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EFFECT OF DIETARY VITAMIN C ON THE HAEMATOLOGICAL PROFILE OF JUVENILE EUROPEAN CATFISH (*SILURUS GLANIS*) REARED INTO RECIRCULATING SYSTEM

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Abstract

A feeding trial was conducted to evaluate the effects of dietary vitamin C (L-ascorbic acid, AA) levels on haematological profile and some biochemical indices of European catfish, reared in a recirculating aquaculture system. A basal commercial diet (40% crude protein and 11.5% lipids) was used as a control (D_0), and three other diets were prepared by supplementing the feed with 50 mg AA kg^{-1} diet (D_1), 100 mg AA kg^{-1} diet (D_2) and 150 (D_3) mg AA kg^{-1} diet. At the end of the feeding trial, blood samples were taken in order to analyse the haematological profile and serum biochemical parameters. Significant differences ($p<0.05$) were recorded among experimental diets in the numbers of red blood cells (RBC), haematocrit (Hct), the mean corpuscular volume (MCV), and the mean corpuscular haemoglobin concentration (MCHC). Regarding the serum glucose, serum total protein, and the concentration of immunoglobulin (IgM) it was observed an insignificant increase ($p>0.05$) with the increasing of the level of vitamin C. In conclusion, supplementation of vitamin C in the diet of Silurus glanis led to good results on fish welfare.

Key words*: Silurus glanis, Vitamin C, hematological profile, serum parameters.*

INTRODUCTION

According to Food and Agriculture Organization (2018), aquaculture continues to be the fastest-growing food sector worldwide. However, aquaculture continues to facing some issues such as diseases (Rahman et al., 2019), feed contamination, and environmental impacts (Crețu et al., 2016).

From all the factors which influence fish growth performance and welfare, in a recirculating aquaculture system, the quality of feed plays a very important role in fish metabolism and welfare (Martinez-Porchas & Martinez-Cordova, 2012). So, for fish, essential nutrients such as proteins, essential fatty acids, vitamins C and E, polysaccharides, and some minerals have pivotal importance to response on the growth, haematological and immunological parameters (Barrows et al, 2008).

In the literature, many research indicates that vitamin C is an important micronutrient that plays a significant role in fish growth by enhancing feed conversion efficiency, protein efficiency ratio (Ai et al., 2006; Eo and Lee, 2008; Alam et al., 2009; Cocan et al., 2018; Dicu et al., 2013), physiological stress (Farahi, 2012), improvement of some haematological parameters like plasma proteins, red blood cell (RBC) count, haematocrit (Hct) value and white blood cell (WBCs) count (Wang et al., 2003; Zhou et al., 2003; Affonso et al., 2007; Nsonga et al., 2009; Pimpimol et al., 2012).

Vitamin C must be supplied in the fish diet, because most of the fish species are unable to synthesize vitamin C since they do not have the enzyme L-gluconolactone oxidase which is responsible for synthesis from glucose (Oprea & Georgescu, 2000; Dabrowski, 2000; Trichet, 2015). Also, addition of vitamin C in fish diet proves to reduce the toxic effects of environmental pollutants on fish organism. Therefore, increasing the bioavailability of vitamin C may reduce the effects of environmental toxins on fish (Mehrpak et al., 2015).

The quantity of vitamin C which has to be added to the fish diet varies according to fish species, size, feeding rates, environmental

factors, nutrient interrelationships, health condition, water quality, feed formulation technique (Gouillou-Coustans and Kaushlic, 2000), culture conditions (NRC, 2011) respectively form of the vitamin C that is supplied (National Research Council, 2011). The addition of some concentrations of AA in the diet enhances the immune status and disease resistance in channel catfish (Li Y. et al., 1985), Indian major carp (Sahoo et al., 2003), rainbow trout (Fazaei et al., 2015), Japanese sea bass (Ai et al., 2004), Nile Tilapia (Mirea et al., 2013), Stellate sturgeon (Dicu et al., 2013), goldfish (Nica et al., 2016).
Therefore, the objective of this study was to evaluate the effects of dietary vitamin C levels on the haematological profile and some biochemical indices of European catfish reared in a recirculating aquaculture system.

MATERIALS AND METHODS

Experimental trial was conducted at the "Dunărea de Jos", University of Galați, in a recirculating aquaculture system (RAS). The RAS system was previously described in our earlier studies (Enache et al., 2011).
The experiment was conducted during five weeks period. 123 fish juveniles with an average weight of 118.96±0.43 g were randomly distributed to the rearing units of the RAS. The food used for the biomass culture was extruded pellets with a diameter of 4.3 mm an adequate content for the age of the fish (Table 1).

