Factors Affecting Fish Blood Profile: B- Effect of Environmental and Genetic Factors

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ABSTRACT

Continued studies were conducted to understand how some factors could affect blood composition of a fish. In this part (B) of the present serial studies, we gave light on the effect of some environmental and genetic factors on hematological and /or the biochemistry parameters of a fish. These factors are water temperature, narcosis, handling stress, poly-culture, farm conditions, starvation, fish species, sex, and size.

INTRODUCTION

Hematological parameters of fish are useful tools that aid in the study immunopotentiators. Such tests are general but not conclusive and must be correlated with biochemical tests of the subject. The classification of leucocytes (WBCs) as in the vertebrates has been done following morphological criteria where by various groups can be distinguished such as lymphocytes, granulocytes and macrophages (Ellis, 1981). Dominguez *et al.* (2004) confirmed that elevating rearing water temperature for 2 weeks increased the circulating of immunoglobulin M (IgM) concentration in Nile tilapia, but rearing the fish at 33 °C resulted in a decrease in IgM concentration, suggesting that the fish possess an appropriate thermal range for production of immune substances. Plasma level of IgM significantly increased with salinity at 12 and 24 g/L. On the other hand, plasma IgM level did not change by exposing the fish to acidification and suspended solids. These results suggest that the specific immune system of tilapia changes by certain factors in aquatic environment. Adam (2007) registered a negative correlation between hematological indices and water quality parameters with moderate regression factors. Relationship between erythrocytes (RBCs) and water quality characteristics was positive and with very low regression factor. However, the present study was designed to evaluate the effect of some factors affecting some blood parameters. These factors included fish species, sex and

age; water salinity and pollution; rearing conditions; dietary treatments; since it was given in Verse 12 of Sura Faatir of Holly Quran concerning the variations in water and fish composition: "And not alike are the two bodies of water. One is fresh and sweet, palatable for drinking, and one is salty and bitter and from each you eat tender meat and extract ornaments which you wear, and you see the ships plowing through [them] that you might seek of His bounty; and perhaps you will be grateful". So, this was also included in the objectives of the present study.

MATERIALS AND METHODS

Random fish samples were taken for blood collected from the caudal peduncle by special syringe, adequate amount of whole blood was withdrawn in small plastic vials containing EDTA (ethylene diamine tetra acetic acid) as anticoagulant and used to obtain the blood plasma by centrifuge at 3500 rpm for 15 min. Plasma samples were used for determination of creatinine (Tietz, 1986), triglycerides (McGowan *et al.*, 1983), total proteins (Tietz, 1990) and albumin (Wotton and Freeman, 1982) concentrations, as well as the activity of aspartate amino transferase (AST) and alanine amino transferase (ALT) using commercial test kits in a private lab. in Kafr El-Sheikh governorate, Egypt. Globulin level was calculated by subtracting albumin from total protein. The other samples of blood were used to determine the hematological parameters as concentration of hemoglobin (Hb), total count of RBCs, and total count of WBCs (Natt and Herrick, 1952) and hematocrit (Hct) using Auto Counter (Decie and Lewis, 2006) in the same lab. The other hematological parameters were mathematically calculated.

To study the effects of water temperature and fish species and size, five fishes from each body weight (50 ± 3 , 150 ± 3 , and 250 ± 3 g) and each freshwater species (Nile tilapia, African catfish, and striped mullet) were collected from a fish farm in Tolompat-7- Alreiad, Kafr El-Sheikh governorate. Concerning saltwater fish, five fishes from each body weight (50 ± 3 , 150 ± 3 , and 250 ± 3 g) and fish species (sea bream and sea bass) were gathered from a fish farm in Mosalas Al-Diba, Damietta governorate. The fish samples were collected during both summer (September, at water temperature 30°C) and winter (January, at water temperature 10°C) seasons. The water quality conditions were as follow:

Water temperature, $^\circ\text{C}$	Water type	pH	Dissolved oxygen (DO), mg/L	Ammonia, mg/L	Salinity, g/L
30	Fresh	8.1	6.1	0.76	1.30
	Salt	8.1	4.9	1.1	20.4
10	Fresh	7.6	8.2	0.43	1.10
	Salt	7.9	6.1	0.93	20.9

To study the effect of some narcotic agents, before and after the anesthesia, on the blood profile of both male and female of Nile tilapia and African catfish, three agents (MS222, Qunaldin, and clove oil) were used. The levels used for narcosis were 75 mg/L MS222 for 20 min., 9 $\mu\text{g/L}$ ethyl alcohol Qunaldin for 15 min., and 1-3 mL clove oil dissolved in 10 mL alcohol added and added to 10 L water for 20 min. This trial was conducted during March 2019. The rearing water conditions were 20.7°C , 7.5 pH, 4.9 mg/L DO, 0.61 mg/L ammonia, and 1.2 g/L salinity. Five fishes from each sex within each fish species were taken from a fish farm in the district of Ard Al-Shorta, Baltim, Kafr Al-Sheikh governorate. The fish body weights were 400 ± 3 g for both males and females Nile tilapia and 250 ± 3 and 300 ± 3 g for males and females African catfish, respectively.

To study the effect of fish species and sex under poly-culture system on the blood picture. Thus, five fishes from each sex (male and female) within each species (Nile tilapia, African catfish, striped mullet, and common carp) from a fish farm in Ma'zoor district, Al-Hamol, Kafr Al-Sheikh governorate working with the poly-culture system were taken. The earthen pond in this farm had an area of 40 Fadden (= 168000 m²), stocked with million tilapia fry, 20 thousand striped mullet, 10 thousand common carp, and 5 thousand African catfish. The samples' fish weights were 300±3 and 250±3 g for males and females of Nile tilapia, 850±3 and 300±3 g for males and females of African catfish, 240±3 and 270±3 g for males and females of striped mullet, and 850±3 g for either males and females of common carp, respectively. The fish rearing water has 21°C, 8.3 pH, 5.1 mg/L DO, 0.74 mg/L ammonia, and 1.6 g/L salinity.

For studying the effect of fish species and sex on the hematological and biochemical parameters under handling stress, during March 2019. Hence, five males and five females from each fish species as Nile tilapia (400±3 g for each sex), African catfish (200±3 and 300±3 g, respectively), and common carp (1000±3 g for both sexes) were sampled. The rearing water conditions were 20.3°C, 6.1 pH, 4.5 mg/L DO, 0.32 mg/ L ammonia, and 1.4 g/ L salinity. Fish samples were taken from a fish farm in the district of Ard Al-Shorta, Baltim, Kafr Al-Sheikh governorate, and then transferred into glass aquaria for 24 h. About six Nile tilapia and African catfish were stocked into each glass aquarium with 200-210 L water, whereas common carp were individually stocked into glass aquaria. Each sex was given in a separate aquarium. After 24 h., blood samples were withdrawing, and fish were returned to aquaria for one hour. Thereafter, fish were net handled out- and in-water 5-6 times, each for 15 sec. and repeated for 20 min., then another blood samples were withdrawing. The water quality criteria in this experiment were as follow:

Treatment	Water temperature, °C	pH	DO, mg/L	Ammonia, mg/L	Salinity, g/L
Males Nile tilapia	19.8	6.8	5.2	0.37	0.31
Females Nile tilapia	20.3	6.3	4.6	0.42	0.29
Males African catfish	20.1	6.5	5.3	0.51	0.30
Females African catfish	20.4	7.1	4.2	0.29	0.31
Males common carp	19.7	6.9	5.6	0.35	0.37
Females common carp	20.0	6.8	4.9	0.32	0.40

To study the effect of farm location on blood profile of different sizes of Nile tilapia. Thus, five fishes from either size (150±3 and 250±3 g) from three different farms concerning the environmental conditions were collected during August 2017. The 1st farm in Baltim, Kafr El-Sheikh governorate, its fishes were normal without mortality and apparently sound. Its water properties had 30.2 °C, 7.5 pH, 6.1 mg/L DO, 0.36 mg/ L ammonia, and 1.1 g/ L salinity. The 2nd farm located at Tolombat-7, Al-Riad (Fathy El-Gammal), Kafr El-Sheikh governorate, it had 100 died fish daily, with external symptoms (protruded eyes). Its water properties had 31.7 °C, 4.7 pH, 3.2 mg/L DO, 2.9 mg/L ammonia, and 1.1 g/ L salinity. The 3rd farm located at Siag-Hamol (Sobhy Halawa), Kafr El-Sheikh governorate, it had more death cases than the former, ca. 300 fish daily, with symptoms: protruded eyes, large red-orange spots on the hind part, the post-mortem examination showed enlarged spleen, intestinal edema, pale-enlarged liver, its water properties had 29.3 °C, 9.2 pH, 4.3 mg/L DO, 1.2 mg/L ammonia, and 2.1 g/L salinity.

