groups C (67.30%, of 0.52 µg g^{-1} total AX), EE (55.90%, of 1.95 µg g^{-1} total AX), EC (61.83%, of 1.31 µg g^{-1} total AX), EA (67.97%, of 1.53 µg g^{-1} total AX), EAX (80.13%, of 4.53 µg g^{-1} total AX) and EβC (51.81%, of 2.49 µg g^{-1} total AX) according to other tissues (Fig. 2D).

The level of βC in hepatopancreas and ovarian were found higher in the groups EE (30.35% and 30.73%), EC (48.76% and 52.51%), EA (101.99% and 94.97%), EAX (99.00% and 73.74%) and EβC (157.21% and 151.96%) compared with control group, respectively (for each of the diets $p<0.001$) (Fig. 1E). In addition, in the this study was determined that βC level in muscle of crayfish fed with the diets EA, EAX and EβC was significantly higher (77.62%, 90.91%, 280.42% respectively) than those of crayfish fed with the control diet. It was found that the βC level in gills of crayfish fed with the diets EA and EAX was higher than the control (14.72%, 26.90% respectively) but this level in EC group was lower (22.34%). Moreover, the present study showed that the level of βC was higher in the ovarian of the groups C (24.86%, of 7.20 µg g^{-1} total βC), EE (26.80%, of 8.73 µg g^{-1} total βC), EC (31.49%, of 8.67 µg g^{-1} total βC), EA (28.26%, of 12.35 µg g^{-1} total βC), EAX (25.20%, of 12.34 µg g^{-1} total βC) and EβC (26.24%, of 17.19 µg g^{-1} total βC) according to other tissues (Fig. 2E).

The levels of MDA in the tissues of the crayfish fed with the control and experimental diet

The percentage of the MDA level in hepatopancreas, ovarian, gills and muscle were found lower in the groups EE (76.77%, 64.23%, 59.90% and 39.02%), EC (62.53%, 60.84%, 66.92% and 42.55%), EA (53.53%, 51.51%, 40.10% and 45.26%), EAX (79.84%, 76.58%, 73.43% and 50.68%) and EβC (57.29%, 52.24%, 49.62% and 26.83%) compared with control group, respectively ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$) (Fig. 1F)..

In this study, MDA level was statistically higher in the hepatopancreas and ovarian of the groups EC, EA, in the ovarian of the groups C, EE, EAX and EβC accor-

