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EFFECT OF ADDING NANO-SELENIUM, VITAMIN E AND THEIR MIXTURE TO THE DIET ON THE PRODUCTIVE AND PHYSIOLOGICAL TRAITS OF COMMON CARP FISH (*CYPRINUS CARPIO* L.)

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Abstract

This study was conducted in the laboratories of the College of Agriculture, University of Basrah for 60 days. This study aimed to study the effect of adding nano-selenium, vitamin E and their mixture to the diet on the productive and physiological traits of common carp fish (*Cyprinus carpio* L.). The fish were divided into four groups according to the feeding system, namely: control treatment and nano-selenium treatment at a concentration of (1 $mg.kg^{-1}$ feed), vitamin E treatment with a concentration of (500 $mg.kg^{-1}$ feed), and their mixture (nano-selenium with a concentration of (1 $mg.kg^{-1}$ feed) + vitamin E at a concentration of (500 $mg.kg^{-1}$ feed)). The following traits were studied: FCR, SGR, D.G.R, albumin, total protein, Globulin, A / G ratio, cholesterol, liver enzymes ALT, AST, ALP, HBV, The antioxidant enzyme (peroxidase and Glutathione), PCV% and Hb and estimating selenium in muscles. The results of the study showed that fish fed on the diet added to it vitamin E was significantly excelled ($P < 0.01$) in the FCR, SGR, and D.G.R. In the physiological traits, significant differences ($P<0.01$) have appeared in albumin and total protein in the treatment of nano-selenium and vitamin E treatment compared to the control treatment. In the mixture treatment, it was showed a decrease in total protein and the percentage of cholesterol and it did not show significant differences in liver enzymes (ALT, AST, and ALP) and in estimating selenium in muscles. As in the nano-selenium treatment and the mixture treatment, they achieved the best results in PCV% Hb concentration. Based on the above, we recommend using vitamin E and nano-selenium as important additives to fish diets to improve some of the productive and physiological traits for carp fish.

Keywords : Nano-Selenium, Vitamin E, Physiological Traits, *Cyprinus carpio* L.

Introduction

Fisheries are considered one of the main pillars for the productive base in livestock. Therefore, the importance of fish farming has increased during the past years due to the great advantages of this activity, where Carp fish are still the most important group among aquatic groups which contribute to increasing production to 72% of production capacity for freshwater, Because it has the ability to withstand the environmental conditions represented by temperature and oxygen which can withstand the decrease to 4.5 $mg.L^{-1}$ (Baldry, 2000; Kuhlwein *et al*., 2014). From the nutrients necessary for growth and increase of production are the mineral elements, which are good elements, including selenium, which is one of the rare mineral elements of high importance to human and animal health, as it enters the installation of some antioxidant enzymes Antioxidant, including the enzyme Glutathione and Peroxidase for its role in improving the immune response Where it has a role in increasing the immune susceptibility of the body (Kim and Mahan, 2003). One of the additions that modern science has turned to it is nano selenium, which has become a new source of selenium because it contains special features such as a large surface area and high surface activity, Nanotechnology is the science and technology of objects with very small sizes, which are smaller than 100 nm, and because of this size, new changes may occur in their physical structure that leads to increase reactivity and solubility (Troncarelli *et al*., 2013). Vitamin E plays an important role in many physiological traits and biochemical processes including growth, reproduction and immune response (NRC, 2011; Lu *et al*., 2016). This vitamin is a necessary vitamin for humans and animals, and the body of fish cannot be manufactured (Li & Gatlin, 2009). Because of the importance of vitamin E, it is used in aquaculture to breeding the Aquatic Organisms as additives to stimulate growth, resistance to stress and disease, as well as the survival of fish and shrimp (Vismara *et al*., 2003). It has a role as an antioxidant agent against lipid peroxidation on cell membranes and the preservation of cells tissue from oxidation and damage (Mocchegiani *et al*., 2014). Vitamin E requirements sometimes depend on the presence of other dietary supplements such as selenium (Poston *et al*., 1976) and linoleic acid (Watanabe *et al*., 1977) and vitamin C (Shiau and Hsu, 2002; Shiau, 2005).

Materials and Methods

Fish of Experiment

In the study, young of the common carp fish (*Cyprinus carpio* L) were sterilized with a saturated salt solution (NaCl) for approximately 35-40 seconds (Herwing *et al*., 1979) to get rid of diseases if they were present. They may be stuck on the skin or nostrils such as fungi and other organisms. Before starting the experiment for 14 days, they were placed in the pre-prepared glass basins for study with sizes (30 x 40 x 60 L3) during the period of the adapting and laboratory-made.

Water tests

The water temperature of the basins was measured using a mercury thermometer from Chinese origin with a grade from 0 to 100 °C, where the temperature amounted to 26-27 °C which could be suitable for the growth of common carp fish, as indicated by (Al-ashaab, 2011). The temperature of the fish of warm water which ranges between 20-30 °C at different levels of nutrition. The dissolved oxygen concentrations ($mg.L^{-1}$) were measured using modified Winkler bottles (APHA, 2005) which recorded a grade of 6-7 (6.5 ± 0.41) $mg.L^{-1}$. Wang et al., (1997) stated that oxygen

concentration should be at least (3 mg.L^{-1}) and pH using PH-009 (1) A Mater ranged from 8-8.4 (8.2 ± 0.16). Boyd and Tucker (1998) indicated that the concentration of pH should not be less than 6.4 and not more than 8.6, and water salinity was between (3.9-2.83 ± 0.5). These measurements were conducted weekly by Electrical connection device (WTW, German origin). Manga & Hundal (2014) demonstrated that common carp fish can be raised in salinity of up to 6 ‰, where showed a percentage of appetite for natural food and thus the ranges that emerged during all experiments are within the natural ranges that allow the growth of common carp fish.

Designing Experiments and feeding diets

The following feed materials were used in the experiments as shown in Table (1). The feed material included in the diet was analyzed by a Rapid Content Analyzer device as shown in Table (2), the diet was prepared according to (Lovell, 1989), Where the used feed materials were crushed and passed through the sieve and all of them have been mixed well to ensure their homogeneity and added to it amount of hot water up to 105 ml per 250 g of the mixture and then mixed, after that, the temperature of the mixture was raised to 75 ºC and leave the mixture to cool down. The recommended food additives were then added to the research and the diet was formed in the pellet form by using the plastic syringe (50 ml). After that, the diet was dried in the laboratory for two days, it was placed in the bags and stored in the refrigerator for feeding fish.