Table1. Chemical composition of feed

Parameter		Quantity
Crude protein		40%
Crude fat		11.5%
Crude ash		7.5%
Crude cellulose (fiber)		4%
Phosphorus		0.7%
Vitamin A		3000 (UI/kg)
Vitamin D		600 (UI/kg)

The ratio used during this experiment was 2% of the fish's body weight. The daily ratio was divided into two equal parts and was fed in the morning and in the evening
Four experimental groups were carried out: D_0-commercial feed, without vitamin C; D_1-commercial feed supplemented with 50 mg vitamin C kg^{-1}; D_2-commercial feed supplemented with 100 mg vitamin C kg^{-1} and D_3-commercial feed supplemented with 150 mg vitamin C kg^{-1}. Ascorbic acid (AA) used was provided from Janssen Chimica Company (Geel, Belgium) and had 99.9% purity. The incorporation technique of AA in commercial feed was described by Plăcintă et al., 2012.

Water quality. The water quality was monitored during the experiment. Dissolved oxygen and temperature were measured daily with Hanah HI 9147-04, the pH was measured with pH meter WTW model 340. One time per week N-NH_4^+, N-NO_2^-, N-NO_3^- concentrations were determined with Spectroquant Nova 400 photometer compatible with Merk kits.

Blood sampling and analysis. Fishes were anesthetized using 2-phenoxyethanol bath (8 ml 40 L^{-1} of water for 5 minutes) before taking blood from the fish. For blood collection, samples were taken at ten fish from each experimental variant from vena caudalis using syringes that previously were rinsed with lithium heparin. For serum collection, blood samples were taken without using anticoagulant and then transferred immediately to Eppendorf tubes. Blood samples taken were centrifuged at 3,500 rpm for 10 min to obtain serum.
The red blood cell counts (RBC × 10^6 μL^{-1}) were determined with Neubauer hemocytometer using a Potain pipette and Vulpian diluting solution.
For the haematocrit (Ht, %) determinations, blood samples (30 µL) were put in micro-haematocrit capillary tubes and then centrifugated for 5 min at 10,500 rpm. Measurements were made by microhematocrit reader and expressed as a percentage.
Haemoglobin (Hb, g dL^{-1}) concentration was determined by the cyanmethaemoglobin method by adding 20 µl of whole blood to 5 ml of Drabkins solution. The absorbance was measured using a spectrophotometer (Specord 210-Analytic Jena), at a wavelength of 540 nm.
The mean corpuscular volume (MCV, μm^3), the mean corpuscular haemoglobin (MCH, pg), and the mean corpuscular haemoglobin concentration (MCHC, g dL^{-1}) were calculated from the values of Ht, Hb, and RBC, according to Ghergariu et al. (1985).

Plasma glucose levels (GLU, mg dL^{-1}) was determined by the glucose oxidase method using a UV-Vis spectrophotometer at a wavelength of 635 nm.
Total protein (TP, g dL^{-1}) was determined according to the Biuret method. The absorbance was read at 546 nm wavelength. The immunoglobulin (IgM, mg dL^{-1}) levels were determinate by the method of Bakopoulos (1997).
Lipid peroxidation or malondialdehyde-MDA, (nmol mL^{-1}) level was determined from tissue, liver, kidney, intestine, and blood plasma, according to Draper & Hadley (1990) method, at an optical density of 532 nm.

Statistical analysis. All data are presented as mean ± standard deviation. Data were subjected to ANOVA test using SPSS 21 version. Before ANOVA, the normality of the data was verified by Kolmogorov-Smirnov test. When the ANOVA reveals a significant difference ($p<0.05$), Tukey's test was used for post-hoc multiple comparisons.