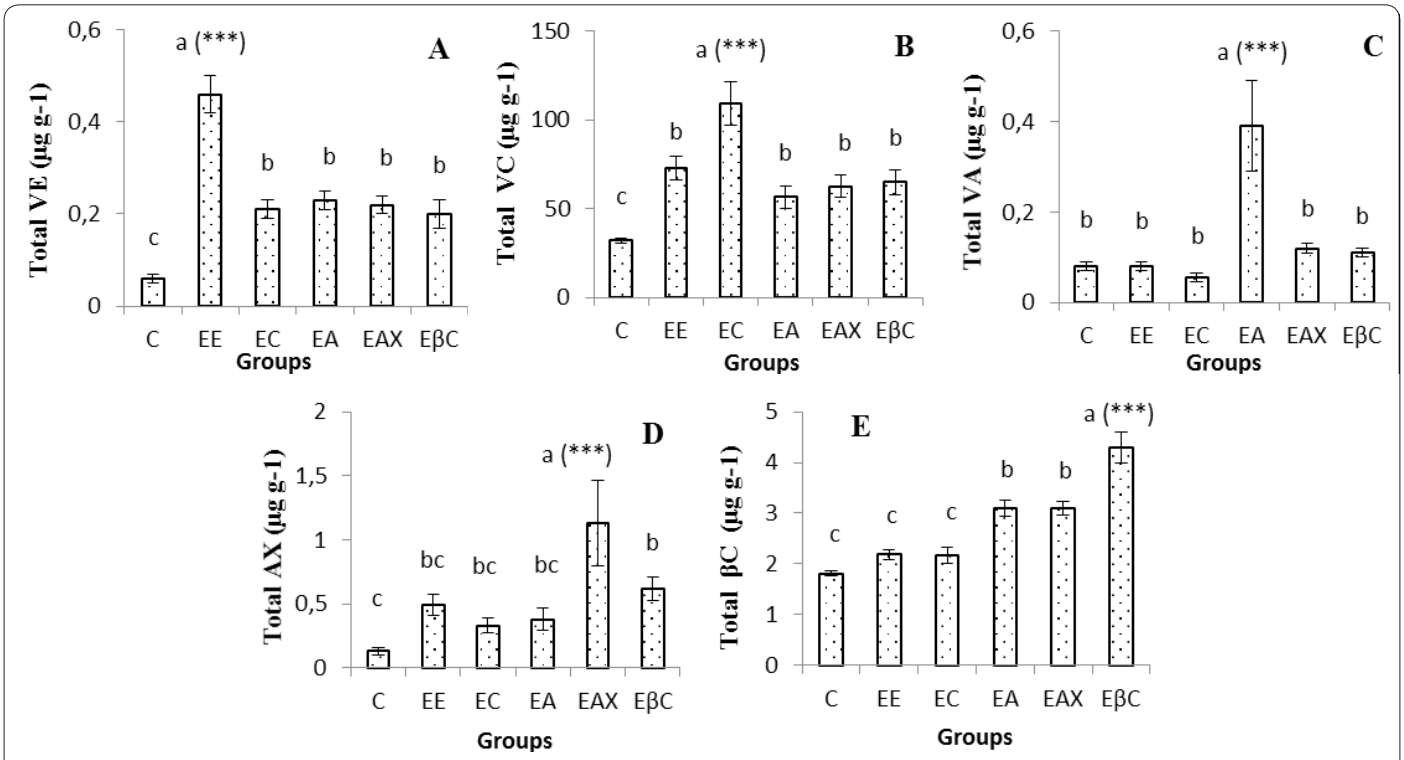

Figure 2. The comparison of the mean total levels of the tested VE (A), VC (B), VA (C), AX (D) and βC (E) according to tissues of the C and experimental groups (EE, EC, EA, EAX, EβC) (Note: ***: p<0.001).

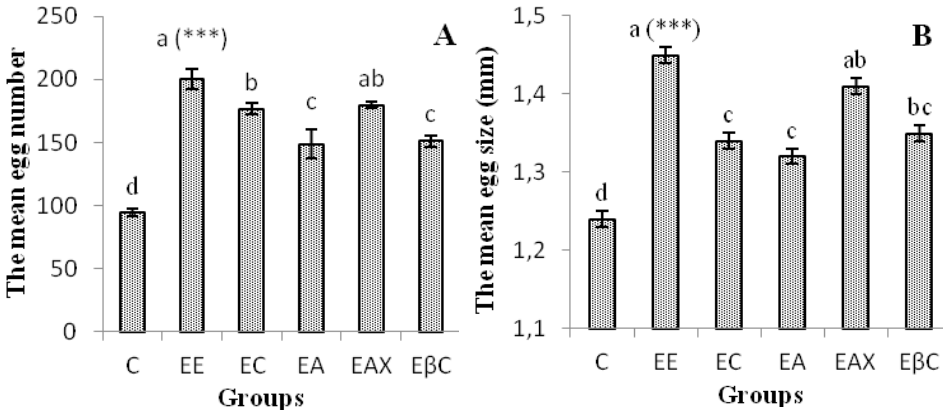

Figure 3. The comparison of the mean ovarian egg number (A) and size (B) of the C and experimental groups (EE, EC, EA, EAX, EβC) (Note: ***: p<0.001).

ding to other tissues.

The ovarian egg number and egg size of the crayfish fed with the control and experimental diet

The present study showed that the use of antioxidants in supplemental feeding gave rise to a significant increase in the number of ovarian eggs. The mean ovarian egg number of crayfish were found higher in the groups EE (111.62%), EC (86.58%), EA (56.96%), EAX (89.69%) and EβC (59,86%) compared with control group (p<0.001) (Fig. 3A). The mean egg size of crayfish was significantly bigger in the groups EE (16.94%), EC (8.06%), EA (6.45%), EAX (13.71%) and EβC (8.87%) compared with control group (p<0.001) (Fig. 3B).

Discussion

A. leptodactylus doesn't eat during the last part of the gonadal development. In addition to, this crayfish moult in September and October when gonad developments in the laboratory condition (14, 3). Previous studies showed that because the levels of nutrients in the ovarian eggs which providing the basis for reproduction is dependent on the maternal body stores, broodstock must be fed a high quality diet in the early months of gonadal maturation to improve their reproductive performance (7, 8). In respect to, in the present study, feeding was started in July and terminated in Nowember.

In this study was determined that the crayfish fed with EE, EC, EA, EAX and EβC diets had statistically highest of the total VE, VC, VA, AX and βC levels, respectively. This result indicated that the levels increased with diet supplementation of the VE, VC, VA, AX and βC can be evaluated by *A. leptodactylus*, and it can store they in the hepatopancreas, ovarian, gills and muscle. In Some researcher have reported that the use of VE in supplemental feding gave rise to increase of VE level in the liver of *S. salar* (28), in liver and muscle tissues of *S. maximus* (29), in the hepatic and muscle of *E. malabaricus* (30), in the body of *M. japonicas* (31). Some researchers showed that the use of VC resulted in

