EFFECT OF ORGANIC SELENIUM AND VITAMIN E ON SOME BLOOD AND BIOCHEMICAL PARAMETERS IN COMMON CARP *CYPRINUS CARPIO* L.

M. A. Mohammad **M. A. Hasan**
Prof. **Researcher**
College of Agriculture, University of Mosul, Iraq
E-mail: dr.mahmoud@uomosul.edu.iq

ABSTRACT

The aim of this research is to add various quantities of organic selenium with or without α-tocopherol to support growth and reduce oxidative stress. A total of 168 fingerlings of common carp *Cyprinus carpio* with an average weight of 31±2 g/fish were distributed in 24 glass aquariums. Fish were fed on eight different experimental diets, including a control, and a diet that contains 0.6, 0.7 and 0.8 mg/kg of organic selenium (2nd, 3rd and 4th diets) respectively. The fifth, sixth and seventh diets contained the same amounts of organic selenium with 300 mg/kg of α-tocopherol, but only α-tocopherol was added to the eighth diets. The results of a statistical analysis found that the use of organic selenium with α-tocopherol enhanced the values of blood parameters included Hb and PCV which reached 10.85 gm/100 ml, 33.03% respectively; biochemical included TP, GLU and GPx which reached 4.13 g/100 ml, 2.08 mg/100 ml and 1.90 pg/ml respectively, while the control diets have 9.35, 27.80, 3.67, 84.50 and 1.34 respectively i.e., the eighth treatment (0.8 organic selenium with Vitamin E) representing the best treatments.

Key Words: α-67, tocopherol, antioxidants , trace elements, Selenomethionine.
***Part of M.Sc. thesis of the 2nd author.**

تأثير إضافة السيلينيوم العضوي وفيتامين E على بعض الصفات الدمية والكيموحيوية في أسماك الكارب الشائع *Cyprinus carpio* L.

محمود احمد محمد **محمد عدنان حسن**
استاذ **باحث**
كلية الزراعة والغابات/جامعة الموصل

المستخلص

هدفت الدراسة الحالية الى إضافة تراكيز مختلفة من السيلينيوم العضوي مع أو بدون فيتامين ألفا توكوفيرول لدعم النمو وتقليل الإجهاد التأكسدي. تم توزيع 168 من إصبعيات الكارب الشائع *Cyprinus carpio* بمعدل وزن 31±2 غم/سمكة في 24 حوضًا زجاجيًا. غذيت الأسماك على ثمانية وجبات تجريبية مختلفة، بما في ذلك عليقة السيطرة (العليقة 1)، وعلائق احتوت على 0,6 و 0,7 و 0,8 ملغم/ كغم من السيلينيوم العضوي (العليقة الثانية والثالثة والرابعة)على التوالي. احتوت العلائق الخامسة والسادسة والسابعة على نفس الكميات من السيلينيوم العضوي مع 300 ملغم /كغم من الألفا توكوفيرول، العليقة الثامنة اضيف لها الألفا توكوفيرول فقط. أظهرت نتائج التحليل الإحصائي للبيانات أن إضافة السلينيوم العضوي مع الألفا توكوفيرول أدت إلى تعزيز قيم معايير الدم,والتي تضمنتHb وPCV والتي بلغت 10,58 غم\100مل و33,33 % على التوالي والكيموحيوية التي تضمنت TP و GLU وGPx والتي بلغت 4,13 غم /100مل و2,08 ملغم /100مل و1,90 بيكو غم/مل على التوالي , بينما بلغت هذه القيم في عليقة السيطرة 9,35 و27,80 و3,67 و84,50 و1,34 على التوالي اي ان العليقة الثامنة (0,8 سيلينيوم عضوي مع فيتامين E) تمثل أفضل المعاملات .

كلمات مفتاحية: ألفا توكوفيرول، مضادات الأكسدة، المعادن النزرة، سلينومثيونين.

