www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 16: 617-627 (2016) DOI: 10.4194/1303-2712-v16_3_14

RESEARCH PAPER

Comparative Studies of Some Haematological and Serological Indices in Rainbow Trout (*Oncorhynchus mykiss* Walbaum., 1792) with Ichthyophthiriasis

Kaveh Azimzadeh[1,*]

[1] Young Researcher and Elite Club, Urmia Branch, Islamic Azad University, Urmia, Iran.

* Corresponding Author: Tel.: +98 9143409583;
E-mail: kn_az@yahoo.com

Received 05 December 2015
Accepted 29 April 2016

Abstract

The present study was aimed to reveal whether Ichthyophthiriasis induces plasma parameters alterations in rainbow trout. Thereby, total sialic acid (TSA), adenosine deaminase (ADA), heat shock protein-27(HSP-27), homocysteine (Hcy), cholinesterase (CholE) activity level and some blood parameters were studied in suffered ones. After diagnosis of severe Ichthyophthiriasis, blood samples were collected from seventy infected and same numbers of healthy ones. The results indicated significant increases ($P<0.01$) in TSA, Hcy, HSP-27, CholE, MDA, Urea, CREA, AST and TPP in plasma along with a remarkable decrease in ADA, glucose (GL), catalase(CAT), glutathione peroxidase (GPx), paraoxonase (PON) and zinc in the infected group compared with healthy ones. In hematology, thrombocytosis, neutrophilia, monocytosis and eosinophilia along with anemia were clarified in infected group than healthy ones.

The obtained results suggested that Ichthyophthiriasis in rainbow trout develops considerable blood profiles alterations and some of them may utilize in the part of fish medicine.

Keywords: Ichthyophthiriasis, rainbow trout, blood parameters.

Introduction

Ichthyophthiriasis (white spot disease, Ich) commonly involves in aquaculture parasitic diseases of fresh water fish in subtropical, tropical and temperate zones which plays an important role in severe mortality and economic detriments. It belongs to ciliate protozoan parasite (as ectoparasite) (*Ichthyophthirius multifiliis*) and is known to be the one of most pathogenic parasites of fish (Mifsud and Rowland, 2008). Crowding stress facilitates Ich occurrence and subsequently leads to innate immune suppress. On the other word, Ich causes fish more prone to bacterial diseases (Witeska *et al*., 2010). Ich-mediated hematological and serological studies are scarce. However, Witeska study in 2010 clarified alterations in hematological indices such as leukocytosis in fish with minor symptoms, leukopenia in ones with severe symptoms along with lack of changes in Hb and PCV during *Ichthyophthirius multifiliis* infection in common carp. In addition, multiple histopathological changes in different tissues reported by Yu in 2012 in grass carp with experimentally infected *Ichthyophthirius multifiliis*. Also, RBC and WBC significant changes have been reported by Ghiraldelli in 2006 in the parasitized Nile tilapia and cultured carp with ectoparasites such as Ciliophora.

Sialic acid (SA) is known to be the one of derivatives of neuraminic acid as acetylated derivative that is widely distributed in all vertebrate tissues, body fluids and in higher invertebrate species (Guzel *et al*., 2008). It perches the terminal location on macromolecules such as glycoproteins and cell membranes (Nazifi *et al*. 2010). Furthermore, it is known as an inflammatory marker (Thougaard *et al*., 1998) and so, the determination of SA may be a valuable indicator for diagnosis and prognosis of inflammatory diseases. (Guzel *et al*., 2008; Citil *et al*., 2004). In many inflammatory and infectious diseases of domestic animals, SA values has been analyzed (Karagenc *et al*., 2005), but, no assessment has been observed regarding SA levels in fish parasitic diseases. Heat Shock Proteins (HSPs) were initially identified in different *Drosophila* tissues (Arrigo and Laundry, 1994 ; Tissieres *et al*., 1974) and also an elevation in their amounts has been reported during undesirable conditions such as ischemia, hypoxia, and stress factors in plasma. It is worth mentioned that among them, small heat shock proteins such as HSP-27 possesses special important. (Guay *et al*., 1997; Mymrikov *et al*., 2011). Hcy, as a sulfur-containing

amino acid, is generated from the intracellular demethylation of methionine (Clarke, 2002) It is involved in endothelial cell damage in experimental animals. Hyperhomocysteinemia participates in oxidative stress occurrence and plays essential role in pathological effects, which has been demonstrated as a mechanism involved in the formation of anaemia (Nazifi *et al*., 2013). Cholinesterases (CholE) are comprised into two main enzymes (acetylcholinesterase, AchE; and butyrylcholinesterase, BChE) and involve in hydrolysis of acetylcholine (ACh). Recently they were determined as inflammatory markers (Da Silva *et al*., 2012). Adenosine deaminase (ADA) catalyzes degradation of adenosine and deoxyadenosine to inosine and deoxyinosine and also is known as a substantial enzyme in the maturation and differentiation of T lymphocytes and its activity is higher in T cells than B cells (Hoshino *et al*., 1994 ; Aydin *et al*., 2010). The ADA activity has been determined higher in diseases with immune response stimulation. (Aydin *et al*., 2010).

To our knowledge, although a little study has reported alterations of different parameters in *Oncorhynchus mykiss* diseases, but no assessment has been performed to investigate the alterations of above mentioned parameters in Rainbow trout Ichthyophthiriasis.

Materials and Methods

Examination of Ich

This study was conducted in Urmia city, Iran during summer 2012. Seventy rainbow trout with Ich from eight aquaculture farms (high crowded) were identified by observation of severe infestation to parasites on the head, fin and gill with white-spot appearance and ciliate protozoan (*Ichthyophthirius multifiliis*) were observed with the horseshoe-shaped nucleus by light microscope. The same numbers without any Ich signs and any other diseases symptoms were selected as the healthy group. Also, we tried to be identical all selected farms based on management, nutrition and geographical conditions.

Preparation of Blood Samples

Anesthesia was not carried out in the fish for its probable effects on blood parameters. (McKnight 1966). Blood samples were collected from all fish via caudal vein into EDTA–contained tubes for hematologic tests and plasma preparation. Thereafter, all tubes were centrifuged with 4000 RPM for 10 minutes at room temperature.

Hematology Indices Determination (CBC)

Hemoglobin (Hb) measurement was performed according to cyano-methemoglobin method with spectrophotometer at 540 nm absorbance (spectrophotometric method). For packed cell volume (PCV) identification, standard micro hematocrit method was used. White blood cells (WBC) and red blood cells (RBC) count were carried out with Dacie`s solution as a diluting fluid. (Blaxhall and Daisley 1973). Determination of erythrocyte indices including mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined by (Haney *et al*., 1992) method. Moreover, differential count was performed for leukocyte percentage identification. Blood smears were stained with Giemsa solution 5% and specification of each cell percentage were counted in one hundred cells.

