www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 16: 283-288 (2016) DOI: 10.4194/1303-2712-v16_2_08

RESEARCH PAPER

The Effects of Dietary Antioxidants on the Arginase Activity and Nitric Oxide Level of Narrow-Clawed Turkish Crayfish (*Astacus leptodactylus*, Esch. 1823) in Moulting Period

Fulya Benzer[1], Mehtap Özçelik[2], Nuran Cikcikoglu Yildirim[3,*]

[1] Tunceli University, Faculty of Engineering, Department of Food Engineering, Tunceli, 62000, Turkey.
[2] Vocational School of Health Services, University of Firat, 23119 Elazig, Turkey.
[3] Tunceli University, Faculty of Engineering, Department of Environmental Engineering, Tunceli, 62000, Turkey.

* Corresponding Author: Tel.: +90.530 3649999; Fax: ;
E-mail: nurancyildirim@tunceli.edu.tr

Received 23 November 2015
Accepted 25 March 2016

Abstract

The effects of dietary antioxidants (vitamine E, vitamine C, Astaxhantin, β carotene) on arginase activity and nitric oxide levels of hepatopancreas and muscle of freshwater crayfish *(Actacus leptodactylus)* were investigated during moulting.150 mg kg^{-1} vitamine E (VE), 200 mg kg^{-1}vitamine C (VC), 200 mg kg^{-1} Astaxhantin and 200 mg kg^{-1} β carotene were added to experimental diets. *A. leptodactylus* with these diets were fed with 2% of their total weight daily, in a day during 75 days. Arginase activity was increased in muscle of crayfish fed the diets VC, VE, astaxanthin and β karoten but in hepatopancreas arginase activity was increased with β carotene when compared to control. NO levels in muscle were decreased when compared to control but this decrease was not statically significant. NO levels in hepatopancreas of crayfish fed the diets VC and astaxanthin were higher than control groups. Our results demonstrated that dietary antioxidants affect the arginase activity and nitric oxide level of freshwater crayfish (Astacus leptodactylus, Esch. 1823) during moulting.

Keywords: *Actacus leptodactylus,* Antioxidants, Arginase , Moulting Period, Nitric oxide.

Kabuk Değişim Döneminde İnce Kıskaçlı Türk Kereviti (*Astacus leptodactylus*, Esch. 1823)'nin Arjinaz Aktivitesi ile Nitrik Oksit Seviyesi Üzerine Diyet Antioksidanların Etkileri

Özet

Diyet antioksidanları (vitamin E, vitamin C, astaxantin, β karoten)'nın tatlı su kereviti *(Actacus leptodactylus)* kas ve hepatopankreası arjinaz aktivitesi ile nitrik oksit seviyeleri üzerine etkileri kabuk değişimi döneminde araştırılmıştır. Deneysel diyetlere 150 mg kg^{-1}vitamin E, 200 mg kg^{-1}vitamin C, 200 mg kg^{-1} Astaxhantin and 200 mg kg^{-1} β karoten eklendi. *A. leptodactylus* 75 gün boyunca bu diyetler ile toplam ağırlığının 2% oranında beslendi. Kontrol ile kıyaslandığında Arjinaz aktivitesi kerevit kasında VC, VE, astaxanthin ve β karoten ile artmıştır ancak hepatopankreasda arjinaz aktivitesi β karotene ile artmıştır. Kas NO seviyeleri kontrolle kıyaslandığında azalmıştır ama bu azalış istatistiksel olarak önemli değildir. VC ve astaxanthin ile beslenen kerevit hepatopankreasından NO düzeyleri kontrol gruplarına göre daha yüksek bulunmuştur. Sonuçlarımız diyetsel antioksidanların kerevitlerin kabuk değiştirme döneminde arjinaz aktiviteleri ve NO düzeylerini değiştirdiğini kanıtlamıştır.

Anahtar Kelimeler: *Actacus leptodactylus,* Antioksidanlar, Arjinaz, Kabuk değiştirme dönemi, Nitrik oksit..

