Chelonian Conservation And Biology

Vol. 19 No. 1 (2024) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI: doi.org/10.18011/2024.01(1).457.467

STUDIES ON THE EFFECTS OF FAT-SOLUBLE VITAMINS E AND K ON THE GROWTH PERFORMANCE AND ANTIOXIDANT ACTIVITY OF LABEO ROHITA

Dr Amara Akhtar[1], Muhammad Munawar Ramzan[1], Misbah tasleem[1], Amina zafar[1], Nimra Naseer[1], Mariam khalid[1], Muntah tariq[1], Musfira jabeen[1], Misha Khalid[1], Muhammad Ahmed Saqib[1]

[1]Department of zoology, wildlife and fisheries, University of Agriculture Faisalabad Pakistan

Corresponding: Muhammad Ahmed Saqib, Department of zoology, wildlife and fisheries, University of Agriculture Faisalabad Pakistan, ahmadsaqib0913@gmail.com

ABSTRACT

An experiment was conducted to find the effects of fat-soluble vitamins E and K on growth performance and antioxidant activity of Labeo rohita (Rohu). There were five treatments, T1 (control treatment) containing 0 mg/kg of vitamin E and K, T2, T3, T4, and T5 (Experimental treatment) containing 200 mg/kg of vitamin E, 500 mg/kg of vitamin E, 200 mg/kg vitamin K and 500 mg/kg vitamin K respectively in diets. Acclimatized Labeo rohita juveniles were transferred to the fisheries research farm of the University of Agriculture Faisalabad and fed twice a day for an experimental duration of six months. Physio-chemical parameters like temperature, dissolved oxygen (DO), pH, total hardness, electrical conductivity, etc. were maintained at their optimum level. Juveniles of Labeo rohita attained maximum growth during the thorough experimental period except in December due to abrupt environmental changes. Experimental treatment T3 (Vitamin E=500 mg/kg) showed maximum weight, fork, and total length that is 11.26g, 2.53cm, and 2.96 cm respectively. At higher concentrations of vitamin E in diet feed intake, feed conversion ratio (FCR) and specific growth rate (SGR) were also increased. Catalase (CAT) and peroxidase (POX) activity increased while superoxide dismutase (SOD) activity was slowed by high vitamin E concentrations supplemented diets in Labeo rohita. In T3 highest catalase activity (134.03U/ml) and peroxidase activity (0.34U/ml) were observed while the highest (SOD) activity (18.01U/ml) was observed in T1. A significant fluctuation ($p \leq 0.01$) in the growth and antioxidant activity of Labeo rohita was determined by statistical analysis during the whole experimental period.

INTRODUCTION

Aquaculture contributes significantly to global efforts to reduce hunger and malnutrition by providing fish and other fish products high in protein, vital fatty acids, vitamins, and minerals. Aquaculture may also make major contributions to development by boosting incomes, creating job opportunities, and increasing the returns on resource utilization[1]. Fish is believed to provide one-

sixth of the world's protein[2]. Catla catla (Catla), Labeo rohita (Rohu), and Cirrhinus mrigala (Mrigal) are three important species of carp indigenous to Asian rivers. These species currently produce roughly 2 million MT per year fish[3].

For healthy cell growth, development, and function, vitamins are necessary nutrients. They are vital to biological functions such as metabolism, immunity, digestion, vision, and bone health. They also support cardiovascular health, lessen stress and anxiety, encourage healthy aging, and meet all dietary needs. Two categories of vitamins,Fat-soluble vitamins are stored in the body's liver, adipose tissue, and muscles. Vitamins A, D, E, and K are the four fat-soluble vitamins. When there is dietary fat present, the body absorbs these vitamins more readily. The body does not store vitamins that are soluble in water. All nine of the B vitamins and vitamin C are water-soluble vitamins[4].