Plasma Biochemical Parameters Detection

ADA activity was determined by the Mybiosource Elisa kit, HSP-27 determination was measured based on a commercial ELISA kit (Biosource, U.S.A), Hcy and cholinesterase activity (Pars azmoon Co.kits, Tehran, Iran) were measured using a Cecil, Italy in accordance with the spectrophotometric method MDA and PON activity was detected by Satoh (1978) and Furlong (1988) method respectively (spectrophotometer, model Cecil, Italy). SOD, CAT, GPx activities were assessed in erythrocyte hemolysis based on manual methods, Abei (1984) for CAT and commercial kits (Ransod® and Ransel, Randox laboratories Ltd. G.B) for SOD and GSH-Px (Auto analyzer, Alcyon-300, USA) as well as, GL, Urea, CREA, TPP, AST, ALT detected by commercial kits (Pars azmoon., Chemical co., Tehran, Iran) with Auto analyzer, Hitachi- 917, Japan. Finally, TSA was determined by Sydow (1985) method (spectrophotometric, Spekol-1500).

Statistical Analysis

Statistical analysis was accomplished in all analyses. Mean ± SD and determination of variation between the data results were carried out with Student's *t*-test with SAS version 9.1 and significance level was specified at ($P<0.01$)

Results

Significant alterations in the majority of plasma biochemical profiles and CBC indices were detected in infected rainbow trout compared to control group ($P\leq0.01$), which have been documented in Table 1 and Table 2 respectively. Considerable increase of TSA, Hcy, HSP-27 and CholE were determined in Ich group rather than control ones. In the case of nitrogen metabolism, an increase in Urea and CREA and TPP was observed. Furthermore, considerable decrease of GL and ADA along with significant elevation of AST, as the liver function index, were exhibited in diseased

Table 1. The following table reveals all parameter alterations in naturally infected groups compared with control ones

Parameter	Control Group 1 (Length) 221 ± 6 mm / Control Group 1 (Weight) 252 ± 19 g	Control Group (Number)	Control Group 2 (Length) 277 ± 6 mm / Control Group 2 (Weight) 369 ± 13 g	Control Group 2 (Number)	Naturally infected Group 1 (Length) 217 ± 4 mm / Naturally infected Group 1(Weight) 248 ± 22 g	Naturally infected Group 1 (Number)	Naturally infected Group 2 (Length) 271 ± 5 mm / Naturally infected Group 2 (Weight) 358 ± 17 g	Naturally infected Group 2 (Number)
TSA (mg/dl)	11.21 ± 0.58	37	14.12 ± 0.61	33	23.14 ± 1.31†	39	41.15 ± 1.48¥	31
MDA(nmol/ml)	1.22 ± 0.72	37	2.34 ± 0.92	33	4.10 ± 1.02†	39	6.45 ± 1.11¥	31
Hcy(mg/dl)	2.07 ± 0.2	37	3.17 ± 0.6	33	7.08 ± 1.14†	39	10.05 ± 1.32¥	31
ADA (U/L)	13.09 ± 0.11	37	15.08 ± 0.14	33	3.23 ± 0.14†	39	5.28 ± 0.19¥	31
HSP-27 (ng/ml)	2.59 ± 0.23	37	4.22 ± 0.16	33	5.89 ± 0.43†	39	10.02 ± 0.79¥	31
CholE (IU/L)	1114.26 ± 52.03	37	1537.58 ± 75.13	33	3309.35 ±32.28†	39	5310.18 ±62.55¥	31
PON(U/L)	38.34 ± 2.48	37	71.52 ± 4.21	33	13.13 ± 1.79†	39	32.46 ± 2.57¥	31
SOD(U/gHb)	721.14 ±76.13	37	717.11 ± 89.15	33	739.12 ± 36.02	39	747.11 ± 63.04	31
CAT(k/gHb)	61.10 ± 2.25	37	82.13 ± 2.74	33	30.29 ± 1.07†	39	41.32 ± 1.25¥	31
GSH-Px(U/mgHb)	52.11 ± 2.19	37	79.18 ± 3.52	33	19.69 ± 1.09†	39	30.23 ±2.13¥	31
TAC(mmol/L)	0.37 ± 0.07	37	0.58 ±1.13	33	0.13 ± 0.05†	39	0.28 ±0.07¥	31
GL(mg/dl)	29.11 ± 0.8	37	40.22 ± 0.79	33	16.15 ± 1.36†	39	27.04 ± 1.13¥	31
CREA(mg/dl)	0.3.02 ± 0.17	37	0.7 ± 0.22	33	1.11 ± 0.06†	39	2.28 ± 0.12¥	31
UREA (mg/dl)	2.21 ± 0.92	37	3.31 ± 0.2	33	5.43 ± 0.86†	39	6.39 ± 1.1¥	31
TPP(g/dl)	2.24 ± 0.04	37	3.12 ±0.07	33	3.32 ±0.16†	39	4.30 ±0.21¥	31
AST (U/l)	47.12 ± 17.13	37	68.10 ± 19.22	33	121.52 ± 9.07†	39	152.39 ± 14.11¥	31
ALT (U/l)	21.23 ± 3.36	37	26.66 ± 2.44	33	51.30 ± 2.13†	39	58.51 ± 1.38¥	31
Zinc (µg/dl)	115. 11 ± 2.66	37	146.18 ± 4.5	33	50.13 ± 1.17†	39	76.18 ± 1.46¥	31

Table 2. The following table denotes hematological alterations in Ichthyophthiriasis compared with healthy ones

Parameters	Control Group	*Ichthyophthirius multifiliis* infected Group
PCV(%)	36.2 ± 2.2	21.2 ± 2.3†
Hb (g/dl)	11.3 ± 2.1	5.2 ± 0.5†
RBC (µl/10^6)	4.1 ± 0.1	1.89 ± 0.2†
MCV (fl)	275± 38	261± 26
MCH (pg)	61.5 ± 6.4	65.3 ± 2.3
NEU (%)	19.4 ± 2.1	39.6 ± 4.2†
LYM (%)	71.6 ± 2.6	37.8 ± 3.8†
MONO (%)	3.6 ± 1.4	10.8 ± 2.6†
EOS (%)	5.3 ± 1.2	11.8 ± 4.1†
PLT (10^3/µL)	32.6 ± 9.7	69.2 ± 4.8

Data are expressed as mean ± standard deviation. †Significantly different from the control group (P<0.01)

fish compared with control ones. In the respect of oxidative stress, remarkable increase in MDA and reduction of antioxidant enzymes were observed in infected fish. According to the hematological results, PCV, Hb amount and RBC count decreased ($P \leq 0.01$). Neutrophilia, lymphopenia, monocytosis and eosinophilia occurred in each group than healthy ones. Moreover, Pearson`s correlation revealed considerable interaction ($P<0.01$) (Tables 3, 4, 5, 6) between fish length, weight and all biochemical parameters in exception of SOD in *Ichthyophthirius multifiliis* naturally infected groups and suggest that increase of fish length and weight affect in all biochemical profiles of naturally infected groups.