Introduction

Crustaceans are generally used as bioindicators in various aquatic systems (Borkovid *et al*., 2007). Freshwater crayfish *A. leptodactylus*, indigenous crayfish species of Europe have been harvested commercially in Turkey since the 1960s (Rahe and Soylu 1989). The only native crayfish species in Turkey*, A. leptodactylus* is widely distributed in lakes and ponds in many parts of the country. It is also known as Turkish, Galicia, swamp or pond crayfish (Harlioglu, 2004). Moulting occurs in all arthropods, from insects to crustaceans, it is essential for growth, reproduction and metamorphosis. Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) catalyses the hydrolysis of L-arginine to form L-ornithine and urea in the final reaction of the urea cycle (Powers and Meister,1982). Arginase enzyme is also found in organs and organisms not synthesizing the urea (Aminlari and Vaseghi, 1992). Arginase activities were measured in the hepatopancreas of 15 species of marine invertebrates and the highest arginase activities have been found in the Crustacea (Hanlon, 1975). Although crayfish are ammonotelic organisms

© Published by Central Fisheries Research Institute (CFRI) Trabzon, Turkey
in cooperation with Japan International Cooperation Agency (JICA), Japan

and do not contain an active urea cycle, they have an active arginase enzyme (Erişir *et al*., 2006). Nitric oxide is produced from arginine by nitric oxide synthase (NOS) (Beckman, 1996). Arginine is the sole substrate of NOS, but it participates in several other pathways that appear to be competitive with NOS (Wu and Morris, 1998). Arginase and NOS interactions are complex. Arginase reacts with the amino acid much more rapidly than with iNOS. Although the requirement for arginine by iNOS is much lower than it is for arginase, variations in extracellular concentrations of arginine affect NOS activity. Thus arginine metabolism by arginase may be a control mechanism for NOS (Broucher *et al*., 1999). In aquatic vertebrates it was established that feeding, hormonal, seasonal and environmental (temperature, moisture, salinity, water deprivation etc.) conditions alter arginase activity (Erisir *et al*., 2006). Food conversion, and hence growth, depend greatly on food intake (Huisman., 1976) and in both teleosts and ureotelics feeding is an important factor affecting arginase activity (Cvancara., 1969). Vitamin E provides homeostasis in living cells. Several studies have indicated that there are relationships between vitamin E and arginase, and between arginase and selenium (Erisir *et al*., 2003). The determined amounts of vitamin E have been supplemented to diets to increase pleopodal egg number in crayfish, to modulate the antioxidant defense system and to provide optimum growth in prawn (Harlioglu and Barim., 2004, Dandapat *et al*., 2000, He and Lawrence, *et al*., 1993).

A. leptodactylus is a native freshwater crayfish species in Turkey. It is widely distributed in lakes, ponds 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 *A. leptodactylus* after 1985 decreased dramatically (from 5000 tons annually to 200 tons) in most Turkish lakes as a result of the crayfish plague, over-fishing, water pollution and water withdraws for agricultural irrigation (Harlioglu and Barim, 2004; Barim, 2005). 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 *A. leptodactylus* populations are require for this good quality diet (Barim, 2009).

This study was designed to investigate whether dietary antioxidants affect the arginase activity and nitric oxide level of freshwater crayfish (*A. leptodactylus*, Esch. 1823) or not.

Materials and Methods

Experimental Protocol

This study was carried out at the aquarium laboratory of Fırat University, Aquaculture Faculty, Elazığ, Turkey. The male crayfish used in the present study was provided from Keban Dam Lake population of *A. leptodactylus.*

Crayfish were housed in 15 glass aquariums (25x25x110 cm). Plastic pipes (15 cm in length and 7cm in diameter) were provided as shelters for the crayfish. Adequate aeration was provided for each aquarium by a simple air pump. *A. leptodactylus* were acclimatized 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. Triplicate groups of crayfish (9 individuals per group) were randomly assigned to each feeding treatment. The carapace length (mm) and weight (g) were recorded for each crayfish. The weight and carapace length of crayfish; Control group (carapace length : 42,75±0,41 mm, weight: 17,69±0,68 g), Vitamin E group (carapace length : 42,38±0,50 mm, weight : 17,18±0,70 g), Vitamin C group (carapace length: 43,13±0,29 mm, weight: 17,69±0,76 g), Astaxanthin group (carapace length: 43,50±0,42 mm, weight :17,19±0,40 g), β carotene group (carapace length : 42,75±0,37 mm, weight :16,53±0,61 g). Crayfish were fed 2% of their total wet weight daily, divided into three separate feedings (Harlıoğlu and Barim, 2004).