Table 1: Percentages of diet ingredients

Feed material	Percentage (%)
Flour	25
soybean	25
Fish powder	25
yellow corn	10
Barley	10
Wheat Bran	5
Total	**%100**

Table 2: Analysis of the diet by Rapid content analyzer

Material	Diet
Humidity%	7.95
Protein%	31.67
Fats%	3.09
Ash%	7.60
Fiber%	1.64
Carbohydrate%	48.05

Each of the nano-selenium particles which imported from the Al-Naqaa Foundation for Scientific Research (Egypt) were added as well as vitamin E (from Jordanian origin). In this experiment, three treatments were used. In addition to the fourth treatment (control treatment), with the rate of three replicates per treatment and two replicates for control treatment, each replicate contained 10 fish, 110 common carp (*Cyprinus carpio* L.) were distributed randomly on 11 glass basins with a weight of (12.01-13.26 g), The diets were included following percentages:

First treatment: control diet (without adding).

Second treatment: A diet containing (1 mg.kg^{-1}) of nano-selenium particles.

Third treatment: A diet containing (500 mg.kg^{-1}) vitamin E.

The fourth treatment: A diet containing (1 mg.kg^{-1}) of nano-selenium particles + (500 mg.kg^{-1}) vitamin E.

Nano-selenium powder was used with the size of (60 nm) in the form of powder with gray color, Transmission Electron Microscope (TEM) was conducted as shown in Figure (1) on the nano-selenium sample in the Central Laboratory of the College of Education for Pure Science (Ibn al-Haitham).

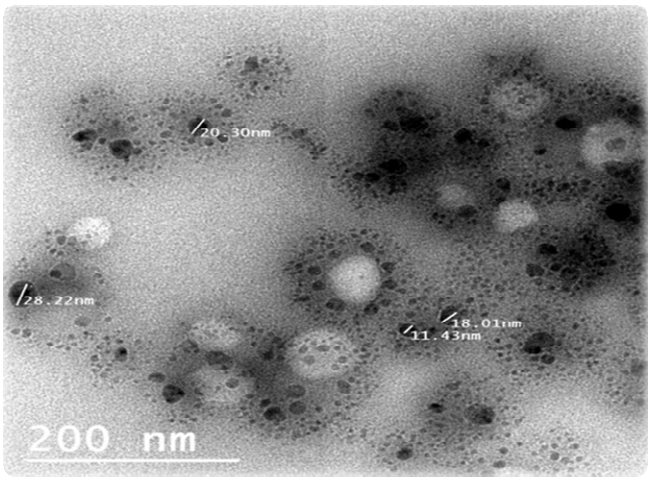

Fig. 1 : Nano-selenium particles.

Nutritional criteria studied in the experiment

1- Qualitative growth rate (S.G.R): (Hepher, 1988)

2- Feed Conversion Rate (F.C.R): (Hepher, 1988)

3- The average of daily weight gain for fish (g /day/thickness) (Uten, 1978)

Cellular blood tests:

The blood was withdrawn from different groups of fish after cutting the caudal vein. The fish were caught in a way where the head to the top to help flow the blood by pressure on the venous vein and the blood was placed in the microhaematocrit tubes containing the anticoagulant, where one end of the tube was closed with artificial clay, The tube was then placed in the Microhaematocrit Centrifuge to separate the serum from the blood at 3000 rpm for two minutes (Yang and Chen, 2003). The tube was then placed in the Microhaematocrit Centrifuge to separate the serum from the blood at 3000 rpm for two minutes (Yang and Chen, 2003). The serum was then kept in a tube and placed in a freezer until the laboratory tests were performed.

Packed Cell Volume (PCV):

The percentage of Packed Cell Volume (PCV) and Hemoglobin (Hb) before freezing. Other measurements were measured as follows:

Microhaematocrit tubes were used 75 mm length, with a diameter of 1.1 mm, containing anticoagulants, the tubes were filled with blood and one of its ends was closed with artificial clay. The tubes were then placed in a Microhaematocrit Centrifuge for 2 minutes at a speed of 3000 rpm. It was read by Microhaematocrit reader which represent the Packed Cell Volume / 100 ml of blood.

Hemoglobin (Hb)

Blood hemoglobin was directly calculated by the Packed Cell Volume, using the law mentioned by (Campbell, 1995).

Blood Biochemical Examination

Estimating albumin level in serum

The albumin level in serum was calculated using the special kit from the German company (Human). Sample reading was measured at a wavelength of 570 nm using the optical spectrometer and according to the work method attached by the company.

Estimating total protein in serum:

The level of total proteins in the serum was estimated using the special kit from German company (Human). Sample reading was measured at wavelength (580) nm using the optical spectrometer and according to the work method attached by the company.

Estimating of Globulin level in serum

The level of serum globulin was estimated by the method of (Wolf and Darlington, 1971) through the following equation

Globulin (mg / 100 ml serum) = total protein – albumin

Estimating the percentage of albumin to the globulin:

The percentage was estimated according to (1979, Richards and Pickering) by dividing the albumin on globulin (A / G).

Estimating the cholesterol level in serum:

The level of cholesterol in the serum was estimated using the kit from a German company (Human), A method of enzymatic color, the models were read at a wavelength of 500 nm.

Measuring the concentration of the Alkaline phosphatase (ALP) enzyme

The concentrations of the enzyme in the serum were measured using prepared solutions and Kit from the German company (Human). The samples were read using a spectrophotometer at a wavelength of 400 nm.

Measuring the concentrations of the Aminotransferases (AST and ALT):

A) Examination of Glutamic Oxaloacetate Transaminase (GOT) also called Aspartate transaminase (AST)

The concentrations of the enzyme in the serum were measured using prepared solutions (kit from German company Human). The samples were read using a spectrophotometer at a wavelength of 340 nm.

B) Examination of Glutamic Pyruvic Transaminase (GPT) is also called Alanine transaminase (ALT):

The concentrations of the enzyme in the serum were measured using a kit from the foreign Randox company. The samples were read using a spectrometer at a wavelength of 550 nm.

Estimating the level of glutathione in the serum:

The concentration of Glutathione in the serum was measured using the modified Ellman detector method (Al-Zamely, 2001).

