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Effects of Diet Supplementation with Zinc Enriched Yeast on Blood Indices and some Biochemical Parameters in Rainbow Trout (*Oncorhynchus mykiss*)

Ahmad Gharekhani*, Ghobad Azari Takami*, Amir Tukmechi**, Mohammad Afshar Nasab and Naser Agh*******

**Department of Health, Aquatic Animal Health and Disease, Faculty of Specialized Veterinary Science, Science and Research Branch, Islamic Azad University, Tehran, IRAN*
***Department of Pathobiology and Quality Control, Artemia and Aquatic Animals Research Institute, Urmia University, Urmia, IRAN*
****Iranian Fisheries Research Organization (IFRO) Head of Shrimp Health and Disease Department, Tehran, IRAN*
*****Department of Ecology and Nutrition, Artemia and Aquatic Animals Research Institute, Urmia University, Urmia, IRAN*

(Corresponding author: Amir Tukmechi)
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ABSTRACT: Zinc (Zn) is an essential trace element in all living organisms, and the first eukaryotic zinc uptake transporter was discovered in the yeast, *Saccharomyces cerevisiae*. Zinc-enriched yeast is considered zinc (Zn) supplements currently available. The purpose of the investigation was to compare and evaluate the effect of zinc enriched yeast blood indices and biochemical parameters in rainbow trout. The fish (mean body weight 10 ± 0.5 g) were fed a commercial diet supplemented with 0 (control), 1×106, 1×107 and 1×108 CFU/g of Zn-enriched yeast for 60-days. Blood samples were taken from the onset and at day 60 of feeding supplemented diet for measuring the blood parameters in rainbow trout. Results showed that significant increase in white blood cells count was seen in all treatment groups during feeding trial when compared to the control group. As well as results documented that diet supplementation with Zn-enriched yeast altered some biochemical parameters in rainbow trout. On the basis of our findings, Zn-enriched yeast could change blood indices and biochemical parameters in rainbow trout.

Keywords: Rainbow trout, zinc enriched yeast, blood indices, biochemical parameters.

INTRODUCTION

Zinc (Zn) is an essential trace element for all living organisms, its role in biology was first recognized by Raulin in 1869 (Prasad, 2009). It acts as a co-factor for a large number of proteins and enzymes (Schneider, 2013). Also, Zinc affects many aspects of the immune system and it is essential for normal development and function of immunity such as phagocytosis, intracellular killing and cytokines production (Prasad, 2009). Zinc also functions as an antioxidant and anti inflammatory agent. Zinc requirements of fish are difficult to determine because fish may in part utilize trace elements that are present in solution. However, zinc is not taken from water in sufficient amounts to meet the needs of fish and most therefore be supplied by the diet to prevent deficiencies. In rainbow trout an adequate zinc content of the diet was estimated to be 15-30 mg per kg. This trace element is readily absorbed from the gastro-intestinal tract, gills, fins and skin of fish. Dietary zinc availability and absorption is reduced in the presence of phytates, and high dietary intakes of calcium, phosphorus and copper (Bury *et al.,* 2006). Zinc could be supplemented in diets as inorganic mineral salts, typically as Zinc oxide or Zinc (Strnadov *et al.,* 2011). Usually, the organic forms of trace minerals have higher bioavailability than inorganic forms. In addition, organic forms of them are less toxic and more environmental friendly than inorganic forms (Yang *et al*., 2012).

Probiotics are live microbial feed supplements with beneficial effects on host by producing inhibitory compounds, competition or chemicals and adhesion sites, immune modulation and stimulation, and improving the microbial balance (Tukmechi *et al.,* 2011). *Saccharomyces cerevisiae* contains various immune stimulating compounds such as β-glucan, nucleic acids, mannan oligosaccharides and chitin, and has been proved to enhance the immune responses and growth in fish (Gopalakannan and Arul, 2010). Many studies of the processes involved in the uptake of trace elements by the *Saccharomyces cerevisiae* have increased considerably in recent years. This yeast has become a model microorganism for studying metal transporters and their accumulation in the cells.

Saccharomyces cerevisiae is known for its ability to accumulate metal ions from aqueous solutions by different physico-chemical interactions, e.g. by adsorption and absorption, or by a metabolism-dependent mechanism (Stehlik-Tomas *et al.,* 2004). Production of yeast biomass rich in organically bound Zn is important to the animal industry because such forms of Zn are readily absorbed by the animal. In a study with growing heifers, found that those fed Zn methionine gained weight 8.1% faster and 7.3% more efficiently (Shet *et al.,* 2011).