RESULTS AND DISCUSSIONS

The results obtained in our experiment for the haematological profile were corroborated with those of fish growth performance (Plăcintă et al., 2012). So, from the technological data at the end of the experiment, it was concluded that fish from the V_1 variant registered a better growth performance. The final weight of fish from the D_1 variant (172.52 g) was significantly higher ($p<0.05$) than those from the control variant (164.22 g), or from V_2 (162.86 g) or V_3 (164.67 g).
Also, in a RAS system water quality have a crucial role and any problems associated with it will result in deterioration of fish health. During the experiment, the water parameters remained in the normal ranges for the species (Bhatnagar et al., 2013) and showed no significant differences (ANOVA, $p>0.05$) across treatment diets. The means values of dissolved oxygen, temperature, and pH were 5.24±0.44 mg L^{-1}, 20.29±1.13ºC, respectively 7.77±0.14 pH units. Regarding the nitrate, nitrite, and ammonium concentrations the means values were 76.24±5.22 mg L^{-1}, 0.56±0.09 mg L^{-1}, respectively 0.77±0.12 mg L^{-1}.

In figures, 1-6 are presented the obtained values of the haematological parameters at the end of the experiment. The results obtained showed significant differences ($p<0.05$) in terms of the number of erythrocytes, haematocrit percentage, mean corpuscular volume, and the mean corpuscular haemoglobin concentration.
The *red blood cell count (RBC)* showed significant differences between the experimental groups (ANOVA, $p<0.05$).
Thus, the post hoc analysis of Tukey's showed that the values of erythrocytes from the control group (1.58±0.06 × 10^6 cells μL^{-1}) were not statistically different from those obtained in D_1 (1.52±0.12 × 10^6 cells μL^{-1}). Significantly higher values of RBC were registered in the D_2 (1.74±0.09 × 10^6 cells μL^{-1}) and at the D_3 variant (1.67±0.08 × 10^6 cells μL^{-1}) (Figure 1).

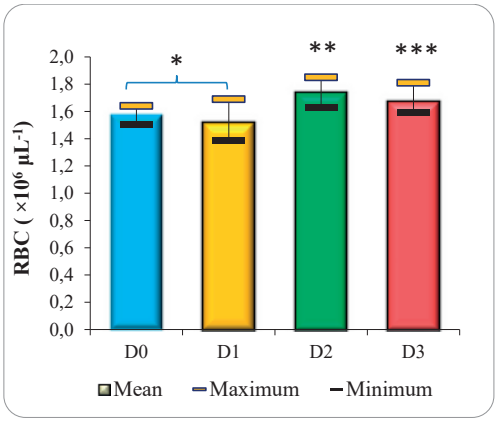

Figure 1. Mean, minimum and maximum values of RBC erythrocytes of *S. glanis*

Haematocrit percentage (Ht) showed significant differences ($p<0.05$) between the experimental variants. The haematocrit percentage from the variant D_3 (27.86±0.69%) was significantly higher than D_2 (26.20±0.78%), while no differences were recorded between the control group (22.02±0.6%) and D_1 (21.66±0.58%) (Figure 2).
The *haemoglobin concentration (Hb)* showed no statistical differences between the experimental variants. However, a slight increase in haemoglobin concentration was registered in the D_3 variant (9.10±0.27 g dL^{-1}), while in the D_0, D_1, and D_2 the mean value of the haemoglobin concentration was 8.64±0.39

g dL^{-1}, 8.42±0.6 g dL^{-1}, 8.52±0.46 g dL^{-1} (Figure 3). Analysing the results of the erythrocyte constants, it can be observed that the administration of vitamin C in feed has induced significant differences ($p<0.05$) in MCV and MCHC.