To study the effect of starvation on the blood profile of males and females Nile tilapia during March 2019, in a fish farm in the Ma'zoor district-77, Al-Hamol, Kafr El-Sheikh governorate. Thus, blood samples were withdrawn from five fishes from each sex (male and female) and size (100 ± 3 and 150 ± 3 g for either sexes) after 2-3 months of starvation. The farm fish were re-fed for 25 days with Aqua-sinking feed 30% crude protein. Thereafter, other blood samples were collected. Water quality criteria before and after fish feeding were:

Water temperature, °C	pH	DO, mg/L	Ammonia, mg/L	Salinity, g/L
Before feeding (after 2-3 months starvation)				
21.0	5.9	7.2	0.46	1.3
After 25 days feeding				
23.1	6.7	8.0	0.74	1.4

To evaluate the blood picture of fish as affected by species and size of different fresh and salt water fishes. Therefore, five fishes were collected from each fish species (Nile tilapia, African catfish, grey mullet, and thin-lipped mullet), from a fish farm at Tolombat-7, Al-Reiad, Kafr El-Sheikh governorate during August 2017. The water quality parameters were 30.1 °C, 8.1 pH, 5.3 mg/L DO, 0.45 mg/L ammonia, and 1.3 g/L salinity). While, sea bream, and sea bass were sampled from a fish farm in Mosalas Aldiba, Damietta governorate during October 2017, where its water properties had 27 °C, 7.5 pH, 6.7 mg/L DO, 0.78 mg/L ammonia, and 22.1 g/L salinity). However, meager fish with size (50 ± 3 , 150 ± 3 , and 250 ± 3 g) was collected from a fish farm at Bar-Bahary, Baltim, Kafr El-Sheikh governorate during August 2017, where its water properties had 31.3 °C, 5.8 pH, 3.4 mg/L DO, 0.46 mg/L ammonia, and 12.9 g/L salinity).

To compare between both sexes for Nile tilapia and African catfish from different locations concerning their blood parameters. Five fishes (250 ± 3 g for each sex, species, and location) from each of males and females of Nile tilapia and African catfish from three different locations (River Nile, Lake Borulus, and a private fish farm) were collected during September 2018. The water quality in these locations were:

Water temperature, °C	pH	DO, mg/ L	Ammonia, mg/ L	Salinity, g/ L
River Nile (Rosetta branch)				
32	7.8	5.3	1.20	2.4
Lake Borulus				
30.9	7.1	7.8	0.64	5.3
Fish farm				
32.1	6.3	4.3	0.71	1.1

All blood analysis data were statistically analyzed according to SAS (2006). When F- test was significant at $P\leq 0.05$, Duncan's multiple range tests (Duncan, 1955) was carried out.

RESULTS

Water temperature and fish species and size:

Fish rearing water temperature significantly affected all tested haematological parameters, except Hb and MCV; whereas, fish species significantly affected all of them. But fish weight significantly affected each of Hct, MCV, MCHC, and WBCs. Most interactions were significant for MCV, MCH, MCHC, platelets, and WBCs (Table 1). Elevating water temperature reduced all values of the tested biochemical parameters. Except ALT, all other biochemical parameters were significantly affected by fish species and weight. The interactions among these variables were also significant, except for ALT, total protein (TP), albumin (AL), and TG (Table 2).

Anesthesia type, before and after anesthesia, and fish species and sex:

Table 3 shows different dimensions for the effect of different narcotic substances, before and after anesthesia, and fish species and sex, as well as their interactions concerning the haematological measurements. It is worth noting that MS222 elevated Hb, Hct, RBCs, and WBCs values comparing with the other two narcotic agents. Yet, clove oil particularly reduced Hct, RBCs, and platelets. But Quinaldin mainly reduced Hb and WBCs. MS222 elevated ALT and urea values, while clove oil increased the level of AL, TCH, and LDL comparing with the other agents. Fish species and sex significantly affected only the activity of both enzymes AST and ALT. Significant interactions were calculated too (Table 4).

Fish species and sex under poly-culture system: All haematological and biochemical parameters were significantly affected by fish species; yet, fish sex significantly affected only MCHC, TG, and LDL values (Tables 5 and 6). Most interactions were significant for the hematological parameters (Table 5), but not for the biochemical parameters (Table 6).

Table 1: Impact of water temperature, fish species and fish weight on haematological parameters

	Hb (g/dl)	Hct (%)	RBCs (X 10 ⁶ /μl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets (X 10 ³ /μl)	WBCs (X 10 ³ /μl)
Temperature (°C)								
10	13.25	35.14 ^a	2809 ^a	126.4	45.99 ^b	38.63 ^b	219047 ^a	76501 ^b
	±0.31	±0.53	±69	±2.78	±1.11	±1.23	±8777	±7814
30	13.14	31.01 ^b	2534 ^b	127.6	53.38 ^a	43.75 ^a	160560 ^b	82149 ^a
	±0.37	±1.02	±109	±2.28	±1.74	±1.32	±10988	±6052
Fish species								
Nile tilapia	10.59 ^d	27.99 ^c	2028 ^d	142.1 ^b	59.45 ^b	42.72 ^c	174983 ^c	66007 ^c
	±0.22	±1.95	±133	±1.65	±2.91	±1.99	±19478	±5737
African catfish	14.08 ^b	27.99 ^c	2302 ^c	121.4 ^d	64.13 ^a	55.00 ^a	79567 ^d	177157 ^a
	±0.34	±0.88	±71	±2.29	±1.15	±1.74	±8616	±10951
Striped mullet	17.90 ^a	38.08 ^a	3919 ^a	91.2 ^e	42.98 ^c	46.13 ^b	178600 ^c	79430 ^b
	±0.27	±0.87	±80	±2.04	±0.42	±0.52	±4851	±2445
Sea bream	12.33 ^c	37.18 ^a	2528 ^b	145.7 ^a	43.78 ^c	31.63 ^d	283733 ^a	40900 ^d
	±0.13	±0.59	±66	±1.70	±0.69	±0.14	±5974	±1838
Sea bass	11.08 ^d	34.13 ^b	2580 ^b	134.5 ^c	38.08 ^d	30.48 ^d	232133 ^b	33133 ^c
	±0.12	±0.43	±45	±0.55	±1.16	±0.14	±9966	±1313
Fish weight (g)								
50	13.01	31.48 ^b	2661	121.3 ^c	50.28	42.59 ^a	184300	90498 ^a
	±0.42	±1.01	±126	±2.94	±1.95	±1.86	±13695	±11146
150	13.32	32.61 ^b	2654	127.1 ^b	49.27	41.93 ^a	188700	76628 ^b
	±0.41	±0.96	±107	±2.94	±1.87	±1.60	±13209	±7834
250	13.26	35.13 ^a	2700	132.6 ^a	49.50	39.06 ^b	196410	70850 ^c
	±0.42	±1.07	±107	±3.27	±1.80	±1.27	±11697	±5658
P value								
Temp	0.6085	0.0001	0.0001	0.2248	0.0001	0.0001	0.0001	0.0025
Species	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Size	0.4047	0.0001	0.7621	0.0001	0.2967	0.0001	0.1612	0.0001
Temp*Species	0.0030	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Temp*Size	0.2226	0.3111	0.4082	0.8630	0.0091	0.0001	0.1998	0.0001
Species*Size	0.7568	0.0177	0.2572	0.0001	0.0001	0.0001	0.0001	0.0001
Temp*Species*Size	0.6923	0.5477	0.4959	0.0001	0.0001	0.0001	0.0004	0.0001

a-e: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, Hb: haemoglobin, Hct: haematocrit, RBCs: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, WBCs: white blood cells.

Table 2: Impact of water temperature, fish species and fish weight on blood biochemical parameters

	AST (u/l)	ALT (u/l)	AL (g/dl)	GL (g/dl)	TP (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	TCH (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Temperature (°C)											
10	120.45 ^a ±5.90	45.03 ±1.61	1.13 ±0.02	3.09 ^a ±0.06	4.55 ^a ±0.06	0.56 ^a ±0.05	11.28 ^a ±0.56	275.67 ^a ±9.36	199.73 ±4.64	119.99 ^a ±5.94	115.63 ^a ±4.18
30	103.33 ^b 4.69	45.72 ±6.87	1.11 ±0.04	2.95 ^b ±0.08	4.31 ^b ±0.09	0.82 ^b ±0.07	9.18 ^b ±0.52	228.80 ^b ±11.25	202.16 ±5.12	161.96 ^b ±7.57	52.88 ^b ±3.31
Fish species											
Nile tilapia	159.10 ^a ±5.21	51.67 ±2.59	1.10 ^a ±0.03	2.64 ^a ±0.15	3.99 ^a ±0.16	0.88 ^a ±0.10	10.22 ^a ±1.14	200.0 ^a ±15.62	187.5 ^a ±8.45	102.7 ^a ±2.70	74.83 ^a ±11.01
African catfish	86.70 ^c 10.97	32.60 ±0.89	0.95 ^c ±0.03	2.78 ^{bc} ±0.08	3.98 ^c ±0.07	1.51 ^a ±0.07	10.57 ^a ±0.60	186.5 ^a ±9.92	204.4 ^a ±9.33	122.1 ^c ±10.60	74.27 ^b ±8.44
Striped mullet	59.20 ^d ±1.46	52.00 ±2.73	1.39 ^a ±0.07	2.86 ^b ±0.09	4.55 ^b ±0.09	0.58 ^c ±0.02	3.93 ^c ±0.17	182.1 ^d ±3.84	172.0 ^d ±2.53	78.27 ^a ±3.17	69.67 ^b ±4.28
Sea bream	124.97 ^b ±1.90	35.33 ±0.91	1.07 ^b ±0.02	3.40 ^a ±0.07	4.82 ^a ±0.06	0.25 ^d ±0.01	13.20 ^a ±0.36	366.7 ^a ±6.35	232.1 ^a ±6.29	215.2 ^a ±4.82	105.0 ^a ±7.62
Sea bass	129.50 ^b ±3.20	55.27 ±16.92	1.09 ^b ±0.02	3.42 ^a ±0.07	4.82 ^a ±0.06	0.23 ^d ±0.00	13.23 ^a ±0.35	325.7 ^b ±3.61	208.6 ^b ±5.44	186.4 ^b ±7.32	97.47 ^a ±6.88
Fish weight (g)											
50	108.28 ^b ±6.66	55.12 ±10.24	1.07 ^b ±0.03	3.06 ^a ±0.10	4.37 ±0.11	0.724 ^a ±0.08	10.99 ^a ±0.70	238.8 ^c ±12.47	187.3 ^b ±6.30	139.3 ^b ±9.06	73.86 ^b ±5.96
150	104.84 ^b ±5.39	41.46 ±1.64	1.08 ^b ±0.02	3.08 ^a ±0.08	4.43 ±0.08	0.713 ^a ±0.08	9.79 ^b ±0.63	252.9 ^b ±14.25	210.6 ^a ±5.52	137.5 ^b ±9.64	88.82 ^a ±6.04
250	122.56 ^a ±7.49	39.54 ±1.49	1.20 ^a ±0.05	2.91 ^b ±0.09	4.49 ±0.08	0.631 ^b ±0.08	9.91 ^b ±0.70	264.9 ^a ±12.38	204.9 ^a ±5.67	146.0 ^a ±7.80	90.08 ^b ±7.02
P value											
Temp	0.0001	.9202	0.4342	0.0207	0.0001	0.0001	0.0001	0.0001	0.6018	0.0001	0.0001
Species	0.0001	.1208	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Size	0.0001	0.1377	0.0001	0.040	0.253	0.0002	0.0001	0.0001	0.0002	0.012	0.0001
Temp*Species	0.0001	0.2414	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Temp*Size	0.0001	0.4321	0.4848	0.0071	0.0890	0.0001	0.0003	0.0001	0.2867	0.0001	0.0070
Species*Size	0.0001	0.4512	0.0001	0.0002	.0015	0.0001	0.0001	0.0001	0.0012	0.0001	0.0001
Temp*Species*Size	0.0001	0.4782	0.1886	0.0267	0.5214	0.0001	0.0001	0.0001	0.0001	0.0058	0.0002

