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Influences of diets supplemented with pomegranate peel on haematology, blood biochemistry and immune status in monosex Nile tilapia, *Oreochromis niloticus*

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ABSTRACT

A total of 360 monosex *Oreochromis niloticus* with an average body weight of 7.1±1 g were used. The fish were randomly divided into 8 equal triplicate groups (15 fish/replicate). A basal control diet was formulated to fulfil the nutrient requirements of the fish that contained 25% crude protein (CP) and 448.3 kcal/100 g. The other 7 diets (treatment diets) were supplemented with pomegranate peel (PP) at rates of 1, 2, 3, 5, 10, 15 and 20%. The fish were fed the diets 3 times daily at a rate of 5 to 7% of the body weight per day, 6 days a week for 90 days. Haematological analysis revealed that red blood cell (RBC) counts and haemoglobin levels (Hb) were significantly lower and that white blood cell (WBC) counts were significantly ($P<0.01$) higher in blood from fish fed the different PP-supplemented diets than in blood from fish fed the control diet. Glucose and total protein levels were increased after 90 days. Cholesterol levels were reduced by PP, while triglyceride levels were significantly increased. In addition, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly reduced in the blood of monosex Nile tilapia fed diets with different concentrations of PP. Immunological parameters (IgM levels and lysozyme activity) were significantly improved by PP supplementation. These results indicate that addition of PP to monosex *O. niloticus* diets improved immune status and liver and kidney function without any adverse effects on health status.

INTRODUCTION

Antibiotics are commonly used to control fish diseases; however, these compounds produce deleterious side effects. Therefore, researchers have sought to find alternative inexpensive, safe and effective natural products such as herbs, vegetables and other edible plants for use as growth promoters or immunostimulants (El-Dakar *et al.*, 2015 and Toutou *et al.* 2018). The cost-effective production of high-quality products from less-expensive agro-industrial by-products is a target within the feed industry in Egypt (El-Sayed *et al.* 2014). A large quantity of pomegranate peel (PP) residues is produced in juice manufacturing, representing a valuable waste of the food industry as they contain bioactive compounds (Toutou *et al.* 2019).

The waste product pomegranate peel (PP) has higher antioxidant levels than pomegranate juice. Thus, PP is an attractive candidate nutritional supplement for animal feed (Badawi and Gomaa, 2016). Approximately 1.5 million tons of pomegranates are produced worldwide (Eikani *et al.* 2012). The PP constitutes 5% to 15% of the total weight of a pomegranate (Orzuaa *et al.* 2009). The disposal of such large quantities of PP waste is therefore an environmental problem (Kanatt *et al.* 2010); however, PP is a valuable waste of the food industry, as it contains bioactive compounds, especially antioxidant polyphenols that mainly include hydrolysable tannins and anthocyanins (Çam and Hışıl, 2010; Ibrahim, 2010). Blood characteristics are very important tools that can be used as effective indices of water balance, nutritional status and overall health condition in fish (Nwani *et al.* 2015; Zaahkook *et al.* 2016). Numerous dietary supplements have measurable effects on blood constituents (Animashahun *et al.* 2006; Bhatti *et al.* 2009). Therefore, haematological and blood biochemical variables have been used as indicators of health status in fish of many species fed different kinds of food. Diet composition and metabolic adaptation are the main factors responsible for changes in haematological and blood biochemical variables in fish (Ighwela *et al.* 2012). Such parameters are reliable indicators of fish physiological status and are often used to evaluate fish health and immune potential (Kondera *et al.* 2017). PP extract has extensively been studied for its strong antimicrobial and anti-inflammatory effects (Jurenka, 2008). PP extract has no side effects and no known drug interactions (Murthy *et al.* 2004). Studies on herbal extracts as food additives have found that they can enhance growth and protect fish from diseases (Johnson and Banerji, 2007), but studies related to the use of PP as an additive in fish feed are scarce. It is necessary to elucidate the effects of this plant product and its extract on fish performance. Hence, the objective of the present study was to examine the effects of dietary supplementation with the natural additive PP on haematology, blood biochemistry and immune status in monosex Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

Preparation of PP

The PP used for this study was locally prepared in the laboratory. Pomegranate fruits was obtained from the local market in Assiut Governorate, Egypt. The fruits were washed with distilled water and peeled, and their edible portions were carefully separated. The peels were air-dried in a hot air oven at 40°C until the moisture content reach approximately 8% (dry basis). The dry ingredients were finely ground by using a Lab Mill, sieved, and then manually mixed in a plastic container for approximately 15 minutes to ensure their homogeneity.

Experimental fish

A total of 360 apparently healthy live monosex Nile tilapia (*O. niloticus*) with an average body weight of 7.1±1 g were obtained from a private fish farm in Fayoum Governorate, Egypt. The fish were kept in a recirculation system. High water quality was ensured, and the system was thermostatically controlled at 25 ±1°C. The fish were divided into 8 groups, each of which was divided into subgroups. Each subgroup had three replicates (15 fish/replicate). The fish were allowed to acclimate to the laboratory conditions for three weeks before the start of the experiment.

Feeding regime

The diets were prepared at the Department of Zoology, Faculty of Science, Al Azhar University, Assiut Governorate, Egypt. The feed was divided into three equal

portions and distributed by hand at one side of each aquarium three times daily (9 a.m., 1 p.m. and 3 p.m.) at a rate of 5 to 7% of the fish body weight, 6 days a week for 90 days. A basal control diet was formulated to fulfil the nutrient requirements of the fish that contained 25% crude protein (CP) and 448.3 kcal/100 g (Table 1). The basal control diet was prepared with 10% yellow corn and 30% rice bran. For the other 7 diets (the treatment diets), the basal diet was supplemented with PP at rates of 1, 2, 3, 5, 10, 15 and 20%.