Fish with low vitamin levels frequently exhibit anemia, fin erosion, gill hyperplasia, scoliosis, poor growth, decreased feed efficiency, hemorrhages, myopathies, extended coagulation time, hyperexcitability, and cataracts[5].Studies using cell-based, pre-clinical, and clinical interventions have examined the processes behind vitamin E's effects on inflammation and the immune system. Vitamin E affects T cell activity in two ways: directly by influencing T cell membrane integrity, signal transduction, and cell division; and indirectly by influencing inflammatory mediators produced by other immune cells. The way that vitamin E modulates immune function has practical implications because it influences the vulnerability of the host to allergy disorders like asthma as well as infectious diseases like respiratory infections. A growing field of research indicates that other forms of vitamin E, such as tocotrienols and other tocopherols, may also have strong immune-modulatory properties. Studies investigating the action of vitamin E in the immune system have generally concentrated on α-tocopherol[6].

In its capacity as a lipid-soluble antioxidant, vitamin E guards against oxidation of lipid reserves, lipoproteins, and cellular membranes. Its major role is to protect unsaturated fatty acids from oxidation caused by free radicals. Before being absorbed into the body, the tocopheryl acetates are hydrolyzed by digestive enzymes rather than functioning as antioxidants[7].Blood clotting, bone metabolism, and blood calcium regulation are all impacted by the fat-soluble vitamin K. Vitamin K benefits include improved bone, cognitive, and cardiovascular health. Prothrombin is a clotting factor and protein that the body needs vitamin K to make it that is crucial for blood clotting and bone metabolism. Among its many advantages, vitamin K may also strengthen the nervous system and inhibit the cancer[8].

Lipid oxidation targets polyunsaturated fatty acids. Two distinct reactions that produce hydroperoxides as the main byproduct can be used to carry out lipid peroxidation. When hydroperoxides break down quickly, a variety of secondary products are produced, including lipid free radicals, which raise the oxidation of other molecules like proteins, nucleic acids, and other lipids. Due to its numerous health risks, lipid peroxidation poses a serious threat to both human health and the food industry but Oxidative damage is decreased by using

antioxidants[9].Objectives of current study are evaluation of fat soluble vitamins supplemented diet effects on the growth performance and antioxidant enzyme activity on different organs like kidney , liver , gills etc. of Labeo rohita (Rohu).

MATERIAL AND METHODS

The present trial was organized to determine the effect of fat-soluble vitamin E and k on growth performance and antioxidant enzyme activity of Labeo rohita. Six months experiment was organized at Fisheries Research farms, Department of Zoology and Fisheries, at University of Agriculture, Faisalabad. During study physiochemical parameters, initial weight, total length and fork length of fish were measured. After completion of trial, fish organs like liver, kidney and gills were extracted for determination of enzymes. For determination of effects of vitamin E and K on the growth and antioxidant enzyme activity following treatments were given to fishes.

- T1-control diet containing 0mg/kg of vitamin E and K
- T2-Diet containing lower concentration (200mg/kg) of vitamin E
- T3-Diet containing higher concentration (500mg/kg) of vitamin E
- T4-Diet containing lower concentration (200mg/kg) of vitamin K
- T5-Diet containing higher concentration (500mg/kg) of vitamin K

Determination of physiochemical parameters

After every two weeks different physiochemical parameters like temperature, dissolved oxygen, PH, total hardness, Electrical conductivity was measured. For determination of water temperature and DO electronic meter HANNA HI-9146 used. A.P.H.A method (1998) used for determination of calcium hardness, magnesium hardness and total hardness.

$$\text{Total Hardness (mg/L)} = \frac{\boldsymbol{volume\ of\ EDTA\ used\ for\ titration \times A \times 1000}}{\boldsymbol{volume\ of\ sample(ml)}}$$

Calcium

$$\text{Total Hardness (mg/L)} = \frac{Volume\ of\ EDTA\ used\ for\ titration \times 400.8}{volume\ of\ sample(ml)}$$

Magnesium

After analyzing, the calcium and total Hardness magnesium was measured by following formula

Where:

A-B=C

A= Total Hardness B=Calcium× 2.50.01

Determination of Morph-metric parameters

Body weight and body length of fish were measured and recorded by following formula Weight Gain (g) =Finalweight (g)-Initial weight (g)