a-e: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GL: globulin, TP: total protein, TCH: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein.

Table 3: Impact of anesthesia, fish species, and fish sex on haematological parameters

	Hb (g/dl)	Hct (%)	RBCs (X 10 ⁶ /μl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets (X 10 ³ /μl)	WBCs (X 10 ³ /μl)
Anesthesia type								
MS222	12.67 ^a ±0.23	33.12 ^a ±1.06	2278.5 ^a ±60.99	148.2 ^b ±1.52	56.25 ^b ±1.16	38.74 ^b ±0.63	179930 ^b ±5776	85375 ^a ±2080
Quinaldin	12.11 ^b ±0.20	31.32 ^b ±0.58	2064.7 ^b ±63.62	150.6 ^a 0.82	59.81 ^a ±0.77	39.77 ^b ±0.58	206431 ^a ±8186	72585 ^c ±1574
Clove oil	12.49 ^{ab} ±0.25	30.94 ^b ±0.97	2049.5 ^b ±51.54	151.6 ^a ±0.73	60.05 ^a ±0.86	41.35 ^a ±0.72	176500 ^b ±10636	80153 ^b ±2138
Period								
Before	12.62 ^a ±0.22	32.64 ^a ±0.83	2107.2 ±52.91	152.6 ^a ±0.79	59.27 ±0.79	40.06 ±0.52	174824 ^b ±6312	81720 ^a ±1682
After	12.23 ^b ±0.14	30.29 ^b ±0.39	2154.6 ±46.32	147.7 ^b ±0.89	58.13 ±0.80	39.85 ±0.57	200417 ^a ±7400	77022 ^b ±1712
Fish species								
Nile tilapia	11.55 ^b ±0.12	27.47 ^b ±0.36	1865.3 ^b ±27.63	149.3 ^b ±0.82	62.16 ^a ±0.71	42.51 ^a ±0.33	206924 ^a ±6659	77770 ±1625
African catfish	13.30 ^a ±0.17	36.46 ^a ±0.39	2396.5 ^a ±42.54	151.0 ^a ±0.96	55.24 ^b ±0.61	37.40 ^b ±0.51	168317 ^b ±6571	80972 ±1795
Fish sex								
Male	12.52 ±0.17	31.72 ±0.32	2164.0 ±53.03	148.4 ^b ±0.78	57.44 ^b ±0.92	40.44 ^a ±0.57	187971 ±6946	80440 ±1501
Female	12.33	32.21	2097.8	151.9 ^a	59.96 ^a	39.47 ^b	187270	78302

	±0.21	±0.84	±45.98	±0.95	±0.61	±0.50	±7204	±1912
<i>P value</i>								
Type*period	0.0120	0.3479	0.0001	0.0001	0.2181	0.0571	0.0003	0.1904
Type*species	0.0496	0.3793	0.1139	0.0001	0.4228	0.3897	0.0001	0.0026
period*species	0.0001	0.3446	0.0001	0.0032	0.1943	0.0004	0.3155	0.0007
Type*period*species	0.0372	0.3722	0.0241	0.0001	0.1305	0.0688	0.0001	0.0172
Type*sex	0.0662	0.3355	0.0001	0.0001	0.0014	0.0001	0.0366	0.0835
period*sex	0.4824	0.3262	0.1314	0.1973	0.0659	0.7260	0.2360	0.5905
Type*period*sex	0.0131	0.3704	0.1131	0.1319	0.6572	0.2736	0.1858	0.0046
species*sex	0.6899	0.3202	0.0008	0.8881	0.5182	0.2801	0.0004	0.3244
Type*species*sex	0.8492	0.3486	0.0223	0.0001	0.0179	0.0001	0.0839	0.2086
period*species*sex	0.3431	0.3353	0.3054	0.4406	0.0001	0.6394	0.3533	0.8111

a-c: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, Hb: haemoglobin, Hct: haematocrit, RBCs: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, WBCs: white blood cells.

Table 4: Impact of anesthesia, fish species, and fish sex on blood biochemicals

	AST (u/l)	ALT (u/l)	AL (g/dl)	GL (g/dl)	TP (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	TCH (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Anesthesia type											
MS222	142.7 ±12.81	74.80a ±7.81	1.05ab ±0.02	3.48 ±0.07	4.53 ±0.07	0.60 ±0.02	5.70a ±0.21	225.1b ±5.46	223.0 ±5.32	91.03 ±1.69	89.98b ±4.64
Quinaldin	141.9 ±7.79	53.30b ±2.29	1.04b ±0.02	3.63 ±0.06	4.66 ±0.06	0.55 ±0.02	5.23ab ±0.17	235.7ab ±6.03	222.0 ±5.42	89.30 ±1.80	96.10b ±4.88
Clove oil	136.4 ±5.23	57.65b ±2.20	1.10a ±0.02	3.58 ±0.07	4.68 ±0.07	0.55 ±0.02	5.10b ±0.15	245.0a ±6.40	213.4 ±4.72	92.78 ±1.87	108.3a ±5.68
Period											
Before	145.8a ±8.37	55.77b ±1.71	1.08 ±0.01	3.46b ±0.05	4.54b ±0.05	0.57 ±0.01	5.45 ±0.17	236.6 ±5.28	221.3 ±3.78	90.33 ±1.65	98.30 ±4.47
After	134.9b ±6.34	68.07a ±5.48	1.04 ±0.01	3.66a ±0.05	4.71a ±0.05	0.56 ±0.01	5.23 ±0.13	233.9 ±4.63	217.6 ±4.63	91.73 ±1.25	97.93 ±4.02
Fish species											
Nile tilapia	157.9a ±7.95	68.12a ±5.47	1.06 ±0.01	3.55 ±0.06	4.60 ±0.05	0.57 ±0.01	5.45 ±0.16	234.2 ±5.12	218.9 ±4.67	89.42 ±1.38	100.40 ±4.56
African catfish	122.8b ±6.14	55.72b ±1.72	1.07 ±0.01	3.58 ±0.05	4.65 ±0.05	0.56 ±0.01	5.23 ±0.14	236.3 ±4.81	220.0 ±3.74	92.65 ±1.52	95.83 ±3.89
Fish sex											
Male	156.9a ±7.29	56.65b ±1.64	1.05 ±0.01	3.53 ±0.05	4.58 ±0.05	0.57 ±0.01	5.35 ±0.15	233.3 ±5.50	219.3 ±4.63	90.77 ±1.69	97.98 ±4.67
Female	123.7b ±6.99	67.18a ±5.53	1.07 ±0.01	3.60 ±0.05	4.67 ±0.05	0.57 ±0.01	5.33 ±0.15	237.2 ±4.37	219.6 ±3.80	91.30 ±1.20	98.25 ±3.78
<i>P value</i>											
Type*period	0.0001	0.0001	0.6117	0.0007	0.0002	0.1799	0.8907	0.1401	0.0024	0.8695	0.0443
Type*species	0.0001	0.0001	0.4149	0.1407	0.1819	0.7631	0.5168	0.0001	0.0294	0.8628	<.0001
period*species	0.7907	0.0001	0.3891	0.8312	0.6296	0.7832	0.3126	0.8117	0.0618	0.0870	0.0495
Type*period*species	0.0009	0.0001	0.6905	0.0753	0.0701	0.4866	0.8281	0.0002	0.6585	0.0006	0.0001
Type*sex	0.0001	0.0001	0.2374	0.0002	0.0012	0.8825	0.2809	0.7184	0.3634	0.3858	0.6639
period*sex	0.0002	0.0001	0.1989	0.1245	0.2617	0.9137	0.4839	0.7653	0.0587	0.3232	0.1595
Type*period*sex	0.0001	0.0001	0.7276	0.0075	0.0064	0.9726	0.6118	0.2907	0.8907	0.3296	0.0033
species*sex	0.1892	0.0001	0.7239	0.9151	0.8302	0.7075	0.6971	0.0092	0.3610	0.5059	0.0802
Type*species*sex	0.0229	0.0001	0.8351	0.6386	0.6217	0.4322	0.5760	0.0322	0.3954	0.9798	0.1245
period*species*sex	0.0001	0.0001	0.9803	0.0723	0.0707	0.2760	0.1875	0.0336	0.9715	0.7200	0.0728

a-b: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GL: globulin, TP: total protein, TCH: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein.