Table 1: The composition and chemical analyses (% on dry matter basis) of the experimental diets

Ingredients composition (g)	**Composition (%) of the experimental diets**							
	Control	%1	%2	%3	%5	%10	%15	%20
Fish meal (65%)	70	70	70	70	70	70	70	70
Soy bean meal	250	250	250	250	250	250	250	250
Corn gluten	80	80	80	80	80	80	80	80
Yellow corn	100	90	80	70	50	0	0	0
Pomegranate peel	0	10	20	30	50	100	150	200
Wheat bran	150	150	150	150	150	150	150	150
Rice bran	300	300	300	300	300	300	250	200
Fish oil	20	20	20	20	20	20	20	20
Premix[1]	30	30	30	30	30	30	30	30
Total	1000	1000	1000	1000	1000	1000	1000	1000
Chemical composition (%)								
Dry matter (DM)	93.1	93	93.4	93.9	93.6	94.3	93.2	93.6
Crud protein (CP)	25	25.1	25.3	25.1	25.1	24.8	24.9	24.8
Ether extract	10.1	10.1	9.6	9.3	9.5	9.3	8.8	8.5
Crude fiber	5.7	5.6	5.8	5.7	5.9	5.9	6.1	6.2
Ash	7.7	7.7	7.8	6.9	7.2	8.6	7.9	7.8
Nitrogen free extract	51.5	51.5	51.5	53	52.3	52.2	52.3	52.7
M. E. (kcal/100g [2])	374.0	374.4	371.3	373.2	372.4	365.5	365.8	364.3

1Premix composition: each 1 kg contains Vit A (400000 i.u.), Vit D3 (100000 i.u.), Vit E (230 mg) Vit K3 (165 mg) Vit B1 (300 mg), Vit B2 (80 mg), Vit B6 (200 mg), Vit B12 (1 mg), Vit C (650 mg), Niacin (1000 mg), Methionine (3000 mg), Choline chloride (10000 mg), Folic acid (100 mg), Biotin (2 mg), Pantothenic acid (220 mg), Magnesium sulphate (1000 mg), Copper sulphate (1000 mg), Iron sulphate (330 mg), Zinc sulphate (600 mg), Cobalt sulphate (100 mg), Calcium carbonate up to 1000 g; [2]Metabolizable energy (M E) was calculated as 4.5, 8.1 and 3.49 kcal/100g for protein, lipid and NFE, respectively according to Pantha (1982).

Haematological analysis

Whole blood was assessed for haemoglobin concentration (Hb), haematocrit (Hct), and red blood cell (RBC) count by using an automated technical analyser (Celltac α MEK- 6400J/K). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and white blood cell (WBC) count were calculated according to the methods of Dacie and Lewis (2002).

Biochemical analysis

Blood samples were collected through cardiac puncture as described by Osman *et al.* (2011). No anaesthetic was given to the fish, as anaesthesia can affect blood parameters. The blood samples were then allowed to coagulate for 15–20 minutes at 4°C prior to centrifugation for 20 minutes at 3000 rpm to separate serum. The serum was stored at -20°C until use for biochemical and immunological analyses. Serum total protein, cholesterol, triglyceride, glucose, urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were calculated according to the methods of Reitman and Frankel (1957), Henry (1964), Trinder (1969), Friedewald *et al.* (1972) and Thomas (1992).

Immunological responses

Lysozyme activity was measured spectrophotometrically according to the method of Ellis (1990). Immunoglobulin M (IgM) levels were determined using an ELISA kit (Catalogue No. CSB-E12045Fh (96k test), Cusabio Biotech Co., Ltd.).

Statistical analysis

Data were presented as mean±SD. The results were subjected to one-way analysis of variance (ANOVA) to test the effect of treatment inclusion on fish performance. Data were analyzed using SPSS (1997) program, Version 16. Differences between means were compared using Duncan's multiple range tests at $p < 0.01$ level.

RESULTS

Haematological variables

The average values for RBCs, Hb, MCV, MCH, MCHC, Hct, and WBCs of the monosex *O. niloticus* fed diets containing different concentrations of PP are presented in Table 2 and Figures 1-3. Statistical analysis of these parameters revealed significant differences between the fish fed diets containing different concentrations of PP and the control diet.

The results indicated that RBCs, Hb and Hct were significantly (P<0.01) lower in PP-supplemented groups than in the control group.

The groups fed diets with high PP concentrations exhibited significantly greater MCV and MCH values than the control group. No significant differences in MCHC were recorded between the control fish and the fish fed different PP concentrations (except for the 20% PP group). WBCs were significantly (P<0.01) higher in the blood of PP-supplemented fish fed than in that of control fish.

Table 2: Haematological parameters (mean ±SD) of *O. niloticus* fed diets containing different concentrations of pomegranate peel for 45 and 90 days

Treatment		**Experimental diet**							
Parameter		Control	1 %	2 %	3 %	%5	10%	15%	%20
RBCs ($\times 10^6$ µl)	45 days	2.2±0.3^{a}	1.9±0.2^{ab}	1.8±0.3^{ab}	1.9±0.2^{ab}	1.6±0.4bc	1.8±0.1^{ab}	1.7±0.2^{bc}	1.4±0.15^{c}
	90 days	2.9±0.1^{a}	2.1±0.2^{b}	2.2±0.2^{b}	2.0±0.15^{b}	2.2±0.1^{b}	1.9±0.2^{ab}	1.6±0.2^{c}	1.6±0.2^{c}
Hb (g/dl)	45 days	9.9±0.4^{a}	9.3±0.2^{b}	8.9±0.1^{c}	8.4±0.1^{d}	7.9±0.2^{E}	7.2±0.1^{E}	7.0±0.1^{F}	6.5±0.2^{F}
	90 days	11.0±0.1^{a}	9.7±0.5^{b}	9.2±0.3^{c}	8.9±0.2^{c}	8.2±0.2^{d}	8.1±0.2^{d}	7.3±0.1^{E}	6.9±0.1^{F}
MCH (pg)	45 days	44.0±4.8^{a}	36.0±0.3^{c}	40.0±0.5^{b}	36.0±0.1^{c}	33.0±2.0^{c}	34.0±4.4^{c}	27.0±12.0^{d}	34.0±2.2^{c}
	90 days	36.0±0.24^{b}	35.0±2.1^{b}	35.0±2.3^{b}	38.0±1.1^{ab}	35.0±0.6^{b}	41.0±2.6^{ab}	42.0±5.2^{a}	37.0±5.0^{ab}
WBCs ($\times 10^3$ µl)	45 days	26.4±0.8^{d}	36.0±2.5^{ab}	32.3±3.3^{c}	38.0±2.9^{a}	37.0±1.8^{a}	35.0±0.8^{abc}	37.0±3.3^{a}	33.0±0.6^{bc}
	90 days	27.1±0.7^{b}	0.7±39.8^{a}	37±4.8^{a}	2.1±39.0^{a}	5.9±34.8^{a}	1.4±37.8^{a}	2.0±36.1^{a}	1.6±37.5^{a}

Means within the same row not sharing a superscript letter are significantly different (P<0.01).
Red blood cells (RBC), haemoglobin concentration (Hb), mean corpuscular haemoglobin (MCH) and white blood cells (WBC).