Determination of Antioxidant Enzyme activity

Different fish organs such as liver, kidney and Gills were extracted from each pond for determination of antioxidant enzymes activity.The organs were weighed. After that, phosphate buffer added 3 times greater than organ weight. The organs were homogenized for 15 minutes and the filtered obtained from the muslin cloth was centrifuged at 10,000 r/m-1 for 15 minutes and stored at 4oC for further analysis.For purification of enzyme a buffer solution having PH 6.5 were added on extracted organ of fish. For stabilization of crude protein low and high concentration of ammonium sulphate (NH4) SO4 salt were added.Ammonium sulphate, 1Mm EDTA, phosphate buffer, 10mM 2-mercaptoethanol used for purification of enzymes.

Antioxidant enzyme assay for SOD, CAT AND POX

Reagents used for SOD activity are 0.067mM potassium phosphate buffer, 0.1MEDTA solution containing 0.3mMNaCN, 0.12Mm Riboflavin and 1.5nM NBT. It is calculated by the following formula;

$$\text{Percentage Inhibition} = \frac{Blank - sample}{Blank} \times 100$$

Reagents used for Antioxidant activity of CAT enzyme are 60mM Phosphate buffer, 10Mm hydrogen peroxide. It can be calculated by following formula;

$$\text{Activity (Unit/mL)} = \frac{\Delta A/min \times dilution \times 2ml}{0.04 mM^{-1} cm^{-1} \times 0.05 mL}$$

Reagents required for Antioxidant activity of peroxidase (POX) enzyme are 0.2 M phosphate buffer, Guaiacol, Hydrogen peroxide. It is calculated by;

$$\text{Activity (unit/mL)} = \frac{\Delta A/3}{26.6 \times 60/3000}$$

Statistical analysis of data

Statistical analysis was used to measure the data conducted for fish growth, antioxidant enzyme and other parameters.ANOVA, comparison of means and correlation analysis were performed to determine the statistical difference of various treatment and relationship of parameters.

RESULTS

The current trial was conducted to determine the effects of fat-soluble vitamins E and K on the growth performance of Labeo rohita and determination of antioxidant enzyme activity of catalase (CAT), peroxides (POX) and superoxide dismutase (SOD). For acclimatization fingerlings, fed with basal diet twice a day and kept under lab conditions for one week and then transferred to ponds.

After that physiochemical parameters and the effect of vitamins on kidney, liver and gills were observed. In this research, maximum weight gain, fork and total length was observed in T3 (VE=500mg/kg) followed by T5 (VK=500mg/kg), T2 (VE=300mg/lg) and T4 (VK=300mg/kg) respectively, while the minimum growth was overserved in T1 (control group).

Growth parameters

After six-month observation, Labeo rohita shows minimum growth in T1 and maximum in T3. Fish increase in size by feeding high vitamin supplemented diet. Different disease occurs in fish due to oxidation of lipid but vitamin K and E have antioxidant nature. Therefore, lipid peroxidation decreases and growth of fish improves.

Fish growth depends upon intrinsic and extrinsic factors. Physiochemical parameters i.e. dissolved oxygen, total hardness, temperature; electrical conductivity and pH were maintained to the optimum limits, which is required for fish growth.

Weight gain ,fork length and total length increased by Labeo rohita was observed during whole study period .Feed conversion ratio (FCR) and specific growth rate (SGR) was calculated. Antioxidant activity of catalase, peroxidase and super oxide dismutase was determined in liver, gills and kidney.

Treatments	Weight Gain	Fork length increase	Final length increase
T1	7.99	1.3	1.51
T2	9.35	2	2.48
T3	11.26	2.53	2.96
T4	8.75	2	2.31
T5	10.5	2.46	2.71

Table1 Mean of weight gain, fork length increase and total length increase by Labeo rohita.