At the end of the research, 9 crayfish were taken from each group of five ration. For biochemical assays, the hepatopancreas and muscle in the crayfish were removed and were stored at -80°C until used.

Experimental Diets

The practical control diet used in this study (Table 1) was originated according to Barım., 2005. The raw nutrient levels of control diet were formulated according to Weende analysis method (AOAC., 1984). Levels of dietary VE (150 mg kg^{-1}) (Barim *et al*., 2005), VC (200 mg kg^{-1}) (conldin *et al*., 1995, Hari *et al*., 2002), VA (240 mg kg^{-1}) (Conldin *et al*., 1995), ASX (200 mg kg^{-1}) (D'abrama and Conklin *et al*., 1995, Meyers and Latscha *et al*., 1995) and βC (200 mg kg^{-1}) (D'abrama *et al*., 1995, Meyers and Latscha *et al*., 1995) were set in relation to levels reported by other researchers in a variety of crustacean species. The VE, VC, VA, ASX and βC concentarions of the ration were determined by using HPLC (Conklin., 1995; Miller *et al*., 1984). The VE, VC, ASX and βC contents of the control were 7.27±2.09 mg kg^{-1}, 34.12±2.01 mg kg^{-1}, 2.05±0.13 mg kg^{-1}, 26.37±0.12 mg kg^{-1}, respectively. In all groups supplemented antioxidants , levels of 139.05±3.27 mg kg^{-1} VE, 174.93±4.28 mg kg^{-1} VC, 192.13±5.25 mg kg^{-1} ASX and 169.25±1.25 mg kg^{-1} βC diet were determined for VE, VC, VA, ASX and βC diets, respectively by HPLC. No VE, VC, VA,

Table 1. Arginase activity and NO levels of hepatopancreas and muscle tissues

	Groups				
	Astaxanthin	Vitamin C	β carotene	Vitamin E	Control
Tissues	Arginase activity				
Muscle	5.15±0.59[ab]	6.77±1.76[a]	6.17±1.25[a]	8.50±1.80[a]	2.53±0.35[b]
Hepatopacreas	0.75±0.19[b]	0.76±0.03[b]	2.15±0.20[a]	1.26±0.31[b]	0.90±0.17[b]
	Nitric Oxide Level				
Muscle	9.49±1.04[a]	9.72±0.31[a]	9.34±0.56[a]	8.94±0.93[a]	10.99±0.98[a]
Hepatopacreas	9.73±1.26[a]	12.4±2.42[a]	4.54±0.32[b]	4.54±1.72[b]	5.54±0.63[b]

Different letters in same line indicate statistically importance according to Duncan's Multiple Range test ($P<0.05$)

ASX and βC was added to the control diet, except that supplied by the feed ingredients. Dietary VE (50% dl-α-tocopheryl acetate), VC (33% L-ascorbic acid monophosphate), VA (1000000 IU per gram retinyl acetate), ASX (8% astaxanthin, Carophyll Pink,) and βC (10% β-Carotene) was donated by DSM.

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 (Harlıoğlu and Barim , 2004) 0.10 Antioxidant (Erisir *et al*, 2006) 0.10 Vitamin E, A, C-free vitamin premix, 0.50 Mineral premix (aminleri *et al*., 1992) 0.18.

Proximate Composition

Crude protein 38.86 Crude fat 8.02 Crude fiber 3.02 Crude ash 14.17 Nitrogen free extract 28.93 Moisture 7.00 Gross energy (kcal/g) 3.32.

1 Kavilamycine **2** Antioxidant (mg/kg dry diet): butylated hydroxytoluene 12.5 **3** Vitamin premix (mg/kg): Menadion 600, Riboflavin 1200, Pridoxin 1000, Cobalamin 3, Niacin 5000, Biotin 8, Folic acid 200, Colin clorid 60, Calcium D-Pantothenate 1600 Calsiferol 400000 **4** Mineral premix (mg/kg dry diet): Mn 80, Fe 35, Zn 50, Cu 5, I 2, Co 0.4, Se 0.15

Sample Preparation and Biochemical Assays

The tissues were weighed and homogenized with 10 volumes of 10 mM Tris-HCl buffer pH (7.4) in a glass Potter Elvehjem homogenizer in an ice bath. The homogenates were centrifuged at 20.000 x g for 10 min at 4ºC. The supernatants were used for the arginase and NO assay.