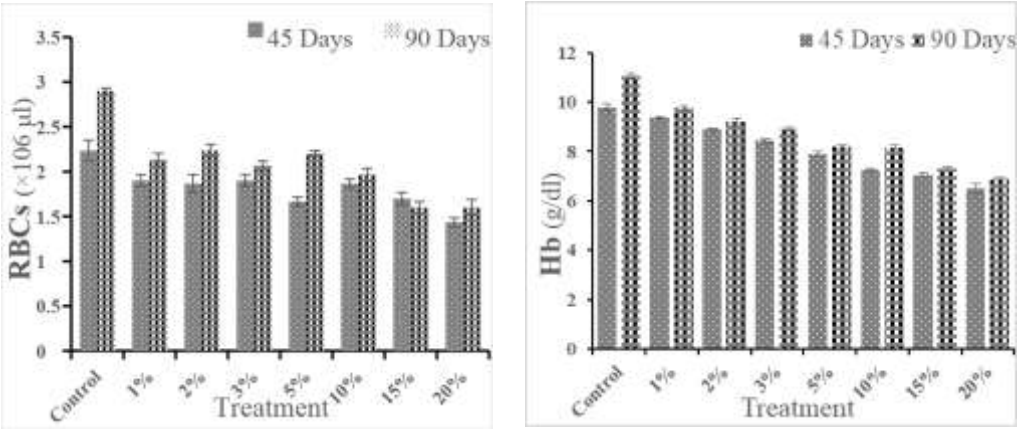

Fig. 1: Mean±SD of RBCs and Hb in the blood of monosex Nile tilapia (*O. niloticus*) fed diets containing different concentrations of pomegranate peel for three months. RBCs: Red blood cells, Hb: Haemoglobin.

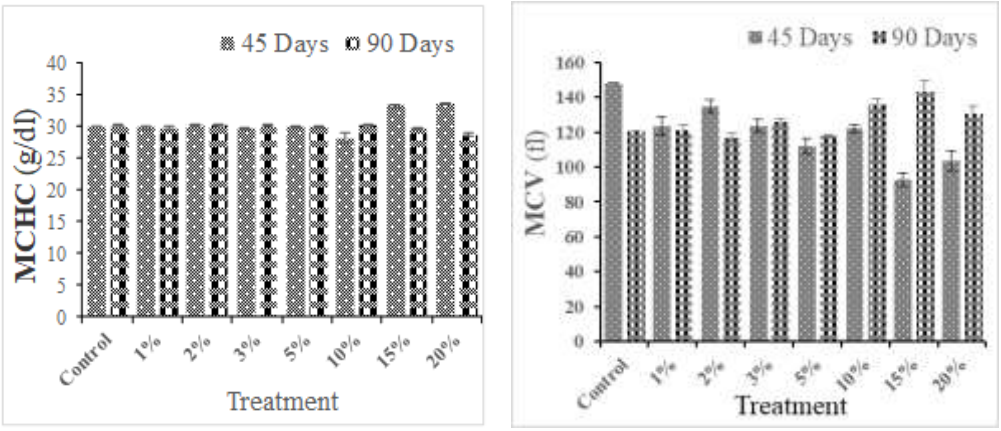

Fig. 2: Mean±SD of MCHC and MCV in the blood of monosex Nile tilapia (*O. niloticus*) fed diets containing different concentrations of pomegranate peel for three months. MCHC: Mean corpuscular haemoglobin concentration, MCV: Mean corpuscular volume.

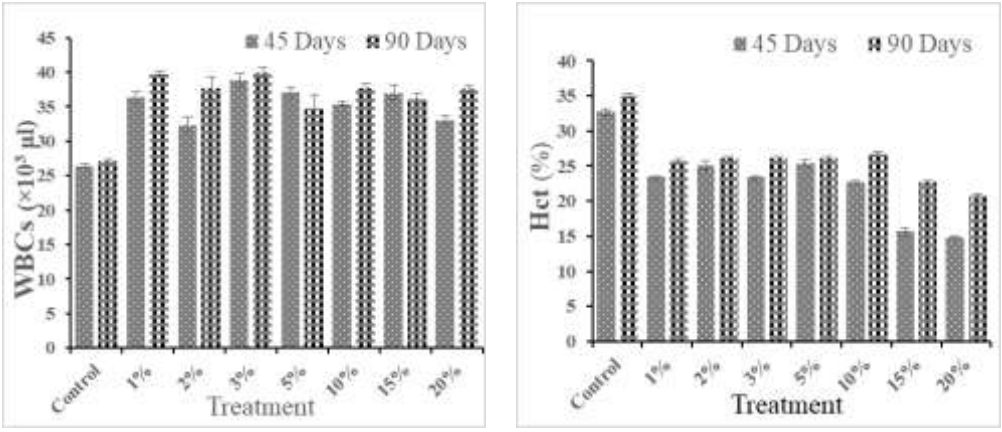

Fig. 3: Mean±SD of WBCs and Hct in the blood of monosex Nile tilapia (*O. niloticus*) fed diets containing different concentrations of pomegranate peel for three months. WBCs: White blood cells, Hct: Haematocrit.

Blood biochemistry

The average concentrations of total protein, glucose, cholesterol, triglyceride, creatinine, urea, ALT, and AST in the blood serum of monosex *O. niloticus* are presented in Table 3 and Figure 4-6. After 90 days of exposure, significant (P<0.01) differences were observed in the average values of all detected biochemical parameters between the fish fed the different PP-supplemented diets and those fed the control diet. The levels of all biochemical parameters (except cholesterol) were significantly (P<0.01) higher in the blood serum of the fish fed the different PP-supplemented diets than in that of control fish (Table 3 and Figures 4-6).

Table 3: Effects of dietary supplementation with pomegranate peel for 45 and 90 days on select biochemical parameters in monosex Nile tilapia (*O. niloticus*).