Table 5: Impact of fish species and fish sex on haematological parameters

	Hb (g/dl)	Hct (%)	RBCs ($\times 10^6/\mu\text{l}$)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets ($\times 10^3/\mu\text{l}$)	WBCs ($\times 10^3/\mu\text{l}$)
Fish species								
Nile tilapia	13.02 ^a ±0.34	31.96 ^c ±0.38	2192 ^c ±34.15	146.00 ^b ±1.12	60.08 ^b ±1.86	40.88 ^c ±1.23	245000 ^a ±8916	85700 ^b ±6662
African catfish	12.92 ^b ±0.18	37.53 ^b ±1.34	2480 ^b ±47.68	151.10 ^a ±2.42	51.45 ^c ±0.75	33.97 ^d ±0.87	150900 ^c ±10640	85700 ^b ±2108
Striped mullet	17.44 ^a ±0.34	43.01 ^a ±1.24	3858 ^a ±45.85	77.60 ^a ±1.18	41.17 ^d ±0.44	46.09 ^b ±1.04	178300 ^b ±1739	75700 ^b ±1407
Common carp	12.20 ^c ±0.24	20.87 ^d ±0.51	1789 ^d ±49.00	119.40 ^c ±2.61	68.69 ^a ±1.47	66.93 ^a ±2.06	133900 ^d ±1709	162400 ^a ±5292
Fish sex								
Male	14.02 ±0.57	33.53 ±1.96	2596 ±173.02	124.40 ±7.14	54.42 ±2.51	45.38b ±3.00	167800b ±10421	100300 ±9681
Female	13.78 ±0.44	33.16 ±2.03	2563 ±188.65	122.65 ±6.46	56.28 ±2.46	48.56a ±2.91±	186250a ±10864	104450 ±7257
Interactions								
Tilapia*Male	12.20 ±0.11	31.55 ±0.63	2168 ±63.51	146.00 ±1.64	55.96 ±1.16	37.88 ±0.54	233600 ±9395	72200 ±9431
Tilapia*Female	13.84 ±0.40	32.38 ±0.40	2216 ±30.43	146.00 ±1.70	64.20 ±2.39	43.88 ±1.41	256400 ±14299	99200 ±4432
Catfish*Male	12.94 ±0.31	40.52 ±1.61	2530 ±69.79	157.80 ±1.39	50.10 ±0.40	32.04 ±0.53	125600 ±9373	84800 ±3734
	12.90	34.53	2430	144.40	52.80	35.90	176200	86600

a-d: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, Hb: haemoglobin, Hct: haematocrit, RBCs: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, WBCs: white blood cells.

Table 6: Impact of fish species and fish sex on blood biochemical parameters

	AST (u/l)	ALT (u/l)	AL (g/dl)	GL (g/dl)	TP (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	TCH (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Fish species											
Nile tilapia	154.8 ^a ±19.48	66.20 ^a ±20.05	1.02 ^a ±0.03	2.16 ^c ±0.11	3.97 ^a ±0.18	0.64 ^b ±0.04	4.40 ^a ±0.56	257.2 ^b ±17.32	248.5 ^a ±14.36	93.90 ^a ±2.02	122.7 ^b ±18.01
African catfish	175.9 ^a ±15.72	37.00 ^{ab} ±1.48	1.07 ^a ±0.04	2.23 ^b ±0.13	3.93 ^a ±0.15	0.73 ^a ±0.04	3.80 ^a ±0.29	323.6 ^a ±13.73	179.5 ^a ±12.98	98.40 ^a ±1.95	169.3 ^a ±20.82
Striped mullet	58.50 ^a ±1.49	39.90 ^{ab} ±0.81	1.90 ^a ±0.08	2.28 ^b ±0.09	4.94 ^a ±0.15	0.46 ^c ±0.01	4.30 ^a ±0.15	182.3 ^c ±5.46	175.7 ^b ±1.49	85.50 ^a ±1.71	61.60 ^c ±6.16
Common carp	80.00 ^a ±1.46	23.80 ^b ±0.83	0.78 ^c ±0.03	2.59 ^a ±0.18	3.79 ^a ±0.13	0.22 ^d ±0.01	12.10 ^a ±0.23	130.4 ^d ±1.80	178.9 ^b ±6.05	46.00 ^c ±1.55	48.30 ^c ±2.08
Fish sex											
Male	106.45 ±10.18	46.50 ±10.46	1.18 ±0.10	2.36 ±0.08	4.27 ±0.15	0.51 ±0.05	6.20 ±0.82	218.1 ±18.12	207.8 ^a ±10.91	80.80 ±4.63	85.65 ^a ±13.39
Female	128.15 ±16.98	36.95 ±3.04	1.21 ±0.10	2.27 ±0.11	4.05 ±0.15	0.52 ±0.05	6.10 ±0.83	228.7 ±18.93	183.5 ^b ±7.86	81.10 ±5.18	115.30 ^a ±15.37
Interactions											
Tilapia*Male	153.8 ±16.69	83.60 ±39.64	1.02 ±0.04	2.04 ±0.07	4.08 ±0.30	0.62 ±0.06	5.00 ±1.05	240.6 ±21.33	280.0 ±17.03	93.00 ±3.13	91.60 ±21.72
Tilapia*Female	155.8 ±37.79	48.80 ±9.35	1.02 ±0.06	2.27 ±0.21	3.86 ±0.23	0.66 ±0.05	3.80 ±0.37	273.8 ±27.51	217.0 ±11.91	94.80 ±2.85	153.8 ±22.46
Catfish*Male	135.8 ±9.60	37.60 ±2.50	1.04 ±0.04	2.38 ±0.20	4.08 ±0.22	0.76 ±0.05	3.60 ±0.40	324.6 ±21.04	191.0 ±10.30	96.60 ±2.77	149.8 ±34.13
Catfish*Female	216.0 ±14.69	36.40 ±1.86	1.10 ±0.07	2.08 ±0.16	3.78 ±0.21	0.70 ±0.07	4.00 ±0.45	322.6 ±20.14	168.0 ±24.22	100.20 ±2.80	188.8 ±24.42
Mullet*Male	56.20 ±2.40	40.60 ±1.21	1.88 ±0.09	2.34 ±0.15	5.14 ±0.07	0.45 ±0.02	4.20 ±0.20	174.2 ±9.15	175.0 ±2.26	85.80 ±2.96	53.40 ±10.08
Mullet*Female	60.80 ±1.28	39.20 ±1.11	1.92 ±0.14	2.22 ±0.11	4.74 ±0.27	0.48 ±0.02	4.40 ±0.24	190.4 ±4.23	176.4 ±2.16	85.20 ±2.08	69.80 ±5.97
Carp*Male	80.00 ±3.05	24.20 ±1.39	0.76 ±0.04	2.68 ±0.10	3.76 ±0.07	0.21 ±0.01	12.00 ±0.32	133.0 ±2.10	185.2 ±9.39	47.80 ±1.74	47.80 ±3.28
Carp*Female	80.00 ±0.55	23.40 ±1.03	0.80 ±0.04	2.50 ±0.35	3.82 ±0.26	0.23 ±0.02	12.20 ±0.37	127.8 ±2.60	172.6 ±7.52	44.20 ±2.48	48.80 ±2.92
P value	0.0449	0.5724	0.9794	0.5294	0.7573	0.6414	0.3527	0.6303	0.0866	0.5601	0.4110

a-d: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GL: globulin, TP: total protein, TCH: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein.

Fish species and sex under handling stress: Handling stress significantly affected all haematological and biochemical parameters, except Hct, WBCs, ALT, and AL, but fish species did not significantly affect MCH, TP, and urea. Fish sex affected only Hb, platelets, and WBCs, AST, creatinine, and TG (Tables 7 and 8).