Table 1 illustrates mean of weight, fork and total length gained by Labeo rohita during the whole study period. In this period,Labeo rohita shows highest weight gain in T3 as 11.26 g while T1,T2,T4 and T5 had 7.99,9.35,8.75 and 10.5 respectively.Labeo rohita shows highest fork length in T3 as 2.53 cm while T1,T2,T4 and T5 had 1.3,2,2 and 2.46 respectively. Labeo rohita shows highest total length in T3 as 2.96 cm while T1, T2, T4, and T5 had 1.51, 2.48, 2.31 and

2.71 respectively.

Treatments	Feed Intake	Weight gain	FCR	SGR
T1	17.36	7.99	2.02	55.51
T2	19.85	9.35	2.14	62.38
T3	22.94	11.27	2.03	75.15
T4	18.1	8.38	2.2	55.88
T5	22.6	10.29	2.16	68.61

Table 2 Mean of feed intake, feed conversion ratio and specific growth rate of Labeo rohita.

Table 2 indicates mean of feed intake, feed conversion ratio and specific growth rate of Labeo rohita during study period. Feed conversion ratio and specific growth rate was maximum in T3 of Labeo rohita as compared to T1,T2,T4 and T5 respectively.

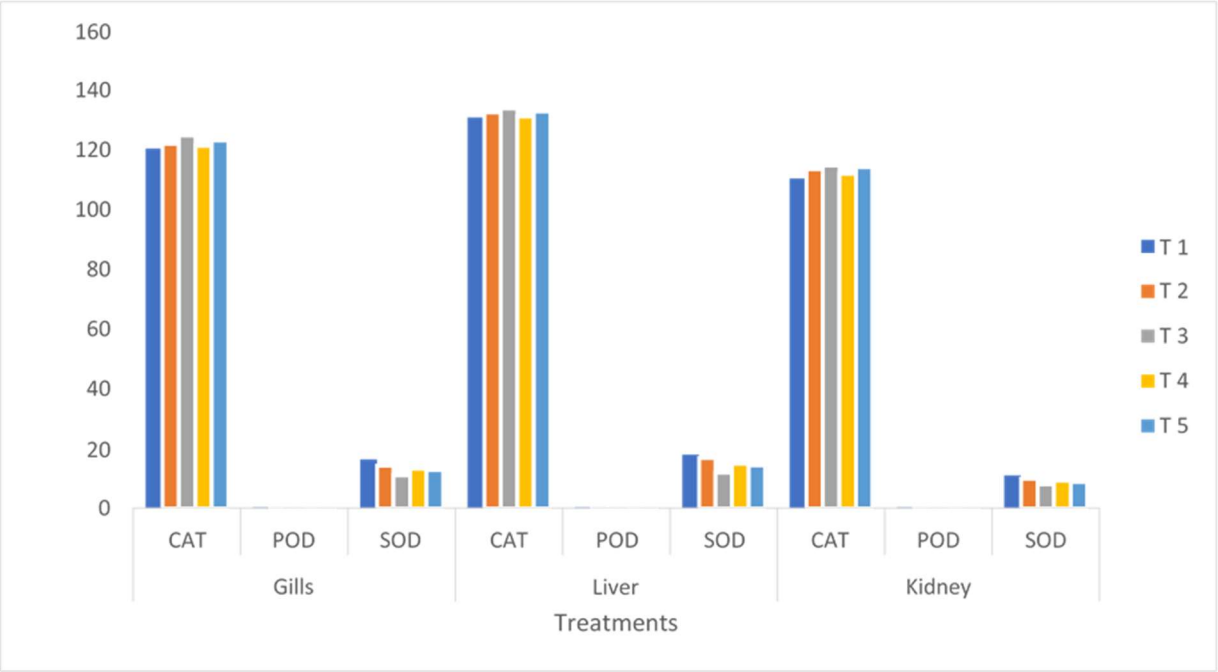
Organs	Gills			Liver			Kidney		
Anti-oxidant	CAT	POD	SOD	CAT	POD	SOD	CAT	POD	SOD
T1	120.57	0.29	16.46	130.9	0.3	18.01	110.54	0.27	11.08
T2	122.16	0.32	14.37	132.75	0.33	16.93	113.67	0.31	10
T3	125.02	0.36	11.13	134.03	0.37	12.07	115.05	0.35	8.06
T4	121.55	0.3	13.33	131.37	0.32	15.05	112.13	0.28	9.41
T5	123.36	0.34	12.9	133.14	0.35	14.52	114.37	0.33	8.86

Table 1 Determine the antioxidant activity of gills, liver and kidney of Labeo rohita.