Treatment		**Experimental diet**							
Parameter		Control	1%	2%	3%	5%	10%	15%	20%
TP (mg/dl)	45 days	2.3±0.15^{ab}	2.7±0.35 ^{a}	2.5±0.12^{a}	2.1±0.35 ^{a}	2.7±0.4^{a}	2.8±0.4 ^{a}	2.8±1.3 ^{a}	1.9±0.43 ^{a}
	90 days	2.5±0.03^{d}	2.6±0.4 ^{d}	3.6±0.03^{c}	4.7±0.55^{ab}	5.2±0.73^{a}	4.6±0.56^{ab}	4.3±.32^{bc}	4.3±0.32^{bc}
GL (mg/dl)	45 days	59.0±8.4^{a}	52.2±0.7^{ab}	47.5±3.5^{ab}	45.6±6.6^{b}	45.3±0.2^{b}	46.0±1.1^{b}	46.4±9.4^{b}	54.0±12 ^{ab}
	90 days	31.5±8.5^{b}	31±8.9 ^{b}	33±3 ^{b}	37.5±4.5 ^{b}	38.7±0.6^{b}	39±2.8^{b}	42±5.9^{b}	73.7±7.4^{a}
Effect of pomegranate peel on lipids									
Chol (mg/dl)	45 days	72.9±3.4^{c}	78.9±3.3^{bc}	79.1±1.5 ^{bc}	95.6±0.8^{a}	89.7±4.9^{ab}	87.3±3.4^{ab}	85.4±13^{ab}	81.7±4.1^{bc}
	90 days	100±12.6^{a}	73.2±5.8^{b}	66.7±0.5^{b}	61.2±6.9^{b}	62.7±3.9^{b}	69.1±1.3^{b}	68.4±8.6^{b}	60.9±8.8^{b}
Tg (mg/dl)	45 days	203.0±5.0^{a}	202.3±4.5^{a}	188.0±4.4^{b}	186.4±1.4^{b}	168.3±2.1^{c}	163.6±9.0^{c}	133±10.4^{d}	138±15.6^{d}
	90 days	259.6±5.4 ^{c}	266.1±9.9^{c}	276.6±3.2^{b}	282.8±1.4^{a} ^{b}	289.2±0.5^{a}	284.8±6.3^{a} ^{b}	131.8±4.1^{d}	128.5±4.6^{d}
Effect of pomegranate peel on kidney function									
Crt (mg/dl)	45 days	0.5±.09^{cd}	0.5±.03 ^{cd}	0.6±.04^{bcd}	0.5±.09^{d}	0.7±.02^{a}	0.8±0.02^{a}	0.5±.07^{cd}	0.6±.12^{bc}
	90 days	0.4±.15^{c}	0.6±.2^{bc}	0.6±0.1^{abc}	0.5±.06^{bc}	0.6±.14^{abc}	0.4±.03 ^{c}	0.8±.06^{a}	0.6±.03^{ab}
Ur (mg/dl)	45 days	5.5±1.3^{a}	4.6±2.2 ^{ab}	3.2±0.32^{bc}	2.3±1.1^{c}	1.5±0.78^{c}	2.0±0.1^{c}	2.2±0.2^{c}	2.1±1.2^{c}
	90 days	3.8±1.1^{abc}	4.5±1.18 ^{ab}	4.8±0.34^{a}	3.3±0.2 ^{bc}	2.9±0.3 ^{c}	4.4±0.2^{ab}	3.9±0.6^{abc}	4.3±0.7^{ab}
Effect of pomegranate peel on liver enzyme activity									
ALT (U/L)	45 days	23.3±1.8^{a}	20±0.66^{b}	17.8±.73^{c}	17.3±.45^{c}	16.9±.65^{cd}	15.6±.45^{d}	13.8±.83^{E}	13.9±.65^{E}
	90 days	20.7±.36^{bc}	23.1±2.02 ^{ab}	20.2±2.4^{c}	23.2±1.2^{ab}	24.8±1.6^{a}	16.5±1.2^{d}	15.6±1.5^{d}	15.4±.98^{d}
AST (U/L)	45 days	21.2±.75^{a}	18.8±1.02^{b}	17.6±0.3^{bc}	15.1±1.1^{dE}	14.3±1.3^{EF}	12.8±1.1^{F}	15.2±1.1^{dE}	16.1±.47^{dE}
	90 days	22.9±3.1^{cd}	26.3±1.2^{abc}	23.8±3.3^{bcd}	26.6±1.3^{ab}	27.8±0.5^{a}	20.8±1.3^{d}	21.1±1.4 ^{d}	21.7±1.5 ^{d}

Means within the same row not sharing a superscript letter are significantly different (P<0.05).
Total protein (TP), glucose (GL), cholesterol (Chol), triglyceride (Tg), creatinine (Crt), urea (Ur), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST).

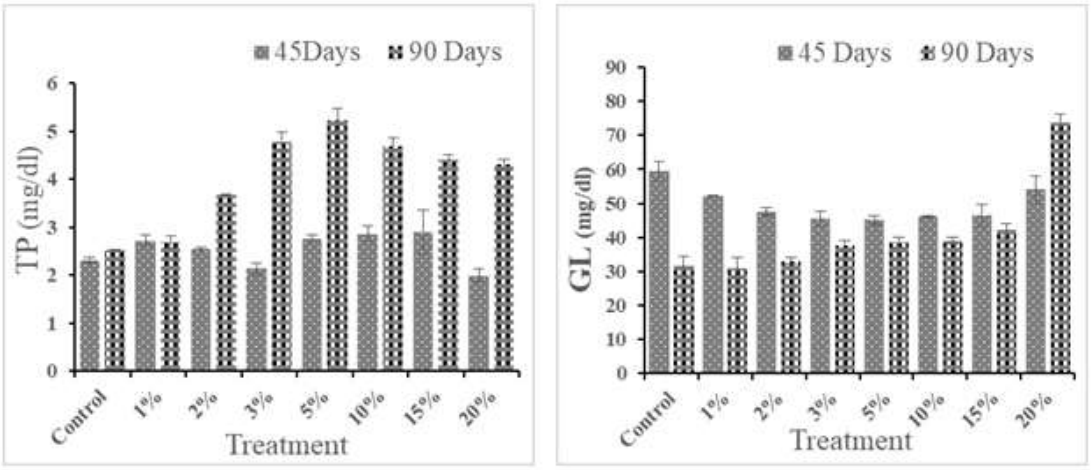

Fig. 4: Mean±SD of TP and GL in the blood of monosex Nile tilapia (*O. niloticus*) fed diets containing different concentrations of pomegranate peel for three months. TP: Total protein, GL: Glucose.

