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Factors Affecting Fish Blood Profile: A- Effect of Nutritional Treatments

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ABSTRACT

Blood profile was studied as affected by different fish species, dietary crude protein, replacements, and additives, as well as diets' types, in laboratorial and field studies. It was clear that most studied treatments (food types, probiotic levels, levels of clover seed wastes, initial body weight, and Teen Barshomy wastes and levels) had been significantly affected most studied haematological and/or biochemical parameters. That means that fish blood constituents are not stable within known ranges, but widely varied according to various environmental conditions.

INTRODUCTION

Press and Evensen (1999) mentioned that species variation in the morphology of the immune system is to be expected, given the large number and diversity of species within the teleost fishes. Several external and internal factors can influence the activity of innate immune parameters. Temperature changes, handling and crowding stress can have suppressive effects on innate parameters, whereas several food additives and immunostimulants can enhance different innate factors. There is limited data available about the ontogenic development of the innate immunological system in fish (Magnadóttir, 2006). Moreover, Sahan and Duman (2008) found that haematocrit, leucocytes, monocytes, and neutrophils were increased in common carp fed beta glucan. Also, Aly *et al.* (2008) showed significant increase in haematocrit values in group of Nile tilapia fed the mixture of *B. subtilis* and *L. acidophilus* comparing with the control. However, the present study was designed to evaluate the effect of different nutritional treatments on some blood parameters.

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samples were taken for blood collected from the caudal peduncle by special syringe, adequate amount of whole blood was withdrawn in small plastic vials containing EDTA (ethylene diamine tetra acetic acid) as anticoagulant and used to obtain the blood plasma by centrifuge at 3500 rpm for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. Blood 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 erythrocytes (RBCs), and total leukocytes (WBC_S) (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. Five fishes from each treatment in each case were chosen to withdraw blood samples to study the effects of different nutritional treatments on blood profile as followings:

1- Field study (in a private fish farm, Hag Aboulenin, in Metubus - Kafr El-Sheikh governorate during season 2017) of feeding Nile tilapia for 96 days on graded levels of a probiotic (PRO-LYNE®) addition of floating and sinking diets.
2- In-door laboratorial study (Aquaculture Research Unit, Sakha, Central Lab. of Aquaculture Research, Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt) on feeding Mono-sex Nile tilapia diets containing graded levels of ground wastes of sieving the Egyptian clover seeds instead of the dietary soybean meal on basis of crude protein content, for 57 days during 2017.
3- Field study in Mahmud Hussein Hatchery, Tolombat-7, Al-Reiad, Kafr El-Sheikh governorate during season 2017 to study the effect of dietary crude protein levels (27, 32, and 38%) on common carp for 6 months during 2018.
4- To study the effect of feeding all-males mono-sex Nile tilapia for 75 days on diets containing 25 and 50% fig skins and leaves instead of the dietary corn.

All obtained data were analyzed according to statistical analysis system software (SAS, 2006) for windows. Duncan's multiple range tests (Duncan, 1955) were used to compare between the parameters of the different nutritional groups. The differences were significant at 0.05 levels.

RESULTS

A study was conducted over 96 days in order to comprise between floating and sinking diets supplemented with and/or without a probiotic and their effects on growth performance chemical composition and food utilization of Nile tilapia (*Oreochromis niloticus*). The two studied factors were the diet type (floating and sinking diets) and probiotic (PRO-LYNE®, at 0%, 1% and 2% of the diet). Food type (floating or sinking diets) significantly affected blood values of Hct, mean corpuscular haemoglobin concentration (MCHC), platelets and WBCs; whereas the probiotic level affected all tested haematological parameters (Table 1), except RBCs, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH). Meanwhile, the only significant interaction (food type × probiotic level) was calculated for Hct and MCH. Concerning the biochemical parameters (Table 2), food type significantly affected on urea, triglycerides (TG), and low density lipoprotein (LDL) only. The probiotic level did not affect the measured biochemical parameters. Yet, the interaction was significant for AST, ALT, and high density lipoprotein (HDL).