a significant increase in hepatopancreas and ovarian of *L. vannemei* (32) and in liver tissues of *M. salmoides* (33). Hu et al. (34) identified that the use of higher level (6000 IU kg^{-1}) of VA increased the level of VA in liver of juvenile hybrid tilapia, but it was't increase below 6000 IU kg^{-1}. In a different study, Hamre and Lie (28) showed that a supplemetation of dietary VE increased 5-7-fold the VE levels in whole body according to the control fish (*S.salar*). Similarly, in this study found that total VE level in the tissues of *A. leptodactylus* fed diets containing VE was statistically higher 8.04-fold than those fed C diet ($p<0.001$). In the present study, the highest levels of VE VC, VA, AX and βC were found in the hepatopancreas and ovarian compared with muscle and gills. 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. For these reason, hepatopancreas represents the status of antioxidants and OS in crustaceans (35, 5). For that matter De Vlaming et al. (36) reported that vitellogenin is synthesized in the liver under the control of estradiol produced by the follicle cells in the ovaries. In a different study, Serrano-Pinto et al., (37) observed that the hepatopancreas is the extra ovarian site of lipoprotein (i.e. vitellogenin) synthesis of *C. quadricarinatus*.

In some aquatic species was reported that the remobilisation of nutritional stores from existing tissues is required during the latter stages of gonadal development (8). Rodriguez-Gonzalez et al. (38) found continuous transfer of nutrients from the hepatopancreas to the gonad for activating and sustaining gametogenesis of female crayfish (*C. destructor*). Cahu et al. (39) determined that that the hepatopancreas and eggs of *P. indicus* fed the vitamin E supplemented diets was the storage part for VE, but this vitamin was lower in the muscle. Lie et al. (40) 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. In addition, Alava et al. (41) determined that VC level in ovarian of *P. japonicas* fed with APM-supplementation diet was higher according to hepatopancreas and muscle. Cavalli et al. (11) showed that feeding increasing VC levels to *M. rosenbergii* resulted in higher contents of this vitamin in the midgut gland and ovary, but not in the muscle. In the same time, it was found that VE level in the eggs *of M. rosenbergii* increased with the high dietary supplementation of VE, but it wasn't in the low dietary VE levels (11). In this study also found that the VE level in hepatopancreas of crayfish fed with the C, EC, EA, EAX and EβC diets was statistically highest than those of other tissues (each for $p<0.001$). But, in the peresent study was determined that VE level in crayfish fed with EE diet was statistically lowest in the muscle, and highest in the ovarian according to other tissues. In this study, VC level was statistically highest in the hepatopancreas of C and EAX diet groups, in the ovarian of EC and EA diet groups and in the hepatopancreas and ovarian of EE and EβC diet groups, and lowest in the muscle of C, EVC and EAX diet groups, in the muscle and gills of EE and EβC diet groups and in the hepatopancreas, gills and muscle of EA diet group. Reason for that, during ovarian maturation, *A. leptodactylus* may be transport VE and VC from the muscle and gills to ovaries when amount of VA and VC in the body increased.

Previous studies found that the carotenoids generally is transfer from muscle to ovary during spawning period (7, 8). Sagi et al. (42) reported that carotenoids are accumulated in the oocyte to suffice the embryonic need of *C. quadricarinatus*. In addition, Sagi et al. (42) found that the level of βC in the hepatopancreas of crayfish was higher according to muscle and ovarian, and the level of AX in the hepatopancreas of crayfish was lower according to ovarian. This findings is in agreement with the results in the present study in which the level of βC in the hepatopancreas of *A. leptodactylus* in the C, EE, EC, EA and EAX diet groups was significantly highest according to ovarian, gills and muscle, but βC level in the hepatopancreas and muscle of crayfish in the EβC diet groups was significantly highest according to ovarian and gills. Pangantihon-Kühlmann et al. (43) reported that the low deposition of AX in the hepatopancreas of the pond tiger shrimp (*P. monodon*) fed with the dietary AX supplementation may suggest transfer of this nutrient to the ovary during gonadal development. Similarly, in this study the level of AX in the ovarian of *A. leptodactylus* both experimental and C diet groups was significantly highest according to hepatopancreas, gills and muscle. The possible reason for this was rewieved by Liñán-Cabello et al. (7) that oogenesis in crustaceans 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, Crustacean eggs accumulate carotenoids in high levels for these functions during vitellogenesis. Nevertheless, Liñán-Cabello et al. (44) found that the concentration of AX, βC and VA in *L. vannamei* were higher in the digestive gland compared to the ovary during gonadic maturation. However, it was found by Sagi et al. (45) that dietary βC was transported to the ovarian after converted to AX in the hepatopancreas. In addition, Hu et al. (34) found that the level of VA in the liver of juvenile hybrid tilapia increased because dietary βC supplementation had been converted to VA. In this respect, the present study also observed that total VA level in the tissues of *A. leptodactylus* in the EAX and EβC diet groups was higher according to the tissues of crayfish in the C, EE and EC diet groups. In addition to, in this study total βC level in the tissues of *A. leptodactylus* in the EA and EAX diet groups was highest according to the tissues of crayfish in the C, EE and EC diet groups.