Arginase Activity Assay

Arginase activity was measured spectrophotometrically in the optimized conditions for crayfish Hartenstein 1971 by the thiosemicarbazide diacetylmonoxime urea (TDMU) method of Geyer and Dabich gever 1971 one unit of arginase activity was expressed as the amount of enzyme catalyzing the formation of one µmole of urea h^{-1} at 37ºC. The results are given as units/mg of protein. Protein was measured by the method of Lowry (Lowry *et al*, 1951) using bovine serum albumin as standard.

NO Levels Assay

NO leves were measured according to Griess method. NO measurement is very difficult in biological specimens, because it is easily oxidized to nitrite (NO_2) and subsequently to nitrate (NO_3) which serve as index parameters of NO production. Samples were initially deproteinized with NaOH and ZnSO4. Total nitrite (NO_2+NO_3) was measured by spectrophotometer at 545 nm after conversion of NO_2 to NO_3 by assay reactive. A standard curve was established by a set of serial dilutions of sodium nitrite. Results were expressed as µmol per gram tissue (Lyall *et al*., 1995).

Statistical Procedures

Results were expressed as mean±SE. Analysis of variance (ANOVA) followed by Duncan test was used to determine whether there were significant differences among the groups. The 5% level of significance was used to establish differences.

Results

Arginase activity and NO levels of hepatopancreas and muscle tissues were shown in Table 1. The arginase activity in muscle of crayfish fed the diets VE, VC and βC were significantly higher than Control group but in NO levels no statistically significant differences were found in muscle of crayfish fed the diets between VE, VC, astaxanthin, βC and control (Table 1). It was found that the arginase activity in hepatopancreas of crayfish fed the diets βC was higher than the control but the crayfish fed astaxanthin and vitamine C were lower than control group. NO levels in hepatopancreas of crayfish fed the diets VE, astaxanthin were increased but this increase was insignificant in vitamin E group.

Discussion

Moulting is a complex process that is affected by

a range of external factors such as temperature, photoperiod, nutrition and eyestalk ablation (Kuballa and Elizur, 2007). Vitamins are organic compounds required in small quantities in diet of fish and crustacean. Because some food substance, especially VE, VA and carotenoids, cannot be synthesized by crustacean, these substances must be added in food (Palace *et al*., 2006). Vitamin E (VE), vitamin C (VC), vitamin A (VA), astaxanthin (ASX) and β-carotene (βC) have been supplemented to diet to increase the reproduction, to modulate the antioxidant defense system and to provide the optimum growth in aquatic organism (Conklin, 1995; Palace and Werner 2006; Harlıoglu and Barim; 2004). The reduction or elevation observed in arginase activity with dietary intake of the vitamins may affect connective tissue formation in tissues. Likewise, it has been known that the presence of sufficient ascorbic acid (vitamin C), a required cofactor for prolylhydroxylase, thus requires the formation of stable collagen (Libby *et al*., 2002). Several studies indicated that there was a relationship between vitamins and arginase (Jekinson *et al*., 1996, Park *et al*., 1991). Erisir *et al*.(2003) investigated the effects of dietary vitamin E and selenium on arginase activity in the liver, kidneys, and heart of rats treated with high doses of glucocorticoid. They found that vitamin E and Se in combination may prevent the changes in arginase activity in various tissues caused by prednisolone. It was also observed that vitamin E supplementation markedly decreased arginase activity in the liver. Barim *et al*. (2009) investigated the effect of dietary antioxidants on the arginase activity and nitric oxide level of freshwater crayfish (*Astacus leptodactylus*, Esch. 1823). No significant differences in arginase activity and NO levels in gill were observed among diets. The arginase activity and NO level in hepatopancreas, muscle and ovarian were changed by dietary supplements of antioxidants. Erişir et. al (2006) investigated the effect of dietary vitamin E on the arginase activity in the females of freshwater crayfish (*Astacus leptodactylus*, Esch. 1823). It was concluded that the presence of vitamin E higher than 150 mg kg^{-1} in ovigerous crayfish and 100 mg kg^{-1} in females with $stage^{-1}$ juveniles in diets may negatively affect connective tissue formation by decreasing muscle arginase activity. Benzer et. al., 2004 investigated the effects of vitamin E and selenium on arginase activity in cypermethrine administered rats. Vitamin E, selenium, vitamin E+selenium combination increased the activity of arginase in several tissues in the same way. Barim *et al*. 2009 determined the effects of vitamin E, C, astaxanthin and β- carotene on oxidative stress in some tissues of freshwater crayfish (*Astacus leptodactylus* esch. 1823) in moulting period. They showed that the supplemental of VE, VC, AX ve βK in diet of *A. leptodactylus* decreased malondialdehyde levels in the hepatopancreas, gonad, muscle and gills tissues. However, SOD, CAT, GSH-Px activity and GSH level changed according to tissue specific.