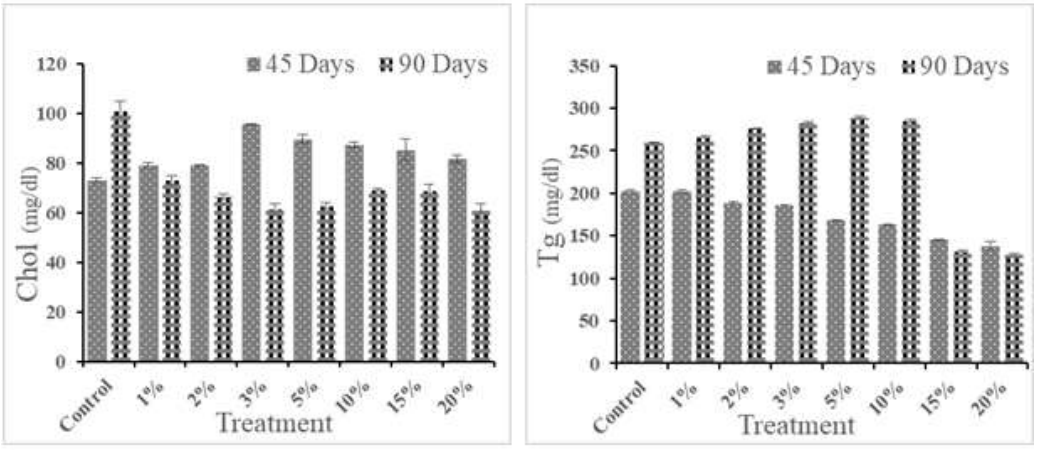

Fig. 5: Mean±SD of Chol and Tg in the blood of monosex Nile tilapia (*O. niloticus*) fed diets containing different concentrations of pomegranate peel for three months. Chol: Cholesterol, Tg: Triglyceride.

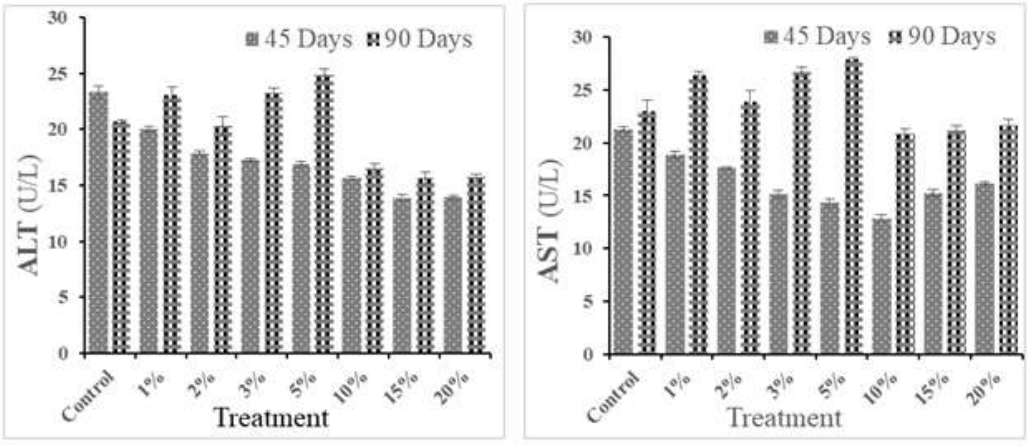

Fig. 6: Mean±SD of ALT and AST in the blood of monosex Nile tilapia (*O. niloticus*) fed diets containing different concentrations of pomegranate peel for three months. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

Immunological responses

Figure 7 shows the effect of PP on select immunological parameters in the blood of monosex Nile tilapia fed a basal diet or diets with different concentrations of

PP. IgM levels and lysozyme activity were found to be significantly greater in PP-supplemented groups than in the control group.

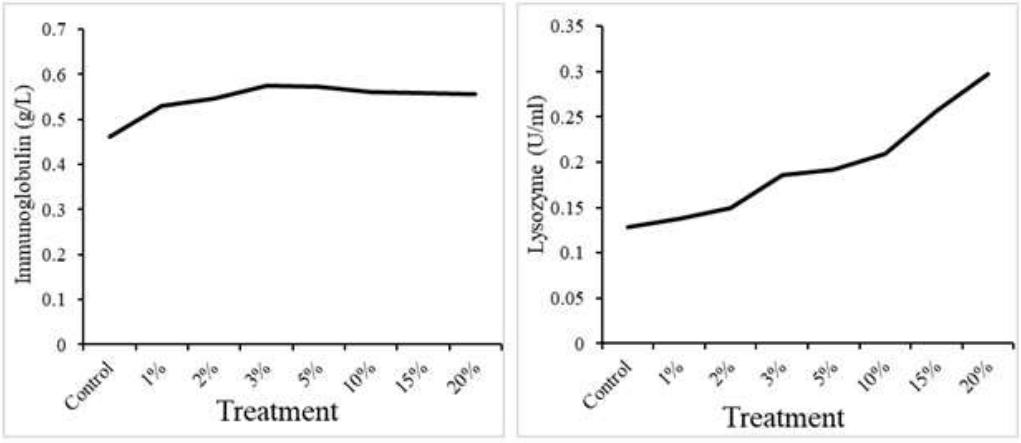

Fig. 7: Effects of a control diet and supplemented diets with pomegranate peel on immune status (IgM levels and lysozyme activity) in *O. niloticus.*

DISCUSSION

Pomegranate has been established to have many pharmacological effects in aquatic and terrestrial species (Vidal *et al.* 2003; Badawi and Gomaa, 2016; Toutou *et al.* 2019). However, little information is available about its effects on blood characteristics and immunity in Nile tilapia. Blood monitoring is important in aquaculture given that cultivation conditions affect the basic physiological functions of farmed fish (Tavares-Dias and Moraes, 2007). Feed-induced changes in blood indices in fish have been reported by many authors (e.g., Kelly, 1979; Kilgour, 1987). In our study, significant reductions in RBCs, Hb and Hct were found in the blood of monosex *O. niloticus* fed diets containing different concentrations of PP compared to the blood of control fish, consistent with the results of Badawi and Gomaa (2016). Such slight changes in RBCs and Hb in this study suggest that supplementation with PP (at the lower levels) may not have affected the health status of the monosex *O. niloticus*. Similar results have been documented in different fish species fed with *Citrus spp.* peel and oil (Acar *et al.* 2015 and Toutou *et al.* 2018). In addition, similar results have been recorded for *Huso huso* (Khajepour and Hosseini, 2012) and hybrid tilapia (Yue and Zhou, 2008) fed soybean meal-supplemented diets. There were no significant differences in MCV, MCH, and MCHC between PP-supplemented fish and control fish. WBCs were significantly higher in the blood of PP-supplemented fish fed than in that of control fish after three months of the feeding trial. Similar results have been recorded for *Oreochromis mossambicus* fed diets supplemented with thyme, rosemary and fenugreek compared to control fish (Gültepe *et al.* 2014). Additionally, Kumar *et al.* (2014) recorded elevated WBCs in Indian catfish (*Mystus montanus*) fingerlings fed herbal diets compared to those fed non-herbal diets. The higher WBC counts in the PP-supplemented fish in this study suggest a possible immunomodulatory effect of PP.