Pearson`s Correlation Test Between Fish Weight, Length and Biochemical Parameters

***Ichthyophthirius multifiliis* Naturally Infected Group 1 (Length)**

ADA possesses significant correlation between length in naturally infected Group 1 as negative correlation. With increasing the length, the activity of ADA compared to the observed correlation coefficient decrease and vice versa. In respect of other parameters, there are not significant correlation between those ones.

***Ichthyophthirius multifiliis* Naturally Infected Group 1 (Weight)**

ADA activity ALT activity and TSA have significant correlations between weight in naturally infected Group 1 as positive correlation. It means that an increase in weight of the fish, causes elevation of those ones in naturally infected Group 1.

***Ichthyophthirius multifiliis* Naturally Infected Group 2 (Length)**

There is not significant correlation between parameters and length in naturally infected Group 2.

***Ichthyophthirius multifiliis* Naturally Infected Group 2 (Weight)**

MDA, TPP, TSA and ADA activity possess significant correlations between weight in naturally infected Group 2. It means that an increase in weight of the fish, take place elevation of them in naturally infected Group.

Table 3. Pearson`s Correlation Analysis between Length of Fish with Parameters in *Ichthyophthirius multifiliis* naturally infected Group 1

Length	Analysis	Parameters	
.123	Correlation Coefficient	TSA	1
.455	Sig		
.132	Correlation Coefficient	MDA	2
.424	Sig		
.030	Correlation Coefficient	Hcy	3
.857	Sig		
-.365*	Correlation Coefficient	ADA	4
.022	Sig		
.117	Correlation Coefficient	HSP	5
.479	Sig		
.148	Correlation Coefficient	CholE	6
.368	Sig		
-.079	Correlation Coefficient	PON	7
.634	Sig		
.077	Correlation Coefficient	SOD	8
.642	Sig		
.151	Correlation Coefficient	CAT	9
.358	Sig		
-.047	Correlation Coefficient	GSH	10
.775	Sig		
.149	Correlation Coefficient	TAC	11
.365	Sig		
-.069	Correlation Coefficient	GL	12
.678	Sig		
.201	Correlation Coefficient	CREA	13
.220	Sig		
.082	Correlation Coefficient	UREA	14
.620	Sig		
.029	Correlation Coefficient	TPP	15
.862	Sig		
-.277	Correlation Coefficient	AST	16
.088	Sig		
.082	Correlation Coefficient	ALT	17
.620	Sig		
-.054	Correlation Coefficient	Zinc	18
.742	Sig		

Table 4. Pearson`s Correlation Analysis between Weight of Fish with Parameters in *Ichthyophthirius multifiliis* naturally infected Group 1:

Weight	Analysis	Parameters	
.389*	Correlation Coefficient	TSA	1
.015	Sig		
.255	Correlation Coefficient	MDA	2
.116	Sig		
.042	Correlation Coefficient	Hcy	3
.799	Sig		
.375*	Correlation Coefficient	ADA	4
.019	Sig		
.134	Correlation Coefficient	HSP	5
.416	Sig		
-.258	Correlation Coefficient	CholE	6
.113	Sig		
.043	Correlation Coefficient	PON	7
.795	Sig		
-.078	Correlation Coefficient	SOD	8
.638	Sig		
.249	Correlation Coefficient	CAT	9
.126	Sig		
.256	Correlation Coefficient	GSH	10
.116	Sig		
.063	Correlation Coefficient	TAC	11
.705	Sig		
.120	Correlation Coefficient	GL	12
.465	Sig		
.100	Correlation Coefficient	CREA	13
.543	Sig		
.164	Correlation Coefficient	UREA	14
.317	Sig		
.196	Correlation Coefficient	TPP	15
.233	Sig		
.190	Correlation Coefficient	AST	16
.245	Sig		
.432**	Correlation Coefficient	ALT	17
.006	Sig		
-.110	Correlation Coefficient	Zinc	18
.506	Sig		

The results in Table 2 indicate that a significant level of correlation coefficient for TSA and ADA is equal to 0.015 and 0.019. So, ADA and TSA possess significant correlations between weight in naturally infected Group 1. On the base of the observed correlation coefficient (0.389) and (0.375) it follows that the correlation between these two variables is positive, meaning that an increase in weight of the fish, happen elevation of TSA and ADA in naturally infected Group 1. Also, further studies showed that with 99% confidence level, there is a significant correlation between ALT activity and weight of the fish and more than 18% shared variance between these two variables. In respect of other parameters, there are not significant correlation between those ones.

Discussion

Sialic acid (SA) may assign valuable marker for diagnosis and prognosis of inflammatory diseases and is associated with acute phase proteins. (Motoi *et al*. 1984). Also, SA concentration has been identified to be high in the course of many diseases. (Citil *et al*. 2004). In present study, TSA levels elevated in infected group than non-infected ones. We could not find any information in literature about SA levels in rainbow trout with Ich. TSA elevation could be ascribed to acute phase proteins enhancement, as acute phase proteins, such as α1-acid glycoprotein, are sialyted glycoproteins. (Motoi *et al*. 1984). Furthermore, relationship between SA and innate immune system of skin mucous is discussed. The fish skin mucous is known to be the major first line defense against pathogens. (Ellis, 2001). Multiple components such as the glycoproteins, lysozyme, immunoglobulin, antimicrobial peptides, lectins, C-reactive protein, are aggregated in fish skin mucous as innate immune system (Shephard, 1994). On the other hand, skin mucous secretions of various fish species contain sialic acid and sialated glycoproteins constitute in skin mucous ingredients. (Sumi *et al*. 1997). It is worth mentioning that they involve in inhibitation and protection against bacterial break down and virus invasion (Nigam *et al*. 2012). Since SA is roboustly linked to bacterial macromolecules and bacteria, thereby impede their adherance to the epithelial cells. (Traving and Schauer 1998). Perhaps, skin mucous SA is bounded with *Ichthyophthirius multifiliis* for hindering its penetration. Therefore, SA is elevated in blood for compensation of SA in skin mucous.