Carbamoyl phosphate synthetase and ornithine transcarbamoylase from urea cycle enzymes were shown to be absent in crustaceans (Hird *et al*., 1986). In addition, crustaceans do not only have an active arginase enzyme but also the enzymic capacity to convert ornithine (the second reaction product of arginine hydrolysis) to proline (Hird *et al*., 1986; Sisini *et al*., 1981). Proline is a fundamental structural element of collagen. Animals such as the earthworm, starfish and mussel evolving to use increased amounts of collagen synthesise proline from the ornithine moiety of arginine (Hird *et al*., 1986; Hird *et al*., 1983). Arginine is the sole substrate of NOS (Wu and Morris, 1998). Arginase and NOS interactions are complex. The arginase (AR) enzymes (ARI and ARII) convert L-Arg into urea and L-ornithine, precursors for polyamines and L-proline compounds, which are vital to tissue homeostasis and wound repair (Gobert *et al*., 2004, Janne *et al*., 1991). Arginase competes with inducible NOS (iNOS; NOS2), the high-output, inducible pathway for increased production of NO, for L -Arg, their common substrate in multiple cell types including endothelial cells. In cases in which L -Arg is limited, NO levels can fall, and this may be the result of enhanced AR activity (Chang *et al*., 1998).

As a result of this study, arginase activity was increased in muscle of crayfish fed the diets VC, VE, astaxanthin and β karoten but in hepatopancreas arginase activity was decreased with astaksantin and vitamine C. NO levels in muscle was decreased VE when compared to control but this decrease was not statically significant. NO levels in hepatopancreas of crayfish fed the diets VC and astaxanthin were higher than control groups. Our results demostrated that the responses of the vitamins to arginase activity and NO levels were different according to the tissue.

References

Aminlari, M. and Vaseghi, T. 1992. Arginase distribution in tissue of domestic animals, Comparative Biochemistry and Physiology B, 103: 385-389. doi: 10.1016/0305-0491(92)90309.

AOAC (Association of Official Analytical Chemists), (1984). Official methods of analysis (14th Ed), Association of Official Analytical Chemists. Inc. Arlington p:1141.

Barim, O. 2005. The effects of different levels of vitamin E added to the ration of freshwater crayfish (*Astacus leptodactylus* Esch. 1823) living in Keban Dam Lake. PhD Thesis.

Firat University Graduate School of Natural and Applied Sciences. Department of Aquaculture, p. 73.

Barim, O. 2009. The Effects of Dietary Vitamin E on the Oxidative Stress and Antioxidant Enzyme Activities in Their Tissues and Ovarian Egg Numbers of Freshwater Crayfish *Astacus leptodactylus* (Eschscholtz, 1823), Journal of Animal and Veterinary Advances, 8:1190-1197. doi: javaa.2009.1190.1197

Barim, O. and Erisir, M. 2009. The Effect of Dietary Antioxidants on the Arginase Activity and Nitric Oxide

Level of Freshwater Crayfish (*Astacus leptodactylus*, Esch. 1823), Kafkas Universitesi Veteriner Fakültesi Dergisi, 15: 745-750. doi:10.9775/kvfd.2009.098-A

Beckman, J.S. 1996. The physiological and pathological chemistry of nitric oxide. In: J.Lancaster (Ed.), Nitric oxide principles and actions. San Diego: Academic Press,7.