Table 7: Impact of handling, fish species and fish sex on haematological parameters

	Hb (g/dl)	Hct (%)	RBCs (X 10 ⁶ /μl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets (X 10 ⁹ /μl)	WBCs (X 10 ³ /μl)
Handling								
Before	13.89 ^a ±0.33	26.79 ±2.13	1989 ^a ±88.48	123.0 ^b ±7.49	94.03 ^a ±7.24	60.58 ^a ±4.76	231400 ^a ±17578	109227 ±5708
After	12.63 ^b ±0.36	26.81 ±1.66	1876 ^b ±87.47	145.5 ^a ±2.16	69.71 ^b ±2.60	49.92 ^b ±2.63	219750 ^b ±11595	111637 ±5936
Fish species								
Nile tilapia	10.74 ^c ±0.24	23.84 ^b ±0.52	1614 ^b ±30.87	138.4 ^b ±8.71	74.83 ±5.85	46.70 ^b ±1.65	226600 ^b ±9077	82205 ^c ±3593
African catfish	14.68 ^a ±0.12	40.23 ^a ±0.49	2588 ^a ±27.61	155.1 ^a ±0.41	83.62 ±26.04	36.91 ^c ±0.31	134200 ^c ±4508	97420 ^b ±575
Common carp	14.37 ^b ±0.18	16.34 ^c ±0.66	1597 ^b ±19.32	109.4 ^c ±5.17	87.16 ±1.56	82.14 ^a ±3.12	315925 ^a ±6981	151670 ^a ±1376
Fish sex								
Male	13.38 ^a ±0.37	27.00 ±1.91	1940 ±87.86	137.7 ±4.59	88.09 ±17.09	55.33 ±4.12	236800 ^a ±14729	114648 ^a ±4891
Female	13.14 ^b ±0.36	26.60 ±1.90	1925 ±89.32	130.8 ±6.90	75.66 ±4.39	55.17 ±3.82	214350 ^b ±14837	106215 ^b ±6539
Probability of the interactions								
Handling	0.0001	0.9653	0.0001	0.0001	0.01747	0.0001	0.0007	0.0965
Species	0.0001	0.0001	0.0001	0.0001	0.8422	0.0001	0.0001	0.0001
Sex	0.0195	0.3200	0.4997	0.2031	0.4848	0.8868	0.0001	0.0001
Handling*Species	0.0711	0.0001	0.2055	0.0060	0.4558	0.0001	0.0001	0.8168
Handling*Sex	0.0002	0.0250	0.0606	0.0793	0.4088	0.0785	0.0001	0.4346
Species*Sex	0.3686	0.1228	0.9242	0.0442	0.2740	0.0090	0.0008	0.0001
Handling*Species*Sex	0.0002	0.0001	0.0001	0.1812	0.2645	0.1069	0.0001	0.0015

a-c: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, Hb: haemoglobin, Hct: haematocrit, RBCs: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, WBCs: white blood cells.

Table 8: Impact of handling fish species and fish sex on blood biochemical parameters

	AST (u/l)	ALT (u/l)	AL (g/dl)	GL (g/dl)	TP (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	TCH (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Handling											
Before	53.60 ^a ±2.89	37.90 ±0.94	0.99 ±0.02	3.44 ^a ±0.06	4.41 ^a ±0.05	0.70 ^a ±0.03	7.37 ^a ±0.60	171.7 ^a ±11.01	85.30 ^a ±2.32	74.50 ^a ±1.75	78.70 ^a ±11.39
After	63.83 ^a ±5.34	38.37 ±1.00	0.98 ±0.03	3.68 ^a ±0.06	4.65 ^a ±0.05	0.79 ^a ±0.02	12.50 ^a ±2.69	186.7 ^a ±9.69	94.80 ^a ±3.67	70.20 ^a ±1.72	97.27 ^a ±9.72
Fish species											
Nile tilapia	75.25 ^a ±6.95	36.30 ^a ±0.93	0.96 ^a ±0.03	3.68 ^a ±0.07	4.59 ±0.08	0.75 ^a ±0.04	6.65 ±0.43	175.0 ^a ±10.93	106.7 ^a ±3.19	75.80 ^a ±2.02	77.75 ^a ±12.16
African catfish	50.35 ^b ±2.71	38.15 ^a ±1.00	1.07 ^a ±0.03	3.43 ^b ±0.07	4.51 ±0.06	0.82 ^a ±0.02	11.30 ±4.17	235.4 ^a ±7.72	78.35 ^a ±2.65	72.80 ^{ab} ±1.75	146.6 ^a ±7.52
Common carp	50.55 ^b ±3.38	39.95 ^a ±1.45	0.93 ^b ±0.03	3.57 ^{ab} ±0.07	4.49 ±0.07	0.67 ^c ±0.03	11.85 ±0.22	127.3 ^c ±3.59	85.10 ^b ±2.53	68.45 ^b ±2.45	39.60 ^c ±3.55
Fish sex											
Male	54.50 ^b ±2.67	37.20 ±0.69	0.99 ±0.02	3.52 ±0.06	4.51 ±0.06	0.77 ^a ±0.03	8.33 ±0.49	178.4 ±9.79	85.50 ^b ±2.51	72.43 ±1.61	88.67 ±10.34
Female	62.93 ^a ±5.51	39.07 ±1.16	0.99 ±0.03	3.60 ±0.06	4.55 ±0.05	0.72 ^b ±0.03	11.53 ±2.76	180.0 ±11.09	94.60 ^a ±3.56	72.27 ±1.94	87.30 ±11.09
Probability of the interactions											
Handling	0.0133	0.6935	0.8464	0.0009	0.0004	0.0007	0.0333	0.0152	0.0001	0.0214	0.0025
Species	0.0001	0.0493	0.0041	0.0125	0.3804	0.0001	0.2350	0.0001	0.0001	0.0066	0.0001
Sex	0.0394	0.1193	1.0000	0.1951	0.5680	0.0313	0.2417	0.7893	0.0001	0.9269	0.8156
Handling*Species	0.0008	0.2089	0.6139	0.1834	0.1334	0.0001	0.2224	0.0001	0.0001	0.0042	0.0001
Handling*Sex	0.2437	0.6125	0.8464	0.0039	0.0079	0.5817	0.3655	0.2638	0.0262	0.0195	0.7372
Species*Sex	0.0004	0.0449	1.0000	0.0019	0.0016	0.1340	0.3418	0.0136	0.0190	0.0271	0.0025
Handling*Species*Sex	0.0054	0.0044	0.7680	0.4432	0.2782	0.6958	0.3801	0.8765	0.0112	0.0001	0.1742

a-c: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GL: globulin, TP: total protein, TCH: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein.

Fish farm location and fish size: Fish farm conditions affected significantly but unexpected that the best-sound farm (1st) fishes reflected lower Hb, Hct, RBCs, AST, ALT, TP, AL, GL, urea, and TCH (Tables 9 and 10) than the worst farm's fish (3rd, Sobhy), which reflected high mortality with pathogenic symptoms, as given in the materials.

Table 9: Impact of fish farm and fish size on haematological parameters

	Hb (g/dl)	Hct (%)	RBCs (X 10 ⁶ /μl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets (X 10 ³ /μl)	WBCs (X 10 ³ /μl)
Farms								
Health fish	10.01 ^a ±0.24	18.05 ^c ±0.71	1344 ^c ±34.71	144.00 ^a ±0.94	74.57 ^a ±0.63	51.65 ^a ±0.38	75600 ^a ±8931	96290 ±2494
Gamal fish	9.68 ^b ±0.23	21.32 ^b ±0.57	1559 ^b ±31.21	139.40 ^b ±3.36	59.86 ^b ±0.68	43.40 ^b ±0.76	144000 ^a ±10150	91570 ±9550
Sobhy fish	11.96 ^a ±0.40	30.06 ^a ±0.36	2200 ^a ±21.96	136.30 ^b ±0.79	54.51 ^c ±1.36	39.80 ^c ±0.85	123280 ^a ±11881	96520 ±4460
Fish size (g)								
50	10.87 ^a ±0.45	23.94 ^a ±1.41	1707 ±108.31	143.20 ^a ±1.49	64.49 ^a ±1.92	45.25 ±1.29	106800 ±13243	103053 ^a ±5248
150	10.23 ^b ±0.21	22.35 ^b ±1.41	1695 ±91.05	136.60 ^b ±1.77	61.47 ^b ±2.72	44.65 ±1.56	121787 ±8521	86533 ^b ±3705
Interactions								
Health*50	9.88 ±0.17	19.02 ±0.26	1338 ±32.77	143.20 ±1.83	74.24 ±0.92	51.90 ±0.51	52800 ±9151	93120 ±1781
Health*150	10.14 ±0.47	17.08 ±1.32	1350 ±65.80	144.80 ±0.58	74.90 ±0.94	51.40 ±0.60	98400 ±3906	99460 ±4448
Gamal *50	9.60 ±0.40	21.81 ±1.15	1522 ±47.79	148.60 ±2.01	60.76 ±1.19	41.62 ±0.67	129000 ±14595	107340 ±15582
Gamal *150	9.76 ±0.28	20.83 ±0.15	1596 ±37.63	130.20 ±2.13	58.96 ±0.53	45.18 ±0.73	159000 ±11752	75800 ±6581
Sobhy *50	13.14 ±0.16	30.99 ±0.14	2262 ±14.63	137.80 ±1.16	58.46 ±0.52	42.22 ±0.46	138600 ±20968	108700 ±2458
Sobhy *150	10.78 ±0.12	29.13 ±0.35	2138 ±5.83	134.80 ±0.58	50.56 ±0.44	37.38 ±0.33	107960 ±8844	84340 ±3048
P value	0.0002	0.7719	0.0546	0.0001	0.0001	0.0001	0.0150	0.0384

a-c: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, Hb: haemoglobin, Hct: haematocrit, RBCs: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, WBCs: white blood cells.

The big sized fish gave significantly lower haematological values than the smaller ones. The interactions were more significant for most haematological (Table 9) than the biochemical parameters (Table 10).