Plasma MDA is known as indicator of lipid peroxidation and oxidative stress index (Elia *et al*. 2003). *I. multifiliis* infection in silver catfish provokes oxidative lipid damage in the liver, and concurrent enhancement in lipid peroxidation products levels with progression of the infection was revealed in silver catfish. Garcia *et al*. (2011). In fish with *I. multifiliis* infection immune responses appeared by localization of epidermal infiltration of granulocytes and neutrophils (Matthews *et al*., 2005). During

Table 5. Pearson`s Correlation Analysis between Length of Fish with Parameters in *Ichthyophthirius multifiliis* naturally infected Group 2

Length	Analysis	Parameters	
-.072	Correlation Coefficient	TSA	1
.699	Sig		
-.001	Correlation Coefficient	MDA	2
.995	Sig		
.234	Correlation Coefficient	Hcy	3
.205	Sig		
.112	Correlation Coefficient	ADA	4
.550	Sig		
-.112	Correlation Coefficient	HSP	5
.550	Sig		
.032	Correlation Coefficient	CholE	6
.866	Sig		
.196	Correlation Coefficient	PON	7
.290	Sig		
-.041	Correlation Coefficient	SOD	8
.825	Sig		
-.043	Correlation Coefficient	CAT	9
.820	Sig		
.116	Correlation Coefficient	GSH	10
.534	Sig		
.195	Correlation Coefficient	TAC	11
.292	Sig		
.180	Correlation Coefficient	GL	12
.331	Sig		
-.041	Correlation Coefficient	CREA	13
.827	Sig		
.050	Correlation Coefficient	UREA	14
.791	Sig		
.237	Correlation Coefficient	TPP	15
.200	Sig		
.225	Correlation Coefficient	AST	16
.224	Sig		
-.006	Correlation Coefficient	ALT	17
.973	Sig		
-.006	Correlation Coefficient	Zinc	18
.973	Sig		

There is not significant correlation between parameters and length in naturally infected Group 1.

inflammatory processes due to macrophages and neutrophils activation, the production of hydrogen peroxide (H2O2) and superoxide (O2 −) are increased, which promote the formation of ROS and free radicals, which can lead to host tissue damage (Jones *et al*., 2000). In present study, serum MDA increased to more than three-fold in rainbow trout with *I. multifiliis* infection which demonstrates that this parasite induces oxidative stress. Arabi, (2005) and Vutukuru *et al*. (2006) revealed MDA increase in *Oncorhynchus mykiss* gill hemogenate and visceral tissue of *Esumus danricus*, respectively. However, some studies have reported low levels of MDA in rainbow trout and Teleost (Ates, *et al*., 2008; Varghese *et al*., 2000). In present study, antioxidant enzymes (CAT, GSH-Px and TAC) were determined to be low. It postulated that they impress on oxidative stress. (Garcia *et al*., 2011) demonstrated larger degree of protection against *Ichthyophthirius multifiliis* through higher antioxidant enzymes activity in silver catfish which is not in accordance with our study.

Low activity of ADA in Ich group may contribute to high level of adenosine. Adenosine is a strong anti-inflammatory agent that regulates the function of cells participated in the inflammatory reactions. On the other word, it inhibits neutrophil-mediated injury to endothelial cells and modulates leukocyte– endothelial interactions (Cronstein *et al*., 1986 ; Rodriguez *et al*., 2012). Consistent with this, the plausible serum ADA activity decrease in infected group may ascribe to disease conditions, wherein could be attributed to the necessity for high concentration of extracellular adenosine for attenuating of Ich mediated-skin endothelial cells inflammation. This process also leads to decreased intracellular ADA activity and consequently reduction in the serum. In addition, our study revealed a remarkable decrease in serum zinc in the infected group compared to non-infected ones. Zinc possesses cellular antioxidant effect (Powell, 2000) and an association between zinc and cellular-immunity (T-cell) has been already determined. Zinc is considered to have an essential role in the immune system (Shankar and Prasad, 1998) and its shortage is strongly disadjusted immune system function, mainly cellular immunity (T-lymphocytes) (Hönscheid *et al*., 2009). Moreover, zinc cation is found in the innermost region of the active site of ADA and contributes to the catalytic mechanism with enzyme residues (Cooper *et al*., 1997; Sideraki *et al*., 1996). Since, zinc plays an important role in ADA

Table 6. Pearson`s Correlation Analysis between Weight of Fish with Parameters in *Ichthyophthirius multifiliis* naturally infected Group 2

Weight	Analysis	Parameters	
.487**	Correlation Coefficient	TSA	1
.006	Sig		
.394*	Correlation Coefficient	MDA	2
.028	Sig		
.078	Correlation Coefficient	Hcy	3
.676	Sig		
.476**	Correlation Coefficient	ADA	4
.007	Sig		
.228	Correlation Coefficient	HSP	5
.216	Sig		
.051	Correlation Coefficient	CholE	6
.785	Sig		
.347	Correlation Coefficient	PON	7
.056	Sig		
.353	Correlation Coefficient	SOD	8
.052	Sig		
.300	Correlation Coefficient	CAT	9
.101	Sig		
.196	Correlation Coefficient	GSH	10
.290	Sig		
.325	Correlation Coefficient	TAC	11
.075	Sig		
.216	Correlation Coefficient	GL	12
.244	Sig		
-.020	Correlation Coefficient	CREA	13
.917	Sig		
.131	Correlation Coefficient	UREA	14
.484	Sig		
.400*	Correlation Coefficient	TPP	15
.026	Sig		
.060	Correlation Coefficient	AST	16
.750	Sig		
.110	Correlation Coefficient	ALT	17
.556	Sig		
.110	Correlation Coefficient	Zinc	18
.556	Sig		

The results in Table 4 indicate that a significant level of correlation coefficient for MDA and TPP is equal to 0.028and 0.026. So, MDA and TPP possess significant correlations between weight in naturally infected Group 2. On the base of the observed correlation coefficient (0.394) and (0.400) it follows that the correlation between these two variables is positive, meaning that an increase in weight of the fish, take place elevation of MDA and TPP in naturally infected Group 2. Also, further studies showed that with 99% confidence level, there is a significant correlation between TSA, ADA activity and weight of the fish and more than 23% and 22% shared variance between these two variables. In respect of other parameters, there are not significant correlation between those ones.

activity.Thus, low ADA activity during the course of Ich might be related to hypozincemia.

Heat shock proteins play a major role in inhibition of stress effects on cells (Aşkar *et al*., 2007). HSP-27 is known to be a stress protein and has an antioxidant effect that is associated to both the decrease of iron quantity in cell and the increase of the intracellular glutathione level (Ferns *et al*., 2007;). Several conditions are involved in high production of HSP-27, such as apoptosis, vascular diseases, hyperthermia, and different types of cancers (Ciocca *et al*., 1993).There is no literature about HSP-27 values in rainbow trout with Ichthyophthiriasis. HSP-27 role, as a biomarker in various diseases, has been reported (Vidyasagar *et al*., 2012).Moreover, HSP-27 overexpression attenuates concanavalin A-induced liver damage in mice (Bao and Liu, 2009). This might have been the case in this study as high levels of HSP-27 in the infected ones may be attributed to the elevation of protein synthesis in cells under stressful conditions, such as fever and inflammation for defending against disease.