Benzer, F. and Yilmaz, S. 2004. The Effects Vitamine E and Selenium on Arginase Activity in Cypermethrin Administered Rats, Kafkas Universitesi Veteriner Fakültesi Dergisi, 10:195-198. doi:2004/61-A

Boucher, J.L., Moali, C. and Tenu, J.P. 1999. Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for L-arginine utilization, Cellular and Molecular Life Sciences, 55:1015. doi: 10.1007/s000180050352

Chang, C.I., Liao, J.C. and Kuo, L. 1998. Arginase modulates nitric oxide production in activated macrophages, The American Journal of Physiology - Heart and Circulatory Physiology, 274: 342-348. doi: 0363-6135/98

Chiu, Y.N., Austic, R.E. and Rumsey, G.L. 1986. Urea cycle activity and arginine formation in rainbow trout (*Salmo gairdneri*), Journal of Nutrition, 116: 1640-1650.

Conklin, D.E., (1995). Vitamins. In: Crustacean nutrition. Advances in World Aquaculture. (L.R. D'Abramo, D.E. Conklin and D.M. Akiyam, eds.) 6:123-149.

Cvancara, V.A. 1969. Studies on tissue arginase and ureagenesis in fresh-water teleosts, Comperative Biochemistry and Physiology, 30: 489-496. doi:10.1016/0010-406X(69)92018-0

Dandapat, J., Chainy, G.B. and Rao, K.J. 2000. Dietary vitamin-E modulates antioxidant defence system in giant freshwater prawn, *Macrobrachium rosenbergii*, Comparative Biochemistry and Physiology-Part C: Toxicology & Pharmacology, 127: 101-115. doi:10.1016/S0742-8413(00)00132-8

D'abramo, L.R. and Conklin, D.E. 1995. Swimming through troubled water. Proceedings of the special session on shrimp farming. In, Browdy CL, Hopkins JS (Eds): New Developments in the Understanding of the Nutrition of Penaeid and Caridean Species of Shrimp. 95-107, Aquaculture'95, World Aquaculture Society, Baton Rouge, Louisiana, USA, 1995.

Erisir, M,, Beytut , E., Ozan S and Aksakal, M. 2003. Effects of dietary vitamin E and selenium on arginase activity in the liver, kidneys, and heart of rats treated with high doses of glucocorticoid, Cell Biochemistry and Function, 21: 331–335. doi: 10.1002/cbf.1032

Erisir, M., Barim, O., Özcelik, M. and Harlioglu, M. 2006. The Effect of Dietary Vitamin E on the Arginase Activity in the Females of Freshwater Crayfish (*Astacus leptodactylus*, Esch. 1823), Turkish Journal of Veterinary and Animal Sciences, 30 :195-199.

Geyer, J.W. and Dabich, D. 1971. Rapid method for determination of arginase activity in tissue homogenates, Analytical Biochemistry, 39: 412-417. doi:10.1016/0003-2697(71)90431-3

Gobert, A.P., Cheng, Y., Akhtar, M., Mersey, B.D., Blumberg, D.R., Cross, R.K., Chaturvedi R, Drachenberg, C.B., Boucher, J.L., Hacker, A., Casero Jr, R.A. and Wilson, K.T. 2004. Protective role of arginase in a mouse model of colitis, The Journal of Immunology, 173: 2109-2117. doi: 10.4049/ jimmunol.173.3.2109

Hanlon, D.P. 1975. The distribution of arginase and urease in marine invertebrates, Comparative Biochemistry and Physiology B, 52: 261-264. doi:10.1016/0305-0491(75)90062-0

Hari, B. and Kurup, B.M. 2002. Vitamin C (ascorbyl 2 polphosphate) requirement of freshwater prawn *Macrobrachium rosenbergii* (de Man), Asian Fisheries Science, 15, 145-154.