Table 10: Impact of fish farm and fish size on blood biochemical parameters

	AST (u/l)	ALT (u/l)	AL (g/dl)	GL (g/dl)	TP (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	TCH (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Farms											
Health fish	131.3 ^a ±8.26	40.40 ^a ±2.40	0.99 ^b ±0.04	2.02 ^b ±0.17	3.31 ^c ±0.15	1.45 ^a ±0.07	4.33 ^b ±0.23	125.50 ^b ±6.23	170.90 ^a ±4.18	97.00 ^a ±3.63	18.10 ^b ±2.16
Gamal fish	262.4 ^a ±5.89	75.00 ^a ±2.84	1.89 ^a ±0.06	3.13 ^a ±0.23	5.25 ^a ±0.22	0.42 ^b ±0.02	7.30 ^a ±0.65	139.40 ^a ±6.62	97.80 ^c ±5.52	65.10 ^b ±4.18	56.50 ^a ±6.25
Sobhy fish	272.0 ^a ±5.93	72.60 ^a ±6.00	2.01 ^a ±0.07	2.49 ^b ±0.28	4.63 ^b ±0.25	0.45 ^b ±0.03	6.20 ^a ±0.80	129.30 ^{a,b} ±4.29	116.40 ^b ±5.62	41.50 ^c ±2.43	64.20 ^a ±2.93
Fish size (g)											
50	222.1 ±19.36	67.73 ^a ±5.28	1.57 ^b ±0.12	2.75 ±0.22	4.50 ±0.29	0.78 ±0.14	5.31 ±0.56	124.67 ^b ±4.47	127.87 ±11.07	69.87 ±7.37	45.67 ±5.52
150	221.7 ±16.52	57.60 ^b ±5.04	1.69 ^a ±0.13	2.35 ±0.21	4.29 ±0.26	0.77 ±0.13	6.57 ±0.57	138.13 ^a ±4.66	128.87 ±7.00	65.87 ±5.89	46.87 ±7.07
Interactions											
Health*50	122.4 ±5.07	43.40 ±4.08	0.96 ±0.05	1.86 ±0.12	3.11 ±0.13	1.48 ±0.06	3.94 ±0.14	114.00 ±9.88	181.80 ±2.60	106.60 ±2.62	20.60 ±3.67
Health*150	140.2 ±15.54	37.40 ±2.16	1.02 ±0.07	2.18 ±0.31	3.50 ±0.26	1.42 ±0.12	4.72 ±0.39	137.00 ±3.27	160.00 ±3.54	87.40 ±2.50	15.60 ±2.11
Gamal*50	264.0 ±8.12	74.60 ±4.32	1.74 ±0.05	3.40 ±0.24	5.36 ±0.24	0.38 ±0.01	6.20 ±0.80	123.80 ±3.97	91.40 ±8.62	56.40 ±5.91	49.00 ±1.41
Gamal*150	260.8 ±9.41	75.40 ±4.20	2.04 ±0.04	2.86 ±0.39	5.14 ±0.40	0.47 ±0.03	8.40 ±0.81	155.00 ±7.72	104.20 ±6.51	73.80 ±2.44	64.00 ±12.06
Sobhy*50	280.0 ±8.94	85.20 ±4.58	2.00 ±0.09	2.98 ±0.32	5.02 ±0.29	0.47 ±0.05	5.80 ±1.39	136.20 ±5.87	110.40 ±8.35	46.60 ±3.19	67.40 ±5.11
Sobhy*150	264.0 ±6.78	60.00 ±7.87	2.02 ±0.11	2.00 ±0.35	4.24 ±0.35	0.43 ±0.04	6.60 ±0.93	122.40 ±4.96	122.40 ±7.37	36.40 ±1.86	61.00 ±2.72
<i>P value</i>	0.2237	0.0346	0.1225	0.1154	0.1600	0.4197	0.6331	0.0038	0.0218	0.0001	0.1370

a-c: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GL: globulin, TP: total protein, TCH: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein.

Starvation and fish sex: Starvation significantly increased each of Hct, MCV, WBCs, urea, and TG (Tables 11 and 12). Males had lower significantly Hct and WBCs, but higher TP, TCH, and LDL values. The interactions were more significant for most haematological (Table 11) than the biochemical parameters (Table 12).

Table 11: Impact of starvation and fish sex on haematological parameters

	Hb (g/dl)	Hct (%)	RBCs (X 10 ⁶ /μl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets (X 10 ³ /μl)	WBCs (X 10 ³ /μl)
Starvation								
Before	13.35 ±0.19	31.35 ^b ±0.68	2065 ±115.4	146.3 ^b ±1.58	59.48 ±1.06	40.12 ±0.58	219100 ^a ±10147	86475 ^b ±2895
After	13.71 ±0.23	34.74 ^a ±0.89	2272 ±49.27	150.8 ^a ±1.41	60.56 ±0.38	40.19 ±0.49	181100 ^b ±9995	100650 ^a ±2383
Fish sex								
Male	13.31 ±0.16	31.70 ^b ±0.61	2137 ±37.73	149.4 ±1.16	59.24 ±1.02	40.10 ±0.45	200850 ±9271	88420 ^b ±3078
Female	13.76 ±0.25	34.32 ^a ±0.99	2201 ±123.9	147.7 ±1.90	60.80 ±0.45	40.21 ±0.61	199350 ±12444	98705 ^a ±2663
Interactions								
Before*Male	13.36 0.29	31.61 ±0.33	2125 ±73.61	151.4 ±0.60	56.90 ±1.76	38.45 ±0.22	196100 ±12181	79870 ±4140
Before*Female	13.34 0.27	31.80 ±0.78	2005 ±223.6	141.1 ±2.07	62.06 ±0.42	41.79 ±0.86	242100 ±12991	93080 ±2926
After*Male	13.25 0.15	32.65 ±0.48	2148 ±23.70	147.3 ±2.10	61.57 ±0.25	41.74 ±0.46	205600 ±14475	96970 ±2575

After*Female	14.17	36.83	2396	154.3	59.54	38.63	156600	104330
	0.40	±1.46	±79.19	±1.12	±0.58	±0.53	±8882	±3788
<i>P value</i>	0.1147	0.1022	0.1490	0.0001	0.0006	0.0001	0.0005	0.3975

a-b: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, Hb: haemoglobin, Hct: haematocrit, RBCs: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, WBCs: white blood cells.

Table 12: Impact of starvation and fish sex on blood biochemical parameters

	AST (u/l)	ALT (u/l)	AL (g/dl)	GL (g/dl)	TP (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	TCH (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Starving											
Before	125.6	68.35 ^a	1.03 ^a	2.96 ^a	3.96 ^a	0.55	4.65 ^a	227.6 ^a	176.5 ^a	103.4 ^a	88.75 ^a
	±11.22	±4.89	±0.03	±0.06	±0.03	±0.02	±0.24	±7.02	±5.76	±2.18	±6.34
After	108.1	47.95 ^b	0.66 ^b	2.29 ^b	3.11 ^b	0.52	5.80 ^a	169.9 ^b	226.5 ^a	66.50 ^b	59.10 ^b
	±12.43	±1.54	±0.02	±0.02	±0.02	±0.02	±0.26	±1.40	±5.33	±1.32	±1.72
Fish sex											
Male	105.6	61.95	0.86	2.68	3.59 ^a	0.54	5.10	207.9 ^a	206.6	84.95	81.65 ^a
	±12.07	5.03	±0.05	±0.11	±0.11	±0.02	±0.26	±9.86	±9.15	±4.97	±6.99
Female	128.1	54.35	0.83	2.57	3.48 ^b	0.53	5.35	189.6 ^b	196.5	84.95	66.20 ^b
	±11.37	±3.22	±0.04	±0.07	±0.09	±0.02	±0.30	±5.72	±6.41	±4.20	±3.34
Interactions											
Before*Male	124.3	77.80	1.06	3.07	4.05	0.54	4.50	245.0	176.5	104.7	105.00
	±20.31	±6.75	±0.05	±0.11	±0.04	±0.03	±0.27	±9.98	±8.10	±4.05	±8.99
Before*Female	126.8	58.90	0.99	2.85	3.86	0.56	4.80	210.1	176.5	102.1	72.50
	±10.88	±5.96	±0.04	±0.04	±0.02	±0.02	±0.42	±6.38	±8.63	±1.82	±5.48
After*Male	86.9	46.10	0.66	2.29	3.12	0.53	5.70	170.8	236.6	65.20	58.30
	±11.20	±2.35	±0.03	±0.03	±0.02	±0.03	±0.37	±2.17	±9.33	±1.05	±2.08
After*Female	129.3	49.80	0.66	2.28	3.09	0.50	5.90	169.0	216.4	67.80	59.90
	±20.67	±1.93	±0.02	±0.02	±0.02	±0.03	±0.38	±1.86	±3.23	±2.43	±2.84
<i>P value</i>	0.2334	0.0229	0.3266	0.0975	0.0047	0.3542	0.8908	0.0101	0.1982	0.3213	0.0040

a-b: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GL: globulin, TP: total protein, TCH: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein.

Fish size and species (salt-and freshwater fishes): Highest Hb, Hct, RBCs, AL, and LDL were reported for thin-lipped mullet (Tobara). Freshwater fishes had higher WBCs counts and creatinine concentration, but lower GL, TCH than the saltwater fishes (Tables 13 and 14). Fish size in the interactions had also significant effects.