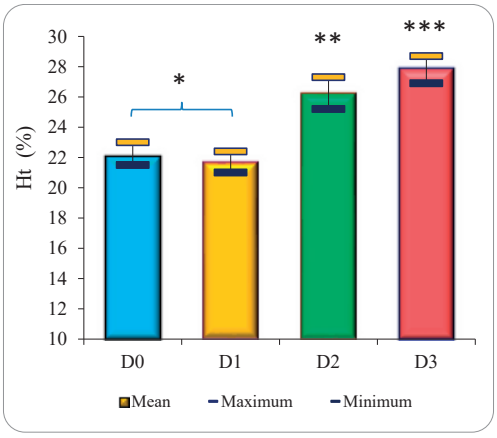

Figure 2. Mean, minimum and maximum values of the hematocrit of *S. glanis*

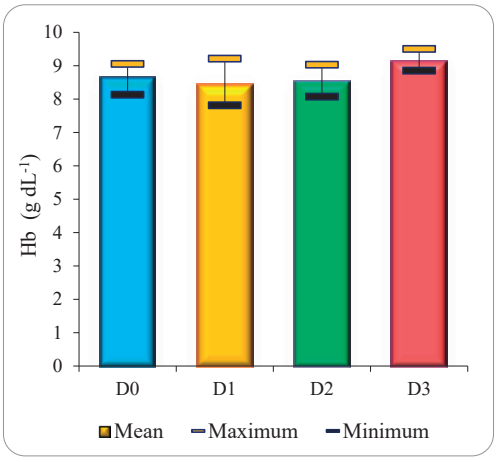

Figure 3. Mean, minimum and maximum values of haemoglobin of *S. glanis*

Regarding *the mean corpuscular volume (MCV)* values the post hoc analysis of Tukey's test showed that the MCV value from D_3 (167.21±1.02 μm^3) was significantly higher in comparison with those from the D_0 (139.63±8.91 μm^3), D_1 (143.56±11.31 μm^3), and D_2 (154.94±5.43 μm^3) (Figure 4).

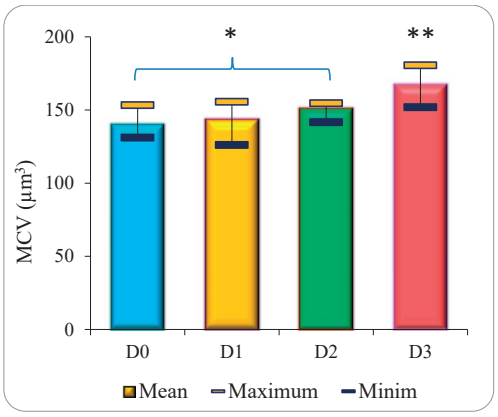

Figure 4. Mean, minimum and maximum values of MCV of *S. glanis*

The *mean corpuscular haemoglobin (MCH)* indicates the erythrocyte loading with haemoglobin. The MCH values were 54.67±0.49 pg in D_0, 55.95±7.04 pg in D_1, 49.20±4.92 pg in D_2, and 54.60±3.16 pg in D_3, without significant differences (ANOVA, $p>0.05$) between the four experimental variants (Figure 5).

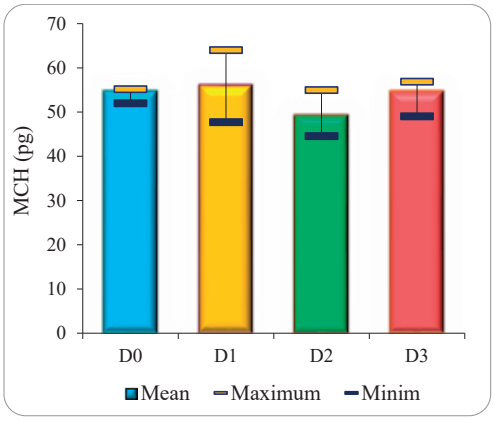

Figure 5. Mean, minimum and maximum values of MCH of *S. glanis*

The mean corpuscular haemoglobin concentration (MCHC) was significantly (ANOVA, $p<0.05$) influenced by the administrated concentration of vitamin C. The post hoc analysis showed that the values of MCHC from the D_2 (32.57±2.66 mg dL^{-1}) and D_3 (32.68±1,17 mg dL^{-1}) were significantly lower than those from D_0 (39.29±2.72 mg dL^{-1}) and D_1 (38.95±3.50 mg dL^{-1}) (Figure 6).

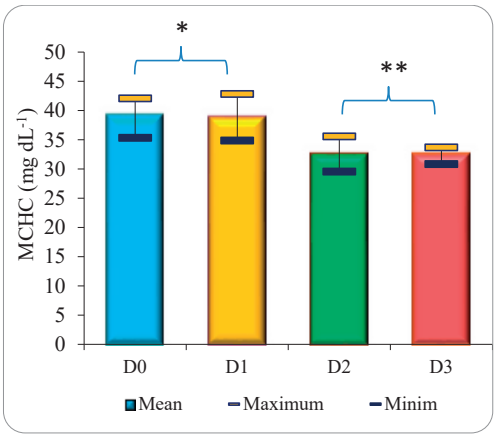

Figure 6. Mean, minimum and maximum values of MCHC of *S. glanis*

Haematological analyses may provide a reflection of the health status of fish (Docan et al., 2011). In our experiment the haematological results obtained for *Silurus glanis* are in the line with those presented in the literature (Table 2).