High level of Hcy was determined in Ich group compared with the healthy ones. It is involved in cardiovascular diseases, oxidative stress generation and endothelial cell damage. (Jacobsen, 2000). Hcy is considered to cause endothelial dysfunction and damage as its deleterious effects on vessels has been characterized (Thambyrajah and Townend, 2000). On the other word, Nazifi *et al*., in 2012 demonstrated deleterious effect of Hcy on erythrocytes that can lead to anemia. Hence, in present study, high level of Hcy may demolish erythrocytes and leading to anemia in Ich group. In addition, the zinc cation possesses key role in Hcy metabolism. The betaine-homocysteine methyl transferase (BHMT) and methionine synthase are known as essential enzymes in Hcy metabolism. These enzymes belong to zinc-containing metalloenzymes and the binding of Hcy to BHMT is carried out by zinc. Moreover, Hcy is activated through zinc for conversion to methionine (Heidarian *et al*., 2009). The zinc deficiency can describe Hcy enhancement. Furthermore, direct effect of possible *Ichthyophthirius multifiliis* toxins on methionine cycle is hypothesized.

Cholinesterases (CholE) are reputed to be as an

inflammatory marker and their high activities have been identified in different diseases (Das, 2007). Wolkmer, 2010 reported alterations in cholinesterase activity in trypanosomosis and *Staphylococcus aureus* infection and ascribed to clinical signs and immune response. In addition, direct relevance between cholinesterases (AChE and BChE) activity and inflammatory markers such as interleukin-6 has been documented, thereby forenamed result assert the concept that cholinesterase activity boost the systemic inflammatory response (Ben Assayag *et al*., 2010). In present study, serum cholinesterase level was found to be high. Since, the cholinesterase is identified as an inflammatory marker, its enhancement may reflect elevated inflammatory responses during Ich. Moreover, liver damage could describe this increase, because it is commonly synthesized in hepatocytes and liberated into blood. It is worth mentioning that remarkable increase of CholE activity is used in discrimination between liver and non-liver diseases (Ogunkeye and Roluga 2006). On the other word, following liver damage, aspartate aminotransferase (AST), as liver function marker, is elevated. We observed considerable levels of aspartate aminotransferase in infected group compared in healthy ones. This might have been the case in this study as another possible in high levels of CholE may be attributed to the Ich-mediated liver damage. Finally, aquatic environment contamination may increase CholE in present study. (Jebali *et al*., 2013).

In recent study, both Urea and CREA as nitrogen metabolism values were found to be high in infected group compared with healthy ones, which more likely due to Ich induced-kidney insufficiency. Higher amount of Urea and CREA were revealed in fish with various species during ecto-parasites infection which is in accordance with our study. (El-seify *et al*. 2011).

Infected fish exhibited an increase in TPP when compared to healthy ones. TPP is considered as health index in aquascience and some factors induce hyperproteinemia, such as metabolism severity, nutrition, health status and high stocking density.(Svoboda *et al*., 2001; Joseph, 2006).This might have been the case in our study as high TPP is attributed to fluid volume disorders, overcrowded stockings and/or elevation of acute phase proteins.

AST and ALT enhancement were clarified in infected group rather than healthy ones which may suggest effects of *Ichthyophthirius multifiliis* at liver. Higher activity of ALT and AST was reported in different species of fish during ecto-parasite infection. (El-seify *et al*. 2011).

The GL concentration as the main analyte of carbohydrate metabolism accompanied with significant decrease. This may have been the case in our study as Ich causes anorexia and/or induction of glycogenesis (glycogen accumulation) in liver. We could not found any information in the literatures about a link between plasma GL with glycogen storage in rainbow trout Ich. Bacterial infections and viral hemorrhagic septicemia diminish GL concentration in rainbow trout, (Rehulka, 2003). Recently, one of the other antioxidant enzymes has been paid more attention, is paraoxonase (PON) which is synthesized in liver and liberated into blood. It belongs to esteric hydrolases that hydrolysis xenobiotics such as organophosphates and it is closely adjoined with Apo lipoprotein AI subtraction of HDL. Furthermore, PON decreases oxidative stress in tissues and cells and its activity has been reported in aquascience of some literatures (Karatas *et al*., 2012; Bastos *et al*., 1998). PON plays a vital role in Xenobiotic biotransformation and protects against lipid peroxidation (James 2006; Aviram and Resenblot 2005). Many studies reported PON activity in human. (Turk et al., 2005), but, in veterinary medicine, it is still scarce. (Bakemkiran *et al*., 2008). In this study, PON concentration was found to be low. Which is in accordance with Karatas study in 2012. This may have been the case in present study as PON involves inhibitory role on lipid peroxidation reaction.

In the recent study, Ich caused marked alterations than healthy ones in haematological parameters including lower values of PCV, RBC count and Hb without significant changes in MCV, MCH, which indicated anaemia. Significant decrease in RBC, Hb and PCV in *Oreochromis niloticus* with gill Ich and saprolegniasis reported by Tavares-Dias in 2002 which is in accordance with present study, while, no evidence of anemia was demonstrated during *Ichthyophthirius multifiliis* infestation in common carp. (Kurovskaya and Osadchaya, 1993). Low count of peripheral RBC may associate with loss of haemapoietic tissue. (Rehulka, 2003) reported that anaemia is commonly characterized with chronic glomerulonephritis and according to Erslev study in 1997, concurrent incidence of chronic renal failure and uremia is regularly accompanied by anaemia and its intensity is correlated with uremia severity. It is postulated that high amount of plasma urea is caused anaemia in Ich group. Many published studies have reported induction of anaemia in salmonides with bacterial and fungal infection (Rehulka 2003; Jamalzadeh *et al*., 2009). (Zaki, 2008) also pointed out an association between cortisol with PCV, Hb and RBC reduction as a result of appetite decrease in *Tilapia nilotica*. It is more likely that occurrence of high cortisol level through disease induced- stress plays another cause in decrease of PCV, Hb and RBC with above mentioned mechanism in Ich.

The significant increase of WBCs such as neutrophil, eosinophil and monocyte with lymphocyte decrease were noticed in the present study, which may denote cellular-immunity system interaction with parasite. In the course of eosinophilia, it has been documented in several studies including helminthes infestation in catfish (*Clarias batrachus)* and also parasite infection in European eel (*A. Anguilla)* (Ali and Ansari, 2012).

Thrombocytosis was determined in infected group than healthy ones. During the stress conditions hypercoagulability and thrombocytosis is mostly observed in fish. Fish thrombocytes possess immune functions and different antigenic stimulation leads to thrombocytosis. Moreover, their phagocytosis function has been reported. This might have been the case in our study, higher thrombocyte count may involve in defense against the parasite (Witeska *et al*., 2010).

It is worth mentioning that lymphopenia is attributed to three different mechanisms (Ishikawa *et al*., 2007; Choi 2004; Dolin *et al*., 1976).