***جزء مستل من رسالة الماجستير للباحث الثاني.**

Received:14/5/2022, Accepted:7/8/2022

INTRODUCTION

As a result of declining fish capture in natural water bodies and rising demand, attention is being given to aquaculture fish farming (5), which employs intensive farming techniques, to meet these high consumption requirements, fish raised in farms are subjected to a variety of stresses, including grading, transfer, crowding, and vaccination, all of which pose risks to the fish (2, 26). As a result, the fish ecosystem suffers from a lack of oxygen and a decline in water quality. This naturally causes stress in fish and reduces their resistance to these unnatural environmental conditions, which leads to an increase in the development of illnesses and adversely affects fish growth (10). Additionally, it exposes fish to oxidative stress (6). Researches has focused on the use of healthy food additives such selenium (24). It is used by living organisms whether they are organic or inorganic (8). Selenium is an example of the trace nutrients required to boost fish health and activity. by entering into the synthesis of antioxidant enzymes and thyroid hormones and the formation of selenoproteins, most notably glutathione peroxidase (GPx) and iodothyronine deiodinase enzymes (ID), selenophosphate (13). Since glutathione peroxidase is a component of selenocysteine, which is produced as a result of selenium's most significant antioxidant action, selenium affects glutathione peroxidase activity (23). Vitamin E is an antioxidant that protects cell membrane lipids against oxidation and the generation of free oxygen radicals (17). Because it enhances the immune system, reduces stress, and increases disease resistance, vitamin E is crucial for promoting fish health (14). Studies have proven elevated vitamin E levels in farmed fish enhance stress resistance, antioxidant characteristics and growth ability (14). The interaction of selenium and vit. E has been found to have a strong synergistic effect (18). The objective of the current study is to reduce oxidative stress by incorporating various levels of organic selenium with or even vitamin E, with the intent of enhancing hematological, biochemical, and antioxidant status.

MATERIALS AND METHODS

Additions to the experience

The experimental diets are supplemented with organic selenium at various levels as well as Vitamin E organic selenium is used in the form of selenomthionine, which is similar to the amino acid methionine in that the selenium atom replaces the sulphuric atom. Organic selenium is known as Seleno-L-methionine, and the approved designation is (S)-2-Amino-4-(methylseleno) butyric acid, which is prepared as a 98% pure powder. Vitamin E is used in the form of α-tocopherol, a golden liquid obtained from Iraqi medical stores.

Experimental fish

A total of 168 common carp fingerlings, *Cyprinus carpio* with average weight of 31± 2 g/fish are randomly distributed in 24 glass tanks with dimensions of 40 x 60 x 40 cm. Fish are acclimated in a tank made of glass setting for 14 day prior to the feeding trial. Throughout the trial, the fish are fed 3–4% of their body weight during the experiment.

Physical and chemical properties of breeding water: The breeding ponds' water temperature ranged from 22 to 24 °C, as measured by a mercury thermometer and room temperature controlled by air conditioners. The amount of dissolved oxygen measured with a field device type dissolved oxygen meter HD 3030 ranged from 4 to 4.9 mg/L during the experiment period. pH values ranged from 7.2 to 7.5 as measured by a pH meter from Eutech instruments and all parameters are within normal limit for warm water fish growth.

Experimental diets

Soybean meal, animal protein concentrate, yellow corn, barley and wheat bran, as well as other feed additives, are among the feed ingredients used. Table 1 shows the diets of the major nutrients that have been chemically analyzed. Eight different experimental diets are created, with each element crushed to a 40-mesh size before being mixed and shaped on pellets.

Table 1. Dietary components and chemical analysis (DM%) of the experimental diets

Diets	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Ingredients	Control							
Animal protein	12	12	12	12	12	12	12	12
Soybean meal	30	30	30	30	30	30	30	30
Local barley	20	20	20	20	20	20	20	20
Yellow corn	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5
Wheat bran	19	19	19	19	19	19	19	19
Food salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vit. and Miner. mix.	1	1	1	1	1	1	1	1
Selenomethionine*	-	0.6	0.7	0.8	0.6	0.7	0.8	-
α-Tocopherol*	-	-	-	-	300	300	300	300
Lime stone	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Binder(Bentonite)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100	100	100
Chemical composition (%)								
7.59 Moisture	25.60 Crude protein	6.34 Ether extract	5.67 Ash	4.75 Crude fiber	54.80 Nitrogen free extract		0 14.35 ME (MJ\Kg)	

*** mg/kg**

Among the diets offered to the fish are the control diet with no additives (indicated by the first diet) and the organic selenium diets (represented by the second, third and fourth diets respectively). Organic selenium mixed in water and applied as a spray to a variety of components, with selenomethionine added in levels of 0.6, 0.7 and 0.8 mg/kg. Organic selenium is provided the same amounts in the fifth, sixth, and seventh diets, but 300 mg/kg of vitamin E is also added to the ingredients as drops. There are only 300 mg/kg of vitamin E in the ninth diet.