Table 2. Normal values of haematological profile of *Silurus glanis*

Haematological parameters	Docan et al., 2010	Docan et al., 2011
RBC (10^6 µL^{-1})	1.36 ± 0.17	1.41±1.77
Ht (%)	22.30 ± 2.7	27.1±2.66
Hb (g dL^{-1})	7.33 ± 0.88	6.82±0.73
MCV (µm^3)	165.66± 5.06	194.28±24.4
MCH (pg)	54.43 ± 8.05	48.67±4.66
MCHC (mg dL^{-1})	32.97 ± 2.38	25.25±2.5

In our study, the administration of vitamin C in the *Silurs glanis* food led to the improvement of the haematological profile. Similar results were obtained by Falatkar (2005) in the case of *Huso huso.* Also, Pimpimol et al. (2012), reported in the case of Mekong giant catfish (*Pangasianodon gigas*) an improvement of haematological profile when the fish feed was supplemented with 500 and 750 mg kg^{-1} vitamin C of feed. According to Sahoo and Mukherjee, (2003) increasing RBC values is due to the powerful antioxidant action of vitamin C that protects various tissues of fish, including RBC, against oxidative damage. Since RBC are involved in the control of immune functions (Madhusudan et al., 2015) and are the main production sites of free radicals, the increase of the number of erythrocytes can be attributed to the administration of vitamin C, respectively to the improvement of the oxidative state of fish. Also, according to Dinning (1962), the addition of vitamin C in the fish diet led to the increase of erythropoiesis.

In Table 3 are presented the mean values±SD for the blood serum at the end of the experimental period.

Table 3. The values of glucose, total proteins, and immunoglobulins at the end of the experimental period

Exp. var.	GLU (mg dL^{-1})	TP (g dL^{-1})	IgM (mg dL^{-1})
D_0	69.80±6.18	3.60±0.20	13.55±0.54
D_1	77.40±3.78	3.46±0.28	13.46±0.72
D_2	74.40±3.97	3.56±0.30	14.03±0.52
D_3	73.40±6.66	3.73±0.22	14.03±0.35
ANOVA	0.26	0.52	0.47

In the present study, blood glucose, total proteins, and immunoglobulins levels showed no significant changes ($p<0.05$) between the experimental variants. However, it was observed a slightly decreasing trend of serum glucose with the increasing concentration of vitamin C in the fish feed, while the TP and IgM, showed a slight decrease.

According to some authors addition of high quantities of vitamin C in fish feed can enhance protein synthesis (Andrade et al., 2007). Pimpimol et al., 2012 reported also a slightly increased after 8 weeks of experiment of total serum protein in Mekong giant catfish fed high vitamin C concentrations (500 and 750 mg vitamin C kg^{-1} feed).

According to Jiang et al. (2013), MDA is widely used indicator of oxidative damage to lipids of cell membrane by lipid peroxidation, which is accompanied by the reduction in antioxidant capacity. Lipid peroxidation evaluations in the present research were determined from muscular tissue, kidney, liver, intestine, and blood serum (Figure 7). Significant differences (ANOVA, $p<0.05$) were recorded between the obtained values in all the experimental variants. The post hoc tests showed that MDA values from kidney and liver from D_1 and D_2 variants were significantly ($p<0.05$) lower than those from D_0 and D_3.

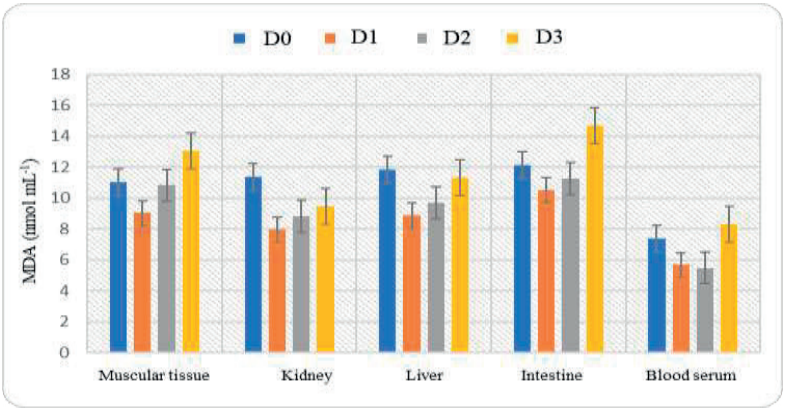

Figure 7. The mean values ± SD of MDA for muscular tissue, kidney, liver, intestine, and blood serum at the end of the experiment

In the case of MDA from the muscular tissue, intestine and blood serum it was observed an intensification of oxidative stress in the case of the highest dose of vitamin C (D3). Malondialdehyde is widely used as an indicator of lipid peroxidation (Esterbauer et al., 1991) and increased levels of MDA are associated with a variety of chronic diseases (Allen-Gil and Martynov, 1995). The improvement of oxidative stress was obtained at lower concentrations of vitamin C (50, 100 mg kg^{-1}), observing an intensification of MDA at a concentration of 150 mg kg^{-1}. However, the values of lipid peroxidation were within the same range of other fresh water fishes (Antache et al., 2013).