Table 1: Impact of diet type and probiotic on haematological parameters of the Nile tilapia

	Hb (g/dl)	Hct (%)	RBCs (X 10^6/µl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets (X 10^3/µl)	WBCs (X 10^3/µl)
Food types								
Floating	11.23 ±0.26	32.42^{a} ±1.30	2224 ±155.0	133.6 ±0.90	20.27 ±0.44	28.73^{b} ±0.66	125000^{a} ±8409	69067^{a} ±3173
Sinking	11.03 ±0.20	28.36^{b} ±0.88	2000 ±36.00	135.0 ±0.81	20.40 ±0.49	30.33^{a} ±0.36	95000^{b} ±7528	56367^{b} ±2315
Additive (%)								
0	11.60^{a} ±0.28	31.44^{a} ±0.93	2075 ±50.69	135.6 ±0.99	20.30 ±0.65	28.20^{b} ±0.92	103000^{ab} ±12806	71850^{a} ±4224
1	10.70^{b} ±0.24	27.92^{b} ±1.14	2235 ±229.1	133.1 ±1.05	20.00 ±0.56	29.80^{ab} ±0.39	127000^{a} ±10651	63700^{b} ±2336
2	11.10^{ab} ±0.28	31.81^{a} ±1.99	2027 ±74.58	134.2 ±1.07	20.70 ±0.50	30.60^{a} ±0.45	100000^{b} ±6791	52600^{c} ±2322
Interactions								
Floating *0	11.70 ±0.46	31.12 ±1.45	2100 ±74.16	134.4 ±1.72	19.00 ±0.63	26.60 ±1.54	130000 ±12450	81700 ±5578
Floating *1	10.70 ±0.37	28.94 ±2.15	2443 ±460.4	131.2 ±1.07	20.00 ±0.63	29.40 ±0.51	146000 ±14265	67400 ±1187
Floating *2	11.30 ±0.46	37.19 ±1.39	2129 ±129.0	135.2 ±±1.46	21.80 ±0.49	30.20 ±0.37	99000 ±9925	58100 ±1592
Sinking*0	11.50 ±0.35	31.76 ±1.32	2049 ±75.75	136.8 ±0.86	21.60 ±0.81	29.80 ±0.37	76000 ±14782	62000 ±806
Sinking*1	10.70 ±0.34	26.90 ±0.84	2026 ±50.18	135.0 ±1.41	20.00 ±1.00	30.20 ±0.58	108000 ±11247	60000 ±4037
Sinking*2	10.90 ±0.33	26.43 ±1.18	1924 ±56.02	133.2 ±1.59	19.60 ±0.51	31.00 ±0.84	101000 ±10416	47100 ±2571
P value	0.8783	0.0016	0.6665	0.1135	0.0085	0.2517	0.0847	0.1511

a-c: Mean superscripted with different letters in the same column and group differ significantly at P≤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 diet type and probiotic on biochemical parameters of the Nile tilapia