Chemical analysis: The estimation of the main chemical components of the shares in the College of Agriculture and Forestry was carried out in the main laboratory located at the University of Mosul using standardized methods efined by the Society of Official Analytical Chemists (3).

Blood Sampling: After eight-week experiment, six fish were taken from each treatment, two fish for each replicate. Blood samples were obtained from fish using plastic syringes 3 ml from the caudal vein. A sample of blood was placed in anticoagulant-free glass tubes tilted at room temperature for two hours to collect serum. In a centrifuge, the tubes were spun for ten minutes at 3000 rpm. The second portion of blood was deposited in glass tubes containing anticoagulant for further blood tests.

Hematological analyses: Hemoglobin and Packed Cell Volume were measured using CBC type Horiba ABX Micros 60 device, which is French-made.

Biochemical analyses of serum

Total protein, albumin, glucose, triglycerides, ALT and AST were measured using the American-made Genotek Chemistry Analyzer Smart-150 by the Italian company Giesse Diagnostics' CT. According to the approach, globulin was calculated by subtracting albumin from total protein.

Estimation of Glutathione peroxidase

The ELISA technique was used to calculate GPx rate in fish plasma. The test is carried out in accordance with the manufacturer's instructions (Sun Long Biotech, China).

Statistical analysis

The Statistical Package for Social Science employed the Complete Randomized Design (CRD) in data analysis to examine the influence of the experimental results on the examined criteria, and the significant differences between the mean characteristics of the multiple-ranged character examination Duncan's test (7).

RESULTS AND DISCUSSION

Blood parameters: The health and physiology of fish can be diagnosed through two vital indicators, which are hematological and biochemical parameters, which is of great importance in forming a clear picture of some positive and negative cases of the impact of environmental and nutritional factors to which fish may be exposed.

Hemoglobin (Hb) and Packed Cell Volume (PCV): The statistical analysis of the hemoglobin parameter, as shown in Table (2), revealed significantly different ($P \leq 0.05$), with the seventh treatment (0.8 mg/kg selenomethionine and 300 mg/kg Vit. E),

resulting in 10.85 gm/l significantly (P≤0.05). The control and second treatments both contained 0.6 mg/kg selenomethionine, giving in 9.35 and 9.58 gm/L respectively. The third treatment, which included 0.7 mg/kg selenomethionine for a total of 10.72 gm/L, outperformed the control diet significantly. In terms of packed cell volume (PCV), the statistical analysis revealed that the fish fed the third and seventh rations were significantly (P≤0.05) superior to the control, second and eighth had values of 33.08, 33.03, 27.08, 28.20 and 28.23 percent. The statistical analysis in Table (2) revealed that the addition of organic selenium and vitamin E increased Hb and PCV. Khan (12) showed that selenium increases the stability of red blood cell membranes and protects them from free radicals, which can induce membrane degradation and hemolysis, resulting in anemia.

Table 2. Effects of organic selenium and vitamin E on Hb content and PCV%

Diets	Hb (gm/ 100ml)	PCV (%)
Control	9.35 ± 0.38 [c *]	27.80 ± 1.08 [b]
Se(0.6)	9.58 ± 0.17 [bc]	28.20 ± 0.53 [b]
Se (0.7)	10.72 ± 0.44 [ab]	33.08 ± 2.54 [a]
Se (0.8)	9.75 ± 0.38 [abc]	29.05 ± 0.94 [ab]
Se (0.6) +E	10.10 ± 0.42 [abc]	30.38 ± 1.36 [ab]
Se (0.7)+E	10.42 ± 0.48 [abc]	31.25 ± 1.44 [ab]
Se (0.8) +E	10.85 ± 0.33 [a]	33.03 ± 1.86 [a]
E	9.78 ± 0.30 [abc]	28.23 ± 1.11 [b]

Means having different alphabets in columns are significant difference (P ≤ 0.05)

The positive findings of our current investigation suggested that the addition of organic selenium resulted in a rise in PCV and hemoglobin. These findings confirmed those of (20). Al-Asha'ab *et al.* (1) did not record significant differences in blood parameters when adding inorganic Se and vit. E to common carp compared to control diet.