Table 13: Impact of fish species and fish size on haematological parameters

	Hb (g/dl)	Hct (%)	RBCs (X 10 ⁶ /μl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets (X 10 ³ /μl)	WBCs (X 10 ³ /μl)
Fish species								
Nile tilapia	10.01 ^f	17.95 ^e	1333 ^e	141.7 ^a	74.95 ^a	53.01 ^b	75400 ^d	95947 ^b
	±0.33	±0.78	±51.40	±2.62	±0.60	±1.00	±6544	±2715
African catfish	14.78 ^e	26.33 ^d	2339 ^d	117.9 ^d	66.61 ^b	57.35 ^a	58867 ^d	167180 ^a
	±0.59	±1.27	±121.5	±2.43	±0.80	±1.23	±4987	±9758
Thin-lipped mullet	25.21 ^a	55.15 ^a	4774 ^a	119.7 ^c	48.99 ^c	42.81 ^d	147640 ^c	96173 ^b
	±0.64	±2.37	±117.0	±1.28	±0.98	±0.84	±12928	±6194
Grey mullet	17.69 ^b	38.70 ^b	4029 ^b	98.3 ^f	44.15 ^d	46.17 ^c	163600 ^e	80753 ^c
	0.51	±1.34	±136.1	±2.88	±0.66	±0.86	±7054	±4348
Meagre	12.80 ^d	38.21 ^b	3206 ^c	124.2 ^c	39.05 ^c	32.68 ^c	208867 ^b	59347 ^d
	±0.23	±1.10	±73.75	±1.70	±0.98	±0.34	±13769	±3862
Sea bass	11.00 ^c	34.24 ^c	2510 ^d	133.9 ^b	37.93 ^c	30.46 ^d	210267 ^b	28600 ^c
	±0.20	±0.63	±64.95	±0.94	±1.22	±0.26	±17037	±1446
Sea bream	12.23d ^c	37.84 ^b	2460 ^d	146.1 ^a	43.24 ^d	31.75 ^{cd}	294667 ^a	38267 ^c
	±0.23	±0.92	±94.52	±2.46	±1.08	±0.23	±8054	±1925
Fish size (g)								
50	14.49	32.54 ^b	2856 ^b	122.1 ^c	50.59	43.38 ^a	166886	88000 ^a
	±0.88	±1.67	±191.7	±3.13	±2.31	±2.03	±14509	±9148
150	14.81	36.02 ^a	2903 ^b	126.2 ^b	50.22	41.75 ^b	161871	73751 ^b
	±0.86	±1.96	±181.8	±2.86	±2.29	±1.68	±15147	±6481
250	15.15	37.91 ^a	3091 ^a	129.6 ^c	51.30	40.96 ^b	168089	80934 ^{ab}
	±0.89	±2.27	±204.7	±2.63	±2.48	±1.58	±14655	±7774
<i>P value</i>								
Species	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Weight	0.2850	0.0001	0.0244	0.0001	0.2368	0.0015	0.7977	0.0011
Species*Weight	0.9894	0.0041	0.5221	0.0001	0.0001	0.1220	0.1063	0.0054

a-f: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, Hb: haemoglobin, Hct: haematocrit, RBCs: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, WBCs: white blood cells.

Table 14: Impact of fish species and fish size on blood biochemical parameters

	AST (u/l)	ALT (u/l)	AL (g/dl)	GL (g/dl)	TP (g/dl)	Creati nine (mg/dl)	Urea (mg/dl)	TCH (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Fish species											
Nile tilapia	144.7 ^b ±8.35	39.93 ^c ±1.62	1.01 ^{cd} ±0.04	1.94 ^c ±0.14	3.20 ^d ±0.13	1.39 ^b ±0.07	4.18 ^c ±0.19	118.40 ^g ±5.12	163.93 ^c ±8.57	99.27 ^d ±3.04	16.60 ^d ±1.62
African catfish	60.60 ^d ±0.47	33.07 ^c ±1.48	0.89 ^d ±0.04	2.95 ^b ±0.08	4.09 ^c ±0.09	1.69 ^a ±0.05	12.00 ^{ab} ±0.45	162.67 ^f ±2.68	220.00 ^a ±15.36	175.3 ^c ±5.15	45.07 ^c ±2.93
Thin-lipped mullet	63.00 ^d ±2.69	53.87 ^b ±3.98	1.77 ^a ±0.12	3.11 ^{ab} ±0.15	5.09 ^{ab} ±0.20	0.54 ^c ±0.04	4.47 ^c ±0.22	212.80 ^d ±9.23	195.73 ^b ±4.50	77.67 ^e ±3.21	95.93 ^a ±9.91
Grey mullet	60.40 ^d ±2.20	51.60 ^b ±4.21	1.51 ^b ±0.11	3.08 ^{ab} ±0.12	4.79 ^b ±0.14	0.56 ^c ±0.03	4.00 ^c ±0.28	180.60 ^e ±7.42	177.00 ^{bc} ±3.80	81.07 ^e ±5.28	64.53 ^b ±7.50
Meagre	166.7 ^a ±9.97	74.87 ^a ±5.31	1.69 ^a ±0.03	3.26 ^{ab} ±0.13	5.19 ^a ±0.16	0.29 ^d ±0.01	11.07 ^b ±0.70	248.40 ^c ±1.99	107.60 ^d ±2.83	163.4 ^c ±3.06	63.33 ^b ±4.00
Sea bass	128.9 ^b ±6.06	35.20 ^c ±4.02	1.09 ^c ±0.03	3.39 ^a ±0.11	4.70 ^b ±0.11	0.22 ^d ±0.01	12.80 ^a ±0.56	331.13 ^b ±6.04	228.27 ^a ±7.34	219.9 ^b ±7.78	65.53 ^b ±5.68
Sea bream	122.0 ^b ±3.61	34.80 ^c ±1.50	1.05 ^{cd} ±0.03	3.38 ^a ±0.10	4.76 ^b ±0.10	0.23 ^d ±0.01	12.93 ^a ±0.63	351.20 ^a ±7.35	221.60 ^a ±8.19	234.3 ^a ±4.08	72.67 ^b ±6.48
Fish size (g)											
50	113.7 ^a ±9.58	49.43 ^a ±4.51	1.21 ^b ±0.06	2.99 ±0.12	4.40 ^b ±0.15	0.71 ±0.09	9.15 ^a ±0.80	230.11 ±14.52	175.00 ^b ±8.16	153.5 ±11.21	58.60 ^{ab} ±6.34
150	99.69 ^b ±5.70	46.46 ^{ab} ±3.20	1.29 ^{ab} ±0.07	3.02 ±0.10	4.55 ^{ab} ±0.14	0.69 ±0.09	8.19 ^b ±0.70	233.40 ±15.53	192.03 ^a ±7.89	149.6 ±11.14	67.63 ^a ±5.74
250	106.5 ^{ab} ±8.01	42.26 ^b ±2.81	1.36 ^a ±0.08	3.05 ±0.11	4.68 ^a ±0.13	0.71 ±0.11	8.99 ^{ab} ±0.73	224.43 ±12.69	196.17 ^a ±9.26	147.2 ±9.89	55.34 ^b ±3.91
P value											
Species	0.0001	0.1737	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Weight	0.0091	0.1626	0.0221	0.8440	0.0507	0.8291	0.0618	0.2482	0.0059	0.3097	0.0480
Species*Weight	0.0001	0.5530	0.0005	0.0038	0.0035	0.0041	0.4618	0.0362	0.0121	0.0145	0.0095

a-g: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GL: globulin, TP: total protein, TCH: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein.

Fish sex in Nile tilapia and African catfish: Fish sex significantly affected only MCH and platelets in Nile tilapia and RBCs in African catfish (Table 15). While, No significant effects of fish sex in both fish species on biochemical parameters were detected (Table 16).

Table 15: Impact of fish species and fish sex on haematological parameters

	Hb (g/dl)	Hct (%)	RBCs (X 10 ⁶ /μl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets (X 10 ³ /μl)	WBCs (X 10 ³ /μl)
Nile tilapia								
Male	11.63 ±0.15	74.48 ±48.08	1948 ±31.98	148.3 ±0.57	62.05 ^b ±0.86	42.88 ±0.53	205661 ^b ±6035	79970 ±1882
Female	11.77 ±0.19	27.47 ±0.56	1981 ±47.52	144.3 ±2.58	66.88 ^a ±1.82	43.87 ±0.61	224331 ^a ±5114	82503 ±1829
P value	0.5653	0.3299	0.5639	0.1403	0.0179	0.2231	0.0197	0.3361
African catfish								
Male	13.74 ±0.16	75.83 ±39.23	2484 ^a ±33.56	150.6 ±1.20	64.34 ±8.75	38.63 ±0.81	164125 ±5522	82718 ±1932
Female	13.40 ±0.18	36.52 ±0.67	2365 ^b ±36.14	150.5 ±1.18	56.48 ±0.43	37.10 ±0.37	152292 ±5647	84071 ±1844
P value	0.1618	0.3184	0.0178	0.9290	0.3715	0.0905	0.1367	0.6133

a-b: Mean superscripted with different letters in the same column and group differ significantly at $P \leq 0.05$, Hb: haemoglobin, Hct: haematocrit, RBCs: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, WBCs: white blood cells.