Mengqing et al. (46) reported that VA requirement for *P. chinensis* increases during the spawning season, because they may shift VA from their tissues to their eggs. In this study was determined that VA level in crayfish fed with C, EE, EC, EA diets was statistically highest in the hepatopancreas according to other tissues, but, in an extraordinary way, this level in ovarian of crayfish fed with EAX diet was highest than those of other tissues. In addition, VA level in crayfish fed with C, EC, EA, EAX and EβC diets was statistically lowest in the muscle according to other tissues. Liñán-Cabello et al. (32) indicated that astaxanthin bioconversion to retinoids may involve further functions during shrimp maturation. It was rewieved by Liñán-Cabello et al. (7)

that if VA in the crustaceans eggs absence, carotenoids may take their place. In addition, Liñán-Cabello et al. (44) suggest that dietary supplementation of carotenoids is important for *L. vannamei* because these molecules are need as both pigment and precursors of VA. But, in a remarkable way in this study, it was determined that the level of the VC and AX in ovarian of crayfish fed with EA diet was statistically highest according to other tissues.

Wilhelm Filho et al. (13) determined that in *P. perna,* the thiobarbituric acid reactive substances content observed in May (reproduction period) were approximately double those found in the rest of the year, and their gonads have a higher lipid, protein synthesis and carbohydrate mobilization. Palacious et al. (12) found that the level of total lipid, acylglycerides, cholesterol in mature ovaries of *Penaeus vannamei* increased. Bell et al. (10) and Cavalli et.al. (11) 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 and organ function (10, 11). Moreower, Agarwal et al. (1) reported that oxidative stress plays a role in multiple physiological processes from oocyte maturation to fertilization and embryo development. It was demonstrated by Liñán-Cabello et al. (7) that ovarian maturated was retarded when diets were deficient in any one of the VE, VA and VC. Cavalli et al. (11) suggested that the dietary supplementation of VC and VE enhanced the synthesis of egg yolk precursors by protecting the hepatic tissues from lipid peroxidation and damage to cell membranes. Several studies have repoted that level of MDA in *A. leptodactylus* decreased with dietary supplementation of VE in the hepatopancreas and muscle during pleopodal eggs development (3) and in the hepatopancreas, ovarian and muscle during ovarian maturation (4). In the present study, MDA levels in the tissues of *A. leptodactylus* fed with EE, EC, EA, EAX and EβC diets have statisticaly lowest than those fed with C diet. Agarwal et al. (1) reported that ROS may have a regulatory role in oocyte maturation and ovarian steroidogenesis. Because OS increaesed during ovarian maturation the antioxidants neutrolize ROS production and protect the oocyte. Moreover, the MDA level in the ovarian of crayfish in the all diet groups have the highest according to other tissues. It was reported that 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. For these reasons, the main cause for these differences could be the different rates of O_2^{*-}generation and different antioxidant potentials in the tissues (1, 44).

Generally, it was determined in vitro studies that the antioxidant activity of these varies according to pressure (low/high) (47, 48). Tsuchihashi et al. (49) noted that beta-carotene was 32 times less reactive toward peroxyl radical than VE. Miki (50) determined that AX are antioxidant approximately 10 times stronger than βC, and 100 times greater than those of VE. In the present study found that the use of AX in supplemental feeding lowered 1.15 times in the hepatopancreas, 1.53 times in the ovarian and 1.51 times in the gills than VE the level of MDA. Moreower, in the crayfish fed with the EAX diet was lowered 2.12 times in the hepatopancreas, 2.04 times in the ovarian and 1.90 times in the gills than EβC diet group the level of MDA.

In an early studies, the importance of vitamin E in reproduction of aquatic animal has been reported (4, 15, 16, 24, 25, 51). For example, the use of VE in supplemental feeding caused better gonad development and spawning in the *C. carpio* (52) and *C. auratus* (53). Tokuda et al. (54) found that *P. olivaceus* fed with added VE spawned eggs 1.5 times more than that of basal diet. This positive effect has been also determined in the study of Du et al. (55), who showed that fertilization rate and hatching rate of eggs of *L. vannamei* increased when the broodstock diets contained at least 350 mg kg^{-1} VE. In a different study, it was determined an increase in the number of pleopodal eggs (15, 16) and stage-1 juveniles (16) and in the number of ovarian eggs (4) for *A. leptodactylus* fed with added VE. Similarly, in this study also found that the use of 100 mg kg^{-1}VE in supplemental feeding increased the number of ovarian eggs according to C diet. But, in the some studies wasn't determined the beneficial effect of dietary vitamin E on reproduction (56, 57, 58).

Alava et al. (41) concluded that supplementation of dietary VC (at least 500 mg kg^{-1}) was necessary for promotion of ovarian maturation of *P. japonicas*. Blom and Dabrowski (59) advocated that the use of ascorbyl monophosphate resulted in a significant increase both fecundity and embryo survival of *O. mykiss*. In similar study, sperm concentration and motility of this species reduced with ascorbic acid deficiency (60). However, in different study maked by Lee and Dabrowski (61) were reported that semen quality can be increased by dietary VC supplementation during maturation. However, Du et al. (62) found that supplementation of ascorbic acid to the basal diet increased the average daily spawns per female, hatching rate and fertilization rate of *L. vannamei.* In this study also found that the use of 100 mg kg^{-1}VC in supplemental feeding increased the number of ovarian eggs according to C diet. Otherwise, the beneficial effect of dietary VC on reproduction wasn't determined in some studies (11, 32).