Estimating selenium in muscles

The analysis was performed using the method of (Plessi *et al*., 2001). The samples were taken from fish muscles after the end of the experiment, It was dried by the convection oven at a temperature of 105 C for twenty-four hours after which the sample was extracted from the oven to weighing it and then returned to the oven for one hour and weighed again with a sensitive balance (XY-8006) until gaining the fixed weight, then grind the sample well for the purpose of digestion. The 4 ml of HNO_3 was added to 0.3 g of the dried muscle at 80 C for one hour and then add 3 ml of HNO_3 with concentration of 65% until the completion of digestion and then added 2 ml of HCl with a concentration of 37% at 100 C for 10 min and then cooling and diluting to 20 mL with ion-free water, 1 ml of the sample took and add to it 9 mL HCl with a concentration of 1.5%. Selenium is then measured using an Inductive Coupled Plasma (ICP) device.

Statistical analysis

The Statistical Analysis System (SAS, 2012) was used in the analysis of data to study the effect of different treatments in the studied traits according to the completely randomized design (CRD). The significant differences between the averages were compared using Duncans' multiple range test (Duncan, 1955).

Results and Discussion

Table (3) shows that there were no significant differences in the average weights at the beginning of the experiment for all the treatments, while at the end of the experiment there were significant differences between the treatments ($P < 0.05$). In the same table, at the end of the experiment, The T1 treatment was excelled on the control treatment and T2 and T3 treatments, the treatments (T3 and T2) were also excelled on the control treatment. while did not find a significant ($P > 0.05$) difference between T2 treatment and T3 treatment, it did not get any Mortality in all treatments during the experiment, In the same table, there were significant differences ($P < 0.01$) between the treatments. The best Feed Conversion Ratio (FCR) was in T1 compared to the fish of other treatments (T2 and T3) and the control treatment. There were significant differences ($P < 0.01$) between treatment T2, which gave the best ratio compared to the control treatment and T3 treatment. In trait of the average of qualitative growth and daily weight gain, The excelling of the T1 treatment continued significantly on the control treatment and T2 and T3 treatments.

Table 3: Effect of adding vitamin E and nano-selenium particles and their interaction to the diet in the average weight of common carp fish.

Treatment	Average ± standard error				
	Primary weight (g/fish)	Final weight (g/fish)	Feed Conversion Rate	Qualitative growth growth rate (g / day)	Daily weight gain (g / day)
The control treatment	120.10 ± 6.59	7.93 ± 179.75**c**	0.08 ± 3.64**a**	0.01 ± 0.290**b**	0.02 ± 0.990**b**
T1Treatment (Vit. E)	6.35 ± 126.83**a**	12.62 ± 259.26**a**	0.11 ± 2.03**c**	0.02 ± 0.516**a**	0.15 ± 2.20**a**
T2 treatment (Nano-Se)	4.86 ± 122.56**a**	9.43 ± 200.93**b**	0.05 ± 2.81**b**	0.003 ± 0.353**b**	0.03 ± 1.30**b**
T3 treatment (Mixture)	6.36 ± 132.63**a**	12.94 ± 211.66**b**	0.23 ± 3.14**a**	0.02 ± 0.336**b**	0.09 ± 1.313**b**
Significant level	**NS**	*	**	**	**

* The averages with different letters within the same column differ significantly between them ($P < 0.05$). ** ($P < 0.01$).

Blood test

Table (4) indicates that the results of the statistical analysis for the albumin showed significant differences (P <0.01) between the treatments, where The treatments T1 and T2 were excelled to the control treatment and T3 treatment which significantly (P <0.01) excelled on the control treatment. In the case of total protein, All treatments were significantly (P <0.01) excelled on the control treatment. As for the Globulin, there are no significant differences between all treatments. In the same table, there were no significant differences between the treatments in the albumin/globulin ratio. In the cholesterol, the table showed a significant reduction in T1 treatment and T2 treatment compared to the control treatment and T3 treatment. Table (4) also shows no significant differences between all treatments in the concentration of Alanine transaminase (ALT). There was no significant superiority in both the Alkaline phosphatase (ALP) and the Aspartate transaminase (AST). The same table for Pyruvic Transaminase (GPT) indicates that all treatments (P <0.05) were significantly excelled on the control treatment.

Table 4: Effect of adding vitamin E and nano-selenium particles and their interaction to the diet in the concentration of albumin, total protein, globulin, cholesterol and enzymes in serum of common carp fish.

Treatment	Average ± standard error								
	Albumin (mg.100 ml^{-1})	Total protein	Globulin (mg.100 ml^{-1})	A/G	Cholesterol (mg.100 ml^{-1})	ALT (UI.L^{-1})	ALP (UI.L^{-1})	AST (UI.L^{-1})	GPX
The control treatment	0.34 ± 2.70**c**	0.21 ± 4.91 **b**	0.46 ± 2.21**a**	0.42 ± 1.46 **a**	13.39 ± 199.99 **a**	± 4.87 0.55**a**	4.83±61.83 **a**	± 43.16 2.21**a**	± 6.43 0.28**b**
T1Treatment (Vit. E)	0.20 ± 5.55**a**	0.38 ± 8.46 **a**	0.55 ± 2.91**a**	0.37 ± 2.15 **a**	3.15 ± 140.03 **b**	± 3.71 0.21**a**	± 60.64 1.82**a**	± 41.14 1.21**a**	± 13.37 3.58**a**
T2 treatment (Nano-Se)	0.63 ± 5.84**a**	± 10.04 0.63**a**	0.44 ± 4.17**a**	0.39 ± 1.68 **a**	2.42 ± 135.25 **b**	± 4.88 0.52**a**	1.57 ± 60.23 **a**	± 42.68 1.24**a**	± 16.49 1.76**a**
T3 treatment (Mixture)	0.21 ± 4.18**b**	0.99 ± 8.03 **a**	0.84 ± 3.84**a**	0.37 ± 1.46 **a**	± 186.26 12.82**a**	± 3.58 0.32**a**	± 61.04 0.87**a**	± 41.63 1.66**a**	± 13.60 0.82**a**
Significant level	**	**	NS	NS	**	NS	NS	NS	*

* The averages with different letters within the same column differ significantly between them (P <0.05).
** (P <0.01).

Table (5) shows significant differences (P <0.05) between the treatments of the experiment in the percentage of P.C.V. The treatment T2 and T3 gave the highest percentage compared to the control treatment and T1 treatment. As for Hb, a significant (P <0.01) differences appeared between the treatments of the experiment. The treatments T2 and T3 achieved the highest percentage compared to the control treatment and T1 treatment. The results of the statistical analysis in Table (6) showed no significant differences between all treatments of the experiment in estimating selenium in muscles.