Generally, the use of vitamins as feed additives is recommended in the diets of farmed fish. Several studies reported that the addition of vitamin C on fish feed had a positive effect on the haematological parameters by increasing RBC, Ht, or Hb concentration (Zafar and Khan, 2020).

In our study, a significant increase of RBC and Ht was found with increasing of vitamin C concentration (100 mg AA kg^{-1} diet and 150 mg AA kg^{-1} diet), indicating that vitamin C protected the RBC membranes from oxidation and improved the ability of oxygen transport. Also, a positive effect on oxidative stress was observed in the variants where vitamin C was supplemented in fish feed at concentration of 50 and 100 mg kg^{-1} feed.

CONCLUSIONS

The results of the present research suggested that the dietary incorporation of vitamin C exerts a positive effect on the haematological profile and oxidative capacity at a dose between 50-100 mg kg^{-1}. However, more research is needed in order to determine the optimum dietary requirement of vitamin C for *Silurus glanis* according to the age and size of the fish, or the breeding system.

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REFERENCES

Ai, Q., Mai, K., Tan, B., Xu, W., Zhang, W., Ma, H., & Liufu, Z. (2006). Effects of dietary vitamin C on survival, growth, and immunity of large yellow croaker (*Pseudosciaena crocea*). *Aquaculture*, 261, 327–336.

Ai, Q., Mai, K., Zhang, C., Xu, W., Duan, Q., Tan, B., & Liufu, Z. (2004). Effects of dietary vitamin C on growth and immune response of Japanese seabass, *Lateolabrax japonicus*. *Aquaculture*, 242, 489–500.

Affonso, E.G., Silva, E., Tavares-Dias, M., Menezes, C. de Carvalho, É. Nunes, D., Ituassú, R., Roubach, E. Ono, J.F., & Marcon, J. (2007). Effect of high levels of dietary vitamin C on the blood responses of matrinxa (*Brycon amazonicus*). *Comparative Biochemistry and Physiology, Part A,* 147, 383–388.

Alam, M.J., Mustafa, M.G., & Khaleque, M.A. (2009). Evaluations of the effects of different dietary vitamin C levels on the body composition, growth performance and feed utilization efficiencies in Stinging Catfish, *Heteropneustes fossilis* (Bloch, 1792). *Journal of American Science*, 5, 31–40.

Allen-Gil, S.M., & Martynov, V.G. (1995). Heavy metal burdens in nine species of freshwater and anadromous fish from the Pechora River, northern Russia. *Science of the Total Environment*, 160–161, 653–659.

Andrade, J.I.A., Ono, E.A., Menezes, G.C., Brasil, E.M., Roubach, E.M., Urbinati, R., Tavares-Dias, M., Marco, J.L., & Affonso, E.G. (2007). Influence of diets supplemented with vitamins C and E on pirarucu (*Arapaima gigas*) blood parameters. *Comparative Biochemistry and Physiology*, 146, 576–580.

Antache, A., Cristea, V., Grecu, I.R., Dediu, L., Docan, A. I., Crețu, M. M., Plăcintă, S., & Coadă, M.T. (2013). The influence of some phytobiotics on oxidative stress at *Oreochromis niloticus* grown in an intensive recirculating aquaculture system. *Lucrări Ştiinţifice-Seria Zootehnie,* 59, 253–257.

Bakopoulos, V., Volpatti, D., Papapanagiotou, E., Richards, R.H., Galeotti, M., & Adams, A. (1997). Development of an ELISA to detect *Pasteurellapiscicida* in culture and in fish tissue. *Aquaculture,* 156(3/4).

Barrows, F.T., Gaylord, T.G, Sealey, W.M., Porter, L., & Smith, C.E. (2008). The effect of vitamin premix in extruded plant-based and fish meal-based diets on growth efficiency and health of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 283,148–155.