Biochemical samples

The statistical analysis of total protein parameters revealed that fish given the seventh diet were significantly (P≤0.05) superior to the control and the second, fourth, fifth, and eighth diets, having values of 4.13, 3.67, 3.77, 3.63, 3.75 and 3.67 gm/100 ml respectively (Table 3). Fish fed the seventh diet outperformed the globulin parameter (gm/100ml) by 2.08 higher than the control, fourth, fifth, and eighth treatments, which were 1.58, 1.63, 1.80 and 1.73 respectively. The statistical analysis of the blood sugar standard (mg/100 ml) revealed that the fish fed on the control and third diets (84.5 and 95.67) outperformed all of the experimental diets represented by the second (63.33), fourth (58.67) and fifth diets (58.33), sixth (77.00), seventh (62.83) and eighth diets (58.17). The second, third, sixth, seventh and eighth treatments were significantly (P≤ 0.05) superior to the control diet in terms of triglyceride levels, which were 286.67, 294, 226, 317, 324 and 212.33 mg/100ml respectively (Table 3). The addition of organic selenium and Vitamin E led to a significant increase (P≤ 0.05) in the parameters of total protein and globulin (P≤ 0.05), and this confirms that these additions had a positive role in obtaining a better health and nutritional status than fish fed a control diet in the current experiment. It is indicated that the absorbed selenium binds to albumin and is transported to the liver for use in the manufacture of selenium proteins (23). According to several studies, these parameters improved in different fish (4). While total protein, albumin and globulin parameters showed no significant differences according to (24). Selenium has the ability to mimic the action of insulin and lower blood sugar, which may explain why triglycerides increased. Blood glucose was reduced by the addition of organic selenium and vitamin E (22). Selenium may have an impact on insulin dependent metabolic pathways since it is thought to be involved in the oxidation and reduction selenoproteins that regulate glucose metabolism and increase the triglycerides criteria (11). When sources of selenium were added, there were no apparent variations in glucose and triglycerides, according to Mushtaq *et al*. (16).

Table 3. Effect of organic selenium and vitamin E on total protein, albumin, globulin, glucose and triglyceride

Diets	TP(gm/ 100ml)	ALB(gm/ 100ml)	GLO(gm/ 100ml)	GLU(mg/ 100ml)	TG(mg/ 100ml)
Control	3.67± 0.07 bc	2.08± 0.05 a	1.58± 0.03 c	84.50± 3.57 ab	212.33±6.81 d*
Se(0.6)	3.77± 0.06 bc	1.93± 0.07 a	1.83± 0.07 abc	63.33± 3.92 c	286.6±15.85 abc
Se (0.7)	3.93± 0.08 abc	1.97± 0.05 a	1.97± 0.07 ab	95.67± 5.84 a	294±3.46 abc
Se (0.8)	3.63± 0.02 c	2.00 ± 0.02 a	1.63± 0.03 c	58.67± 4.84 c	272±13.14 abcd
Se (0.6) +E	3.75± 0.07 bc	1.95± 0.04 a	1.80± 0.17 bc	58.33 ± 5.57 c	254±25.10 bcd
Se (0.7)+E	3.98± 0.14 ab	2.02± 0.03 a	1.95± 0.08 ab	77.00 ± 2.90 b	226±16.81 cd
Se (0.8) +E	4.13± 0.19 a	2.05± 0.06 a	2.08± 0.06 a	62.83 ± 2.30 c	317±30.41 ab
E	3.67± 0.08 bc	1.93± 0.03 a	1.73± 0.05 bc	58.17 ± 3.29 c	324±37.93 a

