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THE ROLE OF DIETARY SUPPLEMENTATION OF TOXIN BINDING (*TOXEBONDE FORTE*) ON SOME PHYSIOLOGICAL PARAMETERS IN JUVENILE COMMON CARP *CYPRINUS CARPIO* EXPOSED OF SUBLETHAL DOSES OF WATER SOLUBLE FRACTION OF CRUDE OIL

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Abstract

This study evaluated the toxicity of water soluble fraction (WSF) of crude oil and dietary antioxidant supplementation (*Toxebonde forte*) on growth performance, feed utilization, carcass composition, water quality, haematological and biochemical parameters in juveniles common carp *Cyprinus carpio* exposed to static renewable bioassay conditions for 8 weeks. After a preliminary determination of the 96 h-LC_{50} of crude oil, fish were exposed to two sub-lethal concentrations (25 and 50 % of the LC_{50} corresponding 3.57 and 7.14 mg/l respectively) of WSF of crude oil for 8 weeks. The results showed a significant decrease in growth parameters in the WSF-exposed groups, whereas the growth parameters improved in the toxebonde forte fed fish. Meanwhile, the whole body moisture and ash contents were significantly decreased, whereas crude protein and crude lipid increased in exposed fish when compared to control group. In addition, total dissolved solid, salinity, total alkalinity and turbidity were significantly ($P<0.05$) increased with contaminated experimental water by WSF of crude oil. The heamatological profile showed concentration-dependent significant decreases ($p<0.05$) in RBC, WBC, LYM, MON, HGB, MCHC, MCH and GRA, whereas MPV count significantly increased ($P<0.05$) in exposed fish when compared with control groups. The effects on the biochemical parameters showed significant decrease ($P<0.05$) in cholesterol, triglyceride and ALP and significant increase GOT in the serum of fish exposed to 25% WSF. However, exposing fish to 50% of WSF of crude oil showed significant increase ($p<0.05$) in cholesterol, GOT, HDL and LDL and significant decrease in triglyceride level compared to the control. On the other hand, the obtained results showed that carcass composition, heamatological and biochemical parameters improved in the toxebonde forte fed fish. The results of the present study concluded that the exposure of common carp *Cyprinus carpio* juveniles to sublethal concentration of WSF of crude oil is capable of inducing homeostatic stress leading to alterations in growth performance, biochemical and haematological indices in the fish, which can in turn bring about various disturbances in the health and wellbeing in the fish. It can also be concluded that toxebonde forte can be used as feed additives to improve growth performance, biochemical and haematological indices in the fish and to reduce the deleterious effects of crude oil pollution.

Keywords **:** WSF of crude oil, common carp *Cyprinus carpio*, growth performance, water quality, haematological parameters, biochemical parameters, exogenous antioxidant (*Toxebonde forte*)

Introduction

Pollution from crude is a growing public health concern worldwide and particularly endemic in countries whose economies are dependent on the oil industry (George *et al*., 2014). Petroleum or crude oil is a naturally occurring liquid found in forming the earth consisting of a complex mixture of hydrocarbons of various lengths. It is usually black or dark brown, but varies greatly in appearance depending on its composition (Islam *et al*., 2013).

The toxic effects of crude oil to the majority of living organisms due to its toxic components such as the polycyclic aromatic hydrocarbons (PAHs), mono-aromatic hydrocarbons like benzene, toluene, ethylbenzene, xylene (BTEX); phenols, heterocyclic compounds and heavy metals have been well documented (Saeed and Al-Mutairi, 1999; Rodrigues *et al.,* 2010; Islas-Flores *et al*., 2013).

The water soluble fraction of crude oil has been described as the small dissolved fraction which is available and may be toxic to the living organisms in the aquatic environment (Olifa, 2012).

In most cases, physical and chemical changes in the aquatic environment are rapidly reflected as measurable physiological changes in fish due to their close association with the environment (Kayode and Shamusideen, 2010).

Crude oil has been known to have adverse effects growth performance and feed utilization in different fish species such as (Omoregie &Ufodike, 1999; Omoregie & Ufodike, 2000; Udofia, 2010: Sharaf & Abdel-Tawwab, 2011), in tilapia (Nwabueze and Agbogidi, 2010; Olaifa, 2012; Onwurah *et al*., 2013; George *et al.,* 2014; Fakolujo *et al*., 2018; Ajah & Ukutt., 2018) African catfish, (Lockhart *et al.,* 1996) rainbow trout.

The changes in water quality after the introduction of water soluble fractions of crude oil have been reported (Nwabueze and Agbogidi, 2010: Eriegha *et al*., 2017; Eriegha, *et al*., 2019).

Haematological parameters such as Hb, Hct, RBC, WBC count and hematological indices like MCV, MCH and MCHC are widely used to evaluate the toxic stress of environmental contaminants (El-Sayed *et al*., 2007, Kavitha *et al*., 2010).

The negative effects of water soluble fraction of crude oil on fish haematology have been investigated in different fish species (Omoregie, 1998; Onwurah *et al*., 2013; Eriegha

et al., 2017) tilapia, (Ajah and Ukutt 2018; Fakolujo *et al*., 2018; Khoshbavar Rostami and Yelghi, 2018) African catfish and (Hantoush, 2010) carp. Blood biochemical parameters were considered as indexes in the physiological state of fish and monitoring the physiological status (Edsall, 1999). Liver function tests have been used as an index of liver function changes to WSF exposure and plasma enzyme glutamic oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) analysis is one of the liver function tests (Abdel-Hameid, 2009). WSF of crude has been shown to alter a variety of biochemical parameters in different fish species such as (Eriegha *et al*., 2017) in juveniles of nile tilapia *O. niloticus*, (Khoshbavar Rostami and Yelghi, 2018) giant sturgeon, *Huso huso* and (Hantoush, 2010) carp.

Toxebonde forte is a complementary feed for all animals. It consists of bentonite, yeast, calcium carbonate, vegetable oil refined (palm). It is one of the MIAVIT Company products. The product obtained from a local company (Kosar Company for Agriculture and Poultry). The positive effects of dietary supplemental exogenous antioxidant of freshwater fish species diets exposed to a water-soluble fraction of crude oil have been investigated to a limited degree. Therefore, dietary supplemental exogenous antioxidant could be one good way to reduce the negative impact of water soluble fraction of crude oil and improve growth performance and physiological status.

For these reasons, the prime purpose of the present study was evaluate the effects of sublethal levels based of water soluble fraction (WSF) of crude oil and dietary supplementation of toxin binding (*Toxebonde forte*) on growth performance, feed utilization, mortality, carcass composition, water quality, haematological and biochemical markers in juveniles common carp *Cyprinus carpio.*

Materials and Methods

Experimental diets: Experimental diets were formulated to the NRC (2011) guidelines on the nutritional requirements for carp. Two diets were formulated with the same composition with the addition of 0.1% toxebonde forte supplementation for one of them. Both diets were formulated to be isonitrogenous (38%), isolipidic (8%) and isoenergetic ($19MJkg^{-1}$).