Table 5: Effect of adding vitamin E and nano-selenium particles and their interaction to the diet in the Packed Cell Volume (PCV) and hemoglobin (Hb) for common carp fish.

Treatment	Average ± standard error	
	PCV %	Hb
The control treatment	0.44 ± 39.23b	0.15 ± 13.07b
T1Treatment (Vit. E)	0.61 ± 40.28b	0.27 ± 13.42b
T2 treatment (Nano-Se)	0.58 ± 44.17a	0.19 ± 14.72a
T3 treatment (Mixture)	0.19 ± 44.36a	0.07 ± 14.78a
Significant level	*	**

* The averages with different letters within the same column differ significantly between them (P <0.05).
** (P <0.01).

Table 6: Effect of adding vitamin E and nano-selenium particles and their interaction to the diet in estimating selenium in muscles for common carp fish.

Treatment	Estimating selenium in muscles (mg.kg^{-1})
The control treatment	0.92 ± 17.81a
T1Treatment (Vit. E)	1.51 ± 17.04a
T2 treatment (Nano-Se)	0.75 ± 19.68a
T3 treatment (Mixture)	0.41 ± 18.12a
Significant level	NS

Productive traits and growth performance

Dietary additives are among the most important methods in fish farming because they make them more resistant to the conditions of intensive breeding and stress that fish can experience. Therefore, dietary supplements with some vitamins and minerals may be an effective way to increase growth performance, immunity and disease resistance (Khan *et al*., 2017; Keen *et al*., 2004). Vitamin E is also functionally metabolized with selenium, where acts as an effective antioxidant in animal tissues (Wise *et al*., 1993). The positive results obtained in the current study for the growth parameters, This proves the importance of vitamin E and its role in the metabolic process in vivo, which works to protect the cell membranes and Mitochondria, it helps in the metabolism of amino acids and It also represents an essential vital factor for antioxidant within the cells of the body and between them through preventing the oxidation of unsaturated fatty acids as well as that The vitamin works to increase palatability by preventing fat oxidation and improving digestion (Gao *et al*., 2012). These results agree with (Sau *et al*., 2004) in their study on rohu fish (*Labeo rohita*) which fed on different levels of vitamin E, where appeared significant differences compared to the control treatment in the final weight and qualitative growth and feed conversion ratio. This result agrees with (Amlashi *et al*., 2011) in their study on Beluga fish (*Huso huso* L.) which fed on different levels of vitamin E, where recorded significant differences compared to the control in the traits of the final weight, qualitative growth, and feed conversion ratio. This result did not agree with (Chen *et al*., 2004)in their study on golden shiner fish (*Notemigonus crysoleucas*) when fed on vitamin E where did not show significant differences compared to the control treatment. The importance of nano-selenium particles in nutrition improves the growth rate

(relative growth and final weight) and also because nanomaterials pass more easily in the cells and tissues of the body compared to non-nanoparticles due to its small size as easily absorbed by the digestive system and the positive effects In the health of the fish where works to resist the stress and diseases that get it (Khan *et al*., 2016; Zhang *et al*., 2008). Our study agreed with (Zhou *et al*., (2009) in their study on carpian carp fish (*Carassius auratus gibelio*) when fed on a diet containing nano selenium at a concentration of (0.550 $mg.kg^{-1}$) where there were significant differences in the final weight compared to the control but did not agree in the trait of feed conversion ratio, which did not show significant differences. The study agrees with the study of (Ashouri *et al*., 2015) when feeding of carp fish on a diet containing nano selenium with a concentration of (1 mg/kg feed) Where it showed a significant superiority in the final weight compared to the control treatment but did not agree in the trait of feed conversion ratio. Nader *et al*. (2017a) found when feeding rainbow trout (*Oncorhynchus mykiss*) on a diet containing vitamin E and nano-selenium particles and their mixture, where it agreed with our results in the use of nano-selenium particles, which did not show significant differences in qualitative growth but differed with our results in the final weight and feed conversion ratio, where appeared to us significant differences compared to the control treatment. As for the mixture between vitamin and nano-selenium, the improvement in fish growth may be due to the effect of the adding (vitamin E + Nano Se) to the experimental diets, which is believed to have a positive effect in strengthening the system of antioxidant in the cell. where the continuous reactions of oxygen within the body leads to the formation of free radicals or peroxides in the absence of defect in the system of self-oxidant oxidation in the cell, which leads directly to the oxidation of lipid membranes, including phospholipids, Which enters into the plasma membranes for the cells (Lehninger, 1972). This result agrees with (Al-Asheb, 2009) When using vitamin E and selenium in the growth of common carp fish in the traits of final weight as well as the average of qualitative growth at a concentration of (330 mg/kg) but the study did not agree in the traits of qualitative growth and daily weight gain. Nader *et al*. (2017a) in their study on Rainbow trout fish *(Oncorhynchus mykiss*) when fed on a diet containing vitamin E and nano-selenium particles and their mixture, where the combination between them enhance the performance of fish growth, which gave positive results in the weight gain and qualitative growth compared to the control treatment. The results of our current study agree with this study in terms of final weigh, where appeared to us significant differences compared to the control treatment. It was also agreed in the trait of feed conversion ratio, where there were no differences compared to the control treatment, But the study did not agree with our study in terms of qualitative growth where we did not have significant differences compared to the control treatment.