Several studies have confirmed that AX, βC and VA may serve some vital roles in reproduction of fish and crustaceans. Alava et. al. (41) determined that VA is necessary for development of the ovaries of *P. japonica* (7). Pangantihon et al. (43) found that tha addition of AX to *P. monodon* diet improves ovarian development. Vassallo-Agius et al. (63) demonstrate that *P. dentex* fed diets supplemented with AX before spawning had increase in egg production. Mengqing et al. (46) determined that the supplementation of VA to the diet may have positive effects on the improvement in fecundity and larval quality of *P. chinensis*. Liñán-Cabello et al. (32) found that oocyte diameter of *C. quadricarinatus* injected with βC was greater than those of crayfish injected with AX and VA. In a different study maked by Liñán-Cabello and Paniagua-Michel (64) was demonstrated that retinoids may be of great importance on the oocyte and embryonic development of *L. vannamei* when retinoic acid, retinol palmitate, βC and AX was provided during the ovarian maturation. . Ahmadi et al. (65) found that feeding increasing AX levels to *O. mykiss* showed positive

effects on the rate of fertilization. Paibulkichakul et al. (66) found that diet with 8% fish oil and 280 mg kg $^{-1}$ AX can improve reproductive performance (maturance, spawning success) in pond-reared *P. monodon* of both sexes. In the present study also showed that the use of VA, AX and βC in supplemental feeding increased the number of ovarian eggs according to C diet. The beneficial action of VE, VC, VA, AX and βC on ovarian development may find in the antioxidant properties of these matters, protecting biological membranes from oxidation and degredation by free radicals. Nevertheless, the beneficial effect of dietary AX, βC and VA supplementation of fish and crustaceans have not found in some studies (67, 68).

The only one study to compare the findings of the present study is that the effect of dietary antioxidant on the ovarian egg number and size of *A. leptodactylus* (4). Barım (4) found that the use of vitamin E (150 mg kg^{-1}) caused an increase in the ovarian egg number and size of *A. leptodactylus*. Similarly, the present study found that the use of vitamin E (150 mg kg^{-1}) in supplemental feeding caused an increase in the ovarian egg number and size.

In summary, the dietary levels of VE, VC, VA, AX and βC had been determined to affect significantly the levels of such nutrients in hepatopancreas, ovarian, gills and muscle. The highest levels of VE, VC, VA, AX and βC were found in the hepatopancreas and ovarian compared with muscle and gills. The highest levels of MDA were determined in the ovarian according to other tissues. In addition, the VE and AX in broodstock diets were the most effective antioxidants on the ovarian egg number and size of *A. leptodactylus*. In particular, the most effect antioxidat during ovarian development for this crayfish can be AX.