Diets were manufactured by initially dry mixing ingredients before homogenising through a commercial food mixer. Diet pellets were extruded through a cold press extruder (SUNRRY, model: SYMM12, China) using a 2mm aperture die. Feeds were subsequently dried in a dehumidifying oven for 24h at 40°C. Test diet formulations and proximate composition are presented in Table1.

Experimental design:

(G1) C= Control

(G2) C+T= Control+1% of Toxebonde Forte of diet

(G3) 25%WSF= exposed to 25% of sub lethal concentration of water soluble fraction of crude oil (3.57 ml\L)

(G4) 25%WSF+T= exposed to 25% of sub lethal concentration of water soluble fraction of crude oil (3.57 ml\L) fed diet supplemented Toxebonde Forte

(G5) 50%WSF=exposed to 50% of sub lethal concentration of water soluble fraction of crude oil (7.14 ml\L)

(G6) 50%WSF+T= exposed to 50% of sub lethal concentration of water soluble fraction of crude oil (7.14 ml\L) fed diet supplemented Toxebonde Forte

Experimental condition: The experiment was undertaken in the Aquaculture unit (Close system), Grdarasha station, College of Agriculture Engineering Sciences - https://academics.su.edu.krd/University of Salahaddin, Erbil, Kurdistan Region-Iraq. The trial was conducted in the experimental closed system. Fish were randomly distributed into $74L^{-1}$ (each measuring30×38×65 cm^3) polyethylene tanks, each of these tanks was equipped with one inlet pipe (to form dissolved oxygen in tanks) all pipes connected with low noise air pump (RESUN, Model: LP-60). After a preliminary determination of the 96 h-LC50 of crude oil by probit regression was found to be 14.34 mg/l, fish were exposed to 2 sub-lethal concentrations (25 and 50 % of the LC50 corresponding 3.57 and 7.14 mg/l respectively) of the WSF of crude oil and a control with and without addition of toxebonde forte for 8 weeks. Dissolved oxygen was measured daily by using DO- meter model (TRANS instruments, HD3030 8403). PH for each was measured daily by an electrometric method using portable pH-meter model (HANNA instruments, HI98129, CE; Made in Romania). Temperature, electrical conductivity (E.C.), total dissolved solids (T.D.S.), salinity, resistivity for each tank were measured daily using meter model (Model SX713, Made in China). A 12h light: 12h dark photoperiod was maintained throughout the experiment. The water of these tanks replaced daily with non-chlorinated water from deep well with the addition the water soluble fraction of crude oil for 8 tanks in total 12 tanks. Water samples from each were collected using 1.5 liter acid washed polypropylene containers in the 12 am in week four for one time and taken to the (Public Health Laboratory in Kurdistan Region of Iraq for the Ministry of Health) for analysis. Turbidity, PH, electro conductivity, total dissolved solid, chloride as (Cl^-), sodium (as Na^+), potassium (as K^+), calcium (as Ca^+), magnesium (as Mg^+), total hardness (as $CaCO^3$), total alkalinity (as $CaCO^3$), Nitrate (as NO^{-3}) and sulfate (SO_4) were determined in the Lab.

Experimental Fish: Common carp (*Cyprinus carpio*) was sourced from Ankawa hatchery station, Erbil, Kurdistan Region, Iraq. Fish were transported to at the Aquaculture unit (Close system), Grdarasha station, College of Agriculture Engineering Sciences, University of Salahaddin, Erbil, Kurdistan Region -Iraq. Fish acclimated to the aquarium system for 14 days before the feeding trial. During that time, fish were fed on a maintenance diet (34% protein and 7% lipid). Fish were graded and randomly distributed into the tanks (n=8, 30.41±2.28 g). Experimental diets were given three times daily at a ration level of ~3% of the fish body weight. Fish were weighed weekly after feeds were withheld for 24h to allow gut clearance, and the amount of diet was adjusted accordingly to the weight.

Preparation water soluble fraction (WSF) of the crude oil: The extraction of the soluble compounds from the crude oil followed the methodology of (Simonta *et al.,* 2006). Briefly, to obtain the WSF one part of commercial brand oil was added to four parts water in a glass container. The composition of crude oil used in the current study was determined in petroleum lab. The composition of crude oil used in the current study was shown in table 2, 3. The mixture was then exposed to sunlight for 4 days. After that,

the upper insoluble phase was discharged and the remaining water phase was collected and used to experiment.

Collection and Transportation of Crude Oil: Crude oil was obtained from the khurmala oil field, in the airtight plastic bottle and transported to the research laboratory of the Fish Resources and Aquatic Animals, Science and Engineering Agricultural College-Salahaddin University-Erbil- Kurdistan Region-Iraq, for subsequent use.

Haematological and Biochemical Analysis: At the end of the trial, fish were euthanised, and blood collected from six fish per treatment. Fish were anaesthetised with buffered tricaine methane sulphate (MS222, Phamaq, Norway) at 200mgL^{-1} followed by the destruction of the brain. Blood was sampled from the caudal vein using a 25-gauge heparinized needle and 1-ml syringe (Campbell, 2015). The blood samples divided into two halves, the first half of each sample placed in heparinised 2 vials for haematological analysis. The other halves of the blood samples placed in clot activator and sun-val then put in ice, then immediately putted in centrifuged at 3,500 rpm for 15 minutes and the supernatant serum collected and placed in labeled in eppendrof tubes stored at -80 °C for biochemical tests. For haematological analysis Leucocyte (WBC), lymphocytes (LYM), monocytes (MON), granulocytes (GRA), hemoglobin (Hb), mean cellular hemoglobin (MCH), mean cellular hemoglobin concentration (MCHC), erythrocyte (RBC), mean cellular volume (MCV), hematocrit (Hct), platelet (PLT) and mean platelet volume (MPV) were measured using fully-auto hematology analyzer (MCL-3800 made in China). Biochemical tests such as cholesterol, glutamic oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), triglyceride, alkaline phosphate (ALP), high density lipids (HDL) and low density lipids (LDL) were measured using Cobas c111 in the Welfare Medical laboratory for Disease Diagnosis in Erbil city.

Proximate composition: Finished test diets and fish samples were analysed according to AOAC (2012) standard methods. All samples were analysed in triplicate. The moisture content was determined after drying material at 105°C with a fan assisted oven until a constant weight was achieved. Similarly, ash levels in the samples were measured by incineration in amuffle furnace at 550°C for period 16h. Crude protein (N×6.25) was performed by the automated Kjeldhal method after acid digestion (Kjeldahltherm microsystem 40, C.Gerhardt GmbH, KG, Germany). Lipid content was determined through a Soxhlet gravimetric method using petroleum ether (1356, Parr Instrument Company, IL, and the USA)

Growth and feed utilization calculations: The growth performance of the fish and feed utilization were measured according to the following formulae;

Specific growth rate (SGR %) = (In FBW-In IBW) / T×100

Feed Conversion Ratio (FCR g) = (Feed intake (g)) / (weight gain (g))

Condition Factor (K %) =FBW/FL^3 x100

Mortality (%) = (Initial Nb - Final Nb)/ Initial Nb×100

Survival (%)= 100–Mortality (%) =Final Nb /Initial Nb×100.