Table 16: Impact of fish species and fish sex on blood biochemical parameters

	AST (u/l)	ALT (u/l)	AL (g/dl)	GL (g/dl)	TP (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	TCH (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Nile tilapia											
Male	147.4 ±7.51	57.00 ±3.40	1.02 ±0.02	3.01 ±0.10	4.26 ±0.07	0.76 ±0.05	5.41 ±0.18	229.6 ±5.61	194.1 ±7.82	90.71 ±1.72	99.30 ±4.99
Female	136.3 ±6.56	60.26 ±5.01	1.02 ±0.01	3.07 ±0.10	4.26 ±0.07	0.74 ±0.04	5.21 ±0.17	221.9 ±5.41	186.1 ±5.49	90.51 ±1.09	96.77 ±4.91
P value	0.2668	0.5917	0.9839	0.6923	0.9538	0.8179	0.4181	0.3214	0.4036	0.9220	0.7186
African catfish											
Male	120.9 ±6.50	46.30 ±1.61	1.05 ±0.01	3.15 ±0.08	4.37 ±0.06	0.80 ±0.05	6.32 ±0.37	247.2 ±5.95	187.5 ±7.78	91.02 ±1.73	115.10 ±5.25
Female	116.1 ±8.20	47.77 ±1.83	1.07 ±0.02	3.19 ±0.10	4.37 ±0.08	0.77 ±0.05	7.82 ±1.44	259.8 ±5.08	182.6 ±7.47	90.92 ±1.67	128.43 ±5.52
P value	0.6496	0.5496	0.4515	0.7496	0.9866	0.6846	0.3152	0.1095	0.6537	0.9669	0.0825

AST: aspartate aminotransferase, ALT: alanine aminotransferase, GL: globulin, TP: total protein, TCH: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein.

DISCUSSION

Hassanen *et al.* (1997) working on sea bass (*Dicentrarchus labrax*), found that neither trash fish nor pelleted feed had significant effect on Hct value, Hb concentration, and MCH. Yet, serum total protein content significantly decreased, when the percentage pelleted feed increased. Daghash and Hussein (1999) found that injecting African catfish, *Clarias gariepinus* with GnRH resulted in increasing the serum glucose and total cholesterol for male and female fish, while serum total protein elevated only in females.

Heat stress significantly increased Hb and Hct values, as well as serum glucose concentration, and serum activity of AST and ALT; but it lowered the serum activity of alkaline phosphatase and serum total protein concentration for the decrease in serum globulin rather than albumin in Nile tilapia. These negative effects of heat stress could be counteracted by dietary supplementation of ascorbic acid (Hussein and Kobeisy, 1999). Moreover, Dominguez *et al.* (2004) confirmed that elevating rearing water temperature (18.4, 23, and 28 °C) for 2 weeks increased the circulating IgM concentration in Nile tilapia, but rearing the fish at 33 °C resulted in a decrease in IgM concentration, suggesting that the fish possess an appropriate thermal range

for production of immune substances. Plasma level of IgM significantly increased with salinity at 12 and 24 g / L. On the other hand, plasma IgM level did not change by exposing the fish to acidification (pH 4.0) and suspended solids (20, 200, and 2000 mg/L). These results suggest that the specific immune system of tilapia changes by certain factors in aquatic environment.

Generally, physiological indicators from blood samples may enable heat stress, health, and welfare to be assessed in Siberian sturgeon, hence Simide *et al.* (2016) conducted routine blood samples, taken from Siberian sturgeon (*Acipenser baerii*), to test the robustness of using only physiological indicators to assess heat stress, health, and welfare. Sampling was performed after 1 month of prebiotic dietary supplementation followed by 4 weeks of sublethal heat stress. The expression of heat shock proteins (HSP) was assessed for the first time in sturgeon erythrocytes. HSP70 and HSP90 expression was triggered by both stress and dietary supplementation. Indicators of non-specific immunity were modified mainly by stress. Complement activity increases with stress while lysozyme activity decreases, but to a lesser extent in supplemented fish. The antioxidant capacity increases with stress while oxidant metabolites decrease and overall oxidative stress was lower for fish that received dietary supplementation. The positive impact of dietary supplementation on health status was observable after a stress challenge.

Moreover, Gaber (2000) concluded that there was no significant effect of dietary inclusion of graded levels of clove oil (as a growth promoter) on RBCs, Hb, PCV, WBCs, and total serum protein. Exposure of fish to phenol, copper sulphate, ammonia, hypoxia, acidic water, starvation and cold water resulted in reduction of serum hemolytic activity as compared to the control values. Small (2004) came to the conclusion that isoeugenol sedated channel catfish (*Ictalurus punctatus*) and lowered plasma cortisol level than the control fish after 15 min. of confinement. No differences in plasma levels of glucose or lactate were observed whether under exposure to high ammonia or sedation with isoeugenol. Fish exposed to high ammonia for 24 h had elevated cortisol levels. Levels of plasma cortisol, glucose and lactate all increased significantly in fish following acute oxygen depletion for 30 min. So, isoeugenol had suppressive effects on channel catfish plasma cortisol levels during confinement and low oxygen conditions, and reduced the plasma lactate response to acute oxygen depletion.

Significant elevation in serum complement activity occurred in sea bass fed alginic acid (Ergosan) and β -glucan (Macrogard) for 15 days. Significant elevation in serum lysozyme, gill and liver HSP concentration was observed, whereas a significant increase of complement activity was only observed in fish that received an Ergosan diet for 15 days. On the long-term period (45 days), no significant differences were observed in innate and specific immune parameters (Bagni *et al.*, 2005). Concentration of plasma glucose significantly increased and concentration of plasma total protein significantly decreased of all groups (with the exception of copper groups concerning glucose) as compared to the control levels. Total WBCs count and differential count was significantly increased of fish treated with copper and water cold stress. While, a non-significant decrease of WBCs was found in fish groups treated with phenol, acidic water, ammonia and starvation. Platelets and RBCs counts tended to reduce in all groups except in the hypoxia fish group. Values of Hct significantly reduced, except in the hypoxia fish group. Concomitant changes in Hb levels were also found. Phenol induced significant decrease in serum lysozyme activity. Hypoxia, cold water, acidic water, and ammonia significantly reduced lysozyme activity. Fish exposed to copper sulphate showed elevated serum lysozyme

activity compared to the control. Starvation for 14 days significantly reduced lysozyme activity in examined samples. This study have shown that both serum lysozyme activity and serum spontaneous activity (SH) can be determined easily in blood samples drawn from live fish which make them potentially useful markers traits for indirect monitoring of disease resistance in fish (Abdelhamid *et al.*, 2006). Phenolic pollution led to significant decrease in serum concentrations of tri-iodothyronine (T_3 , 76.3 vs. 31.4 ng/dL) and thyroxin (T_4 , 8.33 vs. 1.61 ng/dL) hormones, but to significant increase in serum levels of total cholesterol (166 vs. 310 mg/dL) and total lipids (1.82 vs 3.26 g/dL). Thus, phenol is harmful to Nile tilapia (Gad and Saad, 2008).

Adam (2007) registered a negative correlation between hematological indices and water quality parameters with moderate regression factors. Relationship between RBCs and water quality characteristics was positive and with very low regression factor. Gradual (but significant) decrease in Hb and PCV values were reported for Nile tilapia by replacing fish meal by gradual percentages of PPM (PPM: consisted of cottonseed, sunflower, canola, sesame and linseed meals) in their diets; whereas the opposite trend was found for the transaminases activity (Soltan *et al.*, 2008).

Metwalli (2013) gave the following values for Nile tilapia blood, 6.3-6.5 g/dL total protein, 3.2-3.3.4 g/dL albumin, 7.1-7.7.6 mg/dL urea, 1.13-1.18 mg/dL creatinine, 115-124 u/L AST, and 44.0-46.0 u/L ALT. Fish in poly-culture system (Nile tilapia, silver carp, common carp, and African catfish) fed dried sewage sludge did not negatively affect the blood picture in general, although the significantly elevated values of transaminases activity and triglycerides concentration (Abdelhamid *et al.*, 2014).

More recently, Abdelhamid *et al.* (2019) investigated the effect of 5 ng aflatoxin B (AFB) / g (ppb) in the diets of Nile tilapia (*Oreochromis niloticus*) and the detoxification activity of a commercial anti-mycotoxin ARCAVIT[®] Bioacid Forte (0.5 g/kg diet) for 70 days. They reported that AFB is very toxic, even at a low concentration as 5 ng / g diet, for Nile tilapia. It threatens fish hematological and plasma biochemistry parameters. Moreover, the anti-toxic agent (ARCAVIT[®]) at the tested level did not completely overcome the aflatoxicosis symptoms.

CONCLUSION

Fish blood parameters are variable and not constant; so, the interpretation of blood data of fish must be done on light of the specific individual experiment condition because of absence of referenced ranges for fish haematological and biochemical measurements.

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ARABIC SUMMARY

العوامل التي تؤثر على صورة دم الأسماك: (ب) تأثير العوامل البيئية والوراثية

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لاستكمال فهم مختلف العوامل المؤثرة على صورة دم الأسماك، استمرت الدراسة في هذا الجزء (ب) من سلسلة البحوث لإلقاء الضوء على تأثير بعض العوامل البيئية والوراثية، وهي أساسا درجة حرارة المياه، مواد التخدير، إجهاد التداول، الاستزراع متعدد الأنواع، ظروف المزارع، التصويم، نوع الأسماك وجنسها (ذكور وإناث) وحجمها. وكل هذه العوامل قد أثرت في معظم القياسات الهيماتولوجية و/أو البيوكيماوية للأسماك.