	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)
Food types											
Floating	174.5 ±11.40	51.27 ±3.35	5.12 ±0.10	1.23 ±0.03	3.47 ±0.11	0.30 ±0.01	12.27^{b} ±0.91	284.8 ±7.84	338.2^{a} ±16.23	142.5 ±4.13	80.27^{a} ±5.47
Sinking	189.2 ±9.70	52.07 ±3.97	4.84 ±0.10	1.18 ±0.02	3.39 ±0.11	0.31 ±0.01	16.27^{a} ±0.85	272.3 ±6.97	287.2^{b} ±18.04	150.3 ±6.15	64.73^{b} ±5.01
Additive (%)											
0	189.8 ±11.42	46.70 ±3.70	4.94 ±0.16	1.16 ±0.04	3.42 ±0.17	0.31 ±0.01	14.30 ±1.10	283.1 ±9.97	298.6 ±26.72	149.3 ±8.31	75.70 ±7.54
1	186.4 ±11.35	52.40 ±4.24	5.10 ±0.13	1.20 ±0.04	3.50 ±0.12	0.30 ±0.01	13.40 ±1.47	280.7 ±12.04	332.1 ±22.27	150.4 ±5.99	69.60 ±6.92
2	169.4 ±15.97	55.90 ±5.15	4.91 ±0.09	1.25 ±0.03	3.36 ±0.10	0.32 ±0.02	15.10 ±1.21	271.9 ±4.23	307.4 ±17.72	139.4 ±4.47	72.20 ±6.45
Interactions											
Floating *0	187.0 ±16.82	51.00 ±7.14	5.20 ±0.26	1.14 ±0.07	3.64 ±0.25	0.33 ±0.02	12.40 ±1.29	278.6 ±18.12	338.6 ±34.55	129.6 ±8.62	84.60 ±13.33
Floating *1	199.6 ±12.83	58.00 ±5.39	5.22 ±0.14	1.24 ±0.05	3.54 ±0.14	0.29 ±0.01	10.20 ±1.66	298.4 ±14.60	378.4 ±13.84	149.0 ±6.19	84.60 ±6.42
Floating *2	137.0 ±19.24	44.80 ±4.00	4.94 ±0.11	1.30 ±0.04	3.22 ±0.11	0.30 ±0.02	14.20 ±1.53	277.4 ±5.91	297.6 ±23.75	148.8 ±2.08	71.60 ±8.18
Sinking*0	192.6 ±17.33	42.40 ±1.12	4.67 ±0.13	1.18 ±0.04	3.20 ±0.21	0.29 ±0.02	16.20 ±1.39	287.6 ±10.43	258.6 ±34.91	169.0 ±6.53	66.80 ±6.22
Sinking*1	173.2 ±18.12	46.80 ±6.03	4.99 ±0.22	1.16 ±0.05	3.46 ±0.21	0.30 ±0.01	16.60 ±1.36	263.0 ±16.79	285.8 ±31.12	151.8 ±11.05	54.60 ±7.87
Sinking*2	201.8 ±15.92	67.00 ±6.44	4.87 ±0.14	1.20 ±0.03	3.50 ±0.15	0.35 ±0.04	16.00 ±1.95	266.0 ±5.50	317.2 ±28.30	130.0 ±6.44	72.80 ±10.95
P value	0.0374	0.0082	0.4223	0.3154	0.1735	0.1500	0.3450	0.2462	0.1224	0.0021	0.2511

a-b: Mean superscripted with different letters in the same column and group differ significantly at P≤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.

A feeding experiment for 57 days was conducted on Nile tilapia to evaluate the effects of dietary inclusion of graded levels (0, 25, 50, 75, and 100%) of sieving wastes meal of Egyptian clover seeds instead of soybean meal based on crude protein content. The replacement level significantly affected all tested haematological parameters, except MCH (Table 3). It also affects albumin (AL), urea, total cholesterol (TCH), and HDL (Table 4).

Table 3: Impact of dietary inclusion of graded levels of sieving wastes meal of Egyptian clover seed on haematological parameters of the Nile tilapia

Treatments	Hb (g/dl)	Hct (%)	RBCs (X 10^6/µl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets (X 10^3/µl)	WBCs (X 10^3/µl)
0%	11.84^a	26.69^a	2130^a	124.2^a	55.20	43.64^b	121800^a	84600^a
	±0.52	±1.12	±124.5	±3.06	±1.17	±0.36	±11097	±5250
50%	9.12^b	21.22^b	1674^b	127.4^a	53.38	41.60^c	122800^a	64600^b
	±0.44	±1.23	±76.00	±2.50	±1.37	±0.51	±1594	±5240
75%	12.74^a	30.83^a	2472^a	128.0^a	52.32	42.26^{bc}	130400^a	93400^a
	±0.56	±1.77	±159.9	±0.95	±0.66	±0.62	±3400	±6735
100%	8.02^b	18.49^b	1675^b	115.4^b	52.20	46.24^a	98800^b	46600^c
	±0.55	±2.08	±149.3	±3.88	±2.08	±0.41	±2557	±4468

a-c: Mean superscripted with different letters in the same column and group differ significantly at P≤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 dietary inclusion of graded levels of sieving wastes meal of Egyptian clover seed on biochemical parameters of the Nile tilapia