Blood parameters

The albumin, total protein, and Globulin are the main proteins that play an important role in immune responses, therefore when they raise in blood serum is an indicator of health status, disease resistance, and stress. The dietary additives from nano-selenium and vitamin E work to maintain the health of fish as well as enhance their role in removing free radicals, conserving on cells and tissues and Continuing the metabolic operations in the body with a better manner (Bunglavan *et al*., 2014; Kumar *et al*., 2007). The reduction of cholesterol level is due to the vitamin dissolves fatty content or because of the ability of vitamin E to break down the chains leading to fat oxidation (Huang *et al*., 2004), The nano-Selenium particles work to lower cholesterol in the blood through influencing the mechanisms of cholesterol receptor production and the activity of the HMG-CoA enzyme, which is responsible for controlling blood lipid levels. Thus, nano-selenium is an effective antioxidant that improves the health indicators in the serum. this result agrees with our current study, (Yang *et al*., 2010; Dhingra & Bansal, 2006) and Naderi *et al*. (2017b) in their study on Rainbow trout fish (*Oncorhynchus mykiss*) when fed on a diet containing vitamin E and nano-selenium particles and their mixture, where using vitamin E showed decrease in cholesterol compared to the control treatment. In total protein, albumin and Globulin did not agree with the results of our study, where there were no significant differences compared to the control treatment in the total protein, The albumin showed significant differences compared to the control treatment, where the percentage of albumin recorded a decrease compared to the control, Globulin showed significant superiority compared to the control. The results of the current study did not agree with (Zhao *et al*., 2018) when feeding the blotched snakehead fish (*Channa argus* x *Channa maculata*) on different levels of vitamin E, where there was no significant superiority compared to control despite the existence of mathematical superiority, while agreed in the trait of Globulin, meaning no significant differences compared to control. As for cholesterol, the results differed with (Gao *et al*., 2014) when feeding juvenile Japanese flounder (*Paralichthys olivaceus*), which found no significant differences in the level of cholesterol compared to the control treatment. In the nano-selenium particles differed with the study (Naderi *et al*., 2017b) when the rainbow trout (*Oncorhynchus mykiss*) fed on a diet containing vitamin E and nano-selenium particles and their mixture, so when nano-selenium particles were used, there were no significant differences compared to the control treatment. In Total protein and albumin, significant differences showed compared to the control treatment. Where the percentage of albumin decreased compared to control treatment. As for Globulin appeared to be superior on the control treatment, As for Cholesterol is lower than control, and these results agree with our results. The results of our study agree with (Saffari et al., 2018) when different sources of selenium were used to feed common carp fish, where significant differences in the treatment of nano-selenium with a concentration of (1.16 mg/kg feed) at the total protein and albumin levels compared to the control treatment. The level of cholesterol also showed a significant decrease, As well as in Globulin, where no significant differences were observed compared to the control treatment. In their mixture, our study differed with the study of (Naderi *et al*., 2017b) when feeding rainbow trout (*Oncorhynchus mykiss*) on a diet containing vitamin E and nano-selenium particles and their mixture, so When using vitamin E and nano-selenium particles, there were significant differences in cholesterol compared to the control treatment. The percentage of albumin showed a significant decrease compared to the control treatment. Globulin and total protein also showed significant excelling on the control treatment.

Aminotransferases enzymes (AST and ALT) and Alkaline phosphatase (ALP) enzyme

The increase of these enzymes from the normal limit in the serum is the result of tissue breakdown in the liver due to cases of physiological, disease and nutritional (Ebeid, 2005). It agrees with the study of (Naderi *et al*., 2017a) when feeding rainbow trout fish (Oncorhynchus mykiss) on a diet containing vitamin E and nano-selenium particles and their mixture, where using vitamin E showed no significant differences in AST compared to control but this study did not agree in ALP and ALT where showed significant differences compared to control. The results of the current study did not agree with (Zhao *et al*., 2018) when feeding the blotched snakehead fish (*Channa argus* x *Channa maculata*) on different levels of vitamin E, where there was a decrease in ALT and AST enzymes compared to control, where ALP increased compared to the control. It also agreed with the study of (Nader *et al*., 2017a) in their study on Rainbow trout fish (*Oncorhynchus mykiss*) when fed on a diet containing vitamin E and nano-selenium particles and their mixture, where the nano-selenium particles showed no significant differences in AST compared to control but it did not agree with this study in ALP and ALT where there were significant differences which recorded a decrease compared to control. In the mixture treatment, this study agreed with the study of (Nader *et al*., 2017a) in their study on Rainbow trout fish (*Oncorhynchus mykiss*) when fed on a diet containing vitamin E and nano-selenium particles and their mixture, where using vitamin E and nano-selenium particles showed no significant difference in AST compared to control but it did not agree in ALP and ALT where significant differences were observed by recording a decrease compared to the control.

Glutathione Peroxidase GPX

The functional of GPX is the elimination of peroxides through oxidation of GSH (Nordberg & Arner, 2001). Vitamin E inhibits the formation of lipid peroxidation and the presence of the Gpx enzyme, which removes complex peroxides and free radicals (ROS) that are not useful within the body of fish, thus plays an important role in enhancing immunity (Kadry *et al*., 2012; Singh *et al*., 2011) where the effect was positive in this study. Selenium plays an important role in activating the antioxidant defense system through the formation of selenocysteine, which is part of the active concentration of the glutathione peroxidase enzyme, which is very important in the elimination of harmful free radicals (ROS) (Atencio *et al*., 2009; Kohrle *et al*., 2000). Therefore, GPX is expected to increase the strength of antioxidants in the body's biological system. In vitamin E, this study differed with (Nader *et al*., 2017a) in their study on Rainbow trout fish (*Oncorhynchus mykiss*) when fed on a diet containing vitamin E and nano-selenium particles and their mixture, Where using vitamin E did not show a significant difference in GPX compared to the control. It also agrees with (Naderi *et al*., 2017a) in their study on Rainbow trout fish (*Oncorhynchus mykiss*) when fed on a diet containing vitamin E and nano-selenium particles and their mixture, Where using nano-selenium particles showed significant differences compared to the control. Our study differed with (Zhou *et al*., 2009) when feeding crucian carp (*Carassius auratus gibelio*) on a diet containing nano selenium at a concentration of 0.550 mg/kg where no significant differences were observed in the GPX level compared with control. In the case of a mixture, it was similar to (Chen *et al*., 2013) when using different levels of feeding the largemouth bass fish (*Micropterus salmoides*) on a diet containing the combination of vitamin E and selenium which was observed there are significant differences compared to the control. But it did not agree with (Nader *et al*., 2017a) in their study on Rainbow trout fish (*Oncorhynchus mykiss*) when fed on a diet containing vitamin E and nano-selenium particles and their mixture, when using the combination of (vitamin E and nano-selenium particles) showed no significant difference in GPX compared to control.