1) Stress induced lymphopenia with following mechanisms:

a) Lymphocyte re-dispensation to lymphoid organs.

b) Cell destruction or decrease in blood circulation due to high levels of cortisol.

2) Hypoxia-induced lymphopenia.

3) Lymphocyte infiltration to tissues

In conclusion, Ich induces alterations of some blood parameters in rainbow trout. These findings may persuade attempts to expand the importance of biochemistry and clinical hematology in the health screening programs of the rainbow trouts in intensive aquaculture.

References

Aebi,H., 1984. Catalase in vitro. Methods in Enzymology, 105: 121-126.

Ahmdifar,E., Ekrami, R. and Gelichi, A.2010. Effects of different dietary prebiotic inulin levels on blood serum enzymes, hematologic, and biochemical parameters of great sturgeon (*Huso huso*) juveniles. Comparative Clinical Pathology, doi 10.1007/s00580-010-1017-2.

Ali, H and Ansari, K. K. 2012. Comparison of Haematologial and Biochemical indices in healthy and Monogenean infected Common Carp, Cyprinus carpio. Annals of Biological Research, 3 (4):1843-1846.

Arabi, M. and Alaeddini, M.A. 2005. Metal-Ion-Mediated Oxidative Stress in the Gill Homogenate of RainbowTrout (Oncorhynchusmykiss) Antioxidant Potential of Manganese, Selenium and Albumin. Biological Trace Element Research, 108: 155-168.

Arrigo, A., and Landry, J. 1994. Expression and function of the low-molecular-weight heat shock proteins. pp. 335-374. In: (The biology of heat shock proteins and molecular chaperones) Moriomto, R.I. and Georgopoulos, A.T. eds), Cold Spring Harbor Laboratory Press, Plainville, N.Y.

Ates, B., Orun, I. andTalas, Z.S. 2007. Effects of sodium selenite on some biochemical and hematological parameters of rainbow trout (OncorhynchusmykissWalbaum, 1792) exposed to Pb2+ and Cu2+. Fish Physiology and Biochemistry, 34: 53–59. DOI 10.1007/s10695-007-9146-5

Aviram, M. andRosenblat, M. 2005. Paraoxonase and cardiovascular diseases: Pharmacological and nutritional influences. Current Opinion Lipidology, 16: 393–399.

Aydin, I., Bulbul, T., Polat, E.S., Yazar, E. 2010. Serum antioxidant status and adenosine deaminase activity during the gestational period of sheep. Revue de Médecine Vétérinaire. 161: 479-484.

Bademkiran, S., Yokus, B., Cakir,D.U., Durak, M.H. andKurt, D. 2008. Serum Paraoxonase-1 activity in dairy cattle and its association with dystocia. Journal of Animal and Veterinary Advances, 10, 1184-1189.

Bastos, C., Folly, E., Rossini, A., Ceccarelli, P.S., Senhorini, J.A. andBastos, J.C. 1998. Paraoxonase activity in liver of Paco, *Piaractus Mesopotamicus Holmberg* (Characidae).Revista Brasileira De Zoologia, 15:677 – 685.

Blaxhall, P.C. andDaisley, K.W. 1973.Routine Haematological Methods for Use Fish with Blood. Journal of Fish Biology, 5: 771-781.

Brauner, C.J. andWang, T. 1997. The Optimal Oxygen Equilibrium Curve: A Comparison Between Environmental Hypoxia and Anemia. American Zoology, 37:101-108.

Choi, K. 2004. Modulation of Immune Function Parameters in Fish Caused by Sudden Environmental Changes (Under the direction of Mac Law and Gregory Cope).Ph.D thesis. North Carolina: University of North Carolina.

Citil, M.,Gunes, V., Karapehlivan, M., Atalan, G., and Marasli, S. 2004. Evaluation of serum sialic acid as an inflammation marker in cattle with traumatic reticulo peritonitis. Revue de Médecine Vétérinaire, 155: 7, 389-392.

Clarke, R. 2002. Homocysteine and risk of ischemic heart disease and stroke. Journal of American Medical Association, 288: 2015-2022.

Cronstein, B.N., Levin, R.I., Belanoff, J., Weissmann, G. and Hirschhorn, R. 1986. Adenosine: an endogenous inhibitor of neutrophil-mediated injury to endothelial cells. Journal of Clinical Investment, 78(3): 760-770.

Da Silva, C.B., Wolkmer, P., Da Silva, A.S., Paim, F.C., Tonin, A.A., Castro, V.S.P., Felin, D.V., Schmatz, R., Goncalves, J.F.,

Badke, M.R.T., Morsch, V.M., Mazzanti, C.M. and Lopes, S.T.A. 2012. Cholinesterases as markers of the inflammatory process in rats infected with *Leptospira interrogans* serovar *Icterohaemorrhagiae*. Journal of Medical Microbiology, 61: 278–284.

Ben Assayag, E., Shenhar-Tsarfaty, S., Ofek, K., Soreq, L., Bova, I., Shopin, L., Berg, R. M. G., Berliner, S., Shapira, I. 2010. Serum cholinesterase activities distinguish between stroke patients and controls and predict 12-month mortality. Molecular Medicine, 16: 278–286.

Das, U. N. 2007. Acetylcholinesterase and butyrylcholinesterase as possible markers of low-grade systemic inflammation. Medicine Science Monitoring, 13: 214–221.

Dimri, U., Sharma, M.C., Yamdagni, A., Ranjan, R. and Zama M.M.S. 2010. Psoroptic mange infestation increases oxidative stress and decreases antioxidant status in sheep. Veterinary Parasitology, 168: 318–322.

Dolin, R., Reichman, R.C. and Fauci, A.S. 1976. Lymphocyte Populations in Acute Viral Gastroenteritis, Infection and Immunity, 14(2): 422-428.

Ellis, A.E. 2001. Innate host defense mechanisms of fish against viruses and bacteria. Developmental & Comparative Immunology, 25: 827–839

Elia, A.C., Galarini, R., Taticchi, M.I., Dorr, A..

andMantilacci, L. 2003.Antioxidant responses an bioaccumulation in *Icttalurus melas* under mercur exposure. Ecotoxicology and Environmental Safety 55:162–167.

El-Seify, M.A., Zaki, M.S., Desouky, A.R.Y., Abbas, H.H., Abdel Hady, O.K., and Abou Zaid, A.A. 2011. Study on Clinopathological and Biochemical Changes in Some Freshwater Fishes Infected With External Parasites and Subjected to Heavy Metals Pollution in Egypt, Life Science Journal, 8(3).

Erslev, A.J. and Besarab, A. 1997. Erythropoietin in th pathogenesis and treatment of the anemia of chronic rena failure. Kidney International, 51: 622–630.

Furlong, C.E., Richter, R.J., Seidel, S.L. and Motulsky, A.G. 1988. Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the metabolites chlorpyrifosoxon and paraoxon. American Journal of Human Genetics, 43(3): 230-8.