Where In FBW: is the logarithm of the final body weight, In IFW: logarithm of the initial body weight, T: times (number of days), IFW: initial fish weight, FBW: final body weight, WG: Weight gain (g), FI: Feed intake (g), FL: final fork length (cm), Initial Nb: initial number of fish, Final Nb: final number of fish.

Statistical analysis: All results are expressed as mean values ± standard error (±SE). Statistical analyses were conducted by using SPSS statistics version 26 for windows (SPSS Inc., an IBM company, copyright 1989-2019). Data analysis was performed using one-way ANOVA. Duncan's multiple ranged ad- Post hoc LSD test was used to identify where significant differences occurred at the 95 % confidence level (associated probability ≤ 0.05).

Table 1: Formulation and proximate analysis of the experimental diets (dry weight).

Ingredient g kg^{-1}	**C**	**T**
Soybean	530	530
Corn	150	150
Fishmeal	100	100
Premix	25	25
Soya oil	50	50
Wheat flour	100	100
Wheat bran	15	15
Vitamin Premix	11	11
Enzyme	1	1
Mineral premix	20	20
Toxebonde forte	-	1
Proximate composition (%)		
Moisture (%)	7.79±0.42	7.79±0.07
Protein (%)	38.67±0.24	39.51±0.16
Lipid (%)	6.46±0.35	6.81±0.14
Ash (%)	6.4±0.21	6.01±0.05
NFE (%)	39.4±014	39.96±0.01

Vitamin Premix consists of Methionine 5g per kg, Lysine 3g per kg, Thrionine 3g per kg

Mineral premix consists of Mono calicium 5g per kg, salt 1g per kg limestone 14g per kg

Toxebonde forte: Complementary feed for all Animals, Composition Bentonite, yeast, calcium carbonate, vegetable oil refined (palm)

Price: 205 per kg, Toxin Binder, MIAVIT Company, Recommended dosage, 1kg\ton of feed in case of low risk, 2 kg \ton of feed at high risk

Nitrogen-free extracts (NFE %) = 100-(Ash+ moisture+ crud fat+ crude protein)

C= Control diet

T= Diet supplemented 0.1 % of toxebonde forte

Table 2: The analysis of crude oil sample

No	Test description	Unit	Test Method	Results
1	Specific Gravity at 15.56°C	-----	ASTM D1298	0.8530
2	API Gravity at 60F	----	ASTM D1298	34.39
3	Density at 60°C	g/cm³	ASTM D4052	0.8559
4	Total Sulfur	Wt%	ASTM D4294	2.4343
5	Pour point	°C	ASTM D97	-10
6	Kinematic viscosity at 20C	Cst	ASTM D445	11.376
7	Kinematic viscosity at 40C	CSt	ASTM D445	5.634
8	Water content ,free water	vol%	ASTM D4007	0.1
9	Sediments	vol%	ASTM D4007	Nil
10	Flash Point, Tag CC	°C	ASTM D56	Below +18
11	Salt content	Ptb	ASTM D3230	19
12	Salt content	Vol ppm	ASTM D3230	57.1
13	Reid Vapor pressure at 37.8°C	Kpa	ASTM D323	48
14	Copper strip corrosion	-----	ASTM D130	1a
15	Heat of combustion	Btu/IB	Calculated	19622.19

Table 3: The analysis of crude oil sample for determination of elements by ASTM D6595, ASTM D6728

p	Elements	Unit	Result
1	Na	Wt ppm	122.74
2	K	Wt ppm	1.07
3	Li	Wt ppm	84.94
4	V	Wt ppm	46.10
5	Mg	Wt ppm	1.91
6	Ca	Wt ppm	2.68
7	Pb	Wt ppm	0.37
8	Zn	Wt ppm	0.57
9	Si	Wt ppm	0.30
10	Cr	Wt ppm	0.44
11	Ni	Wt ppm	18.61
12	Cu	Wt ppm	0.04
13	Al	Wt ppm	0.00
14	Fe	Wt ppm	0.25
15	Mn	Wt ppm	0.74

Results and Discussion

Growth and feed utilization: The growth performance, feed utilization and a body condition factor (K) parameters are presented in (Table 4). No significant difference in initial body weight for all treatments indicated that the fish were homogeneously distributed among treatments and replicates at stocking. The result showed that the fish in 1% of Toxebone Forte supplement group (G2) had a significant *($P < 0.05$)* increase only in FBW groups compared with the control one. Growth performance parameters and feed utilization were significantly *($P \leq 0.05$)* reduced in fish exposed to sublethal concentrations of water soluble fraction of crude oil. Data revealed that fish of G3,G4,G5 and G6 had a significant *($P < 0.05$)* decrease in mean of final body weights, body weight gain (WG) and a decrease in SGR% compared with the control group and 1% of Toxebonde Forte supplemented groups. The results of weight gain reflected on feed conversion ratio (FCR) which was significantly increased (3.45±0.24) in exposed to 25% of sub lethal concentration of water soluble fraction of crude oil (3.57 ml\L) fed diet supplemented Toxebonde Forte groups compared with control (1.53±0.05). The highest mortality percentage were recorded in G5 (50% WSF) and highest survival percentage were recorded in 1% of Toxebonde Forte supplement groups. No significant difference was found among all groups of in the condition factor (K).

Carcass composition: The terminal carcass composition of fish fed the experimental diets and exposed to both doses of WSF of crude oil is presented in Table 5.

Data in the present study showed that the high significant moisture content (77.44±0.76) was recorded in the group of fish feed on only dietary supplementation of the 0.1% of toxebonde forte for fish diets (C+T) when compared with group 5 and 6. The group of fish feed on only dietary supplementation of the 0.1 % of toxebonde forte for fish diets and group exposed to 25 % of sublethal concentrations of WSF of crude oil led to significantly increase *($P<0.05$)* in whole body protein content when compared with control group. On the other hand, dietary supplementation of the 1% toxebonde forte significantly decrease protein content in groups of fish exposed to 25 % (13.14±0.64) and 50% (10.84±0.65) of sublethal concentrations of WSF of crude oil. The highest whole body lipid content *($P<0.05$)* was recorded significantly in G5 and G6 in comparison with control group. However, lipid percentage was not changed significantly by providing 0.1% toxebonde forte together with 50 % of sublethal concentrations of WSF in G6 when compared with group received 50 % of sublethal concentrations of WSF alone. The significantly lowest whole body ash content (5.96±0.29) was recorded in the group received 25% of sublethal concentrations of WSF of crude oil when compared with control group (7.21±0.1).

Water quality:

The variations in the physico-chemical parameters of the test medium during the experiment are shown in Table 6. PH, electro conductivity (EC), chloride, nitrate, sodium, calcium, temperature, conductivity and resistivity readings revealed no significant differences ($P > 0.05$). However, total dissolved solid, salinity, total alkalinity were significantly ($P > 0.05$) increased by exposing fish to 50% of sublethal concentrations of WSF of crude oil but this trend was not significant in exposing 25% of WSF. Turbidity was significantly ($P > 0.05$) increased by exposing fish to 25% and 50% of sublethal concentrations of WSF of crude oil, but this trend was not significant in exposing 25% of WSF. Nevertheless, dissolved oxygen, total hardness and were significantly ($P > 0.05$) reduced with exposing fish to both doses of WSF of crude oil.