Treatments	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)
0%	314.8	148.0	4.22	1.33^a	2.68	0.12	13.80^a	150.80^{ab}	146.80	65.80^a	55.40
	±1.46	±7.62	±0.21	±0.03	±0.22	±0.00	±1.69	±3.07	±14.58	±1.66	±3.49
50%	324.0	126.4	4.33	1.29^{ab}	3.00	0.12	11.00^{ab}	146.40^{ab}	148.20	62.60^b	53.80
	±7.04	±6.01	±0.17	±0.03	±0.20	±0.00	±1.30	±3.17	±2.01	±0.81	±3.88
75%	311.8	134.8	4.40	1.27^{ab}	2.82	0.12	12.80^a	155.60^a	153.60	58.60^c	65.80
	±3.29	±11.02	±0.19	±0.03	±0.29	±0.00	±1.16	±4.35	±3.04	±0.51	±4.37
100%	311.2	124.2	4.04	1.23^b	2.62	0.11	8.80^b	142.40^b	145.20	58.80^c	54.20
	±0.73	±16.07	±0.14	±0.01	±0.12	±0.00	±0.66	±4.99	±2.27	±0.58	±4.84

a-c: Means superscripted with different letters in the same column differ significantly at P≤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.

A Field study in Mahmud Hussein Hatchery, Tolombat-7, Al-Reiad, Kafr El-Sheikh governorate was conducted to study the effect of dietary crude protein levels (27, 32, and 38%) on common carp for 6 months**.** The 32%-CP-diet reflected the highest (P≤0.05) platelets' count (Table 5); yet, increasing dietary CP% led to gradual increase (P≤0.05) in ALT activity, creatinine concentration, but gradual decreased (P≤0.05) triglycerides level (Table 6).

Table 5: Impact of dietary crude protein levels on haematological parameters of common carp

Treat.	Hb (g/dl)	Hct (%)	RBCs (X 10^6/µl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets (X 10^3/µl)	WBCs (X 10^3/µl)
27%	14.22	22.84	1960	114.8	71.62	65.78	137600^b	183400
	±0.51	±1.30	±102.1	±4.02	±2.11	±3.46	±2379	±7852
32%	13.62	24.07	2018	111.4	66.04	59.24	184000^a	197200
	±0.84	±3.51	±172.6	±6.47	±2.03	±4.85	±13817	±11719
38%	13.62	22.98	1950	113.8	67.44	60.10	151600^{ab}	180200
	±0.17	±0.76	±42.78	±1.66	±1.04	±1.45	±13974	±5014

a-b: Mean superscripted with different letters in the same column and group differ significantly at P≤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 dietary crude protein levels on biochemical parameters of the common carp

Treat.	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)
27%	73.80 ±11.80	19.40^{b} ±0.87	3.58 ±0.16	0.80 ±0.03	2.46 ±0.18	0.25^{ab} ±0.01	12.62 ±0.23	131.60 ±2.09	230.2^{a} ±20.45	41.80 ±0.80	43.60 ±4.79
32%	83.60 ±5.19	23.80^{b} ±1.62	3.52 ±0.22	0.82 ±0.04	2.44 ±0.22	0.23^{b} ±0.01	12.60 ±0.24	139.40 ±9.50	186.8^{ab} ±9.17	45.40 ±1.96	56.40 ±8.31
38%	98.40 ±12.11	30.40^{a} ±1.75	3.37 ±0.11	0.84 ±0.04	2.22 ±0.15	0.27^{a} ±0.01	13.00 ±0.45	133.80 ±3.28	176.4^{b} ±10.71	50.80 ±4.49	47.40 ±2.73

a-b: Means superscripted with different letters in the same column differ significantly at P≤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.