References

1. Agarwal A, Gupta S, Sharma RK.,Role of oxidative stress in female reproduction. Reproductive Biology and Endocrinology 2005; 3: 1-28.
2. Fang Y-Z, Yang S, Wu G. Free radicals, antioxidants, and Nutrition. Nutrition 2002; 18: 872-879.
3. Barım O. The effects of different levels of vitamin E added to the ration of freshwater crayfish (*Astacus leptodactylus* Esch. 1823) living in Keban Dam Lake. Graduate School of Natural and Applied Sciences. Department of Aquaculture PhD Thesis 2005; pp. 73.
4. Barim O. The effects of dietary vitamin E on theoxidative stress and antioxidant enzyme activities in their tissues and ovarian egg numbers of freshwater crayfish *Astacus leptodactylus* (Eschschotlz, 1823). Journal of Animal and Veterinary Advences 2009; 8 (6): 1190-1197.
5. Barim O, Karatepe M. The effects of pollution on the vitamins A, E, C, beta-carotene contents and oxidative stress of the freshwater crayfish, *Astacus leptodactylus*. Ecotoxicol Environ Saf 2010; 73: 138–142.
6. Izquierdo MS, Fernandez-Palacios H, Tacon AGJ. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 2001; 197: 25-42.
7. Liñán-Cabello MA, Paniagua-Michel J, Hopkins PM. Bioactive roles of carotenoids and retinoids in crustacean. Aquaculture Nutrition 2002; 8: 299-309.
8. Palace VP, Werner J. Vitamins A and E in the maternal diet influence egg quality and early life stage development in fish: a review. Scientia Marina 2006; 70S2: 41-57.
9. Maurente G, Bell JG, Tocher DR. Does dietary level affect fatty acid metabolism in fish?. Fish Physiol Biochem 2007; 33: 269-280.
10. Bell JG, McEvoy J, Tocher DR, Sargent JR. Depletion of α-tocopherol and astaxanthin in atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. The Journal of Nutrition (Nutrient Interaction and Toxicity) 2000; 1800-1808.
11. Cavalli RO, Batista FMM, Lavens P, Sorgeloos P, Nelis HJ, De Leenheer AP. Effect of dietary supplementation of vitamins C and E on maternal performance and larval quality of the prawn *Macrobrachium rosenbergii*. Aquaculture 2003; 227: 131-146.
12. Palacious E, Ibarra AM, Racotta IS. Tissue biochemical composition in relation to multiple spawning in wild and pond-reared *Penaeus vannamei* broodstock. Aquaculture 2000; 185: 353-371.
13. Wilhelm Filho,DV, Tribes T, Gaspari C, Claudio FD, Torres MA, Magalhaes ARM. Seasonal changes in antioxidant defenses of the digestive gland of the brown mussel (*Perna perna*). Aquaculture 2001; 203: 149-158.
14. Köksal G. *Astacus leptodactylus* in Europe. In: Freshwater crayfish: biology, management and exploitation (Holdich, D.M. and Lowery, R.S., eds), Croom Helm, London, 1988; pp. 365-400.
15. Harlıoğlu MM, Köprücü K, Özdemir Y. The effect of dietary vitamin E on the pleopodal egg number of *Astacus leptodactylus* (Eschscholtz, 1823). Aquaculture International 2002; 10(5): 391-397.
16. Harlıoğlu MM, Barım Ö. The effect of dietary vitamin E on the pleopodal egg and stage-1 juvenile numbers of freshwater crayfish *Astacus leptodactylus* (Eschscholtz, 1823). Aquaculture 2004; 236: 267-276.
17. Serrano-Pinto V, Vazquez-Boucard C, Villarreal-Colmenares H. Yolk proteins during ovary and egg development of nature female freshwater crayfish (*Cherax quadricarinatus*). Comp Biochem Physiol 2003; 134A: 33-43.
18. Harı B, Kurup BM. Vitamin C (ascorbyl 2 polyphosphate) requirement of freshwater prawn *Macrobrachium rosenbergii* (de Man). Asian Fisheries Science 2002; 15: 145-154.
19. D'Abramo LR, Conklin DE. New developments in the understanding of the nutrition of penaeid and caridean species of shrimp**.** In: Swimming through troubled water, proceedings of the special session on shrimp farming, Aquaculture'95 (Browdy, C.L. and Hopkins, J.S. eds.). World Aquaculture Society, Baton Rouge, Louisiana, USA, 1995; pp. 95-107.
20. Meyers SP, Latscha T. Carotenoids. In: Crustacean nutrition. Advances in World Aquaculture (L.R. D'Abramo, D.E. Conklin and D.M. Akiyama, eds.) 1995; 6: 164-193.
21. Cerhata D, Bauerova A, Ginter, E. Determination of ascorbic acid in blood serum using high performance liquid chromatography and its correlation with spectrophotometric (colorimetric) determination. Caska-Slov-Farm 1994; 43: 166-168.
22. Miller KW, Lorr NA, Yang CS. Simultaneous determination of plasma retinol α-tocopherol, lycopene, β-carotene, and β-carotene by high performance liquid chromatography. Analytical Biochem 1984; 138: 340-345.
23. AOAC (Association of Official Analytical Chemists). Official methods of analysis (14th Ed). Association of Official Analytical Chemists. Inc. Arlington 1984; pp. 1141.
24. Barım-Öz Ö, Beytut E, Kamiloglu NN. The effect of different levels of vitamin E in the diet on vitamins A, E, and β-carotene concentrations in the tissues of mature freshwater crayfish *Astacus leptodactylus* (Eschscholtz, 1823). Turkish Journal of Science & Technology 2011; 6 (1): 25-33.
25. Beytut E, Barim O, Kamiloglu NN. Different levels of dietary DL-α tocopheryl acetate modulate the antioxidant defence system in the hepatopancreas, gills and muscles of the freshwater crayfish,