Hematological parameters changes would be sign of fish physiological responses against environmental stresses e.g. such as pH alteration, salinity of water pollution or bacterial infections (Zorriehzahra, 2010). Thus important internal organs such as kidney, spleen, liver and pancreas that have important duty in fish physiology must be affected acutely by infectious pathogens. Therefore hematological changes would occur subsequently in response to the invading pathogens. In many cases of fish infectious diseases diagnosis could be assisted by hematological study.

There is no information on the effects of dietary zinc enriched yeast in aquaculture industry. Also, no information is available on the effects of zinc enriched *Saccharomyces cerevisiae* in rainbow trout. Therefore, the objectives of this study were to determine the dietary zinc enriched yeast on rainbow trout blood indices and biochemical parameters.

MATERIALS AND METHODS

A. Preparation of Zinc enriched yeast

The zinc nitrate used for the enrichment of Saccharomyces cerevisiae (PTCC 5269) was purchased from Sigma, USA. The growth media used was yeast extract, peptone and glucose (YEPD) which was obtained from Merck, Germany. In present work, Wang *et al.* (2010) method was used for enrichment of yeast with zinc. Briefly, *Saccharomyces cerevisiae* was cultured in 90 ml of YEPD medium at 27ºC, Ph = 5.8 and 160 rpm for 12 h on a shaker incubator (Biotech, South Korea). Then zinc nitrate was added into the medium at concentration of 10 mg ml^{-1} and yeast incubation was continued for 24 h at the same condition. The cells were harvested by centrifugation (3000 rpm for 15 min) and then washed with normal saline to removing the additional zinc nitrate. Finally, Zn-enriched cells were adjusted to the desired concentrations and used for feeding to fish. Atomic absorption spectrophotometer (AAS) (Shimadzu Scientific Instrument Inc., USA) was used to analyze zinc content in yeast cells and diet.

B. Experimental design

Rainbow trout (10 ± 0.5 g) were purchased from a commercial fish farm in Urmia, Iran. Acclimatization to the laboratory condition was performed for 10 days in 1000 L tanks using aerated free-flowing well water that had the following characteristics: temperature (15 ± 1ºC); pH (7.5); dissolved oxygen (8 ± 0.2 mg/l); natural photoperiod (10 h light/14 h dark); flow rate (1.25 l/s). Fish were fed three times daily with commercial fish feed (40% protein), 3% of average initial body weight per day.

C. Diet preparation and feeding trial

Commercial basal diet (Faradaneh, Iran) was used in this study; three experimental diets were formulated to be supplemented at 1×106, 1×107 and 1×108 CFU per g of diet. After spraying the Zn-enriched yeast on commercial feed, pellets were dried at room temperature for 2 h and then the diets were stored at 4°C until use. Fish were divided into 4 groups (in triplicate) of 40 animals distributed per tank and were fed with zinc enriched yeast for a 60-day period. Study continued for 15 days and during this time all fish fed with the control diet without Zn-enriched yeast supplementation. On days 0 and 60, a sample of three individuals per tank (nine per treatment) was taken to measure hematological and biochemical parameters.

D. Hematological indices

Fish were anesthetized with 200 mg/l clove oil, then blood was collected from cardinal vein using heparin coated syringe and transferred into sterile tubes. The blood were allowed to clot at room temperature for 1 h and stored in a refrigerator overnight. The clot was then centrifuged at 1500 × g for 5 min. Then the serum was collected and stored in sterile eppendorf tubes at -20ºC until use for biochemical assays. Also, blood collected by caudalvein puncture in heparinized syringes. Hematocrit values (Ht) were determined by centrifuging fresh blood in heparinised glass capillary tubes for 5 min. Hemoglobin (Hb) level was determined colorimetrically by measuring the formation of cyanomethemoglobin using a commercial kit (Pars Azmoon, Tehran, Iran). Red blood cells (RBCs) and white blood cells (WBCs) were counted under a light microscope using a Neubauer hemocytomter after dilution with phosphate buffered-saline (PBS). Deferential leukocyte counts (neutrophil, lymphocyte and monocyte) were determined using blood smears under a light microscope. Cells were identified on the basis of morphology and cell ultra structure as documented in previous fish leukocyte studies (Jalali *et al.,* 2009).