A laboratory study was conducted to evaluate the effects of replacing 25 and 50% of the diet's corn by meals of fruit skin and leaves of Teen Barshomy (Teen Shoky) on Nile tilapia for 75 days. The replacement type (pee / waste) significantly affected WBCs only; whereas, the replacement level affected significantly all tested haematological parameters, except MCV, MCH, and MCHC. The interaction (replacement type × replacement level) was significant only for platelets (Table 7). The replacement type significantly affected TCH, HDL, and LDL. Moreover, the replacement level significantly affected total protein (TP), albumin (AL), globulin (GL) and LDL (Table 8). The interaction (type × level of replacement) was significant only for TCH.

Table 7: Impact of dietary inclusion of of fruit skin and leaves of Teen Barshomy on haematological parameters of the Nile tilapia

	Hb (g/dl)	Hct (%)	RBCs (X 10^6/µl)	MCV (fl)	MCH (pg)	MCHC (%)	Platelets (X 10^3/µl)	WBCs (X 10^3/µl)
Replacement type								
Skin of fruits	14.14 ±0.47	35.03 ±1.27	2619 ±82.37	138.5 ±1.84	55.74 ±1.07	41.65 ±0.64	255733 ±19412	113333^{a} ±3526
Leaves of fruits	13.83 ±0.53	34.37 ±1.33	2467 ±94.28	133.3 ±1.73	53.63 ±1.67	43.25 ±0.60	242800 ±19172	104767^{b} ±4416
Replacement rate (%)								
0	16.32^{a} ±0.32	40.13^{a} ±0.91	2918^{a} ±11.04	136.6 ±3.15	53.50 ±1.41	42.02 ±0.97	201600^{b} ±6076	123600^{a} ±1893
25	12.75^{b} ±0.31	31.19^{b} ±1.13	2329^{b} ±86.47	135.5 ±1.78	54.08 ±2.53	42.90 ±0.82	311800^{a} ±25595	105200^{b} ±4577
50	12.89^{b} ±0.24	32.77^{b} ±0.89	2381^{b} ±85.25	135.6 ±1.98	56.48 ±0.78	42.43 ±0.59	234400^{b} ±18636	98350^{b} ±4266
Interactions								
Skin * 0%	16.32 ±0.48	40.13 ±1.37	2918 ±16.55	136.6 ±4.73	53.50 ±2.12	42.02 ±1.46	201600 ±9114	123600 ±2839
Skin * 25%	13.10 ±0.36	31.62 ±1.85	2450 ±138.4	139.6 ±1.36	58.00 ±1.97	41.58 ±1.25	285800 ±48653	114600 ±3234
Skin * 50%	13.00 ±0.39	33.32 ±1.25	2488 ±134.2	139.2 ±3.17	55.72 ±1.06	41.34 ±0.76	279800 ±20076	101800 ±7214
Leaves * 0%	16.32 ±0.48	40.13 ±1.37	2918 ±16.55	136.6 ±4.73	53.50 ±2.12	42.02 ±1.46	201600 ±9114	123600 ±2839
Leaves * 25%	12.40 ±0.51	30.77 ±1.51	2208 ±84.58	131.4 ±2.01	50.16 ±4.16	44.22 ±0.78	337800 ±15583	95800 ±6296
Leaves * 50%	12.78 ±0.31	32.22 ±1.36	2274 ±94.69	132.0 ±1.05	57.24 ±1.15	43.52 ±0.65	189000 ±11367	94900 ±4890
P value	0.7060	0.9253	0.3912	0.3928	0.1194	0.4584	0.0185	0.1701

a-b: Mean superscripted with different letters in the same column and group differ significantly at P≤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 dietary inclusion of of fruit skin and leaves of Teen Barshomy on biochemical parameters of the Nile tilapia