Astacus leptodactylus (Eschscholtz, 1823). Journal of Animal and Veterinary Advances 2009; 8 (6): 1177-1184.
26. Ackefors H, Castell JD, Boston LD, Raty P, Svensson M. Standard experimental diets for crustacean nutrition research. II. Growth and survival of juvenile crayfish *Astacus astacus* (Linne) fed diets containing various amounts of protein, carbohydrate and lipid. Aquaculture 1992; 104: 341-356.
27. APHA (American Public Health Association). Standard Methods for the Examination of Water and Wastewater, 16th. Edition. Washington, DC, 1985.
28. Hamre K, Lie O. α-tocopherol levels in different organs of Atlantic salmon (*Salmo salar* L.) - Effect of smoltification, dietary levels of n-3 polyunsaturated fatty acid and vitamin E. Comp Biochem Physiol 1995; 111A (4): 547-554.
29. Tocher DR, Mourente G, Van Der Eecken A, Evjemo JO, Diaz E, Bell JG, Geurden I, Lavens P, Olsen Y. Effects of dietary vitamin E on antioxidant defence mechanisms of juvenile turbot (*Scophthalmus maximus* L.), halibut (*Hippoglossus hippoglossus* L.) and sea bream (*Sparus aurata* L.). Aquaculture Nutrition 200; 8: 195-207.
30. Lin Y-H, Shiau S-Y, Dietary vitamin E requirement of grouper, *Epinephelus malabaricus*, at two lipid levels, and their effects on immune responses. Aquaculture 2005; 248: 235-244.
31. Moe YY, Koshio S, Teshima S, Ishikawa M, Matsunaga Y, Panganiban Jr, A. Effect of vitamin C derivatives on the performance of larval kuruma shrimp, *Marsupenaeus japonicus*. Aquaculture 2004; 242: 501-512.
32. Liñán-Cabello MA, Medina-Zendejas R, Sanchez-Barajaz M, Herrera AM. Effects of carotenoids and retinol in ooctye maturation of crayfish *Cherax quadricarinatus*. Aquaculture Research 2004; 35: 905-911.
33. Chen Y-J, Yuan R-M, Lui Y-J, Yang H-J, Liang G-J, Tian L-X. Dietary vitamin C requirement and its effects on tissue antioxidant capacity of juvenile largemouth bass, *Micropterus salmoides*. Aquaculture 2015; 435: 431-436.
34. Hu C-J, Chen S-M, Pan C-H, Huang C-H. Effects of dietary vitamin A or β-carotene concentrations on growth of juvenile hybrid tilapia, *Oreochromis niloticus* x *O. aureus*. Aquaculture 2006; 253: 602-607.
35. Muriana FJ, Ruiz-Gutierriz V, Bolufer J. Phospholipid fatty acid composition of hepatopancreas and muscle from prawn, *Penaeus japonicus*. J Biochem (Tokyo) 1993; 114: 404-407.
36. De Vlaming V, Wiley HS, Delahunty G, Wallace R. Goldfish (*Carassius auratus*) vitellogenin: induction, isolation, properties and relationship to yolk proteins. Comp Biochem Physiol 1980; 67B: 613-623.
37. Serrano-Pinto V, Landais I, Ogliastro MH, Gutierrez AM, Mejia RH, Villarreal CH, Garcia GA, Vazquez BC. Vitellogenin mRNA expression in *Cherax quadricarinatus* during secondary vitellogenic at first maturation females. Mo. Reprod Dev 2004; 69: 17-21.
38. Rodriguez-Gonzalez H, Hernandez-Llamas A, Villarreal H, Saucedo PE, Garcia-Ulloa M, Rodrigoez-Jaramillo C. Gonadol development and biochemical composition of female crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae) in relation on the gonadosomatic index at first maturation. Aquaculture 2006; 254: 637-645.
39. Cahu CL, Cuzon G, Quazuguel P. Effect of highly unsaturated fatty acids, α-tocopherol and ascorbic acid in broodstock diet on egg composition and development of *Penaeus indicus*. Comp Biochem Physiol 1995; 112A (3/4): 417-424.
40. Lie O, Sandvin A, Waagbo R. Transport of alpha-tocopherol in Atlantic salmon (*Salmo salar*) during vitellogenesis. Fish Phiysiology and Biochemistry 1994; 13(3): 241-247.
41. Alava VR, Kanazawa A, Teshima S-I, Koshio S. Effect of dietary L-ascorbyl-2-phosphate magnesium on gonadal maturation of *Penaeus japonicus*. Nippon Suisan Gakkaishi 1993; 59(4): 691-696.
42. Sagi A, Rise M, Isam K Arad S(M). Carotenoids and their derivatives in organs of the maturing female crayfish *Cherax quadricarinatus*. Comp Biochem Physol 1995; 112B(2): 309-313.
43. Pangantihon-Kühlman MP, Millamena O, Chern Y. Effect of dietary astaxanthin and vitamin A on the reproductive performance of *Penaeus monodon* broodstock. Aquat Living Resour 1998; 11(6): 403-409.
44. Liñán-Cabello MA, Paniagua-Michel J, Zenteno-Savin T. Carotenoids and retinal levels in captive and wild shrimp, *Litopenaeus vannamei*. Aquaculture Nutrition 2003; 9: 383-389.
45. Sagi A, Shoukrun R, Levy T, Barki A, Hulata G, Karplus I. Reproduction and molt in previously spawned and first-time spawning red claw crayfish *Cherax quadricarinatus* females following eyestalk ablation during the winter reproductive-arrest period. Aquaculture 1997; 156: 101-111.
46. Mengqing L, Wenjuan J, Qing C, Jialin W. The effect of vitamin A supplementation in broodstock feed on reproductive performance and larval quality in *Penaeus chinensis*. Aquaculture Nutrition 2004; 10: 295-300.
47. Paola Palozza M.D. Prooxidant actions of carotenoids in biologic systems. Nutrition Reviews 1998; 56(9): 257-265.
48. Zhang P, Omaye ST. β-carotene: interactions with α-tocopherol and ascorbic acid in microsomal lipid peroxidation. Journal of Nutritional Biochemistry 2001; 12: 38-45.
49. Tsuchihashi H, Kigoshi M, Iwatsuki M, Niki E. Action of β-carotene as an antioxidant against lipid peroxidation. Archives of Biochesmistry and Biophysics 1995; 323(1): 137-147.
50. Miki W. Biological functions and activities of animal carotenoids. Pure Appl Chem 1991; 63: 141-146.
51. Barim O, Erisir M. The effect of dietary antioxidants on the arginase activity and nitric oxide level of freshwater crayfish (*Astacus leptodactylus*, Esch. 1823). Kafkas Univ Vet Fak Derg 2009; 15(5): 745-750.
52. Gupta SD, Khan HA, Bhowmick RM. Observations on the effect of vitamin E and growth hormone on the gonadal maturity of carps. Journal of the Inland Fisheries Society of India 1987; 19(2): 26-31.
53. Sutjaritvongsanon S. Level of vitamin E content suitable for gonad developing and spawning of goldfish, *Carassius auratus* (Linn.). Abstracts of Master of Science Theses Fisheries Science, Notes Fac. Fish. Kasetsart Univ. Bangkok, 1987; 12: 2.
54. Tokuda M, Yamaguchi T, Wakui K, Sato T, Ito M, Takeuchi M. Tocopherol Affinity for serum lipoproteins of Japanese flounder *Paralichthys olivaceus* during the reproduction period. Fisheries Science 2000; 66: 619-624.
55. Du S, Hu C, Cai S, Shen Q, Zheng S. Effects of different levels of dietary α-tocopherol on ovarian maturation and reproductive performance of broodstock *Litopenaeus vannamei* (Boone). Journal of Shellfish Research 2006; 25(2): 589-593.
56. King I, Hardy RW, Halver J.E. The effect of dietary vitamin E on the distribution of α-tocopherol in rainbow trout (*Salmo gairdneri*) during ovarian maturation. In: International Symposium on Salmonid Reproduction Ced. By R.N. Iwamoto and S. Sower). Washington Sea Grant Program, University of Washington, Seatle, WA. 1985; pp.111-112.
57. Eskelinen P. Effects of different diets on egg production and egg quality of Atlantic salmon (*Salmo salar* L.). Aquaculture 1989; 79: 275-281.
58. Emata AC, Borlongan IG, Damaso JP. Dietary vitamin C and E supplementation and reproduction of milkfish *Chanos chanos* forsskal. Aquaculture Research 2000; 31: 557-564.
59. Blom JH, Dabrowski K. Reproductive success of female rainbow trout (*Onchorhynchus mykiss*) in response to graded dietary ascorbyl monophosphate levels. Biology of Reproduction 1995; 52: 1073-1080.