Hematological parameters: The results of haematological parameters of common carp *Cyprinus carpio* following exposure to sub-lethal concentrations of crude oil and supplemented toxebonde forte are shown in tables 7.

A significant ($p<0.05$) reduction in the WBC, absolute LYM, LYM %, absolute MON, MON%, Hb and MCHC values were observed in fish exposed to both doses of WSF of crude oil in comparison with control group. Furthermore, RBC significantly ($P<0.05$) decreased by 25%, but this trend was not significant at 50%. No significant change was found in MCH, GRA by exposing to 25%. While exposing fish to 50%, significantly ($P<0.05$) reduced MCH, GRA. A slight reduction was recorded in Hct %, while this reduction was not significant.

PLT significantly ($P<0.05$) increased by 25% and no significant change with 50%. No significant change was found in MPV with exposing 25% while with exposing 50% significantly ($P<0.05$) increased. On the other hand, supplementing T to diet of fish exposed to WSF significantly ($P<0.05$) modulate the toxic effects of WSF by increasing WBC, LYM, Hb, MCHC and PLT. However, MON was not significantly changed.

There was no significant change in Hct value. There was a significant ($P<0.05$) decline in MCV with the addition of T to diets of fish exposed to both doses of WSF. GRA MCH were not significantly affected with the addition of T to diet of fish exposed to 25%, while both of them significantly increased by exposing 50%. MPV was not significantly affected with the addition of T to diet of fish exposed to 25%, while significantly ($P<0.05$) reduced by exposing 50%.

Biochemical parameters: Biochemical parameters in serum of common carp *Cyprinus carpio* following exposure to sub-lethal concentrations of crude oil and supplemented toxebonde forte are shown in tables 8.

GOT and HDL were significantly ($P<0.05$) increased in fish exposed to both doses of WSF of crude oil. No significant change was found in LDL by exposing 25%, while with exposing 50% significantly ($P<0.05$) increased. GPT was not significantly different in fish exposed to WSF of crude oil in comparison with control group.

Triglyceride was significantly ($P<0.05$) decreased in fish exposed to both doses of WSF of crude oil. Cholesterol was decreased significantly ($P<0.05$) with exposing 25 % while increased significantly with exposing 50% in comparison with control group. ALP decreased significantly ($P<0.05$) with exposing 25 %, while this trend was not significant at 50%. in comparison with control group. On the other hand, GOT and GPT significantly ($P<0.05$) decreased with the addition of toxebonde forte to the diets of unexposed (G2) group of fish, while LDL increased significantly when compared with control group.

GPT, GOT and ALP significantly ($P<0.05$) decreased with the addition of toxebonde forte to the diets exposed 25% group of fish in comparison with group (G3), while the addition of toxebonde forte to the diets significantly increased GPT and triglyceride of exposed G6 in comparison with group (G5).

Triglyceride was increased significantly ($P<0.05$) with the addition toxebonde forte for fish diet exposed to 50% of WSF of crude oil. HDL was significantly ($P<0.05$) increased in fish exposed to both doses of WSF of crude oil. LDL was significantly ($P<0.05$) increased with dietary supplementation of antioxidant to diet of unexposed and exposed to 50% WSF (37.33±8.98) group of fish in comparison with control.

The results of this study demonstrated that exposing common carp to WSF of crude oil have significant adverse effects on growth performance, feed utilization, body composition, water quality, haematological and biochemical parameters.

The reduction of growth performance and feed utilization may be attributed to reducing fish's appetite or complete fish fasting, resulted in a lower retention rate of nutrients into the fish's body resulting in exposure fish to WSF of crude oil. Another possible explanation for poor growth performances in the exposed group could be attributed to the effects of the soluble fractions of crude polycyclic aromatic hydrocarbon (PAH) and total petroleum hydrocarbon (TPH) which probably affected the fish eco-physiology (Hodson et al., 1997). The high feed conversion ratio (FCR) observed in the exposed group indicated the inability of the exposed fish to convert feed consumed into required body protein (Sunmonu and Oloyede, 2006).

The relatively high mortality through possible disease contagion observed in test fish exposed to sublethal concentrations of WSF of crude oil was due to the potency of environmental toxicants, which increased disease susceptibility by interfering with immune, reproductive and developmental processes within aquatic animals (Fakolujo *et al*., 2018)

The results of the current study match those observed in earlier studies for example Omoregie and Ufodike (1999) indicated that growth performance and feed utilization significantly reduced in Nile tilapia, *Oreochromis niloticus Trewavas* exposed to 0.313, 0.156, 0.078, and 0.039 mL L^{-1} of water soluble fractions (WSFs) of the Bonny light crude oil.

Furthermore, Omoregie & Ufodike (2000) reported that various sublethal concentrations of water soluble fractions of crude oil for 10 weeks significantly decreased growth of the Nile tilapia *O. niloticus*. Similarly, Sharaf & Abdel-Tawwab (2011) reported that the acute exposure to CPF (commercial petroleum fuel) significantly reduced growth performance and survival of Nile tilapia *Oreochromis niloticus (L.).*

The findings of the current study are consistent with those of Nwabueze and Agbogidi (2010) who indicated that

the water-soluble fractions of crude oil significantly reduced the growth performance of catfish *Heterobranchus bidorsalis* fingerlings. Our results are also in full agreement with data obtained by Fakolujo *et al*. (2018) who found that sublethal levels of WSF of crude oil had adverse effects on the growth performance in African sharptooth catfish *C. gariepinus* juveniles. Nevertheless, the finding of the current study disagrees with (Onwurah *et al*., 2013) finding who indicated that crude oil pollution could be beneficial to tilapia fish at certain sub lethal doses.

The results of the present study indicated that a diet supplemented with 1g kg^{-1} of toxin binding (toxebonde forte) improved growth and survival of juvenile common carp unexposed and exposed of sublethal concentration of WSF. Although there is no information on the toxin binding (*toxebonde forte*) using in aquaculture diets because it is new products, the findings of the current study can be compared with few studies which have been conducted with different types of plant, antioxidant and different species of fish. The results of this study are in agreement with some previous findings.