Table 3 determine the antioxidant activity of gills, liver and kidney of Labeo rohita. Liver shows the highest activity in T3 while gills shows the lowest in control diet. The pattern of increase in catalase activity is Liver>Kidney>Gills.T3 had highest value in liver as 134.03U/mL while T1, T2, T4 and T5 had 130.90, 132.75, 131.37 and 133.14 U/mL respectively.

Highest value of peroxidase in liver of Labeo rohita is during T3 while the lowest in gills. The mean of all treatments are 0.29, 0.32, 0.36, 0.3 and 0.34 respectively. So, the results are T3>T5>T2>T4>T1.The pattern of increase in peroxidase activity is liver>kidney>gills.

Liver shows the highest SOD activity in T1 while gills show the lowest value in T3.The pattern of increase in SOD activity is liver>kidney>gills.T1 had highest value in liver as 18.01U/mL while T2,T3,T4 and T5 had 16.93,12.07,15.05 and 14.52 U/Ml respectively.



Graph 1 shows antioxidant activity of different organs of Labeo rohita in all treatments.

Catalase and peroxidase shows highest significant increase in T3 diet having 500mg/kg of vitamins. Liver shows the highest activity of catalase and peroxidase in T3.Super oxide dismutase shows highest significant increase ($p<0.01$) in T1 .Liver shows highest activity of super oxide dismutase in T1.

DISCUSSION

Aquatic organisms need various micro-nutrients that are essential for their growth, well- being and feed efficiency[10, 11]. A study was conducted to determine the effect of fat-soluble vitamins E and K on the growth performance and antioxidant enzyme activity of fish Labeo rohita. After six months of observation, fish were dissected to determine the effect of vitamins on

organs like gills, kidneys, and liver. Growth of fishes depends on factors that are Intrinsic and extrinsic[12]. Ross et al. (2001) explained that environmental factors directly affect normal growth. These factors involve an optimum Ph range of 6.5 to 8.4, while temperature is from 25 to 35C. During March, maximum growth was observed under favorable conditions while in December growth was decreased due to abrupt environmental changes. During the same period in North 24 Praganas district, the average water temperature varies from 1.66 C to 0.31C during the breeding season from March to September[12]. In diet, vitamin was added which plays an important role in growth and acts as an antioxidant. Growth, immunity, abnormalities, response towards bacterial infection, pro-collagen, and reproduction are synthesized by the involvement of water-soluble vitamins (vitamin C)[13]. In tissue, SOD, CAT, GPX along with vitamins C and E (α-tocopherol) have a potential anti-oxidant impact in neutralizing ROS [14]. In this experiment, out of five treatments T3 (VE=500) shows maximum weight gain, fork, and total length followed by T5 (VK=500) while T1 (0mg/kg VE and VK) shows minimum weight gain. Experimental studies proved that a diet rich in vitamin K improves the immunity and growth of fish. In the intestine and hepatopancreas, the activity of superoxide dismutase increases along catalase, glutathione-S-transferase, anti-hydroxyl radical, anti-superoxide anion, glutathione reductase, and glutathione peroxide by giving a particular amount of vitamin K[15]. The digestive, absorptive ability, growth, and antioxidant activity improved in Labeo rohita which was given a high supplement of vitamin K. The study results show that diets with high concentrations of vitamins E and K, improve feed intake and growth performance and vice versa. The immunity, growth performance, and resistance against diseases are improved by adding vitamin E to the diet of fish [16-18]. Among all the experiments, fish survived rapidly on a diet containing 1000mg of vitamin E. as it increases body weight and growth. Increasing the concentration of vitamins in the diet improves feed conversion ratio (FCR), protein efficiency ratio (PER), and survival growth rate (SGR) among all fish groups. In recent studies, the weight gain, SGR, FCR, and PER were improved in Labeo rohita by giving Zn and vitamin E in the diet[19]. Reactive oxygen species (ROS) decrease in the fish body as the concentration of vitamins is higher in the diet. The Significant differences in FCR and SGR of Labeo rohita were observed in T1 (control), T2 (200mg/kg VE), T3 (500mg/kg VE), T4 (200mg/kg VK) and T5 (500mg/kg VK).