E. Biochemical parameters

In this study glutathione peroxidase activity, alkaline phosphatase (ALP), Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic-pyruvic transaminase (SGPT) were assayed in collected serum samples. Glutathione peroxidase (GPx) activity was assayed following the rate of NADPH oxidation at 340 nm by the coupled reaction with glutathione reductase. The specific activity was determined using the extinction coefficient of 6.22 mM/cm. Glutathione reductase activity was determined spectrophotometrically, measuring NADPH oxidation at 340 nm.

One unit of CAT, GPx, or GR activity is defined as the amount of the enzyme that consumes 1 µmol of substrate or generates 1 µmol of product per min; activity was expressed in international units per ml. An automatic blood enzyme analyzer (Hitachi 704) was used for the following determinations: Alkaline phosphatase, Serum glutamic oxaloacetic transaminase and Serum glutamic-pyruvic transaminase. The apparatus is based upon dry chemical technology and colorimetric reaction. Kits obtained Pras Azmoon, Iran, were used for the determination of all parameters.

F. Statistical analysis

The results were subjected to analysis of variance (ANOVA) followed by Tukey's test to compare different treatments using the SPSS (ver. 19) correlation coefficients were significant with $P< 0.05$.

RESULTS

Hematocrit, hemoglobin and total erythrocyte count were not significantly affected by the addition of Zn-enriched *Saccharomyces cerevisiae* to the rainbow trout diets ($P>0.05$) in any treatment group. Total leukocytes, neutrophil and monocytescounts were significantly higher in the 1×108 CFU/g of Zn-enriched yeast group ($P<0.05$) than in the control group, but a significant decrease was shown in the lymphocyte count in this group in comparison with the control ($P<0.05$) (Table 1 and 2).

Table 1: Hematological parameters in rainbow trout fed diets with Zn-enriched yeast. Each value (X ± SD) is the average performance of nine fish per treatment at start of the study.

Hematological parameters	Control	T1	T2	T3
Hematocrit (%)	34 ± 2.8^{a}	34 ± 3.03^{a}	33.1 ± 1.6^{a}	34 ± 4.55^{a}
Hemoglobin (g/dl)	9.61 ± 0.1^{a}	9.58 ± 0.1^{a}	9.83 ± 0.2^{a}	9.71 ± 0.1^{a}
RBC (10^6 cell/mm^3)	1.33 ± 0.1^{a}	1.31 ± 0.18^{a}	1.41 ± 0.12^{a}	1.32 ± 0.18^{a}
WBC (10^3 cell/mm^3)	5.11 ± 0.2^{a}	5.2 ± 0.31^{a}	5.1 ± 0.31^{a}	5.4 ± 0.09^{a}
Lymphocyte	69.7 ± 8.1^{a}	70.11 ± 6.2^{a}	71.33 ± 1.2^{a}	71.54 ± 6.4^{a}
Neutrophil	20.31 ± 2.1^{a}	20.76 ± 1.9^{a}	20.81 ± 1.41^{a}	21.05 ± 1.7^{a}
Eosinophil	3.21 ± 0.1^{a}	2.89 ± 0.2^{a}	3.2 ± 0.6^{a}	3.4 ± 0.3^{a}
Monocyte	7.48 ± 1.11^{a}	6.24 ± 1.6^{a}	4.66 ± 1.4^{a}	3.94 ± 1.2^{a}
Basophile	0	0	0	0

The same superscript alphabets in the same row are not significantly different at $P<0.05$.
(T1: 1×10^6, T2: 1×10^7 and T3: 1×10^8 CFU/g of commercial pellet)

Table 2: Hematological parameters in rainbow trout fed diets with Zn-enriched yeast. Each value (X ± SD) is the average performance of nine fish per treatment at day 60.