Saei *et al*. (2017) reported that supplementing toxin binder biotox to rainbow trout (*Oncorhynchus mykiss*) fed diets-contaminated with aflatoxin slightly improved growth performance but increased survival. These results also are in agreement with Jiang *et al*. (2016) findings, which showed that dietary curcumin supplementation increased intestinal antioxidant capacity, digestive and absorptive ability thus promoted fish growth in crucian carp (*Carassius auratus*). Similarly, Kumar *et al.* (2017) indicated that fenugreek (*Trigonella foenumgraecum* L.), a seed powder seed at 2% dose is beneficial as dietary supplements for improving growth performance, biochemical and blood parameters in common carp (*Cyprinus carpio*) fingerlings. The current study results were also in agreement with data obtained by Roohi *et al*. (2017) who reported that fenugreek seed meal could be considered as a beneficial dietary supplement for improving the growth performance and blood indices of common carp fingerling. The reasons for the finding of the present study may be due to low level of supplemented toxin binding and high toxicity of WSF of crude oil. However, the findings of the current study do not support the Kim *et al*. (2013) findings who reported that dietary supplementation of Spirulina and Quercetin to juvenile olive flounder *Paralichthys olivaceusis* diet did not improve growth performance.

Whole body moisture, protein, lipid and ash contents of common carp juveniles significantly affected in 50% WSF. While, whole body moisture and lipid contents did not significantly affect in 25% WSF. However, whole body protein was significantly increased whereas ash content significantly decreased in 25% WSF.

Decreasing the moisture content in the current study is disagreement with (Lockhart *et al.,* 1996) study who indicated that exposing fry rainbow trout to crude oil increased fish body water content. The authors also indicated that regulation of body water content is important physiologically and it may offer insight into some of the mechanisms of toxic action of oil on fish. Moreover, the results of the present study are in accordance with data obtained by Majumder and Kaviraj (2019) who found that exposure freshwater fish *Oreochromis niloticus* to sub-lethal concentrations of the insecticide (*Chlorpyrifos*) significantly increase whole body crude protein and lipid. Increasing protein and lipid content in the present study could attributed to decrease the fish's ability to swim and spent little energy, thus fish converted less lipid and protein to energy.

However, increasing protein and lipid content in the present study is disagreement with Amin and Indulkar (2017) who found that exposing freshwater fish *Cyprinus carpio* sublethal toxicity of a synthetic pesticide significantly reduced fish protein, lipid and ash contents.

On the other hand, the moisture and protein contents were significantly decreased by exposing fish to sublethal concentrations of WSF of crude oil and fed a diet supplemented toxebonde forte as a toxin binding in comparison with group feed antioxidant alone. However, lipid content and ash did not improve by administration of antioxidant in both exposed to WSF groups.

All recorded dissolved oxygen concentrations, temperature and pH were adequate to support fish. In the current study pH, electro conductivity, chloride, nitrate, sodium, calcium, temperature, conductivity, resistivity did not significantly affect by exposing fish to WSF of crude oil. These observations were similar to those of other workers (Nwabueze and Agbogidi, 2010; Obasohan *et al*., 2011: Giari *et al*., 2012)

The present study has also demonstrated that the water-soluble fractions of crude oil have a highly significant effect of increasing the total dissolved solid, salinity, total alkalinity and turbidity. Nevertheless, dissolved oxygen, total hardness and were significantly reduced with exposing fish to sublethal concentrations of WSF of crude oil. This observation supports earlier reports by (Nwabueze and Agbogidi, 2010; Eriegha *et al*., 2017: Eriegha *et al*., 2019).

A possible explanation for this might be that increasing biochemical oxygen demand and chemical oxygen demand with exposing fish to WSF of crude oil (Eriegha *et al*., 2019). The reduced dissolved oxygen content of the WSFs with higher concentration may have caused stress in the fish resulting in reduced feeding activity and suffocation, which eventually may have led to the fish kill.

Hematological parameters are sensitive indicators of fish health and physiological status. However, their values may be affected by various environmental pollution by different pollutant (Kondera *et al*., 2019.)

The results of the blood sample in the current study showed significant reduction in WBC, absolute LYM, MON, Hb and MCHC content of the exposed fish compared to control group, while GRA % significantly increased in comparison with control group. Changes in the above haematological parameters might have been affected by WSF crude oil. Leukocytes are involved in the control of immunological function and the changes in WBC counts after exposure to various toxicants may indicate a decrease in nonspecific immunity of the fish (Saravanan *et al*., 2011).

A significant reduction of WBC in the current study after exposure fish to WSF of crude oil is in agreement with previous studies in different fish species (Eriegha et *al*., 2017) on nile tilapia *O. niloticus*, (Khoshbavar Rostami and Yelghi, 2018) african catfish *Clarias gariepinus*, (Omoregie,1998) Nile tilapia *Oreochromis niloticus Trewavas*, (Onwurah *et al*., 2013) *Nile tilapia* (*Oreochromis*

niloticus L.). However, this result disagrees with the finding of Ajah and Ukutt (2018) who found a significant increase in WBC with exposed African sharp tooth catfish *Clarias gariepinus Burchell* juveniles to sublethal levels of water soluble fraction (WSF) of crude oil.

A progressive decrease in absolute LYM, MON in the present study is in agreement with Khoshbavar Rostami and Yelghi (2018).

Reduction in Hb and Hct content in toxicant treated fish may be due to disorders in haemopoietic processes and accelerated disintegration of RBC cell membranes (Svobodova *et al*., 1997). Further lysing of erythrocytes due to toxicant stress may also lead to a reduction in haemoglobin and haematocrit values in the fish (Kori-Siakpere and Ubogu, 2008).

The observed significant variation ($P<0.05$) in hemoglobin value of the exposed fish may be attributed to less oxygen content in the blood of the exposed fish. Moreover, lower haemoglobin values are indication of shrinkage of cell due to toxicant stress on the erythropoietic tissue (Saravanan *et al.*, 2011). The decreases in haemoglobin concentrations also indicate considerable restrictions on the fish's ability to provide oxygen sufficient to their tissues, resulting in a decrease of physical activities of the fish (Nussey, 1994).

These results agree with the findings of other studies. Nasir and Hemtoush (2010) observed a linear reduction in haemoglobin in juvenile common carp *Cyprinus carpio* which suggests an anemic condition in the crude oil treated fishes. Eriegha *et al*. (2017) also reported that reduction in haemoglobin content in exposed fish relative to control signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and will result in a decrease in physical activity.

Nevertheless, this finding disagrees with (Lari *et al*., 2016) study who found that Ht and Hb contents of the blood were not significantly changed with exposing juvenile Caspian roach, *Rutilus caspicus,* to WSF at lethal and sub-lethal level.

The decrease in RBC has been attributed to haemolysis resulting in haemodilution. This result agrees with findings by Eriegha *et al*. (2017) who found a significant ($p<0.05$) reduction in the values of red blood cells, packed cell volume and haemoglobin in juvenile *Oreochromis niloticus* exposed to water soluble fractions of crude oil. Furthermore, according to Rostem and Soltani (2016) crude oil can affect RBC causing a hemolysis by a disruptive effect on the erythropoietic tissues of the spleen and kidney.

The reduction in MCH and MCHC values of was observed with exposed fish to WSF of crude oil in the current study. The variations obtained in these haematological indices (MCH and MCHC) in the present study could be due to a defense against the toxic effect of crude oil through the stimulation of erythropoiesis (Mouse, 1999).

The significant decreases in the MCHC values in the exposed fish are thus probably an indication of swelling of the red blood cells and/or a decrease in hemoglobin synthesis.