60. Ciereszko A, Dabrowski K. Sperm quality and ascorbic acid concentration in rainbow trout semen are affected by dietary vitamin C: an across-season study. Biology of Reproduction 1995; 52: 982-988.
61. Lee K-J, Dabrowski K. Long-term effects and interactions of dietary vitamins C and E on growth and reproduction of yellow perch, *Perca flavescens*. Aquaculture 2004; 230: 377-389.
62. Du S, Hu C, Shen Q. Effect of dietary ascorbic acid levels on reproductive performance of shrimp, *Litopenaeus vannamei*, broodstock. Journal of Shellfish Research 2004; 23: 251-255.
63. Vassallo-Agius R, Imaizumi H, Watanabe T, Yamazaki T, Satoh S, Kiron V. The influence of astaxanthin supplemented dry pellets on spawning of striped jack. Fisheries Science 2001; 67: 260-270.
64. Liñán-Cabello MA, Paniagua-Michel J. Induction factors derived from carotenoids and vitamin A during the ovarian maturation of *Litopenaeus vannamei*. Aquaculture International 2004; 12: 583-592.
65. Ahmadi MR, Bazyar AA, Safi S, Ytrestoyl T, Bjerkeng B. Effects of dietary astaxanthin supplementation on reproductive characteristics of rainbow trout (*Oncorhynchus mykiss*). J Appl Ichthyol 2006; 22: 388-394.
66. Paibulkichakul C, Piyatiratitivorakul S, Sorgeloos P, Menasveta P. Improved maturation of pond-reader, black tiger shrimp (*Penaeus monodon*) using fish oil and astaxanthin feed supplements. Aquaculture 2008; 282: 83-89.
67. Christiansen R, Torrissen OJ. Effects of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon (*Salmo salar* L.). Aquaculture 1997; 153: 51-62.
68. Choubert G, Blanc J-M, Poisson H. Effects of dietary keto-carotenoids (canthaxanthin and astaxanthin) on the reproductive performance of female rainbow trout *Oncorhynchus mykiss* (Walbaum). Aquaculture Nutrition 1998; 4: 249-254.