Hematological parameters	Control	T1	T2	T3
Hematocrit (%)	35.67 ± 0.9^{a}	36.1 ± 1.64^{a}	36.46 ± 2.33^{a}	36.79 ± 1.32^{a}
Hemoglobin (g/dl)	12.22 ± 0.23^{a}	12.41 ± 0.26^{a}	12.5 ± 0.31^{a}	12.67 ± 1.4^{a}
RBC (10^6 cell/mm^3)	1.38 ± 0.23^{a}	1.38 ± 0.51^{a}	1.41 ± 0.19^{a}	1.46 ± 0.67^{a}
WBC (10^3 cell/mm^3)	5.44 ± 0.31^{a}	6.33 ± 0.4^{a}	6.57 ± 0.8^{a}	6.82 ± 0.2^{a}
Lymphocyte	60.7 ± 4.1^{a}	60.51 ± 3.2^{b}	59.39 ± 4.78^{c}	58.54 ± 3.6^{d}
Neutrophil	25.77 ± 2.8^{d}	26.34 ± 2.5^{c}	26.51 ± 1.41^{b}	27.9 ± 2.1^{a}
Eosinophil	1.42 ± 0.2^{a}	1.76 ± 0.4^{a}	2 ± 0.1^{a}	1.8 ± 0.17^{a}
Monocyte	12.11 ± 1.4^{c}	11.39 ± 1.34^{b}	12.1 ± 1.2^{ab}	11.76 ± 1.3^{a}
Basophile	0	0	0	0

The same superscript alphabets in the same row are not significantly different at $P<0.05$. (T1: 1×10^6, T2: 1×10^7 and T3: 1×10^8 CFU/g of commercial pellet)

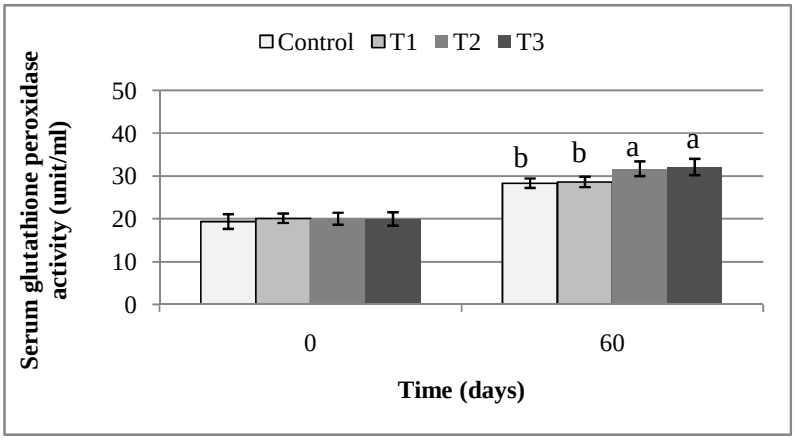

Fig. 1. The glutathione peroxidase activity of rainbow trout fed with Zn-enriched *Saccharomyces cerevisiae*.

Each value (X ± SE) is the average performance of nine fish per treatment. Significance at P<0.05. Fish fed the Zn-enriched yeast supplemented diet showed a significant increase in glutathione peroxidase activity on day 60 compared with the control group (Fig. 1). No significant differences in the serum glutathione peroxidase activity were observed between groups at the start of the study. Biochemical blood plasma profiles of the control and experimental group are documented in Table 3. ALP, SGOT and SGPT were not significantly affected by addition of Zn-enriched yeast to diets (P>0.05) in all treatment groups.

Table 3: Biochemical parameters in rainbow trout fed diets with Zn-enriched yeast. Each value (X ± SD) is the average performance of nine fish per treatment during the study.

Treatment	ALP (unit/ml)		SGOT (unit/ml)		SGPT (unit/ml)	
	Day 0	Day 60	Day 0	Day 60	Day 0	Day 60
Control	88 ± 4.54 [a]	103 ± 1.87 [a]	189 ± 3.98 [a]	199 ± 7.21 [a]	22 ± 1.09 [a]	27 ± 1.2 [a]
T1	89 ± 5.11 [a]	107 ± 1.9 [a]	191 ± 4.22 [a]	202 ± 7.4 [a]	21 ± 1.8 [a]	26 ± 1.1 [a]
T2	91 ± 1.2 [a]	105 ± 2.95 [a]	185 ± 5.41 [a]	197 ± 6.43 [a]	20 ± 1.3 [a]	27 ± 1.05 [a]
T3	90 ± 3.12 [a]	104 ± 3.3 [a]	189 ± 5.31 [a]	201 ± 7.1 [a]	22 ± 1.21 [a]	25 ± 2.1 [a]