Vitamin E and K act as a powerful antioxidant and strengthen the immunity of fish. Increase in concentration of vitamin, increase the catalase and peroxidase activity and decrease superoxide (SOD). The imbalance between the antioxidant defense mechanism and ROS leads to oxidative stress (Nishida, et al 2011).Current research described the lowest activity of gills, liver, and kidney in T1 while higher in T3. Same as catalase, T3 shows the highest peroxidase activity while T1 contains the lowest peroxides. Contrary to catalase and peroxidase, superoxide dismutase shows the highest in T1 and lowest in T3. Catalase activity increases with an increase in vitamin K and E in diet concentration because the antioxidant nature of vitamin donates a proton to convert superoxide free radical into hydrogen peroxide while catalase takes H2O2 as substrate and

converts it to H2O and oxygen. The enzymes like SOD, CAT, and GPX play an important role in neutralizing ROS.In the current research, by adding vitamins in diet the expression of these enzymes was affected. Specifically, when the amount of vitamins E, C, and K was increased the activity of CAT was also increased in the liver[14]. The liver acts as a detoxification centre of the body therefore Labeo rohita shows higher catalase activity in livers than in the kidneys and gills. [20], examined that, when the level of vitamin E was increased in the diet it decreased lipid peroxidation. Gills showed the highest peroxidase activity in T3 0.36U/mL while in the kidney and liver were 0.35 U/mL and 0.37 U/mL respectively. The main site of toxication and unionized ammonia is gills in aquaculture media[21] .The liver microsomal ascorbic acid stimulation was also higher in groups fed with a supplementary diet as compared to a basal diet. Statistical analysis of peroxidase of all organs illustrates significant differences in all treatments. Among all organs higher SOD activity in gills was observed in T1 (control) 16.46 U/mL and lowest in T3. Like gills, kidneys, and liver also show the highest SOD activity in T1 is 11.08 U/mL and 18.01U/mL respectively. Similarly, the result was also explained by who describes the SOD activity in organs of juvenile carpas higher on a feed diet without vitamins while lowest on a diet having a higher concentration of vitamins[22].

SUMMARY

The present study that was conducted to determine the effect of fat-soluble vitamins E and K on growth performance and antioxidant activity of catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) in Labeo rohita.For acclimatization fingerlings, fed with basal diet twice a day and kept under lab conditions for one week and then transferred to ponds. After that physiochemical parameters and the effect of vitamins on kidney, liver and gills were observed.Average weight, fork length and total body length increased significantly ($p<0.01$) with higher concentration of vitamin supplemented diet. Labeo rohita shows highest growth activity in T3 as compared to T1, T2, T4 and T5. Feed conversion ratio (FCR) and specific growth rate (SGR) improved in fish groups provided with supplementary diet.Catalase and peroxidase activity was highest in T3 (500mg/kg vitamin E) 134.03 U/ml and 0.37 U/ml in liver respectively. While SOD shows highest significant increase in T1 diet having 0mg/kg of vitamins and have highest value in liver 18.01U/ml.

REFERENCES

Subasinghe, R., D. Soto, and J. Jia, Global aquaculture and its role in sustainable development.

Reviews in aquaculture, 2009. 1(1): p. 2-9.

Helfman, G.S., Fish conservation: a guide to understanding and restoring global aquatic biodiversity and fishery resources. 2007: Island Press.