	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)
Replacement type											
Skin of fruits	187.1 ±23.95	90.73 ±7.80	4.44 ±0.10	1.11 ±0.03	3.12 ±0.13	0.53 ±0.02	14.93 ±0.75	154.7^{b} ±2.21	174.6 ±3.58	55.73^{b} ±1.90	63.33^{b} ±2.33
Leaves of fruits	190.9 ±22.54	87.40 ±6.87	4.45 ±0.08	1.11 ±0.04	3.08 ±0.09	0.52 ±0.02	15.60 ±0.94	170.9^{a} ±4.63	177.6 ±2.47	61.60^{a} ±1.50	73.73^{a} ±4.37
Replacement rate (%)											
0	169.2 ±27.68	86.00 ±9.84	4.13^{b} ±0.06	1.22^{a} ±0.02	2.70^{b} ±0.05	0.55 ±0.02	14.80 ±1.06	158.0 ±2.08	174.0 ±3.40	61.60 ±2.22	61.60^{b} ±3.80
25	196.2 ±28.14	83.30 ±8.06	4.61^{a} ±0.07	1.06^{b} ±0.05	3.36^{a} ±0.11	0.52 ±0.03	15.00 ±0.86	166.3 ±7.28	173.4 ±4.57	56.60 ±2.43	74.00^{a} ±5.55
50	201.5 ±30.00	97.90 ±8.81	4.58^{a} ±0.12	1.04^{b} ±0.02	3.24^{a} ±0.12	0.50 ±0.03	16.00 ±1.19	164.0 ±4.68	180.9 ±2.93	57.80 ±2.05	±70.00^{ab} ±3.49
Interactions											
Skin * 0%	169.2 ±41.52	86.00 ±14.76	4.13 ±0.08	1.22 ±0.04	2.70 ±0.07	0.55 ±0.03	14.80 ±1.59	158.0 ±3.11	174.0 ±5.10	61.60 ±3.33	61.60 ±5.70
Skin * 25%	236.0 ±44.87	83.40 ±15.38	4.73 ±0.12	1.04 ±0.05	3.52 ±0.17	0.52 ±0.04	14.60 ±0.75	151.4 ±3.06	168.0 ±8.00	52.60 ±2.69	63.20 ±2.69
Skin * 50%	156.0 ±36.96	102.80 ±11.33	4.45 ±0.20	1.07 ±0.04	3.14 ±0.23	0.51 ±0.03	15.40 ±1.63	154.6 ±5.19	181.8 ±4.55	53.00 ±2.57	65.20 ±3.93
Leaves * 0%	169.2 ±41.52	86.00 ±14.76	4.13 ±0.08	1.22 ±0.04	2.70 ±0.07	0.55 ±0.03	14.80 ±1.59	158.0 ±3.11	174.0 ±5.10	61.60 ±3.33	61.60 ±5.70
Leaves * 25%	156.4 ±27.52	83.20 ±7.47	4.49 ±0.05	1.09 ±0.09	3.20 ±0.13	0.51 ±0.03	15.40 ±1.63	181.2 ±10.86	178.8 ±3.93	60.60 ±3.37	84.80 ±8.53
Leaves * 50%	247.0 ±40.61	93.00 ±14.46	4.72 ±0.11	1.01 ±0.02	3.34 ±0.09	0.49 ±0.06	16.60 ±1.89	173.4 ±5.25	180.0 ±4.18	62.60 ±0.93	74.80 ±5.28
P value	0.1153	0.9157	0.1143	0.5917	0.1867	0.9725	0.9260	0.0507	0.4519	0.2138	0.1765

a-b: Means superscripted with different letters in the same column differ significantly at P≤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.