The fluctuation in the MCH values clearly indicates that the concentration of haemoglobin in the red blood cells was much lower in the exposed fish than in the control over the exposure period, thus indicating an anaemic condition.

The significant decreases in the MCHC values in the exposed fish are thus probably an indication of swelling of the red blood cells and/or a decrease in hemoglobin synthesis.

The results of this study are in keeping with previous observational studies by (Khoshbavar Rostami and Yelghi, 2018: Ajah and Ukutt, 2018).

On the other hand, supplementing antioxidant (toxebone forte) to diet of fish exposing to WSF significantly ($P<0.05$) increased WBC, absolute LYM, LYM%, HGB, MCHC and PLT in the present study. Our result disagrees with (Roohi *et al.*, 2017) who reported that the use of fenugreek seed meal in fish diets of common carp (*Cyprinus carpio L.*) did not significantly change haemoglobin, haematocrit and RBC levels and significantly reduced WBC.

The addition of (Toxebonde forte) significantly decreased MCV value in 25% WSF whereas in 50% WSF showed opposite trend. The value of MPV significantly decreased with addition of Toxebonde forte to 50% WSF group of fish diet.

The absolute GRA and GRA % were significantly affect of with the addition of Toxebonde forte in 50% WSF exposed group diet. Although, these results differ from some published studies. Yilmaz (2019) reported that haematological parameters were not influenced by dietary caffeic acid supplementation in Nile tilapia *Oreochromis niloticus*. Similarly, dietary inclusion of transcinnamic acid at levels (250, 500, 750 and 1500 mg kg^{-1}) to rainbow trout diet did not significantly change RBC, Hct and Hb values (Yilmaz *et al.,* 2018).

Cholesterol is a necessary compound of the structure of the cell membrane. It is measured to show the food status in animals (Roohi *et al*., 2017). In the present study, cholesterol level was decreased in the group of fish exposed to 25% of WSF of crude oil. However, significantly increased in groups of fish exposed to 50% of the sublethal level of WSF of crude oil compared with the control group. Increasing concentrations of cholesterol in serum can be a result of damages to liver or kidney syndrome. The results of the current study is inagreement with 25% but disagree with 50% with (Ajah and Ukutt 2018) study who found significant reduction in cholesterol and triglyceride when clariidae catfish juveniles exposed to water soluble fraction of crude oil. The glutamic oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) in serum components can be generally used to assess the tissue damage of the liver and kidney (Agrahari *et al*., 2007).

The glutamic oxaloacetic transaminase (GOT) was significantly increased by exposing fish to 50% of WSF of crude oil while this trend of increasing was not significant with glutamic-pyruvic transaminase (GPT. This is inagreement with Han *et al*. (2019) who found that the GOT and GPT significantly increased in juvenile starry flounder, *Platichthys stellatus* exposed to arsenic. Similarly Shin *et al*. (2016) reported that GOT and GPT in rockfish, Sebastes schlegelii significantly increased during thermal stress by the ammonia exposure. Furthermore, Vedel *et al.* (1998) reported a considerable increase in the GOT and GPT of rainbow trout, *O. mykiss*, exposed to ammonia.

Triglyceride significantly decreased with exposing fish to both doses of WSF of crude oil. This is in agreement with (Ajah and Ukutt 2018) who found a significant reduction in cholesterol and triglyceride when clariidae catfish juveniles exposed to a water soluble fraction of crude oil. Alkaline phosphate (ALP) is the enzyme that has been applied for evaluating hepatocellular damage (Gad and El-Twab, 2009).

Alkaline phosphate did not significantly affect with an exposure of crude oil in the present study. This finding disagrees with previous findings. Eriegha *et al.* (2017) investigated ALP significantly increased when juvenile *Oreochromis niloticus* exposed to four sub-lethal concentrations (30, 45, 60 and 75% of water soluble fractions of crude oil. However, Khoshbavar Rostami and Yelghi (2018) found a significant reduction in ALP when giant sturgeon, *Huso huso* exposed to cute level of crude oil.

High density lipids (HDL) was significantly increased by exposing fish to WSF of crude oil. No significant change found in low density lipids (LDL) by exposing 25%, while with exposing 50% significantly ($P<0.05$) increased.

Cholesterol did not significantly affected with addition of toxebonde forte to exposed and unexposed group of fish. This is coinciding with Saei *et al.* (2017) who observed that inclusion of dietary antitoxic (Biotox) did not significantly affect fingerling rainbow trout (*Oncorhynchus mykiss).*

Although, these results differ from some published studies. Kim *et al.*, (2013) found a significant reduction in the cholesterol with supplementation of dietary Spirulina and Quercetin to juvenile olive flounder *Paralichthys olivaceus*. Kumar *et al.* (2010) also reported that cholesterol significantly decreased in common carp (*Cyprinus carpio L.*) fingerlings fed with differently detoxified *Jatropha curcas* kernel meal. Imanpoor *et al.* (2011) showed that concentration of cholesterol significantly decreased in common carp exposed of chloramine.

GPT was significantly decreased with the addition of toxebonde forte to unexposed and exposed to 25% WSF group, but increased in 50% group of fish. While, APL decreased in both exposed group by proved of antioxidant. This observation is similar with Uncumusaoğlu, (2018) observation who found that the level of ALP significantly decreased in common carp (*Cyprinus carpio Linnaeus, 1758*) fed different levels of zeolite. However, the findings of the current study do not support the Kumar *et al.* (2010) finding who found that ALP significantly increased in common carp (*Cyprinus carpio L.*) fingerlings fed with differently detoxified *Jatropha curcas* kernel meal.

The present study revealed that GOT significantly decreased with the addition of toxebonde forte to control and 25% WSF groups. Trihlyceride increased significantly with the addition of toxebonde forte to fish exposed to both doses of WSF. These results are in agreement with Kumar *et al.* (2010) who indicated that triglycerides significantly increased in common carp (*Cyprinus carpio*) fingerlings fed with differently detoxified *Jatropha curcas* kernel meal. However, this is disagreement with Uncumusaoğlu (2018) who found that the level TRG significantly decreased in common carp (*Cyprinus carpio)* fed different levels of zeolite.

The LDL value significantly increased by adding Toxebonde forte to unexposed group of fish, whereas this increasing was not significant in the exposed group of fish. This is in agreement with Uncumusaoğlu (2018) who indicated that the level LDL increased in common carp (*Cyprinus carpio)* fed different levels of zeolite.

Conclusion

The most obvious finding to emerge from this study is that sublethal concentrations of WSF of crude oil can pose undesirable changes in water quality. The crude oil influenced water quality can, therefore, induce a variety of alterations on haematological and biochemical parameters in juvenile common carp *Cyprinus carpio*. Additionally, growth performance, feed utilization and survival significantly declined in fish exposed to WSF of crude oil. This study also has shown that feeding of toxebonde forte at 0.1 % improves the growth of *C. carpio* juvenile which is affected by exposure to sublethal concentrations of WSF of crude.