Hemoglobin (Hb) and Packed Cell Volume (PCV)

The results of the statistical analysis showed no significant differences in the hemoglobin (Hb) and Packed Cell Volume (PCV) for the treatment of vitamin E compared to the control treatment. It agrees with (Amlashi *et al*., 2011) when feeding Beluga fish (*Huso huso* L.) at different levels (25, 50, 100, 200 mg/kg feed) of Vitamin E, where there are no significant differences compared to the control treatment. But this study differs with the results of (Khara *et al*. (2016) study when using two different levels of (30, 40 mg / kg) vitamin E in feeding Salmo fish (*Trutta caspius*), where appeared a significant excelling in hemoglobin (Hb) and Packed Cell Volume (PCV) compared to the control. Either when using nano-selenium particles, The results of the statistical analysis showed significant differences in hemoglobin (Hb) and Packed Cell Volume (PCV). The powerful antioxidant properties in selenium work to preserve the blood cells in the fish and protect them from hemolysis during normal metabolism and in the extensive breeding conditions (stress) (Khan *et al*., 2016; Molnár *et al*., 2011). Our results agreed with (El-Hammady *et al*., 2007) where it was noted a decrease in PCV values When given a feeding that does not contain selenium or with a small percentage of hybrid tilapia fish (*Oreochromis niloticus* × *Oreochromis aureus*). The results also agree with (Khan *et al*., 2017) when feeding mahseer fish (Tor putitora) on a diet containing nano-selenium particles and vitamin C. where significant differences observed in hemoglobin (Hb) and cell size (PCV) compared to the control. These results also agree with (Saffari *et al*., 2018) When using different sources of selenium in the diet of common carp fish where it found when adding nano-selenium with the concentration of (1.16 mg/kg feed) showed a significant excelling compared to the control. As for the combination of vitamin and nano-selenium, the results of the statistical analysis showed significant differences in hemoglobin (Hb) and Packed Cell Volume (PCV) compared to the control. This is due to the addition of vitamin E and nano-selenium particles, which have the effect of preventing the oxidation of unsaturated fatty acids, especially those found in the cells wall and tissues, Thus preventing oxidation by interacting it with the formed free radicals. and then removing it meaning the prevention of damage caused by unsaturated fatty acids found in the cellular membranes due to the presence of oxygen and the effect of free radicals, Thus, vitamin E and selenium may inhibit free radical interaction with other fatty acids, thus ending the sequent reaction of oxidation, This preserves vitamin E and selenium together with tissue from oxidation and damage and maintains a good level of Hb and packed cell volume (PCV) (Clertin *et al*., 2001; Molnár *et al*., 2011). Vitamin E and selenium may inhibit free radical interaction with other fatty acids, thus ending the chain

reaction of oxidation. Vitamin E and selenium together maintain tissue of oxidation and damage and maintain a good level of Hb and cell volume (PCV Clertin *et al*., 2001; Molnár *et al*., 2011). This study did not agree with (Al-ashaab, 2009) When vitamin E and selenium were used with different concentrations of (150, 300, 450 mg/kg) in the growth of small common carp fish from hemoglobin (Hb) and Packed Cell Volume (PCV), Where showed a statistical differences and not significant compared to the control.

Selenium in muscles

Selenium additives may increase the concentration of selenium in the muscle tissue of some species of fish (Buckley, 2000), While in the current study showed no effect of the addition of nano-selenium in the muscles, where it was observed no significant differences compared to the control, This shows that carp muscles fish did not have a negative effect when adding nano-selenium. This result did not agree with (Zhou *et al*., 2009) When feeding Crucian carp fish (*Carassius auratus gibelio*) on a diet containing nano-selenium at a concentration of (0.550 mg/kg) Where there is a decrease in Selenium level compared to the control. This study also did not agree with (Ashouri *et al*., 2015) when feeding carp fish on a diet containing nano selenium at a concentration of (1 mg/kg feed), which showed significant superiority compared to the control, it was reported that the muscles of carp fish are almost not sensitive to added selenium in feed.

References

Al-ashaab, M.H. (2009). Effect of supplementation of vitamin e and selenium on the growth performance and some blood traits in juvenile common carp *Cyprinus carpio* L.. Journal of Iraqi Agriculture (Special Issue) Proceedings of the Seventh Scientific Conference of Agricultural Research.

Al-ashaab, M.H. (2011). Additions Levels and different Sources of Omega-3 and Omega-6 in diets and their Effect on the Growth of common carp (*Cyprinus carpio* L.) fingerlings. Ph.D. Thesis. College of Agriculture. University of Baghdad: 177 pages.

Al-Zamely, O.M.; Al-Nimer, M.S. and Muslish, R.K. (2001). Detection the level of peroxy nitrite and related to antioxidant status in the serum of a patient with acute myocardial infarction. Nat. J. chem. 4: 625-637.

Amlashi, A.S.; Falahatkar, B.; Sattari, M. and Gilani, M.H.T. (2011). Effect of dietary vitamin E on growth, muscle composition, hematological and immunological parameters of sub-yearling beluga *Huso huso* L. Fish & Shellfish Immunology, 30: 807–814.

APHA (American Public Health Association) (2005). Standard Methods for the Examination of Water and Wastewater. 21st Edition, Washington DC.1193p.

Ashouri, S.; Keyvanshokooh, S.; Salati, A.P.; Johari, S.A. and Pasha-Zanoosi, H. (2015). Effects of different levels of dietary selenium nanoparticles on growth performance, muscle composition, blood biochemical profiles and antioxidant status of common carp (*Cyprinus carpio*). Aquaculture 446: 25–29.

Atencio, L.; Moreno, I.; Jos, Á.; Prieto, A.I.; Moyano, R.; Blanco, A. and Cameán, A.M. (2009). Effects of dietary selenium on the oxidative stress and pathological changes in tilapia (*Oreochromis niloticus*) exposed to a microcystin-producing cyanobacterial water bloom. Toxicon 53(2): 269–282.

Baldry, I. (2000). Effect of common carp (*Cyprinus carpio*) on aquatic restorations. Restor. Reclam. Revi., 6(6): 1-8.

Boyd, C.E. and Tucker, C.S. (1998). Pond aquaculture water quality management. Springer Science & Business Media, 1-7.

Buckley, W.T. (2000). Trace element dynamics. In: Mello, D., J.P.F (Eds.), Farm Animal Metabolism and Nutrition. CAB International, Wallingford, UK, 161–182.

Bunglavan, S.; Garg, A.; Dass, R. and Shrivastava, S. (2014). Effect of supplementation different levels of selenium as nanoparticles/sodium selenite on blood biochemical profile and humoral immunity in male Wistar rats. In: Veterinary World. 7: 1075–1081.

Campbell, T.W. (1995). Avian Hematology and Cytology.2nded. Iowa State Press, Ames Iowa.