Means having different alphabets in columns are significant difference (P ≤ 0.05)

Aminotransferases enzymes (AST and ALT): The first treatment (control diet) and the eighth treatment significantly (P≤0.05) outperformed the other experimental treatments in the ALT criterion (IU/L), with 177.83 and 141.50 respectively, compared to the second (109.67), third (133.67), fourth (114.33), fifth (125.50), sixth (128) and seventh treatments (129.50). The results of the statistical analysis (Table 4) showed a significant superiority (P≤0.05) of the control treatment over all other experimental treatments represented by the second, third, fourth, fifth, sixth, and seventh treatments, which amounted to 205.50, 153.67, 143.33, 142.67, 160.67, 154.17 and 160.00 respectively. The percentages of the liver enzymes ALT and AST in the experimental diets were lower than those in the control diet, which had the highest percentages of these two enzymes, due to the inclusion of organic selenium and vitamin E. The liver enzymes ALT and AST exhibit higher activity when the body is under stress. According to Mushtaq *et al*. (16), selenium added therapies resulted in a significantly lower level of AST and ALT. It was discovered by Saffarri *et al.* (20) that the increase in fish stress resistance was attributable to the selenium's capacity to reduce ALT and AST levels. Dawood *et al*. (6) found no significant differences in the ALT and AST criteria when adding nanoselenium and vitamin E, however Wangkahart *et al.* (24) found no statistically significant differences in the ALT and AST criteria when adding various forms of selenium.

Table 4. Effect of organic selenium and vitamin E on aspartate transaminase (AST) and alanine transaminase (ALT)

Diets	ALT(IU/ L)	AST(IU/ L)
Control	177.83 ±18.40 a	205.50±8.67 a
Se(0.6)	109.67 ± 9.60 b	153.67 ±16.93 b
Se (0.7)	133.67 ± 4.21 b	143.33 ±9.19 b
Se (0.8)	114.33 ± 5.70 b	142.67 ±9.16 b
Se (0.6) +E	125.50 ± 13.45 b	160.67 ±17.35 b
Se (0.7)+E	128.00 ± 14.25 b	154.17 ±13.27 b
Se (0.8) +E	129.50 ± 6.17 b	160.00 ± 7.43 b
E	141.50 ± 7.93 a	172.67 ±8.28 ab

Means having different alphabets in columns are significant difference (P ≤ 0.05)

Glutathione Peroxidase (GPx)

Glutathione peroxidase measurement is an important criterion for identifying oxidative stress in a living cell and an important indicator for evaluating haematological and biochemical results in living organisms, including fish. The statistical analysis data in Figure (1) for glutathione peroxidase, demonstrated a significant superiority (P≤ 0.05) of fish fed on the seventh ration on each of the control, second, and eighth rations, which amounted to 1.90, 1.34 1.50 and 1.45 pg/ml respectively. Due to selenium's entry into the GPx synthesis, the addition of organic selenium and vitamin E increased the amount of glutathione peroxidase GPx. In the cell, GPx has the role of oxidizing GSH to reduce hydrogen peroxide H_2O_2 (19). The main function of selenium is to act as an antioxidant by forming the amino acid selenocysteine, which is a component of the GPx. As a function, selenium regulates the glutathione peroxidase enzyme's activity (23). Selenomethionine is transformed to

selenocysteine, while selenocysteine is turned to to serine and selenide in the liver (21). Selenide is used in the last stage to form a selenide protein such as GPx (9). Wise *et al.* (25) concluded that Vit. E had no effect significantly on GPx activity. Several studies have shown increased activity of GPx when different sources of selenium were added (24). While Mechlaoui *et al.* (15) and Mushtaq *et al.* (16) found no significantly different in GPx.