The same superscript alphabets in the same column are not significantly different at P<0.05. (T1: 1×10^6, T2: 1×10^7 and T3: 1×10^8 CFU/g of commercial pellet)

DISCUSSION

In the present study the supplementation of Zn-enriched *S. cerevisiae* in all treatments altered white blood cell counts in rainbow trout. This result agree with at obtained with administration of whole cell yeast, Zinc methionine and zinc sulfate as sources of dietary zinc in fresh water fish and crustaceans (Tan and Mai, 2001; Tukmechi et al., 2011). The increased rainbow trout white blood cells in treatment groups may possibly be due immune stimulation. As documented in this study highest amount of WBC was shown in 1×108 CFU per g of diet Zn-enriched yeast group. In this treatment whole yeast cell breaks in rainbow trout digestive system and organic zinc release to the lumen and improve host immunity (Pappagianis *et al.*, 2004). The results in Table 2 concerning the effects of Zn-enriched yeast thereof and a basal diet are not supported by the findings of some researchers such as Kobeisy and Hussein (1995), who showed that hemoglobin (Hb) and hematocrit concentrations of *O. Nileoticus* were increased in direct proportion to the percentage of dietary live yeast. They attributed this improvement to the effect of B vitamins in the yeast. Lunger *et al*. (2006) also showed that the PCV% was significantly affected by inclusion of the yeast. Similarly, the PCV value in the present study may be due to a decrease of hemolysis as a result of a high B12 in yeast-treated fish. To the contrary, Siwicki *et al*. (1994), Li and Gatlin (2003), Berge *et al*. (2005) and Lunger *et al*. (2006) reported that the dietary yeast had no significant effect on hematocrit of the fish.

Statistical analysis of hematological findings revealed significant differences (P<0.05) using t-test. In the determination of total WBC count, of 1×108 CFU of Zn-enriched yeast per g of diet significant differences (P<0.05) were observed when compared with the control group. In the fish, anti genic agents such as bacteria or virus, in the first stage the non-specific defense system (cellular) was first stimulated. In these status, first of all the leucocytes will be initially increased in order to protect the fish body with phagocytosis mechanism and produce antibacterial or antiviral chemicals to stop the agent from spreading.

In this study blood serum enzymes analysis revealed that glutathione peroxidase activity ALP, SGOT and SGPT amount not showed obvious significant differences when compared with control (P<0.05).

It is concluded that Zn-enriched *Saccharomyces cerevisiae* positively influenced WBC count and hematological parameters of rainbow trout. In addition, the results of biochemical parameters assays demonstrated that Zn-enriched yeast could be administered for relatively long periods without causing any side effects.

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REFERENCES

Berge, G.M., Baeverfjord, G., Skrede, A. and Storebakken, T. (2005). Bacterial protein grown on natural gas as protein source in diets for Atlantic salmon, Salmo salar, in saltwater. *Aquaculture.* **244**: 233-40. http://dx.doi.org/10.5713/ajas.2013.13200.

Bury, N.R., Walker, P.A. and Glover, C.N. (2006). Nutritive metal uptake in teleost fish. *The Journal of Experimental Biology*. **206**: 11-23. http://dx.doi.org/10.1242/jeb.00068.

Gopalakannan, A. and Arul, V. (2010). Enhancement of the innate immune system and disease-resistant activity in Cyprinus carpio by oral administration of b-glucan and whole cell yeast. *Aquaculture Research.* **41**: 884-892. http://dx.doi.org/10.1111/j.1365-2109.2009.02368.x.

Jalali, M.A., Ahmadifar, E., Sudagar, M. and Azari Takami, G. (2009). Growth efficiency, body composition, survival and haematological changes in great sturgeon (*Huso huso* Linnaeus, 1758) juveniles fed diets supplemented with different levels of Ergosan. *Aquaculture Research.* **40**: 804-809. http://dx.doi.org/10.1111/j.1365-2109.2009.02166.x.

Kobeisy, M.A. and Hussein, S.Y. (1995). Influence of dietary live yeast on growth performance and some blood constituents in Oreochromis Nileoticus. *In the Proceedings of the 5th Scientific Conference of Animal Nutrition, 1995, Ismailia, Egypt*, **1**: 417-625.

Li, P. and Gatlin, D.M. (2003). Evaluation of brewer's yeast (*Saccharomyces cerevisiae*) as a feed supplement for hybrid striped bass (*Morone chrysops* × *M. saxatilis*). *Aquaculture.* **219**: 681-692. http://dx.doi.org/10.1016/S0044-8486(02)00653-1.