Blood biochemical indices are useful for determining fish health status following different feeding trials (Yılmaz and Ergun, 2012). In the present study, Nile tilapia supplemented with different doses of PP exhibited significant increases in blood total protein levels after 90 days of feeding, confirming good growth performance during this experiment. Blood glucose levels have been used as

indicators of environmental stress, as they reflect changes in carbohydrate metabolism under stress conditions (Kamal and Omar, 2011). Glucose levels significantly increased with increasing PP concentrations over the 90-day feeding trial. Increased levels of glucose have previously been recorded in the blood of stressed fish (Levesque *et al.* 2002; Poléo and Hytteørd, 2003; Sayed *et al.* 2007; Adedeji *et al.* 2009; Mekkawy *et al.* 2010; Osman *et al.* 2010b) due to changes in the activity of glucose-6-phosphate dehydrogenase and lactate dehydrogenase, as previously detected by Osman *et al.* (2018). Changes in blood cholesterol and triglyceride concentrations are sensitive indicators of liver dysfunction because lipid homeostasis is one of the principal functions of the liver (Sayed *et al.* 2011). In the present study, PP-supplemented fish demonstrated significantly lower cholesterol levels after the 90-day feeding trial than control fish; the reduction in cholesterol can be explained by the polyunsaturated fatty acids and other constituents in PP. Baba *et al.* (2017) obtained similar results in tilapia (*O. niloticus*) fed argan oil-supplemented diets. PP exerts inhibitory effects on pancreatic lipase activity, inhibiting fat absorption from the intestinal tract (Kumar *et al.* 2018). In contrast to cholesterol concentrations, triglyceride concentrations were significantly elevated after 90 days of feeding in fish supplemented with increasing levels of PP (except at the 15% and 20% levels) compared to control fish. The physiological roles of urea, uric acid and creatinine are not clearly understood. Nevertheless, the levels of these molecules are useful indices for overall gill and kidney health (Campbell, 2004), feed utilization (Tulli *et al.* 2007) and amino acid (arginine) requirements (Tibaldi *et al.* 1995) in fish. Badawi and Gomaa (2016) found low levels of creatinine and urea in fish groups fed diets containing PP extract for 10 weeks. Similar results were recorded in this experiment in the blood of fish supplemented with PP for only 45 days. However, after 90 days of feeding, dietary PP supplementation increased serum urea and creatinine levels in monosex tilapia, indicating that the levels of urea and creatinine increased with increasing feeding trial duration. Different results have been obtained with diets supplemented with thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*) and fenugreek (*Trigonella foenum-graecum*) (Mostafa *et al.* 2009; Yılmaz *et al.* 2012). PP supplementation did not significantly change serum urea and creatinine levels, confirming that the use of PP did not affect the health status of monosex Nile tilapia. In both feeding trials, significantly lower ALT and AST levels were found in the blood of PP-supplemented fish than in that of control fish. Similar results were previously recorded by Badawi and Gomaa (2016). El-Sayed *et al.* (2014) suggested that dietary supplementation with pomegranate has no adverse effects on different fish organs or on fish health status. These results suggest an evident protective effect of PP on the tilapia liver and are consistent with the results of a recent study by Badawi and Gomaa (2016), who studied the effects of diets supplemented with PP extract at rates of 0.1, 0.2, 0.3, and 0.5% on *O. niloticus*. A marked protective effect of PP on liver function, as demonstrated by decreased AST and ALT, has also been shown by Ibrahim (2010). Decreases in enzyme activity can be regarded as indicators of the protective effect of PP on cells, tissues, and organs (Babalola *et al.* 2009). The non-specific immune system of fish is considered to be the first line of defence against invading pathogens. IgM levels and lysozyme activity are important indices of non-specific immunity in fishes. Lysozyme activity is an important parameter in the immune defence of both invertebrates and vertebrates. In the present study, fish fed diets containing 1, 2, 3, 5, 10, 15 and 20% PP had significantly higher IgM levels and lysozyme activity than control fish. The present results are consistent with those of Badawi and Gomaa (2016), who observed

significantly higher final IgM levels and lysozyme activity in the blood of fish fed diets containing 0.5, 0.3, and 0.2% PP extract than in that of fish fed other dietary treatments or the control diet. Additionally, our results are consistent with those of (Harikrishnan *et al.* 2012) who reported that serum lysozyme activity was significantly enhanced in fish fed pomegranate-enriched diets compared to control fish. Our results and those of (Harikrishnan *et al.* 2012 and Badawi and Gomaa 2016) shows that feeding pomegranate-enriched diets may be an important strategy to reduce cumulative mortality and protect fish from diseases.

CONCLUSION

We suggest that pomegranate-enriched diets improve the innate immune system in monosex *O. niloticus* to help protect against infection. The detected increases in immune response in the groups of monosex tilapia fed PP-supplemented diets compared to the control group may have been due to strengthening of the immune system mediated by the high proportions of antioxidants in PP.

REFERENCES

Acar, Ü.; Kesbiç, O.S., Yılmaz, S., Gültepe, N. and Türker, A. (2015). Evaluation of the effects of essential oil extracted from sweet orange peel (*Citrus sinensis*) on growth rate of tilapia (*Oreochromis mossambicus*) and possible disease resistance against Streptococcus iniae. Aquacult., 437: 282-286.

Adedeji, O.B.; Adeyemo, O.K. and Agbede, S.A. (2009). Effects of diazinon on blood parameters in the African catfish (*Clarias gariepinus*). Afr. J. Biotechnol., 8(16): 3940-3946.

Animashahun, R.A.; Omoikhoje, S.O. and Bamgbose, A.M. (2006). Hematological and biochemical indices of weaner rabbits fed concentrates and Syndrella nodiflora forage supplement. Proc. 11th Annual Conference of Animal Science Association of Nigeria. Institute of Agricultural Research and training, Nigeria, 29-32.