Bhatnagar, A., & Devi, P. (2013). Water quality guidelines for the management of pond fishculture. *International Journal of Environmental Sciences*, *3*(6), 1980–2009.

Cocan, D., Popescu, F., Latiu, C., Uiuiu, P., Coroian, A., Raducu, C., Coroian C.O., Miresan, V., Kokkinakis, A., & Constantinescu, R. (2018). Effects of thermal stress on hematological and metabolic profiles in Brown bullhead, Ameiurus nebulosus (LESUEUR, 1819). *AgroLife Scientific Journal*, 7(1), 33–41.

Crețu, M. Dediu, L., Cristea, V., Zugravu, A., Turek Rahoveanu, M. M., Bandi, A.C., Turek Rahoveanu, A., & Mocuta, D.M. (2016). Environmental Impact of Aquaculture: A Literature Review. *International business information management Assoc-IBIMA, Innovation Management and Education Excellence Vision 2020: Regional Development to Global Economic Growth,* 3379–3387.

Dabrowski, K. (2000). *Ascorbic acid in aquatic organisms: status and perspectives*. Washington, DC, USA: CRC press Publishing House.

Dicu (Stroe), M. D., Cristea, V., Mirea (Ciortan), C., Plăcintă, S., Petrea, M.Ş., & Coadă, M.T., (2013). Effects of Different Levels of Dietary Vitamins C on Growth Performance of Stellate Sturgeon (*Acipenser stellatus,* Pallas, 1771). *Animal Science and Biotechnologies*, 46, 244–249.

Dinning, J.S. (1962). Nutritional requirements for blood cell formation in experimental animals. *Physiological Reviews*, 42, 169–180.

Docan, A., Cristea, V., Grecu, I., & Dediu, L. (2010). Haematological response of the European cat fish, *Silurus glanis* reared at different densities in "flow-through" production system. *Archiva Zootechnica, 13*(2), 63–70.

Docan, A., Cristea, V., Dediu, L., & Grecu, I. (2011). Hematological parameters as indicators of toxic stress produced by mycotoxin food contamination in the european catfish (*Silurus glanis* L.). *Journal of Environmental Protection, 12*(4), 1898-1903.

Draper, H.H., & Hadley, M., (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology,* 186, 421–431.

Enache, I., Cristea, V., Ionescu, T., & Ion., S. (2011). The influence of stocking density on the growth of common carp, *Cyprinus carpio*, in a recirculating aquaculture system. *Aquaculture, Aquarium, Conservation & Legislation International Journal of the Bioflux Society,* 4(2), 146-153.

Eo, J., & Lee, K. (2008). Effect of dietary ascorbic acid on growth and nonspecific immune responses of tiger puffer, *Takifugu rubripes*. *Fish Shellfish Immunology*, 25, 611–616.

Esterbauer, H., Schaur, R.J., & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology & Medicine*, 11, 81.

Falahatkar, B. (2005). The effect of dietary vitamin C on some of hematologhc biochemistry and growth indexes of great sturgeon (*Huso huso*). Ph. D. Thesis, *Tarbiat Modaarres University*, Tehran, Iran.

Farahi, A., Kasiri, M., Sudagar, M., Soleimani, I.M., & Zorriehzahra, S.M.J. (2012). Effect of dietary supplementation of *Melissa officinalis* and *Aloe vera* on hematological traits, lipid oxidation of carcass and performance in rainbow trout (*Oncorhynchus mykiss*). *Online Journal of Animal and Feed Research*, *2*(1), 1–5.

Fazaei, Z., Sajjadi, M. M., Sourinejad, I., & Asadi, R. (2015). Vitamin C supplementation to rainbow trout (*Oncorhynchus mykiss*) diet and analysis of growth indices, survival and carcass composition at two different stocking densities. *Journal of Veterinary Research*, *70*(1), 55–62.

Food and Agriculture Organization- FAO. (2018). The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome.

Gouillou-Coustans, M.F., & Kaushlic, S. J. (2000). *Ascorbic acid requirement in freshwater and marine fish: Is there a difference*. In: Ascorbic Acid in Aquatic Organisms, (pp. 49-68). Dabrowski, K. (ed.). Washington, DC, USA: CRC press Publishing House.

Ghergariu, S., Pop, A., & Kadar, L. (1985). *Veterinary clinical laboratory guide*. Bucharest, RO: Ceres Publishing House, 82–90.