Chen, R.; Lochmanna, R.; Goodwina, A.; Praveenb, K.; Dabrowskic, Kyeong-Jun Leed (2004). Effects of dietary vitamins C and E on alternative complement activity, hematology, tissue composition, vitamin concentrations and response to heat stress in juvenile golden shiner (*Notemigonus crysoleucas*). Aquaculture, 242: 553-569.

Chen, Y.J.; Liu, Y.J. and Tian, L.X. (2013). Effect of dietary vitamin E and selenium supplementation on growth, body composition, and antioxidant defense mechanism in juvenile largemouth bass (Micropterus salmoide) fed oxidized fish oil. Fish Physiol Biochem., 39: 593–604.

Clertin, P.; Troutaud, D.; Verlhac, V.; Gabaudan, J. and Deschaux, P. (2001). Dietary Vitamin E and rainbow trout *Oncorhynchus mykiss* phagocyte function: effect on the gut and head kidney leucocytes. Fish and Shellfish Immunology, 11(1):1-13.

Dhingra, S. and Bansal, M.P. (2006). Modulation of hypercholesterolemia-induced alterations in apolipo protein B and HMG-CoA reductase expression by selenium supplementation. Chem Biol Interact 161: 49–56.

Duncan, D.B. (1955). Multiple Rang and Multiple F-test. Biometrics. 11: 4-42.

Ebeid, T.A.; Eid T.A. and El-Habbak, M.M. (2005). Liver and Kidney function parameters in Avian species.Review, Proceedings of the 3rd International Poultry Conference, 4- 7 April, Hughada- Egypt.

El-Hammady, A.K.I.; Ibrahim, S.A. and El-Kasheif, M.A. (2007). Synergistic reactions between vitamin E and selenium in diets of hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) and their effect on the growth and liver histological structure Egypt J Aquat Biol Fish, 11: 53–58.

Gao, J.; Koshio, S.; Ishikawa, M.; Yokoyama, S.; Mamauag, R.E.P. and Han, Y. (2012). Effects of dietary oxidized fish oil with vitamin E supplementation on growth performance and reduction of lipid peroxidation in tissues and blood of red sea bream Pagrus major. Aquaculture, 356: 73–79.

Gao, J.; Koshio, S.; Ishikawa, M.; Yokoyama, S.; Roger, E. and Mamauag, P. (2014). Interactive effects of vitamin C and E supplementation on growth performance, fatty acid composition and reduction of oxidative stress in

juvenile Japanese flounder *Paralichthys olivaceus* fed dietary oxidized fish oil. Aquaculture 422–423: 84–90.

Hepher, I.E. (1988). Nutrition of pond fish Cambridge university press,385pp

Herwing, N.; Garibaldi, L. and Walke, R.E. (1979). Handbook of drugs and chemicals used in the treatment of fish disease. Charles, C. Thomas publisher, illionis. 272.

Huang, C.H.; Higgs, D.A.; Balfry, S.K. and Devlin, R.H. (2004). Effect of dietary vitamin E level on growth, tissue lipid peroxidation and erythrocyte fragility of transgenic Coho salmon*, Oncorhynchus kisutch* – *Comp. Biochem. Physiol.* Part A139: 199-204.

Kadry, S.M.; Marzouk, M.S.; Amer, A.M.; Hanna, M.I.; Azmy, A.H. and Hamed, H.S. (2012). Vitamin E as an antioxidant in female African catfish (*Clarias gariepinus*) exposed to chronic toxicity of atrazine. Egyptian Journal of Aquatic Biology and Fisheries, 16: 83–98.

Keen, C.L.; Uriu-Adams, J.Y.; Ensuma, J.L. and Gershwin, M.E. (2004). Trace elements /minerals and immunity. In: Gershwin ME, Nestel P, Keen, CL, editors. Handbook of Nutrition and Immunity. Totowa, NJ, USA: Humana Press, 117-140.

Khan, K.U.; Zuberi, A.; Nazir, S.; Fernandes, J.B.K.; Jamil, Z. and Sarwar, H. (2016). Effects of dietary selenium nanoparticles on physiological and biochemical aspects of juvenile *Tor putitora*. Turk J Zoo, 40: 704–712.

Khan, K.U.; Zuberi, A.; Nazir, S.; Ullah, I.; Jamil, Z. and Sarwar, H. (2017). Synergistic effects of dietary nano selenium and vitamin C on growth, feeding, and physiological parameters of mahseer fish (*Tor putitora*). Aquaculture Reports, 5: 70–75

Kim, Y.Y. and Mahan, D.C. (2003). Biological aspects of selenium in farm animals. Asian-Australas. J. Anin. Sci., 16: 435-444.

Kohrle, J.; Brigelius-Flohe, R. and Bock, A. (2000). Selenium in biology: facts and medical perspectives. Biol. Chem., 381: 849-864.

Kühlwein, H.; Merrifield, D.L.; Rawling, M.D.; Foey, A.D. and Davies, S.J. (2014). Effects of dietary β-(1, 3)(1, 6)-D-glucan supplementation on growth performance, intestinal morphology and haemato-immunological profile of mirror carp (*Cyprinus carpio* L.). Journal of animal physiology and animal nutrition, 98(2): 279-289.

Kumar, V.; Sahu, N.P.; Pal, A.K. and Kumar, S. (2007). Immunomodulation of 605 *Labeo rohita* juveniles due to dietary gelatinized and non-gelatinized starch. Fish 606 and Shellfish Immunology, 23: 341–353.

Lehninger, A. L. (1972). Biochemistry.Worth Publishers.INC New York.

Li, P. and Gatlin, D.M. (2009). Dietary vitamin E requirement of the red drum *Sciaenops ocellatus*. Aquaculture Nutrition, 15: 313–319.

Lin, Y.H. and Shiau, S.Y. (2005). Dietary vitamin E requirement of grouper, *Epinephelus malabaricus*, at two lipid levels and their effects on immune responses – Aquaculture, 248: 234-244.

Lovell, R.T. (1989). Nutrition and feeding of fish. Van Nostra and Reinhoil New York. 260.

Lu, Y.; Liang, X.P.; Jin, M.; Sun, P.; Ma, H.N.; Yuan, Y. and Zhou, Q.C. (2016). Effects of dietary vitamin E on the growth performance, antioxidant status and innate immune response in juvenile yellow catfish (*Pelteobagrus fulvidraco*). Aquaculture, 464: 609–617

Mangat, H.K. and Hundal S.S. (2014). Salinity tolerance of laboratory-reared fingerlings of common carp, *Cyprinus carpio* (Linn.) during different seasons, International Journal of Advanced Research ARTICLE, 2(11): 491-496.