DEEPTI, M., Interactive Evaluation of Olive Barb (Systomus sarana) as Alternative to Rohu (Labeo rohita) and Mrigal (Cirrhinus mrigala) in Carp Polyculture System. 2021, CENTRAL AGRICULTURAL UNIVERSITY.

Mason, J., Vitamins, trace minerals, and other micronutrients. Goldman L, Ausiello D. Cecil textbook of medicine, 2007. 23: p. 1626-39.

Wang, K., et al., Effects of dietary vitamin E deficiency on systematic pathological changes and oxidative stress in fish. Oncotarget, 2016. 7(51): p. 83869.

Lewis, E.D., S.N. Meydani, and D. Wu, Regulatory role of vitamin E in the immune system and inflammation. IUBMB life, 2019. 71(4): p. 487-494.

Hung, S.S., et al., Uptake, transport and distribution of dl-α-tocopheryl acetate compared to d- α-tocopherol in rainbow trout (Salmo gairdneri). The Journal of Nutrition, 1982. 112(8): p. 1590-1599.

Sau, S., et al., Dietary vitamin E requirement, fish performance and carcass composition of rohu (Labeo rohita) fry. Aquaculture, 2004. 240(1-4): p. 359-368.

Prisacaru, A.E., Effect of antioxidants on polyunsaturated fatty acids–review. Acta Scientiarum Polonorum Technologia Alimentaria, 2016. 15(2): p. 121-129.

Saffari, S., et al., Effects of dietary organic, inorganic, and nanoparticulate selenium sources on growth, hemato-immunological, and serum biochemical parameters of common carp (Cyprinus carpio). Fish Physiology and Biochemistry, 2018. 44: p. 1087-1097.

Dawood, M.A., et al., The evaluation of growth performance, blood health, oxidative status and immune-related gene expression in Nile tilapia (Oreochromis niloticus) fed dietary nanoselenium spheres produced by lactic acid bacteria. Aquaculture, 2020. 515: p. 734571.

Dey, S., et al., Impact of climate change on the breeding of Indian major carps in West Bengal.

J. Inland Fish. Soc. India, 2007. 39(1): p. 26-34.

Kong, Y., et al., Types of carbohydrate in feed affect the growth performance, antioxidant capacity, immunity, and activity of digestive and carbohydrate metabolism enzymes in juvenile Macrobrachium nipponense. Aquaculture, 2019. 512: p. 734282.

Ruiz, M.A., et al., Dietary combination of vitamin E, C and K affects growth, antioxidant activity, and the incidence of systemic granulomatosis in meagre (Argyrosomus regius). Aquaculture, 2019. 498: p. 606-620.

Yuan, J., et al., Effects of dietary vitamin K levels on growth performance, enzyme activities and antioxidant status in the hepatopancreas and intestine of juvenile J ian carp (Cyprinus carpio var. Jian). Aquaculture Nutrition, 2016. 22(2): p. 352-366.

Lu, Y., et al., Effects of dietary vitamin E on the growth performance, antioxidant status and innate immune response in juvenile yellow catfish (Pelteobagrus fulvidraco). Aquaculture, 2016. 464: p. 609-617.

Zhao, H., et al., Evaluation of dietary vitamin E supplementation on growth performance and antioxidant status in hybrid snakehead (Channa argus× Channa maculata). Aquaculture Nutrition, 2018. 24(1): p. 625-632.

Gao, J., et al., 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, 2014. 422: p. 84-90.

Rohani, M.F., et al., Dietary zinc in association with vitamin E promotes growth performance of Nile tilapia. Biological Trace Element Research, 2022. 200(9): p. 4150-4159.

Ai, C., et al., Effect of Dietary Vitamin E on the PO, U~ a and U~ L Activities of Chinese Mitten-Handed Crab, Eriocheir Sinensis. Oceanologia et Limnologia Sinica, 2008. 39(2): p. 123.

Boyd, C.E., Water quality for pond aquaculture. 1998.

Li, J., et al., Effects of vitamin E on growth performance and antioxidant status in juvenile grass carp Ctenopharyngodon idellus. Aquaculture, 2014. 430: p. 21-27.