Lunger, A.N., McLean, E. and Craig, S.R. (2007). The effects of organic protein supplementation upon growth, feed conversion and texture quality parameters of juve Nilee cobia (*Rachycentron canadum*). *Aquaculture.* **264**: 342-52. http://dx.doi.org/10.1016/j.aquaculture.2006.12.012

Papagiannis, I., Kagaloub, I., Leonardos, J., Petridis, D. and Kalfakakou, V. (2004). Copper and zinc in four freshwater fish species from Lake Pamvotis (Greece). *Environment International.* **30**: 357-362. http://dx.doi.org/10.1016/j.envint.2003.08.002.

Prasad, A.S. (2009). Zinc: role in immunity, oxidative stress and chronic inflammation. *Current Opinion Clinical and Nutrition Metabolic Care.* **12**: 646-652. http://dx.doi.org/10.1097/MCO.0b013e3283312956

Schneider, T., Persson, D.P., Husted, S., Schellenberg, M., Gehrig, P., Lee, Y., Martinoia, E., Schjoerring, J.K. and Meyer, S. (2013). A proteomics approach to investigate the process of Zn hyper accumulation in Noccaea caerulescens. *The Plant Journal.* **73**: 131-142. http://dx.doi.org/10.1111/tpj.12022

Shet, A.R., Patil, L.R., Hombalimath, V.S., Yaraguppi, D.A. and Udapudi, B.B. (2011). Enrichment of *Saccharomyces cerevisiae* with zinc and their impact on cell growth. Biotechnology and Bioinformatics Bioengineering 1(4): 523-527.

Siwicki, A.K., Anderson, D.P. and Rumsey, G.L. (1994). Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Veterinary Immunology and Immunopathology*. **41**: 125-139. http://dx.doi.org/10.1016/0165-2427(94)90062-0

Stehlik-Tomas, V., Gulan Zeti, V., Stanzer, D., Grba, S. and Vahi, N. (2004). Zinc, copper and manganese enrichment in yeast Saccharomyces cerevisiae. *Food Technology and Biotechnology*. **42**(2): 115-120.

Strnadov, P., Svobodova, V., Pavlata, L., Misurova, L. and Dvorak, R. (2011). Effect of inorganic and organic zinc supplementation on coccidial infections in goat kids. *Acta Veterinary Brno.* **80**: 131-137. http://dx.doi.org/10.2754/avb201180020131.

Tan, B. and Mai, K. (2001).. Zinc methionine and zinc sulfate as sources of dietary zinc for juvenile abalone, *Haliotisdiscus hannai* Ino. *Aquaculture.* **192**: 67-84. http://dx.doi.org/10.1016/j.aquaculture.2013.04.026

Tukmechi, A., Rahmati Andani, H.R., Manaffar, R. and Sheikhzadeh, N. (2011). Dietary administration of beta-mercapto-ethanol treated *Saccharomyces cerevisiae* enhanced the growth, innate immune response and disease resistance of the rainbow trout, Oncorhynchus mykiss. *Fish and Shellfish Immunology*. **30**: 923-928. DOI: 10.1016/j.fsi.2011.01.016. Epub 2011 Feb 3.

Wang, Z., Zhang, L. and Tan, T. 2010. High cell density fermentation of *Saccharomyces cerevisiae* GS2 for selenium-enriched yeast production. *Engineering*. **27**: 1836-1840. http://dx.doi.org/10.1007/s11814-010-0300-x.

Yang, R., Meng, F.C., Wang, P., Jiang, Y.B., Yin, Q.Q., Chang, J., Zuo, R.Y., Zheng, Q.H. and Liu, J.X. (2012). Effect of organic and inorganic selenium supplementation on growth performance, meat quality and antioxidant property of broilers. *African Journal of Biotechnology*. **11**(12): 3031-3036. http://dx.doi.org/10.5897/AJB11.3382

Zorriehzahra, M.J., Hassan, M.D., Gholizadeh, M. and Saidi, A.A. (2010). Study of some hematological and biochemical parameters of Rainbow trout (*Oncorhynchus mykiss*) fry in western part of Mazandaran province, Iran. *Iranian Journal of Fisheries Sciences.* **9**(1): 185-198.