Mocchegiani, E.; Costarelli, L.; Giacconi, R.; Malavolta, M.; Basso, A.; Piacenza, F. and Monti, D. (2014). Vitamin E–gene interactions in aging and inflammatory age-related diseases: Implications for treatment. A systematic review. Ageing research reviews, 14: 81-101.

Molnár, T.; Biró, J.; Balogh, K.; Mézes, M. and Hancz, C. (2011). Improving the nutritional value of Nile tilapia fillet by dietary selenium supplementation. Isr J Aquacult Bamidgeh, 63(744): 9.

Naderi, M.; Keyvanshokooh, S.; Salati, A.P. and Ghaedi, A. (2017b). Combined or individual effects of dietary vitamin E and selenium nanoparticles on humoral immune status and serum parameters of rainbow trout (*Oncorhynchus mykiss*) under high stocking density. Aquaculture 474: 40–47.

Naderi, M.; Keyvanshokooh, S.; Salati. A.P. and Ghaedi, A. (2017a). Proteomic analysis of liver tissue from rainbow trout (*Oncorhynchus mykiss*) under high rearing density after administration of dietary vitamin E and selenium nanoparticles. Comp Biochem Physiol Part D Genom Proteom 22: 10–19.

NRC (2011). Nutrient requirements of fish and shrimp. Washington D.C.: National Academies Press

Plessi, M.; Bertelli, D. and Agar, M. (2001). Mercury and Selenium Content in Selected Seafood. Journal of Food Composition and Analysis 14: 461-467.

Poston, H.A.; Combs, G.F. and Leibovitz, L. (1976). Vitamin E and selenium interrelations in the diet of Atlantic salmon (*Salmo salar*): Gross, histological and biochemical deficiency signs – J. Nutr. 106: 892-904.

Richards, R.H. and Pickering, A.D. (1979). Changes in serum parameters of *Saprolegnia* infected brown trout, *Salmo trutta* L. J. Fish Dis., 2: 197-206.

Saffari, S.; Keyvanshokooh, S.; Zakeri, M.; Johari, S.A.; Pasha-Zanoosi, H. and Mozanzadeh, M.T. (2018). Effects of dietary organic, inorganic, and nanoparticulate selenium sources on growth, hemato-immunological, and serum biochemical parameters of common carp (*Cyprinus carpio*). Fish Physiol. Biochem., 44: 1087–1097.

SAS (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1^{t}ed.SAS. Inst. Inc. Cary. N.C. The USA.

Sau, S.K.; Paul, B.N.; Mohanta, K.N. and Mohanty, S.N. (2004). Dietary vitamin E requirement, fish performance, and carcass composition of rohu (*Labeo rohita*) fry. Aquaculture, 240: 359–368.

Shiau, S.Y. and Hsu, C.Y. (2002). Vitamin E sparing effect by dietary vitamin C in juvenile hybrid tilapia, *Oreochromis niloticus*×*O. aureus*. *Aquaculture*. 210: 335–342.

Singh, M.; Sandhir, R. and Kiran, R. (2011). Effects on antioxidant status of the liver following specific immune response, nonspecific resistance factors and disease resistance of healthy and aflatoxin B1-induced

immunocompromised Indian major carp, *Labeo rohita* (Hamilton). Aquaculture Nutrition, 8: 159–167.

Troncarelli, M.Z.; Brandao, H.M.; Gern, J.C.; Guimaraes, A.S. and Langoni, H. (2013). Nanotechnology and antimicrobials in veterinary medicine. In: Mendez-Vilas A, editor. Microbial pathogens and strategies for combating them: science, technology, and education. Badajoz: Formatex Research Center; 543-556.

Uten, F. (1978). Standard methods and terminology in finfish nutrition. proc. World sump. on fin fish nutrition and fish feed technology. 11: 20 –23.

Vismara, R.; Vestri, S.; Kusmic, C.; Brarsanti, L. and Gualtieri, P. (2003). Natural vitamin enrichment of *Artemia salina* fed freshwater and marine microalgae. J. Appl. Phycol., 15: 75-80.

Watanabe, T.; Kiron, V. and Satoh, S. (1997). Trace minerals in fish nutrition. Aquaculture 151(1): 185–207.

Watanabe, T.; Takeuchi, T.; Matsui, M.; Ogino, C. and Kawabata, T. (1977). Effect of α-tocopherol deficiency on carp: VII. The relationship between dietary levels of linoleate and α-tocopherol requirement – B. Jpn. Soc. Sci. Fish. 43: 935-946

Wise, D.J.; Tomasso, J.R.; Bai, S.C. and Blazer, V.S. (1993). Effects of dietary selenium and vitamin e on red blood cell peroxidation, glutathione peroxidase activity, and macrophage superoxide anion production in channel catfish. J. Aquat. Anim. Health, 5: 177–182.

Wolf, K. and Darlington, R.W. (1971). Channel catfish virus: a new herpes virus of ictalurid fish. J. Virol., 8: 525-533.

Yang, J. and Chen, H. (2003). Serum metabolic enzyme activities and hepatocyte ultra-structure of common carp after gallium exposure. Zool. Stud., 42(3): 455-461.

Yang, K.C.; Lee, L.T.; Lee, Y.S.; Huang, H.Y.; Chen, C.Y. and Huang, K.C. (2010) Serum selenium concentration is associated with metabolic factors in the elderly: a cross-sectional study. Nutr Metabol 7:38.

Zhang, J.S.; Wang, X.F. and Xu, T.W. (2008). Elemental selenium at nano size (Nano-Se) as a potential chemo preventive agent with reduced risk of selenium toxicity: comparison with Semethyl selenocysteine in mice. Toxicol Sci., 101: 22–31.

Zhao, H.; Ma, H-J.; Gao, S-N.; Chen, X-R.; Chen, Y-J.; Zhao, P-F. and Lin, S-M. (2018). Evaluation of dietary vitamin E supplementation on growth performance and antioxidant status in hybrid snakehead (*Channa argus* × *Channa maculata*). Aquacult Nutr. 24: 625–632.

Zhou, X.; Wang, Y.; Gu, Q. and Li, W. (2009). Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance, muscle composition, and glutathione peroxidase enzyme activity of crucian carp (*Carassius auratus gibelio*). Aquaculture (In press). Aquaculture 291: 78–81.