DISCUSSION

Results in the present study detected that the seriously affected of different nutritional treatments on hematological and biochemical parameters, which confirmed the potentially relationship between feeding systems and physiological responses not only in fish, but also in all farm or experimental animals. In this respect, Hussein *et al.* (2001) found that canola meal increased significantly Hb, serum total protein, globulin, triglycerides, AST, ALT, and thyroid hormones levels. Dietary yeast strains increased Hb, serum total protein, globulin, triglycerides, glucose, AST, ALT, and thyroid hormones levels. They gave the following ranges: Hb (6.4-9.3 g/dL), PCV (22.0-25.3 %), serum total protein (4.83-10.5 g/dL), albumin (1.74-2.64 g/dL), globulin (2.57-8.68 g/dL), triglycerides (248-375 mg/dL), glucose (93.5-128 mg/dL), total cholesterol (146-165 mg/dL), AST (38.8-66.4 u/L), and ALT (22.5-37.9 u/L). El-Ebiary and Zaki (2003) registered increases in the Hb (6.36-8.39 g/dL), PCV (23.1-25.0 %), serum total protein (4.85-9.46 g/dL), albumin (2.26-264 g/dL), globulin (2.59-6.85 g/dL), glucose (92.5-148 mg/dL) and triglycerides' (255-356 mg/dL) concentrations, but decrease of the total cholesterol level (156-143 mg/dL) by increasing the level of active yeast in the Nile tilapia diets. Red tilapia fed sesame hulls by-product reflected lower plasma glucose (78.6-67.2 mg/dL) and total protein concentrations (5.10 vs. 2.69 g/dL) and ALT activity (39.5-10.9 u/dL) comparing with the control (Abd Elmonem *et al.*, 2004). Inversely, El-Houssiny *et al.* (1999) reported no significant (P≥0.05) differences in non-specific immunity factor (serum proteins) nor humoral immune response in Nile tilapia fed different protein levels (20, 25, and 30 %), feeding levels (3 and 5 %), and feeding frequency (2 and 3 times) for 170 days. They gave the following ranges for serum total protein 2.51-2.91 g/dL, albumin 0.99-1.17 g/dL, globulin 1.48-1.83 g/dL, and albumin/ globulin 0.57-0.86.

Ayyat *et al.* (2004) fed Nile tilapia different levels of fish meal and zinc. They found that serum total protein (5.00/5.69 g/dL), albumin (2.90/3.65 g/dL), and AST (30.4/36.6 u/L) values significantly increased with increasing fish meal level in the fish diet, while urea-N (3.83/3.65 mg/dL) and ALT (16.2/12.8 u/L) values significantly decreased. Also, serum total protein (4.60/5.84 g/dL), albumin (2.68/3.39 g/dL), and creatinine (0.90/1.02 mg/dL) concentrations and AST activity (29.6/37.2 u/L) significantly increased with increasing Zn level in the fish diet, while ALT activity significantly decreased. Moreover, El-Dakar *et al*. (2004) found that dietary inclusion of graded levels of fennel seeds meal as a feed additive significantly affected some serum biochemical parameters, particularly total lipids (3.33-5.51 g/dL) and AST (72-116 u/dL) but did not affect glucose (34.3-59.6 mg/dL), total protein (5.03-5.43), and ALT (34-45 u/dL). Abdel-Tawwab *et al.* (2005) represented that Nile tilapia reflected slightly lower Hb and PCV values under crowding-stress condition but there was no significant on them due to dietary CP level. They added that RBCs count and glucose concentration in chronically stressed fish were elevated but plasma cortisol value was reduced particularly with increasing the dietary protein level. However, both transaminases (AST & ALT) were not affected. That means that Nile tilapia may quickly adapted to high rearing density by enhancing feed quality, especially protein level in the diet to prevent the deleterious effect in fish farm. The recorded the following ranges: RBCs 1.26-1.61 × 10^6 µ / L, Hb 6.06-8.40 g / dL, PCV 13.8-16.5%, glucose 64.9-128 mg / dL, protein 2.07-3.45 g / dL, ALT 6.08-8.75 u / L and AST 17.4-65.9 u / L.