The stress mitigating effect of dietary toxine binding (toxebonde forte) was also observed in *C. carpio* when exposed to sublethal concentrations of WSF of crude. This was reflected on growth and survival rate of WSF exposed, but fed with toxebonde. Hence, supplementation of dietary toxebonde forte at 0.1% may be taken as a strategic measure to augment growth and immunity in *C. carpio* juvenile exposed to WSF of crude.

A further study could assess the long-term effects of WSF of crude oil and use different concentration of WSF of crude oil for different age of carp and for other fish species of high economic importance. On the other hand, considerably more work will need to be done to determine the effect of dietary supplementation of different types of natural and artificial antioxidants in reducing the adverse effects of exposure different fish species to the toxicity of crude oil.

Table 4: Growth performance and feed utilization of *Cyprinus carpio* fed the experimental diets and exposed of WSF for 8 weeks. (n=3).

Parameters	C	C+ T	25% WSF	25%WSF +T	50% WSF	50%WSF+T
Initial Body Weight (g)	30 ± 1.25^{a}	30 ± 2.5^{a}	30.62 ± 3.12^{a}	30.62 ± 0.62^{a}	30.62 ± 3.12^{a}	30.62 ± 0.62^{a}
Final Body Weight (g)	83.31 ± 1.06^{d}	88.18 ± 1.06^{e}	66.5 ± 1.5^{ab}	70.62 ± 0.62^{c}	65.83 ± 0.83^{a}	69.75 ± 0.25^{bc}
Weight Gain (g)	53.31 ± 0.19^{b}	58.18 ± 1.43^{b}	35.87 ± 4.62^{a}	40 ± 0.0^{a}	35.2 ± 3.95^{a}	39.12 ± 0.37^{a}
Specific Growth Rate %	1.82 ± 0.05^{b}	1.92 ± 0.12^{b}	1.12 ± 0.17^{a}	1.19 ± 0.01^{a}	1.11 ± 0.16^{a}	1.17 ± 0.02^{a}
Feed Conversion Ratio	1.53 ± 0.05^{a}	1.47 ± 0.08^{a}	2.73 ± 0.6^{ab}	3.45 ± 0.24^{b}	2.54 ± 0.61^{ab}	2.58 ± 0.22^{ab}
Mortality %	6.25 ± 6.25^{a}	00 ± 00^{a}	50 ± 12.5^{b}	37.5 ± 12.5^{b}	56.25 ± 6.25^{b}	43.37 ± 6.25^{b}
Survival %	93.75 ± 6.25^{b}	100 ± 0.00^{b}	50 ± 12.5^{a}	62.5 ± 12.5^{a}	43.75 ± 6.25^{a}	56.25 ± 6.25^{a}
Condition Factor %	1.8 ± 0.19^{a}	2.1 ± 0.24^{a}	1.85 ± 0.07^{a}	1.84 ± 0.15^{a}	1.72 ± 0.12^{a}	1.66 ± 0.06^{a}

Data are presented as mean ± S.E.

Data in the same row with different superscript are significantly different ($P<0.05$).

Table 5: Carcass composition of *Cyprinus carpio* fed the experimental diets and exposed of WSF (n=2)

Parameters	C	C + T	25% WSF	25%WSF +T	50% WSF	50% WSF+ T
Moisture (%)	76.02±0.36^{ab}	77.44±0.76^{b}	76.48±1.15^{ab}	75.13±0.18^{ab}	73.21±2.34^{a}	72.8±0.54^{a}
Crude protein (%)*	11.62±0.16^{ab}	17.95±0.55^{d}	15.79±0.29^{c}	13.14±0.64^{b}	12.78±0.28^{b}	10.84±0.65^{a}
Crude lipid (%)*	8.99±0.13^{ab}	8.73±0.29^{b}	9.69±0.47^{ab}	10.70±0.07^{bc}	11.65±1.01^{c}	12.44±0.24^{c}
Ash (%)*	7.21±0.1^{b}	6.74±0.22^{ab}	5.96±0.29^{a}	6.29±0.04^{ab}	7.00±0.61^{ab}	6.8±0.13^{ab}

Data are presented as mean ± S.D.
Data in the same row with different subscript are significantly different ($p<0.05$)
*Dry matter basis.

Table 6: Mean values for water quality parameter at lab (n=2)

No	Characteristics in mg\L	C	C+ T	25% WSF	25%WSF +T	50% WSF	50% WSF+T
1	Temperature	24.37±0.11^{a}	24.56±0.08^{a}	24.20±0.005^{a}	25.10±0.37^{a}	25.25±0.64^{a}	24.60±0.13^{a}
2	Turbidity in NTU	4.20±0.5^{b}	2.40±0.2^{a}	8.4±0.1^{c}	8.30±0.3^{c}	16.50±0.4^{d}	15.80±0.5^{d}
3	Conductivity	402.25±2.25^{a}	399.5±5^{a}	405.25±2.12^{a}	409.25±2.12^{a}	408.61±3.24^{a}	407.25±5.7^{a}
4	PH	7.62±0.15^{a}	7.73±0.15^{a}	7.65±0.15^{a}	7.59±0.15^{a}	7.54±0.05^{a}	7.66±0.2^{a}
5	Electro Conductivity (EC) ms\cm	0.25±0.004^{a}	0.25±0.004^{a}	0.25±0.005^{a}	0.25±0.004^{a}	0.26±0.005^{a}	0.25±0.002^{a}
6	Total Dissolved Solid (ppm)	160.6±0.64^{a}	159.92±0.72^{a}	161.84±0.56^{a}	161.88±0.68^{a}	167.97±0.93^{b}	166.77±0.27^{b}
7	Chloride as (Cl^{-})	21.0±1.0^{a}	19±0.5^{a}	23.0±2.0^{a}	21.25±0.75^{a}	24.0±2.5^{a}	23±1^{a}
8	Sodium (as Na+)	96±3^{a}	96±1^{a}	96±4^{a}	96±5^{a}	96±6^{a}	103±1^{a}
9	Potassium (as K+) opposite	5.20±0.4^{ab}	3.95±0.45^{a}	5.65±0.45^{b}	6.30±0.2^{b}	8.15±0.25^{c}	8.80±0.4^{c}
10	Calcium (as Ca+)	20±0.5^{a}	19±1.5^{a}	20±1.25^{a}	19±0.25^{a}	20.02±0.07^{a}	19±0.35^{a}
11	Magnesium (as Mg+)	39.12±0.87^{d}	36.55±0.4^{c}	32.27±0.37^{a}	35.10±0.6^{bc}	33.24±0.25^{ab}	35.07±0.67^{bc}
12	Total Hardness (as $CaCO^{3}$)	213.5±4.5^{b}	198.5±6.5^{ab}	182.5±6.5^{a}	194±5^{a}	186.5±4.5^{a}	191±1^{a}
13	Total Alkalinity (as $CaCO^{3}$)	160±4^{ab}	156±3^{a}	156±5^{a}	158±4^{a}	168±3^{ab}	172.5±3^{b}
14	Nitrate (as NO^{-3})	20.94±1.01^{a}	19.27±1.32^{a}	19.77±0.87^{a}	19.95±0.75^{a}	23.20±1.35^{a}	22.20±1.05^{a}
15	Sulfate (SO4)	129.65±1.55^{b}	122.4±1.25^{a}	122.05±1^{a}	123.5±0.4^{a}	120.95±2^{a}	125.8±1.85^{ab}
16	Salinity	0.195±0.0021^{ab}	0.19±0.000^{a}	0.195±0.0037^{ab}	0.191±0.0031^{ab}	0.193±0.0006^{ab}	0.198±0.000^{b}
17	Resistivity	2.51±0.04^{a}	2.54±0.03^{a}	2.49±0.01^{a}	2.47±0.02^{a}	2.49±0.015^{a}	2.48±0.025^{a}
18	O2	7.51±0.097^{b}	7.46±0.045^{b}	6.51±0.41^{a}	6±0.015^{a}	6.58±0.36^{a}	6.1±0.05^{a}