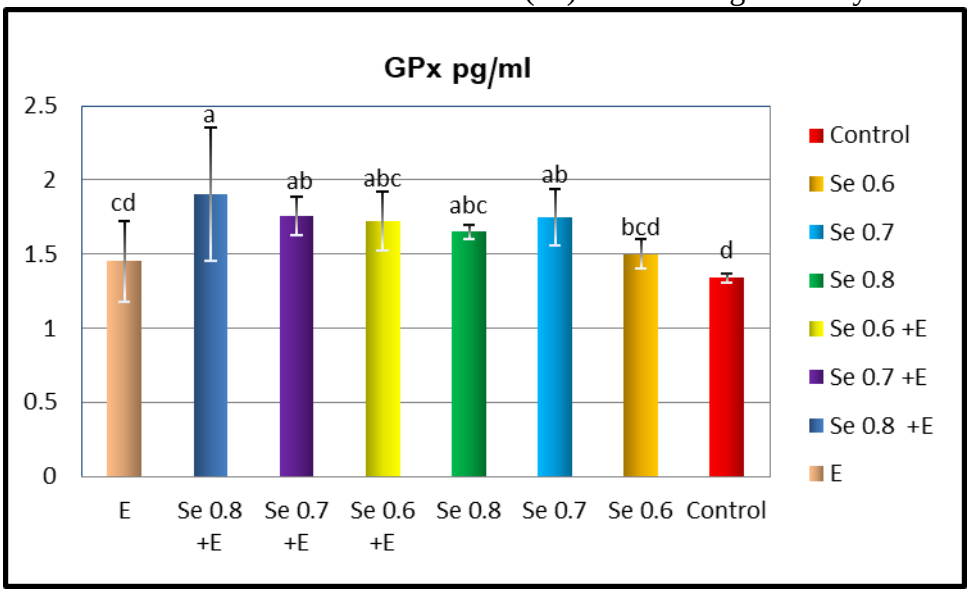

Figure 1. Effect of organic selenium and vitamin on glutathione peroxidase

CONCLUSION

The results showed that the feed additives represented by organic selenium and vitamin E have positive effects on Hematological, biochemical, and glutathione peroxidase parameters, especially when organic selenium 8 mg/kg was added with 300 mg/kg of feed with vitamin E.

ACKNOWLEDGMENT

The researcher would like to thank the College of Agriculture and Forestry for their assistance in completing this research.

REFERENCES

1. Al-Asha'ab, M. H., A.H. Salman and S.D. Mohammad .2009. Effect of supplementation of Vitamin E and selenium on the growth performance and some blood traits in juvenile common carp *Cyprinus carpio* L. Iraqi Journal of Agricultural Research. 14(1):135-143.

2.Ashley, P. J. 2007. Fish welfare: current issues in aquaculture. Applied Animal Behaviour Science. 104(3-4): 199-235.

3. AOAC .2000. Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA.

4.Ashouri, S.,S. Keyvanshokooh, A.P. Salati, S.A. Johari and H. Pasha-Zanoosi .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.

5.Al-Khshali, M. S., and A. M. Mohammed. 2023. Effect of fertilization on growth characteristics of *Cyprinus carpio* cultured in rice fields in Iraq. Iraqi Journal of Agricultural Sciences, 54(2):447-4542. https://doi.org/10.36103/ijas.v54i2.1719

6.Dawood, M. A., M. Zommara, N. M. Eweedah and A. I. Helal. 2020. Synergistic effects of selenium nanoparticles and Vitamin E on growth, immune-related gene expression, and regulation of antioxidant status of Nile tilapia (*Oreochromis niloticus*). Biological Trace Element Research. 195(2): 624-63.

7.Duncan, D. B. 1955. Multiple Range and Multiple F Tests. Biometrics. 11(1): 1-42.

8. El-Sayed, A. F. M. and M. Izquierdo.2022. The importance of Vitamin E for farmed fish—A review. Reviews in Aquaculture. 14(2): 688-703.

9.Favier, A. E. 1993. Nutritional and clinical factors affecting the bioavailability of trace elements in humans. Bioavailability. 93: 202-211.

10. Gaunt, P. S., R. Endris, A. McGinnis, W. Baumgartner, A. Camus, J. Steadman and F. Sun. 2010. Determination of florfenicol dose rate in feed for control of mortality in Nile tilapia infected with *Streptococcus iniae*.

Journal of Aquatic Animal Health. 22(3): 158-166.