Garcia, L.O., Beckera, A.G., Bertuzzia, T., Cunhaa, M.A., Kochhannb, D., Finamora, I.A., Riffel , A.P.K., Llesuyc, S., Pavanatoa, M.A., Baldisserotto, M. 2011. Oxidative stress parameters in silver catfish (*Rhamdia quelen*) juveniles infected with Ichthyophthirius multifiliis and maintained at different levels of water pH. Veterinary Parasitology, 178: 15–21.

Ghiraldelli, L., Martins, M.L., Yamashita, M.M. 2006. Ectoparasites Influence on the Haemarological parameters of Nile Tilapia and Carp Cultured in the State of Santa Catarina, South Brazil. Journal of Fisheries and Aquatic Science, 1(3): 270-276.

Guay, J., Lambert, H., Gingras-Breton, G., Lavoie, J.N., Huot, J., and Landry, J. 1997. Regulation of actin filament dynamics by p38 map kinase-mediated phosphorylation of heat shock protein 27. Journal of Cell Science, 110: 357-368.

Guzel, M.,Askar, T.K., Kaya, G., Atakisi, E.and Erbil Avci, C 2008. Serum Sialic Acids, total antioxidant capacity, an adenosine deaminase activity in cattle with therliriosis an anaplasmosis. Bulletin of the Veterinary Institute i Pulawy, 52: 227-230.

Haney, D.C., Hursh, D.A., Mix, M.C. and Winton, J.R. 1992. Physiological and hematological changes in chum salmon artificially infected with erythrocytic necrosis virus. Journal of Aquatic Animal Health, 4: 48-57.

Heidarian, E., Amini, M Parham, M., and Aminorroaya, A. 2009. Effect of Zinc Supplementation on Serum Homocysteine in Type 2 Diabetic Patients with Microalbuminuria. The Review of Diabetic Studies, 6:1

Hoshino, T., Yamada, K., Masuoka, K., Tsuboi, I., Itoh, K., Nonaka, K., Oizumi, K. 1994. Elevated adenosine deaminase activity in the serum of patients with diabetes mellitus. Diabetes Research Clinical Practice, 25: 97-102.

Ishikawa, N.M., Ranzni-Paiva, M.J.T., Lombardi, J.V. andFerreira, C.M. 2007. Hematological Parameters in Nile Tilápia, *Oreochromis niloticus* Exposed to Sub-lethal Concentrations of Mercury. Brazilian Archives of Biology and Technology, 4: 619-626.

Jacobsen, D.W. (2000). Hyperhomocysteinemia and Oxidative Stress Time for a Reality Check Arteriosclerosis Thrombosis and Vascular Biology, 20:1182-1184

Jamalzadeh, H.R., Keyvan, A., Ghomi, M.R. andGerardi, F. 2009. Comparison of blood indices in healthy and fungal infected Caspian salmon (*Salmo trut tacaspius*). African Journal of Biotechnology, 8: 319-322.

James, R.W. 2006. A long and winding road: defining th biological role and clinical importance of paraoxonases Clinical Chemistry and Laboratory Medicine, 44:1052–9 DOI:10.1515/CCLM.2006.207

Jebali, J., Khedher, S.B., Sabbagh, M., Kamel, K., Banni, M Boussetta, H. 2013. Cholinesterase activity as biomarke of neurotoxicity: utility in the assessment of aquati environment contamination. Journal of Integrated Coasta Zone Management 13(4):525-537.

Jones, R.D., Hancock, J.T., Morice, A.H. 2000. NADPH oxidase: a universal oxygen sensor? Free Radical Biology and Medicine, 29: 416–424.

Karatas, T. and Kocaman, E.M. 2012. Comparison o Paraoxonase Activity, Malondialdehyde and High-Densit Lipoprotein Levels in Cultuvated Normal and Albin Rainbow Trout Reared in the Same Condition KafkasUniversitesiVeterinerFakultesiDergisi,18: 87-90

Karagenc, T.I., Kiral, F.K., Seyrek, K., Bildik, A. and Eren, H. 2005. Detection of serum total sialic acid in cattle with natural tropical theileriosis. Revue de Médecine Vétérinaire, 156: 578-582.

Karaman, U., Beytur, L., Kıran, T.R., and Çolak, C. 2009. Adenosine deaminase level in the serum of the patients *Toxoplasma gondii* seropositive and Giardia intestinalis. African Journal of Microbiology Research, 3(10): 654-657.

Knox, D., Cowey, C.B. and Adron, J.W. 1984. Effects of dietary zinc intake upon copper metabolism in rainbow trout (Salmo gairdneri). Aquaculture, 40(3): 199-207. doi.org/10.1016/0044-8486(84)90187-X

Kurovskaya, L.N., Osadchaya, S.A. 1993. The influence of Ichthyophthirius multifiliis on underyearling carp, Cyprinus

carpio. Journal of Ichthyology, 33: 81-92.

Luskova, V. 1997. Annual cycles and normal values o hematological parameters in fishes. Acta Scientiarum Naturalium Academiae Scientiarum bohemoslovacae Brno, 31: 70.

Mathew, S., Kumar, K.A., Anandan, R., Viswanathan, Nair.and P.G. andDevadasan, K. 2007. Changes in tissue defense system in white spot syndrome virus (WSSV) infected Penaeusmonodon, Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology, 145(3):315-20. doi.org/10.1016/j.cbpc.2007.01.001

McKnight, I.M.A., 1966.Hematological study on the mountain whitefish Popiumwillasemi.Journal of the Fisheries Research Board of Canada, 23: 45–64. doi: 10.1139/f66-005

Matthews, R.A. 2005. Ichthyophthirius multifiliis Fouquet and ichthyophthiriosis in Freshwater teleosts. Advances in Parasitology. 59: 159–240.

Motoi, Y., Kimura, Y., Wakamatsu, H., Shimbayashi,K. 1984.Determination and clinical evaluation of sialic acid and mucoproteinin bovine blood. National Instittute Animal Health, 37: 643-649.

Mifsud, C., Rowland, S.J. 2008. Use of salt to control ichthyophthiriosis and prevent saprolegniosis in silver perch.

Bidyanus bidyanus Aquacult Res, 39: 1175-1180.

Martinez-Álvarez, R.M., Morales, A.E., Sanz, A. 2005. Antioxidant defenses in fish: biotic and abiotic factors. Reviews in Fish Biology and Fisheries, 15: 75–88

Mymrikov, E.V., Seit-Nebi, A.S., and Gusev, N.B. 2011. Large potentials of small heat shock proteins. Physiology Review, 91: 1123–1159.

Nazifi, S., Haghkhah, M., Asadi, Z., Ansari-Lari, M. Tabandeh, M., Esmailnezhad, Z. and Aghamir, M. 2010. Evaluation of Sialic Acid and Acute Phase Proteins (Haptoglobin and Serum Amyloid A) in Clinical and Subclinical Bovine Mastitis. Pakistan veterinary journal, 2074-7764.