Jiang, W., Liu, Y., Jiang, J., Hu, K., Li, S., Feng, L., & Zhou, X. (2013). In vitro interceptive and reparative effects of myo-inositol against copper-induced oxidative damage and antioxidant system disturbance in primary cultured fish enterocytes. *Aquatic Toxicology*, 132–133, 100–110.

Li, Y., & Lovell, R.T. (1985). Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. *Journal of Nutrition*, 115, 123–131.

Madhusudan, R.N., Kodimyala, R., Rudra, R.R., Venkateswara, J. R., & Ghousia, B. (2015). The role of vitamin C as antioxidant in protection of biochemical and haematological stress induced by chlorpyrifos in freshwater fish *Clarias batrachus. Chemosphere,* 132, 172–178.

Martinez-Porchas, M., & Martinez-Cordova, L.R. (2012). World Aquaculture: Environmental Impacts and Troubleshooting Alternatives. *The Scientific World Journal*, 1–9.

Mirea, C.C, Cristea, V., Grecu, I.R., Dediu, L., & Vasilean, I. (2013). Hematological and biochemical characterization of Nile tilapia (*Oreochromis niloticus,* Linnaeus, 1758) reared intensively in a recirculating aquaculture system in relation to water temperature. *Scientific Papers: Animal Science and Biotechnologies*,46 (2), 234–237.

Mehrpak, M., Banaee, M., Haghi, B.N., & Noori, A. (2015). Protective Effects of Vitamin C and Chitosan against Cadmium-Induced Oxidative Stress in the Liver of Common Carp (*Cyprinus carpio*). *Iranian Journal of Toxicology*, 9(30),1360–1367.

Nica, A., Vasilean, I., Popescu, A., & Ibănescu, D.C. (2016). Effects of vitamin C supplementation on growth performance of goldfish (*Carassius auratus*). *Scientific Papers-University of Agricultural Sciences and Veterinary Medicine, Animal Husbandry Series,* 66, 170–173.

National Research Council (NRC), 2011. Nutrient Requirements of Fish and Prawn, pp 207-209. Washington, DC, USA: National Academies Press Publishing House.

Oprea, L., & Georgescu, R. (2000). *Nutrition of fish*, (272 p). Bucharest, RO: Tehnică Publishing House.

Placintă, S., Cristea, V., Cretu M. M., Antache, A., Coadă, M. T., & Petrea, S. M. (2012). Effects of Dietary Vitamin C Supplementation on Growth Performance and Tissue Chemical Composition of *Silurus Glanis* (Linnaeus, 1578). *Animal Science and Biotechnologies*, 45(2), 88–92.

Pimpimol, T., Phoonsamran, K., & Chitmanat, C. (2012). Effect of dietary vitamin C supplementation on the blood parameters of Mekong giant catfish (*Pangasianodon gigas*). *International Journal of Agriculture and Biology*, 14, 256–260.

Rahman, M.D.A., Akter, S., Mominuzzaman, M.K., & Rahman, M.D.K. (2019). Relation between aquaculture with fish disease & health management: A review note. *Bangladesh Journal of Fisheries*, *31*(2), 253–260.

Sahoo, P.K., & Mukherjee, S.C. (2003). Immunomodulation by dietary vitamin C in healthy and aflatoxin B1-induced immunocompromised rohu (*Labeo rohita*). *Comparative Immunology, Microbiology & Infectious Diseases*, 26, 65–76.

Trichet, V.V., Santigosa, E., Cochin, E., & Gabaudan, J. (2015). The Effect of Vitamin C on Fish Health. *Dietary Nutrients, Additives, and Fish Health*, 151–171.

Wang, X., Kim, K., Bai, S.C., Huh M., & Cho, B. (2003). Effects of the different levels of dietary vitamin C on growth and tissue ascorbic acid changes in parrot fish (*Oplegnathus fasciatus*). *Aquaculture*, 215, 203–211.

Zhou, X., Xie, M., Niu, C., & Sun, R. (2003). The effects of dietary vitamin C on growth, liver vitamin C and serum cortisol in stressed and unstressed juvenile soft-shelled turtles (*Pelodiscus sinensis*). *Comparative Biochemistry and Physiology*, 135, 263–270.

Zafar, N., & Khan, M.A. (2020). Effect of dietary iron on growth, haematology, oxidative stress and hepatic ascorbic acid concentration of stinging catfish, *Heteropneustes fossilis*. *Aquaculture,* 516, 1–9.