Baba, E.; Acar, Ü., Yilmaz, S., Öntaş, C. and Kesbiç, O.S. (2017). Pre-challenge and post-challenge haemato-immunological changes in *Oreochromis niloticus* (Linnaeus, 1758) fed, argan oil against *Lactococcus garvieae*. Aquacult. Res., 48(8): 4563-4572.

Babalola, T.O.O.; Adebayo, M.A., Apata, D.F. and Omotosho, J.S. (2009). Effect of dietary alternative lipid sources oh haematological parameters and serum constituents *Heterobranchus longifilis* fingerlings. Trop. Anim. Health. Prod., 41(3): 371.

Badawi, M.E. and Gomaa, A.M. (2016). Influence of diets supplemented with pomegranate peel extract on performance in *Oreochromus niloticus*. JPN. J. VET. RES., 64(Supplement 2): 87-94.

Bhatti, J.; Younas, M., Abdullah, M. and Babar, M. (2009). Feed intake, weight gain and haematology in Nili -Ravi buffalo heifers fed on mott grass and Berseem fodder substituted with saltbush (*Atriplex amnicola*). Pak. Vet. J., 29(3): 133-137.

Çam, M. and Hışıl, Y. (2010). Pressurized water extraction of polyphenols from pomegranate peels. Food Chem., 123(3): 878-885.

Campbell, T.W. (2004). Clinical chemistry of fish and amphibians. Veterinary Hematology and Clinical Chemistry. Pennsylvania, Lippincott Williams and Wilkins : 499-517.

Dacie, S.J.V. and Lewis, S.M. (2002). Practical haematology 11th edition. UK, Churchill Livingstone.

Eikani, M.H.; Golmohammad, F. and Homamis, S. (2012). Extraction of pomegranate (*Punica granatum L*) seed oil using superheated hexane. Food bio product process, 90(1): 32-36.

El-Dakar, A.; Shalaby, S., Nemetallah, B., Saleh, N., Sakr, E., Toutou, M., (2015) Possibility of using basil (*Ocimum basilicum*) supplementation in Gilthead sea bream (*Sparus aurata*) diet. Egypt. J. Aquat. Res. (41): 203–210.

Ellis, A.E. (1990). Lysozyme assays. Techniques in Fish Immunology, 1: 101-103.

El-Sayed, A.F.M.; Dickson, M.W. and El-Naggar, G.O. (2014). Value chain analysis of the aquaculture feed sector in Egypt. Aquacult., 4(37): 92–101.

Friedewald, W. T.; Levy, R. I. and Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18(6): 499-502.

Gültepe, N.; Bilen, S., Yılmaz, S., Güroy, D. and Aydın, S. (2014). Effects of herbs and spice on health status of tilapia (*Oreochromis mossambicus*) challenged with Streptococcus iniae. ACTA VET, BRNO., 83(2): 125-131.

Harikrishnan, R.; Kim, J., Kim, M., Balasundaram, C. and Heo, M. (2012). Pomegranate enriched diet enhances the hematology, innate immune response and disease resistance in olive flounder against Philasterides dicentrarchi. Vet. Parasitol., 187(1-2): 147-156.

Henry, R.J. (1964). Clinical chemistry, New York and Row Publish.

Ibrahim, M.I. (2010). Efficiency of Pomegranate Peel Extract as Antimicrobial, Antioxidant and Protective Agents. W. J. A. S., 6(4): 338-344.

Ighwela, K.A.; Ahmad, A.B. and Abol-Munafi, A.B. (2012). Haematological changes in nile tilapia (*Oreochromis niloticus*) fed with varying dietary maltose levels. W. J. F. M. S., 4(4): 376-381.

Johnson, C. and Banerji, A. (2007). Influence of Extract Isolated from the Plant Sesuvium portulacastrum on Growth and Metabolism in Freshwater Teleost, *Labeo rohita* (Rohu). Fish. Technol., 44(2).

Jurenka, M.T. (2008). Therapeutic applications of pomegranate. A Review. Altern. Med. Rev., 13(2): 128.

Kamal, S.M. and Omar. W.A. (2011). Effect of Different Stocking Densities on Hematological and Biochemical Parameters of Silver Carp, *Hypophthalmichthys molitrix* Fingerlings. Life. Sci., 8(4): 580-586.

Kanatt, S.R.; Chander, R. and Sharma, A. (2010). Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. Int. J. Food. Sci. Technol., 45(2): 216-222.

Kelly, D. (1979). Motion and vision. II. Stabilized spatio-temporal threshold surface. Josa, 69(10): 1340-1349.

Khajepour, F. and Hosseini, S.A. (2012). Citric acid improves growth performance and phosphorus digestibility in Beluga (*Huso huso*) fed diets where soybean meal partly replaced fish meal. ANIM. FEED. SCI. TECH., 171(1): 68-73.

Kilgour, O.F.G. (1987). Mastering Nutrition. Macmillan Education Limited, London.

Kondera, E., Kościuszko, A., Dmowska, A. and Witeska, M. (2017). Haematological and haematopoietic effects of feeding different diets and starvation in common carp *Cyprinus carpio L*. J. APPL. ANIM. RES., 45(1): 623-628.

Kumar, K.; Dasgupta, C.N. and Das, D. (2014). Cell growth kinetics of Chlorella sorokiniana and nutritional values of its biomass. Bioresour. Technol., 167: 358-366.

Kumar, K.; Reddy, V.R. and Prakash, M.G. (2018). Effect of supplementing pomegranate (*Punica granatum*) peel extract on serum biochemical parameters and immune response in broilers during summer. Pharma. Innovation., 7(3): 597-601.

Levesque, H.M.; Moon, T.W., Campbell, P.G.C. and Hontela, A. (2002). Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. Aquat. Toxicol., 60(3-4): 257-267.

Mekkawy, I.A.A.; Mahmoud, U.M., Wassif, E.T. and Naguib, M. (2010). Effects of cadmium on some haematological and biochemical characteristics of *Oreochromis niloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and vitamin E. Fish. Physiol. Biochem., 37(1): 71-84.

Mostafa, A.A.Z.M.; Ahmad, M.H., Mousallamy, A. and Samir, A. (2009). Effect of using dried fenugreek seeds as natural feed additives on growth performance, feed utilization, whole-body composition and entropathogenic Aeromonas hydrophila-challenge of monsex Nile Tilapia *O. niloticus* (L) fingerlings. A. J. B. A. S., 3(2): 1234-1245.