Data are presented as mean ± S.D.
Data in the same row with different subscript are significantly different ($p<0.05$)

Table 7: Mean values for some hematological parameters in *Cyprinus carpio* blood fed the experimental diets and exposed of WSF for 8 weeks (n=4).

Parameters	C	C + T	25% WSF	25%WSF +T	50% WSF	50%WSF +T	Total mean
Leucocyte ($\times10^{9}$/L)	98.30±0.58^{b}	98.55±0.51^{b}	82.30±6.06^{a}	96.32±1.44^{b}	90.67±3.42^{ab}	94.32±2.91^{b}	93.41
Lymphocytes# ($\times10^{9}$/L)	67±0.73^{d}	67.2±0.36^{d}	47.62±1.31^{b}	55.8±3.65^{c}	22.47±0.46^{a}	46.78±0.66^{b}	51.14
Monocytes# ($\times10^{9}$/L)	12.29±0.33^{c}	12±0.5^{c}	10.15±0.5^{b}	9.59±0.36^{b}	5.42±0.4^{a}	5.82±0.42^{a}	9.21
Granulocytes # ($\times10^{9}$/L)	20.14±0.57^{b}	19.82±0.81^{b}	19.69±0.61^{b}	18.92±0.50^{b}	14.25±0.36^{a}	19.48±0.86^{b}	18.72
Lymphocytes %	66.97±0.54^{c}	67.02±0.65^{c}	47.95±0.47^{a}	65.13±1.41^{c}	47.57±0.8^{a}	61.59±1.26^{b}	59.37
Monocytes %	11.95±0.4^{c}	12±0.12^{c}	10.47±0.43^{b}	10.3±0.44^{b}	7.11±0.26^{a}	7.36±0.69^{a}	9.86
Granulocytes %	20.07±0.6^{a}	19.95±0.56^{a}	25.96±0.87^{b}	41.07±0.53^{d}	30.86±0.7^{c}	40.70±0.4^{d}	29.77
Haemoglobin g\dL	7.69±0.07^{d}	7.77±0.07^{d}	5.48±0.06^{a}	6.68±0.16^{c}	5.52±0.07^{a}	6.23±0.25^{b}	6.56
Mean Cellular Haemoglobin pg	67.54±4.19^{ab}	68.11±3.26^{ab}	72.16±6.3^{b}	72.77±4.65^{b}	54.99±4.16^{a}	69.13±7.11^{ab}	67.45
Mean Cellular Haemoglobin concentration g/dL	37.31±0.85^{c}	38.39±0.93^{c}	27.56±0.96^{a}	32.46±0.81^{b}	27.11±0.59^{a}	30.28±1.18^{b}	32.18
Erythrocyte ($\times10^{6}$/L)	1.15±0.07^{a}	1.14±0.05^{a}	0.78±0.07^{b}	0.92±0.05^{ab}	1.02±0.08^{a}	0.92±0.09^{ab}	0.99
Mean Cellular Volume fL	181.07±10.81^{a}	178.02±11.07^{a}	264.05±27.74^{b}	224.29±13.89^{ab}	203.43±17.27^{a}	227.81±20.06^{ab}	213.11
Hematocrit %	20.65±0.48^{a}	20.27±0.42^{a}	19.97±0.75^{a}	20.57±0.07^{a}	20.40±0.33^{a}	20.6±0.31^{a}	20.41
Platelet ($\times10^{9}$/L)	4819.25±30.53^{ab}	4857.75±39.6^{b}	5199.37±113.99^{c}	7041.25±63.71^{d}	4546.50±191.04^{a}	7040.00±12.77^{d}	5584.02
Mean Platelet Volume fL	7.05±0.06^{a}	7.05±0.11^{a}	7.63±0.47^{a}	7.05±0.17^{a}	8.60±0.30^{b}	6.80±0.14^{a}	7.36

Data are presented as mean ± S.D.
Data in the same row with different subscript are significantly different ($p<0.05$)

Table 8: Mean values ± SE for some biochemical parameters in serum of *Cyprinus carpio* fed the experimental diets and exposed of WSF (n=3).

No	Parameters	C	C+T	25% WSF	25%WSF +T	50% WSF	50% WSF+T	Total Mean
1	Cholesterol mg/dl	99.49 ± 1.25^{b}	$108.99\pm.6^{bc}$	78.63 ± 2.86^{a}	63.75 ± 1.31^{a}	117.04 ± 4.64^{c}	116.35 ± 4.21^{c}	97.38
2	Glutamic Oxaloacetic Transaminase U/L	318.66 ± 13.29^{bc}	$116.33\pm.96^{a}$	329.66 ± 12.87^{c}	297.33 ± 4.97^{b}	490.66 ± 7.83^{d}	488.66 ± 6.17^{d}	340.22
3	Glutamic-Pyruvic Transaminase U\L	21.5 ± 0.34^{c}	8.53 ± 0.2^{a}	20.66 ± 0.88^{c}	15 ± 0.57^{b}	23.33 ± 1.85^{c}	30 ± 0.57^{d}	19.83
4	Triglyceride mg\dL	333 ± 16.92^{cd}	315 ± 24.26^{cd}	221.66 ± 26.62^{b}	253 ± 44.74^{bc}	112.66 ± 13.24^{a}	376.33 ± 4.91^{d}	268.61
5	Alkaline Phosphate U\L	35.13 ± 2.18^{c}	32.13 ± 1.6^{bc}	32.3 ± 0.47^{bc}	26.15 ± 0.5^{a}	35.15 ± 1.193^{c}	30.55 ± 0.42^{b}	31.90
6	High Density Lipids mg\dL	13.61 ± 1.64^{a}	15.3 ± 1.23^{ab}	21.71 ± 1.66^{bc}	19.58 ± 1.56^{abc}	23.61 ± 0.53^{c}	19.90 ± 3.97^{abc}	18.95
7	Low Density Lipids mg\dl	21.33 ± 0.88^{a}	35 ± 1.52^{b}	17.74 ± 1.25^{a}	20 ± 1.52^{a}	37.33 ± 0.88^{b}	37.33 ± 8.95^{b}	28.12

Data are presented as mean ± S.D.

Data in the same row with different subscript are significantly different ($p<0.05$)

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