Nazifi, S., Razavi, S.M., Safi, N., and Rakhshandehroo, E. 2012. Malignant Ovine Theileriosis: Alterations in the Levels of Homocysteine, Thyroid Hormones and Serum Trace Elements. Journal of Bacteriology and Parasitology. 3: 7

Nigam, A.K., Kumari, U., Mittal, S. and Mittal, A.K. 2012. Comparative analysis of innate immune parameters of the skin mucous secretions from certain freshwater teleosts, inhabiting different ecological niches.Fish Physiology and Biochemistry,38: 1245–1256. doi: 10.1007/s10695-012-9613-5.

Ogunkeye, O.O., Roluga, A.I. 2006. Serum cholinesterase activity helps to distinguish between liver disease and non-liver disease aberration in liver function tests. Pathophysiology 13: 91–93.

Pescador, N., Villar, D., Cifuentes, D., Garcia-Rocha, M., Ortiz-Barahona,A.,Vazquez,S., Ordonez, A., Cuevas, Y., Morales, D.S., Garcia- Bermejo, M.L., Landazuri, M.O., Guinovart, J. andDel Peso, L. 2010. Hypoxia Promotes Glycogen Accumulation through Hypoxia Inducible Factor (HIF)-Mediated Induction of Glycogen Synthase, PLoS ONE, 5(3): 9644. Doi: 10.1371/journal.pone.0009644

Rehulka, J. 2003.Haematological analyses in rainbow trout Oncorhynchusmykissaffected by viral haemorrhagicsepticaemia (VHS). Diseases of Aquatic Organisms, 56: 185–193. Doi:10.3354/dao056185.

Rodriguez, L.F.S., Oliveirab, M.E.F., Teixeirab, P.P.M., Cavalcantec, J.M., Valec, M.R. 2012. Adenosine deaminase activity as a biochemical marker of inflammatory response in goats infected by caprine arthritis–encephalitis virus. Small Ruminant Research, 108:120– 126.

Satoh, K. 1978. Serum lipid peroxide in cerebrovascular disorders determined by new colorimetric method. Clinica Chimica Acta, 90(1):37-43. Doi:10.1016/0009-8981(78)90081-5.

Shephard, K.L. 1994.Functions for fish mucus. Reviews in Fish Biology and Fisheries, 4: 401–429.

Sumi, T., Hama, Y., Maruyama, D., Asakawa, M. and Nakagawa, H. 1997. Isolation and properties of a sialoglycoprotein from the skin mucus of the stingray Dasyatisakajei. Fish Science, 63: 453–458

Svoboda, M., Kouril, J., Hamackova, J., Kalab, P., Savina, L., Svobodova, Z.,Vykusova,B. 2001. Biochemical Profile of Blood Plasma of Tench (TincatincaL.) During Pre- And Postspawing Period. ActaVeterinaria Brno, 70: 259–268.

Sydow, G. 1985. A simpllifield quick method for determination of sialic acid in serum. Biomedica Biochimica Acta, 44(11-12): 1721-1723.

Tavares-Dias, M., de Morales, F.R., Martins, M.L., Santana, A.E. 2002. Haematological changes in Oreochromis niloticus (Osteichthyes: Cichlidae) with gill ichthyophthiriasis and saprolegniosis. Boletim do Instituto de Pesca, 28: 1-9.

Thougaard, A.V., Hellmen, E. and Jensen, A.L. 1998. Total serum sialic acid is a general disease marker rather than a specific tumour marker in dogs. Journal of Veterinary Medicine Series A, 45:471-479.

Traving, C. and Schauer, R. 1998. Structure, function and metabolism of sialic acids. Cellular and Molecular Life Sciences, 54: 1330–1349.

Turk, R., Juretic, D., Geres, D., Turk, N., Rekic, B., Simeon-Rudolf, V.Svetina, A. 2004. Serum paraoxonase activity and lipid parameters in the early postpartum period of dairy cows. Research in Veterinary Science, 76: 57-61. doi.org/10.1016/j.rvsc.2003.08.001

Thambyrajah, J., and Townend, J.N. 2000. Homocysteine and atherothrombosis — mechanisms for Injury. European Heart Journal, 21: 967–974

Varghese, S., and Oommen, O.V. 2000. Long-Term Feeding of Dietary Oils Alters Lipid Metabolism, Lipid Peroxidation, and Antioxidant Enzyme Activities in a Teleost (*Anabas testudineus Bloch*). Lipids, 35: 757–762.

Velisek, J., Svobodova, Z., Piackova, V. and Sudova,E. 2009. Effects of Acute Exposure to Metribuzin on Some Hematological, Biochemical and Histopathological Parameters of Common Carp (*Cyprinus carpio*). Bulletin of Environmental Contamination and Toxicology, 82: 492–495. Doi: 10.1007/s00128-009-9648-1

Vutukuru, S.S., Chintada, S., Madhavi, K.R., Rao, J.V. and Anjaneyulu, Y. 2006.Acute effects of copper on superoxide dismutase, catalase and lipid peroxidation in the freshwater teleost fish somusdanricus. Fish Physiology Biochemistry, 32, 221–229. Doi: 10.1007/s10695-006-9004-x

Witeska, M., Kondera, E., Logowska, K. 2010. The effects of ichthyophthiriasis on some haematological parameters in common carp. Turkish Journal of Veterinary Animal Sci, 34(3): 267-271.

Wolkmer, P., Lopes, S. T. A., Franciscato, C., Da Silva, A. S., Traesel, C. K., Siqueira, L. C., Pereira, M. E., Monteiro, S. G. and Mazzanti, C. M. A. 2010. Trypanosoma evansi: cholinesterase activity in acutely infected Wistar rats. Experimental Para1sitology 125: 251–255.

Yu, H., Biao, Z., Ying-Jie Lu, W., Tan, S.W., Yan, Q.G., and Li, H. 2012. Histopathological study of grass carp (*Ctenopharyngodon idella*) experimentally infected with *Ichthyophthirius multifiliis*. African Journal of Microbiology Research, 6(14): 3539-3544.

Zaki, S.M., Fawzi, O.M. and Jackey, E.J. 2008. Pathological and Biochemical Studies in *Tilapia nilotica* Infected with Saprolegnia parasitica and Treated with Potassium Permanganate. American-Eurasian Journal of Agriculture & Environmental Science, 3: 677-680.

Zikic, R.V., Stajn, A.S., Pavlovic, S.Z., Ognjanovic, B.I. and Saicic, Z.S. 2001.Activities of Superoxide Dismutase and Catalase in Erythrocytes and Plasma Transaminases of Goldfish (Carassius auratus gibelio Bloch.) Exposed to Cadmium. Physiology Research, 50: 105-111.