Harlioglu, M.M., K.PR.C., K., Ozdemir, Y. 2002. The effect of dietary vitamin E on the pleopodal egg number of *Astacus leptodactylus* (Eschscholtz, 1823), Aquaculture International, 10:391-397. doi:10.1023/A:1023376705651

Harlioglu, M.M. and Barim, O. 2004. The effect of dietary vitamin E on the pleopodal egg And stage-1 juvenile numbers of freshwater crayfish *Astacus leptodactylus* (Eschscholtz,1823),Aquaculture, 236: 267-276. doi:10.1016/j.aquaculture.2004.01.022

Hartenstein, R. 1971. Charecteristics of arginase from the freshwater crayfish, *Cambarus bartoni*, Comparative Biochemistry and Physiology B, 40:781-795. doi:10.1016/0305-0491(71)90152-0

He, H. and Lawrence, A.L. 1993. Vitamin E requirement of *Penaeus vannemei*, Aquaculture, 118: 245. doi:10.1016/0044-8486(93)90460-G

Hird, F.J., Cianciosi, S.C., Mclean, R.M. and Niekrash, R.E. 1983. On the possible significance of the transamidination reaction in evolution, Comparative Biochemistry and Physiology B, 76: 489-495.doi:10.1016/0305-0491(83)90281-X

Hird, FJ, Cianciosi, S.C. and Mclean, R.M. 1986. Investigations on the origin and metabolism of the carbon skeleton of ornithine, arginine and proline in selected animals, Comparative Biochemistry and Physiology B, 83: 179-84. doi:10.1016/0305-0491(86)90350-0

Huisman, E.A. 1976. Food conversion efficiencies at maintenance and production levels for carp, *Cyprinus carpio* L., and rainbow trout, *Salmo gairdneri* Richarson, Aquaculture 9: 259-273. doi:10.1016/0044-8486(76)90068-5

Janne, J., Alhonen, L., Leinonen, P. 1991. Polyamines: from molecular biology to clinical applications, Annals of Medicine, 23: 241-259. doi:10.3109/07853899109148056

Jenkinson, C.P., Grody, W.W., Cederbaum, S.D. 1996. Comparative properties of arginases, Comparative Biochemistry and Physiology B, 114, 107132. doi:10.1016/0305-0491(95)02138-8

Kuballa, A. and Elizur, A. 2007. Novel molecular approach to study moulting in crustaceans, Bulletin of Fisheries Research Agency, 20: 53-57.

Libby, P. and Aikawa, M. 2002. Vitamin C, collagen, and cracks in the plaque, Circulation 105, 1396-1398. doi:10.1161/01.CIR.0000012513.58079.EA

Lyall, F., Young, A. and Greer, I.A. 1995. Nitric oxide concentrations are increased in the fetoplacental circulation in preeclampsia, American Journal of Obstetrics & Gynecology, 173: 714-718. doi:10.1016/0002-9378(95)90328-3

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurements with the folin phenol reagent, Journal of Biological Chemistry, 193: 265-275.

Meyers, S.P. and Latscha, T. 1995. Carotenoids. In, D'Abramo LR, Conklin DE, Akiyam DM (Eds): Crustacean Nutrition 6: 164-193.

Miller, K.W., Lorr, N.A. and Yang, C.S. 1984.

Simultaneous determination of plasma retinol α-tocopherol, lycopere, β- carotene, and βcarotene by high performance liquid chromatography, Analytical Biochemistry, 138:340-345.

Powers, G.S., and Meister, T. 1982. Urea synthesis and ammonia metabolism.. In: Arias I, Popper H, Schachter D, Shafrits DA Ed., the Liver: Biology and Pathobiology Raven Pres, New York, 251-263.

Palace, V., and Werner, J. 2006. Vitamins A and E in the maternal diet influence egg quality and early life stage development in fish: A. review, Scientia Marina, 70S2, 41-57. doi:10.3989/scimar.2006.70s241

Park, J.R. and Tappel, A.L. 1991. Protein damage and lipid per-oxidation: Effects of diethyl maleate, bromotrichloromethane and vitamin E on ammonia, urea and enzymes involved in ammonia metabolism, Toxicology Letters, 8: 29-36. doi:10.1016/0378-4274(91)90187-B

Rahe, R. and Soylu, E. 1989. Identification of the pathogenic fungus causing destruction to Turkish crayfish stocks (*Astacus leptodactylus*), Journal of Invertebrate Pathology, 54: 10-15.

Sisini, A., Pinna, G.G.and Virdis-usai, R. 1981. Arginase characteristics in an ammoniotele, Bollettino- Societa Italiana Biologia Sperimentale, 57: 1510-1516.

Wu, G., and Morris, S.M. 1998. Arginine metabolism: nitric oxide and beyond, Biochemical Journal, 338:1