Murthy, K.N.; Reddy, V.K., Veigas, J.M. and Murthy, U.D. (2004). Study on wound healing activity of *Punica granatum* peel. J. Med. Food., 7(2): 256-259.

Nwani, C.D.; Ifo, C.T., Nwamba, H.O., Ejere, V.C., Onyishi, G.C., Oluah, S.N. and Odo, G.E. (2015). Oxidative stress and biochemical responses in the tissues of African catfish *Clarias gariepinus* juvenile following exposure to primextra herbicide. Drug Chem. Toxicol., 38(3): 278-285.

Orzuaa, M.C.; Mussattob, S.I., Contreras-Esquivela, J.C., Rodrigueza, R., Garzaa, H., Teixeirab, J.A. and Aguilara, C.N. (2009). Exploitation of agro industrial wastes as immobilization carrier for solid -state fermentation. IND. CROP. PROD., 30(1): 24-27.

Osman, A.G.; Koutb, M. and Sayed, A.H. (2010b). Use of hematological parameters to assess the efficiency of quince (Cydonia oblonga Miller) leaf extract in alleviation of the effect of ultraviolet--A radiation on African catfish *Clarias gariepinus* (Burchell, 1822). J. Photochem. Photobiol. B., 99(1): 1-8.

Osman, A.G.; AbouelFadl, K.Y., Abd El Baset, M., Mahmoud, U.M., Kloas, W. and Moustafa, M.A. (2018). Blood Biomarkers in Nile tilapia *Oreochromis niloticus niloticus* and African Catfish *Clarias gariepinus* to Evaluate Water Quality of the River Nile. Journal of FisheriesSciences. Com, 12(1): 1-15.

Osman, A.G.; Abd El Reheema, A.E.B.M., Moustafa, M.A. and Mahmoud, U.M. (2011). In situ evaluation of the genotoxic potential of the river Nile: I. Micronucleus and nuclear lesion tests of erythrocytes of *Oreochromis niloticus niloticus* (Linnaeus, 1758) and *Clarias gariepinus* (Burchell, 1822). Toxicol. Environ. Chem., 93(5): 1002-1017.

Pantha, B. (1982). The use of soybean in practical feeds for Tilapia niloticus. MSc Thesis, Univeristy of Stirling, Scotland, UK.

Poléo, A. and Hytteørd, S. (2003). The effect of aluminium in Atlantic salmon (*Salmo salar*) with special emphasis on alkaline water. J. Inorg. Biochem., 97(1): 89-96.

Reitman, S. and Frankel, S. (1975). Colorimetric Determination of Glutamic Oxaloacetic and Glutamic Pyruvite Transaminase. J. Clin. Pathol., 28-56.

Sayed, A.H.; Ibrahim, A.T., Mekkawy, I.A.A. and Mahmoud, U.M. (2007). Acute effects of Ultraviolet-A radiation on African Catfish *Clarias gariepinus* (Burchell, 1822). J. Photochem. Photobiol., 89(2-3): 170-174.

Sayed, A.H.; Mekkawy, I.A. and Mahmoud, U.M. (2011). Effects of 4-nonylphenol on metabolic enzymes, some ions and biochemical blood parameters of the African catfish *Clarias gariepinus* (Burchell, 1822). Afr. J. Biochem. Res., 5(9): 287-297.

SPSS. (1997). Statistical package for the social sciences, Versions16, SPSS in Ch, Chi-USA.

Tavares-Dias, M. and Moraes, F.R. (2007). Leukocyte and thrombocyte reference values for channel catfish (Ictalurus punctatus Raf), with an assessment of morphologic, cytochemical, and ultrastructural features. Vet. Clin. Pathol., 36(1): 49-54.

Thomas, K.W. (1992). Conflict and conflict management: Reflections and update. J. Organ. Behav., 13(3): 265-274.

Tibaldi, E.; Tulli, F. and Lanari, D. (1995). A note on the use of plasma urea level to validate the arginine requirement assessed by growth data in seabass (*Dicentrarchus labrax*). J. Appl. Ichthyol., 11(3-4): 297-301.

Toutou, M.M.; Osman, A.G., M., Farrag, M.M.S., Badrey, A.E.A., Moustafa, M.A. (2019) Growth performance, feed utilization and gut histology of monosex Nile tilapia (*Oreochromis niloticus*) fed with varying levels of pomegranate (*Punica granatum*) peel residues. AACL Bioflux 12(1):298-309.

Toutou, M.M.; Soliman, A.A., Elokaby, M.A., Ragaa, A.A., Baghdady, E.S. (2018). Growth performance and biochemical blood parameters of Nile tilapia, *Oreochromis niloticus,* and thinlip mullet, *Liza ramada*, fed a diet supplemented with lemon (Citrus aurantifolia) peel in a polyculture system. Egyptian Journal of Aquatic Biology and Fisheries 22(3):183-192.

Trinder, P. (1969). Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. Clin. Biochem., 6(1): 24-27.

Tulli, F.; Vachot, C., Tibaldi, E., Fournier, V. and Kaushik, S.J. (2007). Contribution of dietary arginine to nitrogen utilisation and excretion in juvenile sea bass (*Dicentrarchus labrax*) fed diets differing in protein source. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 147(1): 179-188.

Vidal, A.; Fallarero, A., Pena, B.R., Medina, M.E., Gra, B. and Rivera, F. (2003). Studies on the toxicity of *Punica granatum* L. (Punicaceae) whole fruit extracts. J. Ethnopharmacol., 89(2-3): 295-300.

Yılmaz, S. and Ergün, S. (2012). Effects of garlic and ginger oils on hematological and biochemical variables of sea bass *Dicentrarchus labrax*. J. Aquat. Anim. Health., 24(4): 219-224.

Yılmaz, S.; Ergün, S. and Türk, N. (2012). Effects of cumin-supplemented diets on growth and disease (*Streptococcus iniae*) resistance of tilapia (*Oreochromis mossambicus*). Isr. J. Aquac., 64:IJA_768.

Yue, Y.R. and Zhou, Q.C. (2008). Effect of replacing soybean meal with cottonseed meal on growth, feed utilization, and hematological indexes for juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus.* Aquacult., 284(1/4): 185–189.

Zaahkook, S.A.M.; Hesham, G., Mohamed, H.G.and Salah, M.A. (2016). Physiological and oxidative Stress biomarkers in the freshwater catfish (*Clarias gariepinus*) exposed to pendimethalin-based herbicide and recovery with edta. Int. J. Adv. Res., 4(10): 243-264.