11.Jablonska, E., E. Reszka, J. E. Gromadzinska, Wieczorek, M. B. S. Krol, Raimondi and W. Wasowicz. 2016. The effect of selenium supplementation on glucose homeostasis and the expression of genes related to glucose metabolism. Nutrients. 8(12): 772.

12.Khan, K. U., A. Zuberi, S. Nazir, J.B.K. Fernandes, Z. Jamil and H. Sarwar .2016. Effects of dietary selenium nanoparticles on physiological andbiochemical aspects of juvenile Tor putitora. Turkish Journal of Zoology. 40(5): 704-712.

13.Labunskyy, V. M., D.L. Hatfield and V.N. Gladyshev .2014. Selenoproteins: molecular pathways and physiological roles. Physiological Reviews. 94(3): 739-777.

14.Liu, B., P. Xu, J. Xie, X. Ge, S. Xia, C. Song and R. Chen. 2014. Effects of emodin and Vitamin E on the growth and crowding stress of Wuchang bream (*Megalobrama amblycephala*). Fish and Shellfish Immunology. 40(2): 595-602.

15.Mechlaoui, M., D. Dominguez, L. Robaina, P.A. Geraert, S. Kaushik, R. Saleh and M. Izquierdo . 2019. Effects of different dietary selenium sources on growth performance, liver and muscle composition, antioxidant status, stress response and expression of related genes in gilthead seabream (*Sparus aurata*). Aquaculture. 507: 251-259.

16.Mushtaq, M., M. Fatima, S.Z.N. Shah, N. Khan, S. Naveed and M. Khan .2022. Effects of sodium selenite, selenium methionine, and selenium yeast on growth performance, carcass composition, blood biochemistry, and antioxidant status of intensively reared Hypophthalmichthys molitrix. Aquaculture Reports. 24: 101-182.

17.Naderi, M.,S. Keyvanshokooh, A. Ghaedi and A.P. Salati. 2019. Interactive effects of dietary Nano selenium and Vitmin E on growth, haematology, innate immune responses, antioxidant status and muscle composition of rainbow trout under high rearing density. Aquaculture Nutrition. 25(5): 1156-1168.

18.National Research Council (NRC). 2011. Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington, DC, USA. pp:207–209.

19.Rached, L. A., m.f. McDermott, and p. Pontarotti . 1999. The MHC big bang. Immunological Reviews. 167(1): 33-45.

20.Saffari, S., S.M. Keyvanshokooh, M. Zakeri, S.A. Johari and H.J.A.N. Pasha-Zanoosi. 2017. Effects of different dietary selenium sources (sodium selenite, selenomethionine and nanoselenium) on growth performance, muscle composition, blood enzymes and antioxidant status of common carp (*Cyprinus carpio*). Aquaculture Nutrition. 23(3): 611-617.

21.Schrauzer, G. N. 2000. Selenomethionine: a review of its nutritional significance, metabolism and toxicity. The Journal of Nutrition. 130(7): 1653-1656.

22.Stapleton, S. R. 2000. Selenium: an insulin mimetic. Cellular and Molecular Life Sciences CMLS. 57(13):1874-1879.

23.Sunde, R. A. 2001. Regulation of selenoprotein expression. In Selenium (pp. 81-96). Springer, Boston, MA.

24.Wangkahart, E., B. Bruneel, A. Chantiratikul, M. de Jong, N. Pakdeenarong and P. A. Subramani. 2022. Optimum dietary sources and levels of selenium improve growth, antioxidant status, and disease resistance: re-evaluation in a farmed fish species, Nile tilapia (*Oreochromis niloticus*). Fish and Shellfish Immunology. 121: 172-182.

25. Wise, D. J., J. R. Tomasso, D. M. Gatlin III, S.C. Bai and V.S. Blazer .1993. Effects of dietary selenium and Vitamin E on red blood cell peroxidation, glutathione peroxidase activity, and macrophage superoxide anion production in channel catfish. Journal of Aquatic Animal Health. 5(3): 177-182.

26. Yousef, T.A., and M. S. Al-Khshali. 2023. Relationship of growth hormone receptor gene with some of productive traits of common carp *Cyprinus carpio*. Iraqi Journal of Agricultural Sciences, 54(3):777-783. https://doi.org/10.36103/ijas.v54i3.1760