Several food additives and immunostimulants can enhance different innate factors. There is limited data available about the ontogenic development of the innate immunological system in fish (Magnadóttir, 2006). In this regard, Osman *et al.* (2010) registered that feeding with probiotics improved the RBC_s count (1.159 × 10^6 *vrs*. 0.999 × 10^6 / µL), Hb concentration (5.42 *vrs*. 4.77 g/dL), phagocyte activity, A/G ratio, and serum total protein level of Nile tilapia. Also, Nile tilapia fed spirulina (*Arthrospira fusiformis*) supplementation significantly decreased plasma AST (108 *vrs*. 115 u/dL) and ALT (41 *vrs* 46 u/dL) activity, but significantly increased plasma glucose (63.87 *vrs*. 58.11 mg/dL), total protein (7.58 *vrs* 5.10 g/dL), and total lipid (5.28 *vrs* 4.23 g/dL) (Belal *et al*., 2012). Shortage of food supply and animal feedstuff gape forced the nutritionists to find out and evaluate novel or unconventional food resources such as corn gluten meal (Metwalli, 2013) and insect (black solider fly) larvae to replace the expensive fish meal in aquafeed (Makker, 2015). Al-Ashrm (2017) mentioned among the properties of an ideal narcotic agent, it must be not toxic to fish and human being, do not affect fish behavior or its physiological functions, and to be cheap. He added that clove oil (extracted from the plant *Eugeaia caryaphyllata*) is a local product, safe, effective, and cheap narcotic agent well recommended for fish narcosis.

Moazenzadeh *et al*. (2017) investigated the effect of dietary Zn levels (0, 5, 10, 20, and 40 mg Zn /kg) on hematological parameters of juvenile Siberian sturgeon (*Acipenser baerii*, Brandt 1869). Erythrocyte number, Hb concentration, and Hct were increased with raising dietary Zn level (P≤0.05), whereas a regular trend was not observed in blood indices, including MCV, MCH, and MCHC. A significant increase was also found in WBCs number followed by their differential counts, except for eosinophil percentage. In spite of well correlation with dietary Zn level, no break point was observed to estimate the juvenile Siberian sturgeon requirement based on RBCs numbers. More recently, Hassaan *et al.* (2018) fed Nile tilapia, *Oreochromis niloticus* (average initial weight, 5.91 ± 0.04 g) isonitrogenous and isolipidic diets for 84 days. The diets contained four levels of yeast extract rich in nucleotides and β-glucan: 0 (control), 5, 10 and 15 g/kg diet. No significant (P>0.05) differences were found in Hct, Hb or total protein contents among the treatment groups. Blood sample profiles showed an increase in WBCs and RBCs of fish fed 15 g/kg yeast extract in comparison with the other treatments. Fish fed the diets supplemented with 10 and 15 g/kg yeast extract had significantly higher albumin and globulin levels than the control group, whereas decreased levels of cholesterol and triglycerides, AST and ALT, which were noted in fish, fed the diet supplemented with 15 g/kg yeast extract.

CONCLUSION

Based on the previous data it could be concluded that the possible relationship between different nutritional treatments and fish physiological status. Additionally, 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 of different fish species.

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ARABIC SUMMARY

عوامل تؤثر على صورة دم الأسماك: أ) تأثير المعاملات الغذائية

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تم دراسة تأثير بعض العوامل البيولوجية على تركيب دم الأسماك، مثل نوع الأسماك ، مستوى البروتين الخام فى العليقة ، مواد إحلال وإضافات علفية ، إضافة إلى نوع العليقة ، وذلك فى دراسات معملية وحقلية. واتضح أن معظم المعاملات المختبره (نوع العليقة ، مستويات البروبيوتيك فى العليقة ، مستويات مخلفات غربلة بذور البرسيم المصرى فى العليقة ، وزن الأسماك الأولى ، مستويات وأنواع مخلفات التين الشوكى فى العليقة) قد أثرت معنويا فى معظم القياسات الهيماتولوجية والبيوكيماوية محل الدراسة. وهذا يعنى أن مكونات دم الأسماك ليست ثابتة فى مدى معلوم ، لكنها تتغير بشدة طبقا للظروف البيئية المختلفة ، لذا تُفسر النتائج فى ضوء ظروف كل تجربة منفردة ، لعدم وجود قيم مرجعية لقياسات دم الأسماك.